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Nanomaterials and Worker Health

Medical Surveillance, Exposure Registries, and Epidemiologic Research Conference
Sponsored by the National Institute for Occupational Safety and Health (NIOSH)
Keystone, Colorado, July 21-23, 2010
Selected Papers

Wolters Kluwer Health | Lippincott Williams & Wilkins

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Selected Papers

Guest Editors

Paul A. Schulte, PhD

Douglas B. Trout, MD, MHS

Laura L. Hodson, MSPH, CIH

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Introduction to the *JOEM* Supplement Nanomaterials and Worker Health

Medical Surveillance, Exposure Registries, and Epidemiologic Research

This issue presents selected articles from the Nanomaterial Workers' Health Conference held in Keystone, Colorado, July 21 to 23, 2010. The conference addressed three critical and related topics: medical surveillance; formation of exposure registries; and the conduct of epidemiologic research. Each topic was introduced with a plenary session followed by group breakout sessions to obtain input from the approximately 120 attendees. This supplement issue of the *Journal of Occupational and Environmental Medicine* includes selected peer reviewed articles from the conference and summaries of the breakout sessions.

The conference was initiated with a general session, and there are articles that provide an overview of the topics (Schulte and Trout) and describe lessons from air pollution particulate epidemiology (Peters et al) and the state-of-the-art of nanotoxicology (Castranova), both of which contributed to the initial concern about potential hazards of nanomaterials. The opening session also included an overview of medical surveillance in the context in which occupational physicians must regularly work and at a time when uncertainties about hazards and risks make decisions about medical surveillance of workers difficult (Nasterlack).

After the opening session, the conference began addressing each of the critical topics beginning with medical surveillance. There is an overview article on the various component elements of medical surveillance, which distinguishes individual surveillance from population surveillance (Trout). This is followed by three examples of nanomaterial workers surveillance programs: one in university research laboratories (Sng et al); the second in a small thriving start-up company (Gause et al), and the third in a multinational corporation that produces more than 50 different nanomaterials (David et al). This is followed by a summary of the surveillance breakout sessions (Fischman et al). Also included is an article that illustrates the role of state agencies in tracking emerging hazards such as those that could occur from nanomaterials (Roisman et al).

In the exposure registry session, the history, utility, and critical issues of exposure registries are described (Schulte et al) and an example from the World Trade Center Registry is presented (Cone and Farfel). Also, experiences collecting registry-type data in the synthetic fiber industry are presented as lessons that may be useful in considerations of registries for nanomaterial workers (Marchant and Crane).

The epidemiological research session begins with a commentary on epidemiologic challenge for studies of occupational exposure to engineered nanomaterials (Eisen et al). Aspects of future epidemiologic studies are addressed in five articles. Two are from feasibility studies of carbonaceous nanomaterial manufacturers and users who describe the nature of the materials, the size of the workforce (Schubauer-Berigan et al), and the extent of preventive control use (Dahm et al). Since cross-sectional and prospective studies utilizing biomarkers have been identified as useful approaches to identify potential adverse effects in workers, two papers are presented that describe some of the most promising biomarkers that may be used in these studies (Li and Nel; Eardley et al). This is followed by a summary of the breakout session on issues in designing strategies for studies of nanomaterial workers (Laney et al).

Meanwhile, as society waits for the results of epidemiologic research, it is still possible to assess risks to workers. To illustrate this, there is an article describing how animal data for carbon nanotubes can be modeled to assess risks (Kuempel). This is followed by an article illustrating how the Environmental Protection Agency has used the Toxic Substance Control Act to assess occupational risks (Sayre et al).

Finally, two articles summarize the conference. One is a pioneering effort by French government investigators to develop a program to register nanomaterial workers, conduct medical surveillance, and initiate epidemiologic research (Boutou-Kempf et al). The other is a reflection that Dr William Halperin gave to close the meeting (Kreider and Halperin). Dr Halperin has more than 30 years of experience addressing the surveillance and epidemiologic research of workers at risk, and he applied that experience to nanomaterial workers and how we may avoid the mistakes of the past while dealing with this emerging technology.

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The organizers are grateful for all who participated in the conference and wrote the articles included in this issue. As Dr Halperin noted, someday society may look back on the early stage of nanotechnology and ask whether appropriate caution was taken. This conference and the resultant articles may contribute to an affirmative answer to society's question.

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Nanomaterials and Worker Health

Medical Surveillance, Exposure Registries, and Epidemiologic Research

Paul A. Schulte, PhD and Douglas B. Trout, MD

Objective: This article provides an overview of the issues that arise with medical surveillance, exposure registration, and epidemiologic research involving nanomaterial workers. **Methods:** An occupational health perspective is applied to detecting risks in nanomaterial workers individually and as a group. **Results:** General principles for medical surveillance, exposure registration, and epidemiologic research are identified. A model Nanomaterial Worker Health Study is for consideration. **Conclusions:** The Nanomaterial Worker Health Study can be developed as a tangible action in assuring the public that steps are being taken to learn of any adverse effects from exposure to nanomaterials.

Increasing numbers of workers are involved in research, manufacture, use, and disposal of nanomaterials, but it is not known whether these workers are at risk for adverse health effects, despite a coalescing body of evidence that exposure to some nanomaterials can cause adverse health effects in animals.[1] To protect these workers, precautionary risk management guidance has been issued worldwide.[2–7] To further support the precautionary approach, it is necessary to consider what medical surveillance is warranted for nanomaterial workers and the issues that arise in establishing epidemiologic studies and exposure registries. Critical in protecting the health of workers involved with a new technology, such as nanotechnology, is the need to assess their risks and determine whether risk management programs are functioning effectively. Medical surveillance, exposure registries, and epidemiologic research are three related ways to provide such risk-related ascertainments.[8] The evidence for a precautionary approach to preventing adverse effects from engineered nanomaterials includes research concerning health effects from exposure to small-particle air pollution, incidental nanoparticles in welding and diesel engines, as well as studies in the last 10 years, specifically addressing engineered nanoparticles.[4,9–17] Underlying knowledge of the health effects of particles and fibers also supports concern over worker exposure to nanomaterials.[17] A precautionary approach includes following the hierarchy of prevention (substitution, engineering controls, administrative controls, personal protective equipment, and training) and supporting that hierarchy requires industrial hygiene evaluation to determine whether controls are working and whether there is any residual risk.[18] Also, incumbent in the precautionary approach is the need to anticipate hazards of nanomaterials and develop material screening and testing strategies and guidance for controlling categories of nanomaterials.[19–21]

EXTENT OF EXPOSURE TO NANOMATERIALS

There is an extremely small (but growing) published literature base on the extent of exposure to nanomaterials.[22–29] In part, this is due to a variety of issues, including the relative newness of exposure scenarios, the inconsistencies over how to identify and classify nanomaterials, questions about metrics and practical instrumentation, and difficulty finding and gaining access to workplaces. For the most part, the published literature shows relatively low mass (weight) exposure to nanomaterials compared with bulk counterparts.[27,28] Nevertheless, this finding must be qualified since low mass concentrations can represent high numbers of airborne nanoparticles, and the methods for sampling and analyzing these materials are still evolving.[29] Many companies where nanomaterials have been investigated, manufactured, or utilized have operations that are controlled (isolated, contained, or exhausted).[30,31] Nevertheless, some do not and relatively high, process specific, short-term exposures have been reported.[28,31] Thus far, there has not been a wide range of operations assessed, and in many cases, personal breathing zone measurements are lacking. There is virtually no published information to date on exposures of workers using engineered nanomaterials downstream from their manufacturing (eg, repackaging of dry nanoparticles spray application involving nanomaterials). Nevertheless, simulations indicate that exposures can occur.[32] A more complete understanding of toxic potential and the extent of exposure within and across companies is required and will be the foundation on which occupational health surveillance programs will be based.

OCCUPATIONAL HEALTH SURVEILLANCE

Occupational health surveillance includes hazard surveillance, which involves identifying potentially hazardous practices or exposures in the workplace and assessing the extent to which they can be linked to workers, the effectiveness of controls, and the reliability of exposure measures.[8,18,33,34] Occupational health surveillance is also an umbrella term that includes monitoring of health outcomes or biological changes,[35,36] including medical surveillance of effects at the group and individual level. At the individual level, medical screening involves examination of the health status of an exposed person or persons by tracking of illness or change of biologic functions to detect early signs of work-related disease by administering tests to asymptomatic workers.[37] Numerous Occupational Safety and Health Administration standards and National Institute for Occupational Safety and Health (NIOSH) recommendations specify this type of medical surveillance of workers when there is exposure to a specific workplace hazard.

Occupational health surveillance is part of the standard practice of occupational safety and health.[38,39] National Institute for Occupational Safety and Health guidance issued in 2009 concerning surveillance for workers exposed to engineered nanomaterials included the general recommendation that occupational health surveillance is an important part of a risk management program.[39] In that guidance, a strong recommendation for the conduct of hazard surveillance was made, however, no specific medical screening recommendation was given. The evolving evidence base about potential hazards of occupational exposure to engineered nanomaterials most likely will increase the need to include specific medical surveillance and screening programs as part of the complete occupational health surveillance program. For example, animal studies on carbon nanotubes have shown that pulmonary fibrosis can be a significant health effect of exposure.[13,15,16] In January 2011, NIOSH posted

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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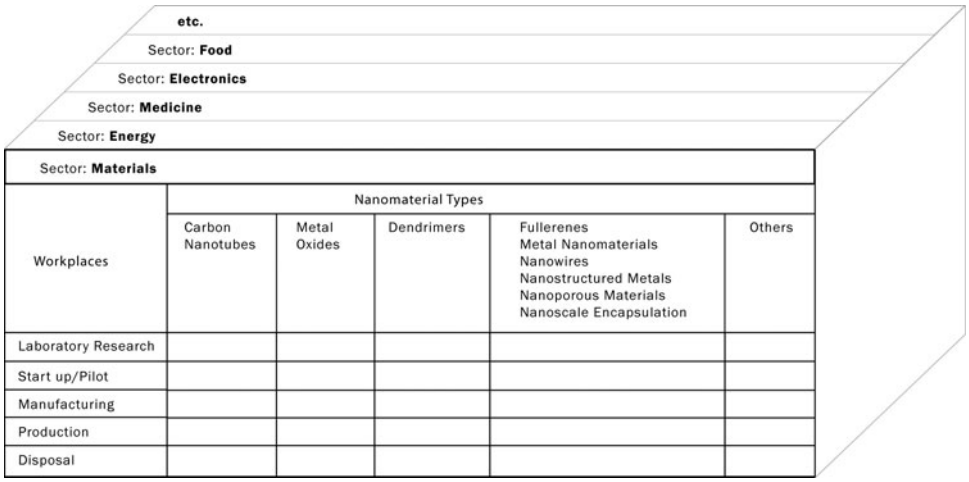

FIGURE 1. Framework for identifying worksites with occupational exposure to engineered nanoparticles (Adapted from reference 42).

on its Web site for public comment a draft Current Intelligence Bulletin on carbon nanotubes/carbon nanofibers.[40] In it, in addition, to risk assessment, recommended exposure limits, and control recommendations, NIOSH recommended baseline and periodic medical surveillance. They included x-ray and spirometry among other assessment techniques.

As nanotechnology permeates the various economic sectors, more products will be manufactured and more occupational exposure will be likely. The workplaces where worker exposure can occur can be depicted by a three-axis matrix of workplace types (functions) × nanomaterial types × business sectors as shown in Fig. 1. The number of cells in this matrix is vast due to the many different types of potential nanoparticles and nanomaterials and the broad array of products and uses.[41,42] While the recommendation for hazard surveillance and precautionary risk management applies across the matrix, specific medical surveillance guidance will need to be tailored categorically. Implementing occupational health surveillance at work sites will allow for the development of baseline and then, if the surveillance is ongoing, periodic assessments and analysis of data, which can serve to alert workers, employers, governmental authorities of any failures of prevention.

EPIDEMIOLOGIC RESEARCH AND EXPOSURE REGISTRIES

Two tools will be useful to augment the implementation and impact of occupational health surveillance. These are the conduct of epidemiologic research and the formation of exposure registries. Epidemiologic research can involve the analysis of occupational health surveillance data to identify potential health effects of exposures, and it can include etiologic investigations of the relationship between exposure to specific nanomaterials and resultant health effects. While epidemiologic investigation of the health effects of nanomaterials is not inherently different from assessing the effects of other potential occupational hazards, there are some factors that are more pronounced.[41] These include heterogeneity of nanoparticles, temporal factors, difficulty identifying a study population, and difficulty obtaining exposure information. The heterogeneity of nanoparticles is the result of a large number of physicochemical parameters and production conditions that can lead to a vast number of different types of nanoparticles. These parameters and conditions include combinations of such factors as size, shape, solubility, surface change, surface coating, crystal structure, and contaminants. The potential toxicity of a nanoparticle can vary, depending on the combination of these factors. Thus, it may be difficult to find study population with similar enough exposures to form cohorts of adequate size for epidemiologic study.

Another issue is that engineered nanomaterials have only been in commerce for limited time. The current size and location of the nanomaterial workforce is difficult to ascertain; although growing, the nanomaterial workforce still could be relatively small currently; however, there are few useful published estimates. Nanotechnology and nanoscience, while having historical precursors, did not readily begin to emerge until the 1980s with the development of techniques to "visualize" nanoparticles and the understanding of scientific and commercial properties of matter at the nanoscale. Commercial production of "nano-enabled" products generally began in the late 1990s. Clearly the first workers to be exposed are those in scientific laboratories in academia and commercial enterprises. The next workers exposed included those involved in pilot and start-up operations.[42] As these efforts become viable on larger scales, manufacturing will increase in volume. Nanomaterials will likely be provided to an increasingly wide array of users who will incorporate them in an increasing variety of products. An increase in occupational exposure of workers involved in the handling, machining, or otherwise processing products containing nanomaterials should be expected. Finally, workers involved with all aspects of end of life of products containing nanomaterials may have increasing exposures to nanomaterials in the future.

As discussed previously, large industrial cohorts (which were the source populations for occupational epidemiology in the past) do not exist for nanomaterials currently. The difficulty in obtaining exposure information and characterizing study populations is exacerbated by the fact that the necessary information for epidemiologic research is often viewed as proprietary. Employers may not be willing to make such information available, because it may affect their competitive edge. New approaches for identifying and characterizing study populations will be needed. One approach that may be useful in setting the stage for epidemiologic research is the use of exposure registries. An exposure registry is the enumeration and identification of exposed individuals for the purpose of providing them information and guidance about potential risk from exposures.[43] Exposure registries also may be sampling frames for epidemiologic research. While exposure registries have been used in public health for more than 50 years, they are costly and have various positive and negative aspects. On the positive side, they may provide for timely information

to workers and fostering development of epidemiologic studies. On the negative side, they may raise undue expectations among workers about medical monitoring and treatment and may be a vehicle for premature legal action. Another article in this issue provides a comprehensive overview of the history of exposure registries and their positive and negative aspects.[44]

PROSPECTUS FOR A NANOMATERIALS WORKER HEALTH STUDY

Rationale

The growing body of evidence about the potential health risks of nanomaterials demands that industry, labor, and government take concerted action to protect the health of workers. Workers are the first people in society with significant exposure to a new technology such as nanotechnology. It is critical that the potentially highly beneficial impact of harnessing phenomena at the nanoscale is not delayed or impaired because society did not take the appropriate anticipatory steps. First and foremost is the need, already begun, to take precautionary steps to control engineered nanomaterial exposures in all workplaces throughout the life cycle of the material. While further investigation is still required, effective control knowledge is available and has been recommended by many governments and organizations.[2–8] Nevertheless, to ensure that all efforts are being taken to learn of any deleterious effects that can occur from exposure to nanomaterials, there is need for a program of workforce medical surveillance and epidemiologic investigation that will indicate any failures of the preventive efforts that are in place. This program can serve as a model effort that combines exposure registration, medical surveillance, and epidemiologic research in a coordinated effort. Already such a program is being proposed in France (Boutou-Kempf et al).[45]

Scope of the Study

A health surveillance and epidemiologic investigation program can be envisioned to include a registry of a large number (perhaps at least 5000) of workers from companies handling different nanomaterials. This registry would serve as a source group in which various analytical studies will be conducted. The exposure and health of the entire group would be monitored initially and periodically over a 5- to10-year period (Fig. 2). In addition, parallel registries and studies in other countries would be promoted by using common metrics and health endpoints. The addition of these workers may allow for the development of cross-national cohorts with common exposures that can be studied prospectively. There are many questions that would need to be resolved in the planning of such a study. These include such issues as participation, coordination, access to data, confidentiality, funding, representativeness, and many more.

Partnership—Funding, Planning, and Initiation

The critical issues in the success of this endeavor are the participation of all three of the major stakeholders: industry, labor, and government. It is envisioned that there would be a transparent tripartite partnership that will be the governing body for this study. The National Institute for Occupational Safety and Health could serve as a coordinator of the study in collaboration with investigators from other agencies and organizations. The governing body of this partnership should consist of business trade associations, government agencies, labor unions, and academia.

Industry, government, and labor, all have responsibility in ensuring that workers are protected from a new technology such as nanotechnology, and all would have an interest that responsible efforts are made to consider and prevent potential health effects in workers. It is envisioned that industry and government would provide funds for this partnership and for studies that are developed.

To initiate this process, a working group (with representatives of industry, labor, government, and academia) would be established to formulate the basis of the partnership (including such aspects as a governing council and plans for initial funding) and plan for future studies. It is beyond the scope of this article to identify the details and estimate the costs of initiating and maintaining such a study; that will be a function of the working group.

STRENGTHS AND WEAKNESSES

The idea of a Nanomaterial Workers Health Study is outlined in brief terms. The strength of this approach is that it would serve as a useful resource to assess questions about worker exposure to

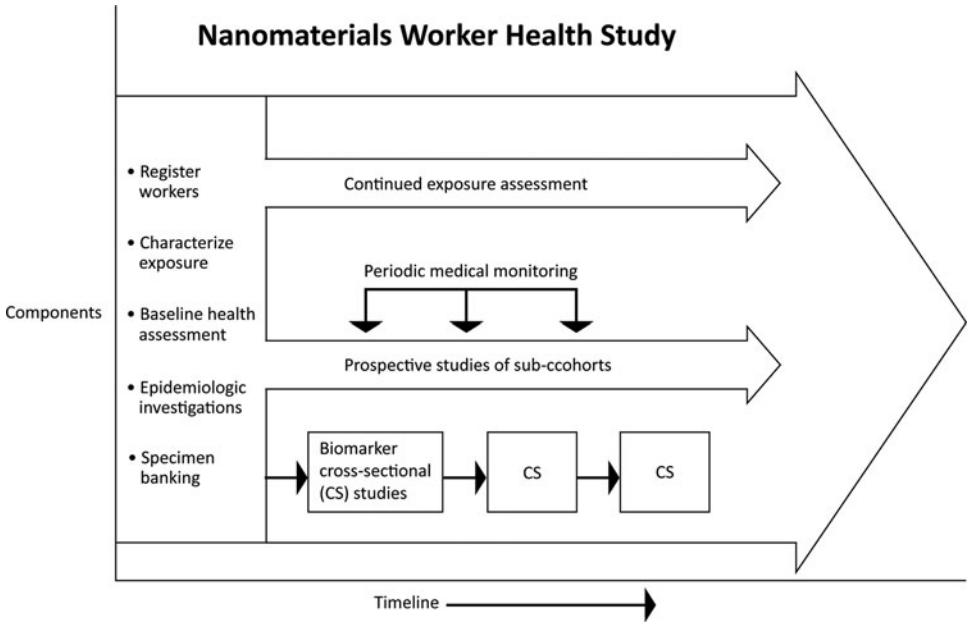

FIGURE 2. Schematic of a nanomaterials worker health study.

nanomaterials, risks from exposure, and utility of controls. It can inform investigators of issues in conducting further studies, and it can provide leads for assessing early indicators of effects. In terms of weaknesses, it may not be representative of the range of exposures and controls since the companies willing to participate in such a multiorganization study may well be those with the best occupational hygiene performance. This can influence the prospect of detecting any health risks, if a causal relationship between nanomaterials and adverse health effects exists. Consequently, it will be important to recruit a range of companies to participate in the study.

CONCLUSION

Implementing occupational health surveillance with focused medical surveillance components is of growing importance as more is learned about the hazards of occupational exposure to various nanomaterials. Since there are current and future workforces that have or will have exposure, precautionary approaches to controlling exposure are warranted. Along with these approaches is the need for developing an investigational strategy for assessing risks to groups of workers through consideration of exposure registries and epidemiological research. Both occupational health surveillance and epidemiologic research will help to identify risks to workers from uncontrolled or poorly controlled exposures. This will allow for further refinement of controlled procedures. If society is to benefit from nanotechnology, it is critical that all steps to protect and assure worker safety are taken, including occupational health surveillance, consideration of exposure registries, and epidemiologic research.

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Lessons From Air Pollution Epidemiology for Studies of Engineered Nanomaterials

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Objectives: This article discusses evidence from epidemiological studies on air pollution for assessing engineered nano-sized particles in workplace environments. **Methods:** Results from epidemiological studies on health effects of fine and ultrafine particles are summarized. These findings are applied to workplaces exposed to engineered nanoparticles. **Results:** Ultrafine or nano-sized particles smaller than 100 nm represent potential health hazards. Because of their short half-lives in ambient air and their large spatial variability, individual exposures in population-based studies are likely to be misclassified. **Conclusions:** Studies of health effects of nanoparticles in occupational settings seem mandated for adequate worker protection but face several challenges, including exposure quantification and adequate confounder characterization. Inclusion of personal measurements of ultrafine particles in future studies will allow exploiting the full scale of temporal-spatial variation of both ambient and engineered nanoparticles.

Ambient particulate matter has been a long-standing concern to induce short-term as well as long-term health effects.[1–3] The size, shape, and density of the particles determine their behavior in the gas phase of the aerosols. As the airways are the major surfaces of interaction, particles with a diameter of less than 10 μm (PM_{10}) entering the airways and with a diameter less than 2.5 μm (fine particles, $PM_{2.5}$) entering the lungs are of primary concern. Nano-sized particles, also called ultrafine particles (UFP), with a diameter less than 100 nm have different properties than larger particles.

1. They deposit with high efficiency in the alveolar region and to a lesser extent in the larger airways.[4]
2. Their motion is defined by diffusion rather than their aerodynamic properties.[4]
3. They have little mass but high number and surface area concentrations.[5]
4. They are not well recognized and are cleared by macrophages in the alveolar space.[6]
5. They potentially translocate into cells through diffusion mechanisms.[7]

In addition to these physical and toxicological properties, the UFP may have a different composition than larger particles in urban atmospheres.[8] In particular, their major sources are local combustion sources, while other sources such as secondary aerosol formation through regional transport or resuspension of dusts do not generally contribute substantially to the fraction of UFP in ambient air.[9] Therefore, UFP have a higher content of soot and organic carbon, while sulfates and nitrates are predominantly found in the accumulation mode range.

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Because of the different properties of ultrafine or nano-sized particles, they are often characterized by number concentration, whereas fine particles are most frequently characterized by the measurements of mass concentration. The measurement of number concentrations for ambient UFP captures their underlying mechanism, which is surface activity based rather then mass based.[10] Also, there is usually too little mass of UFP in ambient air to be measured on an hourly or a 24-hour basis. Toxicological studies often choose to use both metrics, the number and the mass concentrations, to be able to compare the effects.

While a lot of information is available on the health effects of the mass of $PM_{2.5}$ or PM_{10}, substantially fewer studies have assessed the health effects of UFP. In this article, we will briefly summarize the evidence available on health effects of fine particles, highlight findings from studies that have assessed health effects of UFP, and provide an outlook on the potential of applying these findings to workplace settings, where employees may be exposed to engineered nanoparticles.

HEALTH EFFECTS OF FINE PARTICLES

Air pollution not only affects the lungs, as one may intuitively expect, but can have negative impacts on several parts of the human body, as shown in Fig. 1. Mortality is the most studied health endpoint in association with air pollution due to the widespread availability of mortality data for large populations and the importance of mortality in estimating health impacts.

Long-term studies compare mortality rates across populations that vary in their long-term exposure to air pollution, usually using a cohort design. The Harvard Six Cities study[11,12] and the American Cancer Society study,[13,14] first published in the mid 1990s, show a clear increase in all-cause mortality, especially in cardiovascular or cardiopulmonary mortality in association with $PM_{2.5}$. The extended reanalysis of the Harvard Six Cities study by Laden et al[11] showed that a reduction in $PM_{2.5}$ levels resulted in a reduced long-term risk of cardiovascular and respiratory disease mortality over the 16-year period of the study. These associations are mostly attributable to cardiovascular disease mortality.[15] Detailed analyses by Pope et al[15] showed that the largest specific cause of death was ischemic heart disease, which represented almost 25% of all deaths. Myocardial infarction accounted for about half of this category. In addition, statistically significant associations were found for the combined category of dysrhythmias, heart failure, and cardiac arrest.

Short-term studies, usually time-series and case-crossover studies, explore associations between short-term changes in air pollution exposure and daily mortality rates. There are a large number of time-series studies on the association between daily mortality rates and PM_{10} or $PM_{2.5}$ published. In the United States, for example, the "National Morbidity, Mortality and Air Pollution Study," originally conducted in 20 and later in 90 of the largest cities and metropolitan areas in the United States from 1987 to 1994, reported small but constant positive associations between PM_{10} and death.[16] These findings were confirmed in several reanalyses.[17–19] With a comparable approach, hospital admission data have also been analyzed, indicating that on days with elevated PM concentrations, hospital admissions for cardiovascular and respiratory diseases are more frequent.[20–23]

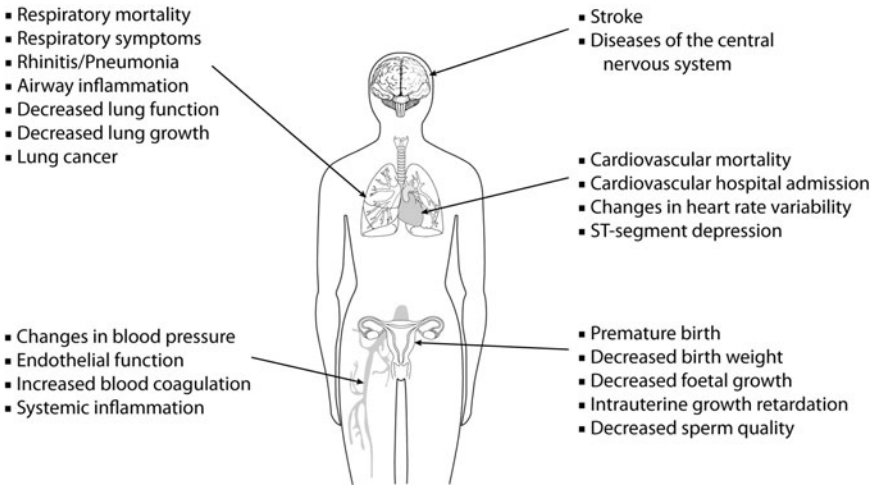

FIGURE 1. Organs of the human body that can be affected by air pollution.

To further establish these associations, small cohort studies, so called panel studies, employing repeated measurements of intermediate phenotypes, for example, measurements of lung function, blood biomarkers, or electrocardiograms, were conducted. These studies provide consistent evidence that on days with high ambient particulate matter exposures or after cumulative exposures over several days, a deterioration of pulmonary[24,25] and cardiovascular function[26] can be observed. In particular, susceptible subgroups are affected, including children and individuals with pulmonary or cardiovascular disease and diabetes or individuals of old age. These findings have been interpreted as being coherent with the both the short-term exacerbation and the long-term effects observed in association with particulate matter exposures.[27] Overall, the growing evidence on the cardiovascular effects of ambient fine particles in urban areas has provided the basis for an update of the global air-quality guidelines and a call for more stringent standards still to be met all over the world.[28]

HEALTH EFFECTS OF ULTRAFINE PARTICLES

Considerations Regarding Exposure Assessment

Studying health effects of UFP pose several challenges. First, the concentrations of UFP are generally not being monitored for regulatory purposes, so that additional air-monitoring efforts are needed to characterize outdoor concentrations of UFP for epidemiological studies. An often used, proxy for UFP is the (total) particle number concentration (PNC). Measurement of UFP requires additional equipment, exposure assessment expertise, and quality assurance measures. Second, the spatial distribution of UFP is substantially more heterogeneous than for fine particles, as shown in the schematic drawing of Fig. 2. In particular, major roads are hot spots for UFP exposures as well as other traffic-related pollutants, such as carbon monoxide, nitrogen oxides, or organic hydrocarbons, originating from incomplete combustion. Therefore, thoughtful selection of the measurement site(s) and multiple measurement sites may be needed under the consideration of a particular epidemiological study design.

Despite high variability within an urban area, reasonable correlations over time have been observed for UFP when measured at urban background stations.[29–32]

Associations Between Mortality and Ultrafine Particles

Long-term health effects of UFP have not been published to date. Nevertheless, studies assessing the role of major roads on long-term health have indicated that effects of fine particles are larger in these settings,[33,34] suggesting that potentially also the UFP contribute to this strengthened association.

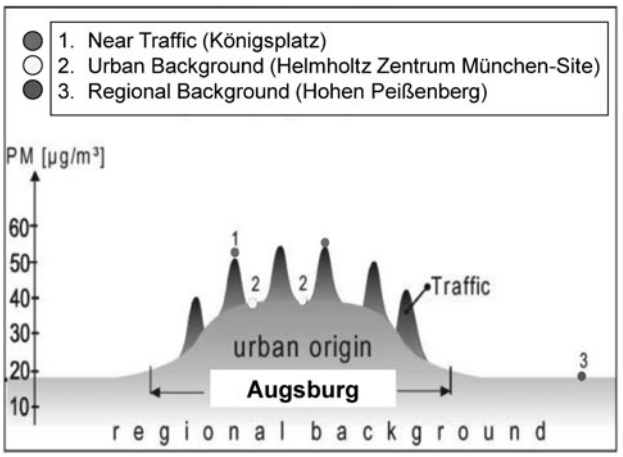

FIGURE 2. Schematic two-dimensional representation of the spatial variation of ambient particles, using three measurement sites in Augsburg, Germany,[29,80] as an example (adapted from Lenschow et al[81]). On the *x* axis, the diameter of the region is given, which is approximately 10 km; on the *y* axis, PM_{10} is shown on typical winter days.

Short-term studies on UFP and mortality are still rare (Table 1). One of the first short-term studies on UFP and mortality was published by Wichmann et al[35] in 2000. They analyzed all-cause, cardiovascular, and respiratory mortality in Erfurt, Germany, and found independent effects of both fine and ultrafine particles. Results suggested a more delayed association for UFP than for fine particles, and the overall association was slightly stronger for respiratory diseases than for cardiovascular diseases. When Stölzel and colleagues[36] reanalyzed the data by using an extended data set (September 1995 to August 2001) as well as an alternative modeling approach similar to the Air Pollution and Health: A European Approach (APHEA) 2 study,[37] they also found a small increase in total and cardiopulmonary mortality in association with different size ranges of UFP for a lag of 4 days. In contrast to the first study in Erfurt, they did not see associations for fine particle mass with total or cause-specific mortality. A recent reanalysis by Breitner et al,[38] moreover, evaluated changes in the association between daily mortality and UFP, as air quality substantially improved during the study

TABLE 1. Short Term Studies on Selected Air Pollutants and Mortality

Authors, Year, Reference Number	City, Country	Study Period	Outcome	Association
Breitner et al, 2009[38]	Erfurt, Germany	Oct 1991–Mar 2002	All-cause	$PM_{2.5}$‡ UFP*↑
Stölzel et al, 2007[36]	Erfurt, Germany	Sept 1995–Aug 2001	All-cause	$PM_{2.5}$‡ UFP* ↑
			Cardiorespiratory	$PM_{2.5}$‡ UFP* ↑
Wichmann et al, 2000[35]	Erfurt, Germany	Sept 1995–Dec 1998	All-cause	$PM_{2.5}$* ↑; UFP* ↑
			Respiratory	$PM_{2.5}$* ↑; UFP* ↑
			Cardiovascular	$PM_{2.5}$* ↑; UFP ↑
Ostro et al, 2007[42]	Six counties in California	Jan 2000–Dec 2003	All-cause	$PM_{2.5}$† ↑; EC† OC†
			Cardiovascular	$PM_{2.5}$* ↑; EC* ↑ OC* ↑
			Respiratory	$PM_{2.5}$‡† ↑ EC† OC† ‡Only among those >65 years of age
Ostro et al, 2008[43]	Six counties in California	Jan 2000–Dec 2003	Cardiovascular	$PM_{2.5}$* ↑; EC* ↑ OC* ↑
Forastiere et al, 2005[39]	Rome, Italy	1998—2000	Cardiovascular	PM_{10}* ↑; PNC* ↑
Samoli et al, 2005[41]	22 (PM_{10}) and 15 (BS) European cities from the APHEA Project	At least three consecutive years between 1990 and 1997	All-cause	PM_{10}* ↑; BS* ↑
			Cardiovascular	PM_{10}* ↑; BS* ↑
			Respiratory	PM_{10}* ↑; BS* ↑

APHEA, Air Pollution and Health: A European Approach; BS, black smoke; EC, elemental carbon; OC, organic carbon; $PM_{2.5}$, particle mass <2.5 μm in diameter; PM_{10}, particle mass <10 μm in diameter; PNC, particle number concentration; UFP, ultrafine particles, particles <100 nm.
↑ *Significant association.
↑ †Small non-significant association.
‡No association.

period. Overall, relative risk estimates were consistent but somewhat smaller than in the previous analyses. Results further suggested that the relative risks for short-term associations of UFP decreased as pollution control measures were implemented in Eastern Germany. In addition to the German study, results from a case-crossover study in Rome indicate an association between fatal coronary events and PNC, PM_{10}, and CO, which appeared strongest for the age group greater than 65 years.[39] A group of experts estimated that a reduction of 1000 particles cm^{-3} would result in a 0.3% reduced risk of mortality, with a 95% confidence interval ranging from 0.1% to 0.9%.[40]

A big European multicity study found positive associations for black smoke and all-cause, cardiovascular, and respiratory mortality.[41]

The few studies considering the associations between mortality and elemental carbon (EC) and organic carbon indicate a positive association, especially for low-educated people.[42,43]

Associations Between Respiratory Disease Exacerbation and Ultrafine Particles

One of the first studies examining the association between UFP and respiratory health in a group of adults with asthma was published by Peters et al.[44] Participants kept a symptom diary and measured peak expiratory flow (PEF) daily for a period of 6 months. The authors found small but consistent associations between elevated fine particles and UFP and a decrease in PEF, an increase in cough, and feeling ill during the day. Associations for PEF were more pronounced for the ultrafine fraction. Analogue results for PEF were seen in a similar Finnish study,[45] however, no associations were observed with respiratory symptoms or medication use. Another panel study on adult asthma patients in Germany, on the contrary, showed that an increase in UFP was associated with the use of corticosteroids and β_2-agonists.[46]

A more recent study in London, England, compared lung function parameters in adults with mild or moderate asthma, walking along Oxford Street, a busy shopping street with a lot of diesel-powered bus traffic, and walking in a nearby park. The authors found that reductions in the forced expiratory volume in 1 second, forced vital capacity, forced expiratory flow at 25% to 75% of vital capacity, and exhaled breath condensate pH were associated with UFP exposure at most measured time points. Results were similar for EC, while there were no consistent associations for $PM_{2.5}$. This fact may indicate that the carbon core of the particles is responsible for the health effects. The authors concluded that UFP and EC might only be sensitive proxy for roadside diesel exposure, a complex mixture of diesel exhaust and resuspended particles.[47] Nevertheless, other studies found no or only small associations between ambient air pollutants and lung function.[48–50] Up to now, only one study on hospital admission for respiratory diseases has been carried out.[51] This study, conducted in Copenhagen, Denmark, extracted daily counts of hospital admissions for respiratory diseases in the elderly (≥65 years) and asthmatic children (5 to 18 years) for 3.5 years and associated the daily counts with air pollution data from a central monitoring site. The authors found significant associations between hospital admission for respiratory diseases and total number concentrations; however, associations diminished after additional adjustment for PM_{10} or $PM_{2.5}$. Taken together, the few epidemiological studies conducted on effects of UFP indicate an adverse relationship on respiratory outcomes; however, results are not consistent.

A study by Heinrich et al[52] used traffic intensity estimated from residential street type as a proxy for combustion-related particle exposure in a cross-sectional study in almost 7000 German adults. They found that living at extremely or considerably busy roads was associated with chronic bronchitis. Positive but not statistically significant associations were seen for nocturnal coughing attacks, wheeze during the past 12 months, and hay fever, while no increases were seen for asthma.

Additional studies in Sweden,[53] Switzerland,[54] and California,[55] demonstrated associations between living close to a major road and symptoms and diagnosis of asthma, chronic bronchitis, and hospital encounters in asthmatic children. Recent analyses of

The California Children's Health Study also showed that new-onset asthma is associated with traffic-related pollution near homes and schools.[56]

Associations Between Cardiovascular Disease Exacerbation and Ultrafine Particles

Several studies have examined the association between hospital admission due to cardiovascular disease and indicators of UFP. A multicenter cohort study on myocardial infarction (MI) survivors showed an increased risk of cardiac readmission to hospital during days with elevated concentrations of urban air pollution, including PNC.[57] In addition, an association was found between exposure to traffic and the onset of MI within 1 hour in a study in Augsburg, Germany.[58] While traveling in a car was the most common source of exposure, associations did not differ much for people who had used public transport. The overall effect estimate also did not change in multivariate analyses adjusting for stress (anger), strenuous activity, or getting up in the morning—factors that are also considered to transiently increase the risk of MI. Results from the APHEA study also indicate an association between black smoke and hospital admission for cardiac events, especially in people older than 65 years.[23]

In addition to comparatively rare severe events such as myocardial infarction or death, more and more studies use parameters, which reflect subclinical physiological responses possibly related to the risk of cardiovascular disease to examine the impact of air pollution. Studies on these more subtle responses support the credibility of the observed associations and provide insight into possible mechanisms that link the inhalation of particles with adverse health outcomes. Air pollution may influence different elements of heart function.[59] An imbalance in the autonomic nervous system is, for example, reflected by changes in heart rate variability (HRV). Regarding HRV, a recent study reported an association between being in traffic in the previous 2 hours and a decrease in the high-frequency component of HRV.[60] Timonen et al[61] found an association between PNC and the ratio of low frequency to high frequency during a period of paced breathing up to 3 days after exposure in a panel of cardiac patients in three European cities. Park et al,[62] on the contrary, did not see any association for HRV with PNC 4, 24, or 48 hours after exposure. In a small study on ten and five participants, respectively, an association between personal $PM_{2.5}$ as well as PNC measurements and HRV parameters was found.[63] Associations were more delayed but more pronounced for PNC despite the smaller number of observations.

In 2002, Pekkanen et al[64] reported an increased risk of exercise-induced ST-segment depression, a marker for myocardial ischemia, in association with fine particles and UFP two days before the clinical visit among subjects with coronary heart disease in Helsinki, Finland. Since then, several studies on ST-segment depression have been conducted (Table 2). Other examined parameters include QT interval prolongation as well as T-wave amplitude and T-wave complexity,[65] both repolarization parameters that play a critical role in arrhythmogenesis, and the number of ventricular and supraventricular runs[66] reflecting an increased risk of arrhythmia by traffic. Zanobetti et al[67] detected an association between being in traffic in the previous 2 hours and T-wave alternans, a marker of cardiac electrical instability in a panel of patients with documented coronary artery disease. Ibald-Mulli[68] found no association between UFP and blood pressure. More recent results from Delfino et al[69] showed an association only during periods of high exertion.

Concerns for Other Outcomes

Recent evidence suggested that also in utero growth may be impaired by ambient particulate matter.[70–73] Ultrafine particles may be of concern for their potential to transgress the placenta.[74]

In addition, there is growing concern that systemic effects of ambient particles may also involve the central nervous system.[75,76]

TABLE 2. Examples of Epidemiological Studies on Ischemia and Repolarization Abnormalities, Including Measurements of Ambient Ultrafine or Carbonaceous Particles

Outcome Variables	Exposure Variables
ST-segment depression	
Chuang et al, 2008	BC, $PM_{2.5}$
Lanki et al, 2008	$PM_{2.5}$ (outdoor + personal), UFP
Mills et al, 2007	Diluted diesel exhaust, exposure study
Gold et al, 2005	BC
Pekkanen et al, 2002[64]	$PM_{2.5}$, UFP
QT-interval prolongation	
Baja et al, 2010	$PM_{2.5}$, O_3, BC, NO_2, CO, SO_2
Henneberger et al, 2005[65]	UFP, ACP, $PM_{2.5}$, OC, EC, NO_2, CO, NO
T-wave amplitude and complexity	
Henneberger et al, 2005[65]	UFP, ACP, $PM_{2.5}$, OC, EC, NO_2, CO, NO
Arrhythmias	
Berger et al, 2006[66]	UFP, ACP, $PM_{2.5}$, PM_{10}, SO_2, NO_2, CO, NO

ACP, accumulation mode particles, raging from 100 nm to 1000 nm; BC, black carbon; BS, black smoke; CO, carbon monoxide; EC, elemental carbon; NO, nitric oxide; NO_2, nitrogen dioxide; O_3, ozone; OC, organic carbon; $PM_{2.5}$, particle mass $<2.5\mu$m in diameter; PM_{10}, particle mass $<10\mu$m in diameter; PNC, particle number concentration; SO_2, sulfur dioxide; UFP, ultrafine particles, particles <100 nm.

Again, the potential of the UFP to translocate[77] may provide a mechanism that could present an additional risk for disease development as inflammatory processes in the central nervous system are crucial in neurodegenerative diseases such as Alzheimer disease and Parkinson disease, the two most prevalent neurodegenerative diseases.[78]

LESSONS LEARNED FROM AIR POLLUTION EPIDEMIOLOGY FOR NANOPARTICLE RESEARCH IN OCCUPATIONAL SETTINGS

Overall, epidemiological studies that have assessed health effects of UFP provide evidence that early transient effects may be induced by elevated exposures. While the picture is not entirely consistent, it warrants sufficient concern about UFP in various settings through the inherent difficulties of estimating population average or individual exposures. It is important to note that in ambient settings, a fraction of the UFP may be present as droplets rather than solid particles.[79] Nevertheless, only the findings that are considered to be attributable to the nonvolatile portion of the UFP are directly transferable to occupational exposures of engineered solid particles. Therefore, the additional consistency between studies of UFP and carbonaceous particles, which are predominately ultrafine, is an important observation.

Epidemiological long-term studies on fine particulate air pollution are mainly cohort studies, which follow a well-defined cohort of participants for several years. Because of the long follow-up time and repeated examinations of the participants, cohort studies are expensive to conduct and take a long time. On the contrary, they yield reliable data and make it possible to study a wide range of exposure-disease associations. For UFP, long-term health effects have not been systematically studied and the difficulties in occupational settings are even larger than in environmental settings. Besides the challenge of estimating the cumulative exposure to either ambient or engineered nanoparticles, the occupational setting is also highly demanding when assembling cohorts, adequately characterizing confounding exposures and avoiding loss of follow-up.

Short-term associations are usually examined by using time series studies, which associate time-varying exposure to time-varying event counts such as mortality or hospital admission. Time

series studies are a type of ecologic study, because they analyze population-averaged health outcomes and exposure levels. Nevertheless, due to the temporal nature of the design, confounding concerns that usually come up with ecological studies such as reverse causation fallacy are avoided in time-series studies. In an occupational setting, routinely collected data are often not readily available and a panel study might therefore be the better design. A panel study is a small prospective cohort study consisting of individual time-series of repeated measurements.

Panel studies provide the advantage of examining individuals repeatedly over a time period of several weeks or months. In addition, each individual is his or her own control. A potentially important conclusion of the expanded work on exposure assessment is to include personal measurements of UFP as epidemiological studies of ambient traffic-related pollution are starting to do. With personal measurements, the full scale of temporal spatial variation can be exploited and associations can be observed, which otherwise may have been overlooked.[47] These studies may consider respiratory as well as cardiovascular function as health outcomes. It is important to note that changes in respiratory function may require underlying disease or bronchial hyperresponsiveness[44,47] to be observable in response to moderate changes of PNC. Similarly, induction of electrocardiogram signs of ischemia in response to elevated UFP require underlying coronary artery disease and may be even an exercise challenge to be observable.[64] This might be a challenge, as people with the respective underlying diseases might be less likely to be working in an occupational setting that involves a high exposure to nanomaterials. In addition, changes in cardiac function or systemic blood markers may be a consequence of a large number of intermediate steps for which not only the particles themselves but also their composition or surface activity may be important. Lastly, it is important to establish whether one is conducting a study for monitoring a highly likely association or for initiating novel research in the face of uncertainty. In the first case, health-monitoring programs might be of the largest benefit for the employees and might indeed be warranted for workers exposed to nanotubes in their occupational setting. In the second case, when an extrapolation from toxicological or epidemiological research carries great uncertainty, well-designed panel studies may be able to quantify the potential for health effects associated with specific and potentially unique occupational exposure scenarios.

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Overview of Current Toxicological Knowledge of Engineered Nanoparticles

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Objective: Nanotechnology is the manipulation of matter on a near-atomic scale to produce nanoparticles with unique properties, allowing new commercial applications. Since nanoparticles exhibit unique physicochemical properties, they are likely to exhibit biological activity significantly different from fine-sized particles of the same chemical composition. Therefore, evaluation of the biological effects of nanoparticles is critical. **Methods:** The article lists the major objectives of nanotoxicology and briefly reviews the literature concerning biological responses to pulmonary exposure. **Results:** Interactions of nanoparticles with biological systems depend on particle size, shape, oxidant generation, surface functionalization, and rate of dissolution. Pulmonary, cardiovascular, and central nervous system responses to pulmonary exposure to nanotitanium dioxide and carbon nanotubes are described. **Conclusions:** Significant biological responses occur in animal models after pulmonary exposure to certain nanoparticles. Control of exposure appears prudent to protect worker health. **Clinical Significance:** Nanotechnology is synthesizing a wide range of nanoparticles, which exhibit unique physicochemical properties. These unique properties make unique biological activity likely. If certain nanoparticles induce adverse effects in vitro or in animal models, then occupational health surveillance and exposure control may be prudent steps in the protection of worker health.

During the Clinton administration, Congress enacted the National Nanotechnology Initiative to foster research in a new field, nanotechnology, and to stimulate the commercial development of new products resulting from such research. Nanotechnology is the manipulation of matter on a near-atomic scale to produce new structures, materials, and devises. Nanotechnology is projected to grow into a trillion dollar industry employing millions of workers worldwide within the next decade.[1] Indeed, a wide variety of novel applications and products are being developed for commercial use in cosmetics, electronics, sensors, structural materials, sporting goods, sunscreens, antimicrobial products, paints, coatings, energy storage devices, conductive fabric, bone grafting, medical imaging, and targeted drug delivery.[2]

At the core of nanotechnology is the synthesis of engineered nanoparticles, which are defined as particles having one dimension less than 100 nm. Engineered nanoparticles are created with tightly controlled size, shape, surface features, and chemistry. Since a large fraction of the particle's atoms are on its surface, nanoparticles exhibit unique physicochemicals, which are distinctly different from those of fine-sized particles of the same chemical composition. Because of the small size and low density of nanoparticles, aerosolization is likely during energetic processes, such as voxtexing, weighing, sonication, mixing, and blending. Therefore, worker exposure via inhalation is anticipated during production, use, and disposal of nanoparticles.[3]

The unique physiochemical properties of nanoparticles are driving nanotechnology and the development of unique products and applications. Nevertheless, these unique physiochemical properties are likely to result in unique bioactivity. *Nanotoxicology* is the systemic evaluation of the interaction of nanoparticles with biological systems, the quantification of resulting responses, and the elucidation of mechanisms determining the interactions and responses to nanoparticles on the molecular, cellular, tissue, organ, and whole body levels. The objectives of nanotoxicology are to

1. Determine the relationships between physicochemical properties of nanoparticles and their bioactivity,
2. Identify responses at the primary site of exposure as well as in distal organs, and
3. Determine the dose and time dependence of these biological responses.

The following is a brief review of selected areas of knowledge development in nanotoxicology.

RELATIONSHIP BETWEEN NANOPARTICLE CHARACTERISTICS AND BIOACTIVITY

A major challenge for toxicological assessment in nanotechnology is the large and rapidly growing number of possible nanoparticles to be tested for biological activity. It is not feasible to conduct a full assessment of bioactivity for every possible nanoparticle. Therefore, it is critical to develop a matrix of relationships between specific physicochemical properties and resultant bioactivity. An understanding of such relationships would allow the prediction of possible health effects in the absence of complete toxicity data. This knowledge can be applied to develop prevention strategies to protect worker health.

At this point in the development of a knowledge base in nanotoxicology, the following physicochemical properties are believed to be important determinants of biological response:

1. Particle size
2. Particle shape
3. Oxidant generation
4. Surface functionalization
5. Rate of dissolution

A growing body of data indicates that particle size is an important factor in driving the biological response to particles. The National Institute for Occupational Safety and Health (NIOSH) laboratory has evaluated the pulmonary response to intratracheal instillation of well-dispersed fine versus nano titanium dioxide (TiO_2) particles.[4] On an equal-mass exposure basis, nano-TiO_2 was as much as 41-fold more potent than fine TiO_2 in causing lung inflammation, lung damage, inflammatory cytokine/chemokine production, and oxidant generation by alveolar macrophages. If lung burden were normalized to total particle surface area deposited, the potency of nano and fine TiO_2 was not significantly different. Particle size also affected the fate of the particles after pulmonary exposure.[4] Fine-sized TiO_2 was avidly phagocytized by alveolar macrophages, while nano-TiO_2 exhibited a significantly greater ability to evade phagocytosis and enter the alveolar walls. The importance of particle size to bioactivity also impacts the pulmonary response to agglomerated versus more dispersed nanoparticles. The NIOSH laboratory reported that intratracheal instillation of a well-dispersed suspension of

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carbon black nanoparticles resulted in an 8-fold greater response on an equal mass burden basis than a poorly dispersed suspension of agglomerated carbon black nanoparticles.[5] Furthermore, the agglomeration status of single-walled carbon nanotubes has been shown to affect both deposition site and pulmonary response.[6] On aspiration, micrometer-sized agglomerates deposit at the proximal alveolar region of mouse lungs and induce granulomatous lesions. In contrast, aspirated well-dispersed single-walled carbon nanotube structures deposit in the distal alveoli, rapidly enter the alveolar walls, and induce interstitial fibrosis. These data indicate that bioactivity of a nanomaterial is dependent not only on the primary size of the nanoparticle but also on the degree at which the nanoparticles are agglomerated, that is, the physical size of the nanoparticle structures, as they interact with biological systems.

Data from NIOSH studies indicate that nanoparticle shape is also a critical determinant of bioactivity. Porter et al[7] reported that TiO_2 nanoparticles in the form of long belts were significantly more toxic in vitro and more inflammatory in mice at 1 day postexposure than an equal mass of TiO_2 nanospheres of the same chemical composition and diameter. Similarly, Shvedova et al[8] reported that high aspect ratio single-walled carbon nanotubes were 23-fold more inflammatory 1 day after aspiration in mice than an equal mass of spherical carbon nanoparticles (carbon black). The high aspect ratio of long, thin carbon nanotubes has raised concern that carbon nanotubes may induce pulmonary responses similar to asbestos.[9]

Nel et al[10] has proposed that oxidant stress may be a critical parameter determining bioactivity. Indeed, a strong correlation ($R^2 = 0.95$) has been demonstrated between the ability of eight different spherical particles to stimulate oxidant production by alveolar macrophages in vitro and their potency to cause pulmonary inflammation 1 day after intratracheal instillation in a rat model.[11] Nevertheless, carbon nanotubes appear to be an exception to the oxidant stress paradigm. Raw single-walled carbon nanotubes, containing 30% iron by weight, generate a substantial hydroxyl radical signal measured by electron spin resonance spectroscopy in an acellular system in the presence of hydrogen peroxide. In contrast, purified single-walled carbon nanotubes (0.2% iron) do not generate hydroxyl radicals. In agreement with the oxidant stress paradigm, raw single-walled carbon nanotubes were highly toxic and caused oxidant stress to cells in culture, while purified single-walled carbon nanotubes were significantly less cytotoxic.[12] Nevertheless, the oxidant stress paradigm does not predict the pulmonary response to single-walled carbon nanotubes in a mouse model. Indeed, Shvedova et al[8,13] found that the level of pulmonary inflammation 1 day after aspiration of raw single-walled carbon nanotubes by mice was not significantly different than the inflammation reported after pulmonary exposure to an equal mass (10 μg per mouse) of purified single-walled carbon nanotubes. Therefore, while oxidant generation appears to be an important factor to determine the pulmonary response to some types of nanoparticles (nanometals and nanospheres), it appears to be of minor importance to the pulmonary response to carbon nanotubes, where particle shape or aspect ratio appears to drive bioactivity.

A critical step in the expression of bioactivity of a nanoparticle is the biophysicochemical interaction of the nanoparticle surface with biological systems.[14] Since the surface activity of nanoparticles is considered critical to bioactivity, it has been proposed that functionalization of the surface of nanoparticles would alter bioactivity and that this may be a practical approach in the development of "safe" nanoparticles. This concept received support from the work of Sayes et al,[15] in which hydroxylization [C_{60} $(OH)_{24}$] significantly decreased the cytotoxicity of fullerenes (C_{60}) in fibroblast, lung epithelial cell, and astrocyte in vitro models. Unfortunately, such functionization of fullerenes did not alter their inflammatory potential in rat lungs 1 day to 3 months after intratracheal instillation, that is, both C_{60} and C_{60} $(OH)_{24}$ exhibited similar levels of transient inflammation.[16]

The pulmonary and systemic response to pulmonary exposure to nanoparticles is believed to be related to the rate at which the particle dissolves. For example, fiber pathogenicity is related to the durability of the fiber, which impacts the biopersistence of such particles in the lung.[17] In contrast, many of the effects of residual oil fly ash have been associated with its soluble metal component.[18] Sager et al[19] have demonstrated that zinc oxide nanoparticles exhibit a high rate of dissolution, which accounts for the rapid clearance of zinc from the lung and translocation to systemic organs. Doping zinc oxide nanoparticles with iron results in a substantial decrease in the rate of dissolution.[20] Iron-doped zinc oxide nanoparticles are far less toxic to cells in culture and cause significantly less lung damage and inflammation in rat lungs at 1 to 30 days after intratracheal instillation.

In summary, there is a growing nanotoxicology database relating bioactivity to a specific physicochemical property of a nanoparticle. As such information is expanded, it will allow one to predict the relative pathogenicity of a given nanoparticle with given properties. This will allow control banding approaches for developing prevention strategies for worker protection.

RESPIRATORY AND SYSTEMIC RESPONSES TO PULMONARY EXPOSURE TO SELECTED NANOPARTICLES

Significant airborne levels of nanoparticles have been associated with various processes (vortexing, weighing, sonication, mixing, blending, and reactor cleanout) in nanotechnology workplaces.[21–24] The following section will briefly review the pulmonary, cardiovascular, and central nervous system responses resulting from pulmonary exposure to TiO_2 nanoparticles or multi-walled carbon nanotubes (MWCNT).

Sager et al[4] have reported that intratracheal instillation of rats to a well-dispersed suspension of nano-TiO_2 caused a dose- and time-dependent pulmonary inflammation and damage. Substantial pulmonary responses were observed after exposure to 0.26 mg of TiO_2 per rat, with responses increasing in a near-linear manner through 1.04 mg per lung. Responses were maximal at 1 to 7 days postexposure and only partially returned toward control (a decrease from the peak response of 25% to 50%) at 42 days postexposure. Substantial pulmonary fibrosis was not noted over this time period.

Inhalation exposure of rats to nano-TiO_2 has also been reported to cause systemic microvascular dysfunction at 1 day postexposure.[25] Intravital microscopic analysis of the ability of arterioles in the shoulder muscle to respond to dilators indicates that significant inhibition of normal dilatory response after inhalation of nano-TiO_2 at lung burdens from 7 to 40 μg. Complete inhibition of dilatory function of systemic arterioles was observed at a lung burden of 400-μg nano-TiO_2, at which dose, no gross changes in bronchoalveolar lavage markers of pulmonary inflammation or damage were noted. Le Blanc et al[26] have reported similar, sensitive inhibition of the ability of coronary arterioles to respond to dilators in rats 1 day after inhalation of 10-μg of nano-TiO_2. These results suggest that pulmonary exposure to nano-TiO_2 may result in elevated peripheral resistance and decreased oxygen delivery to the heart, which may have adverse impact under exercise conditions.

Sriram et al[27] reported that aspiration of TiO_2 nanobelts (30μg per mouse) in mice caused pulmonary inflammation 1 day postexposure. Associated with this pulmonary exposure was a significant elevation of messenger ribonucleic acid (mRNA) levels for markers of inflammation and blood–brain barrier injury in selected regions of the brain.

Aspiration of MWCNT (10 to 40 μg per mouse) in mice has been reported to cause a rapid but transient pulmonary inflammatory and damage response.[28] Response peaked 1 to 7 days postexposure and returned toward control levels at 28 and 56 days postexposure. In

contrast to the transient inflammatory reaction, a persistent (through 56 days) fibrotic response of early onset (7-day postexposure) was noted. Results indicate that acute pulmonary responses to short-term inhalation of MWCNT are similar to those reported after a bolus exposure via aspiration at the same lung burden of MWCNT.[29]

Data from the NIOSH laboratory indicate that inhalation exposure of rats to MWCNT at a lung burden of 17 μg per rat resulted in significant pulmonary inflammation and damage 1 day postexposure. Associated with this pulmonary exposure to MWCNT was complete inhibition of the ability of coronary arterials to respond to dilatory signals.

Aspiration of 80 μg of MWCNT in mice significantly elevated mRNA for inflammatory mediators (interleukin [IL]-1β, IL-6, tumor necrosis factor [TNF]-α, and colony-stimulating factor [Csf]-3) in the olfactory bulb and other selected brain regions at 1 day postexposure.[30] Induction of mRNA for E-selection (a marker of blood–brain barrier injury) was also noted.

Possible mechanisms by which pulmonary nanoparticle exposure results in systemic effects include the following:

1. Translocation of the nanoparticle from the lung to the systemic organ
2. Systemic inflammation
3. Neurogenic signals

Evidence suggests that nanoparticles can translocate to systemic organs. Nevertheless, the rate of translocation is low.[31] Indeed, nano-TiO_2 or MWCNT were below the level of detection in cardiovascular and brain tissue in the NIOSH studies described previously. In contrast, there is evidence that pulmonary exposure to nano-TiO_2 results in potentiation of peripheral blood polymorphonuclear leukocytes, adherence of polymorphonuclear leukocytes to the microvessel walls, and generation of oxidants at the vessel wall.[32] These events have been linked to particle-induced systemic and coronary microvascular dysfunction.[32,33] Lastly, particle-induced systemic and coronary microvascular dysfunction has been linked to neurogenic signals from airway sensory neurons to the cardiovascular tissue.[34]

CONCLUSION

The nanotoxicology literature indicates that the unique physicochemical properties of nanoparticles dictate the interaction with biological systems at the molecular, cellular, organ, and whole body level. Results indicate that nanoparticle size, shape, oxidant-generation capacity, surface functionalization, and rate of dissolution are critical determinants of bioactivity. Structure, function, and mechanistic studies are ongoing with the goal of constructing a matrix of relationships between physicochemical properties and biological response. Such correlations will allow preliminary assessment of relative health hazard for nanoparticles in the absence of a complete toxicological evaluation.

Studies evaluating responses to pulmonary exposure to selected nanoparticles, such as TiO_2 and MWCNT, indicate that reactions are noted both in the organ of exposure, that is, the lung, and in distal organs, such as the cardiovascular and central nervous system. Data indicate that systemic reactions can often be measured at low exposure doses where lung effects are minimal. Therefore, markers of cardiovascular and central nervous response may prove useful biomarkers for worker surveillance. Indeed, volunteers exposed to diesel exhaust exhibit electroencephalography changes, that is, an increase in fast wave activity in the frontal cortex,[35] and microvascular changes, that is, impaired forearm vascular response to dilators,[36] within hours after exposure.

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Role of Medical Surveillance in Risk Management

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Objective: Occupational physicians face increasing pressure by health authorities, employers, and employees to provide practical, targeted, and meaningful medical surveillance to workers handling nanoparticles. **Methods:** On the basis of experience and literature review, examples were identified for successful medical surveillance activities. Consideration was given to the respective context in which they provide benefit, and whether these examples may be extrapolated to the present situation with nanoparticles. **Results:** Occupational medical surveillance based on existing knowledge of hazards and potentially associated health effects is both feasible and useful. In the absence of sufficient knowledge, results from surveillance programs may still provide new insights into exposure–response relationships or help to identify new hazards. In some situations, however, medical surveillance may also produce harm. **Conclusions:** Medical surveillance provides benefits on the individual, company, and societal level, provided that it is planned and performed with its limitations in mind.

In the management of occupational health hazards, medical surveillance is usually considered an established tool for secondary prevention of adverse health effects through early detection. Nevertheless, the term "medical surveillance" is used differently in various contexts and by different institutions or authors, and the borders between "surveillance", "screening," and "examination" are not always clear-cut. This fuzziness of terms has sometimes impacted on discussions regarding sensible and feasible medical measures targeted at managing health risks in occupationally exposed persons and very much so in the ongoing discussion about the risks for employees handling nanomaterials. To get a grip on this topic, a distinction must be made between the two types of surveillance: personal and public health surveillance. While personal surveillance focuses on the individual, public health surveillance is performed with the intention to monitor the overall health experience at population level. Furthermore, we have to differentiate between the management of known health risks associated with a given exposure and the identification of "as yet unidentified" new health risks. A useful set of definitions has been compiled by Trout and Schulte[1]:

- Occupational health surveillance is the ongoing systematic collection, analysis, and dissemination of exposure and health data on groups of workers for the purpose of early detection of disease and injury.
- Medical surveillance examines health status through tracking of illnesses or a change in a biological function in an exposed person or persons. It essentially involves a process of looking for health trends in a worker population.
- Medical screening is one form of medical surveillance that is designed to detect early signs of work-related illness by conducting tests in apparently healthy persons to detect those with early stages of disease or those at risk of disease.

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There is thus no clear border, which strictly separates surveillance from screening, the latter being a subcategory of the former. According to these definitions, occupational health surveillance and medical surveillance may involve medical examinations, while medical screening must do so. A further distinction should be made between general and exploratory approaches on the one hand, which are more often used in broad surveillance programs, and specific or "targeted" approaches on the other hand, with a comparatively narrow focus on occupational exposures and their potential effects on health.

There are some prerequisites for targeted occupational medical screening: (a) knowledge about the existence or at least possibility of an exposure to a health hazard; (b) knowledge about specific health effects caused by such an exposure; (c) the availability of tests with a known sensitivity and specificity to detect such health effects; and (d) knowledge about the strength of an association between exposure and effect.[2]

At the individual level, an apparent benefit may result from any kind of medical surveillance, and specifically from screening, either if the target outcome of screening serves as an early marker of effect but is not itself a pathological condition or if the health condition expected is both diagnosable at an early stage and treatable at this point in time. A moderate suppression of cholinesterase activity after exposure to organophosphates may serve as an example for the former condition. The benefit may be less clear if no treatment option exists for the disease of interest, but it may be arguable with regard to securing a basis for compensation claims. There may, however, be no benefit at all to the individual if there is no therapeutic option and no established causal relation between exposure and health finding. In the following section, several examples for existing surveillance and screening approaches will be provided and an attempt will be made to extrapolate the usefulness and applicability of such approaches to the present situation of employees handling engineered nanomaterials.

Targeted Screening

There exists a wealth of knowledge about the health risks associated with a wide spectrum of occupations; job tasks; and chemical, biological, or physical exposures. Occupational physicians have always used their detailed knowledge about workplaces to develop and perform bespoke examination programs for exposed individuals with the aim to detect work-related health effects at the earliest possible point in time. In some instances, even individual risk factors causing enhanced individual susceptibility to work-related health hazards may be identified and appropriate preventive measures may be suggested. Existing guidelines, often issued by national competent authorities, can be hazard oriented (eg, chemicals, radiation, noise, and infectious agents), job task oriented (eg, driving, controlling, monitoring, and logging), or aimed at specific health endpoints (eg, chronic obstructive pulmonary disease, skin disease, and hearing loss).[3,4]

Those guidelines directed at chemical exposures often contain recommendations regarding biomonitoring, which, in the narrow sense, is the determination of chemicals or their metabolites or adducts in human tissues or body fluids. This method is particularly useful as it complements ambient air measurements by providing information about the actual uptake into the body of an exposed worker of a chemical at a workplace. It thus helps to assess the effectiveness of technical measures of exposure reduction, use of personal protective equipment and safe working practices, and to identify potential

for improvement at both the individual behavioral and at a general organizational level.[5] Biomonitoring is thus the part of occupational medical screening, which most obviously contributes to primary prevention, potentially triggering action to avoid or to reduce hazardous exposures even in the absence of detectable health effects.

Occupational medical screening is not only directed at current workplace hazards and ongoing exposures but, for example, in the case of carcinogens, can also be offered to formerly exposed employees even after the end of employment. This latter approach requires the establishment of registries, which exist in different countries on a variety of past exposures. Their establishment and maintenance can either be mandated by law or result from company or trade union initiatives.[6] In Germany, several registries are kept under the auspices of the Employer's Liability Insurance Association (Deutsche Gesetzliche Unfallversicherung). It covers all substances classified as category one or two carcinogens under German or European Union regulations. Persons registered are offered regular screening examinations, where the screening interval and the examinations performed are chosen on the basis of knowledge about the typical target organs for the exposure in question and on experience about the natural course of such cancers.

The usefulness and feasibility of this approach has been assessed repeatedly, notably in high-risk populations with past exposure to aromatic amines for the endpoint of bladder cancer.[7–10] The individual benefit of participating in bladder cancer screening is obvious for those persons, who were by means of screening diagnosed with early stage bladder cancer, because without screening this diagnosis would have been obtained later. The benefit lies in the fact that in early stage bladder cancer, therapy is more successful and the outcome more favourable than in later stages. In a cohort of more than 1700 workers from the chemical industry, we found evidence that rehabilitation costs for cohort members were 15% to 20% lower if compared with noncohort cases with bladder cancer covered by the same insurance provider.[9] Another result of this study, however, was the confirmation of a deplorably poor positive predictive value of the screening protocol applied, notwithstanding its sufficient sensitivity. Consequently, an uncomfortably high number of cystoscopies were performed in the members of this cohort, where no cancer was diagnosed. This shed a somewhat different light on the perceived benefit for the latter group due to the potential for unwanted side-effects of this diagnostic procedure, such as infections and physical trauma, not to mention the fact that it is usually perceived as less than comfortable. While this situation may be deemed acceptable for a high-risk population, it certainly calls for improvement. In cooperation with urologists, epidemiologists, insurance association, and providers of diagnostic markers, we therefore started a prospective study in this cohort with the aim to evaluate alternative and innovative markers for the early detection of bladder cancer.[11] We are confident that the results of this study will help to identify useful new marker panels and to redefine the cutoff values for the established ones. This example may serve as an illustration of how existing registries may yield benefits not only to the respective individuals under surveillance but also through their scientific exploitation to the general population, where they may help to optimize and improve the cost-effectiveness of screening strategies.

This optimistic view on the usefulness of targeted occupational medical screening can unfortunately not be translated to every kind of targeted screening. This is perfectly illustrated by the ongoing controversy surrounding prostate-specific antigen testing, where overdiagnosis and overtreatment of clinically insignificant prostate cancer are considered a major potential drawback by some—but not all—authors.[12–16] From a patient perspective, the question is to decide, hopefully, after having received sufficient information on the pros and cons of prostate-specific antigen testing and on the preferability of either dying from prostate cancer or running the risk of impotence, incontinence, hospital-borne infection, and other possible complications after unwarranted surgery. Even after the availability of interim results from two large randomized trials, this conundrum remains unresolved.[17–19]

Untargeted Surveillance

In a situation with suspected but, as yet, unproven health risks due to existing exposures, new insights may be expected from surveillance at a population level. Unrecognized health risks can be systematically researched by comparing across different subsets of employees with defined exposures medical findings obtained through or available to the occupational physician. This analysis of aggregate data can be especially useful for the identification of new sites for known health hazards.[3] An unexpected exposure to isocyanates can, for instance, become apparent through measured 1-second forced expiratory volume trends in a group of workers, in which the average effect size would be insignificant for an individual but meaningful for a population if compared with an unexposed group. An apparent shortcoming of this approach is that it relies on observational data not obtained for systematic comparisons in first place. In the previous example, the analysis could be meaningless if it turned out that the group with a higher decline in 1-second forced expiratory volume consisted of heavily smoking shiftworkers while the comparison group consisted mostly of clerical workers. The feasibility of such a company-confined study is further often limited by group size, which, in most industrial settings, may be too small to enable meaningful statistical comparisons. Even more challenges result, at least from the statistician's perspective, from the fact that the frequency of and intervals between examinations may vary considerably, thus introducing potential detection bias, lead time bias, and other pitfalls to data interpretation. These limits can sometimes be overcome by purposefully designing and performing studies in exposed workers, where questions of comparability, group size, information needed on confounders, etc, can be addressed in advance. Such systematic approaches have long been used in some industries, like in the historical example, in which by data pooling across companies, it was possible to verify a preexisting suspicion regarding the manufacturing process of auramine and magenta, but not exposure to the final products, as causative in the development of bladder cancer.[20]

Beyond what has been said previously, basically every prospective or retrospective cohort study concerned with occupational or environmental exposures can be considered as an example for untargeted surveillance. Such research can be carried out in cohorts like the Agricultural Health Study, in which detailed information on exposure has been obtained and documented in advance of the occurrence of the outcome of interest, and socioeconomic differences are not likely to bias the comparisons to a major extent.[21] In the environmental sector, the cohorts set up after the infamous Seveso accident or the studies carried out in atomic bomb survivors continue to contribute to our understanding of the effects of dioxins or radioactive radiation, respectively.[22,23]

It is important to keep in mind the difference between confirmatory testing of a preexisting hypothesis in an existing or—even better—newly assembled data set and an exploratory analysis, in which the possibility of chance findings is sometimes not adequately addressed by some researchers. It seems an unfortunate development that with the increasing availability and user friendliness of statistical packages, which can be used on every desktop computer, the number of studies appears to increase, where the most intricate and sophisticated statistical procedures were employed on data sets not really designed for it. This potentially leads to a plethora of "new findings" resulting from exploratory studies and "creative data modeling", regarding old and new exposures, sometimes calling for preventive action (but at least for more funding, because further studies are needed) even before the scientific discussion about the potential significance of these findings has started. This development

and a passionate "plea for epistemological modesty" has recently fuelled a lively discussion among epidemiologists.[24–26] Irrespective of this controversy, it goes without saying that false alarms and thereby triggered unnecessary responses and expenses, not to mention the distress in allegedly concerned individuals, must be counted among the potential drawbacks associated with untargeted surveillance.

Having said that there shall be no doubt that each unusual pattern or frequency of health findings in a screened population warrants a closer look and thorough workup. Such observations may at first be indiscernible from chance clusters, and the significance of some of these observations has remained a subject for controversial debate for many years to come. Often, we can only in hindsight classify some of these clusters as true "sentinel health events."

Sentinel Health Events

A special role in the detection of hitherto unknown health risks is often ascribed to "sentinel health events." This refers to medical findings or diseases, which are unexpected either by their nature or by their frequency of occurrence, in the screened population. Admittedly, up to now, completely new insights into occupational health hazards have rarely, if ever, been obtained through purposefully designed monitoring strategies but often resulted from accumulating case series, which at length stirred suspicion in vigilant physicians or—unfortunately—pathologists. Notorious historical examples are bladder cancer resulting from aromatic amine exposure, lung cancer from hexavalent chromium, hepatic toxicity from polychlorinated naphthalenes, or even the infamous asbestos case.[27–30]

Sometimes, such index cases may present in a very unsuspicious manner, and it takes the specific knowledge of an experienced plant physician to find the unusual aspect in a seemingly common appearance. The following case report may serve as an example:

A technician presented himself at the site medical clinic, complaining predominantly of cough and breathing difficulties. That morning he had experienced nausea and one bout of vomiting. He had been dismantling reactors and pipes in a propionic acid plant over a period of several days during periodic maintenance activities. No specific exposure event was reported by the employee. On the basis of the knowledge of the plant operations (where nickel tetracarbonyl is used as catalyst), a urine sample was collected for the determination of the urinary nickel concentration—just in case! This examination revealed a high level of nickel in the urine and led to the diagnosis of nickel tetracarbonyl intoxication. The same diagnosis was established in retrospect for two additional employees found to have similar symptoms. They were currently being treated by their family physicians as cases of common cold and incipient pneumonia, respectively. Chest radiographs showed peribronchial infiltration in all three cases, without the signs of bronchial obstruction. Laboratory blood analyses were consistent with a nonspecific inflammatory response. The symptoms resolved, and the clinical examination findings returned to normal in all three persons within 1 week.

These were the first cases of clinically relevant nickel tetracarbonyl intoxications in BASF over a period of more than 40 years. A search in our archives identified a report on a similar incident in 1958, where a total of seven persons had been exposed, resulting in two fatalities.[31] The nickel urine concentrations found in our current cases came close to the lower range observed in these historical fatal cases. Routinely performed carbon monoxide measurements at the beginning of the dismantling and maintenance work had failed to provide a clear warning sign. In retrospect, prolonged off-gassing from insoluble residue cakes formed on the reactor wall was identified as the most likely cause for this unusual exposure. As a consequence, similar tasks will be performed in the future by using self-contained breathing equipment until the absence of potentially hazardous residues on the equipment parts has been positively confirmed. The cases have been published to make responsible persons in other industries with similar processes aware of this unusual exposure scenario.[32]

Medical Surveillance: What Should We Not Do?

The definitions of surveillance and screening quoted at the beginning of this article are somewhat academic in that they direct a view from external on the worker involved (at least, this "unidirectional" interpretation is not explicitly ruled out in these definitions). In this context, the worker may be the object of the examination, and the information obtained on him, and from him, may primarily be used to create knowledge about the interactions between workplace exposures and individual or group health status. While such an approach can have its scientific merit and can indeed produce results that benefit working populations as a whole, it does not account for the fact that the interaction between the occupational physician at a given plant or site and the worker is "bidirectional" by default. The worker is at the same time the subject involved and may rightfully request that each and every finding obtained on him should be interpreted with regard to his current and future health and to the potential consequences for his employability. Thus, in not only occupational but also medical daily practice and outside scientific studies, the primary rule for choosing diagnostic parameters is: "Never use a method where you cannot interpret the results." This attitude is sometimes denounced as misusing an ethical argument as a pretext for a "do-nothing" policy. Nevertheless, it has nothing to do with ethics but is simply derived from the experience of practical occupational physicians who have to answer very personal questions and concerns regarding medical findings and who often have to provide advice that may finally trigger decisions that go as far as giving up a job or leaving an employment for perceived health reasons. It is important to remember that the key question for an individual is not whether screening is effective but whether it does more good than harm.[19]

Medical surveillance is thus one of the cornerstones of occupational health surveillance and, as such, a vital part of the efforts to secure just and favorable working conditions in keeping with the human rights declaration. The examples discussed so far illustrate the role of occupational medical surveillance in various aspects of managing risks at workplaces and beyond. It specifically helps to

- Target known workplace-specific hazards, help to reduce exposure, detect health effects at the earliest possible point in time,
- Identify hitherto unknown health hazards or exposure possibilities,
- Enhance understanding of the significance of personal behavior for risk reduction through communication of findings to individuals under survey,
- Communicate aggregate findings to staff and management to provide the full picture of the occupational hygiene situation, to facilitate targeted intervention,
- Allow employees and management to develop informed conclusions regarding compatibility between individual health status and workplace-related health risks,
- Make employees aware of nonoccupational health risks, which are identified as a "side effect" of occupational medical screening, and
- Translate experience gained from occupational cohorts to diagnostic strategies or toxicological assessments relevant for the general population.

Nevertheless, occupational medical surveillance must be planned and performed keeping the limits, potential pitfalls, and shortcomings in mind.

Extrapolation to "Nano": Should We Screen?

Much has been said about the potential health effects of nanoparticles, where the ongoing discussion leaves no doubt that there is no uniform common or single specific endpoint in human

health. On the contrary, the health hazards associated with nanomaterials will most probably have to be assessed differently for different classes of nanomaterials, while not for any single material itself. Nevertheless, quite a bit of basic research has been carried out to date, and it has provided important clues on what may be expected. The effects may be mediated by oxidative stress, inflammation, and fibrogenesis in the widest sense, and the target organs most often mentioned are respiratory, circulatory, and central nervous system and liver.[33–35] Given the lack of specificity of these endpoints and the high prevalence of respective findings in the general population, most authors agree that—while there is no evidence base for targeted "nano-specific" screening—general medical screening with methods aimed at some of the health outcomes under discussion may be performed in exposed workers.[1,2,36] Such screening should be devised weighing the risk to benefit ratio for the tests in consideration, keeping in mind the risks associated with untargeted medical surveillance. The results of such screening may, after aggregate evaluation on group level, provide future insights into relevant health risks associated with the handling of nanomaterials in workplaces.

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General Principles of Medical Surveillance

Implications for Workers Potentially Exposed to Nanomaterials

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Objective: As potential occupational exposure to nanomaterials becomes more prevalent, it is important that the principles of medical surveillance be considered for workers in the nanotechnology industry. **Methods:** The principles of medical surveillance are reviewed to further the discussion of occupational health surveillance for workers exposed to nanomaterials. **Results:** Because of the rapid evolution of nanotechnology, information may not be available to make a well-informed determination of all factors needed to evaluate risk of health effects from occupational exposure to nanomaterials. **Conclusion:** Every workplace dealing with engineered nanomaterials should conduct hazard and exposure assessments as part of an overall surveillance needs assessment for nanotechnology workers. In workplaces where risk is felt to be present, or at least cannot be ruled out, initiation of medical surveillance is prudent to protect workers' health.

The principles of medical surveillance are an essential component of occupational health practice.[1–3] As the production of (and potential occupational exposure to) nanomaterials becomes more prevalent, it is important that these principles be considered for workers in the nanotechnology industry.

DEFINITIONS AND BACKGROUND

Occupational health surveillance is the ongoing systematic collection, analysis, and dissemination of exposure and health data on groups of workers for the purpose of preventing illness and injury. Occupational health surveillance can help to define the magnitude and scope of occupational health issues among groups of workers, with the ultimate goal of prevention; occupational surveillance data are used to guide efforts to improve worker safety and health and monitor trends over time. The general term *occupational health surveillance* includes hazard and medical surveillance. Although the focus here concerns medical surveillance, integration of hazard and medical surveillance is key to an effective occupational health surveillance program, and surveillance for disease or other health endpoints should not proceed without having a hazard surveillance program in place.[4]

The terms *medical surveillance* and *medical screening* have sometimes been used interchangeably (and sometimes inconsistently) in the past, and it is important to understand distinctions between these activities.[5] *Medical surveillance* describes activities that target health events or a change in a biologic function of an exposed person or persons. A surveillance program involves recurrent longitudinal examinations and data analysis over time. *Medical screening* is a complementary activity, sometimes considered one form of medical surveillance, that is designed to detect early signs of work-related illness by administering tests to apparently healthy persons in a cross-sectional approach.[5] The term *medical monitoring* has been assigned different meanings in the past, but it is most appropriately seen as analogous to screening. Screening activities generally have a more clinical focus when compared to surveillance (the screened person may be directly treated in response to the screening test), but medical screening data, collected in a standardized manner, aggregated, and evaluated over time, can also be evaluated as a part of a surveillance program.

Both medical surveillance and screening are second lines of defense behind the implementation of engineering, administrative, and work practice controls (including personal protective equipment). Surveillance and screening activities should be seen as mechanisms that occupational health care professionals can use to determine whether the usual prevention activities in the hierarchy of occupational health controls are effective.[6] Although both are the examples of secondary prevention, if the results of surveillance and screening efforts are extended to make interventions in the workplace, both may also represent primary prevention activities.

ELEMENTS OF A MEDICAL SURVEILLANCE PROGRAM

The elements of a medical surveillance program generally include the following:

1. Identification of the group(s) of workers for which surveillance or screening activities will be appropriate.
2. An initial medical examination and collection of medical and occupational histories.
3. Periodic medical examinations at regularly scheduled intervals, including specific medical screening tests when warranted.
4. More frequent and detailed medical examinations, as indicated on the basis of findings from these examinations.
5. Postincident examinations and medical screening after uncontrolled or nonroutine increases in exposures such as spills.
6. Ongoing data analyses to evaluate collected information for surveillance and/or screening purposes.
7. Worker training to recognize symptoms of exposure to a given hazard.
8. A written report of medical findings.
9. Employer actions in response to the identification of potential hazards and risks to health.

These elements are present in many surveillance programs currently in use, including those based on medical screening and surveillance recommendations from the National Institute for Occupational Safety and Health (NIOSH). General information concerning surveillance may be found at the NIOSH Web site: www.cdc.gov/niosh/topics/surveillance/. Examples of specific information from NIOSH related to surveillance can be found in resources devoted to specific hazards, such as coal mining (www.cdc.gov/niosh/topics/surveillance/ords/CoalWorkersHealthSurvProgram.html). The Occupational Safety and Health Administration also places great emphasis on surveillance and screening. Mandatory and nonmandatory medical surveillance programs used by the Occupational Safety and Health Administration are

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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compiled at the following Web site: http://www.osha.gov/SLTC/medicalsurveillance/.

OTHER CONSIDERATIONS FOR MEDICAL SURVEILLANCE PROGRAMS

Clear Definitions of Purpose and Availability of Tests/Tools

A medical surveillance program should have a clearly defined purpose/objective and a defined target population, and testing modalities must be available to accomplish the defined objective. Testing modalities may include such tools as questionnaires, physical examinations, and medical testing. These types of evaluations are used within the target population to gain data concerning specific organ system(s) and more general information concerning potential health effects or exposure. Consideration given to potential routes of exposure is a logical means of helping to target medical evaluations. For example, if the route of potential exposure is thought to be inhalation, the pulmonary system may be targeted for medical evaluation. When considering specific testing modalities, existing toxicity information about a given nanomaterial on a larger scale can provide a baseline for anticipating the possible adverse health effects that may occur from exposure to that same material on a nanoscale.

Test Characteristics

Data collected in a surveillance program should be interpreted with some knowledge of the characteristics of the tools being used. Typically, ideal medical screening tests have high sensitivity (the test is positive in a high percentage of persons with the disease). Nevertheless, tests with high sensitivity often have low specificity (some workers with positive test results are actually free of disease [false positives]). In interpreting nonspecific tests, a careful examination with attention to occupational as well as known nonoccupational factors is necessary. The positive predictive value of a test is also of particular importance and will be dependent on the prevalence of the condition being evaluated in the target population.

Ongoing Data Analysis

Those conducting medical surveillance and screening should understand the concepts of sentinel events[4,7] and should be alert for unusual patterns of findings. In some instances, results of data analyses will alert practitioners to elevated rates of common diseases or common symptoms that warrant follow-up investigation. In other instances, data analyses will signal when a disease or illness occurs in excess or in a "cluster" in time and space. Expertise in epidemiologic principles is essential when analyzing and interpreting medical surveillance data and disease rates.[3,8,9]

Availability of Intervention

The availability of effective interventions is an important consideration in establishing a medical surveillance or screening program. The importance and effectiveness of a medical surveillance or screening program may be assessed by determining whether it was successful in leading to interventions that could decrease disease or illness.

Communication

An effective medical surveillance or screening program will require communication with a number of individuals or groups. On the basis of the identified purpose of the program, a clear plan should be established for interpreting the results and presenting the findings to workers and management of the affected workplace(s) in a manner that avoids creating false anxiety or false assurance. An explanation of the level of uncertainty associated with measurements should be routinely included in presentations to workers and management. Workers should be given a summary of the information in accordance with appropriate privacy and confidentiality protections.

Program Evaluation

An important part of any medical surveillance or screening program is assessing the overall program efficacy by evaluating the program in a number of ways. Quality assurance and control should be considered for all workplace sampling and medical testing. For medical tests, review or direct assessment of the laboratory's quality assurance procedures should be considered. Another component of program evaluation is assessing the appropriateness of the target populations. For example, for those workers at risk of exposure to nanomaterials, what percentage actually participated in the medical surveillance program? Conversely, how much excess testing was done on workers without specific risk factors warranting the testing?

Management, Coordination, and Integration With Other Programs

Hazard or medical surveillance or screening and its individual components will not provide for effective occupational health surveillance without coordination of all aspects by a program manager. The occupational health surveillance program manager has the duty of integrating the surveillance components and providing input to maximize the effectiveness of all aspects of the program.

CHALLENGES TO MEDICAL SURVEILLANCE/SCREENING OF NANOTECHNOLOGY WORKERS

A number of the elements of a standard medical surveillance program represent unique challenges when applied to surveillance for nanotechnology workers. Identification of workers potentially exposed to a hazardous substance, an important first step in the initiation of a surveillance program, may be challenging in the "field of nanotechnology." A standard approach for the initiation of surveillance with known hazards (such as substances with a documented evidence base related to biomedical effects and an occupational exposure limit [OEL]) is to utilize the concept of an "action level," which is some fraction of the OEL. Common practice has included triggering of various preventive actions such as a medical surveillance program based on worker exposure at or above the action level. Currently, in many situations, data concerning exposure are not available for properly assessing the need for medical surveillance or screening related to occupational exposure to nanomaterials. In the absence of OELs and attendant action levels for nanomaterials, medical surveillance for groups of potentially exposed workers should be considered on the basis of qualitative job hazard exposure analyses.[8] In workplaces where risk (based on an assessment of the best-available information concerning hazard and exposure) is felt to be present, or at least cannot be ruled out, initiation of medical surveillance is prudent to protect workers' health. Such medical surveillance may consist, at a minimum, of collecting medical history information on a targeted population. A determination of whether medical surveillance is instituted, the components of the medical surveillance, and how frequently data are collected should be made on a workplace by workplace basis, influenced by the possible nature of the health effects associated with the nanomaterial, as derived from available information. When information concerning the degree of hazard associated with a nanomaterial is not known, as with many nanomaterials, various other approaches may need to be utilized-–for example, by determining whether toxicity information exists for a similar type of nanomaterial or larger-scale particles of the same composition that can be used as a surrogate for triggering action.[10] Periodic reassessment of hazard and exposure will be a critical part of this needs assessment for a medical surveillance program.

The lack of specific screening tests for exposure or health endpoints related to nanomaterial exposure is a second important challenge. The utility of nonspecific medical screening is limited, because the health endpoints that may be linked to nanomaterials are not well known or confirmed at this time. Nonetheless, general medical screening may serve as an early warning system for possible, yet to be determined, health effects linked to exposure. This determination will require that the data be continually analyzed on a group basis and, if possible, linked to exposure and compared to appropriate comparison population rates. The limitation of this approach is that it may identify health effects unrelated to nanomaterial exposure (and in some cases, false positives, which may require follow-up and further diagnostic evaluation). It may also give screened employees a false sense that such procedures would be sensitive to any health risk associated with exposure to nanomaterials.

Our ability to address these and other challenges will be improved as our knowledge related to occupational exposure to nanomaterials grows. Some of these challenges can be partially addressed in current worksites where workers are monitored through existing programs whether they work in areas with both regulated hazards (or hazards which may not be regulated but for which well-accepted medical monitoring procedures exist) and nanomaterials. For example, three such types of medical surveillance that may be occurring in a workplace include assessment of the worker's ability to wear or use required respiratory or other personal protective equipment, medical examinations pertaining to job placement, and medical examinations as part of emergency medical care after a work-related exposure or incident. Employers should continue using these established applications of medical surveillance as appropriate and keep in mind that analyses of these data in the future with respect to current nanomaterial exposure may provide useful information concerning health effects potentially related to exposure to those nanomaterials.

CONCLUSIONS

Application of the principles of medical surveillance is essential in creating appropriate occupational health surveillance programs to fit the needs of workers and organizations involved with nanotechnology. Every workplace dealing with nanomaterials should conduct hazard and exposure assessments as part of an overall surveillance needs assessment for nanotechnology workers. In many situations currently, because of the rapid evolution of nanotechnology, information may not be available to make a well-informed determination of all the factors needed to evaluate risk of health effects from occupational exposure to nanomaterials. In workplaces where risk is felt to be present, or at least cannot be ruled out, initiation of medical surveillance is prudent to protect workers' health. Periodic modifications to any initial medical surveillance programs for nanotechnology workers are likely to be necessary, as the knowledge base relative to potential hazards of occupational exposure to nanomaterials grows.

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Current Surveillance Plan for Persons Handling Nanomaterials in the National University of Singapore

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Objective: The number of research projects involving engineered nanomaterials within the National University of Singapore is increasing. We aim to characterize typical exposures in our laboratories and to develop a health surveillance protocol for persons working with nanomaterials in this project that has recently been launched. **Methods:** Our surveillance project builds on existing occupational safety and health risk assessment systems in the National University of Singapore. **Results:** Environmental monitoring will be conducted in all laboratories handling nanomaterials, encompassing airborne nanomaterial concentrations, characterizing chemical and physical properties and assessing dermal exposure potential and significance. Health surveillance will initially follow the occupational health program already in place, to be progressively fine-tuned as more nanotoxicity data become available. **Conclusion:** Our vision is to build an adequate base for a cohort study that can provide good data on the health outcomes of nanomaterials-exposed persons.

The National University of Singapore (NUS) has seen a steady increase in the number of research projects involving nanomaterials over recent years. There are currently more than hundred projects dealing with nanomaterials, with even more expected over the next few years. The types of nanomaterials used range widely from simple substances such as zinc oxide to highly complex functional molecules. Table 1 lists some of the most commonly encountered nanomaterials within NUS research laboratories.

There is concern that researchers handling nanomaterials in free form may be at high risk of exposure, with as yet unknown long-term health consequences. A recent online survey by Balas et al[1] among various university-based and public laboratories around the world revealed that many researchers did not use any type of protection, even among those who recognized the possibility of the nanomaterials becoming airborne. Results of toxicity research to date points to potential adverse health outcomes from exposure to some nanomaterials.[2]

National University of Singapore has a comprehensive occupational safety and health program currently in place that includes a standard operating procedure for safe handling of nanomaterials; but at present, there are no environmental or health surveillance requirements specifically for people handling nanomaterials that are not composed of regulated chemicals. The challenge is to build a comprehensive database that adequately accounts for the great diversity in nanomaterials types and handling methods, while at the same time maintaining convenience, acceptability, and sustainability over the long term.

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Result of Survey of NUS Researchers Handling Nanomaterials

During a nanomedicine and nanotoxicology workshop for researchers held in February 2010, we conducted a self-administered questionnaire survey on the researchers' perceptions of nanomaterials-related risk. Forty-four of 85 individuals who attended the workshop responded (52% response rate), 39 (89% of respondents) of whom were currently working with nanomaterials.

Of those who responded, only 5% agreed with the statement that working with nanomaterials posed no health risk at all, while 60% disagreed and 35% were unsure. A total of 73% agreed that all nanomaterials should be treated as hazardous until proven safe (18% unsure, 9% disagreed). Most (72%) were aware of a code of practice on safe handling of nanomaterials in their laboratory (16% unsure, 12% disagree). More than half (52%) did not think that the same safety data sheet could be used for the bulk chemical and their nanomaterial derivatives (25% unsure, 23% thought it could).

Developments in Singapore Labor Legislation

In the 2006 revision of Singapore's labor law, there were new requirements for employers and stakeholders to take "reasonably practicable measures" to reduce occupational health risks at source to ensure that their employees are not at risk of adverse health effects.[3] The identification of such measures is through a process of activity based risk assessment. With the pressing need for occupational health care for our nanomaterials laboratory researchers and the new labor legislations in mind, a multidisciplinary project team was established in NUS. The team consists of occupational health, environmental monitoring, and laboratory safety specialists from the Department of Epidemiology and Public Health, Department of Civil & Environmental Engineering, and the Office of Safety, Health and Environment, respectively.

Thus, in this project, we aim to

1. characterize typical exposures in our research laboratories by assessing
   a. concentration and physical properties of airborne nanoparticulate levels and
   b. potential for dermal exposure and the likely significance.
2. Develop a health surveillance protocol for persons working with nanomaterials in NUS, building on the existing occupational safety and health risk assessment systems.

METHODS

All persons working with nanomaterials in NUS laboratories will be included. As there is at present no clear exposure definitions or limits for nanosized particles, any individual working directly with nanomaterials or working in the same room where processes involving nanomaterials are ongoing will be classified as potentially exposed.

The basic registry structure consists of two main elements: detailed exposure assessments and health surveillance.

TABLE 1. Common Nanomaterials Used in the National University of Singapore, 2008 to 2010

Type of Nanomaterial	No. of Projects Using
Carbon (mainly nanotubes)	7
Antibody-conjugated nanoparticles of biodegradable copolymers for targeted chemotherapy	6
Quantum dots	3
Silicon	3
Iron oxide minerals	2
Titanium dioxide	2
Zinc oxide	2
Magnetic nanoparticles	2

Exposure Characterization

At present, the university requires all principal investigators involved in laboratory-based research projects to submit risk assessment details to the Office of Safety, Health and Environment for approval before commencement of work via an online project risk assessment system.

In addition to routine laboratory project risk assessment details, the nanomaterials research laboratory database will collect information on the chemical and physical form of the nanomaterials being handled, as well as details on the work processes that take place. This would include information on the types of benchwork being carried out such as mixing, pouring, centrifuging; and also whether the processes take place in a fume hood or on open bench tops.

Environmental monitoring will be conducted in all laboratories handling nanomaterials. Measurements will be taken before, during, and after experiments (or selected activities) to allow for correction for background airborne nanomaterial levels, which consist of naturally occurring nanomaterials from sources such as resuspension of airborne particles due to activities not directly involving engineered nanomaterials.

Two main aspects will be studied-–(1) monitoring and detection of airborne nanomaterial concentrations in the laboratories and (2) characterization of chemical and physical properties of airborne nanomaterials.

A handheld condensation particle counter will provide concentration counts of airborne particles, which will be complemented with chemical measurements of airborne nanoparticles collected in various size stages such as using inductively coupled plasma mass spectrometry.

Analysis of the deposition of nanosized particles on surfaces such as gloves and possibly bench tops will also be conducted to assess the potential for dermal exposure.

By studying the types of materials used, the processes involving nanomaterials in the laboratories with the accompanying environmental measurement data, we hope to stratify the laboratories into several levels of risk for inhalation and risk of skin exposure-–the two main routes through which nanomaterials are currently thought to enter the body. This is akin to the control banding concept[4,5] and will form the initial basis for a job exposure matrix, which may prove a useful tool in subsequent epidemiologic studies on nanomaterials workers.[6] Environmental monitoring data will also be used to research the effectiveness of current control measures used within the laboratories.

Health Surveillance

At present, researchers in contact with known hazards (based on chemical composition and regardless of particle size) are already under regular statutory medical surveillance by occupational health professionals from the Office of Safety, Health and Environment. To streamline processes and maximize acceptability, this component of the project will build on the existing NUS health surveillance program.

Prescribed hazardous chemical exposures for which medical surveillance is required:

a. Fumes, dust, or vapor for arsenic and its compounds
b. Asbestos dust
c. Benzene fumes/vapor
d. Cadmium and its compounds
e. Fumes, dust, or vapor for Lead and its compounds
f. Fumes, dust, or vapor for manganese and its compounds
g. Fumes, dust, or vapor for mercury and its compounds
h. Organophosphates fumes/vapor
i. Perchloroethylene fumes/vapor
j. Silica dust
k. Tar, pitch, bitumen, and creosote
l. Trichloroethylene fumes/vapor
m. Vinyl chloride monomer fumes/vapor

Researchers handling nanomaterials containing any of the 13 chemicals in the list would already be required to undergo statutory medical surveillance. One such group would be those using cadmium-containing quantum dots. During the health surveillance visits, focused physical examination and laboratory tests specific to the exposure and its known health effects will be conducted. For example, persons working with cadmium would be specifically screened for renal, respiratory, and bone problems. Laboratory tests for them would include blood cadmium level and urine beta-2 microglobulin. Typically, exemption from statutory medical surveillance is allowed only when environmental monitoring shows levels to be consistently below 10% of permissible exposure limits (PELs). However, there is evidence to suggest that some nanomaterials may exert toxic effects at levels far below permissible exposure limits,[7] highlighting the need to review the relevance of PELs for nanosized materials.

Materials which are relatively nonreactive in bulk form have also been shown to exert toxic effects at the nano level (such as gold,[8] zinc oxide[9]). The aim of the project is thus to encompass all persons handling any form of nanomaterials in NUS, regardless of quantity. This will be achieved in stages, the first of which is extending the health surveillance program to include those handling nanomaterials in the prescribed hazards list at levels below the 10% PEL threshold for bulk materials.

The next group to be targeted for health surveillance would be researchers handling nanomaterials, where there is strong suspicion of possible adverse health effects—such as carbon nanotubes, where animal studies have linked exposure to asbestos-like pathogenicity.[10] Other examples are nano-gold[8] and zinc oxide.[9] The protocol for health surveillance will be regularly reviewed and revised, as new evidence on health effects become available. For example, if in the future, some nanomaterials were to be confirmed as nonhazardous to human health, health surveillance for persons only handling these could be deemed unnecessary.

Currently, there are no official guidelines or consensus on the specific types of health surveillance programs nanomaterial-exposed employees should undergo. Thus, the health surveillance component of our project will initially follow the occupational health program that is already in place in the University. This encompasses basic health information such as prior or present medical problems or symptoms; history of cigarette smoking; and general physical

examination and investigations such as blood counts, liver and renal function, chest x-ray, spirometry, and specific toxicology tests if necessary (eg, blood cadmium level, urine mercury level).

CONCLUSION

By collecting detailed information on exposure and baseline health status and eventually expanding the registry to include other research and educational institutions both locally and internationally, we hope that in time there will be an adequate base for a cohort study that can provide good data on the exposure characteristics and health outcomes of nanomaterial-exposed persons.

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A Small Business Approach to Nanomaterial Environment, Health, and Safety

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Objective: Integral to the commercialization process for nanotechnology enabled products is the methodology for protecting workers potentially exposed to nanomaterials during product development. Occupational health surveillance is a key aspect of protecting employees and involves both hazard identification and surveillance of known medical data. However, when the health effects and exposure pathways of both new and existing "nano-scale" chemical substances are not yet well understood, conservative hazard controls and baseline data collection can facilitate both immediate and long-term worker protection. **Methods:** Luna Innovations uses a conservative approach based on risk assessment and the OSHA General Duty Clause. **Results:** To date, Luna's approach has been effective for our business model. **Conclusions:** Understanding and managing potential hazards to our nanotechnology workers is key to the success and acceptance of nanotechnology enabled products.

The impact of nanotechnology is universal with both advocates and critics in agreement: nano has potential to become the 21st century's transformative technology. In fact, with a convergence of sciences now occurring in the name of nano, this technology could easily become key to a future in which one does not simply add knowledge; one achieves mastery over matter at the molecular level. Each entity driving nanotechnology commercialization has a specific approach to bring nanotech products to market. Integral to this process is the methodology for protecting workers potentially exposed to nanomaterials during product development. Occupational health surveillance is a key aspect of protecting employees and involves both hazard identification and surveillance of known medical data. However, when the health effects and exposure pathways of both new and existing "nano-scale" chemical substances are not yet well understood, conservative hazard controls and baseline data collection can facilitate both immediate and long-term worker protection.

Luna Innovations Incorporated (Luna) is a Virginia-based small business (as defined by the Small Business Administration) with a diverse coalescence of scientists, engineers, and business professionals developing and manufacturing new-generation products for the health care, telecommunications, energy, aerospace, and defense markets. Luna focuses on researching, developing, and commercializing innovative technologies through our contract research groups. With nearly 200 people in four locations across Virginia, Luna Innovations utilizes a disciplined and integrated business model designed to accelerate the process of bringing to market innovative new products. Luna diligently identifies technologies to fulfill large and unmet market needs taking these technologies from the applied research stage through commercialization.

One of Luna's core technologies is the production and modification of carbonaceous nanomaterials for potential use in applications such as diagnostics, therapeutics, and solar energy. This research is mainly conducted at Luna's nanoWorks Division site in Danville, Virginia. In addition, a variety of nanomaterials are explored through Luna's contracts research division for various applications such as multifunctional composites and coatings, remediation, and antitamper technologies. This work is primarily conducted at Luna's technology development division sites in Blacksburg and Charlottesville, and the nanomaterials in question may be commercially available or synthesized on site in small quantities for internal use or use by select research partners. Given the diversity of nanomaterial use, Luna must consider two types of scenarios when evaluating potential worker exposure to engineered nanoparticles: a research setting where a variety of new or familiar nanomaterials may be involved albeit in extremely limited quantities and perhaps only used a single time for screening purposes; and a production setting where larger quantities of familiar nanomaterials are synthesized regularly and potential for exposure may be present daily or weekly. This presents a challenge for not just Luna but many small research and development businesses. How does a business identify potential significant exposure threats without becoming bogged down in evaluating a vast number of small "research only" nanomaterial events consisting of a particular material being used a single time in a quantity on the milligram scale for a single well controlled reaction or formula?

Luna's Approach to Nanomaterial EHS

Luna has a designated component for environment, health, and safety (EHS) management. In 2007, Luna hired an EHS Manager with 20 years EHS consulting and compliance experience for industry and government to oversee EHS for the company's diverse activities. The EHS Manager is responsible for development and maintenance of the EHS management system for Luna and interacts with each location on a regular basis to implement and continually improve various EHS programs. In addition, facility managers and lab researchers with other primary responsibilities have been designated and trained as EHS representatives to assist in the day-to-day implementation of EHS programs at each location. The EHS Steering Committee, chaired by the EHS Manager, comprising senior managers and technical experts within the company provides management support for commitment to EHS compliance and employee safety for all activities at Luna Innovations. Like other small nanotechnology companies,[1] Luna seeks responsible risk management strategies to protect its employees working with nanomaterials.

Luna's overall approach to protecting workers involved in nanomaterial research and manufacturing follows OSHA's (Occupational Safety and Health Act) General Duty Clause,[2] which assigns the responsibility to the employer to furnish each employee a place of employment free from recognized hazards with the potential to cause physical harm.

Sufficient evidence to support the presence of legally "recognized" hazards of nanomaterial(s) is not yet available for all nanomaterials with which Luna works. Luna's internally produced carbonaceous nanomaterials represent an unknown hazard in our laboratories; therefore, Luna has implemented several controls for minimizing or eliminating exposures. For instance, there is scientific basis for recognizing hazards associated with multiwall carbon nanotubes but there is currently no definitive evidence for fullerenes similar to those the nanoWorks Division in Danville, Virginia, is producing.

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Luna uses a traditional risk management process and a hierarchy of control methods to accomplish this goal in a cradle to grave approach.

Risk Management at Luna Innovations

Basic risk management steps used at Luna include the following:

- Assess potential and known hazards,
- Assess potential or known exposures,
- Assess potential and known risk
  - Potential or known hazard + exposure = potential or known risk,
- Control risks through hazard control selection and implementation, and
- Monitor and review controls regularly through program reviews and collect baseline medical surveillance information for use when exposure risks are better defined.

The risk management methodology begins by assessing potential risks through various hazard and exposure assessment activities. For instance, Luna conducts initial hazard assessments of research facilities and new project-specific EHS analysis at the genesis of the project. Internal checklists have been developed and are used at all locations. This checklist requires the principal investigator (PI) to consider and answer questions on various aspects of the forthcoming work, including the identity of potential hazardous materials (nanomaterials included) and whether aspects of the project may impact the environment through changes in Luna's waste stream (by significantly changing the quantity of an existing waste material, introducing a new material to the current waste stream or identification of materials whose effect on the environment is ill defined and whose method of safe disposal is in question or undefined). The answers provided by the PI are reviewed internally to determine the suitability of the approach to be conducted at Luna's laboratories with existing engineering controls.

Once a program is initiated, or a request for material is received, a new project EHS consideration/hazards analysis worksheet is completed by the Luna PI. A portion of this form asks specifically if nanomaterials will be used, which ones, and whether the Material Safety Data Sheet forms have been entered into the electronic database Luna maintains. The PI also must answer questions related to known hazards, whether dust or vapor potential exists, and whether the recommended engineering controls for the materials are already in place at Luna. Disposition of the waste and residual raw materials is also addressed, as well as potential for air emission and waste water discharge. Through this review, Luna attempts to ensure the PI has considered new and current chemicals from cradle to grave. In addition, the amounts of materials expected to be brought on-site may be addressed at this point. If the amounts are large, meetings may be held to specifically review engineering controls and waste generation. This review may be conducted annually for programs lasting more than 1 year. Otherwise, a closeout meeting is conducted to ensure the waste and remaining raw materials have been accounted for and proper disposal is planned.

The continuous production scenario at Luna's nanoWorks facility is managed in a similar but slightly different fashion. Because numerous new projects and materials are not the major concern here and the initial hazard assessment was conducted before production commenced, hazard assessments and periodic nano-EHS program reviews are conducted and reviewed on an annual basis. During these reviews, typical hazard identification and characterization occurs and potential worker exposure is reassessed. Engineering controls are evaluated to ensure proper operation, and personal protective equipment (PPE) selection is reviewed to ensure that the appropriate selection is available for the operators. Documentation of good work practices is revisited, and improvements and updates to worker training are conducted. Luna also conducts a medical surveillance review and determines whether follow-up testing is warranted for its personnel. Finally, Luna reviews its procedures against recent regulatory developments to ensure environmental, OSHA, and DOT compliance.

Any issues or concerns are logged into a matrix consisting of requirements and actions taken along with appropriate dates in a formal tracking system. In addition to the periodic reviews, both the EHS Steering Committee and individual employees may bring issues to the attention of senior management through this corrective action matrix throughout the year. Luna's EHS program includes regular reviews of chemical hazards and regulatory developments throughout the year to implement recommended or required changes as necessary with respect to the nano-EHS program. In addition, operations are continually surveyed to identify potential exposures to nanomaterials to better select or improve engineering controls or identify needed administrative controls.

Exposure Assessment at Luna Innovations

Exposure assessment has been a critical part of Luna's risk management plan from conception, especially at the nanoWorks facility. For instance, early exposure assessment determined possible exposure to the prototype fullerene product by technicians during nanomaterial generation and processing, and during dust collector maintenance. Luna has voluntarily participated in monitoring studies conducted by Virginia Tech (VT) and Oak Ridge National Laboratories (ORNL) as well as hosted National Institute for Occupational Safety and Health (NIOSH) and OSHA voluntary compliance assistance visits. These external reviews have been conducted to better assess potential exposure routes during production of Trimetaspheres nanomaterials (Luna nanoWorks, Danville, Virginia) and related carbonaceous nanomaterials.

Luna periodically monitors work environments for engineering control performance. A Dust Trak Aerosol Monitor uses a laser photometer to measure particle concentrations at the site in the range of 100 nm to 10 microns. Data collected assists Luna in determining whether current controls are functioning properly or if new control methods should be considered or implemented. In addition, hoods are regularly calibrated and their airflow tested to ensure proper face velocities are present.

Instrumentation R&D with ORNL

Many current industrial processes for production of engineered nanomaterials do not have effective monitoring systems to ensure high-precision nanomaterial production. This lack of instrumentation can lead to inconsistent product quality, which hinders technology development, and can result in significant waste of precious raw materials. Luna is working with DOE's ORNL Nanoapplications Center to research the manufacturing of advanced engineered nanomaterials. The objective is the development of novel on-line monitoring systems of the manufactured nanoparticles in real time. These new instruments are targeted to monitor an array of variables relevant to the quality of nanoparticles, such as size distribution, size dispersion, density, surface charge, and material-specific data such as ionic, elemental, and molecular composition. Efficient fusion of such data can be performed by advanced chemometrics methods to yield high-valued information such as size-resolved chemical composition as a function of time and space in a nanoparticle reactor. The suite of instruments will be applicable to characterize a wide range of nanomanufacturing processes involved, and Luna has served as a test site for the evaluation of new technologies for this type of monitoring, which may be utilized for assessment of nanoparticle characterization outside of the reactor as well.

CONTROL OF POTENTIAL RISKS AT LUNA INNOVATIONS

Potential risk of nanomaterial exposure at Luna is handled through hazard control selection and implementation. Three main types of controls are used—administrative, engineering, and PPE.

Effective administrative controls can go a long way in minimizing potential exposure. The first of the administrative controls actually goes all the way back to the initial risk review: is the facility equipped to handle the nanomaterial in question, and if not, should the project be pursued? In other words, can the work plan be accomplished through the use of another material (one less hazardous or one that Luna is familiar with handling for instance)?

Once Luna has determined the risk of bringing the material on-site or the production of a particular nanomaterial is acceptable and is in Luna's business interests, other administrative controls become important in managing risks such as reducing or eliminating potential nanomaterial exposures. This includes having a robust hazard communication program and chemical hygiene plan that address nanomaterials, conducting hazard assessments to determine PPE requirements, having an appropriate waste management program, and having proper standard operating procedures (SOPs) for both production equipment and research laboratories that handle the nanomaterials.

Engineering controls are another very important aspect of risk management and are intertwined with the laboratory SOPs. Engineering controls are only effective if the SOPs are followed and the equipment is properly maintained and operating correctly. In the research laboratories, Luna relies mainly on chemical hoods and a specially designed integrated dust collection system to contain any airborne nanomaterials in dust or aerosol form. In laboratories where nanomaterials are in use, all process equipment is moved into these hoods so that the materials do not have to be dispensed or transported outside of the hood, thereby minimizing the potential for contamination of surrounding bench tops and equipment. In some cases, special handling techniques to minimize particulate generation are used by researchers in the hoods. At the nanoWorks facility, a Thiel Air Technologies Dust Collection System has been installed with high-efficiency, self-cleaning cartridge filters to minimize airborne particulates within and outside of the production facility. This unique exhaust system has been verified to be able to capture particles down to 1 nm.

Personal protective equipment is the final element of risk management to be considered. Luna employs laboratory coats, impervious gloves, safety glasses, and NIOSH-approved elastomeric half-face respirators with P100 cartridges where appropriate to further minimize the possibility of exposure to the worker. Respirators are approved and used in accordance with OSHA's Respiratory Protection Standard.[3]

Medical Surveillance of Nanomaterial Handlers at Luna Innovations

Interim guidance issued by the NIOSH in 2009 concluded, "Currently there is insufficient scientific and medical evidence to recommend the specific medical screening of workers potentially exposed to engineered nanoparticles."[4] Luna has decided to follow prudent recommendations for its workers at the nanoWorks facility including maintaining strict exposure controls, detailed worker record keeping, and characterization of baseline and periodic health status of workers. For workers at Luna's other sites, it has been determined that the potential for nanomaterial exposure is extremely low because of the amounts and frequencies in use combined with the implemented controls, whereby medical surveillance is currently not necessary. This, of course, would be reevaluated if during risk assessment of new programs it is determined that the potential for exposure or repeated exposure is higher.

For the nanoWorks facility, the EHS and human resources group at Luna maintains medical records and job descriptions for each employee. The job description includes the workers primary job function and tasks associated with it. Medical records include baseline chest radiographs and pulmonary function test results. All laboratory activities on a daily basis are required to be recorded in log books. A respiratory protection program exists as well and within its record keeping are medical questionnaires and medical approvals, pulmonary function test results, and fit testing.

Future Concepts for Data Handling at Luna Innovations

Luna is a small business, where less than 20% of the employees currently have the potential for nanomaterial exposure. Therefore, data collection, storage, and analysis of the related documents are straightforward. For larger entities, an on-line system may be needed to allow a program to sort and store data sets and information securely. One possibility would be for Luna to use a system similar to other internal tracking systems used for logging laboratory activities. A simple daily spreadsheet is being considered to record the employee's identification, the amount and type of nanomaterial in use, and the potential exposure time. At a later date, the EHS department could then determine total potential exposure time between a particular set of dates for an individual.

A more complex system could be envisioned where not only the daily log activities were recorded but also the medical testing, engineering control testing, and training records could be stored along with general EHS information related to safety and the materials themselves. Luna has worked on a prototype Web portal for the Air Force that could support such a system called WINGS-**W**eb **I**nterfaced **N**anotechnology Environmental, Safety, and Occupational Health Guidance System.[5] In its current Beta site form, it serves to provide comprehensive guidance modules related to regulations and industry's best practices, while also serving as a repository for related literature. It also includes tools for risk assessment and for searching trusted sites but is intended to be expandable to include tools and questionnaires related to medical tracking and medical surveillance. By centralizing the entire EHS program into a single portal, the WINGS program has the capability to simplify risk management assessment while storing data in a form that could be exported to another program for plotting trends or creating formalized reports.

CONCLUSIONS

The success and public acceptance of nanotechnology-enabled products will depend upon the nanotechnology community's ability to understand and manage potential hazards to our nanotechnology workers. The immediacy of the need for responsible and sustainable development of engineered nanomaterials cannot be overstated. Therefore, building an EHS knowledge base is essential to ensure the future of nanotechnology and the safety of those working in this emerging field.

In the current absence of formal guidance and well-defined toxicological and health data, Luna has chosen to pursue a conservative approach to nanomaterial safety. This approach is centered on using administrative and engineering controls, as well as PPE, to diligently identify nanomaterial uses and minimize or completely eliminate exposures. This systematic approach has been institutionalized into a flexible platform for the production, characterization, and development of nanotechnology enabled products.

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Developing a Registry of Workers Involved in Nanotechnology

BASF Experiences

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Objective: To assist BASF in the establishment of a registry of workers involved in nanotechnology. **Methods:** The initial step was a complete inventory of nanomaterials and sites of use. Guidance was developed to clarify which particulate nanomaterials were to be included in the survey. Site management was then contacted by the medical department to obtain a list of workers. **Results:** The time line for collecting data ranged from several months to a year, depending on the information needed, and presented challenges based on the lack of global definition and labeling of nanomaterials. Less than 50 nanomaterials are used as raw materials in less than 10% of the sites globally. In North America, less than 5% of sites and 5% workers use nanomaterials. **Conclusions:** Further work is required to integrate the inventory, registry, and exposure assessments.

BASF is a global company headquartered in Ludwigshafen, Germany, with more than 90,000 employees worldwide. One of its subsidiaries, BASF Corporation, is the US entity headquartered in Florham Park, New Jersey, with approximately 20,000 employees. BASF manufactures nanomaterials and nano-enabled products. The use of nanomaterials (particles between 1 and 100 nm) has sparked much discussion about their safe handling in the manufacturing workplace. The options for medical surveillance of potentially exposed workers have repeatedly been discussed over the last years, with no specific recommendations resulting until this point in time.[1–4] A possible strategy would be to supplement baseline examinations with additional testing for endpoints that are associated with the pathologic conditions, for example, observed after exposure to ambient small particles[5,6] or derived from animal experiments.[7] Unfortunately, the applicability of such endpoints to engineered nanomaterials is uncertain, as is the sensitivity of the endpoints recommended. Until tests can be determined to be specific, easily implemented, and interpretable, BASF has elected to take only the first step of establishing a registry of workers who can be followed for changes in health status.[1]

Development of a registry is, in principle, a straightforward task, and such an activity has been used for many years to evaluate individuals with specific pathologies such as cancer. Registries have also been used for individuals exposed in the workplace, for example, the beryllium registry maintained by Oak Ridge National Laboratory. Nevertheless, in the case of exposure to nanomaterials, developing a registry requires an understanding of what is meant by "nanomaterial." For substances that use the prefix "nano," such as carbon nanotube, there is little question, but for substances that have been in use for years, asking plant managers "do you work with nanomaterials" will not elicit the response needed. For example, silica has been used for many years in construction materials. Without a designation on the label that the silica is nanoscale, production workers would not be able to identify it as such. So, members of the global Environment, Health, and Safety (EHS) community within BASF engaged in a process to develop the information and structure to identify those substances of interest, that is, an inventory of nanomaterials used in the company. This inventory was developed by using the International Organization for Standardization (ISO) definition[1,8] supplemented by the German Chemical Industry caveats[2].[9] This inventory also led to the development of BASF internal exposure assessment criteria and methodology that would be needed to supplement the exposure registry. The process, results, and pitfalls encountered are described.

METHODS

The strategy developed by BASF for occupational safety and health incorporated three major efforts of information gathering: an inventory of nanomaterials used in the workplace; a registry of workers handling these nanomaterials; and assessments of exposure to those nanomaterials. This information was entered into separate data capture systems, which were intended to interconnect so that individuals could be associated with specific nanomaterials in use and be associated with specific exposure levels (Fig. 1). The source of information was different for each set of data, as was the owner of the information. For example, business-specific information on the nanomaterials used in products (ie, the inventory) was obtained from the product stewards who had the best understanding of their products; these data were then "owned" by this group, who would be responsible for updating the information on a periodic basis. Information for the registry was obtained from plant/site managers but was "owned" or maintained by the medical department. And information on the exposure levels was obtained by the occupational safety department, which was responsible for maintaining and updating the information. While these three pieces of information are independent, they need to be linked.

Inventory

To develop a registry, an inventory of nanomaterials used in the workplace had to be established. The source of the inventory data were the product stewards (EHS affiliates to the business), who were given guidance on how to identify the substances of interest. Guidance included definition, manufacturing methods, and physical

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[1] Nano-objects are discrete particles with one, two, or three dimensions between 1 and 100 nm.

[2] In addition, the following criteria were applied, that is, *nanomaterials* are defined as intentionally manufactured, solid, particulate substances, either in powder form or as dispersions or as aerosols, consisting of nano-objects and their aggregates and agglomerates. In addition, nanomaterials are distinguished from larger-sized particles because they must contain, when measured by standardized and recognized methods, at least 10% by weight of nano-objects, or have, when measured by appropriate methods, a volume-specific surface area larger than $6 \times 1/100\ nm^2$. For an unequivocal particle size characterization, BET measurement data are valid for only nonporous, monodisperse particles. To compensate for the different substance densities, the measured surface area needs to be normalized to a substance density of 1 g/cm^3 by multiplying the substance density (g/cm^3), which must be known, with the measured surface area (m^2/g). The resulting value is then called "volume specific surface area" (1/nm). Following the considerations of Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), a meaningful threshold the for volume-specific surface area could be $6 \times 1/100$ nm, corresponding to a surface area of 60 m^2/g of perfect spheres of 100-nm diameter at a substance density of $1 g/cm^3$.

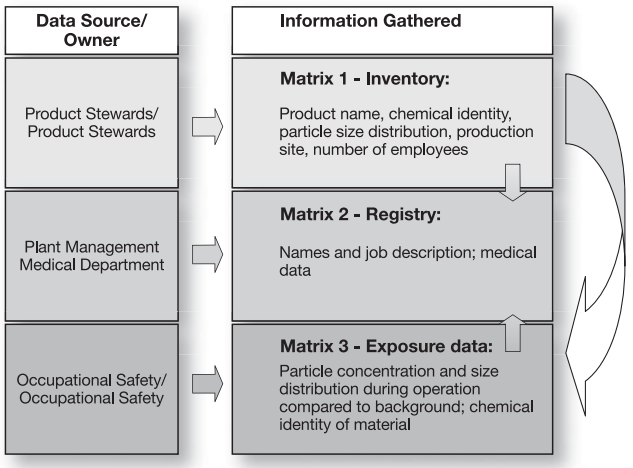

FIGURE 1. Matrices of information needs. Sources and owners of information are listed in the boxes on the left. This information proceeds laterally to the information matrix (boxes on right). The arrows on the right indicate how information is shared among matrices.

properties associated with nanomaterials. The definition used was the ISO definition[8] supplemented by the German Chemical Industry caveats.[9] Information collected included product name, chemical identity, particle size distribution, affected division, production site and plant, and number of affected employees. These data were captured in a simple spreadsheet for subsequent conversion to a database. The product stewards were identified as the data owners and were charged with updating information on nanomaterial use.

Registry

Once a list of nanomaterials in use was compiled, questionnaires were sent to managers of sites identifying these substances to confirm their use and to identify the individuals and tasks involved. The initial registry encompassed only sites in North America (~210 sites; ~11,000 workers). The time line for data collection from inventory to registry is provided in Fig. 2. This registry was compiled and maintained by the medical department. Furthermore, the medical history and findings from previous medical examinations are maintained by the medical department under strict medical confidentiality. No specific surveillance will be initiated until clear criteria and endpoints are identified. This does not preclude, however, that the persons included into this registry may be subject to medical surveillance examinations for other reasons than exposure to nanomaterials.

Exposure Assessment

Information on manufacturing sites involved with nanomaterials obtained from the inventory data collection was used to establish qualitative and quantitative exposure assessments of the workplace. Qualitative data include the production plant, work area, and operation, while exposure data include particle concentration and size distribution in the nanoscale and in the microscopic range during operation, compared to background, and chemical identity of captured material. While the source of the information on sites is derived from the inventory, the exposure assessments are conducted and maintained by the Industrial Hygiene/Occupational Safety Department. These data comprise the last module and can be integrated with the registry to be used to evaluate the risks to the workforce and to develop a prospective study of health effects.

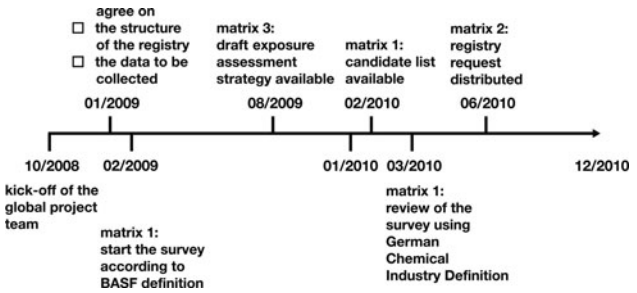

FIGURE 2. Timeline for collection of information in various matrices. Figure shows the timeline for activities to gather information. Total process required nearly 24 months from agreement.

RESULTS

The data collection for the inventory phase required education of the site managers and product stewards, involvement of the research community, and answers to practical questions about which particulate nanomaterials might qualify. One of the challenges for collecting these data was the lack of a definition that is universally accepted. The National Nanotechnology Initiative has defined *nanoparticles* as an engineered or designed particle with at least one dimension less than 100 nm. This definition likely represents the most simplified description. Nevertheless, there are many groups wrestling with defining all the various particles that are considered "nano," and each group has labored to develop precise wording on what constitutes a nanomaterial. The ISO divides the realm of nanomaterials into nano-objects, that is, discrete particles, and nanostructured materials, that is, agglomerates, composites, etc. Before launching a survey to develop an inventory, a decision needed to be made on which materials were of greatest concern. While the ISO definition of nano-objects served as a starting point, there was concern that it would not encompass all the particulate nanomaterials that might be of interest to regulators, once a definition was promulgated. Therefore, an industry definition was used that might be equivalent to a regulatory definition.

It was recognized that a virtual, global interdisciplinary team had to be established. This team included members from industrial hygiene, product stewardship, occupational medicine, metrology, and production. Their combined expertise was used to develop all three information matrices of information. Of particular challenge was coordination of schedules to allow for real-time discussions on progress of the project. Once the initial inventory was completed, a global effort, each region was responsible for developing a registry and exposure assessment program. The results of the registry presented here are the experiences of the BASF Corporation, the US subsidiary.

The results of the survey indicated that most of the nanomaterials were used in the United States and Europe, with less than 100 different nanomaterials (products containing nano-objects or having nanostructures) produced; most of the nanomaterials on the global inventory were in use in the United States. In addition, less than 50 different nanomaterials are used as raw materials. Approximately 5% of BASF sites use nanomaterials. A challenge was to decide to what extent legacy products were included, that is, products that now fall into the category of nanomaterial that have been manufactured for decades. Because a regulatory definition might well include products on the market for decades, it was decided to include such products in the inventory. Most of the materials on the inventory were dispersed in either a liquid or solid matrix, with few free particles in use or sold as product. The product stewards (the EHS contacts with the business groups) were the best source of information on which nanomaterials were used, and this group was charged with periodic updates.

Collection of data proceeded onto a spreadsheet format, which captured the name of the material, CAS number, volume used, site where used, final product, and estimate of worker population. At some point, this information will be loaded in a unique database that can crosslink with the registry and exposure matrices.

On the basis of the information on "where used" in the inventory, sites were identified for the development of the registry. For the site managers, a questionnaire was developed that provided a drop-down list of substances and product names that had been identified in the inventory. Site managers needed to only check their use of the substance/product name. Names of workers and job descriptions were then provided to the medical department. Currently, 5% of the US BASF workforce is engaged in handling nanomaterials. These individuals may be subject to additional examination once definitive tests are identified. Furthermore, this list of workers will be provided additional training and provided nano-specific procedures.

Workplace sites will also be included into exposure assessment programs. Unfortunately, discussion within national and international bodies involved in industrial hygiene and occupational safety is ongoing about suitable approaches to exposure assessment.[10–14] Furthermore, health-based occupational exposure level values as assessment criteria have not yet been established. Thus, industrial hygiene was challenged to establish and roll out appropriate methodology, measurement strategy, and assessment criteria for exposure assessment, which could be utilized at all affected production sites worldwide.

CONCLUSION

The efforts of this global team successfully identified nanomaterials used within BASF, and a registry of workers in the United States was established. Further work needs to done to establish a harmonized reporting system that can integrate the exposure assessment with the worker registry. In addition, a process is required to update the inventory, especially as regulatory definitions are promulgated.[14,15] And finally, a consistent method to identify nanomaterials needs to be established.

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National Institute for Occupational Safety and Health Nanomaterials and Worker Health Conference—Medical Surveillance Session Summary Report

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Objectives: The goal of these sessions was to identify current practices and recommendations regarding medical surveillance for nanomaterial workers. **Methods:** Conference participants met in three discussion groups. **Results:** There were few existing programs directed to nanomaterial workers. Participants expressed a range of views, from feeling that comprehensive medical surveillance is important currently to suggesting that targeted medical surveillance will become important when more complete data are available to assess risks. **Conclusions:** Results of health outcomes research for ultrafine air pollution and toxicological information about specific nanomaterials should inform the design of medical surveillance programs. Groups with high exposures should be identified and targeted. Overall, because of uncertainties in the health effects of concern, investments in control measures, exposure assessment efforts, and exposure registries are currently most likely to be important prevention strategies.

In an effort to address questions about the appropriate role of medical surveillance in an overall preventive program for workers with nanomaterials, approximately 120 participants at the National Institute for Occupational Safety and Health conference broke up into three discussion groups after some presentations on this topic. The participants were diverse in terms of their primary discipline (eg, physicians, epidemiologists, and health and safety specialists), affiliation (eg, academic, consulting, public health, industry, and labor), region or country of origin, and their experience with health concerns related to nanomaterials. The following summary distills the feedback received in these breakout sessions. These breakout sessions were useful in brainstorming ideas and approaches and permitting some preliminary discussion. The statements that follow should not be construed to represent the viewpoints of all or most of the participants, but they do reflect opinions of some speakers.

CURRENT SITUATION

Of the participants' organizations, some were conducting general medical surveillance on employees, though this was not specific to potential health effects from nanomaterial exposures. Input from the groups suggested that represented organizations were not performing specific medical screening or surveillance, either directed primarily to nanomaterial exposures or with endpoints chosen because of concerns about particular nanomaterials.

One organization's representative reported that he and other members had a medical surveillance program directed, at least in part, to nanomaterial concerns, although it was not specific to nanomaterials. This representative noted that a requirement from their governmental funding agency drove this program. This surveillance included baseline examinations, including routine laboratory tests, and annual surveys. The annual surveys included job hazard questionnaires that would capture work with nanomaterials and some medical questions regarding pulmonary conditions that might result from nanoparticle exposures. Interestingly, detailed responses regarding the types of nanomaterials used were part of a separate industrial hygiene survey/database, which was not connected with the medical survey.

Similarly, many individuals who work with nanomaterials are enrolled in occupational health surveillance programs, which include annual medical history, pulmonary function tests, and, in some case, other tests, such as chest radiography, as a result of other aspects of their work and other hazardous exposures. In some countries, for example, Germany and Switzerland, occupational health surveillance is mandatory for all workers with at least annual examinations, although the focus is on general workplace exposures, not specifically on nanomaterial exposures.

Some organizations did exposure tracking only at this point (with no medical surveillance or screening component). This approach potentially permits future medical evaluation of exposed workers, should a hazard be identified.

IMPLICATIONS OF LIKELY EXPOSURES AND EXPOSURE DOCUMENTATION

Several participants provided a rationale for not conducting occupational health surveillance for nanomaterial workers based upon some evidence that the use of nanomaterials did not result in any exposure, largely because of engineering controls in place, the use of personal protective equipment, and/or knowledge regarding the physical form of materials (i.e., in solution or suspension). These participants did acknowledge that there is some uncertainty regarding the efficacy of controls, such as fume hoods, in preventing any exposures.

There was some agreement that careful documentation of potential exposures to nanomaterials with specificity as to type is essential and should be a part of the health record or accessible to occupational health professionals, permitting an awareness of potential exposures when evaluating workers. Business units in some organizations currently report some information about nanomaterials being handled in the workplaces, but the definition of which parameters (eg, size, shape, agglomeration, and coating) ideally need to be reported is unclear. A goal should be to have improved tracking of where nanomaterials are in use. Optimally, appropriate exposure

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measurements should be conducted for potentially exposed workers. Ultimately, appropriate quantification of exposures, combined with adequate information about exposure–response relationships, is necessary for health risk assessment and to design appropriate targeted medical surveillance programs. Much of this information is not currently available. Some organizations reported that they use a control-banding approach, a method of estimating exposure and hazard, when precise exposure and hazard information is not available.

PROS AND CONS EXPRESSED REGARDING MEDICAL SURVEILLANCE FOR NANOMATERIALS CURRENTLY

Pros

- In the face of uncertainties, conduct of medical surveillance would be viewed as proactive and represent a commitment to employee health and safety.
- Conduct of medical surveillance may help to establish boundaries on the nature and occurrence of potential problems and uncertainty.
- Data collected may serve a risk management function.

Cons or Difficulties

- Medical surveillance may pose resource issues (cost, time involved, etc) for occupational health/environment, health, and safety programs.
- There is lack of clarity as to the health endpoints of concern, particularly in the medium and long term, making design of rational surveillance programs particularly challenging.
- While nonmalignant and malignant pulmonary conditions and certain cardiovascular conditions have been appropriately suggested as potential health effects of exposure to nanomaterials based on other scientific knowledge, such conditions would likely be common in the populations engaged in this work as they age, independent of nanomaterial exposures. There would be difficulties in sorting out the cause of any abnormalities identified through medical surveillance. Participants expressed concern related to separating abnormalities that might be related to nanoparticle exposures from those associated with nonoccupational (or other occupational) causes.
- Markers of physiological changes or health effects that may be related to nanomaterial exposure are nonspecific, with multiple potential causes. Similarly, markers of exposure or of inflammation may be affected by exposures to other small particles, such as ultrafine particles, for example, diesel exhaust. Confounding effects of other exposures need to be taken into account in designing surveillance schemes that attempt to evaluate short- and long-term effects of engineered nanoparticles.
- Concern was expressed regarding the use of medical screening tests, which subject workers to potential harm, such as computed tomographic scans with consequent radiation, or which generate data of uncertain significance, leaving the occupational health care professional and the worker without guidance as to the appropriate action in the face of a "positive" result. The cost of false-positive results, in terms of unnecessary anxiety and costs of follow-up tests, should be considered.
- Assessment of endpoints that may reflect potential central nervous system effects of exposures, if warranted, will likely raise employee concerns as to the kinds of information that should be collected in surveillance programs at baseline and throughout employment. Some of this information would likely be perceived as falling within the realm of mental health, with the attendant sensitivities to the collection and management of this kind of information.
- Medical surveillance may provide a false sense of security for employees, suggesting, perhaps incorrectly, that testing is sufficiently sensitive to detect all potential adverse health effects from nanomaterial exposures.

OTHER CONSIDERATIONS RELEVANT TO MEDICAL SURVEILLANCE FOR NANOMATERIAL EXPOSURES

Recognizing many data gaps regarding workplace exposures and likely health effects, some participants expressed concern that the expanding development, production, and the use of engineered nanomaterials could be considered a large and largely uncontrolled experiment, engaging increasing numbers of workers across the United States and the globe. This recognition suggests a need for proactive assessment and control of exposure and serious consideration of medical surveillance for potential health outcomes, especially when exposures may not be fully controlled.

It was pointed out that many workers, particularly those working for smaller employers, have no access to occupational health care services and are not currently participating in any form of medical surveillance. Exposure assessment is likely nonexistent in these settings as well. Discussions of the need for medical surveillance or registries need to take these workers into account. These underserved workers may, in fact, account for the largest number of potentially exposed workers, based on survey data presented at the conference.

There is likely a perception that the work environment is safe and free of risk among large segments of the nanomaterials workforce, particularly among those in research and development, who have available engineering controls and personal protective equipment. Such individuals will likely have little interest in participating in medical surveillance programs. Training programs that provide a strong rationale for participation in medical surveillance will be needed for these groups. Engaging the workforce, with a clear explanation of the potential risks and the levels of uncertainty, is essential to establishing meaningful surveillance programs and ensuring compliance with them. A partnership between those potentially exposed and those interested in assessing risk and outcome needs to be the context for work in this area.

The point was made that the legal status of health records needs to be carefully set out. Any connection between employer-collected records and larger state or national registries needs to be explicit with clear safeguards for confidentiality and job security. Confidentiality and privacy concerns that may arise, for example, with prolonged retention of data, problems in securing data, and appropriately limiting access to data, must be addressed.

Some participants felt that it is important, when designing medical surveillance programs, to avoid making assumptions about mechanisms of disease, dose–response relationships, and latency in an area of new and evolving exposures. Given the situation in the United States in which medical surveillance programs tend to end at the conclusion of employment, it was suggested that a European approach be considered, in which information about exposures/jobs and medical examination results is provided to employees leaving employment.

SUGGESTED APPROACHES

The experience with research regarding ambient air pollution and cardiovascular and pulmonary effects should inform the design of medical surveillance programs for nanomaterials. Similarly, toxicological information of concern about the adverse effects of specific types of nanomaterials, for example, carbon nanotubes, should be considered in the decision to initiate and design the medical surveillance programs. It was suggested that groups with high exposures be identified, based on air monitoring. Such groups could be initially targeted for medical surveillance.

Participants indicated that efforts to identify and test for appropriate markers for likely or known effects, for example, targeting certain inflammatory mediators, would be more promising

than untargeted general medical surveillance programs, for example, questionnaires and physical examinations. Some participants indicated that, when medical surveillance will be utilizing methods of unproven utility, sensitivity, and specificity, it should be done in a research mode with full, informed consent and appropriate oversight.

Generally, participants felt that efforts are warranted now to identify and contemporaneously document the salient features of work activities, work areas, types of nanomaterials used, and controls, ideally in a consistent and easily retrievable fashion across organizations. Such efforts will facilitate the conduct and interpretation of medical surveillance for groups of nanomaterial workers, whether it is initiated now or in the future. Moreover, these efforts will be of great value to any future implementation of exposure registries and epidemiologic studies.

Some participants suggested that surveillance of these populations of workers for morbidity and mortality patterns is essential. Observation of differences in rates or age of onset of certain conditions that may be plausibly connected to nanomaterial exposures may be informative, particularly if there is accompanying exposure information.

Many participants felt that investments in control measures, exposure assessment efforts, and exposure registries are likely to be more effective prevention strategies at this time than investments in medical surveillance and that these approaches should probably be of higher priority currently, especially if resources are limited.

The Role of State Public Health Agencies in National Efforts to Track Workplace Hazards and the Relevance of State Experiences to Nanomaterial Worker Surveillance

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Objective: This essay examines the role state public health agencies could play in the surveillance of emerging workplace hazards including nanotechnology. **Methods:** This essay describes existing state occupational health surveillance programs in order to demonstrate their potential applicability, and limitations, in regards to nanomaterial worker surveillance. **Results:** State public health agencies have access to information and an ability to put surveillance information to use in ways that complement those of industry, academia, regulatory agencies, and federal partners. **Conclusions:** Some state public health agencies have significant experience with occupational health surveillance and are therefore valuable partners in the development and implementation of nanotechnology worker surveillance programs. Including states in emerging hazard surveillance enhances surveillance activities and builds state capacity to help workers.

Public health surveillance, the ongoing systematic collection, analysis, and interpretation of health data for the purpose of improving safety and health, is an essential public health function.[1,2] Occupational health surveillance is the tracking of occupational injuries, illnesses, fatalities, and hazards to monitor trends and progress over time and guide efforts to improve worker safety and health.[1] State public health agencies have been recognized as critical partners in efforts to conduct public health surveillance since 1951 when the Centers for Disease Control and Prevention asked the Association of State and Territorial Health Officials to convene a group of state epidemiologists and have them develop a list of diseases that should be reported to the public health service.[3] This group later became the Council of State and Territorial Epidemiologists, which currently recommends diseases and conditions for reporting within states and to the Centers for Disease Control and Prevention and develops recommendations for state-based public health surveillance.

The states are important partners in all aspects of public health surveillance, and they play a unique and well-described role in surveillance for work-related injuries, illnesses, fatalities, and hazards.[4–8] The National Institute for Occupational Safety and Health (NIOSH) has recognized the role of state public health agencies in occupational health surveillance and has incorporated them as principal partners in the NIOSH surveillance strategic plan.[9] NIOSH has fostered state capacity by providing funding for state-based occupational health programs since the 1970s. In 2010, NIOSH awarded 5-year cooperative agreements to 23 states (public health agencies and labor departments) to enhance state-based occupational health and safety surveillance capacity.[10] Current NIOSH-supported state-based surveillance programs include both basic occupational safety and health surveillance ("Fundamental") programs and in-depth ("Expanded") programs. The Fundamental Program enables states to establish an occupational safety and health program and to carry out basic surveillance using existing data sets (eg, occupational health indicators[11,12]). The Expanded Program enables states to focus on one or more priority health conditions, injuries, hazards, or worker populations (eg, occupational pesticide illnesses,[13] work-related fatalities[14]) in addition to conducting basic surveillance activities. These cooperative agreements serve the dual purposes of building state occupational health surveillance capacity and augmenting national surveillance of occupational conditions.

In July 2010, NIOSH and the Mountain and Plains Education and Research Center jointly sponsored the conference, Nanomaterials and Worker Health: Medical Surveillance, Exposure Registries, and Epidemiologic Research. Attendees discussed both the growing evidence that exposure to engineered nanomaterials may cause adverse health effects in workers and the need for occupational health surveillance of nanomaterial workers to better characterize the hazards and guide prevention efforts. The importance of helping societies "act in the face of uncertainty in a precautionary manner," and the role that surveillance plays in these efforts, have been described elsewhere.[15] Options for tracking workers exposed to engineered nanomaterials, including medical surveillance, exposure registries, and epidemiologic studies, were discussed at the conference and are described in this journal and in previous publications.[15–17]

As discussions about implementing new surveillance systems for this emerging hazard take place, it is important to remember that state public health agencies have been identified as uniquely able to (1) provide critically needed data on occupational diseases, (2) generate information necessary to evaluate the conventional occupational data sources, (3) actively link surveillance findings with intervention efforts at the state and local levels, and (4) integrate occupational heath into mainstream public health practice.[6] This essay aims to characterize the occupational health surveillance that state public health agencies currently conduct, describe the limitations with existing systems for detecting the potential hazards associated with nanomaterial exposure, and demonstrate that state public health agencies should be included as essential partners in the development and implementation of nanotechnology worker surveillance programs.

STATE PUBLIC HEALTH AGENCY OCCUPATIONAL HEALTH SURVEILLANCE

The California Department of Public Health's (CDPH) authority to conduct surveillance of work-related injuries and illnesses was expanded in 1985 with legislation (Chapter 1394, Statutes of 1985) mandating the development of an occupational health and disease prevention program that includes data collection, investigations, technical assistance, prevention efforts, and other components of occupational health surveillance. Today, CDPH occupational health surveillance programs for several specific health endpoints are supported by cooperative agreements with NIOSH. The existence of a legislative mandate and the amount of state and federal support

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varies among states, and some states do not have any occupational health surveillance capacity, but many of the CDPH occupational health surveillance programs described below have counterparts in other states.

The CDPH Occupational Lead Poisoning Prevention Program is supported both by a state-funded mandate and by NIOSH as the California component of the NIOSH Adult Blood Lead Epidemiology and Surveillance program.[18] The California Health and Safety Code requires laboratory reporting of blood lead levels (Section 124130) and that CDPH develop and maintain an occupational lead poisoning prevention program (Sections 105185 to 105195); funding is provided by a fee on employers in industries where there is documented evidence of potential occupational lead poisoning. The Occupational Lead Poisoning Prevention Program staff maintain an occupational blood lead registry and track adult blood lead levels to determine who is exposed to lead in California, identify lead-poisoned workers and help them get proper medical care, assist employers to improve their lead safety practices, provide information to help health care providers care for lead-poisoned workers, and help clinical laboratories comply with adult blood lead reporting requirements.[19] Lead is one of the few occupational hazards for which there is a state-funded mandate requiring a surveillance program. Lead is also one of a select number of occupational hazards for which the California Department of Industrial Relations, Division of Occupational Safety and Health (Cal/OSHA), has a comprehensive standard governing workplace exposures and medical surveillance. Also, lead is atypical in that it is one of the few exposures for which valid and reliable environmental and personal (biomonitoring) test methods are available. As a result of decades of research demonstrating, characterizing, and quantifying the hazards from lead exposure, occupational health surveillance programs benefit greatly from legislation that guides workplace activities and provides dedicated funding for surveillance efforts; few occupational surveillance programs have this sort of support.

California has been conducting multisource surveillance of work-related asthma (WRA) since 1993. The current CDPH program is partially funded by NIOSH and aims to identify primary and secondary causes of WRA, characterize exposures and disease, and devise prevention strategies.[20] CDPH collects and analyzes mandatory physician reports of occupational injuries and illnesses, workers' compensation data, and hospital data and uses key word searches and International Statistical Classification of Diseases (ICD-9) and other codes to identify cases of WRA. Information from these reports is supplemented by telephone interviews and review of medical records. These data are used to generate state-based prevalences of WRA by industry and occupation to guide intervention activities. Interviews of workers with WRA also serve as an opportunity to provide individuals with educational materials and technical assistance related to their condition. When a review of the data reveals a high-risk worksite or industry, worksite visits and interviews with employees and other stakeholders are conducted to guide development of targeted interventions to prevent WRA. In contrast to lead surveillance where surveillance efforts are based on mandatory reporting of blood lead levels, state surveillance for WRA, and other disease-specific endpoints (eg, occupational carpal tunnel syndrome surveillance), relies on passive reporting using multiple secondary sources of data that are not primarily collected for the purpose of occupational health surveillance. Although there are limitations with these types of surveillance systems (eg, clinician recognition that the injury or illness is work-related is critical for detection), they are efficient, timely, cost-effective, and supply meaningful data.[7]

The unfolding story of diacetyl-related lung disease ("popcorn lung") offers another example of the ways in which a state public health agency conducts occupational health surveillance and links data to public health interventions.[21] Between 2004 and 2006, Cal/OSHA received reports of two index cases of bronchiolitis obliterans among California flavor manufacturing workers. CDPH collaborated with Cal/OSHA, NIOSH, employees, employers, and medical providers to initiate industry-wide medical surveillance based on lung function screening spirometry and respiratory health questionnaires. The information obtained was used to characterize the flavor manufacturing workforce, identify employees with obstructive lung disease, determine risk factors associated with obstruction, calculate the increased risk of obstruction associated with working in the flavor manufacturing industry, work with clinicians to ensure that employees received enhanced medical surveillance and proper medical care, and make recommendations regarding workplace interventions for primary and secondary prevention. In September 2010, an occupational diacetyl regulation was approved by the standards board responsible for promulgating Cal/OSHA regulations. The surveillance results provided important information establishing the need for a standard and outlining appropriate requirements. Distinct from the long-term surveillance systems for lead and WRA, in this case, two sentinel cases led to the recognition of an emerging hazard and prompted collaboration with Cal/OSHA to develop a new active surveillance system.

The three state-based occupational health surveillance systems described earlier are quite successful, but different occupational health conditions require different surveillance systems and not one system described earlier could be easily adapted for nanomaterial worker surveillance. Lead surveillance is based on mandatory blood lead testing and reporting, but nanomaterial biomonitoring methods are in too early a stage of development to be used as the basis for a surveillance program. WRA surveillance depends on clinician awareness and reporting of the work-relatedness of a particular condition, but nanomaterial-related health effects are just beginning to be recognized and no pathognomonic sign or symptom has yet been identified. In the absence of clinician recognition and documentation of a particular nanomaterial-related health outcome, neither the passive surveillance for WRA, nor the active surveillance for obstructive lung disease in flavor manufacturing workers, would be applicable for nanomaterial workers.

POTENTIAL PUBLIC HEALTH CONTRIBUTIONS TO NANOMATERIAL WORKER SURVEILLANCE

Surveillance of nanomaterial workers presents many challenges, and state public health agencies have much to offer these efforts. Nevertheless, state public health capabilities are of little use in the absence of dedicated staff and resources. New legislation that requires medical surveillance and/or industry participation in an exposure registry, establishes a role for public health, and provides a funding mechanism would improve the success of a new project. Our experience establishing a new surveillance program for flavor manufacturing workers demonstrated the challenges of operating in the absence of a funded mandate and compulsory industry participation. In this section, we will describe some of the ways in which states could contribute to nanomaterial worker surveillance, assuming the infrastructure for occupational health surveillance, and the resources to support such programs, are available.

An initial challenge that medical surveillance programs, exposure registries, and epidemiologic studies face is the identification of exposed employees. The nanotechnology workforce crosses many industry and occupation sectors and has been difficult to characterize.[16] Nanotechnology applications are already used for soil remediation, personal care products, paints, electronics, fabrics, sports equipment, and energy technologies, and research is underway for applications in agriculture, medicine, and many other sectors. The workforce is quite variable across states depending on the nanomaterial resources, research, manufacture, use, disposal, and regulations in each state. State public health agencies can work with

academia, regulatory agencies, trade associations, and employee groups to characterize the nanomaterials workforce. For instance, the California Environmental Protection Agency's Department of Toxic Substances Control (DTSC) has taken an active interest in nanotechnology and has developed partnerships with relevant industries in an effort to develop an "industrial ecology of manufacturing" that will protect public health and the environment.[22] DTSC has used California legislation (Chapter 699, Statutes of 2006) to request information relevant to determining environmental fate and transport from manufacturers who produce or import carbon nanotubes, nanometals, and nanometal oxides in California. In doing so, DTSC has made significant inroads into identifying these companies and establishing a dialogue with them, both useful first steps in the development of an occupational health surveillance program such as an exposure registry. DTSC and CDPH are in the process of establishing a memorandum of understanding to address collaboration and data sharing. As was the case with the flavor manufacturing worker surveillance program, state-based characterization of the nanomaterial workforce is necessary and state public health agencies can work with relevant partners to accomplish this first step.

Once the relevant workplaces have been identified, state public health agencies have several mandates that enable them to work with employees, employers, and medical providers to ensure cooperation with medical surveillance, exposure registries, and epidemiologic studies. State public health agencies are vested with the legal authority to require disease reporting and the authority to request health data including medical records. In many states, including California, there is required reporting of occupational injuries and illnesses to public health agencies. While there is no specific legal authority to require reporting of nanomaterial-associated injuries or illnesses, the legislature, or OSHA, could enact requirements for reporting illnesses associated with nanomaterial exposure. Even in the absence of a nanomaterial-specific reporting requirement, state public health agencies can play a key role as a repository of individual data. Nanomaterial worker medical surveillance and exposure data will be collected at the level of a particular workplace, but state public health agencies could serve as central reporting sites where information from all relevant workplaces could be collected and compiled, sentinel cases could be identified, and trends could be established. State public health agencies are familiar with accessing individual-level data and are experienced with the human subjects review and confidentiality issues that arise when tracking ill workers. Perhaps most importantly, state public health agencies, by virtue of their enduring presence in state government, can develop and sustain ongoing injury and illness surveillance systems to track nanomaterial workers, if adequate and long-term resources are made available to support this work.

State occupational health staff can also work with other public health partners and utilize other public health data for the purpose of nanomaterial worker surveillance. Several innovative projects integrating occupational health into mainstream public health have been described[8] and new collaborations may be helpful for nanomaterial worker surveillance. For instance, public health agencies maintain cancer registries and conduct statewide population-based cancer surveillance. Partnerships with cancer registries and use of their data represent a potential way of identifying individual cases, or using data for epidemiologic studies, assuming that robust industry and occupational data are collected and coded. Efforts are currently underway, by NIOSH and others, to make improvements in industry and occupation data and to develop software that will make coding feasible and cost-effective.

State public health agencies also have unique relationships and experience communicating with health care providers. Communication is particularly important in the setting of an emerging hazard where there is potential for the emergence of a new occupational disease. Occupational health staff can alert health care providers to an emerging hazard through state medical boards, occupational medicine clinics, and other networks. This sort of outreach also serves as an opportunity to encourage health care providers to report cases to the state health department so that sentinel cases can be detected and trends can be identified. State occupational health physicians and nurses can also provide technical assistance to providers regarding appropriate medical treatment and follow-up.

Any new nanomaterial worker surveillance program must include mechanisms for acting on the information that is obtained. As mentioned above, states consolidate individual data into trends that can be used to identify and prioritize high-risk industries, occupations, and populations. This information can be used to aid enforcement actions by regulatory agencies and to develop public health prevention strategies. Many state public health agencies have the authority to investigate workplaces if a problematic worksite or high-risk work practices are identified from individual reports. State public health agencies have experience referring identified workplaces and/or employers to OSHA for technical assistance or enforcement actions and are well positioned to collaborate with other state agencies (eg, state environmental agencies) in efforts to reduce or eliminate hazards.

Several state public health agencies have the multidisciplinary staff, including health educators, occupational physicians and nurses, industrial hygienists, epidemiologists, and toxicologists, needed to design and implement prevention programs. States typically network with the employee groups, employers, trade associations, community groups, health and safety professionals, academics, and environmental and occupational regulatory agencies necessary to develop and disseminate feasible and effective interventions to prevent workplace injuries, illnesses, fatalities, and hazards. Occupational health staff can also work with partners in other areas of public health on prevention efforts. For instance, state public health departments are taking a lead role in developing worksite-based wellness programs. These programs sometimes tend to focus on behavioral changes to improve general health, but the recognition of a new occupational hazard would provide further impetus to ensure that injury and illness prevention efforts are integrated into workplace wellness activities. States participating in NIOSH cooperative agreements currently collaborate to standardize data, thus demonstrating that state-based information can be transmitted to national partners to help establish national trends and to serve as the basis for national intervention programs.

CONCLUSIONS

The role of state public health agencies in occupational health surveillance has been well described. Many states have significant and relevant experience and bring unique capacities to the surveillance of occupational injuries, illnesses, fatalities, and hazards. Other states, without existing occupational health programs, will require resources for capacity building in order for them to participate in these efforts. The legal authority afforded to state public health agencies to require disease reporting, obtain individual-level data, and investigate workplaces aids their ability to detect sentinel cases and monitor trends. The use of multidisciplinary staff and experience working with a variety of stakeholders supports enforcement activities and promotes prevention efforts. Individual employers and industries may conduct medical surveillance or contribute to an exposure registry, but this information should be tracked and evaluated by states for consolidation, evaluation, and action. State public health agencies can also work to support legislation and regulations that support occupational health surveillance and protect workers. For these reasons, information from new surveillance programs should not bypass state public health agencies on their way from workplaces to national partners, rather state public health agencies should be included as critical partners from the beginning.

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Exposure Registries

Overview and Utility for Nanomaterial Workers

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Objective: This article provides the background for consideration of exposure registries to address potential disease risks in nanomaterial workers. **Methods:** The history of exposure registries is reviewed with a focus on their purpose and criteria for establishment. **Results:** A rationale is presented for developing registries of nanomaterial workers, and unresolved obstacles and challenges are identified. These include issues on inclusion criteria, funding, potential for legal risks, access to data, confidentiality of business information, privacy, and workers' expectations. **Conclusion:** If society is to gain the benefits from nanotechnology, it must take precautions and demonstrate care for those, such as workers, who may be most at risk of adverse effects. Establishing exposure registries is a part of such a precautionary and caring approach.

Innovations in nanotechnology have generated hundreds of diverse nanomaterials with novel properties and unknown potential to enhance or harm human health. Current toxicology studies in animals indicate hazards, which may be present for exposure to certain types of engineered nanomaterials.[1,2] Whether adverse health effects will result from occupational exposures to nanomaterials throughout their life cycle may not be known for years. Therefore, pragmatic and effective measures are needed to (1) preserve essential data elements for future epidemiologic evaluation, (2) establish mechanisms for early identification and communication of health hazards, and (3) protect employee health among workers potentially exposed to nanomaterials. This article explores the potential for exposure registries of nanomaterial workers to meet these growing needs.

The uncertainty that characterizes the hazards and risks of occupational exposure to engineered nanomaterials is a legitimate concern to workers, employers, and entrepreneurs. Because of their remarkably small size, nanoparticles may be difficult to detect unless using advanced aerosol monitoring equipment; they may move and disperse in unusual ways and therefore may be encountered and unwittingly inhaled or absorbed. For workers, these uncertainties can be reflected in the extent to which effective controls are implemented and recognized in their workplaces, and whether they realize that they have a right to know about occupational hazards. For employers, the uncertainty can result in inadequate protection of the workforce and inefficient or inappropriate use of control resources. These impacts on workers and employers leave entrepreneurs and investors in nanotechnology concerned about its growth potential and future liabilities. In the end, society could feel an adverse impact not only in the workplace and production but also in limitations to obtaining the potentially significant benefits of nanotechnology.

To address these uncertainties, government agencies and others have advocated precautionary approaches to workplace control of nanomaterials.[3–7] Nevertheless, further assurance of worker health and safety is warranted because the degree of compliance with precautionary guidance is unknown, as is the degree to which such guidance is effective across the large number of scenarios characterized by workplace types, nanomaterials types, and business sectors.[8] Although it is yet unknown the extent to which any nanomaterials currently being used or produced may pose health risks to humans, an argument can be made in support of examining the issues relevant to establishing nanomaterials workers registries, which in effect can be viewed as occupational exposure registries, even though actual exposures to any specific material, as well as any potential hazards of that material, remain unknown.[8]

Generally, an exposure registry is a system for collecting and maintaining in a structured record, comparable information on persons with known or suspected occupational or environmental exposure to a hazardous substance.[9,10] The ultimate purposes of exposure registries are to provide services and feedback to registrants and facilitate the development of new scientific knowledge. Exposure registries are not warranted in every situation in which uncertainty about hazards and risks is an issue. This is because exposure registries may have unintended consequences and high costs to workers, employers, and society, which on balance would vitiate the rationale for their establishment. A registry would be warranted if it meets established criteria or rationale, but in the case of nanotechnology workers with unknown exposures and unknown hazards, there might be good reasons to consider registration of nanotechnology workers. While no widely sanctioned set of criteria exist for occupational exposure registries, the history and use of occupational exposure registries provide a basis for determining their applicability to nanomaterial workers.

HISTORY OF EXPOSURE REGISTRIES

Exposure registries have been used for more than 50 years to help to identify and evaluate occupational and environmental health problems.[9,11,12] They have often been used to identify workers and residents exposed to known hazards (eg, kepone, 2-naphthylamine, beryllium, lead, benzene, Agent Orange, ionizing radiation), but in other cases, they have been established to address exposure to suspected hazards (eg, World Trade Center dust, Gulf War/Operation Iraqi Freedom, and tremolite asbestos). In addition, exposure registries have been established in which the hazard is known, but the actual exposure of risk is not (eg, Three Mile Island). While exposure registries are not epidemiologic studies per se, they form the basis for such studies by helping to identify populations potentially exposed to materials of known or unknown hazard and possibly at increased health risks. They also serve to provide a structured, orderly approach to identifying and maintaining communication with workers exposed to known or suspicious hazards.[9]

Because of the need to track and follow-up individuals over long time periods between exposure and various resultant chronic diseases, the registration of workers based on their exposures to

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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chemical and physical agents can be efficient.[9,10,13,14] The forerunner of contemporary exposure registries is the long-term follow-up of atomic bomb survivors that began in 1950, which involved the registration and prospective study of the populations of Hiroshima and Nagaski.[15] One component of this effort to trace atomic bomb survivors, the Life Span Study, was organized to include 100,000 individuals who were followed to determine the long-term effects of radiation exposure.[9] Similarly, the National Dose Registry of Canada was established in 1951, which contains dose records of people who are monitored for occupational exposure to ionizing radiation.[16]

Radiation dose registries for monitoring occupational exposure continue to be the most widely developed registries in the world.[16–18] One survey identified 21 of the 28 European countries, with a central registry for recording dose data from occupational radiation exposure. In North America, there is the National Dose Registry of Canada, which was established in 1951 (http://www.hc-sc.gc.ca/ewh-semt/occup-travail/radiation/regist/index_e.html). In the United States, there is no central radiation dose exposure registry but there are various ad hoc registries that exist as a function of prospective epidemiologic studies of workers exposed to ionization radiation.[18,19] The US Department of Energy, Nuclear Regulatory Commission, and Department of Defense records of workers and/or soldiers may continue *de facto* or explicit exposure registries.

Registration of exposed persons is a standard procedure in public health for addressing some infectious disease exposures, as well as for following patients treated with various therapeutics.[13] For example, in the early 1950s, the notifying, following, and screening of individuals at risk of thyroid cancer due to therapeutic thymus irradiation constituted an exposure registry.[20] There is also a long history of registries for outcomes in women exposed to drugs in pregnancy.[21]

A classic example of an environmental exposure registry was the Michigan polybrominated biphenyl follow-up registry established in 1976.[22] About 4600 persons were initially enrolled, interviewed, and studied for acute and subacute adverse health outcomes. This represents the ideal in exposure registries because the exposure occurred over a relatively short period; polybrominated biphenyl is toxic to animals both acutely and chronically, is persistent for a lifetime, and is measurable.[9]

In the occupational health field, a number of explicit or *de facto* registries have been established. Workers exposed to aromatic amines have been followed by corporations or government agencies and have been screened for bladder cancer.[23,24] The National Institute for Occupational Safety and Health maintained for various times registries of workers exposed to kepone, dibromochloropropane, and dioxin. In addition to these formalized registries, *de facto* exposure registries have been created in the lists of surviving members of retrospective cohort mortality studies complied by scientific investigators.[25] These lists, the results of vital status determinations, inherently constitute registries; however, the registrants are not aware of their risks or membership in such *de facto* registries.[9,26]

Various pieces of legislation, in addition to the Occupational Safety and Health Act of 1970, have supported the establishment of exposure registries. These include the Health Services Research, Health Statistics, and Health Care Technology Act of 1978 (Public Law 95-623) and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) of 1980 (Public Law 96-510). The Health Services Research, Health Statistics, and Health Care Technology Act mandated that the National Center for Health Statistics (NCHS) study the issues in establishing a federal system to assist in locating individuals who have been or may have been exposed to hazardous substances, determining the effect of such exposures on their health, and helping them obtain access to appropriate medical care and treatment.[9,27,28] There were five recommendations in the study that were pertinent to the establishment or operation of exposure registries:

1. Develop adequate documentation of data files and computer programs that have widespread research utility.
2. Improve the timeliness of epidemiologic studies of exposed populations.
3. Develop improved risk assessment methodologies and analytical technology for the detection and monitoring of hazards in the environment.
4. Institute efforts for coordinating environmental and health monitoring programs and for expanding the study of population subgroups that display an unusually high or low incidence of disease-specific morbidity or mortality.
5. Study the resource requirements involved in establishing a network of coordinated screening and diagnostic services for individuals with suspected exposure to hazardous substances.

Public Law 95-623 also mandated the study of incidence, prevalence, distribution, and effects of environment-related disease in populations. Subsequently, a report was released that presented a plan for these studies. The report described the major data collection systems within the environmental health area and discussed problems inherent in using this database to associate human health effects with environmental exposures.[9]

In 1980, exposure registries were mandated by the legislation that created the CERCLA, often referred to as "Superfund." The CERCLA also created a new agency of the Public Health Service, the Agency for Toxic Substances and Disease Registry (ATSDR). This agency is required to implement the health-related authorities of the act, among which is the requirement, "in cooperation with the States, establish and maintain a national registry of serious diseases and illnesses and a national registry of persons exposed to toxic substances."[29]

The top priorities for registries for the ATSDR included the following:

1. Persistent, measurable levels of hazardous agents in which animal studies or other evidence predict significant adverse effects in humans, or
2. Hazardous agents for which current methods exist to prevent an adverse outcome; or
3. Persons with outcomes of interest, where measurements of exposure to hazardous agents are available.[29]

In another document entitled "National Registry Proposal," ATSDR in 1987 provided an interpretation of when an exposure registry should be established. "The ultimate purposes of a registry of persons exposed to toxic substances are to provide service to registrants and to facilitate the development of new scientific knowledge. Besides identifying and keeping track of exposed persons, a registry should coordinate the clinical and research activities that involve its registrants. Since many researchers may propose using registrants as study participants, the registry should be the focal point of coordination. By maintaining a comprehensive data base on all exposed persons, a registry should try to collect information that satisfies multiple needs. Besides playing an important role in assuring uniformity and quality of the collected data, a registry should ensure that data collection is not duplicative in studies in order to reduce the overall burden to exposed or potentially exposed persons."[30]

This document also proposed criteria for when a registry should be established, who should be included, levels of follow-up, and when follow-up of a registry cohort should be terminated.[9] Subsequently, the National Exposure Registry was created, and a ranking scheme was developed by ATSDR to select substances for which specific subregistries would be developed.[31,32] The National Exposure Registry established four general subcategories: volatile organic compounds; dioxins; heavy metals; and radioactive materials (http://www.atsdr.cdc.gov/substances/ToxChemicalClasses.asp).

The most recently established exposure registry is the World Trade Center Health Registry, which was established in 2003 by the New York City Department of Health and Mental Hygiene, and enrolled more than 40,000 persons exposed in the World Trade Center disaster.[33] Registry eligibility included workers and residents and was triggered by a person's location on September 11, 2001, and included those who were involved in subsequent rescue, recovery, clean-up, and other activities at the World Trade Center site or the World Trade Center recovery operation on Staten Island, New York. The World Trade Center Health Registry criteria involved exposure (or its surrogate location), and the registry was established to document physical and mental health effects.[34]

Overall, exposure registries have been a useful tool of public health to address situations of uncertainty regarding hazards and risks. When used as a basis for medical screening, registries have generated useful clinical, psychosocial, and epidemiologic data and have been a source of aid to workers and residents at risk.[35] The cost of maintaining such registries has not been widely reported in the literature but appear to be extensive.[10] Nevertheless, the costs of formal epidemiologic investigations-–both in monetary terms and in terms of the lost informational value where historical reconstructions of cohorts and exposures estimates are required—can be enormous, but having an exposure registry in place may help to limit the costs of epidemiologic research.

RATIONALE FOR DEVELOPING REGISTRIES OF NANOMATERIAL WORKERS

As summarized previously, exposure registries have traditionally been implemented among groups of people exposed to known or suspected hazards. Although the extent to which any nanomaterials currently being produced or used actually are hazardous, the health risks they may pose to humans remains unresolved. However, on the basis of preliminary findings, an argument based on good occupational health practice can be made in support of establishing a nanomaterials worker exposure registry.[2,3,8]

The overall objectives for nanoworker registration, depending on intended use, include standardization and preservation of essential employee materials, and work history records in anticipation of future epidemiologic research efforts. However, timing may be the most critical issue, as it has been shown in other industry sectors that historical data collection is extremely difficult to do accurately, especially where the necessary records are not standardized or worse, have not been preserved. The rationale for developing a nanoworker registry includes the following aspects.

First, the nanotechnology platform represents a broad and rapidly expanding capacity for the development of and applications for new nanomaterials. The rate at which materials are developed and commercialized for a wide range of beneficial applications that enhance quality and performance of consumer products, improve medical diagnosis and treatments, reduce energy consumption, and expedite environmental remediation is likely to far outpace our ability to understand potential hazards and control risks. Therefore, the need to identify, follow, and evaluate large groups potentially exposed to specific types of nanomaterials is growing. Because risks remain unknown, we do not know which, if any, diseases or conditions may be associated with nanomaterial exposures. Therefore, formal epidemiologic studies may be impractical or difficult to conduct.

Nevertheless, even without knowing the disease outcomes that might become of interest, the identification and enumeration of members of potentially exposed cohorts could occur immediately. Because basic personnel/administrative records routinely generated and maintained by most businesses, research organizations, and government (especially branches of the military in the United States) typically serve as the starting point for forming epidemiologic study cohorts, these data should be readily obtainable and could be easily preserved for future research purposes.

Second, in the event that toxicological or early epidemiologic research or cluster investigations find health effects with exposure to certain types of nanomaterials, an exposure registry can facilitate the identification of the most relevant industries and employees that might be impacted and minimize the otherwise protracted process of identifying and enumerating an appropriate cohort to evaluate for risks. Establishment of a broad-based exposure registry now can substantially reduce the time and effort required in the future to identify, enumerate, and track individuals occupationally exposed to specific types of nanomaterials in response to an urgent or focused need. Because the data on potentially exposed workers will be derived from many companies (including academic research laboratories, start-up and pilot facilities, manufacturing, and production companies) and geographic regions and countries, identification and development of industry-wide cohorts for epidemiologic studies would be possible. Should serious health effects be associated with a certain type of nanomaterial, the registry would allow rapid identification of relevant occupational subgroups that would be most efficient to evaluate further.

Through the registry, communication of hazards, risks, and necessary warnings; recommendations for primary prevention (ie, engineering controls), industrial hygiene monitoring, and personal protective equipment use; and targeting of medical surveillance, all will be enhanced. Again, because a registry would include workers with similar potential exposures across many (possibly including very small) employers, relevant groups of workers and their employers-–based on their registered workplaces and exposures-–may be identified and contacted with the most recent and relevant information.

Fourth, while the generation of new science and more effectively protecting the health of the registered population are the two basic purposes of exposure registries, participating companies and institutions will also benefit. Participation is consistent with other well-established company-based health promotion and product stewardship efforts, and registration can be viewed as an extension of both. Through registry participation, companies will have the capacity to provide current and former employees with longitudinal reports on their nanomaterial exposure as they are generated or available. Evaluation of registry data further will allow corporate health and safety officials to track the nature of nanomaterial exposure among the workforce over time, evaluate potential trends in individual and aggregate data, and compare company patterns to aggregated and anonymized data from other companies in the same or related industry sectors. Employer obligations for communication of new science and any necessary hazard and risk warnings may be enhanced and expedited through a registry, as relevant recipients of information of specific interest or importance can be readily targeted.

UNRESOLVED ISSUES—OBSTACLES AND CHALLENGES

Despite the compelling arguments favoring the establishment of some form of nanotechnology worker registry, there remain several challenges and unresolved issues. Many of these were recently explored with participants in three panel discussion groups at the Exposure Registries session at the Nanomaterials and Worker Health: Medical Surveillance, Exposure Registries, and Epidemiologic Research Conference, July 21–23, 2010, at the Keystone Conference Center, Keystone, Colorado. Highlights from each group are structured under several thematic questions and summarized later.

Which Nanomaterial Workers Should be Registered?

Answering this question from a traditional perspective of exposure registries would depend on the degree of hazard and exposure

by the type of nanomaterial and whether epidemiologic studies were anticipated. However, for many, if not most nanomaterials, both the degree of hazard and the level of exposure likely remain unknown. The early findings of pulmonary fibrosis in animals exposed to various carbon nanotubes would suggest that workers exposed to them might be good candidates to be in exposure registries.[36,37] Even at the limit of quantification (7 $\mu g/m^3$) for organic carbon, there have been estimates of risk of pulmonary fibrosis as 1 of 1000 over a working lifetime.[38]

Before deciding more broadly which workers should be registered, defining who constitutes a "nanotechnology worker" will need to be determined. Several options are possible, including characterizing workers by industry sector, type of exposure, or materials handled or by job tasks performed. Inclusions of end users or "transients" through the industry pose an additional challenge to defining a nanoworker as well and may be deferred for later inclusion. In addition, worker mobility and inclusion of the international workforce are potential obstacles that could be alleviated in part through a web-based, secure system. With these above stated issues and uncertainties, the simplest option would be to enroll nanomaterial workers in registries based on a case-by-case basis and where possible dependent on: 1) whether nanomaterial exposure is likely, 2) whether it is reasonably anticipated that the exposure is hazardous and that an epidemiologic study would be conducted, or 3) whether a specific medical screening of exposed workers might be recommended.

What Information Should be Required to be Maintained?

Registration focused on identification of workers manufacturing or manipulating nanomaterials is of primary interest, with specific exposure levels identified of secondary concern. Particularly, if an epidemiologic study is possible or under consideration, unexposed as well as exposed workers in the nanomaterial manufacturing or similar facility will be important to include. In the early stages of registry development, fact and duration of employment may be all the information that is available, and whether this limited amount of information is adequate for either epidemiologic or screening purposes is an issue requiring further discussion. As workers are registered, however, some consistency in the information obtained would facilitate evaluation, particularly for epidemiologic study-–even if not all data are collected immediately.

As employment classification as a nanotechnology worker is relatively new (and may not be explicit)-–though expanding rapidly—information maintained at this point in time could be as basic as "yes/no" employment in a facility in which nanomaterials are manufactured and/or used. Prospectively, more information could be added, especially if the registry was web based that could be easily modified. Practical issues of the kinds of data to be immediately captured should surpass the need for perfect data collection. As mentioned earlier, exposure levels, measurements, etc, eventually would be ideal to include—especially if quantitative risk estimation efforts were anticipated. Nevertheless, these kinds of data are likely premature to require, and their absence does not preclude more rudimentary registry functions (including the identification of critical data gaps) to proceed. Thus, flexibility in required content could be critical to initiating data collection. Initially, it may be important to collect data on individuals with a reasonable probability of exposures, regardless of whether this is definitively known, as well as those who perceive that they have been exposed.

How Will the First Registrants be Included?

Once the core data elements of a nanoworker registry are determined, the next challenge will be how to recruit and register the first participants. As noted, some employers already have a registration system through the employment records maintained. If these records could be centralized, such information might provide the basis for adding new member companies. If the registry is to include individuals either working independently or as part of a company or institution that chooses not to participate at the institutional level, alternative recruitment approaches will be needed, including communication of the availability and objectives of the registry—and which incentives would be in place to ensure future registrants.

Who Should Manage the Registry?

Most exposure registries have been managed by government agencies, in part, because they involve more than one company, a company that is no longer in business, or residents related to a specific environmental exposure. This does not preclude that a company or consortia can manage a registry. In any case, worker input is an important component. In some ways, employee rosters and related occupational safety and health data are *de facto* exposure registries. It may be advisable, given that the focus is an emerging technology with broad potential societal benefit that a tripartite (business, labor, and government) oversight structure be considered. Such a group can provide the overall guidance for a registry and reflect the needs of various stakeholders.

An oversight committee or other similar structure can also manage other of the issues listed later, including measures to protect worker's privacy, employers' confidential business information, and interest in accessing registry information, which is likely to be diverse, depending on the purpose for requesting access. Ultimately, legal ownership of the data may pose some important obstacles, as the source information for a registry would include individuals and employers.

Who Should Fund the Registry?

Generally, industry and government should fund registries, since they represent both public health and corporate areas of responsibility. Employers have the responsibility for providing a safe and healthy workplace, and government has the responsibility to provide employers with the necessary information and determine that they are meeting their responsibilities. While employers are not mandated by law to establish registries, they are mandated to maintain components such as records, health and safety information, and a workplace free of hazardous exposures. Moreover, the support (or establishment) of an exposure registry for nanomaterial workers may demonstrate good product stewardship and risk management practice.

While logistically more complicated, there are some clear advantages to having broad support for a registry, that is, multiple funding sources. These include a reduced budgetary burden to any one funding agency, a broader and potentially more diverse collection of perspectives for oversight, more parties with a vested interest in its successful use, and greater probability of sustainability across economic and political turns. In addition to government and major corporate sponsors, other sources of financial support may include company membership fees, user fees, and grants for start-up (as initial costs are expected to be greatest).

Is There a Legal Risk to Employers?

Whether a registry poses a legal risk to employers may depend on its purpose—the semantics of "registry" and "exposure" may have legal implications administratively. Such questions do require the input and consideration of the legal community. Nevertheless, it could pose more of a risk for employers not to consider, including their employees in a registry. Supporting a registry is also a good liability-reducing practice because it provides enhanced defense against claims of corporate indifference or inaction; in the event, a particular nanomaterial is found to cause harm. Medical directors would likely find utility in a registry to protect workers and provide immediate feedback of new knowledge. Such proactivity on

the part of employers would hopefully prove to be beneficial and not a liability.

Apart from occupational health and safety benefits, companies may actively reduce or avert legal liability by looking for, identifying, and controlling occupational health hazards. In the event in which litigation arises, participation in the registry will provide an enhanced defense against claims of corporate indifference or inaction. Exposure registration may also help to document whether or not exposures to specific materials were likely to have occurred, and if so, when, where, or under which workplace circumstances they would have occurred.

Would Employees Have Undue Expectations of Service?

Whether employees would have undue expectations is a difficult issue to address, without a fully conceptualized registration process implemented. The employer's approach to communicating "up front" the goals and expectations-–as well as the limitations and rationale for participation-–would hopefully instill reasonable expectations on the part of employees. Incentives and disincentives for participation, if clarified and communicated appropriately at the onset, would also provide a framework for what employees may or may not expect. No doubt some reasonable disclaimer will need to be part of any registry, specifically indicating that the registry serves research and communication purposes and does not replace or reduce other obligations of the employer and the employee to uphold high standards for occupational health and safety.

Would Employers' Confidential Business Information be in Jeopardy?

As indicated previously, a management structure that was designed to oversee the registry would provide a mechanism to protect confidential information. The challenge to constructing a registry of those working with nanomaterials is to maintain information on the individuals' employment history, with adequate record of tasks and types of materials handled, rather than to elaborate the complex utilization or inherent "uniqueness" of a particular material.

Can the Privacy of Workers' Records be Maintained?

Again, the advantage to an oversight team to manage the registry would be to maintain the workers' privacy, as well as the employers' confidential business information. In addition, it will be important not only to ensure privacy but also to avoid inducing anxiety or any negative stigma attached to participation.

Nevertheless, because the primary function of a registry is to preserve and enhance the ability to link information, including individually identifiable data, from various sources, the potential always exists for breach of confidentiality or even misuse of personal identifying information. These aspects must be taken very seriously if a registry is to be trusted and ultimately to be effective in recruiting and retaining participants that provide valid and complete information.

The challenge, however, would be for workers who were highly mobile and how to track them among the various nanomaterial workplace settings-–which could be as diverse as a university laboratory, start-up company, or large corporation. How to maintain linkages through the individual's career and maintain private information may pose a challenge; however, in the age of high technology, it should be possible.

Who Would Have Access to the Registry?

The answer to this question is critical to determining the long-term success of the registry. It will be inevitable that researchers will want to use the registry to identify increased risks among nanotechnology workers and to evaluate the potential relationship between risks and exposures. This is a mandate for governmental researchers. While such research interests should be of broad interest, manufacturers of nanomaterials may prefer that they (or their scientists) play a major or even exclusive role in conducting or overseeing the research. As active participants in the registry, companies reasonably should expect access to the data for research purposes, especially because these employers have primary responsibility for their employees. On the contrary, many research interests are academically based, and these investigators may or may not have a vested interest in the research findings. Should these groups also expect to have open access to the data (especially if it is wholly or even in part funded publically)?

It is clear that different potential users would have different expectation for accessing registry data-–and would require different utility—and once developed, ideally, all would have access. Once again, central and multidisciplinary management might facilitate review and control of database access and use. The design, and guidance for access, should envision all types of users.

Use of the registry for dissemination of information to nanotechnology workers (or a subset of targeted because of the nature or location of their work) also poses challenges. The proper balance between open access to a nanomaterials registry and adequate protections against misuse and dissemination of scientifically unsound or potentially disturbing information will need to be struck.

While answers to all of these questions may not be available, the overall scientific and public health rationale for establishing a nanotechnology workers registry is sound, and the approaches and methodologies required for a minimally functional registry are straightforward and readily available. The ultimate challenges to establishing a registry of nanotechnology workers, however, may have more to do with important political and legal obstacles rather than scientific or occupational health concerns. Nevertheless, further refinement of a blueprint for a nanotechnology worker registry, continued dialogue with the stakeholders, and initiation of modest pilot and feasibility phases may lead to stronger acceptance of and participation in a full-scale registry in the future.

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World Trade Center Health Registry—A Model for a Nanomaterials Exposure Registry

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Objective: To describe the development of and some of the early results from the World Trade Center Health Registry (WTCHR). Is the WTCHR a model for a nanomaterials exposure registry? What lessons may be learned from the WTCHR? **Methods:** We describe the steps involved in creation of the WTCHR, from design through implementation. **Results:** The lessons learned from the WTCHR include thorough documentation of exposure early in the registry, using multimode surveys to maximize response rate, establishing an institutional home with sufficient resources for core as well as in-depth longitudinal and intervention studies, meeting with stakeholders regularly, making data accessible, and timely publication of findings, including wide dissemination of clinical guidelines. **Conclusions:** The process of creating and maintaining the WTCHR provides important lessons for the possible creation of a nanomaterials exposure registry.

The September 11, 2001 (9/11/01), terrorist attack on the World Trade Center (WTC) killed thousands and exposed hundreds of thousands to horrific events resulting from the collapsing towers and immense dust/debris cloud that followed. Types of hazardous chemical exposures associated with this disaster included gypsum, concrete, wood, paper, man-made fibers, chrysotile asbestos (0.8% to 3.0% of mass), quartz, metals, jet fuel, combustion products, diesel exhaust,[1] and aerosols containing sulfuric acid, polycyclic aromatic hydrocarbons, and silicon.[2] Nanosized particles were likely present, including diesel emissions and ultrafine dust containing carbon nanotubules.[3] This article will describe the development of the largest exposure registry for those exposed to the 9/11 disaster and discuss the extent to which this may be an appropriate model for a nanomaterials exposure registry.

CREATING AN EXPOSURE REGISTRY

The WTC Health Registry (WTCHR) is hosted by the New York City (NYC) Department of Health and Mental Hygiene in collaboration with Centers for Disease Control and Prevention (CDC)/Agency for Toxic Substance and Disease Registry (ATSDR)/National Institute for Occupational Safety and Health (NIOSH). With more than 71,000 registrants, it is the largest postdisaster exposure registry in US history.[4] It was established to prospectively monitor the long-term (20 + yrs) health of workers, residents, and other persons with a high probability of direct exposure to the September 11 terrorist attack and its aftermath.

Establishing an exposure registry of the magnitude of the WTCHR required close coordination between governmental agencies at the local and federal level, multiple institutional review board approvals (CDC, contractor, local health department), timely development of eligibility criteria and questionnaires, extensive outreach and multimodal data collection (phone and in-person interviews and later web and paper surveys).

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Discussions about the need to create an exposure registry for those exposed to the WTC disaster began within a few weeks after 9/11/01. The magnitude of the exposure to both physical and stress-related risk factors and the large estimated eligible population[4] ($N = 410{,}000$) were factors in the decision-making process. The NYC Commissioner of Health believed that a 9/11 registry would protect impacted people from being repeatedly being sought out by researchers for different studies, and at the same time, encourage legitimate research, serve a public health purpose, be comprehensive, and include all those exposed and willing to participate. A scientific advisory committee (SAC) first met in February 2002, including representatives of the local health department, academic institutions, clinical groups, and relevant Federal agencies. The decision to create an exposure registry was made by NYC Department of Health and Mental Hygiene in conjunction with representatives from the CDC, including NIOSH and the National Center for Injury Prevention and Control.

A few weeks after 9/11/01, a CDC assignee was designated as lead scientist responsible for drafting an initial protocol. Changing the health code to require participation was considered, but a decision was made to make it a voluntary registry, largely due to concerns that a health code amendment might delay the initiation of the registry and difficulty determining which geographically diverse groups or individuals might be required to register. The study protocol was modeled the basis of the registry created after the Oklahoma City bombing of 1996, as well as the ATSDR's National Environmental Registry, a voluntary registry of persons exposed to contaminated water supplies or exposure to dioxins and other chemicals. The registry was determined to be "research" rather than "public health surveillance". The specific aims included the following: (1) expand knowledge about the long-term health effects of the 9/11 disaster; (2) conduct community activities to respond to the health concerns and specific needs of enrollees and others exposed to 9/11; (3) maintain the registry as a valuable public health resource for future research.

The initial WTCHR protocol included sections on identifying target populations, methods of recruitment and enrollment, data collection instrument development, data collection, management of psychological distress, training of personnel conducting the survey, informed consent, tracking registrants over time, information management, data analysis, roles and responsibilities of investigators, oversight of the registry, and constitution of the SAC. The first SAC meeting occurred in February 2002. The study protocol was delivered to the director of the CDC by the NYC Commissioner of Health. Evaluation of potential chronic physical as well as mental health effects, cancer, and mortality, was planned from the beginning of the WTCHR.

In July 2002, ATSDR announced a $20 million award for establishment of the registry authorized by the Federal Emergency Management Agency. The ATSDR was identified as the lead Federal agency through which funding would be managed. Subsequent funding, including several million dollars per year for the analysis of the Wave 1 survey, creation of a Wave 2 survey (2006 to 2008), and ongoing maintenance of the WTCHR, was obtained from the Environmental Protection Agency and the ATSDR. The NYC provided additional funding for an in-depth respiratory study of residents and area workers, and preparation for future cancer and mortality studies. Future funding for maintaining the WTCHR is scheduled to

come from the James Zadroga 9/11 Health and Compensation Act of 2010.

The primary focus of recruitment in the beginning was through requesting lists of potentially exposed employees, residents, or persons with security badges for the damaged and destroyed buildings, for example, a Port Authority listing of over 90,000 badge holders. Over 200 key employers, unions, and agencies provided lists, or access via E-mail to their respective populations. Innovative recruitment efforts were required to reach specific target populations, including extensive use of the media, point of purchase stands with flyers, door-to-door recruitment, attendance at police department roll calls and visits to fire houses throughout NYC.

Three separate institutional review board applications were submitted. One, for the NYC Department of Health, one for the CDC, and one for Research Triangle Institute, the contractor engaged to conduct the Wave 1 interview survey. The three institutional review board approvals took more than 1 year from original date of submission.

Enrollment interviews were conducted from September 2003 through November 2004 yielding 71,437 enrollees from all 50 states.[5] Eligibility groups included persons most likely to have had direct exposure to the events of 9/11. Persons who were present in lower Manhattan south of Chambers Street ($n = 43,487$), rescue and recovery workers and volunteers who worked at least one shift on the WTC site ($n = 30,665$), residents of Lower Manhattan south of Canal Street ($n = 14,665$), and Lower Manhattan school students ($n = 2,075$) and school staff ($n = 571$).

In addition to the SAC created during the initial phase of the WTCHR, a community advisory board and later a labor advisory committee were created to ensure that input from community and labor groups and communication of the registry findings would be achieved throughout the remaining life of the registry.

One of the primary tasks of the registry was to document the extent of self-reported exposure of registrants to the events of 9/11/01. Determinants of exposure considered in developing the Wave 1 WTCHR survey include

- dust cloud exposure, home and workplace dust exposure
- proximity to WTC site or occupant of collapsed or damaged buildings
- atmospheric dispersion patterns and building canyon effect
- history of injury on 9/11/01
- reported witnessing of horrific events on 9/11/01
- onset and duration of exposure
  - rescue and recovery workers
    - location of work and specific tasks performed on 9/11, 9/12, 9/13–17, 9/18–12/31/01, and 1/1/02–6/30/02
    - no. of days worked at WTC site, no. of hours/day, and use and adequacy of respiratory protection
  - residents, area workers, and students
    - evacuation, date of return, condition of buildings, thickness of settled dust, methods and timing of cleaning, and adequacy of cleaning
  - passersby
    - location, time of exposure to dust cloud.

CLARIFYING EXPOSURE, SOCIAL SUPPORT, AND IMPACT OF 9/11 ON OVERALL HEALTH AND DISABILITY—THE WAVE 2 SURVEY, 2006 to 2008

The WTCHR was designed to conduct periodic surveys of registrants. To this end, a second health survey was conducted 2006–2008.[6] This survey gathered self-reported information from registrants on medical disease diagnoses, self-reported cancer incidence, and chronic mental health effects including psychological distress as well as diagnosed Posttraumatic Stress Disorder (PTSD), depression, and anxiety.

The 30-minute Wave 1 survey did not allow for sufficient details on exposure in three following main areas that were added to the Wave 2 survey: (1) mask and respirator type and use, training, and cleaning; (2) condition of residence, details on evacuation and residential cleanup; and (3) condition of workplace for area workers who returned after evacuation. The Wave 2 survey also asked questions regarding social support, unmet health care needs, and general health and disability. The Wave 2 survey benefited from extensive input from the WTCHR's SAC, community advisory board, and labor advisory committee.

MAINTAINING AN EXPOSURE REGISTRY

Much of the work in maintaining an exposure registry involved contacting registrants with information and requests for updated contact information, and offering assistance with locating services and referrals. The WTCHR maintains regular contact through an annual report of findings, an annual card, a frequently updated Web site (http://nyc.gov/html/doh/wtc/html/registry/registry.shtml), and specific mailings with resource guides, clinical guidelines, and invitations to forums or other presentations regarding the registry.

On the Wave 2 survey, registrants reported unmet health care needs, including both physical and mental health needs. Individualized follow-up has been undertaken, starting with residents, area workers, and passersby, to insure that each registrant with PTSD and/or reported physical health problems is referred to appropriate care. Customization and personalization of the outreach letters was important to improve the response rate to this intervention.

To achieve the first goal of the registry, expanding knowledge about the long-term health effects of the 9/11 disaster, periodic surveys of registrants have been conducted, to update health information and monitor potential health effects and health needs. In addition, the registry conducts mortality and cancer studies, matching registrant data with the National Death Index and state cancer registries. In the future, surveillance for chronic health effects such as cardiovascular diseases or other illnesses that likely involve hospitalizations will include matching with hospital registries.

Adverse respiratory health effects from 9/11 reported by the WTCHR have included increased incidence of asthma[7] and increased symptoms of cough, wheezing, and shortness of breath[5,6] associated with increased exposure on and after 9/11/01. Registry research is also informed by other 9/11-related research, for example, reports of significant declines in forced expiratory volume in 1 second and forced vital capacity[8–10]; increased symptoms of cough, wheezing, chest pain, and shortness of breath[9–11]; case reports of acute eosinophilic pneumonia[12] and sarcoid-like granulomatous lung disease.[13] Carbon nanotubules have been found in lung biopsies of WTC-exposed workers with interstitial lung disease.[3] Other nonrespiratory adverse physical health effects have been reported, including increased gastroesophageal reflux disease,[14,15] sinusitis,[15] and vocal cord dysfunction.[16]

Mental health consequences reported among WTCHR registrants after exposure to the disaster have included PTSD symptoms and diagnosed PTSD, depression, and anxiety.[17–20]

A LONG-TERM PERSPECTIVE

Follow-up surveys of WTCHR registrants are planned every 3 to 4 years, until at least 20 years have elapsed from the date of enrollment. Assessing the long-term consequences of a large environmental disaster of this type requires ongoing commitment of funding agencies, governmental agencies, and advisors to sustain core research (eg, periodic surveys), as well as core communication and tracing activities to maintain an updated and engaged cohort. The WTCHR informs registrants, the public, and policy makers

about the health impacts of 9/11 through peer-review publications, clinical guidelines, and a dedicated Web site.

LIMITATIONS

The voluntary nature of the WTCHR means that some populations may have been underrepresented and selection bias may influence some of the results. We did recruit from over 200 lists of those most likely exposed to the events associated with 9/11 and have adjusted for source of enrollment in many WTCHR analyses. The lack of objective exposure data, particularly during the first week after the disaster, and the self-reported nature of WTCHR exposure and most health outcome data (except for mortality and cancer) means that over- or under-reporting may have occurred. Recall bias may have been present because the Wave 1 survey was conducted several years after the events of 9/11/01. We are currently conducting validation studies of selected health outcomes (eg, sarcoidosis, self-reported cancers) to address possible over- or under-reporting. Since WTCHR surveys have been conducted using multiple modes (paper, web, and computer-aided telephone or in-person interviews), registry analyses have had to address potential mode effects.

IN WHAT WAYS IS THE WTCHR A POTENTIAL MODEL FOR A NANOMATERIALS EXPOSURE REGISTRY?

The WTCHR has several characteristics that make it an appropriate model for a nanomaterials exposure registry, including its large size, focus on a relatively unique set of exposures, national scope with regional emphasis, planning for long-term follow-up of diverse health outcomes, provision for external research collaboration, public use data sets, and multiple sources of funding.

The lessons learned from the WTCHR that might apply to a nanomaterials registry include documenting potential exposure hazard and actual exposure levels, as thoroughly as possible early in the registry, and addressing physical as well as mental health issues, although for a nanomaterials registry, neuropsychological and neurobehavioral issues will likely be important to measure as well. Although multimode surveys may be needed to maximize response rate and enrollment in a nanomaterials registry, potential mode effects may need to be addressed in the analysis of the data.

The WTCHR has found that one key to the successful retention of registrants is the timely release of findings through publications. In addition, regular communications, maintaining an up-to-date dedicated Web site, and insuring transparency through timely and regular communications with labor and community advisory committees have all contributed to the high retention rate (only several hundred registrants had withdrawn from the WTCHR by the end of 2010). Similarly, keeping results accessible through public access databases, with an easy-to-use interactive Web application has facilitated involving the broader scientific community, media, and the public in a better understanding of the data. The WTCHR, in response to public and medical community needs, has translated scientific findings into clinical guidelines, with updates as the science develops.

It is essential to establish an exposure registry of this magnitude within an institutional home with adequate resources for core funding for periodic surveys and maintaining contact with enrollees. Additional dedicated funding has been needed to support ancillary longitudinal in-depth studies and interventions to respond to enrollee concerns.

Finally, recognizing the complexity of the exposure and outcome measures used in a large and diverse registry such as the WTCHR, it has been important to collaborate and coordinate with other researchers: Establishing effective means of communication and coordination of methods from the beginning of an exposure registry will improve comparability of analyses. Consistent reports of findings between researchers will strengthen the potential impact of the registry findings on policy.

CONCLUSION

As the WTCHR approaches the 10th anniversary of the 9/11/01 disaster, it can serve as a potential model for future exposure registries, not just for tracking the long-term health effects of environmental disasters such as the Gulf of Mexico oil spill of 2010, but also making possible early and ongoing documentation of effects of exposure to workers engaged in emerging technologies, such as the nanomaterials industry. Establishing such an effective exposure recording and health tracking system will ensure that the health of workers is being effectively protected, and reassure the public that such technologies live up to their promise of progress and innovation without undue health risks.

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The Benefits and Challenges of a Voluntary Occupational Exposure Database

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Objective: This article describes the experience of creating and implementing an occupational exposure database for synthetic vitreous fibers (SVFs). The lessons learned and benefits achieved through this experience may be instructive to government and industry when assessing the need, utility, and design of an occupational exposure database for nanomaterials. **Methods:** This article consists of an empirical account of the issues faced during the construction and maintenance of an occupational exposure database for SVFs. **Results:** The occupation exposure database for SVF proved to be beneficial and successful but encountered several challenges relating to data consistency, data quality, and other problems. **Conclusions:** The SVF database provides a good case study to illustrate the potential benefits and challenges of creating and administering an occupational exposure database.

Voluntary or cooperative programs between government and industry have become a popular alternative or supplement to traditional regulations. Voluntary programs will likely continue to flourish because the high cost of formal rulemaking hinders government agencies charged with addressing an ever-growing number of regulatory targets and priorities. The North American Insulation Manufacturers Association (NAIMA), a trade association of companies manufacturing fiberglass, rock wool, and slag wool insulation products, recently completed an 8-year voluntary occupational safety program for synthetic vitreous fibers (SVFs) in partnership with the Occupational Safety and Health Administration (OSHA). The centerpiece of this program was the creation of an SVF occupational exposure database.

In May 1999, NAIMA began implementing a comprehensive voluntary work practice partnership with OSHA in response to OSHA's Priority Planning Process. This NAIMA–OSHA partnership program, known as the Health and Safety Partnership Program (HSPP), promoted the safe handling and use of insulation material and incorporated education and training for workers involved in the manufacture, fabrication, installation, and removal of fiberglass, rock wool, and slag wool insulation products. As a result of the HSPP, a voluntary permissible exposure limit (PEL) of one fiber per cubic centimeter (1 f/cm^3) was established and, most relevant for this article, an extensive worker exposure database was created.

This article describes the experience of creating, implementing, and completing an exposure database for SVFs. The lessons learned and benefits achieved through this experience may be instructive to government and industry when assessing the need and utility of an occupational exposure database for substances such as nanomaterials. This article summarizes the background and creation of the SVF exposure database; describes the benefits achieved to date, followed by discussion of the challenges involved in the creation of any industry-wide exposure database, and how those challenges were addressed. This article concludes that the SVF database provides a good case study to illustrate the potential benefits of an exposure database as well as the potential challenges and pitfalls in creating such a database.

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BACKGROUND AND HISTORY OF THE SVF EXPOSURE DATABASE

Synthetic vitreous fibers are a class of inorganic fibrous materials including glass wool or fiberglass, mineral wool (also known as rock and slag wool), textile glass fibers, and refractory ceramic fibers. Historically, this class of fibers has also been described as man-made mineral fibers, man-made vitreous fibers, and manufactured vitreous fibers. Fiberglass and rock and slag wool fibers are used primarily in a variety of thermal and acoustic insulation products, but also have numerous filtration, fireproofing, and other applications.

Human exposure to SVFs occurs almost exclusively in the occupational context, because installed product usually do not result in exposure to airborne fibers.[1] Synthetic vitreous fibers are used in a variety of applications. Insulating homes, other buildings, and industrial processes against heat loss and heat gain represents the largest single use for glass and rock and slag wools; up to 70% of industry output is for these applications. These wools can be blown into structural spaces, such as in walls and attics. Rock wool and glass fiber are also incorporated into ceiling tiles to provide fire resistance and thermal and sound insulation. Batts, blankets, and semirigid boards made of glass, rock wool, or slag wool fibers are used in both residential and commercial buildings. Pipe and board insulations are used extensively in industrial processes. In addition, glass, rock wool, or slag wool can be used to insulate cold and hot pipes both indoors and outdoors and in many climates. They are also used on sheet-metal ducts and plenums for thermal and acoustic insulation, resulting in quieter and more energy-efficient heating and air conditioning systems. Glass, rock, and slag wools are effective thermal and acoustic insulators and improve energy efficiency in many electrical appliances and other types of machinery. Vehicles or carriers (cars, ships, aircraft, and spacecraft) are fitted with glass wool insulation to enhance their performance and provide the appropriate thermal and acoustic conditions for the goods or passengers being transported. Glass and rock wools are also used in sound-absorbent barrier panels alongside motorways and railways. Glass and rock wools are used as growing media and for soil conditioning in agriculture. Rock wool mats are used for insulation of railway and tramway tracks against vibration. The unique properties of special-purpose fibers make them ideal for use in battery separator media and as filtration medium.

THE SVF HSPP

Before 1995, occupational exposure to SVFs was regulated primarily as a nuisance dust. In 1995, OSHA published the results of its Priority Planning Process, a multiyear process to develop a list of 18 occupational safety and health issues that the agency deemed needed additional attention because of either the seriousness of the hazard or the number of workers potentially exposed. Recognizing that it lacked the resources to conduct formal rulemaking on all 18 substances or issues on its list, OSHA prioritized five of the issues

for rulemaking and announced its intention to address the other 13 issues through voluntary or other measures. Synthetic vitreous fibers were among the list of 18 work-related issues identified by OSHA as a priority, but were designated for voluntary measures rather than rulemaking. The Occupational Safety and Health Administration listed SVFs as a priority largely because OSHA estimated that more than 225,000 workers were exposed to SVFs, and that projections indicated that the total number of workers handling SVFs in the coming years would increase.

In early 1996, NAIMA approached OSHA to discuss a voluntary worker protection program in response to the agency's announcement of the Priority Planning Process listing SVFs as a nonregulatory priority. The North American Insulation Manufacturers Association and its member companies had already instituted their own product stewardship program, and were eager to share the results of these efforts with OSHA, in hope of resolving agency concerns about workplace safety for SVFs. For example, NAIMA member companies had funded tens of millions of dollars of health research on SVFs at leading independent laboratories and universities in the United States and abroad. In addition, NAIMA and its member companies had developed safe work practices to protect workers against exposures to SVFs, including an internal recommended worker 8-hour time-weighted average (TWA) exposure limit of 1 f/cm^3.

From 1996 to 1999, NAIMA negotiated with OSHA to create and implement a voluntary program for SVFs, known as the HSPP (http://www.naima.org/pages/benefits/hspp/hspp.html). The HSPP was formally adopted by OSHA on May 18, 1999, and applied to the manufacture, fabrication, installation, and removal of fiberglass, rock wool, and slag wool insulation products. The HSPP contemplated a 3-year implementation period (1999 to 2002), followed by a 5-year compliance period (2002 to 2007), which has now been successfully completed. The HSPP included a number of specific commitments imposed upon NAIMA and its member companies that were designed to educate and encourage compliance with the HSPP guidelines by other employers and their workers.

SPECIFIC PROVISIONS OF THE HSPP

A significant feature of the HSPP was the establishment of a voluntary 1 f/cm^3 PEL for fiberglass and rock and slag wools fibers. The HSPP committed NAIMA member companies to use product design, engineering controls, work practices, respiratory protection, or a combination of any or all of these measures to bring fiber exposure to the voluntary 1 f/cm^3 PEL. To strengthen these control measures, the HSPP specified comprehensive work practices for those working with fiberglass, rock wool, and slag wool insulation. NAIMA also undertook sponsorship of training sessions to help educate workers and employers about the consolidated work practices. To do so, NAIMA gave its members and other employers educational tools such as video tapes and literature to further explain the recommended work practices.

A fundamental aspect of the recommended work practices dealt with when and where to use respiratory protection. The HSPP recommended respiratory protection whenever exposures on a job exceeded the 1 f/cm^3 8-hour TWA PEL. The N95 series dust respirators certified by NIOSH were the approved type of respirators recommended by the HSPP.

Most important, the HSPP committed NAIMA to provide an exposure database to help contractors and workers determine the level of potential exposure to fiberglass, rock wool, or slag wool for a given task. NAIMA also committed to supplement the database with additional exposure data collected from various sources and studies. Exposure monitoring and an exposure database are closely related to the respiratory protection guidelines, thereby offering contractors standardized methods for determining whether respiratory protection is needed for a particular task. This helps contractors reduce the burden of compliance under the OSHA Respiratory Protection Standard.

When OSHA endorsed the HSPP, OSHA supported the ability of contractors to rely on the NAIMA exposure database as the means for determining exposure levels. Specifically, the preamble to OSHA's 1998 Respiratory Protection Rule states that "OSHA recognizes that there are many instances in which it may not be possible or necessary to take personal exposure measurements to determine whether respiratory protection is needed."[2] In addition, OSHA's rule preamble states that the "Final rule permits employers to use other approaches for estimating worker exposures." Consistent with this incentive for voluntary compliance in the OSHA regulations, the agency approved the use of "[d]ata from industry-wide surveys by trade associations" and noted that such information is "often useful in assisting employers . . . to obtain information on employee exposures in their workplaces." In fact, OSHA specifically cited NAIMA's database in the preamble as an example of industry data that could be relied upon by employers.

RESULTS

NAIMA met or exceeded all of the commitments set forth in the HSPP. First, NAIMA organized an HSPP committee to oversee the program's implementation, and soon thereafter formed an Occupational Health and Safety Subcommittee to govern the development of an exposure database and establish a quality assurance/quality control (QA/QC) auditing team to oversee population of the exposure database. The QA/QC auditing team included a faculty member from Arizona State University, certified industrial hygienists from several NAIMA member companies, a corporate officer of NAIMA, and a third-party computer expert. Professor Marchant of Arizona State University manages the database.

An important aspect to the creation and maintenance of the exposure database was the establishment of QA/QCprocedures for data submittal. The QA/QC procedure describes the steps that must be taken in approving data for the database and identifies the specific information that must be available for a data point. Specifically, the following details are required about all data points: (1) sample identifier; (2) sample date; (3) SVF type (fiberglass, rock wool, or slag wool insulation); (4) product type; (5) type of manufacturing/use (primary manufacturing, fabrication, etc); (6) job description (packer, installer, feeder, etc); (7) sample type (personal or area); (8) number of samples for TWA; (9) TWA quantifier; (10) results to two decimal places; (11) sampling and analytical methods employed; and (12) sample duration.

NAIMA committed to format and categorize by product and task 3000 to 5000 samples of pre-1990 exposure measurements. In the first year of implementation, the database had 4200 exposure samples. By the end of the second year (2003), the exposure database had expanded to include over 7000 exposure samples. According to the HSPP, 400 data points were to be added to the database each year after the first 2 years. This target of 400 additional exposure samples was exceeded each year. All new data entered into the database was first reviewed and approved by the QA/QC auditing team pursuant to its written procedures. By the end of the HSPP, the database had in excess of 14,000 exposure measurements, including exposure data on more than 35 different products and more than 60 different jobs. NAIMA is committed to maintaining the database beyond the HSPP, and thus the database is expected to continue to grow over the years.

BENEFITS OF SVP EXPOSURE DATABASE

The HSPP has officially been completed. In general, the HSPP successfully accomplished its goals. Creating the extensive exposure database was the element of the HSPP that required the most time, effort, and resources. Although the industry had abundant exposure data, those data had not been archived in one location and it was therefore not easily accessible. The HSPP created an ideal

opportunity to assemble a robustand high-quality database that could be relied upon by workers, agency staff, and independent researchers. In that regard, the SVF database has proven to be very successful and useful.[3,4]

Contractors can rely on NAIMA's database without conducting their own exposure monitoring. It helps contractors and workers determine the level of potential exposure to fiberglass, rock wool, or slag wool for a given task. The exposure database contains sample data about exposure levels categorized by product type and specific work task. Furthermore, NAIMA has analyzed exposure data involving typical exposure levels for many common jobs, and documented that most of these jobs currently can be completed without exceeding the exposure limit of 1 f/cm^3 for an 8-hour TWA.

The SVF database is clearly valuable, as demonstrated by various government agencies and other entities who have relied upon it, such as the 2002 International Agency for Research on Cancer monograph on SVFs cites both the HSPP exposure database and the HSPP itself.[5] Similarly, when the Agency for Toxic Substances and Disease Registry of the U.S. Department of Health and Human Services created a toxicological profile on SVFs, that agency also relied upon the SVF database.[1] Data from the SVF database have also been summarized in several user-friendly formats for use by employers, workers, and other interested parties. An example of such a data summary is shown in Table 1, showing that most exposures in the industry are below the 1 f/cm^3 voluntary PEL.

CHALLENGES FACING EXPOSURE DATABASES

Exposure databases such as the SVF database provide important benefits and applications, but creation of such databases do present a number of challenges and potential problems. The experience with the SVF database provides some insight on some of these issues and how they have been addressed in this context.

Consistency Issues

A major challenge involves ensuring the consistency of the data submitted to and accepted into the database. It is important that individual data points be consistent and comparable because the strength of the database is found in its ability to provide information and estimates based on collective results. There are several potential problems that can adversely impact the consistency of data in any database.

One of the biggest problems encountered by the SVF database was that data submitted to the database had sometimes been collected using different analytical methods to measure SVF exposure. Some exposure samples used mass-based exposure measurements (eg, μg/m^3) whereas more recent exposure data use a fiber-counting analytical method. Although North American industry has now agreed on the NIOSH 7400B analytical method, some data points were submitted to the database using other fiber counting rules (eg, NIOSH 7400A, phase contrast optical microscopy). Data submitted from other countries (eg, Australia) were often collected using a different analytical method than NIOSH 7400B. For the SVF database, exposure data collected using different analytical methods were entered into the database while preserving information about the analytical method recorded in the relevant data field. This maximized the amount of relevant data entered into the database but made it impossible to undertake analyses using all the data in the database because of the incompatibilities between the different methods ("apples to oranges" comparisons). This problem of diverse analytical methods may become significant for a nanotechnology exposure database, in the absence of a standard analytical method for nanomaterials.

The inherent differences in product types pose another problem. Many SVF products may present different exposure profiles because of various factors such as product use and quantities, fiber characteristics, and application environments. An exposure database, such as the SVF database, can provide greater resolution and hence

TABLE 1. Fiberglass, Rock Wool, and Slag Wool Respirable Fiber Exposure Data

Product Description	Time-Weighted Average*Exposure Levels (fiber/cm^3)†
Fiberglass	Mean (Average)
Acoustical panel‖	
Cutting/sawing with power tools	0.06
Handling	0.02
Aircraft insulation‖	
Cutting/sawing with power tools	0.11
Fabrication/assembly	0.14
Appliance insulation‖	
Fabrication	0.12
Installation	0.07
Automotive insulation‖	
Fabrication/assembly	0.03
Installation	0.01
Batts/blankets‡‖	
Lamination	0.04
Installation	0.13
Cutting/sawing	0.17
Blowing wool with binder‡‖††	
Installation	0.26
Blowing wool	
Without binder‡‖**††	
Installation	0.83
Cavity fill insulation‖	
Installation	0.21
Flex duct‖	
Installation/assembly	0.01
Fiberglass mat‖	
Forming	0.01
Fiberglass residential¶‖	
Removal	0.40
Compressed air cleanup	0.56
Filtration products‖	
Fabrication	0.52
Duct board‡¶‖	
Fabrication	0.10
Installation	0.02
Handling	0.01
Cutting/sawing with power tools	0.06
Duct liner‡‖	
Fabrication	0.06
Installation	0.09
Duct wrap‡	
Installation	0.35
Industrial board/blanket¶‖	
Fabrication/installation	0.05
Removal	0.44
Cutting/sawing with power tools	0.07
Pipe insulation¶‖	
Installation	0.04
Removal	0.04
Fiberglass	
Metal building insulation	

(*Continues*)

TABLE 1. (*Continued*)

Product Description	Time-Weighted Average*Exposure Levels (fiber/cm^3)†
Installation	0.10
Miscellaneous‡‖	
Fabrication with handheld power cutting tools	0.32
Manufacturing	0.05
Rock and slag wools	
Batts/blankets‡§	
Installation	0.09
High density batts§	
Installation	0.09
Blowing wool with binder‡¶	
Installation	0.34
Cavity fill insulation‡‖	
Installation	0.11
Ceiling tiles‡§	
Installation	0.23
Industrial board/blanket‡‖	
Removal	0.07
Mobile home insulation‖	
Installation	0.13
Cutting/sawing	0.12
Lamination	0.03
Pipe insulation§¶	
Installation	0.02
Safing§	
Installation	0.10
Spray-on fire proofing§	
Installation	0.09
Feeding	0.05
Manufacturing‖	
Bulk	0.07
Commercial and industrial	0.07
Ceiling panels and tiles	0.20
Filtration	0.21
Spray-on fire proofing	0.20
High-density board	0.06
Pipe insulation	0.03
Rock and slag residential¶	
Removal	0.13
Miscellaneous‡‖	
Fabrication with handheld power cutting tools	0.15

*Sample duration of 240 min or longer.
†As evaluated by the NIOSH 7400 "B" sampling and analytical methodology.
‡Johns Hopkins University Study
§Rock and Slag Wool Installers Study
¶Fluor Daniel Study of Worker Exposures During Removal of SVF
‖NAIMA member company studies
**Insulation Contractors Association of America Installers Study
††NAIMA/Clayton Study
Source: Data provided to NAIMA by Arizona State University after a thorough review and analysis by Arizona State University on October 24, 2004.

greater utility if it therefore classifies exposure data points by product type. This presents challenges, however, that may also be anticipated for nanotechnology. Specifically, there are many different products in the SVF industries (as there is in the nanotechnology field), with subtle differences within product lines and across companies. For example, two similar product types made by different manufacturers might use a slightly different binder formula that could affect the likelihood and duration that fibers stay aloft and potentially inhaled, thus potentially affecting exposure levels.

More generally, different companies might define and categorize similar products differently, and those definitions might change over time. For example, one category of fibers in the SVF database is "special application fibers," a category requested by OSHA and potentially subject to different interpretations by different companies if not clearly defined. Moreover, the nature of SVF products has changed over time, as many of the fibers have been reformulated, often to reduce any potential health concerns. Thus, comparing exposure to levels of a specific category such as glass batt insulation in different time periods may once again involve products with different exposure characteristics and potential risks (despite having the same nomenclature).

A final consistency issue concerns tasks assigned and their actual job description. In the SVF industry, and possibly when applying nanotechnology, exposure levels can vary significantly across different job categories. Accordingly, stratifying data points by job type is important for making the database useful and relevant. Such categories, however, raise questions about definition and consistency. Each facility is configured differently, and it may use different products or input materials, creating diverse sets of working conditions. All of these variables can impact exposure levels for workers assigned to the same job category. In addition, each company has the right to their own definition of their job types. For example, during the course of developing the SVF database, it was discovered that many companies defined the "general room" job category differently. Because the database managers kept a coded source list for all data points in the database, they were able to go back to the original data sources and ask for clarification, in order to correct the data entered.

Data Quality Issues

Data quality may remain a problem even when definitions are clear and ambiguous. Data in the SVF database were collected from a wide variety of different sources, with different assurances and reliability. A vigorous QA/QC process helped to screen out, correct, or resolve uncertainties about many of the questionable data points submitted to the database (eg, samples with overloaded filters). Nevertheless, some data points were so problematic that they could not be resolved by the QA/QC committee. Some data points submitted to the database were missing mandatory data fields. In such cases, the QA/QC committee would follow up with the original source of the data to determine if the missing data fields could be completed. If those missing data fields cannot be completed (often because the original records could not be located or did not contain the required information), the data points were not entered in the SVF database, but rather were maintained in a separate file called "Valid Data Not Otherwise Meeting Database Criteria." Data in this file could potentially be used for other research purposes, but are kept separate from and not included in database analyses.

Other quality-related problems include reliability of the data, confidentiality and public access to the data, and representativeness of information in the database. In one case, for example, a set of exposure data points was submitted with exactly 480-minute duration of sampling time. This uniformity of exposure time did not seem consistent with the normal variation observed in the field, and thus follow-up by the QA/QC committeeand database administrator were necessary to resolve this issue.

Additional Complexities

The SVF database operates by entering all data points available that meet the database criteria and QA/QC review. Thus, data cannot be representative of the industry as a whole. Samples are not

taken randomly across workplaces but tend to concentrate on job tasks or product types where exposures are known or suspected to be the highest in the facility. Thus, exposure sampling tends to occur where the exposures occur (or at least believed to occur). This factor would tend to inflate average exposure levels in the database relative to the real-world average levels industry-wide but omits gaps in the analysis where exposures therefore remain unknown. In addition, companies differ in how often and when they sample exposure. Larger companies tend to collect and submit more data than smaller companies, so the data may disproportionately represent exposure levels in larger rather than smaller companies. If larger companies with more resources and expertise tend to control exposures better than smaller companies, this factor would tend to underestimate overall exposure levels, thereby skewing the data to reflect their experience and needs.

The exposure data also inevitably include some gaps or limited samples for some occupational contexts. To address these gaps, NAIMA commissioned and paid for outside consultants to obtain data points necessary to fill in these gaps. This can be an expensive undertaking, however, and unless there is an entity associated with the database prepared to make such investments, the gaps in exposure data may remain unfilled.

Several additional issues are raised by the experience from the SVF database. First, database issues can raise competitiveness issues. For example, the definition of certain categories such as special application fibers can create competitive advantages or disadvantages for certain companies. If these concerns are not handled effectively, they can create controversies associated with the database. In the case of the SVF database, these potential concerns were largely addressed, and resolved when they did arise, by a QA/QC committee that included respected experts from most of the major companies or industry sectors involved. These individuals were capable of identifying potential competitiveness issues early and taking proactive actions to resolve such issues before they became a significant problem.

Another potential problem concerns data confidentiality. Assurances of confidentiality of the identity of the company submitting the data were essential to ensure submission of the data (and attendant legal issues are beyond the scope of this article). This issue was resolved in the SVF database by coding the company name and facility submitting the data in the database. Only the database can decode this information. The confidentiality of this code key is strictly protected, but there is always a possibility that future litigants may request the key in a third-party subpoena. This risk could be eliminated by destroying the code key, but maintaining the key has been critical for the database manager to go back to original submitters when questions or ambiguities arise about some of the definitions used in a data submission.

Another issue is whether and how the database is made publicly available. Some databases are made accessible on the web, whereas others are kept more proprietary. The SVF database is not made publicly available in raw form, but summaries of the data are prepared and made widely available to stakeholders and other interested parties. Requests for more complete access to the raw data are considered on a case-by-case basis.

A final problem faced by the SVF database is the lack of participation by some entities with available data. The SVF database enjoys strong participation by all companies within the sponsoring trade association (NAIMA); those companies outside the trade association likely had relevant exposure data but chose not to participate. Beyond requests to such entities, there does not seem to be any incentive that could be used to bring them into the fold.

CONCLUSION: KEYS TO SUCCESS

The SVF database provides a good case study to illustrate the potential benefits of an exposure database as well as the potential challenges and pitfalls in creating such a database. Despite many challenges, the SVF database has generally been successful. In retrospect, some critical factors can be identified as key contributors to this success. First, and probably foremost, the existence of an active QA/QC team staffed with committed and highly respected experts from the industry greatly enhanced the technical accuracy and external credibility of the database. Second, the development and regular updating of a clear and thorough database dictionary to guide data submitters and which carefully defines all database fields and criteria helped to maximize the accuracy and consistency of the data submissions. Third, maintaining careful records of all data submissions, including the coded identity of the company and location associated with each data point, permitted follow-up, correction, or verification of any data point for which subsequent issues or questions may have arisen. Finally, the success of the database was made possible by the commitment and funding of the trade association and its member companies, from the top of each organization down. This combination created a high-quality, objective, and comprehensive exposure database that may serve as a model for nanotechnology and may continue in its own right.

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Epidemiologic Challenges for Studies of Occupational Exposure to Engineered Nanoparticles; A Commentary

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Objective: Identify most likely health effects of occupational exposure to engineered nanoparticles (ENP). Recommend analytic approaches to address epidemiologic challenges. **Methods:** Review air pollution and occupational literature on health effects of fine particulate matter (PM). Provide example of mortality study of exposure to PM composed of metalworking fluid. Apply standard Cox models and g-estimation to adjust for potential healthy worker survival effect (HWSE). **Results:** In contrast with standard methods, g-estimation suggests that exposure to PM may cause chronic heart and lung disease; longer exposure reduces survival. HWSE appears stronger for chronic disease than for cancer. **Conclusions:** We recommend hazard surveillance, short-term panel studies of biomarkers, and prospective cohort studies of cardiovascular and respiratory diseases. Building research capacity in g-estimation methods to reduce HWSE is necessary for future studies of chronic disease and ENP.

Many complex and unresolved issues related to the characterization of engineered nanoparticles (ENP) need to be confronted in the planning of an epidemiologic study. Here we propose three recommendations intended to address the major challenges to designing epidemiologic studies of occupational exposure to ENP.

First, there is the question of specifying the exposures of interest. To date, nanotoxicology has focused on a limited number of engineered nanomaterials including carbon nanotubes, but there are many types of nanomaterials. Second, the relationships between physiochemical properties and bioactivity are not yet well understood, leaving an open question about the most biologically relevant exposure metrics of these particles. Third, study populations of workers employed in the manufacture or use of specific classes of nanoparticles need to be characterized. Hazard surveillance offers a framework for the systematic collection and analysis of exposure data to resolve these issues and provide a basis for future health studies.

Recommendation 1: Implement hazard surveillance of a broad range of ENP with regular monitoring of both number concentrations and mass concentrations to identify exposed cohorts and to document job and exposure histories for future studies.

Anticipating the most likely adverse health effects presents another challenge. In the absence of any human data, it is reasonable to turn to known health effects of similar exposures in the ambient or occupational environment. We begin by reviewing the extensive literature on health effects of fine particulate matter ($PM_{2.5}$) and ultrafine particles (UFP) in urban air pollution. (UFP and nanoparticles are used synonymously to describe particles less than 0.1 μm in aerodynamic diameter.) We then review what we know about those same health effects in relation to workplace exposures similar in particle size distribution and composition to urban traffic PM. Although the novel physiochemical properties of ENP may cause new mechanisms of injury, studies of workers exposed to $PM_{2.5}$ with a high UFP component (boilermakers, welders, and autoworkers) provide a reasonable basis for identifying the most likely health effects of ENP.

LEARNING FROM AIR POLLUTION STUDIES OF FINE AND ULTRAFINE PARTICLES (NANOPARTICLES)

Cardiovascular disease (CVD) was first associated with air pollution in a mortality study of the Six Cities cohort initially designed to assess pulmonary function. Comparing the most polluted to the least polluted of the six US cities, Dockery et al[1] reported an adjusted mortality rate ratio for CVD of 1.26 (95% confidence interval [CI] = 1.08 to 1.47). Since that time, a wide and compelling literature has evolved on the basis of hospital admissions[2,3] and mortality,[1,4–8] establishing that exposure to ambient air pollution increases the risk of CVD. Mounting evidence suggests that the primary cause of this increased risk is PM—especially $PM_{2.5}$ generated from combustion sources, that is, urban traffic.[9] Attention is now shifting to the smaller UFP in traffic-derived pollution; recent studies of daily cardiopulmonary mortality[10] and biomarkers of platelet activation[11] suggest that UFP ($PM_{0.1}$) may be more toxic than $PM_{2.5}$. It is the UFP fraction of traffic emission that appears to contain most of the polycyclic aromatic hydrocarbons (PAHs), a carcinogenic component of oil that is also generated by combustion.[12] Potential pathways have been identified to explain how exposure to UFP in traffic pollution may cause CVD.[13] The association between outdoor air pollutants and exacerbation of preexisting chronic obstructive pulmonary disease (COPD) is also supported by reasonable evidence; ambient PM has been linked with hospital admissions[2,14] and emergency department visits for respiratory disease[3] and COPD,[15] as well as with COPD mortality.[16] Extrapolating from the ambient environment, the most likely health outcomes of exposure to ENP are chronic heart and lung diseases.

Daily exposure has been linked to CVD and COPD hospitalizations and mortality in numerous time-series studies.[17] Attention has now shifted to studies of long-term exposure to air pollution, defined as 1 year or more.[18] Concerns about potential confounding by sociodemographic characteristics have led to the development of sophisticated graphical methods for characterizing small-scale spatial gradients in urban air pollution. Less attention has been devoted to measuring changes in the concentrations of ambient exposures over time at the individual level, although age is a convenient measure of duration of exposure.

OCCUPATIONAL STUDIES OF RESPIRABLE AND ULTRAFINE PARTICLES

By contrast, exposure to workplace hazards can generally be well characterized by combining employment records with industrial hygiene sampling data. UFP, however, have not been regularly monitored in the workplace. Although most mechanical processes that generate dusty occupational environments are unlikely to produce significant number concentrations of UFP, hot processes that involve vaporization and inevitable cooling may.[19] A cohort of Norwegian asphalt production workers and pavers was reported to be exposed to UFP, at a concentration of $3.4 \times 10^4/cm^3$, as well as to mineral oils

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and PAHs.[20] In addition to such hot processes as asphalt paving, UFP can also originate from combustion, for example in diesel engines, and high-speed mechanical processes such as grinding.[21] In a recent exposure survey of seven Swedish industries, UFP was measured by number concentration and surface area in combustion processes such as diesel engines, hot processes such as welding and smelting (30×10^3 to 100×10^3 n/cm), and high-speed grinding (10×10^3 n/cm).[21]

There are no studies of the potential health effects of UFP in the workplace, and the literature on occupational exposures to respirable $PM_{3.5}$ (or fine $PM_{2.5}$) is limited to short-term studies of acute effects. In recent studies of boilermakers, biomarkers of preclinical cardiovascular effects, including markers of inflammation[22,23] and heart-rate variability[24] have been associated with exposure to metal-rich $PM_{2.5}$ containing PAHs. By design, these were panel studies with multiple measures of outcome and exposure collected over several days. Biomarkers of cardiovascular effects were measured frequently, simultaneous with continuous $PM_{2.5}$ exposure monitoring in a small number of subjects (typically fewer than 30). Because subjects can be compared with themselves, confounding can only occur by factors that vary over time. Thus, this is an efficient design for studying physiologic pathways of biologic effects.

Recommendation 2: Study biomarkers of short-term cardiovascular and pulmonary responses in small panel studies of workers exposed to specific types of ENP.

By observing repeated measures of biomarker outcomes at regular intervals and monitoring real-time exposure, even studies with small sample size can have sufficient statistical power to detect small health effects. This design will allow alternative exposure metrics of ENP to be examined in relation to outcomes and provide new insights on disease mechanisms.

STUDIES OF CHRONIC DISEASE IN WORKER COHORTS EXPOSED TO UFP

The occupational literature on both CVD and COPD incidences or mortalities is limited and surprisingly inconclusive, given the magnitude of workplace exposure to PM and PAHs.[25,26] There are a few studies, however, that suggest that workers are also at risk of chronic disease. In a large cohort of European asphalt pavers, a 60% increase in fatal myocardial infarctions was associated with an average exposure of 273 ng/m^3 benzo(a)pyrene, a specific PAH, relative to in those unexposed.[27] In a cohort of Canadian aluminum smelter workers, an elevated hazard ratio (HR) for ischemic heart disease (IHD) was found in the highest categories of both past and current exposures to benzo(a)pyrene, a marker of coal tar pitch volatiles, among the actively employed.[28] Workplace PM exposure is currently regulated under a generic standard for particles not otherwise classified, at 5 and 15 mg/m^3 for respirable and total particulate matter, respectively, as an 8-hour daily time-weighted average.[29] Even after adjusting for differences in the length of a day, permissible exposure limits in occupational settings are up to three orders of magnitude higher than that allowed in the general community.[30,31]

In a recent study based on the American Cancer Society cohort of more than 1 million US adults, Pope and colleagues[32] analyzed data on cardiovascular mortality, ambient $PM_{2.5}$, and both active and secondhand cigarette smoke by using a common daily exposure metric. Results suggest a log linear exposure–response relationship with excess risk even at low exposure levels. The occupational exposures to respirable PM or fine $PM_{2.5}$ in many US manufacturing plants lie in the exposure gap between ambient air pollution and active smoking. Filling in the missing range with adjusted HRs for cardiovascular mortality and daily exposure to fine $PM_{2.5}$ in occupational settings will potentially identify new worker populations at risk. Moreover, studying chronic disease in worker cohorts exposed to fine or respirable PM (including UFP) will be relevant for planning future health studies of ENP.

CHRONIC HEART AND LUNG DISEASE AND PM EXPOSURE IN OCCUPATIONAL SETTINGS

To highlight the challenges posed by studying chronic disease in occupational cohorts, new results are presented for a cohort of United Autoworkers-General Motors (UAW-GM) workers exposed to PM composed of metalworking fluids (MWF). This cohort has been observed for mortality from 1941 to 1995.[33] An extensive retrospective exposure assessment for oil-based MWF was conducted on the basis of size-selective gravimetric sampling data. The (unmeasured) PAH content of straight MWF has declined since the 1980s but may still be present.[34] The PM generated when straight MWF are sprayed to cool machining and grinding operations contains a high proportion of respirable particles. Although UFP have not been measured, the rapid heating and cooling of mineral oils potentially generates significant number concentrations of UFP.[19] Long-term personal exposures have been estimated by combining employment records with historical exposure monitoring data. Figure 1 presents the average annual concentration (mg/m^3) of PM composed of straight MWF, by particle size, in one of the three automobile manufacturing plants in the UAW-GM study. The graph shows that $PM_{3.5}$ accounts for about 30% of average annual concentration of total PM, across all jobs in each year over the study period.

To date, several cancers have been associated with straight MWF on the basis of internal analysis of quantitative estimates of past exposure.[33,35–41] We have also reported standardized mortality ratios for COPD mortality of 0.94 (95% CI = 0.87 to 1.00) for all white male population and 0.78 (0.64 to 0.95) for all African American male population in the cohort compared with the general US population.[33] Here we present new exposure–response results, based on 2659 deaths due to IHD (Fig. 2) and 306 deaths due to COPD (Fig. 3). HRs for IHD and COPD mortality were modeled as smoothed functions of cumulative exposure to straight MWF (total PM mg/m^3-years). Smoothing was implemented using penalized splines in Cox models adjusted for gender, race, plant, and calendar year, with age as the time metric. For CVD, the exposure–response curve suggests a modest rise in relative risk over the densest portion of the exposure range, with a plateau in the relative risk at HR equal to 1.2. For COPD, the exposure–response increases across the entire range. For both outcomes, the confidence bands are wide and include the null.

One might expect more conclusive results, given the range of PM exposures in the autoworkers study. There are several possible explanations for why the CVD curve plateaus and the confidence bands are so wide, but two are most relevant here. First, the exposure–response models were based on cumulative exposure to *total* PM; the air pollution literature has shown that the smaller particles have greater cardiovascular toxicity. Thus the exposure–response curve for $PM_{3.5}$ may be steeper and (or) have tighter CIs. The second explanation is the healthy worker survivor effect (HWSE).

It is important to keep in mind that associations in occupational studies are attenuated because of HWSE.[42] Downward bias arises when less healthy workers reduce exposure by transferring jobs, taking time off work, or terminating employment, leaving healthier workers with more exposure. It is plausible that HWSE impacts chronic diseases-–with long survival and a lot of symptoms–more than more rapidly fatal diseases such as cancer. We recently applied g-estimation to address HWSE in the autoworkers cohort.[43,44]

G-ESTIMATION TO REDUCE HWSE BIAS

Causal methods contrast outcomes that would have been observed in scenarios where exchangeable (or the same) individuals are subjected to different levels of exposure. Robins[45] has shown that standard conditional epidemiologic models (Cox, logistic, and Poisson regression) will be biased if past exposure predicts future values of a time-dependent variable, which is both a risk factor for

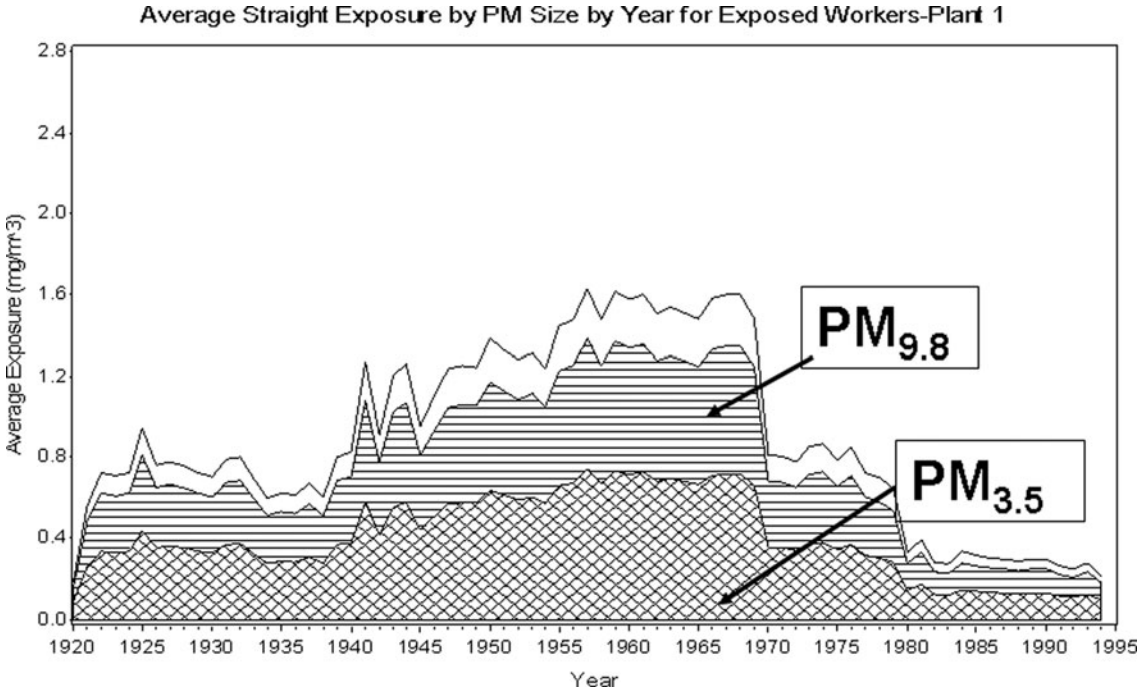

FIGURE 1. Annual mean straight metalworking fluids exposure (mg/m^3) by particle size (PM) in exposed autoworkers. PM indicates particulate matter.

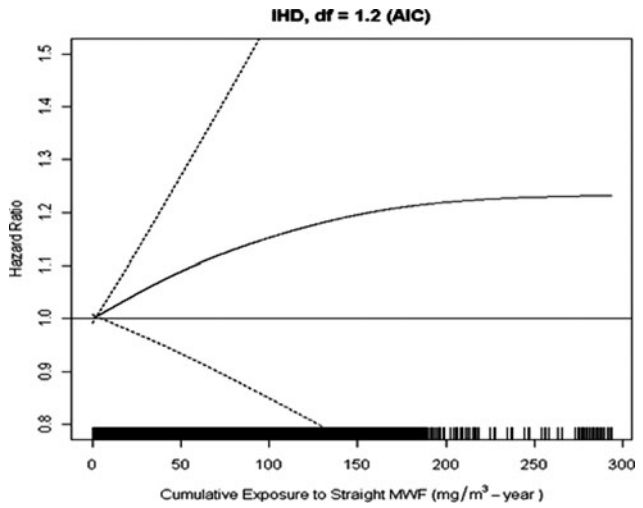

FIGURE 2. Adjusted hazard ratio for ischemic heart disease (IHD) as a smoothed function of exposure to straight metalworking fluids (MWF) (mg/m^3-yr) as estimated in a Cox regression model. Dashed lines are 95% confidence bands. AIC indicates Akaike's Information Criteria.

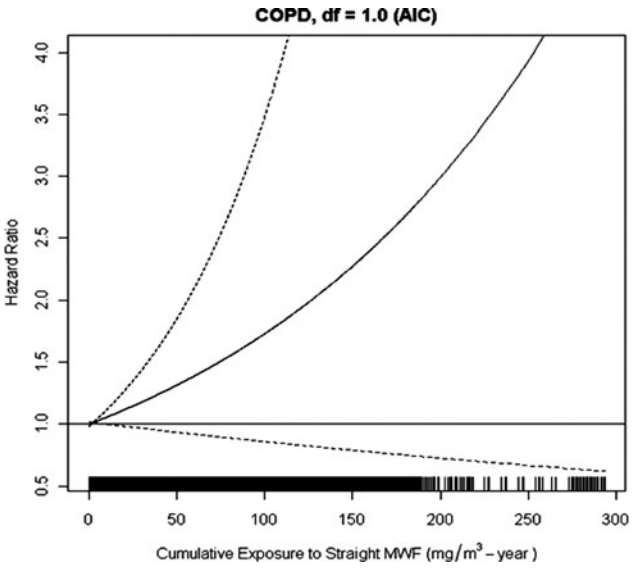

FIGURE 3. Adjusted hazard ratio for chronic obstructive pulmonary disease (COPD) as a smoothed function of exposure to straight metalworking fluids (MWF) (mg/m^3-yr) as estimated in a Cox regression model. Dashed lines are 95% confidence bands. AIC indicates Akaike's Information Criteria.

survival and predicts subsequent exposure. In such situations, causal models are needed to provide unbiased dose–response estimates. Health status is such a time-dependent variable, so we need causal models to avoid bias due to HWSE.[45,46] Most causal methods other than g-estimation of accelerated failure time models require that all levels of exposure occur in all strata of the confounders. In occupational studies, however, those not actively employed are unexposed by definition. Thus, g-estimation is a causal approach that can be applied to adjust for HWSE in the occupational setting. Although causal models are becoming part of the mainstream epidemiologic literature,[47] they had not been applied at all in occupational studies until recently.

We have applied g-estimation to the autoworkers cohort study as reported by Chevrier and Eisen.[43,44] The results are briefly summarized here and extended (J.C., S.P., E.A.E., unpublished data, September 2010). The autoworker cohort was restricted to 38,747 subjects hired after start of follow-up. A total of 2595 subjects died of

IHD (International Classification of Diseases, Ninth Revision: 410 to 414) over the follow-up period. Exposure to straight MWF was treated as a binary variable (ever vs never exposed) in each year and the g-estimated survival ratio (SR) compares survival if everyone had been exposed for the first 5 years with survival if no one had ever been exposed. We used the results from g-estimation to calculate survival curves for IHD mortality under alternative exposure histories. The solid line in Fig. 4 represents observed survival, where each worker's actual exposure is included. The other three curves are hypothetical, based on our g-estimation analysis. The dotted line represents survival that would have been observed if no one had been exposed at all, the dashed line represents survival that would have been observed if everyone had been exposed for the first 5 years of follow-up, and the dash-dotted line represents survival that would have been observed if everyone had been always exposed. Because exposure can cause IHD, the curve representing survival if no one were exposed shows greater survival at each time than under any other scenario (including the observed); and survival if always exposed is less than under any other scenario. The curves indicate that longer exposure is more harmful, and that long follow-up is required to see the effect of MWF on IHD mortality.

As described in Chevrier and Eisen,[43,44] the SRs were transformed into HRs so that we could compare g-estimation results with standard methods. Adjusted HRs estimated in standard Cox models (with time on follow-up as the metameter) were 0.97 (95% CI = 0.94 to 1.00) for both IHD and all cancers combined per 5 years of exposure, and 0.99 (95% CI = 0.91 to 1.07) for COPD. The g-estimated HRs were higher for all three outcomes. The application of g-estimation reversed the direction of the HR for each outcome, from below the null to an elevated HR with a CI that excluded the null. The two methods differ in several respects; however, the difference between the g-estimated and standard HR for each outcome may provide some information about the magnitude of HSWE bias. The differences, expressed as a percentage of the g-estimate, were 22% for IHD, 33% for COPD, and 13% for all cancers combined. These results suggest that the downward HWSE bias may be stronger for heart disease and chronic lung disease than for cancer.

Early studies of ENP exposure should focus on cardiovascular and respiratory diseases incidence rather than mortality. Occupational mortality studies, however, are retrospective studies and require many years of follow-up. By contrast, incidence studies can be prospective, based on hospital discharge data or data collected on biomarkers of subclinical arteriosclerosis, such as carotid wall intima-medial thickness,[18] for example. Past-exposure information needed for these chronic disease studies at the individual level may be available from the hazard surveillance recommended earlier. In any occupational study of chronic disease, whether prospective or retrospective, HWSE is a challenge to study validity and must be addressed.

Recommendation 3: Plan prospective studies of CVD and COPD incidences in relation to occupational exposure to ENP, and make sure to measure a time-varying health status variable (eg, time off work) so that g-estimation can be applied to address downward bias.

To anticipate challenges of studying worker cohorts exposed to ENP, we considered studies of workers exposed to small particles. In the absence of any epidemiologic studies of occupational exposure to UFP, we turned to our own studies of respirable PM with PAH. The relevance of the challenges identified in the autoworkers study to the future study of ENP is speculative. ENP may cause health effects other than the respiratory and cardiovascular effects related to combustion-generated PM. Our experience

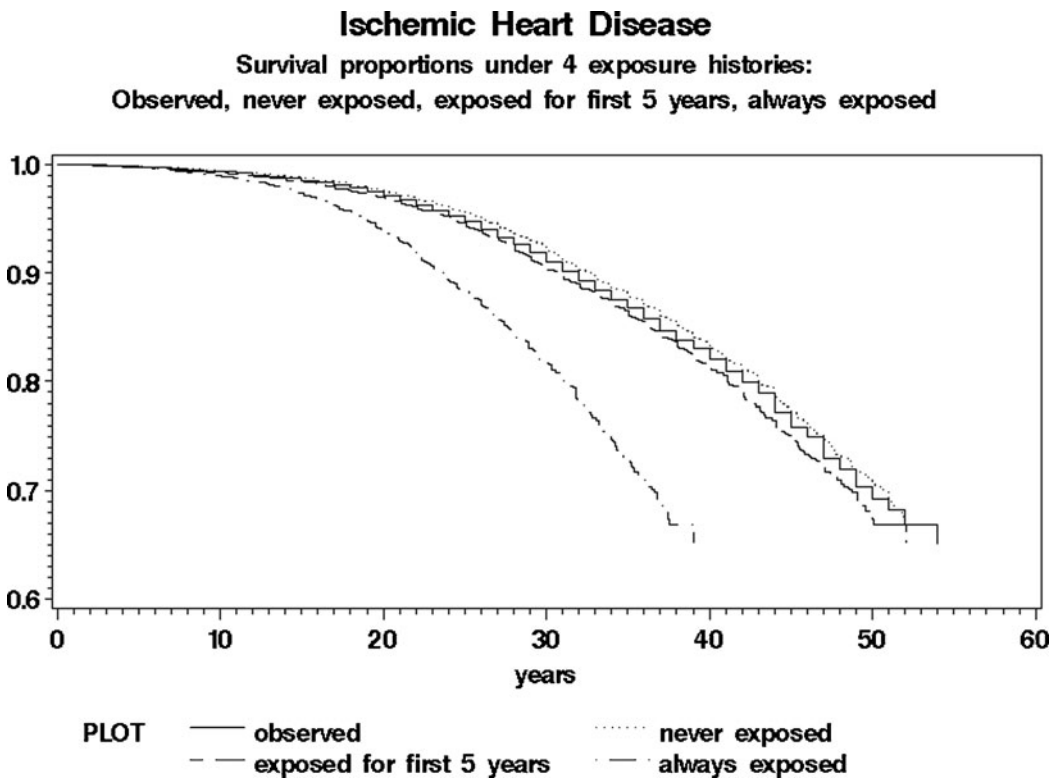

FIGURE 4. Survival curves for ischemic heart disease (IHD) under the observed exposures and 3 hypothetical exposure scenarios: never exposed, exposed to straight metalworking fluids (MWF) for 5 yrs, and exposed to straight MWF throughout follow-up, based on g-estimation in the autoworkers cohort study.

in occupational studies of chronic disease-–whether retrospective or prospective—highlights the importance of taking account of healthy worker survivor bias in study design.

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Engineered Carbonaceous Nanomaterials Manufacturers in the United States

Workforce Size, Characteristics, and Feasibility of Epidemiologic Studies

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Objective: Toxicology studies suggest that carbon nanotube (CNT) exposures may cause adverse pulmonary effects. This study identified all US engineered carbonaceous nanomaterial (ECN) manufacturers, determined workforce size and growth, and characterized the materials produced to determine the feasibility of occupational ECN exposure studies. **Methods:** Eligible companies were identified; information was assembled on the companies and nanomaterials they produced; and the workforce size, location, and growth were estimated. **Results:** Sixty-one companies manufacturing ECN in the United States were identified. These companies employed at least 620 workers; workforce growth was projected at 15% to 17% annually. Most companies produced or used CNT. Half the eligible companies provided information about material dimensions, quantities, synthesis methods, and worker exposure reduction strategies. **Conclusions:** Industrywide exposure assessment studies appear feasible; however, cohort studies are likely infeasible because of the small, scattered workforce.

Uses of engineered nanomaterials represent a fast-growing but ill-characterized aspect of many industrial sectors. Application of nanotechnology is spreading unevenly across these sectors, with manufacturing proliferating earliest, followed by electronics and information technology applications and, lastly, health care and life sciences applications. On the basis of a recent report by the International Council on Nanotechnology,[1] more than 30% of nanomanufacturers worldwide participating in their voluntary survey create and handle engineered carbonaceous nanomaterials (ECN) [eg, carbon nanotubes (CNT), fullerenes, graphene, and carbon black]. Human health effects from workplace exposures to ECN are uncertain, but toxicological studies suggest that they may include harmful pulmonary and extrapulmonary effects. Studies in mice and rats suggest that single-walled and multiwalled CNT exposures may result in pulmonary inflammation and fibrosis[2–4] and some may also penetrate the pleural mesothelium.[5] Possible extrapulmonary effects under investigation include cardiovascular inflammation,[6] immunological effects,[7] systemic exposure (accompanied by lack of clearance from the body),[8,9] and penetration of the blood-brain barrier.[10–12] A recent systematic review of laboratory toxicology studies suggests that in experimental treatments, cell viability is lower and cell death is higher than among controls.[13]

Unique properties of engineered nanomaterials, such as high particle number per equivalent mass, size, surface area, surface charge, and shape, may be of greater importance than particle mass and bulk properties in determining exposure and toxicity. Despite the growing evidence for possible hazards from occupational exposure to ECN, little information exists on actual workplace exposure,[14–17] and no epidemiologic studies have been conducted.[18] Future evaluation of potential health risks, such as cancer and cardiovascular or immunological disease, associated with occupational exposure to engineered carbonaceous nanomaterials will require improved characterization of the workforces and workplaces involved in this industry.

The purpose of this study was to enumerate the companies directly manufacturing (or using in other manufacturing processes) engineered carbonaceous nanomaterials in the United States, and to estimate the US workforce size and characteristics of nanomaterials manufacturers. The project was initiated on the basis of evidence of pulmonary fibrosis and other lung effects observed in experimental animal studies exposed to carbon nanotubes and nanofibers. The information gathered through these surveys will be used to identify possible candidate industries or workplaces for occupational epidemiology and worker exposure assessment studies.

The specific objectives of the overall study were threefold: (1) to collect and compile information on US ECN workforce size and growth in recent years; (2) to estimate, by workplace and year, the quantity and type of nanomaterials produced by manufacturers of ECN; (3) to collect and compile available information concerning presence or absence of exposure controls and on types of controls in place, to the extent feasible. The findings related to the third goal are described elsewhere.[19] This information was then used to determine the feasibility and potential timing of industrywide exposure assessment and epidemiology studies, as well as health outcome surveillance, in the ECN industry.

METHODS

Identification of Potentially Eligible Companies

Industry profiles[20,21] and internet searches were used to identify companies potentially producing ECN in the United States. Information from these and other sources was assembled on the basis of characteristics of the companies and of the nanomaterials produced as well as the workforce size, location, and estimated growth. Information needed to carry out the feasibility study was collected and compiled using the following sources: (1) Volume I of The Nanotech Report, Vols 4 and 5,[20,21] a comprehensive nanotechnology industry characterization report, used to identify the key companies and personnel producing or using ECN; (2) internet searches for suppliers and manufacturers of each type of ECN in the United States; and (3) information from personal contacts and colleagues. The completeness of this original search, which was conducted in late 2008, was assessed by comparing the list of CNT producers to

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the companies identified by the state of California during mandatory registration of companies producing CNT there, and also by the NIOSH nanotechnology coordination group, which was separately identifying companies willing to participate in field surveys of ECN production.

Collection and Confirmation of Information on Companies, Materials, and Workforce

It was then determined whether companies initially identified were eligible for participation. To be eligible, at the time of the survey (October 2008 to May 2009), the company must be manufacturing (or applying in other manufacturing processes) ECN in the United States either at full-scale, at pilot-scale, or at research scale with plans to scale-up within 5 years. Redistributors and repackagers, and companies exclusively operating at research-scale, were excluded. Volume II of The Nanotech Report, Vols 4 and 5, supplemented with a search of Dun & Bradstreet (www.dnb.com), was used to obtain information for each of these companies on company size, location, and materials produced. This source was also used to determine whether the manufacturing conducted by each of these companies was primary (ie, directly manufacturing ECN), secondary (ie, using ECN in a separate manufacturing process), or both.

Process engineers, chief technical officers, or health and safety managers at each company were contacted to confirm the information collected from the Lux and Dun & Bradstreet reports. A telephone questionnaire (see Appendix for the data collection form, http://links.lww.com/JOM/A53) was administered to collect additional information: types of engineered carbonaceous nanomaterials produced; size, shape, and quantities of nanomaterials produced; location of facility and pertinent contact information; size of population working with ECN; mass of ECN materials produced or used. In a few instances, a written survey was administered at the request of the company contact. Information was compiled using a relational database to track company contacts and survey results. The annual industry growth among all facilities was estimated by measuring annual change in workforce size over a 3-year period (2004 to 2006 for eligible companies that did not participate in the survey or 2006 to 2008 for companies that did participate in the survey). Overall growth was collated into Year 1, Year 2, and Year 3 estimates to combine information across participating and nonparticipating eligible companies with workforce estimates.

The work practices and engineering controls reported by these companies, associated with their engineered carbonaceous nanomaterials production processes, are described in the companion article.[19]

RESULTS

A total of 139 potentially eligible companies were identified, of which 61 (44%) were determined to be eligible. Of the 78 ineligible companies, 32 (41%) were not handling ECN, 14 (18%) were doing bench-scale research and development work only, 11 (14%) had non-US carbon nanomaterials manufacturing operations only, 9 (12%) were solely distributors or repackagers, 8 (10%) were no longer in business at the time of the survey, and 4 (5%) were nanomaterials consultants or handling intellectual property and patents.

The most common material produced by eligible companies was CNT (72%), followed by graphene (16%), fullerene (15%), and carbon or polymer nanofibers (15%) (Table 1). Other carbon nanomaterials produced in the United States less commonly included diamond films, nanoporous carbon, carbon quantum dots, and dendrimers. Eligible companies were most likely to be manufacturing or using ECN in the northeastern and western United States (Table 1), and were most common in California, Massachusetts, Texas, and Ohio. Geographic heterogeneity in the production and use of different types of engineered carbonaceous nanomaterials was also observed. For example, CNT and fullerene manufacturers were predominantly located in the northeastern and western regions of the United States, while graphene manufacturing occured primarily in the midwest and west. Vapor-grown carbon nanofibers were most frequently produced in the midwest and west, and electrospun polymer fibers and "other" carbonaceous nanomaterials were produced most frequently in the midwest.

Overall, 34% of eligible companies were reported to be exclusively primary manufacturers of ECN, while 26% were solely secondary manufacturers, and 39% were both primary and secondary manufacturers. These percentages did not vary substantially by ECN type (Table 2). Regarding the scale of manufacturing among the companies, 59% were at full manufacturing scale (either primary

TABLE 1. Locations of Companies Manufacturing or Using Engineered Carbonaceous Nanomaterials in the United States

	US Region (%)*				
Nanomaterial Type	**Northeast**	**Southeast**	**Midwest**	**West**	**Total†**
Carbon nanotubes	18 (41%)	4 (9%)	5 (11%)	17 (39%)	44
Graphene	1 (10%)	1 (10%)	4 (40%)	4 (40%)	10
Fullerenes	4 (44%)	1 (11%)	1 (11%)	3 (33%)	9
VGCNF	1 (17%)	0	2 (33%)	3 (50%)	6
EPF	0	1 (33%)	2 (67%)	0	3
Other‡	4 (27%)	1 (7%)	6 (40%)	4 (27%)	15
Total§	21 (34%)	6 (10%)	15 (25%)	19 (31%)	61§

EPF, electrospun polymer fibers; VGCNF, vapor-grown carbon nanofibers

*Northeast includes CT, DE, MA, NH, NJ, NY, PA, VT; southeast includes GA, MD, NC, SC, TN, VA; midwest includes IL, MI, MN, OH, WI; and west includes AZ, CA, NM, OK, TX, WA. No eligible manufacturers or users were identified in AK, AL, AR, CO, DC, FL, HI, IA, ID, IN, KY, KS, LA, ME, MI, MO, MS, MT, ND, NE, NV, OR, RI, SD, UT, WV.

†Companies were counted once for each type of material produced and for each region in which it was produced.

‡Includes dendrimers, diamond-like, nanoporous carbon, carbon quantum dots, and others.

§Each company producing multiple materials was counted only once.

TABLE 2. Primary or Secondary Manufacturing Status by Nanomaterial Type

	Manufacturing Status			
Nanomaterial type	**Primary**	**Secondary**	**Primary & secondary**	**Total***
Carbon nanotubes	14 (33%)	18 (42%)	11 (26%)	43
Graphene	4 (40%)	4 (40%)	2 (20%)	10
Fullerenes	3 (38%)	3 (38%)	2 (25%)	8
VGCNF	3 (50%)	3(50%)	0	6
EPF	0	1 (33%)	2 (67%)	3
Other†	6 (40%)	5 (33%)	4 (27%)	15
Total‡	21 (34%)	16 (26%)	24 (39%)	61‡

EPF, electrospun polymer fibers; VGCNF, vapor-grown carbon nanofibers.

*Companies were counted once for each type of material produced.

†Includes dendrimers, diamond-like, nanoporous carbon, carbon quantum dots, and others.

‡Each company producing multiple materials was counted only once.

or secondary), 11% were at pilot-scale, 11% were at research and development-scale with plans to scale up, and 18% (primarily, the nonparticipating companies) operated at unknown scale.

About half ($n = 30$) of the eligible companies agreed to be interviewed and provided information about material quantities, dimensions, and methods employed to reduce workers' exposures. The number of employees reported to be handling ECN at the 36 manufacturers with workforce size estimates (from all sources) ranged from three to 100 (476 total, not including 11 companies for which estimates were not available) and at the pilot scale operations from one to 30 employees (144 total, not including 1 company for which estimates were not available). Thus, the eligible companies employed a total of about 620 workers directly using ECN (Table 3). Most ($n = 375$) worked with single-walled or multiwalled CNT. Companies handling carbon nanomaterials in manufacturing, pilot scale, and R&D operations preparing to scale-up in the near future have all seen growth in the industry during 2006, 2007, and 2008. Employee numbers increased by roughly 14% in manufacturing, 74% in pilot plant operations, and 44% in R&D operations preparing to scale-up in the next 5 years. The estimate for manufacturers includes participating and nonparticipating companies with employee data available over a 3-year period. Though the time periods for ascertaining the number of workers employed differed by 2 years for the participating (2006 to 2008) and nonparticipating companies (2004 to 2006), only one out of the five nonparticipating companies had a notable change in employee numbers over the 3-year period. Twenty-two participating companies and five nonparticipating companies were used for the growth estimate. Employee counts for the nonparticipating developmental/small sales companies were only available for 1 year, so they were not included in the above growth estimates.

The overall ECN workforce growth between Year 1 and Year 3 was 34%: 17% from Year 1 to Year 2, and 15% from Year 2 to Year 3 (Table 4). Comparing the growth in CNT manufacturing operations with that in those involving all other types of ECN, the industry growth picture is similar. The number of employees in manufacturing operations involving CNT increased 44% over the 2-year period; from 192 employees to 276 in 22 companies. Manufacturers of other ECN experienced a 16% increase in growth over the 2-year period: from 109 to 126 employees in nine companies.

The growth in pilot operations is due primarily to CNT operations. Throughout the 3 years of interest, approximately 90% of employees working in pilot operations worked for companies involved with CNT. In research and development operations preparing to scale-up, both fullerenes and CNT play a role in the growth. There was an 88% increase in the number of employees in CNT operations and a 13% growth in fullerene operations.

The quantities produced annually as reported by the manufacturers ranged from 0.9 to 10,000 kg (roughly 18,000 kg total; Table 3). Quantities handled at the pilot plant sized operations were reported to range from 0.2 to 3000 kg (3350 kg total). No quantity data were available for the nonparticipating developmental scale companies. Three companies considered the quantities they produced to be business sensitive information; one would not provide any quantity information though the other two were willing to provide "less than" estimates. Although CNT producers comprised the largest group of ECN manufacturers, they produced the second-highest quantity of material: greater than 3472 kg (14 CNT manufacturers did not provide quantity information). Electrospun polymer fibers were produced in 40,000 linear yard quantities, and 10,000 kg of carbon nanofibers were reported to be produced annually. Quantities of other ECNs were much lower: 700 kg of dendrimers, 40.3 kg of graphene, and 14.6 kg of fullerenes. At least 4000 kg of "other" nanomaterials were produced annually.

Overall, 87% of companies completing the questionnaire provided information on synthesis methods. Among CNT manufacturers ($n = 13$), 62% reported chemical vapor deposition, 23% reported using arc discharge, 15% reported flame combustion, 8% reported using laser ablation, and one company did not report (some reported more than one method).

Our comparisons of the list of CNT producers against both the contacts made by the NIOSH nanotechnology coordination group and in response to the state of California's request for notification of CNT production in the state found no companies that were not captured by our study.

DISCUSSION

This study attempted to identify all companies producing ECN in the United States. The number of companies is large, with more

TABLE 3. Number of Employees and Quantity Produced by Nanomaterial Type Eligible Companies (Participants and Nonparticipants)

	Manufacturers		Pilot/Developmental Scale	
Type of Carbon Nanomaterial	**No. of Employees Per Company**	**Quantity (kg/yr) Produced Per Company**	**No. of Employees Per Company**	**Quantity (kg/yr) Produced Per Company**
Carbon nanotubes	2–100	0.2–2500	1–30	0.1–300
Vapor grown nanofibers	5	10,000	NA	NA
Polymer fibers electrospun	10–18	40,000 linear yards	NA	NA
Fullerenes	4–23	1–13.5	NA	0.1
Graphene	8	ND	3–20	0.001–40
Dendrimers	3–19	700	NA	NA
Diamond-like	5	1200 wafers/yr	NA	NA
Nanoporous carbon	NA	NA	ND	3000
Other	9	1000	NA	NA
No data	5–100	ND	NA	NA

ND, no data; NA, not applicable.

TABLE 4. Industry Growth-–Employee Count by Manufacturer and Nanomaterial Type, Among Eligible Companies With Workforce Size Information

Nanomaterial CNT or Other	Production Scale	Employee Count, Year 1*	Employee Count, Year 2† (% Change From Year 1)	Employee Count, Year 3‡ (% Change From Year 2)
CNT	Manufacturing	172	196 (14%)	214 (9.2%)
CNT	Pilot	20	43 (115%)	62 (44%)
Total CNT	All combined	192	239 (24%)	276 (15%)
Other	Manufacturing	89	80 (−10%)	100 (25%)
Other	Pilot	20	32 (60%)	26 (−19%)
Total other	All combined	109	112 (2.7%)	126 (13%)
Total ECN	All combined	301	351 (17%)	402 (15%)

CNT, carbon nanotubes; ECN, engineered carbonaceous nanomaterials.
*Year 1 was 2004 for nonparticipating companies ($n = 5$) and 2006 for participating companies ($n = 26$).
†Year 2 was 2005 for nonparticipating companies and 2007 for participating companies.
‡Year 3 was 2006 for nonparticipating companies and 2008 for participating companies.

than 60 represented. The ECN workforce identified in this study, however, is small, with an average of about 10 workers handling ECN per company. By far, single-walled and multiwalled CNT were the most common substances produced, with the 43 eligible companies employing at least 375 workers by our estimate (see Table 2). The strengths of this study include the systematic evaluation of ECN producers in the United States in 2008 and 2009. Consistent information was collected on workforce size and characteristics across the entire industry. Comparison to data collected by other groups suggests that this study's sampling method adequately captured carbon nanotube producers.

However, this study has a number of limitations that affect interpretations of the feasibility of epidemiologic studies in this workforce and that could be used to design improved surveys in the future. The participation rate was suboptimal. This may have been affected by companies' concerns over recent interest among regulatory agencies in listing CNT as a hazardous substance. Responsiveness might have been improved if NIOSH rather than a contractor had made the contacts (eg, several nonparticipants reported concerns in sharing data with potential competitors). It was necessary to rely on self-report for information. In addition, the time period of survey (October 2008 to May 2009) occurred during a severe global economic recession, which may have affected companies' plans to expand at the time of the survey. This likely led to an underestimate of the workforce size in future years, as the rate of growth may appear lower than reality with economic recovery. It is also clear that this study underestimated the research and development workforce size, because such groups were excluded if they did not express a plan to move to at least pilot scale in the next 5 years. Furthermore, a number of research and development institutions in government, academia, and private industry were excluded at the outset (ie, they were not captured in our initial examination of 139 potentially eligible companies) because they were known to be involved only in research and development and were thought likely to have smaller workforces exposed to a wide variety of materials. It appears that 34 companies initially queried that were ineligible for this study are actually handling ECN either in the United States or elsewhere. Lastly, the number of companies no longer in business at the time of the survey illustrates the fact that the ECN workforce is small and fluid: many new companies either fail or are acquired by other companies if successful. Business relationships (eg, company buyouts and supply chains) were recorded when possible in our database, which may be valuable for tracking these workers for any future epidemiologic studies.

Industrywide exposure assessment studies are likely feasible at this time; however, they are subject to a number of challenges. Studies of the US ECN workforce will likely be hampered by limitations in the measurement capabilities for these materials. While a mass-based method has been developed for assessing elemental carbon exposure in diesel-exposed occupations,[22] it cannot differentiate between nano-sized particles and those of larger size (a potentially important toxicological consideration). In addition, the method's limit of quantitation for single-walled and multiwalled carbon nanotubes may approach workplace equivalent mass concentrations that have been shown to cause adverse effects in animal studies.[23] At present, investigations are being carried out on the most appropriate particle size–based analysis methods for CNT and other ECNs.[15–17] Fiber counts and dimensions may also prove useful as metrics, as has recently been described in the asbestos literature.[24] Exposure assessment studies will provide important information regarding tasks and conditions most likely to result in elevated occupational exposures, which will be critical for designing future epidemiologic studies in these emerging industries. Furthermore, these methods should ideally be translated to the toxicology studies, so that findings can be compared between human and animal studies.

In order for optimized exposure assessment methods to be applied by industry-based exposure assessment personnel (which would be required for large-scale epidemiologic research), they must be focused and cost-effective while also measuring toxicologically relevant aspects of exposure. In addition, employers should be encouraged to collect, retain, and share such exposure measurements over the long term, as they may be needed for future epidemiologic studies.[25] Exposure registries and large exposure databases may be useful tools to retain institutional knowledge about ECN workers and exposures.

A challenge affecting both exposure assessment and epidemiologic studies of occupational exposures to ECN will be consideration of concomitant exposures at these facilities. ECN synthesis methods may involve exposures to other hazardous materials, such as heavy metals used as catalysts, or hydrocarbons used as feedstock or precursor.[17] A recent study estimated that 11 potentially hazardous exposures existed within a carbon nanofiber manufacturing facility, including several types of carbon nanofibers, the precursor material and intermediates of pyrolysis (often, simple polycyclic aromatic

hydrocarbons), methane, propanol, and high heat.[26] Not all of these are hazards for the same outcome (eg, cancer or nonmalignant respiratory disease). Furthermore, this phenomenon is not unique to the study of ECN—most studies of long-term health impacts from occupational exposures must contend with multiple exposures. However, epidemiologic studies should assess exposures to other substances that potentially cause the disease of interest, so that they may be treated statistically as confounders, or (more importantly) that their joint effects with ECN may be investigated.

Large-scale cohort studies of the US ECN workforce are likely infeasible because of the small workforce (causing low power) and short follow-up time available for diseases with long latency.[18] However, potential cross-sectional studies and utility of pooling internationally, particularly of CNT and carbon nanofiber manufacturers, should be considered. At least ten additional companies, many of them large multinational corporations, are involved in ECN manufacturing outside the United States. It is unclear how large the US workforce may become, given the typical patterns of manufacturing in the high-technology manufacturing;[27,28] thus, international pooling of cohorts may offer the most promise for large-scale studies. In the meantime, cross-sectional studies may be the most feasible epidemiologic design. Such studies should consider evaluating pulmonary function as well as biomarkers of exposure or early effect, in relation to various aspects of measured exposure. Relevant biomarkers of early effect could include endpoints related to genotoxicity (eg, spectral karyotyping or multiphase fluorescence in-situ hybridization), given recent evidence regarding interference of CNT with mitotic spindle formation, biomarkers of oxidative stress (glutathione and malondialdehyde in mouse studies[29]), or markers of pulmonary fibrosis. These could include serum immunoproteins IL-6, KL-6, SP-A, and SP-D, which have shown promise as markers of pulmonary inflammation and fibrosis in studies of workers exposed to indium compounds and cobalt-tungsten-carbide.[30,31] Of great need are biomarkers of systemic exposure, such as genes expressed in circulating lymphocytes or soluble serum proteins[32] that have been identified from animal studies. Such biomarkers could complement industrial hygiene-based sampling to identify workers at higher risk of effects from exposure, as well as confirm results from toxicology studies.

CONCLUSIONS

This study identified a small (>600) but growing workforce within 61 companies manufacturing and using engineered carbonaceous nanomaterials in the Unites States. The materials most commonly produced include single-walled and multiwalled carbon nanotubes. Industrywide exposure assessment studies will be useful in identifying the extent of occupational exposure to these materials. Epidemiologic researchers should consider the feasibility of cross-sectional studies using biomarkers of exposure and early pulmonary effect, along with industrial hygiene sampling, as well as the potential for pooled international studies of carbon nanotube manufacturing workers.

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Exposure Control Strategies in the Carbonaceous Nanomaterial Industry

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Objective: Little is known about exposure control strategies currently being implemented to minimize exposures during the production or use of nanomaterials in the United States. Our goal was to estimate types and quantities of materials used and factors related to workplace exposure reductions among companies manufacturing or using engineered carbonaceous nanomaterials (ECNs). **Methods:** Information was collected through phone surveys on work practices and exposure control strategies from 30 participating producers and users of ECN. The participants were classified into three groups for further examination. **Results:** We report here the use of exposure control strategies. Observed patterns suggest that large-scale manufacturers report greater use of nanospecific exposure control strategies particularly for respiratory protection. **Conclusion:** Workplaces producing or using ECN generally report using engineering and administrative controls as well as personal protective equipment to control workplace employee exposure.

Nanotechnology has emerged at the forefront of science research and technology development over the past decade. The nanotechnology sector has already achieved a multibillion dollar US market and is widely expected to grow to a 1 trillion dollar market in the United States by 2015.[1] As the mass production of engineered carbonaceous nanomaterials (ECNs) continues to grow, increased numbers of workers will be exposed to these materials.

Concurrent to the growth of the ECN market there is a coalescing level of evidence, which indicates that exposure to some forms of ECNs may cause adverse health effects. Although there are many active toxicology programs assessing the potential health effects of ECN, no epidemiologic studies are yet available, as they require long time periods and a sizeable workforce to be informative.[2] As with most particles in the workplace, inhalation is considered to be the main route by which free unbound nanomaterials can enter the bodies of workers, although data supports the possibility of dermal exposures as well.[3]

Studies have shown that long carbon nanotubes possess asbestos-like pathogenicity, which has raised even greater concerns about the possibility of exposures to such ECNs.[4–6] Other animal studies have linked ECNs to possible adverse health effects, such as pulmonary inflammation, oxidative stress, onset of early interstitial fibrosis, and granulomas.[7,8] Genotoxicity may result from ECN exposure: single-walled carbon nanotubes have been found to induce aneuploidy in human respiratory epithelial cells through interference with mitosis.[9] Some evidence suggests that, once inhaled, nanomaterials can pass from the lungs into the bloodstream and might present a systemic health hazard. Inhaled carbon nanomaterials have been shown to rapidly clear rat lungs and translocate to other organs including the liver and spleen.[10] Therefore, because of the current scientific evidence concerning the potential health hazards associated with nanomaterials, appropriate steps should be taken in the workplace to minimize worker exposure to ECN.

Safe occupational handling approaches and exposure control strategies for ECN, including administrative and engineering controls as well as personal protective equipment (PPE), are still developing. Nevertheless, several guidelines for working with nanomaterials have been issued by various countries[11–15] and other guidelines from various stakeholders have been released as well.[16,17] Nevertheless, the extent to which these exposure control strategies are being used during the manufacturing of nanomaterials in the United States has been relatively unknown.

As part of an investigation of the feasibility of industrywide exposure assessment and epidemiologic studies of ECN workers,[18] the authors conducted a survey of companies manufacturing ECN in the United States, to identify types and quantities of materials produced and factors related to workplace exposure reductions. Several other studies, similar in nature to this project, have been conducted internationally[19,20] to assess workplace health and safety and product stewardship practices for nanomaterials. The main objective of this manuscript is to describe current ECN manufacturing exposure control strategies, specifically engineering and administrative controls and PPE being used in the US ECN manufacturing industry.

METHODS

The methods used to identify companies participating in the study are described in detail elsewhere.[18] Briefly, study participants were identified by using the Lux Nanotech Reports, fourth and fifth editions,[21,22] as well as Web searches for manufacturers of ECN. The number of companies initially found totaled 139. Inclusion criteria for this study focused on companies manufacturing (or using during manufacturing) in the United States some type of ECN, which was defined as elemental carbon particles purposefully engineered to have specific properties or composition with at least one dimension less than 100 nm. Of the 139 companies originally identified from the initial list of prospective participants, 78 did not meet the inclusion criteria, because they were not handling ECN (41%), were involved solely in bench-scale research and development work (18%), had non-US carbon nanomaterials manufacturing operations only (14%), were solely repackagers (12%), or for other reasons (15%).[18]

Introductory letters explaining the purpose of the study along with data collection forms were sent to company contacts prior to contact via e-mail or mail. This allowed participants advance notice of the type of questions that would be asked as well as the data being collected. Initial contacts were made to explain the aims and goals of the study, and formal interview times with knowledgeable company personnel were arranged. Phone surveys were conducted from October 2008 to May 2009. All phone interviews were administered by a certified industrial hygienist. Company representatives participating in the interviews included environmental health and safety personnel, scientists, and managers. The certified industrial hygienist conducting the phone interview preferentially scheduled

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the phone survey with a representative of the environmental health and safety staff or, if unavailable, the chief technical officer or other knowledgeable technical contact.

Data were collected to identify facility location, types, and quantities of materials produced as well as work practices and exposure control strategies from the participating companies manufacturing ECNs at or below 100 nm. Nevertheless, data were also collected for materials in the diameter size range greater than 100 nm as long as the company produced one form of ECN under 100 nm. Information was also ascertained on the size of worker populations at each facility as well as the change in the industrywide work force size from 2006 through 2008.[18] Because there was no measure for response accuracy on the collected data, responses are described as reported. Potential participants were informed at the time of contact that participation was completely voluntary, and that results would be published only in aggregate form. The information gathered from this survey is being used to evaluate the feasibility of an industrywide exposure assessment and epidemiology study for US manufacturers and users of ECN. The challenges and opportunities for designing surveillance work are further discussed in the companion paper.[18]

Participating respondents included companies that were self-described as currently manufacturing or using ECN, companies performing pilot scale work, and companies performing research and development (R&D) activities with plans of scaling up within the next 5 years. Potential participants who were strictly involved with R&D work with no plans to scale up were excluded. The participants were then classified into three groups for further examination on the basis of trends seen in production and exposure controls methods already in place. The groups consisted of companies performing manufacturing using production-based exposure controls, companies performing manufacturing using laboratory-based exposure controls, and companies performing R&D or pilot scale work. Proportions are calculated per group use of the specific exposure control strategy found in Tables 2 through 5. Ninety-five percent confidence intervals were calculated for all proportions in SAS 9.2, using the Wilson interval for estimation of binomial proportions.[23]

RESULTS

Sample Characteristics

From the 61 eligible companies, 30 agreed to participate in the study, resulting in a response rate of 49.2%. The eligible participating companies consisted of 15 manufacturers, as well as 15 companies performing pilot scale or R&D type work with plans to scale up within the next 5 years. The 30 participating companies were further divided into three groups for closer examination, on the basis of trends seen in production and exposure control methods already in place for each facility. Group 1 consisted of eight companies performing manufacturing using production-based exposure controls. Group 2 was composed of seven companies performing manufacturing using laboratory-based exposure controls. The 15 pilot-scale or R&D companies composed group 3. The eight group 1 companies described systems and programs more typical of large-scale manufacturing operations, such as enclosed systems, comprehensive ventilation with pollution control devices, and automated packing operations. Most of these manufacturers also provided work clothing along with change facilities to their employees. The group 2 manufacturers appeared to employ laboratory practices (nonspecific laboratory hoods, biological safety cabinets (BSC), benchtop glove boxes, or benchtop vented boxes) with some specialized modifications to contain the ECN being handled. The group 3 companies consisted of several large corporations as well as small start-up companies performing R&D or pilot scale work with ECN. Their use of controls was a mixture of laboratory- and production-based methods.

ECN Characteristics

Nearly half of all participating companies reported manufacturing more than one type of ECN ($n = 11$), while several companies made different variations of the same type of material ($n = 4$). A total of 56 different types of ECN were reportedly produced by all respondents (Table 1). The most frequently produced types of nanomaterials were multiwalled carbon nanotubes (MWCNT;$n = 18$, 32.1%), followed by single-walled carbon nanotubes (SWCNT; $n = 17$, 30.4%), graphene ($n = 6$, 10.7%), nanofibers ($n = 5$, 8.9%), fullerenes ($n = 4$, 7.2%), and others, which included carbon quantum dots, dendrimers, diamond like films, and nanoengineered carbon black ($n = 6$, 10.7%).

The mean quantity produced for each nanomaterial ranged from 4.1 kg for fullerenes to 5001.8 kg for nanofibers (Table 1) with cumulative production total from all participants of roughly 15,000 kg of ECN. The mean diameter for the reported nanomaterials ranged from 0.6 nm for the fullerenes to 157 nm for the nanofibers. The mean particle length for reported nanomaterials of nonspherical shape ranged from 58.4 μm for nanofibers to 187.9 μm for SWCNT to 773.3 μm for MWCNT. The calculated mean aspect ratio (AR) was largest for SWCNT at 186,936, while MWCNT and nanofibers had mean ARs of 68,704 and 424, respectively. Agglomerates of ECN were reported for all types of nanomaterials surveyed with average sizes ranging from 26.5 nm for the group consisting of other types of ECN to 209.3 nm for MWCNTs. Functional groups were reported to be present on 44.6% ($n = 25$) of all types of ECN. Common functional groups reported by participating companies were carboxylic acids, alcohols, and amines. Metal impurities were also reported for 23 (41.1%) of the 56 different types of ECN, all of which were either SWCNT or MWCNT. The most common types of metal impurities reported were Co, Ni, Fe, Mo, Y, and Al.

Engineering Controls

All participating companies reported using some sort of engineering control to reduce worker exposure to ECN and used multiple forms of engineering controls to reduce worker exposure as well ($n = 30$, 100%). Overall, the most common forms of controls used to minimize workplace exposures to ECN were that of chemical fume hoods ($n = 25$, 83%), seen in Table 2. This trend was true for both group 3 ($n = 13$, 87%) and group 2 ($n = 7$, 100%). A total of 3 of the 25 companies which reported using fume hoods also reported having HEPA filters associated with those hoods; however, this question was not directly asked as part of the original survey and cannot be considered representative.

The most commonly used form of engineering controls set in place by the group 1 exposure control group was local exhaust ventilation (LEV; $n = 8$, 100%), which was often reported to be custom built for the specific process or task. Two companies (25%) from group 1 reported using LEV with a HEPA filtration system, while only one company (14%) reported using this control for group 2 and five companies (33%) from group 3 reported using LEV with a HEPA filtration system.

The least common type of engineering exposure control strategy used by all three groups was BSC ($n = 2$, 7%). One BSC each was reportedly used by groups 2 and 3, while none were used in group 1. Group 1 also reported using the highest percentage of ventilated enclosures and glove boxes ($n = 6$, 75%) closely followed by group 3 ($n = 10$, 67%), while group 2 used this form of control the least ($n = 2$, 29%). Of the 18 total companies reportedly using ventilated enclosures and glove boxes, half reported that they were designed with HEPA filters. Nevertheless, this question was not directly asked during the phone survey, and it was not included on

TABLE 1. Descriptive Information on ECN From Participating Respondents

	Total Number Produced	Diameter/Size, mean (range) (nm)	Length, mean (range) (μm)	Mean Aspect Ratio	Mean Agglomerate Size (μm)	Mean Quantity Produced (kg)
SWCNT	17 (30.4%)	5.04 (0.5–50)	187.9 (0.5–1,000)	186,936	68.3	44.9
MWCNT	18 (32.1%)	29.3 (1.2–200)	773.3 (0.1–18,000)	68,704	209.3	21.6
Nanofibers	5 (8.9%)	157 (20–300)	58.4 (1–200)	424	100	5,001.8
Graphene	6 (10.7%)	133 (2–500)	N/A	N/A	100	10.07
Fullerene	4 (7.2%)	0.6 (0.1–1)	N/A	N/A	200	4.1
Others	6 (10.7%)	52.9 (5–100)	N/A	N/A	26.5	1,175.02

MWCNT, multiwalled carbon nanotubes; N/A, not applicable; SWCNT, single-walled carbon nanotubes.
Ranges and percentages are represented in parentheses. Means calculated with values provided from survey. Mean aspect ratio was calculated by averaging the individually calculated aspect ratios.

TABLE 2. Number, Proportion (95% Confidence Interval) of Companies Using Various Engineering Control Methods for ECN

	n	LEV	LEV W/HEPA	Chemical Fume Hoods	Biological Safety Cabinets	Ventilated Enclosures/ Glove Boxes	Enclosed Production Processes	Separate Ventilation for Office
Manufacturing Type								
Group 1: production-based exposure controls	**8**	**8**, 1.0 (.68, 1.0)	**2**, .25 (.07, .59)	**5**, .63 (.31, .86)	**0**, 0.0 (0.0, .32)	**6**, .75 (.41, .93)	**3**, .38 (.14, .69)	**6**, .75 (.41, .93)
Group 2: laboratory-based exposure controls	**7**	**3**, .43 (.16, .75)	**1**, .14 (.03, .51)	**7**, 1.0 (.65, 1.0)	**1**, .14 (.03, .51)	**2**, .29 (.08, .64)	**3**, .43 (.16, .75)	**3**, .43 (.16, .75)
Group 3: pilot and R&D scale operations	**15**	**8**, .53 (.3, .75)	**5**, .33 (.15, .58)	**13**, .87 (.62, .96)	**1**, .07 (.01, .3)	**10**, .67 (.42, .85)	**5**, .33 (.15, .58)	**11**, .73 (.48, .89)
Total	**30**	**19**, .63 (.46, .78)	**8**, .27 (.14, .44)	**25**, .83 (.66, .93)	**2**, .07 (.02, .21)	**18**, .6 (.42, .75)	**11**, .37 (.22, .54)	**20**, .67 (.49, .81)

HEPA, high-efficiency particulate air filtration; LEV, local exhaust ventilation.
Cells report number of companies (bold) as well as proportions. Corresponding 95% confidence intervals are represented in parentheses.

the data collection forms provided to the companies and may not be representative.

Overall, a total of 11 companies (37%) reported having completely enclosed production processes. Five (33%) of the enclosed production processes came from group 3, while three each came from groups 1 (38%) and 2 (43%). Most companies ($n = 20$, 67%) reported the overall use of a separate ventilation system for any office space that was near or connected to the manufacturing areas of ECN.

Some respondents described specialized or modified engineering controls such as walk-in hoods for high exposure tasks, or sonicators in closed containers (in some cases, the enclosed sonicators were placed inside chemical fume hoods). Most companies that reported using a HEPA filtered ventilated hood or other ventilated enclosures indicated using these devices when the exposure potential was deemed to be the greatest.

Work Practice and Administrative Controls

Overall, most companies reported providing some form of Health and Safety (H&S) training to employees ($n = 21$, 70%) (Table 3). Group 2 reported providing the least amount of H&S training ($n = 4$, 57%), while group 1 were the most likely to provide H&S training to their employees ($n = 6$, 75%) closely followed by group 3 ($n = 11$, 73%).

A majority of respondents, overall, had a housekeeping program in place ($n = 25$, 83%) as well as standard operating procedures for equipment maintenance ($n = 21$, 70%). A majority of companies also used wet methods for clean up ($n = 21$, 70%) as well as using some form of restricted or isolated access during the production or handling of ECN ($n = 22$, 73%). Group 3 companies reported using wet methods for clean up the most ($n = 13$, 87%), while group 2 reported using restricted access or isolated operations to control employee exposures most frequently ($n = 6$, 86%). Group 3 also reported using HEPA-filtered vacuums most often to clean spills or for routine cleaning ($n = 9$, 60%). Group 1 reported that three (38%) of the companies used HEPA vacuums and group 2 reported using HEPA vacuums the least often ($n = 2$, 29%).

Overall, a minority of companies provided change facilities or laundering programs for employee work clothing respectively ($n = 9$, 30%; $n = 13$, 43%). Nevertheless, group 1 reported providing both services to employees ($n = 4$, 50%; $n = 4$, 50%) more often than do the other groups.

Many companies described specific administrative controls such as placing carbon nanotubes in solution as soon as possible to minimize employee exposure. Two companies mentioned internal policies of carbon nanotubes only being allowed out of ventilated work areas when they were in solution. A few companies mentioned placing sticky mats at all entrances and exits of any room where ECN was stored or handled to reduce possible cross contamination.

TABLE 3. Number, Proportion (95% Confidence Interval) of Companies Using Various Work Practice and Administrative Exposure Control Methods for ECN

	n	H&S Training Training	House Keeping Program	Wet Method for Clean up	HEPA Filtered Vacuum	Restricted/ Isolated Operations	Equipment Maintenance SOPs	Change Facilities	Uniforms Supplied/ Laundered
Manufacturing Type									
Production-based exposure controls	**8**	**6**, .75 (.41, .93)	**7**, .88 (.53, .98)	**4**, .5 (.22, .78)	**3**, .38 (.14, .69)	**6**, .75 (.41, .93)	**7**, .88 (.53, .98)	**4**, .5 (.22, .78)	**4**, .5 (.22,.78)
Laboratory-based exposure controls	**7**	**4**, .57 (.25, .84)	**6**, .86 (.49, .97)	**4**, .57 (.25, .84)	**2**, .29 (.08, .64)	**6**, .86 (.49, .97)	**4**, .57 (.25, .84)	**0**, 0.0 (0.0, .35)	**2**, .29 (.08, .64)
Pilot and R&D Scale Operations	**15**	**11**, .73 (.48, .89)	**12**, .8 (.55, .93)	**13**, .87 (.62, .96)	**9**, .6 (.36, .8)	**10**, .67 (.42, .85)	**10**, .67 (.42, .85)	**5**, .33 (.15, .58)	**7**, .47 (.25, .7)
Total	**30**	**21**, .7 (.52, .83)	**25**, .83 (.66, .93)	**21**, .7 (.52, .83)	**14**, .47 (.3, .64)	**22**, .73 (.56, .86)	**21**, .7 (.52, .83)	**9**, .3 (.17, .48)	**13**, .43 (.27, .61)

H&S, health and safety; HEPA, high-efficiency particulate air filtration; SOP, standard operating procedures.
Cells report number of companies (bold) as well as proportions. Corresponding 95% confidence intervals are represented in parentheses.

Also, several companies reported that the weighing and transferring operations for dry powders occurred in isolated or restricted access areas and workers who entered these areas were required to complete nanospecific hazard training.

Two manufacturers and two R&D/pilot scale operations reported performing routine monitoring for airborne particulates. Nonspecific, total particulate counters were reported as the instruments employed. These questions were not directly asked as part of data collection efforts so may not be representative of the numbers of participating manufacturers performing air monitoring.

PPE Controls. Every company surveyed reported using some form of PPE to minimize worker exposure to ECN (Table 4). The most common form of protective clothing reported was the use of gloves ($n = 29$, 97%), which was reported by all of the companies in groups 1 and 2 (100%) and by 14 companies in group 3 (93%). The next most common form of protective clothing reported was the use of aprons ($n = 14$, 47%), which was most often reported by group 3 ($n = 8$, 53%). Full Tyvek suits were reportedly used most often by group 1 ($n = 7$, 88%), while group 2 used this form the least ($n = 1$, 14%). Nearly all surveyed companies reported using safety glasses ($n = 28$, 93%), while companies reportedly provided footwear and boot covers to employees less often ($n = 12$, 40%).

Respiratory Protection

A majority of companies reported providing some kind of respiratory protection to employees when working with ECN as well ($n = 23$, 77%) (Table 5). Most of the companies that reported using respiratory protection stated using either a half face negative pressure respirator ($n = 13$, 43%) with P100 or N100 cartridges and P100 or N95 filtering facepieces ($n = 6$, 20%). Several companies, mostly in group 1, reported providing multiple types of respiratory protection depending on the possibility and level of exposure. Three companies (10%), overall, reported the use of some type of respirator but did not specify the type. One company each from groups 2 (14%) and 3 (7%) reported using only nuisance dust masks. For this study's purpose those, two companies were counted as not using respirators because dust masks do not provide adequate respiratory protection for nanoparticles.[14,24] A total of seven companies (23%), six from group 3 (40%) and one from group 2 (14%) reported not using any type of respiratory protection. One of the seven companies that reported not using respiratory protection stated the reason was due to the advanced ventilation controls in place at the facility. Two of the seven companies that did not use respiratory protection during ECN production or use reported having enclosed production processes. Only one company mentioned an OSHA 29 CFR Part 1910.134 compliant fit-test program, although the question was not directly asked as part of the survey given to participating companies.

DISCUSSION

The results of the survey generally indicate that use of exposure control strategies, including engineering and administrative controls as well as personal protective equipment, in US industry is being reported by US manufacturers and end users of ECN. Most of the participating companies from this survey employed some type of airborne particulate control method such as the use of HEPA-filtered hoods, custom designed LEV systems, or enclosed production processes to control work place exposures to ECN. Also, technical contacts at all manufacturing, pilot plant, and R&D scale operations expressed awareness of the importance of controlling exposures to airborne carbon nanomaterials through the use of administrative controls and PPE. Nevertheless, room for improvement exists in areas such as respirator selection as well as engineering control selections.

The most important finding was that nearly one in four companies surveyed manufacturing or using ECN in the United States reported not using any type of respiratory protection or reported using an ineffective form of protection such as a dust mask. One of the seven companies not using respiratory protection stated that it was not needed due to the operations being fully enclosed, and one other company reported having enclosed production processes but did not state that this was their reason for not using respirators. Similar trends on respirator usage have been seen in previous international surveys on exposure control strategies and PPE uses.[19,20] NIOSH has recently recommended that respirator use be considered even for enclosed processes if measurement data indicate that nanomaterial exposure is not well controlled.[14] As recommended exposure limits become available for airborne nanoparticles, it will be possible to use the traditional NIOSH respirator selection logic to select respiratory protection with an assigned protection factor that is sufficient to provide protection against the actual airborne concentration of nanoparticles in the workplace.[25] In January 2011, NIOSH posted on its Web site for public comment, a recommended exposure limit for carbon nanotubes and carbon nanofibers of 7 $\mu g/m^3$ as an 8-hour time weighed average.[26]

It is difficult to generalize about what types of exposure control strategies are appropriate for each individual company. Factors that influence selection of engineering controls and other exposure control strategies include the physical form of the nanomaterial, task duration, frequency, and quantity of ECN being handled. Nevertheless,

TABLE 4. Number, Proportion (95% Confidence Interval) of Companies Using Personal Protective Equipment for ECN

	n	Aprons	Tyvek Suits	Work Boots/Boot Covers	Safety Glasses	Gloves	Respirators
Manufacturing Type							
Production-based exposure controls	**8**	**3**, .38 (.14, .69)	**7**, .88 (.53, .98)	**3**, .38 (.14, .69)	**7**, .88 (.53, .98)	**8**, 1.0 (.68, 1.0)	**8**, 1.0 (.68, 1.0)
Laboratory-based exposure controls	**7**	**3**, .43 (.16, .75)	**1**, .14 (.03, .51)	**3**, .43 (.16, .75)	**7**, 1.0 (.65, 1.0)	**7**, 1.0 (.65, 1.0)	**6**, .86 (.49, .97)
Pilot and R&D scale operations	**15**	**8**, .53 (.3, .75)	**5**, .33 (.15, .58)	**6**, .4 (.2, .64)	**14**, .93 (.7, .99)	**14**, .93 (.7, .99)	**9**, .6 (.36, .8)
Total	**30**	**14**, .47 (.3, .64)	**13**, .43 (.27, .61)	**12**, .4 (.25, .58)	**28**, .93 (.79, .98)	**29**, .97 (.83, .99)	**23**, .77 (.59, .88)

Cells report number of companies (bold) as well as proportions. Corresponding 95% confidence intervals are represented in parentheses.

TABLE 5. Number, Proportion (95% Confidence Interval) of Companies Using Respirator for ECN

	n	Dust Mask	Filtering Facepiece	Half Face	Full Face	PAPR	Did Not Specify	None
Manufacturing Type								
Production-based exposure controls	**8**	**0**, 0.0 (0.0, .32)	**2**, .25 (.07, .59)	**5**, .63 (.31, .86)	**1**, .13 (.02, .47)	**2**, .25 (.07, .59)	**0**, 0.0 (0.0, .32)	**0**, 0.0 (0.0, .32)
Laboratory-based exposure controls	**7**	**1**, .14 (.03, .51)	**1**, .14 (.03, .51)	**4**, .57 (.25, .84)	**0**, 0.0 (0.0, .35)	**0**, 0.0 (0.0, .35)	**1**, .14 (.03, .51)	**1**, .14 (.03, .51)
Pilot and R&D scale operations	**15**	**1**, .07 (.01, .3)	**3**, .2 (.07, .45)	**4**, .27 (.11, .52)	**0**, 0.0 (0.0, .2)	**1**, .07 (.01, .3)	**2**, .13 (.04, .38)	**6**, .4 (.2, .64)
Total	**30**	**2**, .07 (.02, .21)	**6**, .2 (.1, .37)	**13**, .43 (.27, .61)	**1**, .03 (.01, .17)	**3**, .1 (.03, .26)	**3**, .1 (.03, .26)	**7**, .23 (.12, .41)

PAPR, powered air purifying respirator.
Cells report number of companies (bold) as well as proportions. Corresponding 95% confidence intervals are represented in parentheses.

given the limited information about the human health risks associated with occupational exposure to ECN, appropriate steps should be taken to minimize the risk of worker exposure through the implementation of risk management programs.[13,27] When controlling potential exposures within a workplace, NIOSH has recommended a hierarchical approach to reduce worker exposures.[28] The basis for the hierarchy of controls is to eliminate the hazard when possible by substituting it with a less hazardous material or, if not feasible, control the hazard at or as close to the source as possible through engineering controls. If those measures are not successful, then administrative controls and PPE, respectively, should be used as last efforts.

There were several limitations to this study that are worth mentioning. One limitation is the possibility of a selection bias, which could have occurred for the survey responses from participating companies. This bias could not be avoided because all contributing participants of the survey provided information on a voluntary basis. Companies that chose to participate might have been more aware of the health and safety issues with ECN. If this were true, it still provides some perspective into the differences between the various types of manufacturing groups because of the varying range of responses received regarding the exposure control strategies already in place across all three groups.

In addition, the survey was conducted through the months of October 2008 to May 2009 during a severe economic recession, which may have affected the participation rates of companies receiving the survey. It should also be noted that the number of companies were most likely underestimated because of the exclusion of repackagers, as well as bench scale research and development companies that did not express a plan to move to at least pilot scale in the next 5 years.

Also, there was no way to verify survey results from respondents. Nevertheless, given the assurance that data would be published only in aggregate form, there was little motivation for or any indication of dishonest responses, as company answers seemed generally consistent across the two groups of manufacturers and the R&D/pilot scale operations group as well. Still, it is unknown to what extent the reported engineering controls and PPE were adequately deployed within the work environment. Nevertheless, since this original survey, we have conducted several site visits at participating companies to assess possible exposures in the workplace to carbon nanotubes and nanofibers. This has allowed direct, visual confirmation of the reported survey results for the uses of exposure control strategies.

Although there have been several best practice guidelines for managing the risks of nanomaterials published, there are no widely accepted exposure limits for ECN, and there are no readily available and cost-effective instrumentation to assess workplace exposures. Much of this has to do with the diversity of ECNs being produced and their varying sizes, shapes, and compositions, which makes it difficult to develop any standard exposure limits. Another significant step to overcome is that the scientific community is still searching for the most relevant aspect of airborne nanomaterials that should be measured: number, surface area, mass concentration, or a combination of these.[29]

For the most part, this survey indicates that the current controls used are still relatively underdeveloped or in the process of being developed by some companies manufacturing or using ECN in the United States. This is likely because of the fact that organizations worldwide have not come to a consensus regarding the existence of risks or accepted exposure limits. This unique situation can make it difficult for industry to justify reducing exposures and thus might

slow the adoption and dissemination of best practice exposure control strategies. Nevertheless, until widely accepted exposure limits with validated air monitoring procedures become readily available, the general best practice guidelines provided by trusted organizations should be followed to control workplace exposures to ECN.

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Feasibility of Biomarker Studies for Engineered Nanoparticles

What Can Be Learned From Air Pollution Research

Ning Li, PhD and Andre E. Nel, MD, PhD

Objective: Occupational exposure to engineered nanoparticles (NP) may pose health risks to the workers. This article is to discuss the feasibility of identifying biomarkers that are associated with NP exposure. **Methods:** Scientific literature on the adverse health effects of ambient ultrafine particles (UFP) and NP was reviewed to discuss the feasibility of conducting biomarker studies to identify NP-induced early biological changes. **Results:** Various approaches for biomarker studies have been identified, including potential injury pathways that need to be considered and the methodologies that may be used for such studies. **Conclusions:** Although NP may have novel mechanisms of injury, much can be learned from our experience in studying UFP. Oxidative stress-related pathways can be an important consideration for identifying NP-associated biomarkers, and one of the most effective approaches for such studies may be proteome profiling. **Clinical Significance:** Biomarker studies will provide valuable information to identify early biological events associated with the adverse health effects of engineered nanomaterials before the manifestation of clinical outcomes. This is particularly important for the health surveillance of workers who may be at higher risk due to their occupational settings.

The introduction of nanotechnology has brought great benefits to a wide span of areas in today's society and will continue to do so in the future, but it also brings many unknowns about its potential adverse health effects. The specific physicochemical characteristics of engineered nanoparticles (NP) may introduce health risks, which differ significantly from fine particles of the same chemical composition.[1] Therefore, it is important to realize that certain groups of people, such as workers in nanotechnology-related fields, are at higher risk than the general population because of their close and constant contact with these materials and begin to take protective measures before an outbreak of serious clinical outcomes.

One of the strategies for preventing serious nanotoxicity from happening is to identify early biological events associated with exposure to harmful NP and then use that information for prevention. This can be achieved through biomarker studies in NP target organs/tissues or preferably in the biological fluid. While biomarker studies for NP toxicity are currently at their early stage, our experience in biomarker research for the incidental or ambient NP, aka, ultrafine particles (UFP), can be used to facilitate this process due to some similarities between UFP and certain NP. One of the injury mechanisms that are common to UFP and certain NP is the induction of oxidative stress and inflammatory responses by particles. In this communication, the feasibility of conducting NP-associated biomarker studies, based on what has been learned from air pollution research, will be discussed.

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IMPORTANCE OF BIOMARKER STUDIES FOR NANOPARTICLE-RELATED OCCUPATIONAL SAFETY

Nanoparticles are less than 100 nm in size and are intentionally produced with specific characteristics required for their applications. Because of their unique size and physicochemical properties, such as surface area, shape, crystallinity, surface charge, reactive surface groups, dissolution rate, state of agglomeration, or dispersal, etc, NP are potentially more dangerous than larger particles of the same composition and may cause unanticipated adverse health effects to people who are exposed to these particles.[1]

Nanoparticle exposure can take place in almost all economic sectors, but occupational exposure in research laboratories and industries that manufacture, handle, use, and dispose these particles place the workers at potentially higher risk.[2–4] Although there has been no report that link NP exposure to a definitive disease outcome, epidemiological studies have found hazardous respiratory effects through occupational exposure to carbon black and fumed silica.[5–7] Another example of potential occupational hazard is the exposure to metal or metal oxide NP.[8–10] Metal oxide NP are often used as industrial catalysts, and increased levels of these particles have been found in areas surrounding factories.[8] There has been reported incidence of bronchitis, metal-fume fever, changes in lung function, and increased lung infection among welders.[9,10] Metal-fume fever is a clinical syndrome that is presented as a flu-like illness characterized by self-limiting inflammation and oxidative stress response in the lung.[11] It has been suggested that this condition is caused by the inhalation of highly concentrated metal oxide particles, particularly zinc oxide (ZnO).[11–15] Given the growing use of NP and so many unknowns about their potential health effects, it is imperative to develop effective methods for assessing health risks associated with NP exposure. This is particularly important for the health surveillance and monitoring of workers who may be exposed to NP in the occupational setting.

Because of the short history of nanotechnology, currently, there is no published report that has established a definitive link between a disease outcome and exposure to a specific type of NP in humans. As it is almost certain that the growth of nanotechnology will outpace epidemiological studies, instead of waiting for these reports, an active approach would be to take precaution now so that the people at higher risk can be properly protected. One effective strategy to achieve this goal is to identify biomarkers associated with NP exposure. A "biomarker" is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."[16] Therefore, the role of biomarkers in assessing the health effects of NP is to link exposure to the disease outcomes by providing mechanistic indicators that are associated with early adverse effects of NP (Fig. 1). Although it is expected that it may take a long period of time to develop a panel of biomarkers that can be used as indicators of exposure-specific disease outcomes, identification of early biological responses related to injury pathways, based on our knowledge in air pollution research, would be a good starting point at this time.

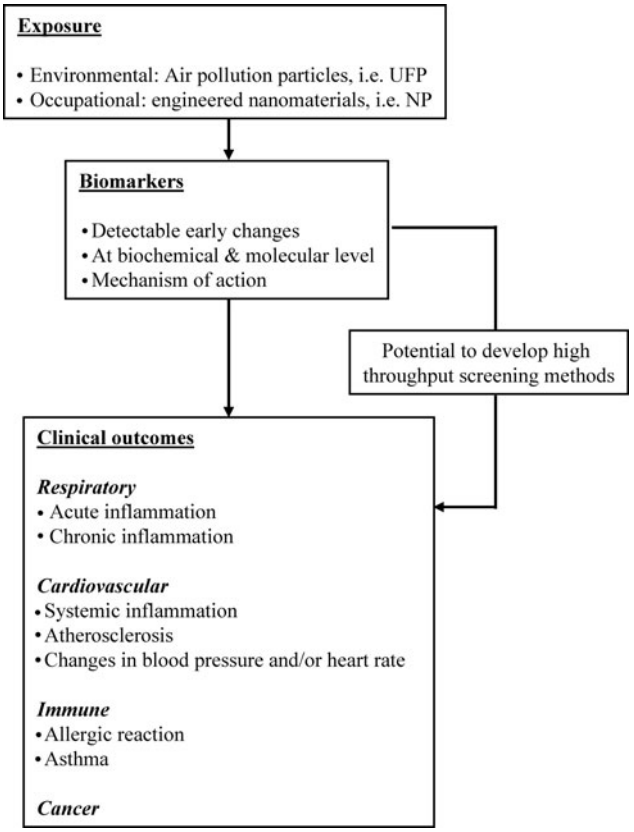

FIGURE 1. The role of biomarkers in assessing the adverse health effects of nanoparticles is to link particle exposure to the clinical disease outcomes. Biomarkers are the detectable characteristic changes that reflect exposure-associated early events usually at biochemical and molecular levels. Biomarker studies may provide mechanistic explanation to the adverse health effects of nanoparticles as well as the potential to develop high throughput screening methods for medical surveillance of workers who are exposed to nanoparticles in occupational settings.

OXIDATIVE STRESS-RELATED INFLAMMATION AS AN INJURY MECHANISM FOR THE TOXICITY OF ULTRAFINE PARTICLES AND CERTAIN NANOPARTICLES

In the last few years, in response to the rapid growth of nanotechnology, nanotoxicology has emerged as a unique field to study the toxicity of engineered nanomaterials (ENM), including NP, and to understand the injury mechanisms that are specifically related to the scale, dimension, and physicochemical characteristics of these materials.[17,18] The concept of nanotoxicology is basically evolved from air pollution research, especially that on the incidental NP (ie, UFP). Although there are significant differences between UFP and NP in many aspects of their characteristics, there are certain similarities in the mechanisms of action and potential to produce adverse health effects between these two types of nanoscaled particles (Table 1).[1,12,19]

For example, two mechanisms that are common to UFP and several types of NP are the induction of oxidative stress and inflammatory response.[1,12,19,20] That particle-induced oxidative stress is one of the major mechanisms for the adverse biological effects of UFP has been demonstrated in cellular, animal, and human studies.[19,21–33] Inhaled UFP are capable of inducing oxidative stress in the lung as well as in systemic circulation. Particle-generated reactive oxygen species (ROS) and subsequent oxidative stress have been shown to be involved in many pathological conditions associated with respiratory and cardiovascular disease outcomes, including lung inflammation, asthma exacerbation, atherosclerosis, and thrombosis.[19,30,32,34–38] Similarly, increasing evidence from cellular and animal studies has indicated that a number of NP also exert their proinflammatory and toxic effects through the same mechanisms.[39–42]

The prooxidative and proinflammatory properties have been observed in a number of metal oxide NP. Titanium dioxide (TiO_2) NP, which have a number of industrial applications, are capable of generating ROS, inhibiting reduced glutathione (GSH), activating several Nrf2-mediated antioxidant enzymes (ie, heme oxygenase-1, thioredoxin reductase, GSH tranferase, and catalase), and upregulating inflammatory cytokine gene expression in human airway epithelial cells (BEAS-2B) and in rats.[39,43–46] These prooxidative and proinflammatory effects of TiO_2 are correlated to particle size, surface area, and composition.[8,39,43,44,46–48] Copper oxide (CuO) NP that also have widespread applications have been shown to cause oxidative stress-mediated toxicity in a number of cultured cells.[8,49–51] Exposure of airway epithelial cells to CuO NP induced a significant increase in 8-isoprostanes and the ratio of oxidized to total GSH in these cells, which was accompanied by decreased viability; this prooxidative effect of CuO NP could be effectively inhibited by coexposure to antioxidant resveratrol.[8] Moreover, antioxidants, including N-acetyl cysteine (NAC) and catalase, could significantly attenuate the effect of CuO NP on the expression of plasminogen activator inhibitor-1, a protein involved in several cardiovascular diseases, in mouse pulmonary microvascular endothelial cells.[52] The ROS generation by ZnO NP, the NP that are considered responsible for the metal-fume fever, in mouse macrophage and human bronchial epithelial cells could lead to oxidant injury, inflammatory response, and cell death.[53] It has been suggested that the prooxidant activity of ZnO NP is the result of particle dissolution.[54] Prevention of ZnO NP dissolution through Fe doping could effectively reduce the prooxidative and proinflammatory effects of these particles.[54] In animal studies, long-term inhalation exposure to nickel hydroxide NP induced oxidative stress and inflammation in the lung and cardiovascular system in hyperlipidemic apoprotein E-deficient ($ApoE^{-/-}$) mice.[55] Intratracheal instillation of iron oxide NP in mice could lead to a significant decrease in GSH and an increase in proinflammatory cytokines in the bronchoalveolar lavage (BAL) fluid during acute response, while formation of microgranuloma, an indicator of chronic inflammation, was observed 28 days after exposure.[56] In addition to metal or metal oxide NP, other NP such as silica, cationic polystyrene, and C60 fullerene have also been reported to exert prooxidative and proinflammatory in vitro and in vivo, including increased ROS production, induction of oxidative stress, activation of antioxidant and signaling pathways, and apoptosis.[57–59]

It is necessary to point out that not all NP cause inflammation via a mechanism involving oxidative stress.[60,61] For example, it has been reported that while purified single-walled carbon nanotubes failed to generate ROS in cultured mouse macrophages, pharyngeal aspiration of these materials could induce progressive fibrosis and granuloma formation in mouse lung.[60] Recently, Crouzier et al[61] have demonstrated that intranasal instillation of purified double-walled carbon nanotubes elicited an inflammatory response in mice, which was accompanied by a decreased ROS production.[61] Nonetheless, currently available evidence suggests that there are quite a few types of NP that exert their adverse health effects through similar mechanisms as UFP, including generation of oxidative stress and induction of inflammation. Thus, it is not impossible to initiate biomarker studies for these NP by focusing on these two well-defined injury pathways.

TABLE 1. Comparison of Ultrafine and Nanoparticles

Particle Properties	Ultrafine Particles	Nanoparticles
Source	Incidental (eg, combustion)	Engineered (controlled synthesis)
Size	<100 nm	<100 nm
Surface area/volume	High	High
Uniformity	Low	High
Organic chemical content	High	Low
Metal content	High	High to low
Ability to generate reactive oxygen species	Strong	Varies
Other contributing factors to toxicity	Content of other chemicals	Surface area and shape
	Climate variation	Crystallinity
	Local traffic activity	Surface charge
		Reactive surface groups
		State of dissolution, aggregation, or dispersal
Exposure routes	Inhalation	Inhalation, skin, ingestion, medical use
Adverse health effects	Yes	Potential
Major risk factors	Distance to source	Occupational exposure (manufacture, handling, waste disposal,
	Time to commute	research laboratories)
		Commercial use

FEASIBILITY OF BIOMARKER STUDIES TO ASSESS NANOPARTICLE EXPOSURE-ASSOCIATED ADVERSE HEALTH EFFECTS

From the long history of air pollution research, various biomarkers linking air pollution exposure to its adverse effects in the respiratory and cardiovascular systems have been identified in human studies, and most of these biomarkers are associated with two major toxicological response pathways, oxidative stress and inflammation.[62,63] For example, increased 8-isoprostane and melondialdehyde in exhaled breath condensate have been reported as biomarkers for local oxidative stress in the lung, whereas systemic oxidative stress markers include alteration in the levels of antioxidant enzymes and GSH in the blood.[64–67] While increased levels of proinflammatory cytokines, cytokine receptors, and C-reactive protein have been considered the biomarkers associated with air pollution-induced systemic inflammation, platelet activation and increased expression of adhesion molecules have been identified as the biomarkers for the adverse cardiovascular effects of air pollution.[62,64,65,68] For the NP that share similar injury mechanisms (ie, oxidative stress and inflammation) with UFP, it is theoretically feasible to conduct biomarker studies starting with similar approaches. For example, to assess the early events associated with exposure to these NP, the choice of potential biomarkers to be studied can include the changes that indicate local and systemic oxidative stress, systemic inflammation, and inflammatory response in NP target organs, such as those in respiratory, cardiovascular, and immune system.

Currently, there is no report of any definitive human disease that is caused by NP exposure. Therefore, it would not be practical and efficient to begin NP-associated biomarker identification in human studies. A more effective strategy would be using the step-wise approach to evolve NP-associated biomarker identification from cellular to animal and eventually to human studies, the same approach that has been used for studying air pollution-associated biomarkers. The advantage of cellular studies is that they will allow us to rapidly identify NP-induced early changes at biochemical and molecular levels, which may not be detected as disease endpoints in animal or in human studies, but may provide valuable information about the mechanistic basis for disease outcomes and help to guide further studies. In addition, cellular studies may also provide great potential for developing high throughput screening methods to accelerate biomarker studies. As the second step of studying NP exposure-related biomarkers, animal studies can further validate the findings from in vitro studies and have the advantage of being more physiologically relevant to disease outcomes in humans. Finally, biomarkers identified by cellular and animal studies will be validated in human studies, which have the ability to directly demonstrate the "real-life" disease endpoints and to guide the development of surveillance strategies for the workers who are potentially at higher risk of exposure to the adverse health effects of NP.

USE OF PROTEOMICS TO IDENTIFY BIOMARKERS ASSOCIATED WITH NANOPARTICLE EXPOSURE

While many biomarker studies are still carried out by using traditional biochemical and immunological assay methods, the technologies of mass spectrometry, high throughput screening, cell- and tissue-based DNA microarrays, and proteomics have provided great potential to accelerate this process. Among these new techniques, use of proteomics has been shown to be an effective approach for studying biomarkers induced by air pollutants, including ambient UFP.[69–72] Proteomics uses high-throughput methodologies to study the complete profile of proteins in a given cell or tissue.[73] Its ability to analyze global cellular response has made it possible to identify potential biomarkers that are associated with exposures to various environmental stimuli and stress.[74] Thus, the discovery of new biomarkers by proteomics, combined with the traditional biological response endpoints, can become a powerful tool to assess the health effects and susceptibility factors related to environmental pollutants, including certain NP.[73]

Proteomics has been used as an analytical approach for identifying markers that are linked to exposures to environmental agents, as well as in disease conditions in both animals and humans, and proteome changes related to oxidative stress and inflammation have been identified under many of these conditions.[71,75–78] Our own experience of using proteomics to study the biological effects of particulate air pollutants has allowed us to develop a oxidative stress response model that may explain the adverse health effects of particulate matter in the respiratory, cardiovascular, and immune system and to identify potential biomarkers associated with the adjuvant effect of UFP on allergic airway inflammation.[71,77] Using this technology, we are able to study the biochemical and immunological

changes associated with exposure to incidental NP (ie, diesel exhaust particles [DEP] and UFP), focusing on oxidative stress and inflammatory response. We have demonstrated that organic DEP extract is capable of inducing stratified oxidative stress responses in mouse macrophages and human bronchial epithelial cells that include the activation of antioxidant and detoxification defense systems, inflammation, and toxicity in cultured cells.[71] This series of response is in parallel with a linear increase in newly expressed proteins measured by two-dimensional gel electrophoresis.[71] By liquid chromatography-tandem mass spectrometry analysis, more than 30 proteins were identified as responsive to DEP-induced oxidative stress, suggesting that some of these proteins may serve as markers for exposure to prooxidative DEP chemicals.[71,79] Other DEP-induced proteome changes include protein modification by nitrotyrosine, activation of the unfolding protein response, and increased expression of ATF4, an endoplasmic reticulum stress-associated transcription factor.[69,71] In animal studies, we are able to identify oxidative stress-induced proteome changes in the BAL fluid and lung tissue in mouse asthma models.[72,77] Our most recent study demonstrates that the expression of polymeric immunoglobulin receptor, complement C3, neutrophil gelatinase-associated lipocalin, chitinase 3-like protein 3 (Ym1), chitinase 3-like protein 4 (Ym2), and acidic mammalian chitinase (AMcase) in the lung is associated with the adjuvant effect of UFP on the primary immune response (allergic sensitization) and particulate oxidant potential.[29,77] Increased Ym1 expression is also associated with the boosting effect of UFP on the secondary immune response in the "real-life" inhalation exposure study conducted near downtown Los Angeles.[27] Moreover, our most recent study on NP has demonstrated oxidative stress-associated proteome changes in the BAL fluid from C57BL/6 mice that were exposed to ZnO NP via pharyngeal aspiration, suggesting that proteomics may also be used to identify biomarkers related to the exposure of certain NP (unpublished data). As it is evident that oxidative stress and inflammatory responses are also responsible for the toxicity of a number of NP, there is a great potential to use the technology of proteomics to identify the biomarkers associated with exposure to those NP that exert their adverse effects through these two injurious pathways.

In summary, the complicated physicochemical characteristics of ENM have brought an urgent need to study their potential adverse health effects, especially among workers who are exposed to these materials through daily work. While it will take a long period of time to link human disease outcomes to specific ENM exposures, we can take the advantage of our experience in air pollution research and available new technologies to study NP exposure-associated biological responses at biochemical, molecular, and cellular levels, a process known as biomarker studies. The ideal biomarkers for assessing environmental and occupational exposures should be able to provide strong mechanistic, molecular, or biochemical basis for the diseases, be exposure specific, reflect early adverse health effects, have clinical relevance, and easy to use. Although we are not able to identify the biomarkers that meet all these criteria at this time, it is feasible to study NP exposure-associated early biological events focusing on well-defined injury mechanisms such as oxidative stress and inflammation, which may be used as indicators of exposure to the hazardous NP.

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Identification of Systemic Markers from A Pulmonary Carbon Nanotube Exposure

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Objective: Interest exists for early monitoring of worker exposure to engineered nanomaterials. Here, we highlight quantitative systemic markers of early effects after carbon nanotube (CNT) exposure. **Methods:** Mice were exposed by pharyngeal aspiration to 40-μg CNT and harvested 24 hours, 7 days, and 28 days postexposure for measurements of whole blood, lung and extrapulmonary tissue gene expression, blood and bronchoalveolar lavage (BAL) differentials, and serum protein profiling. **Results:** Early effects included increased inflammatory blood gene expression and serum cytokines followed by an acute phase response (eg, CRP, SAA-1, SAP). Beyond 24 hours, there was a consistent increase in blood and BAL eosinophils. At 28 day, serum acute phase proteins with immune function including complement C3, apolipoproteins A-I and A-II, and α_2-macroglobulin were increased. **Conclusions:** Carbon nanotube exposure resulted in measurable systemic markers but lacked specificity to distinguish from other pulmonary exposures.

Inhalation of airborne particles results in adverse cardiovascular outcomes in humans. In fact, epidemiological data shows that increased cardiovascular morbidity and mortality correspond to high levels of airborne particulate matter (PM), and at-risk populations appear to be more susceptible to these effects.[1] In humans and animals, pulmonary exposure to PM results in increased atherosclerosis, impaired fibrinolysis, and reduced vascular function.[1] Evidence also suggests that the smaller the particle, from PM_{10} to $PM_{2.5}$ to PM less than 0.18 μm, the greater the cardiovascular risk.[1,2] Consequently, these findings have led to the assessment of cardiovascular effects of other inhaled particles, particularly nanoparticles.

Carbon nanotubes (CNT) are engineered nanomaterials. Because of their small size, large surface area, and high reactivity, CNT are hypothesized to potentially elicit systemic effects if inhaled. Studies have shown significant endpoint effects directly related to cardiovascular disease, including vascular oxidative stress, increased prothrombotic potential, and progression of atherosclerosis, occur after exposure.[3,4] Carbon nanotubes–related immune effects have also been described.[5,6] A key mechanism proposed to contribute to these observed downstream effects of CNT is the release of soluble mediators from the lung into the circulation.[6,7] To date, the pulmonary response to CNT is well described and is characterized by a granulomatous or interstitial fibrosis, dependent on the particle dispersion, inflammation, and biopersistence.[8–14] Therefore, the potential exists not only to measure markers of the lung response but also to identify those that could promote endpoint extrapulmonary effects.

Currently there is expanding interest, from the perspectives of occupational health surveillance and future epidemiological research, in early monitoring of worker exposure to engineered nanomaterials including CNT.[15,16] Recently, we showed that within 4 hours after a CNT exposure, systemic inflammation as indicated by whole blood cell gene expression occurred along with elevated inflammatory and procoagulant serum proteins.[7] A generalized stress response in various extrapulmonary tissues, including acute sensitivity in the aorta, was also found. The systemic markers measured directly reflected the ongoing lung response to CNT.[7] Here, we highlight quantitative systemic markers of early effects in mice from 4 hours to 28 days after a single CNT exposure. Results from NIOSH indicate that pulmonary and systemic responses are qualitatively similar in mice exposed to single-walled CNT (SWCNT) or multiwalled CNT (MWCNT). However, we observed that the MWCNT produced a greater magnitude of response than SWCNT at an equal mass dose.[7] Therefore, our studies focused primarily on MWCNT.

METHODS

C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) 10 weeks of age were used in this study. All mice were provided food (Teklad 7913) and tap water *ad libitum* in ventilated cages in a controlled humidity and temperature environment with a 12-hour light/dark cycle. Animal care and use procedures were conducted in accordance with the "PHS Policy on Human Care and Use of Laboratory Animals" and the "Guide for the Care and Use of Laboratory Animals" (NIH publication 86-23, 1996). These procedures were approved by the National Institute for Occupational Safety and Health Institutional Animal Care and Use Committee.

MWCNT used in this study were obtained from Mitsui & Company, courtesy of Dr Endo, Shinshu University, Japan (MWNT-7; average 49 nm in diameter and 3.9 μm in length; 0.27% iron). Comparative data for SWCNT (Carbon Nanotechnologies, Inc, Houston, TX; 1 nm in diameter and 0.1 to 1 μm in length; 8.8% iron), at 24 hours postexposure only, will be included. Dispersion of CNT with the vehicle dispersion media (DM; phosphate buffered saline with 0.6 mg/mL serum albumin and 0.01 mg/mL 1,2-dipalmitoyl-sn-glycero-3-phosphocholine) and characterization (degree of dispersion and distribution of MWCNT lengths and widths) were described previously.[7,12] Mice were exposed by pharyngeal aspiration[17] with 40 μg of CNT in a total of 50 μl, and blood and tissues were harvested at 24 hour, 7 days, and 28 days postexposure. In our ongoing studies, both male and female mice have been studied with no observable sex differences. For the illustration of systemic markers, the data presented here utilized male mice at 24 hour (DM, $n = 6$; SWCNT, $n = 5$; MWCNT, $n = 6$), 7 days (DM, $n = 6$; MWCNT, $n = 6$) and in reference to 4 hour[7] and female mice at 7 days

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(DM, $n = 6$; MWCNT, $n = 6$) and 28 days (DM, $n = 6$; MWCNT, $n = 6$) post-MWCNT exposure. Mice were sacrificed by carbon dioxide asphyxiation, and blood was collected for serum antigen analysis and whole blood messenger RNA expression. The lung, heart, aorta, and a consistent section of the liver were harvested and frozen in liquid nitrogen. All samples were stored at -80°C before analysis.

Measurements of blood and bronchoalveolar lavage (BAL) cell differentials by flow cytometry were done on separate groups of exposed mice (groups detailed in Results) by the following methods. Mice were sacrificed, and BAL was collected as previously described.[18] Differential counts of BAL cells were done as previously described[19] with the following modifications. The BAL cells were resuspended in 250 μL PBS, and 100 μL was added into a flow cytometry tube with 100 μL of 10% rat serum in FACS buffer for 10 minutes. Then, 50 μL of premixed antibodies in FACS buffer was added, and cells were stained for 30 minutes at room temperature with agitation. The mixture contained a final concentration of 5 μg/mL of the following antibodies: Fc block, Ly6G-FITC, Siglec-F-PE, CD45-PerCp, and CD11c-APC. All the antibodies were purchased from PharMingen (Becton Dickinson, San Diego, CA). The Caltag counting beads (PCB-100, Invitrogen, Carlsbad, CA) were added for cell enumeration before analysis in the FACSCalibur (BD Biosciences, San Diego, CA). Samples were acquired through a live gate without compensation. After collecting 4000 counting beads, the data of all cells were exported to the analysis software, FlowJo (Treestar, Costa Mesa, CA). The leukocytes were identified by cells that expressed CD45+. Neutrophils were defined as cells that expressed CD45+Ly6G+, eosinophils as CD45+Siglec-F+, and macrophages as CD45+CD11c+. For blood, collected in EDTA, 100 μL was added into a flow cytometry tube with 100 μL of 10% rat serum in FACS buffer for 10 minutes. Then, 50 μL of premixed antibodies in FACS buffer was added, and cells were stained for 30 minutes at room temperature with agitation. The mixture contained the following monoclonal antibodies in these final concentrations: MHC II-FITC (2.5 μg/mL, 2G9), Gr-1-APC (2 μg/mL, RBC-8C5), CCR3-PE (0.625 μg/mL, 83.101.111), CD3-Per-CP (10 μg/mL, 145–2C11), B220-Per-CP (2 μg/mL, RA3–6B2), and NK1.1-PE (2 μg/mL, PK136). All the antibodies were purchased from PharMingen (Becton Dickinson, San Diego, CA) except CCR-3, which was purchased from R&D Systems (Minneapolis, MN). To prevent nonspecific binding to Fc receptors, 2.4G2 blocking reagent (6 μg/mL) was added to the monoclonal antibody mix. Red blood cells were lysed with 100 μL of Caltag Cal-lyse lysing solution (GAS-010, Invitrogen, Carlsbad, CA) for 10 minutes in the dark followed by 1 mL of deionized water. The Caltag counting beads (PCB-100, Invitrogen, Carlsbad, CA) were added for cell enumeration before analysis in the FACSCalibur (BD Biosciences). Samples were acquired through a predefined gate in Cellquest, and the compensation was done afterward by FlowJo (Treestar, Costa Mesa, CA) analysis software. After collecting 3500 counting beads, the data of all cells were exported to FlowJo. The data were then analyzed according to the following gating strategy. First, leukocytes were separated by side scattering and forward scattering into three gates: lymphocytes, monocytes, and eosinophils plus neutrophils. Lymphocytes were identified by FSC/SSC and expression of CD3 or B220. B cells were distinguished from T cells by MHC-II expression in the lymphocyte gate. Eosinophils were defined as cells expressing the CCR3 receptor. Neutrophils were defined as those cells expressing the myeloid differentiation antigen Gr-1 and lacking CCR3. Monocytes were identified by FSC/SSC and expression of Gr-1.

Gene expression changes were measured as previously described utilizing the same custom designed TaqMan array profile (Supplemental Digital Content, Table S1, http://links.lww.com/JOM/A52).[7] Serum antigen measurements were determined by Rules Based Medicine (Austin, TX) using the multiplex immunoassay RodentMAP v2.0. Total plasminogen activator inhibitor 1 (PAI-1) levels were determined by ELISA (Molecular Innovations). For PAI-1, male C57BL/6J mice ($n = 6$ vehicle and $n = 6$ MWCNT) were sacrificed 24 hour postexposure, and blood was collected into 3.2% sodium citrate at a 9 to 1 ratio, respectively. After centrifugation at 1500 g for 12 minutes, plasma samples were collected and frozen for PAI-1 determination.

Proteomics and subsequent analysis were performed by Protea Biosciences (Morgantown, WV) utilizing Isobaric Tags for Relative and Absolute Quantitation technology. Given the volume required for the analysis, a pooled serum sample from the sham ($n = 6$) was compared with serum from MWCNT treated mice ($n = 6$). The P value is representative of the effect of contributing peptide ratios (treated/sham) for a specific protein. This method was chosen not only because of sample volume limitations but also as a pilot approach to initially find treatment effects.[20]

All data are presented as means ± standard errors. Analyses were performed using JMP Statistical Discovery Software. Serum protein analysis and quantitative real-time reverse transcriptase polymerase chain reaction confirmation of the Taqman arrays and any additional genes were analyzed by one-way analysis of variance generating a least squares mean table by Student t test. Analysis of Taqman arrays was done by Student t test comparing only control to treatment. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Previously, our laboratory reported that cytokines and chemokines involved in inflammation including IL-6, IL-5, CCL11, CCL22, and CXCL1 were elevated in the serum 4 hour after CNT exposure.[7] By 24 hours, these proteins returned to baseline and others were reduced compared to sham. At 24 hours, levels of acute phase proteins including C-reactive protein (CRP), haptoglobin, and serum amyloid P (SAP) were increased in the serum (Table 1). Further analysis showed significant elevations of serum amyloid A1 (SAA-1), SAP, and haptoglobin gene expression in the liver (Fig. 1),

TABLE 1. Serum Protein Analysis 24 Hour Post-CNT Exposure

	DM ($n = 5$)	SWCNT ($n = 4$)	MWCNT ($n = 5$)
C-Reactive protein (μg/mL)	6.24 ± 0.44	7.63 ± 0.60*	7.68 ± 0.17*
Haptoglobin (μg/mL)	41 ± 3	105 ± 36*	144 ± 15*
Serum amyloid P (μg/mL)	31 ± 2	41 ± 4*	43 ± 2*
Timp1 (ng/mL)	1.01 ± 0.05	1.89 ± 0.21*	2.73 ± 0.32**
CCL7 (MCP-3) (pg/mL)	151 ± 14	130 ± 8	98 ± 13*
CSF1 (macrophage) (ng/mL)	5.68 ± 0.28	5.41 ± 0.27	4.32 ± 0.18**
CCL2 (MCP-1)	64 ± 7	51 ± 4	44 ± 8
CXCL2 (MIP-2) (pg/mL)	20 ± 2	15 ± 1*	13 ± 2*
Lymphotactin (pg/mL)	129 ± 16	103 ± 2	59 ± 7**

CCL2 (MCP-1), chemokine (C-C) ligand 2 (monocyte chemotactic protein 1); CCL7 (MCP-3), chemokine (C-C) ligand 7 (monocyte chemotactic protein 3); CSF1, colony stimulating factor 1 (macrophage); CXCL2 (MIP-2), chemokine (C-X-C) ligand 2 (macrophage inflammatory protein 2); DM, dispersion media; MWCNT, multi-walled carbon nanotubes; SWCNT, single-walled carbon nanotubes; Timp1, tissue inhibitor of metalloproteinase 1.

*$P < 0.05$ vs DM

**$P < 0.05$ vs all groups.

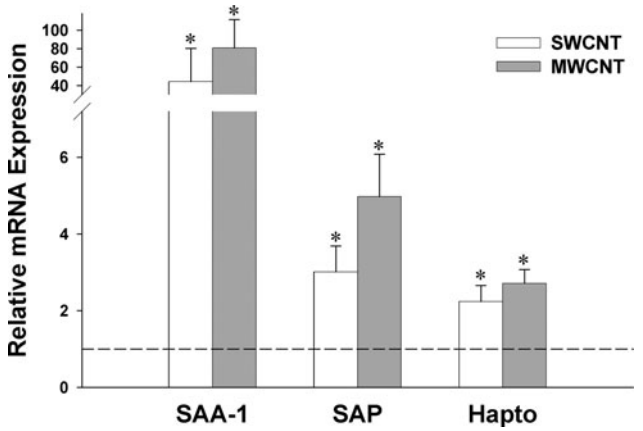

FIGURE 1. Effect of CNT exposure on liver gene expression of acute phase proteins 24 hours postexpsoure. Levels of mRNA are expressed relative to sham (arbitrarily set to 1.0 and indicated by dotted line) for SWCNT (open bars) and MWCNT (gray bars). *$P < 0.05$ versus sham. Abbreviations include serum amyloid A-1 (SAA-1), serum amyloid P (SAP), and haptoglobin (Hapto).

which confirmed an acute phase response. At 24 hours, proteins associated with activation and recruitment of macrophages such as CCL7 and colony stimulating factor 1 (CSF1—macrophage) and neutrophil and lymphocyte chemoattractants, CXCL2 and lymphotactin, respectively were reduced with MWCNT exposure (Table 1). Plasma levels of plasminogen activator inhibitor 1 (PAI-1), a procoagulant cardiovascular risk marker that inhibits plasminogen activator thereby reducing the conversion of plasminogen to plasmin and resultant fibrinolysis,[21] were shown to be elevated 4 hours post-CNT exposure[7] and remained increased at 24 hours (1.12 ± 0.06 ng/mL DM vs 1.74 ± 0.12 MWCNT; $P < 0.01$). Lung particulate exposure data have shown examples of both increased PAI-1 and reduced plasminogen activator systemically,[7,22–24] indicating that this pathway is acutely affected. At 4 hours postexposure, the ratio of matrix metalloproteinase 9 (MMP-9), an extracellular matrix remodeling protein, to tissue inhibitor of metalloproteinase 1 (TIMP-1) showed an increasing trend in the MWCNT-exposed mice (160 ± 29 DM; 104 ± 7 SWCNT; 268 ± 81 MWCNT) because of increased levels of MMP-9.[7] At 24 hour, MMP-9 levels had returned to control levels in the MWCNT group while TIMP-1, a primary inhibitor of MMP-9,[25] was elevated in both the SWCNT and MWCNT groups (Table 1), likely in a compensatory mechanism. This significantly reduced the ratio of MMP-9 to TIMP-1 (187 ± 43 DM; 99 ± 16* SWCNT; 50 ± 2* MWCNT; *$P < 0.05$). There was a significant time-dependent effect with respect to MMP-9, TIMP-1, and the ratio from 4 to 24 hours in mice exposed to MWCNT. Alterations in circulating levels of MMP-9, TIMP-1, and/or the MMP-9/TIMP-1 ratio are implicated in the pathogenesis of cardiovascular disease including left ventricular remodeling, atherosclerotic plaque stability, and inflammatory cytokine production.[26–31]

Utilizing a custom designed TaqMan array (Supplemental Digital Content, Table S1, http://links.lww.com/JOM/A52), aorta gene expression levels, elevated at 4 hours, were reduced or returned to baseline by 24 hours (Supplemental Digital Content, Table S2, http://links.lww.com/JOM/A52). Levels of metallothionein 1 (MT-1) and hypoxia inducible factor 3 alpha (Hif-3α) remained elevated at 24 hours after MWCNT exposure. TIMP-4 was increased at both 4 and 24 hours in SWCNT exposed mice (Supplemental Digital Content, Table S2, http://links.lww.com/JOM/A52). In the MWCNT groups, TIMP-4 showed further induction at 24 hours compared to 4 hours. Analysis of gene expression from the heart and liver at 24 hours also showed reduced levels for genes elevated at 4 hours (Supplemental Digital Content, Table S2, http://links.lww.com/JOM/A52).

Previous data from isolated whole blood cell RNA showed that at 4 hours after MWCNT exposure, several stress response and inflammation-related genes were increased.[7] We applied the same custom-designed TaqMan array and found that none of the ~100 genes tested were elevated at 24 hours (data not shown). Additional analysis of blood differentials was examined at all time points, and a consistent feature was an increase in eosinophils. This occurred after 24 hours lasting through 28 days and was most prominent at 3 to 7 days postexposure (Fig. 2). In the BAL, increased eosinophils were found at 24 hour (data not shown), which could explain the initial decline in blood eosinophils. Reflecting the consistent increase in blood eosinophils, BAL analysis by flow cytometry showed at 7 days eosinophils comprise 50% of the lavage cells by differential counts (Fig. 2). This was confirmed by manually counted cytospins, which also showed more than 50% of cells were eosinophils (data not shown). At 28 days post MWCNT exposure eosinophils in the BAL remained elevated, ~15 fold greater than sham mice (Fig. 2). Regarding other cell types, at 24 hours there was a significant decrease in total lymphocytes and monocytes that returned to sham levels by 3 days (Table 2). Blood neutrophils were increased in males at 4 hours,[7] 3 days and in 7 days females, but not in the other groups (Table 2), suggesting this measurement was not a consistent marker of exposure.

Comparison of gene expression changes in the lung between 4 and 24 hours post-CNT exposure is shown in Supplemental Digital Content, Table S3 (http://links.lww.com/JOM/A52). Significant inflammation was observed at 4 hours and was maintained through 24 hours with a greater response in MWCNT-exposed compared with SWCNT-exposed mice. Several genes related to macrophage function (eg, CCL2, osteopontin, and arginase I) were increased at 24 hours compared with 4 hours. Macrophage-dependent gene expression was more prominent at 7 days when compared with 4 hours and 28 days (data not shown). Lactate dehydrogenase (LDH) activity, a marker of cellular toxicity, was significantly increased by CNT exposure in a time dependent fashion (Supplemental Digital Content, Fig. S1, http://links.lww.com/JOM/A52).

By 28 days, primary inflammatory serum proteins, PAI-1, and blood gene expression returned to baseline levels (data not shown). Subsequent serum proteomic analysis showed increased levels of acute phase proteins associated with inflammation and the innate immune response such as complement C3 (C3), apolipoproteins A-1 and A-II, hemoglobin subunits alpha and beta-1, alpha-2-macroglobulin (A2M), serotransferrin, and liver carboxylesterase N (LCN) (Table 3). The same proteins were elevated following MWCNT exposure in a separate ongoing study, thus strengthening these initial observations (data not shown).

DISCUSSION

Rapidly following a pulmonary exposure to CNT, we found that the response of the lung was translocated to the periphery via the blood. This response was measured by increased inflammatory whole blood gene expression and increased circulating factors including primary cytokines, chemokines, and markers of coagulation. Many observed changes returned to baseline by 24 hours with a subsequent rise in systemic inflammatory markers, such as acute phase proteins. This also occurred in the extrapulmonary tissues, which showed an early stress response followed by a resolution. The presence of eosinophils was a consistent feature in the BAL and blood following exposure. Beyond the acute systemic inflammatory response, serum proteomics data revealed markers of an ongoing systemic inflammatory response related to an innate immune response 1 month after a single exposure. Taken together, our data suggest

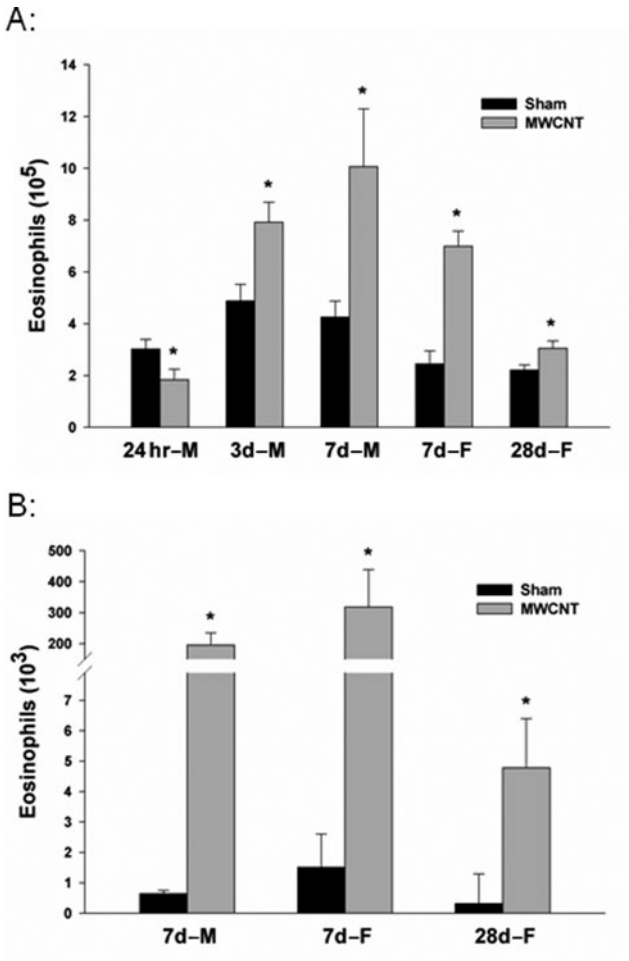

FIGURE 2. Effect of MWCNT exposure on blood and bronchoalveolar lavage eosinophils. (A) Effect of MWCNT on blood levels of eosinophils in sham (black bars) and MWCNT (gray bars) exposed mice. Abbreviations include male (M) and female (F). *$P < 0.05$ versus respective sham. (B) Effect of MWCNT on bronchoalveolar lavage levels of eosinophils in sham (black bars) and MWCNT (gray bars) exposed mice. Abbreviations include male (M) and female (F). *$P < 0.05$ versus respective sham.

that a systemic signature results from a single CNT exposure. The early effects we measured, however, were not unique in comparison to other exposures, such as PM.

To date, several studies have shown systemic endpoint effects following pulmonary CNT exposure. These effects include vascular oxidative stress, increased progression of atherosclerosis, enhanced prothrombotic potential, and immunosuppression.[3–6] These studies, along with the known pulmonary fibrotic and allergic effects,[8–14,32–34] could provide the ability to systemically monitor effects of CNT exposure. Our initial studies exposed mice to 40 μg of CNT. This is equivalent to approximately 4 months of exposure[12] utilizing peak measurements from a research laboratory of 400 $\mu g/m^3$;[35] thus, it was a high dose exposure, but representative of that currently used in the literature. Specifically, this dose was used as a positive control to verify the initiation of a systemic response and potential markers of exposure. Furthermore, because of the biopersistence of CNT, it cannot be assumed that lower doses over a longer period of time would not initiate similar responses.

Early effects of CNT exposure increased serum proteins of well-established markers of systemic inflammation and cardiovascular disease.[7] These included IL-6 with subsequent elevated levels of acute phase proteins, such as CRP and SAA-1. Although serum SAA-1 levels were not determined because of the lack of a specific SAA-1 ELISA, it was the most prominent of the measured acute phase genes expressed in the liver and, therefore, circulating levels were likely increased. The systemic inflammatory markers, although CNT-nonspecific because they are also increased after PM exposure,[1] could directly promote negative cardiovascular outcomes. For example, all of these markers are known to be associated with the development, progression, and/or stability of atherosclerotic plaques.[36] In addition, both vascular dysfunction and prothrombotic potential were eliminated following PM exposure in mice lacking IL-6.[37,38] C-reactive protein directly quenches nitric oxide thereby promoting vascular dysfunction.[39,40] Also, serum amyloid A induced endothelial dysfunction by increasing reactive oxygen species and decreasing endothelial nitric oxide synthase.[41] Therefore, the endpoint measurements of vascular oxidative stress, increased progression of atherosclerosis, and enhanced coagulation potential following CNT exposure could be proposed from the systemic inflammatory response markers.

A response of interest was the marked eosinophil influx into the lung. This response was predicted by increased markers of eosinophil recruitment and activation, which included IL-5 and CCL11 in the lung and serum.[7] Most particle exposures, such as PM, silica, and welding fume, induce a neutrophil-dominated lung response, although some studies have shown an increase in eosinophils.[42–46] Similar to CNT, asbestos exposure can induce a marked eosinophil response,[47,48] which may be the result of a similarity in physical properties. Recent studies have shown that eosinophils play an important role in the early development of allergic airway inflammation.[49] In addition, CNT pulmonary exposure enhanced an allergic inflammatory response.[32–34] Therefore, the data suggests that CNT exposure may not only exacerbate, but potentially induce allergic airway inflammation.

Within hours after CNT exposure, alterations in inflammatory blood gene expression were evident.[7] By 24 hours, blood gene expression changes from our panel had returned to baseline. This suggests a rapid and transient effect, but however, does not include changes that could have been discovered by global gene expression analysis. In parallel, a reduction of acute stress response genes seen in various extrapulmonary tissues was evident when comparing the response at 4 and 24 hours. Interestingly, we found increased TIMP-4 in the aortas of CNT-exposed mice at both 4 and 24 hours. TIMP-4, with suggested specificity to cardiovascular tissues,[50] was recently proposed as a systemic marker for vascular inflammation.[51] Cardiovascular disorders in both human and animal models including atherosclerosis, arterial balloon injury, and heart allograft rejection all showed increased TIMP-4.[50,51] Therefore, the early and sustained expression of TIMP-4 in the aorta following CNT exposure was likely a surrogate marker for a vascular inflammatory response.

In this study, we found a select group of acute phase proteins linked to activation of the immune response at 28 days after MWCNT exposure. With regard to biomedical applications, studies have shown direct complement activation by CNT.[52,53] Therefore, in the lung, CNT have the potential to directly activate complement in a similar manner especially if translocation occurs. Also, it is possible that the systemic inflammatory response was the result of increased C3 levels. While the mechanisms regarding changes in C3 should be explored, increased levels were found in the serum of individuals exposed to high levels of PM[54–56] and were associated with the development of diabetes and cardiovascular disease.[57–59] Furthermore, increased C3c, a marker of subclinical inflammation and a cleavage product resulting from activation of C3, was an independent predictor of PM associated risk of diabetes.[60] Apolipoproteins

TABLE 2. Blood Leukocyte Differentials Examined by Flow Cytometry After MWCNT Exposure

		Total Leukocytes (10^6)	Lymphocytes (10^6)	Monocytes (10^5)	Neutrophils (10^5)
Sham ($n = 8$)	24 hr—Male	11.83 ± 0.88	9.89 ± 0.77 (83%)	5.94 ± 0.44 (5%)	10.47 ± 0.99 (9%)
MWCNT ($n = 8$)		6.81 ± 0.35*	5.31 ± 0.28* (78%*)	2.50 ± 0.32* (4%*)	10.67 ± 1.57 (16%*)
Sham ($n = 4$)	3 d—Male	16.14 ± 1.45	13.97 ± 1.32 (86%)	8.81 ± 1.10 (6%)	7.93 ± 0.66 (5%)
MWCNT ($n = 4$)		13.18 ± 0.63	10.65 ± 0.53 (81%*)	7.24 ± 0.61 (5%)	10.14 ± 0.23* (8%*)
Sham ($n = 6$)	7 d—Male	12.25 ± 1.17	9.82 ± 1.02 (80%)	9.83 ± 1.15 (8%)	10.29 ± 1.03 (8%)
MWCNT ($n = 7$)		14.02 ± 2.14	10.77 ± 1.68 (77%*)	10.96 ± 1.33 (8%)	11.44 ± 1.60 (8%)
Sham ($n = 6$)	7 d—Female	12.45 ± 0.96	11.06 ± 0.92 (89%)	4.09 ± 0.41 (3%)	7.32 ± 0.59 (6%)
MWCNT ($n = 6$)		13.88 ± 0.43	11.68 ± 0.33 (84%*)	3.69 ± 0.29 (3%)	11.33 ± 1.47* (8%)
Sham ($n = 5$)	28 d—Female	10.54 ± 1.38	9.17 ± 1.22 (87%)	5.39 ± 0.68 (5%)	6.08 ± 1.13 (6%)
MWCNT ($n = 6$)		14.13 ± 2.80	12.18 ± 2.51 (86%)	6.71 ± 0.85 (5%)	9.73 ± 2.12 (7%)

MWCNT, multi-walled carbon nanotubes
Values are represented as total leukocyte numbers extrapolated from the concentration of counting beads. The differential, by percent, is shown in parenthesis.
*$P < 0.05$ vs respective sham.

TABLE 3. Summary of Serum Proteomics Results 28 Day Post-MWCNT Exposure

Protein	Ratio (MWCNT/DM)	*P*
Complement C3	1.50	<0.0001
Apolipoprotein A-I	1.29	<0.0001
Apolipoprotein A-II	1.23	0.0288
Hemoglobin subunit alpha	1.59	0.0117
Hemoglobin subunit beta-1	1.33	0.0033
Alpha-2-macroglobulin	1.22	0.0049
Serotransferrin	1.27	0.0338
Liver carboxylesterase N	1.28	0.0467

DM, dispersion media; MWCNT, multi-walled carbon nanotubes.

TABLE 4. Summary of Systemic Markers Following A Single MWCNT Exposure

	<24 hr	24 hr	>24 hr
Primary cytokines (eg, IL-6)	↑	↔ (↓)	↔
Inflammatory blood gene expression	↑	↔	↔
Coagulatory marker PAI-1	↑	↑	↔
Acute phase proteins (eg, CRP, SAA-1)	↔	↑	↔
BAL eosinophils	↔ (↑)	↑	↑
Blood eosinophils	↔	↓	↑
Acute phase proteins related to immune activation	nd	nd	↑

CRP, C-reactive protein; MWCNT, multi-walled carbon nanotubes; nd, no data; PAI-1, plasminogen activator inhibitor 1; SAA-1, serum amyloid A1.

A-I and A-II have anti-inflammatory actions on circulating leukocytes and protect endothelial cells lining the vascular wall from complement activation.[61–63] Also, if serum SAA-1 levels were increased, as predicted by liver gene expression, SAA-1 could displace apolipoproteins A-I and A-II creating an acute phase HDL resulting in a proatherogenic state.[64,65] The hemoglobin subunits were increased possibly as a reflection of the hemolytic activity of complement. Liver carboxylesterase N and A2M also have immune functions related to surfactant. Liver carboxylesterase N cleaves surfactant protein B converting more active large to less active small aggregate surfactant. This action is considered pathologic in acute inflammation.[66] Alpha-2-macroglobulin represents a conserved arm of the innate immune system that inactivates proteinases (eg, MMP-9) and decreases surfactant protein D degradation to increase innate immune function.[67,68] Lastly, transferrin has a well-characterized immune function of iron binding. Therefore, at 28 days postexposure, a group of acute phase proteins were increased that suggested immune activation.

In summary, exposure to CNT results in a measurable systemic inflammatory response. As summarized in Table 4, early effects include increased serum levels of primary cytokines and inflammatory gene expression in blood cells. This was followed by a reduction in the initial inflammatory markers and a predicted acute phase response. Beyond 24 hours postexposure, a consistent eosinophilic response as well as a series of proteins related to immune activation was evident. The markers correlated well with existing literature showing endpoint measurements of pulmonary CNT exposure mainly related to adverse cardiovascular effects. Of note is the general lack of specificity of the markers. Many of the markers (eg, IL-6, acute phase proteins, PAI-1) would not be easily separated from other pulmonary exposures. Therefore, additional studies are underway to determine the potential of a specific systemic signature of CNT exposure, which will aid in the early monitoring of human exposure.

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Workshop Summary: Epidemiologic Design Strategies for Studies of Nanomaterial Workers

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Objective: The potential health consequences of exposure to nanomaterials have yet to be elucidated though increasing evidence points to the potential for nanomaterials to cause adverse human health effects. This workshop addressed the feasibility of developing studies to measure health risks among nanomaterial workers. **Methods:** Breakout groups discussed different epidemiologic designs and methods to encourage companies to collect and retain exposure and health data. **Results:** Major challenges include defining and recruitment of appropriate study populations and obtaining adequate exposure data. Both prospective cohort studies and small cross-sectional panel studies utilizing biomarkers of exposure and effect offer approaches to study occupational groups. **Conclusions:** Potential exists to assemble cohorts to study the human health effects associated with nanomaterial exposure. Stakeholder partnerships are critical to the success of these studies and international partnerships hold great potential.

The potential adverse health consequences of exposure to engineered nanomaterials have yet to be elucidated. However, a growing body of toxicologic research suggests that exposure to some forms of engineered nanomaterials has the potential to cause serious adverse human health outcomes. Given the evidence to date, it is generally accepted that precautionary measures are important to prevent human exposure to nanoparticles. Despite this agreement, it remains unclear whether exposure to nanomaterials has in fact resulted or will result in any cases of human morbidity or mortality. Epidemiologic studies are needed to adequately address the extent to which exposure to nanomaterials is associated with adverse health outcomes and to quantify the risk of specific health outcomes in subsets of workers currently exposed to nanomaterials.

In July 2010, the National Institute for Occupational Safety and Health (NIOSH) and the Mountain and Plains Education and Research Center sponsored a conference on Nanomaterials and Worker Health: Medical Surveillance, Exposure Registries, and Epidemiologic Research. Following the panel of speakers describing epidemiologic design challenges, exposure assessment, use of biomarkers, and risk assessment for nanomaterials, approximately 120 attendees participated in breakout sessions to consider four key questions as follows:

1) Which epidemiologic design strategies are most promising?
2) How do we encourage companies to collect and retain the necessary information?
3) Are there international networks for research collaboration?
4) What are immediate opportunities for epidemiologic studies?

The ideas generated about these topics during the breakout session and group discussion are summarized below.

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EPIDEMIOLOGIC DESIGN STRATEGIES

No epidemiologic studies of workers exposed to engineered nanomaterials have yet been conducted, primarily because of feasibility issues such as access to exposed populations and exposure characterization.[1] However, the lessons learned from studies of other particulates (eg, asbestos, fine particulates in air) suggest that early attention to health effects in the context of epidemiologic studies should at the very least be considered.[2–6] Epidemiologic studies have the potential to be quite valuable in determining links between different types of occupational exposure to nanomaterials and the development of health problems. In addition, if properly designed, these studies could provide the ability to identify adverse health outcomes much earlier than if not conducted.

Several participants suggested that the time is right to begin cohort studies, while others suggested that at the present, small cross-sectional or panel studies might be more appropriate and feasible. These two approaches could be done separately or, combining these ideas, one could start by enumerating a cohort and then use smaller, transitional studies to validate biomarkers within the framework of the cohort study. Some participants suggested that, on the basis of animal toxicology data, studies of pulmonary fibrosis in the carbon nanotube (CNT) workforce might be most promising at this time. Others felt that studies of cardiovascular effects would be most informative.

Important challenges to designing a successful epidemiologic study of nanomaterial workers were identified by the participants and will be summarized in this article.

Challenges in Defining and Recruiting an Appropriate Study Population

A major challenge in conducting an epidemiologic investigation is finding a study population to assemble for a cohort study. The industry is not well defined and there are distinct challenges in identifying groups of individuals exposed to nanomaterials (both manufacturers and end users) particularly with respect to exposure to similar types of nanomaterials.

To adequately design and conduct a study of nanomaterial workers requires a working definition of "nanomaterials worker." Because the types of nanomaterials and their uses are so diverse, accurately understanding the lifecycle of the typical nanomaterial and tracking through how many workers' hands that material may have passed is difficult. It is likely that the initial epidemiologic studies will be unable to adequately define every worker who may have been exposed to a given nanomaterial throughout the lifecycle of that material or the significance of such exposure. Therefore, to the extent possible, initial studies should focus on workers who are aware they are handling "raw" nanomaterials. These would include production, research and development, and laboratory workers. Although there are strengths to restricting the study population to production workers, there are also limitations. In studies of exposure and disease, misclassification of exposure is a legitimate concern. Any comparative health effect could be obscured if a segment of the production workforce defined as exposed to nanoparticles was actually unexposed through the adequate use of engineering controls or personal protective equipment.

Traditionally, companies involved in manufacturing have constituted the ideal study population, as their workers' exposures (which are often monitored in the workplace) may be higher than those of other workers. Nevertheless, the numbers of employees involved in manufacturing may be insufficient for long-term follow-up studies. It was suggested that workers involved in nanoparticle research at federal laboratories (eg, department of energy, department of defense) could be important groups to study. The extensive health registries already existing for these federal employee cohorts and detailed information on coexisting exposures also make these populations attractive for study. University populations could also be a potential source for recruitment of occupational cohorts. Given the small numbers of workers in any given occupational site, novel techniques to recruit study participants may be required and could include the use of mass media to invite individual workers, or the recruitment of recent nanotechnology technician graduates from community colleges. Small companies are also quite ephemeral; startups will lose out to or be acquired by larger producers. Workers from small companies may move to larger companies that will be able to conduct medical surveillance programs and within which epidemiologic studies might be easier.

Once an occupational cohort is identified the numbers of individuals to be recruited will depend on the type of health endpoint to be examined. The minimum latency after exposure may be short for some diseases, such as the sarcoidosis observed in workers involved in cleanup after the World Trade Center disaster on September 11, 2001.[7] However, a worker registry consisting of thousands of workers who are followed-up repeatedly would likely be necessary to detect this type of endpoint. To obtain thousands of nanomaterial workers might take a 10- to 20-year timeframe, which would be a strong downside to this design. It was also noted that, in ambient air pollution studies, a long-term (eg, 20-year) follow-up study with thousands of subjects is typical for cardiovascular and respiratory diseases. Even given these limitations and the possibility that an assembled cohort will be insufficient to detect rare outcomes, it seems prudent, given the current uncertainties, to begin to assemble cohorts of workers in order to be able to adequately examine health outcomes in the future.

Challenges in Exposure Assessment

At present, measurement of nanoparticulates is difficult, and in many real-world settings the levels of exposure are likely transient or very low. Nevertheless, lack of ideal measures of exposure does not prevent the design and initiation of epidemiologic studies.

Methods for measuring nanomaterials exposure are rudimentary at present. Exposure concentrations are not known, and potentially important metrics such as surface area and size are difficult to measure using available equipment. Methods are also needed for personal exposure monitoring because area samples may not provide enough specificity or accuracy. For certain types of nanoparticles, such as CNTs, particle number by size may be an important metric, as it has been for coal dust and asbestos.

Ideally, exposures could be assessed over specified times (eg, 15 minutes, hour-long, 40-hour work week). One could use a combination of measures, particle counting, and subjective impression of work activities to classify activities at greater risk for exposure. This would allow the classification of workers into high, medium, and low categories. However, requiring this amount of data could unduly limit participation in a cohort study. Participants from different industries agreed that broad categories were reasonable at this point, and that methods of exposure assessment will probably improve with time. In a given work site, workers' job functions (eg, packaging, material harvesting, and clean-up) are starting points for classifying exposure. However, job classifications will not always determine exposure levels because there are human risk factors that confound that relationship. Still, uncertainty around exposure levels should not preclude the design and initiation of studies.

Novel technologies, such as use of access card records or global positioning system technology could allow researchers to combine the worker's distance from the source with an area sample to estimate exposure; however, such tracking may be unacceptable to the worker or infeasible in practice.

Challenges in Defining Appropriate Endpoints

A key feature of most epidemiologic studies is a clear statement and understanding of the disease or health outcome being assessed. For case-control designs this is through establishing a case definition. Similarly for cross-sectional studies, at least some *a priori* knowledge of the expected disease is required. It is currently unknown whether exposure to engineered nanoparticles increases one's risk for developing any health outcome. Nevertheless, evidence from animal studies indicate that it is biologically plausible. Also, important information can be gleaned from epidemiologic studies of air pollution and of workers exposed to welding fumes, diesel fumes, and ultrafine carbon particles. By analogy, these studies coupled with animal studies implicate respiratory and cardiovascular diseases outcomes as the most important endpoints of concern.

No specific biomarker of early effect for nanomaterials exists at this time, but workshop participants discussed several promising markers and agreed that additional studies should be conducted. In the case of CNT exposure, pulmonary endpoints (eg, early markers of pulmonary fibrosis or lung cancer) may be the most sensitive. Once workers are classified according to exposure, the development of interstitial lung disease should be monitored. Some participants felt that it will be difficult to determine the most useful biomarker until it is certain that there will be disease development in exposed populations. Nevertheless, others disagreed that knowledge of the disease endpoint was required prior to initiating studies of biomarkers of effect. Epidemiologic studies, including biomarkers, will help determine what diseases are of concern. Biomarkers used in epidemiologic studies (particularly for hypothesis-generating studies) would require less stringent standards for validation than those used clinically for disease screening but would ideally be affordable, accessible, and noninvasive.

There was substantial discussion of the need to incorporate markers of early cardiovascular effect, such as heart rate variability, reperfusion rate with blood pressure cuff, and markers of oxidative stress, given the substantial data from ultrafine particle exposures. The design of studies should incorporate the possibility that individuals with preexisting cardiac conditions may be more susceptible to the effects of nanoparticle exposure. In addition, the many confounding factors associated with cardiac endpoints would need to be accounted for in the design of epidemiologic studies.

Some other potentially useful markers of early effect identified by participants include exhaled breath condensate, serum markers of early interstitial disease, and high-resolution computed tomography or spiral computed tomography. Concerns identified with these biomarkers include lack of specificity, unclear clinical significance, potential harm from radiation exposure, and lack of clinical validation.

Summary: Epidemiologic Study Designs

The general conclusion from the participants was that prospective cohort studies may be useful, but for many nanomaterials, workforce size has not been determined and it is likely that for a given site, the numbers of individuals working with nanoparticles may still be quite small. In addition to cohort designs, cross-sectional or small-panel studies could be useful to detect early markers of disease from nanoparticle exposure and to provide preliminary evidence for larger hypothesis-testing studies. Smaller studies would permit the collection of more detailed information related to exposure, the

use of exposure controls, and personal protection. However, the limitations of a cross-sectional study design, including representativeness and inability to establish causality, are important to consider. With the uncertainty associated with accurate classification of exposure and the uncertainty related to what endpoint is most relevant to measure, it may be that cross-sectional studies are inherently more susceptible to spurious associations than other study designs.

Clearly, the gold standard epidemiologic study design would be a large prospective cohort. Nevertheless, the feasibility of such an endeavor is questionable and much discussion still needs to occur between interested stakeholders (eg, employer, worker, medical, academic, governmental, and international communities) to initiate such a process. One potential approach for amassing an adequately sized cohort would be for investigators to assemble smaller study cohorts in multiple locations, but to participate in common protocols determined by a consortium agreement that would include common laboratory and exposure determination methods. So while there are different design approaches that the scientific community could take to begin to provide empirical evidence of the risk of nanoparticle exposure to worker health, both long-term and short-term studies are necessary and it is important to begin these investigations.

STAKEHOLDER PARTNERSHIPS NECESSARY FOR OCCUPATIONAL STUDIES

As described earlier, epidemiologic studies of nanomaterials workers will be important in answering questions regarding the human health impacts of these exposures. Nevertheless, participants expressed concern that information needed for epidemiologic studies is not routinely being collected by employers. Barriers were identified, including concerns about sharing "business sensitive" information in the private sector. Incentives may be needed to encourage companies to assist in determining potential health effects associated with the materials their personnel are producing or using. It was also pointed out that most researchers do not have right of entry into companies; therefore, time and great effort will be required to build trust with the industry. There may also be concerns on the part of companies about potential legal ramifications of study findings. The cost of participating in a study is also a potential concern for industry, particularly for small manufacturers.

The participants identified several methods to increase the availability of employment data. One idea was to require any recipients of federal nanoparticle research and development funds to address this need in their applications for funding and to agree to participate in registries or health studies. It was expressed that government nanomaterial research entities should be at the forefront of this effort. Participants noted that department of energy and department of defense facilities already have worker registry systems. This may provide incentive to private industry if information could be pooled from multiple sources of both primary and secondary manufacturers. It was noted that department of defense, in particular, may have thousands of workers exposed to nanomaterials in grinding, sanding, and spray-painting operations.

Other possible avenues to encourage participation include working through insurance companies, which were not represented at this meeting. Insurance companies might be encouraged to turn down coverage for nanoparticle workers compensation claims if companies do not agree to participate in research on health effects. It was also suggested that companies participating in health studies be awarded some benefit (eg, reduced legal liability, tax incentive, or workers compensation relief). Analogy was made to the asbestos industry, which has seen many bankruptcies as a result of findings of adverse health effects from exposure.

Some of the information needed for occupational epidemiology studies consists of rosters of workers handling nanomaterials, along with their job histories. It was pointed out that US law requires companies that go out of business to offer their personnel and exposure records to National Institute for Occupational Safety and Health for future epidemiologic studies. This is important for the nanomaterials industry, which has a high attrition rate, but many nanotechnology companies may be unaware of this requirement. Effort should be made to inform these industries and solicit these records from expiring companies.

Participants advocated the need to engage industry about health, safety, and environmental aspects of nanotechnology and to encourage them to value a healthy workforce. The need for health studies should be put on the agenda of big industry conferences so that trade associations become aware and will put together programs on this topic. A partnership among all the stakeholders (government, employers, etc) was suggested as a key component in overall feasibility. These may be critical in developing a centralized registry, which could serve as the starting point for a cohort.

POTENTIAL INTERNATIONAL NETWORKS FOR RESEARCH COLLABORATION

It was pointed out by participants that international collaborations are already occurring on many aspects of nanotechnology; occupational health studies would be a valuable and natural addition. Possible populations or research opportunities could include those presented in Table 1.

It was recommended that participants in this conference work together with international contacts (eg, International Agency for Research on Cancer) and share information. Multinational companies may be a particularly good source population for epidemiologic studies. It was stated that many individual companies already require collaboration between factories. Working model consortia have been established in other industries (eg, cobalt, nickel) that could be models. It was pointed out that some countries (eg, France) require extensive documentation that may be useful for epidemiologic research. In the end, participants agreed that international cooperation was needed to combine resources and link together small studies.

IMMEDIATE OPPORTUNITIES FOR EPIDEMIOLOGIC STUDIES

Upon discussing the challenges and barriers to conducting epidemiologic studies (outlined earlier) the session participants considered what immediate opportunities for epidemiologic studies were

TABLE 1. Potential Populations or Research Opportunities to Target for Occupational Health Studies of Nanomaterial Workers

A proposed NIOSH study of all US carbon nanotube producers and users
Registries of nanomaterials users at US Department of Energy facilities
International studies of nanoparticle exposures among military workers in Taiwan
Identification (and possible epidemiologic study) of nanotechnology workers in France
Other medical monitoring and biomarker work being conducted in Taiwan, Singapore, and elsewhere in Southeast Asia
The United Kingdom Nanotechnology Safety Forum is considering studies
Studies of nanomaterials workers in Scotland
The REACH program, a new European Community Regulation on chemicals and their safe use, may require health-effects studies for nanomaterial producers or users in Europe[8]
The NanoimpactNet, a multidisciplinary European network on the health and environmental impact of nanomaterials is consolidating information on health effects and exposure, which could serve as a collaborative platform

most reasonable. Although broad consensus was not reached on specific actions or study designs, consensus was reached with regard to one point-–something should be done. Although the limitations of all the study designs discussed were highlighted throughout the session, the overriding conclusion was summed up by invoking Voltaire's "let not the perfect be the enemy of the good." Session participants were in agreement that a proactive approach was necessary and that it would be ill-advised to not pursue epidemiologic research of some form among nanomaterial workers.

However, because of the many unknowns that exist, the question of what can be done immediately is not easily answered. Some favored small incremental approaches while others advocated large-scale international cohort studies. The session concluded with Dr Paul Schulte[1] of National Institute for Occupational Safety and Health proposing a specific nested hybrid design that could potentially allow for a number of different study designs to occur simultaneously as part of a larger cohort or registry.

In general, given a strong enough exposure/disease relationship and/or adequate sample size, an observational cohort design would not necessarily require *a priori* knowledge of a specific disease endpoint if a population of exposed individuals could be adequately identified and observed over time. In addition, a large observational cohort design would not preclude sampling within the cohort or recruiting new worker populations to conduct panel studies, case-control studies, cross-sectional studies, or studies focusing on biomarkers.

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Carbon Nanotube Risk Assessment

Implications for Exposure and Medical Monitoring

Eileen D. Kuempel, PhD

Objective: Quantitative risk estimates using toxicology data provide information for risk management to protect workers with potential exposure to carbon nanotubes (CNTs). **Methods:** Dose–response data from subchronic inhalation studies in rats were used in benchmark dose modeling. Dose was airborne mass concentration of multiwalled CNTs. Responses included pulmonary inflammation, lipoproteinosis, and fibrosis. **Results:** Estimated human-equivalent concentrations to the rat lowest observed adverse effect levels were similar to some workplace airborne concentrations of CNTs. Working lifetime risk estimates of early-stage adverse lung effects were more than 10% at the limit of quantification (7 $\mu g/m^3$) of the National Institute for Occupational Safety and Health analytical method for measuring CNT airborne concentrations. **Conclusions:** Exposure monitoring and control are the primary occupational health measures to protect workers from potential exposure to CNT. Medical monitoring for early detection of occupational respiratory diseases may also be warranted.

Although carbon nanotubes (CNTs) may be thought of as a recent discovery, the first images of CNTs were apparently first published in Russia in the early 1950s.[1] In the 1990s, enhanced production methods were developed,[2] enabling commercial production and interest in applications of CNTs. CNT structures consists of single or multiple graphene sheets, resulting in single-wall or multiwall CNTs (SWCNTs and MWCNTs, respectively). The diameter of individual SWCNT is approximately 1 nm and the diameter of individual MWCNT is approximately 2 to 100 nm. Both SWCNTs and MWCNTs tend to form agglomerated structures of up to several micrometers in diameter. The length of individual CNT can be a few micrometers up to several millimeters. Nanotubes have been constructed with length-to-diameter ratio of 132 million,[3] substantially greater than any other material. There are many variations of CNTs including different metal content. CNTs are of high commercial interest due to their unique properties. CNTs are several times stronger than steel at the same weight; and they provide excellent thermal and electrical conductivity. CNTs are used in composites, aerospace, electronics, and energy applications. Production volumes are anticipated to increase,[4] and consequently the number of workers with potential exposure to CNTs is also likely to increase.

National Institute for Occupational Safety and Health (NIOSH) is a leading institute in assessing workplace hazards including that from CNTs. NIOSH is authorized by the Occupational Safety and Health Act of 1970 to develop recommended occupational safety and health standards.[5] NIOSH conducts toxicological research, risk assessment, exposure assessment, and health surveillance, and develops criteria for recommended standards. These recommended standards are formally transmitted to Occupational Safety and Health Agency, which is the agency responsible for promulgating occupational safety and health regulations in the United States. The risk assessment process provides input to developing occupational safety and health, including occupational exposure limits (OELs).

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METHODS

Critical Dose Estimation

Two examples of using toxicological data from animal studies in risk assessment are illustrated in this article, which involve estimating a critical dose of either (1) a lowest observed adverse effect level (LOAEL) or (2) a benchmark dose (BMD). The LOAEL approach is used here to estimate equivalent exposures in workers to those associated with adverse effects observed in animal studies. This approach provides estimates of the level of exposure that indicates potential adverse effects in humans. Comparison with occupational exposure data provides information on whether the potential exposure in workers may be sufficient to indicate the need for medical monitoring.

The identification of a LOAEL or NOAEL (no observed adverse effect level) is dependent on the probability of detecting an effect, which depends on the sample size (number of individuals), sensitivity of the analytical method, and the probability of disease (which depends on dose and potency). A *LOAEL* is the lowest dose associated with a statistically significant increase in an adverse response in an exposed group. A LOAEL depends on the dose spacing in the experiment and the number of animals. An adverse effect is often (but not always) nonreversible and associated with a functional impairment or development of a chronic adverse health outcome. The effect concentrations (eg, LOAELs) in subchronic (13-week) studies tend to be higher than those in chronic studies.[6] In the analyses shown here, the subchronic effect concentrations are converted to the estimated equivalent lung doses, accounting for duration of exposure, so may better estimate the chronic effects than did concentrations without consideration of duration as reported by Kalberlah et al.[6]

BMD methods are used to estimate doses associated with specified risk (eg, 10%) and to provide a standardized method of estimating a point of departure for extrapolation to lower risk levels (which may be acceptable or feasible), and to estimate exposure limits for up to a full working lifetime. A benchmark response (BMR) is an adverse effect level (eg, 10%) that is considered biologically and statistically significant, and which may include early subclinical effects linked to increased risk of developing chronic adverse effects and disease. A BMD is the dose associated with a specified level of risk of the BMR. Thus, although the response endpoint associated with a LOAEL may be qualitatively similar to a BMR, a BMR is linked to a risk estimate, and is less dependent on the dose spacing and the slope of the dose–response relationship. BMD methods can be used in quantitative risk assessment, which is defined as an estimation of the severity and likelihood of an adverse effect associated with exposure to a hazardous agent.[7,8]

The steps in estimating occupational risks from animal dose–response data are illustrated in Fig. 1. For occupational aerosols, such

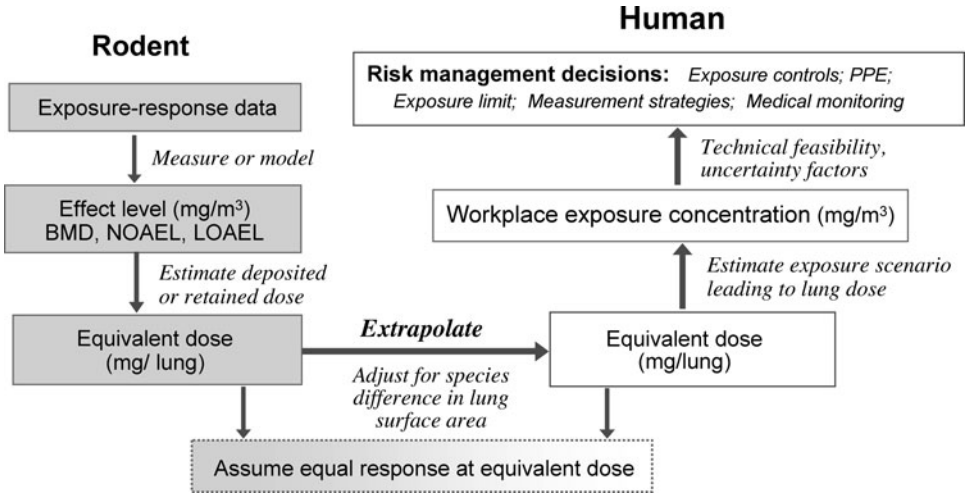

FIGURE 1. Risk assessment methods using animal data of airborne particles, for example, carbon nanotubes. BMD, benchmark dose; LOAEL, lowest observed adverse effect level; NOAEL, no observed adverse effect level; PPE, personal protective equipment.

as airborne CNTs, the animal dose–response data are extrapolated to predict risk in workers if exposed up to a full (45-year) working lifetime. This requires estimation of the human lung dose corresponding to a critical (adverse) effect (BMR or LOAEL response) or absence of effect (NOAEL) in the animal. The animal lung dose (measured or estimated) is extrapolated to humans using data on factors that influence species-specific lung dose (particle size-specific regional deposition in the lungs, breathing rates, exposure scenario). In the absence of other data, it is assumed that, at an equivalent dose, the human and animal response is equal. The workplace exposure scenario (concentration and duration) that would result in the human-equivalent lung dose is estimated using a human lung dosimetry model. Currently, these models have not been evaluated for CNTs. Nevertheless, according to aerosol physics principles, for particles larger than approximately 500 nm in diameter, the aerodynamic diameter (which accounts for inertial behavior regardless of density and shape) accurately predicts the particle deposition efficiency in the respiratory tract regions.[9,10] Although individual CNTs have diameters from 1 to 10s of nanometers, the airborne CNT structures are often large heterogeneous agglomerates made up of individual CNTs. The physical size of these airborne CNT agglomerates are typically in the micrometer size range. For example, the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of the aerosolized CNTs in the Ma-Hock et al[11] study were approximately 1.2 and 2.7, respectively, and in the Pauluhn[12] study were approximately 2.74 and 2.11, respectively. Furthermore the aspect ratio, based on the overall envelop size of the CNT structures, is typically less than 10.[13] Therefore, it is reasonable to assume that the spherical particle-based lung deposition models, though not yet validated for CNT, should provide reasonably accurate estimates of their deposition efficiency. The lung clearance model predictions may be more uncertain, however, given that CNT clearance has been shown to be slower than expected for a given mass of poorly soluble low-toxicity spherical particles.[12,14]

The estimated equivalent workplace exposure concentration (to that associated with a critical effect in the animal studies) is used to develop OELs and to develop other risk management strategies such as engineering control requirements, use of personal protective equipment, and need for medical monitoring. For example, if workplace exposures are demonstrated to be considerably below the health-based OEL, then the engineering controls typically would be considered to be effective, and periodic exposure monitoring may be sufficient to verify the continued effectiveness of controls. However, if exposures are above the OEL, then additional measures are clearly needed to reduce exposures including improved engineering controls and interim use of respirators (until exposures are demonstrated to be maintained below OELs). In addition, medical monitoring may be indicated for early detection of any adverse effects from exposure. Medical monitoring decisions depend on many factors, including the availability of appropriate medical tests, the potential for accidental exposures (even in a well-controlled workplace), and the concerns of workers.[15]

Animal Data

Risk assessment methods are illustrated using two recent subchronic (13-week) inhalation studies of MWCNTs in rats.[11,12] The exposure concentrations in Ma-Hock et al[11] were 0, 0.1, 0.5, and 2.5 mg/m^3; the LOAEL (as reported by the authors) was 0.1 mg/m^3 for granulomatous inflammation, of which 30% of rats had developed a minimal or higher grade based on histopathology. At 0.5 mg/m^3, 85% of the rats had developed lipoproteinosis (0% at 0.1 mg/m^3). The exposure concentrations in Pauluhn[12] were 0, 0.1, 0.45, 1.62, and 5.98 mg/m^3. A NOAEL was identified at 0.1 mg/m^3 and the LOAEL was 0.4 mg/m^3 for pulmonary inflammation (based on elevated polymorphonuclear leukocytes in bronchioalveolar lavage fluid) and alveolar interstitial thickening (a measure of pulmonary fibrosis) of which 90% of rats had developed a minimal or higher grade based on histopathology. Although alveolar interstitial thickening was not evaluated in the Ma-Hock et al[11] study, the findings of granulomatous inflammation and lipoproteinosis are consistent with the development of pulmonary fibrosis (silicosis) in rodents and humans from exposure to respirable crystalline silica.[16–18]

Concerning the severity of biological response, it is useful to evaluate where along the biological continuum from exposure to disease[19] would these subchronic responses in rats (or human equivalent) lie. Pauluhn[12] showed the persistence of alveolar interstitial thickening at 26 weeks after the end of the 13-week exposure (ie, at week 39). On the contrary, these effects are relatively early-stage (minimal or mild fibrosis in Pauluhn[12] and Ma-Hock et al[11] studies, and there has not been an evaluation of whether these effects are associated with functional impairment in the animals or would be clinically significant in humans. The rat exposures were only for 13 weeks and there is uncertainty about the chronic consequence of these persistent effects. Nevertheless, alveolar interstitial

thickening observed in animal studies has been considered relevant to humans and to indicate "fundamental structural remodeling."[20,21] Thus, these effects observed in the rat subchronic studies appear to be early biological effects that could result in altered structure and function.[19]

Risk Assessment Examples

The following examples of risk estimation methods are intended to describe simple, data-based estimates using minimal assumptions.

Example 1: Human-Equivalent LOAEL

The purpose of this exercise is to estimate the exposures in workers that are equivalent to the rat subchronic LOAELs, using data from the Ma-Hock et al[11] study as an example.

The first step is to estimate, as follows, the deposited lung dose of MWCNTs in rats at the end of the 13-week exposure:

$$\text{airborne concentration} \times \text{duration} \times \text{ventilation rate} \times \text{deposition fraction} = \text{deposited dose} \quad [1]$$

eg, 0.1 mg/m^3 × (6 hr/d × 5 d/wk × 13 wk) × 0.013 m^3/hr × 0.072 = 0.035 mg/rat lung, where the ventilation rate in the rat is 0.21 L/min × 0.001 m^3/L × 60 min/hr. The ventilation rate is based on species and body weight,[22,23] assuming 300 g average body weight for male and female rats in Ma-Hock et al.[11] The deposition fraction was estimated on the basis of particle size distribution (MMAD and GSD) in the Multiple-path Particle Dosimetry (MPPD) 2.0, lung dosimetry model, assuming unit density (to be consistent with the definition of aerodynamic diameter).[24] [Note: These same calculations were applied to the Pauluhn,[12] except using values of 0.015 m^3/hr (ventilation rate for rat of body weight 369 g at 0.25 L/min) and 0.046 deposition fraction based on the reported MMAD and GSD. In addition, these MPPD 2.0 estimates of MWCNTs retained in rat lungs after 13 weeks of inhalation exposure were found to be fairly similar (about 15% to 40% higher) than those approximated from a graph of the measured matrix-bound cobalt that was retained in rat lungs[12]].

The next step, as follows, is to extrapolate the rat lung dose (Equation 1) to humans:

$$\text{human lung dose} = \text{rat lung dose} \times \text{human/rat alveolar surface area } (102\ \text{m}^2/0.4\ \text{m}^2) = 9.0\ \text{mg in human lungs}, \quad [2]$$

where average human and rat alveolar epithelial surface area estimates are from morphometric analyses[25] (although estimates vary for the average adult human alveolar surface area, for example, US Environmental Protection Agency[22] cites 54 m^2). Normalizing on surface area of the respiratory tract region(s) is typically used for insoluble particles, which deposit and clear along the respiratory tract surface.[22] In this case, the alveolar surface area is used because it is a primary site of respirable particle deposition and also the target tissue for development of pulmonary fibrosis.

Finally, the workplace exposure scenario that would result in the human-equivalent lung dose is estimated. In this example, the occupational duration approximately equivalent to a 13-week exposure in animals is used in estimating the human-equivalent exposure scenario to that associated with the rat LOAEL. That is, 13 week is to 104 week (2-year chronic bioassay in rats) as 5.6 years is to a 45-year working lifetime (given that an animal chronic bioassay is typically assumed in occupational risk assessment to be equivalent to a 45-year working lifetime). The estimated human 8-hour time-weighted average (TWA) concentration over 5.6 years that would result in the human-equivalent lung dose in the pulmonary (alveolar) region is then calculated as follows:

$$\text{human-equivalent lung burden (mg)}/[\text{air intake} \times \text{exposure} \times \text{deposition fraction}] = 9.0\ \text{mg}/[9.6\ (\text{m}^3/\text{d}) \times (5\ \text{d/wk} \times 50\ \text{wk/yr} \times 5.6\ \text{yr}) \times 0.099] = [0.00676\ \text{mg/m}^3 \quad [3]$$

where the human-equivalent lung burden is from (Equation 2); the air intake is for the reference worker;[26] and the alveolar deposition fraction is based on the MMAD (GSD) as in (Equation 1), estimated in MPPD 2.0 (Yeh and Schum deposition model).[24]

Thus, exposure to 6.8 μg/m^3 (as 8-hour TWA concentration in air) for a duration of 5.6 years is estimated to be equivalent to a subchronic (13-week) LOAEL of 0.1 mg/m^3 in rats (for granulomatous inflammation of minimal or greater severity) in Ma-Hock et al,[11] based on the estimated deposited lung dose. Using this same approach, the human-equivalent concentrations to the other LOAEL responses were estimated at approximately 6 to 35 μg/m^3 (Table 1). Concerning potential workplace exposures, it is relevant to note that the limit of quantification of the method used to measure airborne CNTs is 7 μg/m^3 (as an 8-hour TWA airborne concentration).[27] This finding indicates a critical need to develop more sensitive methods for measuring workplace airborne exposures to CNTs.

Example 2: BMD Estimation

The subchronic inhalation data in rats[11,12] are used to illustrate BMD modeling and estimation of working lifetime risk estimates. Figures 2 and 3 show the fit of a multistage (polynomial degree 2) model to the rat exposure-response data to estimate a 10% excess (added) risk of the BMR (granulomatous inflammation or pulmonary fibrosis of minimal or higher grade). The multistage model was the only one of the BMD software dose–response models[28] that converged and provided adequate fit ($P > 0.1$ in a goodness of fit test)[29] to these sparse data (which have only one dose each between 0% and 100% response) (Figs. 2 and 3).

In this example, the BMD is an exposure concentration (also known as benchmark concentration) because it is based on exposure data rather than lung dose data. The BMD is the maximum likelihood estimate, and the BMDL is the lower 95% confidence limit estimate.

TABLE 1. Human-Equivalent Estimated Concentrations to Effect Levels Observed in Rat Subchronic Inhalation Studies of MWCNTs

Compound and Study	Adverse Effect	Rat Effect Concentration (mg/m^3)	Human- Equivalent 8-hr TWA Concentration in 5.6 yrs (μg/m^3)
MWCNT 9.6% Al_2O_3, 0.5% Co (Ma-Hock et al[11])	Granulomatous inflammation	0.1 LOAEL	6.8
	Lipoproteinosis	0.5 LOAEL	35
MWCNT 0.5% Co (Pauluhn[12])	Pulmonary inflammation	0.1 NOAEL	5.9
	Alveolar interstitial thickening	0.45 LOAEL	27

LOAEL, lowest observed adverse effect level; MWCNT, multiwalled carbon nanotube; NOAEL, no observed adverse effect level; TWA, time-weighted average.

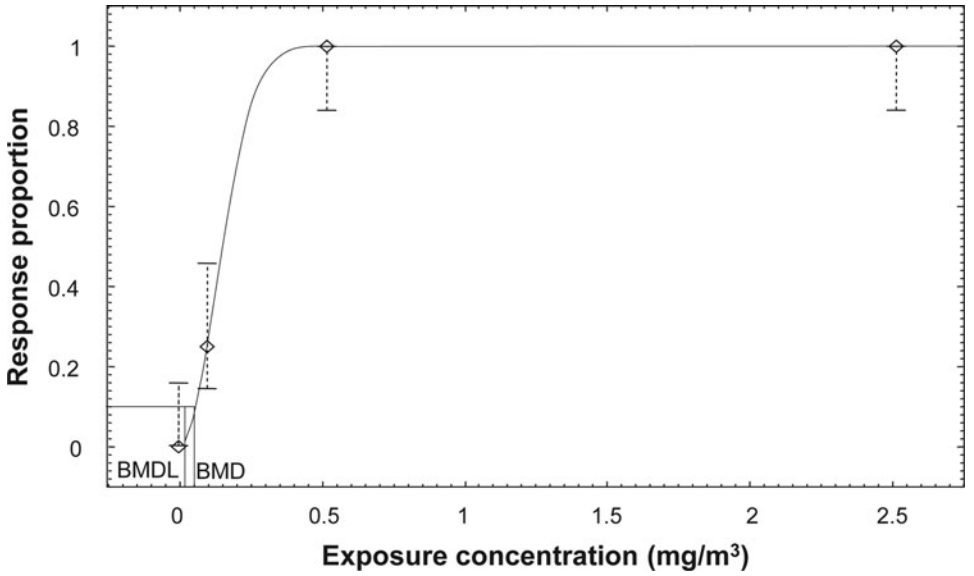

FIGURE 2. BMD estimation—granulomatous inflammation in rats.[11] Multistage model, polynomial degree 2; $P = 0.99$. BMD(L), 10% excess risk = 0.06 (0.02) mg/m^3. BMD, benchmark dose maximum likelihood estimate; BMDL, BMD lower 95% confidence limit.

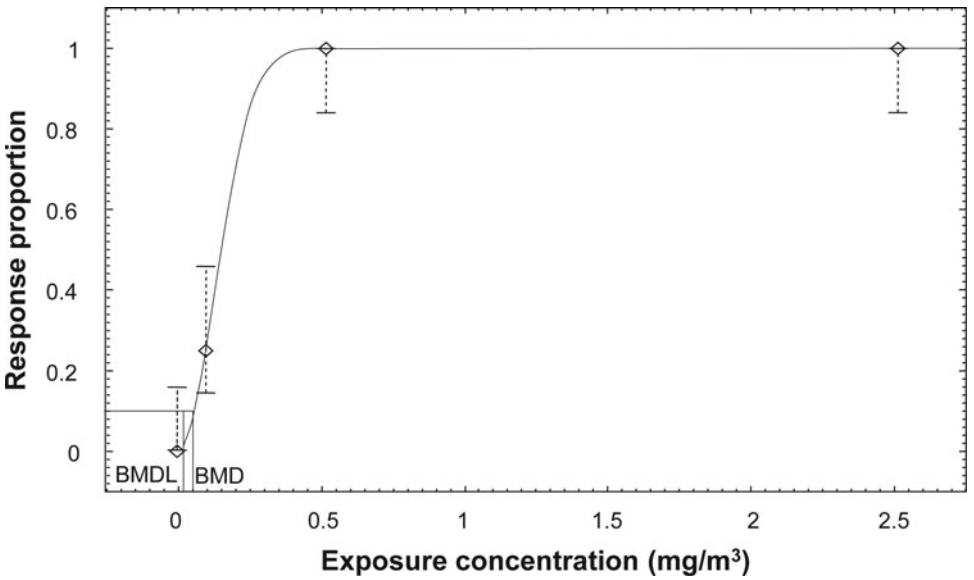

FIGURE 3. BMD estimation—alveolar interstitial thickening in rats.[12] Multistage model, polynomial degree 2, $P = 0.88$. BMD(L), 10% excess risk = 0.1 (0.05) mg/m^3. BMD, benchmark dose maximum likelihood estimate; BMDL, BMD lower 95% confidence limit.

BMD(L) is used here to indicate both the BMD and the BMDL estimates. The BMD(L) estimates corresponding to a 10% BMR are 0.06 (0.02) mg/m^3 in Ma-Hock et al[11] and 0.1 (0.05) mg/m^3 in Pauluhn[12] (Figs. 2 and 3). Because these dose–response data are sparse near the 10% BMR, especially in the Pauluhn,[12] it is necessary to evaluate further whether these BMD(L) estimates are reasonable. Given that the model optimization algorithm seeks the best fit to all the data including the maximum (100%) responses, a common approach is to drop the highest dose group and refit the model to evaluate the effect on the BMD(L) estimates.[29] The results of these model refits showed little effect (up to four decimal places) on the BMD(L) estimates. In addition, as would be expected, these BMD(L) estimates are similar to or lower than the LOAELs and NOAEL in those studies; LOAEL of 0.1 mg/m^3 in Ma-Hock et al;[11] and LOAEL of 0.4 mg/m^3 and NOAEL of 0.1 mg/m^3 in Pauluhn.[12] Thus, the BMD(L) estimates appear to be reasonable despite the less-than-ideal dose–response relationships (Figs. 2 and 3).

Next, the BMD(L) estimates (as exposure concentration) are used to estimate an equivalent lung dose in rats, in this case, either the deposited or the retained lung burden at the end of the 13-week study. These lung dose estimates were obtained by using data on the MWCNT particle size MMAD (GSD) and unit density in the rat model in MPPD 2.0;[24] and the rat lung doses were extrapolated to humans by normalizing on lung surface area (as in example 1).

In this example, a 45-year working lifetime exposure concentration associated with human-equivalent lung doses (deposited dose or retained dose) was estimated using the same particle size data (as in the rat model) for the human model in MPPD 2.0 (Yeh and Schum deposition model).[24] This method provides estimates of the working lifetime exposure concentration associated with a 10% excess risk of early-stage adverse lung effects.

In the final step to develop a health-based OEL, the 10% BMDL would be used as the point of departure to extrapolate to lower doses and risks (eg, model-based or linear extrapolation, or uncertainty factors). However, this step is beyond the scope of this example.

RESULTS

On the basis of the methods and assumptions described in example 1, Table 1 gives the estimated human-equivalent concentrations to the NOAEL or LOAEL from the rat subchronic inhalation studies of multiwall CNTs.[11,12] No uncertainty factors were applied to these estimates. These are not considered safe levels, but are the levels estimated to be associated with the effect levels in the animal studies. These estimates are based on the equivalent deposited lung dose.

Results from example 2, based on BMD(L) estimation and extrapolation to humans over a full (45-year) working lifetime are shown in Table 2. In this case, the human-equivalent BMDL estimates indicate that working lifetime exposure concentrations to approximately 0.2 to 2 $\mu g/m^3$ would be associated with a 10% excess risk of early-stage adverse lung effects (pulmonary inflammation and fibrosis) in workers.

Published exposure measurements of workplaces producing or using CNTs are provided in Table 3. These airborne concentrations ranged from not detected to more than 8000 $\mu g/m^3$, with many concentrations in the 10s of micrograms per cubic meter. Although most of these samples were short-term (eg, 30-min), task-based measurements of total carbon (not specifically CNTs), these data indicate the potential for workers to be exposed to airborne concentrations at or above those associated with early-stage adverse lung effects in rats.

DISCUSSION

Workplace exposures to 8-hour TWA airborne concentrations of approximately 6 to 35 $\mu g/m^3$ MWCNTs over 5.6 years were estimated to be equivalent to the LOAEL or NOAEL in the two rat subchronic inhalation studies[11,12] (Table 1). Working lifetime (45-year) equivalent airborne concentrations associated with 10% excess risk of early-stage adverse lung responses were approximately 0.2 – 2.0 $\mu g/m^3$ (BMDL estimates) (Table 2). Limited workplace exposure data indicate the potential for workers to be exposed to MWCNTs, SWCNTs, and carbon nanofibers (CNFs) at these concentrations or higher (Table 3). Although the MWCNT data from the rat subchronic inhalation studies are used in this paper to illustrate risk estimation, additional animal studies of SWCNTs and CNFs (with routes of exposure by pharyngeal aspiration, intratracheal instillation, or short-term inhalation) have shown similar lung responses also at low mass doses (recently reviewed by NIOSH[27]). Thus, until more data are available, it is considered prudent to use extra precaution in controlling exposures to all types of CNT and CNF.[27]

The risk estimates for MWCNTs are based on early-stage adverse effects (e.g., alveolar interstitial thickening indicating early-stage fibrosis); however, those effects persisted up to four months after the end of exposure.[12] These findings indicate the potential for

TABLE 2. Working Lifetime 8-Hour TWA Concentration Associated With 10% Excess Risk

		Human-Equivalent 8-hr TWA Concentration Over 45-yr Working Lifetime	
Study	**Response**	**BMD_{human} ($\mu g/m^3$)**	**$BMDL_{human}$ ($\mu g/m^3$)**
Deposited lung dose*			
Ma-Hock et al[11]	Granulomatous inflammation	0.51	0.19
Pauluhn[12]	Alveolar interstitial thickening	0.77	0.38
Retained lung dose*			
Ma-Hock et al[11]	Granulomatous inflammation	2.7	1.0
Pauluhn[12]	Alveolar interstitial thickening	4.2	1.9

*Using aerodynamic size data reported in subchronic studies, assuming spherical particle lung deposition and clearance kinetics (Multiple-path Particle Dosimetry version 2.0).[24]

Limit of quantification of analytical method to measure exposure is approximately 7 $\mu g/m^3$ as an 8-hr TWA concentration.[27]

BMD, benchmark concentration maximum likelihood estimate; BMDL, BMD lower 95% confidence limit; TWA, time-weighted average concentration.

TABLE 3. CNT Occupational Exposure Data

Material and Process	**Concentration($\mu g/m^3$)***	**Reference**
SWCNT—production facility	10–53	Maynard et al[30]
MWCNT—research laboratory, before and after controls	37–434 ND–39	Han et al[31]
CNF composite—weighing, mixing, cutting	64–1094	Methner et al[32]
MWCNT composite—wet or dry cutting	54 2110–8380	Bello et al[33]

*Most are short-term (eg, 30-min) samples of total carbon.

CNF, carbon nanofiber; CNT, carbon nanotube; MWCNT, multiwalled carbon nanotube; ND, not detected; SWCNT, single-walled carbon nanotube.

chronic lung disease and need for effective measurement and control of workplace exposures. They also suggest that medical monitoring may be needed to detect early-stage adverse lung effects, including pulmonary inflammation and fibrosis, which workers may be at risk of developing at potential workplace exposures in certain jobs.

It should be noted that no uncertainty factors were used in these risk estimation examples. According to standard occupational health practice, a human-equivalent exposure to a NOAEL, a LOAEL, or a BMD(L) associated with a 10% excess risk would not be used directly to develop an OEL for humans. Instead, these estimates would typically be used as points of departure to estimate lower levels of risk or to apply uncertainty factors.

To reduce the uncertainty in risk estimation of CNTs, additional information and research are needed in several areas. For example, additional information is needed to assess whether the observed rat subchronic lung responses would correspond to functionally or clinically significant responses in humans. In addition, it is unclear whether these early responses would be detected in standard medical tests. Fibrosis in human lungs is generally detected by chest radiography or computed tomography. Yet, there is no information at this time to determine whether the amount of pulmonary fibrosis (alveolar interstitial thickening) observed in the animal studies (eg, Pauluhn[12]) would be readily detectable in humans. More sensitive medical screening or biomarker tests may be needed to detect these early effects and to intervene to prevent potential development of occupational lung disease in workers producing or using CNTs.

Research is needed on the chronic effects of exposure to CNTs, including potential carcinogenic effects. Studies are needed on the effect of dose rate on the development of adverse lung effects from CNT exposure. For example, it is not known whether the cumulative lung doses associated with adverse lung effects in the rat subchronic studies would be associated with more or less severe responses if the same dose were received over a longer period of time (eg, biological adaptation and/or residence time may affect the long-term lung response to a given dose). It is also uncertain whether human lungs and rat lungs have similar sensitivity to CNTs, as humans are known to be more sensitive to some pulmonary toxicants.[6]

Because CNTs are produced with varying physical-chemical characteristics, data are needed on the extent to which these various factors may influence the hazardous properties of the CNT, in addition to the effect of the carbon composition of all CNTs. Evidence that particle shape, size, and surface area influence the lung response to carbon particles is seen in the comparison between the lung responses to ultrafine carbon black (ufCB)[34] versus MWCNTs[11,12] based on the same study design (13-week inhalation) and animal species (rat). Comparing the study LOAELs, the MWCNT was at least 10 times more potent than the ufCB (LOAEL of 7 mg/m^3 for ufCB vs 0.1 or 0.4 mg/m^3 for MWCNTs).[11,12] Although LOAELs and NOAELs are dependent on dose spacing, the NOAEL of 0.1 mg/m^3 in one study of MWCNTs[12] was an order of magnitude lower than the NOAEL of 1 mg/m^3 in a study of ufCB.[34]

Measurements of CNT airborne characteristics are needed to determine the extent to which CNT particle size and morphology may influence lung deposition and retention, after accounting for aerodynamic diameter. It would be useful to know how the characteristics of CNT materials in the workplace compare with those in the animal studies, and to have sufficient data to link those characteristics to the hazardous properties of the CNTs in order to prevent adverse health effects in workers. For example, in a recent study in mice, dispersed SWCNT structures were associated to a greater extent with interstitial fibrosis whereas the agglomerated structures were more clearly associated with granulomas.[35] Until specific particle size characteristics are linked to qualitative and quantitative differences in toxicity, it would be prudent to apply the available CNT data (which include studies in rats and mice exposed to SWCNTs or MWCNTs with different metal content) to the risk assessment and risk management of other CNT materials, erring toward greater precaution in the absence of more specific information.

Finally, there is a critical need for more data on worker breathing zone concentrations of CNTs, including in workers who are using products containing CNTs or the structurally-similar CNFs. Published exposure measurements of airborne concentrations of CNTs, or total carbon in work areas producing or using CNTs (Table 3), indicate the potential for workers to be exposed to levels of CNTs associated with granulomatous inflammation, lipoproteinosis, and early-stage, persistent pulmonary fibrosis in animal studies (Table 1). Workplace exposures to airborne concentrations of approximately 7 to 35 mg/m^3 CNT over 5.6 years were estimated to be equivalent to the LOAELs in the two currently published rat subchronic inhalation studies.[11,12] These limited exposure data in workers indicate the potential for workers to be exposed at airborne concentrations of CNTs exceeding the 8-hour limit of quantification (7 μg/m^3) of the measurement method,[27] which is associated with more than 10% excess risk of early-stage adverse lung effects based on the animal data (Table 2). These findings support the need for effective monitoring and control of CNT exposures as the primary occupational health measure to prevent adverse health effects. Medical monitoring may also be needed as a secondary prevention measure to detect early inflammatory and fibrotic lung effects in workers. Finally, there is a need to develop and validate biomarkers for early adverse biological effects of CNTs.

CONCLUSIONS

MWCNT exposure in rats caused adverse lung effects at exposures at least an order of magnitude lower than did ufCB in subchronic inhalation studies. Current workplace exposures in some jobs or tasks involving production or use of CNTs indicate the potential for early-stage adverse lung effects based on similar estimated lung doses in animals studies. Risks of more than 10% for early-stage pulmonary fibrosis are estimated from animal dose–response data at the limit of quantification of 7 μg/m^3 (as 8-hour TWA concentration) of the measurement method for airborne elemental carbon including CNTs (NIOSH method 5040). These findings have implications regarding the need to develop OELs, improved workplace exposure measurement, and effective engineering controls, and to consider medical monitoring programs for early detection of occupational respiratory diseases in workers producing or using CNTs.

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Nanomaterial Risk Assessment and Management Experiences Related to Worker Health Under the Toxic Substances Control Act

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Objectives: This paper examined the data and experiences gathered through the review of over 100 nanomaterial submissions for industrial nanomaterials, and what these data indicate for worker health at industrial facilities where nanomaterials are synthesized, and/or incorporated into final products for the marketplace. **Methods:** The types of nanomaterials, their uses, potential health effects and worker exposures, methods for examining worker and general population exposures, and risk management actions taken under the Toxic Substances Control Act (TSCA) prior to their manufacture are summarized. **Results:** There is a diversity of nanomaterials are currently entering the marketplace, but there are certain materials reviewed under TSCA such as carbon-based nanomaterials and metal oxides that are more likely to be commercialized than others. There are health and monitoring data that have been received by EPA that are useful in determining potential risks, and risk management approaches such as limiting uses of the nanomaterials and embedding nanomaterials in polymer matrices that reduce concerns for worker exposures. Certain EPA data gathering tools such as those used to collect nanomaterial use and worker exposure information, and screening level approaches for estimating worker exposures are useful and could be enhanced to better estimate worker risks. **Conclusions:** The data and experiences with nanomaterials under TSCA should prove useful when considering worker exposure registries, medical surveillance and epidemiological research.

The Toxic Substances Control Act (TSCA) of 1976 provides the U. S. Environmental Protection Agency (EPA) with authority to regulate certain industrial chemical substances.[1] Under the TSCA, chemicals used in industrial applications are reviewed for their risks to human health and the environment while other substances (such as foods, drugs, cosmetics, and pesticides) are excluded. The scope of the TSCA reviews includes an evaluation of the potential risks to workers and other populations, and the environment. The TSCA definition of a chemical substance is based on molecular identity, not on physical properties such as particle size.[2] Given this definition and the types of uses covered, many nanomaterials are chemical substances subject to TSCA. Nanomaterials based on chemical substances already on the TSCA Inventory are considered existing chemicals. Examples of nanomaterials based on existing chemicals are metals like iron and gold, and some metal oxides such as titanium dioxide and silicon dioxide. Nanomaterials that are not on the TSCA Inventory are considered new chemicals. Examples of nanomaterials that are new chemicals are carbon nanotubes and fullerenes. Because carbon nanotubes and fullerenes are different allotropes of carbon, the EPA considers them to have different molecular identities from other forms of carbon and to be new chemical substances.[3] New chemical substances are subject to reporting and review prior to commercialization. Nanomaterials based on existing chemical substances are not subject to reporting before commercialization.

New chemicals are examined by the EPA before manufacturing as part of the Premanufacture Notice (PMN) process under section 5 of the TSCA.[4] Under section 5 of the TSCA, the EPA has the authority to evaluate the potential risks in a PMN and to take actions to prevent any unreasonable risk including banning production of the chemical substance. This includes potential unreasonable risks to workers. The Occupational Safety and Health Administration (OSHA) does not have rulemakings, regulations or Permissible Exposure Limits (PEL) that would specifically apply to the new chemical nanomaterials described in this article. However, OSHA does have a framework of existing requirements that covers nanomaterials. These include section 5(a)1 of the OSH Act at 29 USC 654 known as the General Duty Clause, the Hazard Communications Standard at 29 CFR 1910.1200, the Personal Protective Equipment Standard at 29 CFR 1910.132, the Respiratory Protection Standard at 29 CFR 1910.134, the Hazardous Chemicals in Laboratories at 29 CFR 1910.1450, and several substance-specific standards at 29 CFR 1910 Subpart Z.

Basic data required in a PMN submission include chemical identity, use information, anticipated production volume, byproducts, exposure and release information, disposal practices, and existing available health and environmental effects test data. Exposure monitoring data are not required since the submission is completed before the substance is manufactured or imported. The Agency evaluates these data in the open literature, and data submitted with earlier analogous PMN materials in making its determination of potential risks. This process involves a team of reviewers, including chemists, engineers, toxicologists, ecotoxicologists, exposure assessors, and risk assessors. Where there are sufficient concerns for risks to human health or the environment, the EPA will require additional information on substance identification, effects information, and/or exposure information. Since 2005, the Agency has reviewed over 100 nanomaterials as part of the PMN process, and other nanomaterials as part of its Nanoscale Materials Stewardship Program.[5]

The purpose of this article is to discuss some of those experiences, recognizing that many of the specific data and materials reviewed are regarded as confidential business information by the companies who submitted the PMN information. Early publication of PMN information will enable stakeholders to have improved understandings of the directions of emerging data and its implications for risk assessment. These TSCA approaches and experiences related to worker health are relevant to gauging the scope of materials in commerce and extent to which workers are exposed, the approaches to gathering exposure data and estimating worker exposures for exposure registries, and routes of exposure most likely relevant to any potential adverse effects.[6] While the focus of this article is on worker exposure, the EPA has also established interim technical guidance for assessing environmental fate and transport, general population, environmental, and consumer exposures.[7]

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In this article, aspects of the characterization of materials proposed for commercialization, use and exposure information for these materials, and the EPA's methods for estimating worker and general population exposures based on this information are reviewed. Next, toxicity information relevant to worker exposures is addressed, and general approaches to estimating concern levels for workers are examined. Finally, risk management methods to address potential concerns for worker health will be discussed.

PHYSICOCHEMICAL CHARACTERISTICS

A good understanding of the physicochemical characteristics of the nanomaterials under evaluation is critical to an accurate assessment of potential risks to workers, consumers, the general population, and environmental receptors such as aquatic species. Furthermore, such factors are important if exposure registries are developed, because the associations between the physicochemical characteristics of specific nanomaterials and workers who are exposed to them should be understood. Many of the questions posed by the EPA to commercial submitters as part of the reviews of nanomaterials under the TSCA new chemicals process are aimed at better understanding the type, and size distributions, of nanomaterials to which humans and environmental receptors may be exposed. Such questions are often pivotal to the overall risk assessment conclusions made by the Agency on these materials. The importance of understanding the physicochemical characteristics of nanomaterials, before drawing toxicological conclusions based on the testing of such materials, is illustrated in the literature: for example, an inhalation study in mice tested the effects of what was described as multiwalled carbon nanotubes (MWCNTs), and found that while the concentrations tested did not result in significant lung inflammation or tissue damage, they were correlated with systemic immune function alterations.[8] This was a novel finding at the time, given that the most sensitive endpoints in similar studies were associated with adverse lung effects rather than effects on the immune system; such a finding could have altered the overall risk assessment approaches for MWCNTs in terms of the endpoints considered in the risk assessment and the associated toxicity values on which an occupational exposure limit would be based. However, later examinations of the materials used indicated that the material tested was more likely a mixture of carbon nanofibers and carbon nanotubes.[9,10] Beyond the chemical characterization of the nanomaterial itself, it is also critical to the analysis of potential worker risks to understand whether the nanomaterial is associated with other materials such as resins which are used to encapsulate nanomaterials such as CNTs for certain commercial applications. If nanomaterials are associated with resins and are respirable, then different occupational exposure limit considerations come into play, as opposed to those applicable to the nanomaterial in isolation. For example, if the nanomaterial is encapsulated in an insoluble resin then the resin characteristics and size distribution would be important to consider in the assessment of the overall risks to workers.

In the case of workers, it is also important to understand the particle size distribution to which workers may be exposed, and what fractions of that distribution would deposit in different portions of the respiratory tract. For example, risks to workers due to effects of particles that lodge in the alveolar region of the lung are greatly lessened if nanomaterials are present in size ranges above that which would be inhaled into the deep lung. The EPA[11] and others[12] note an increased concern for respirable poorly soluble particulates with sizes less than 10-μm aerodynamic diameter; this same size fraction is noted as having high deposition rates in the human alveolar region. The PMN submissions for nanomaterials can contain limited information on the nanomaterial product size distribution or airborne particle size distribution, and the methodology used can impact the usefulness of the data in characterizing exposures. For example, the EPA prefers condensation and optical particle counting methods involving direct air sampling to provide estimates of respirable airborne particle fractions in the workplace, rather than a dynamic light scattering method that measures particle sizes in solution since measurements done in solution may not be representative of size distributions in exposure situations. The EPA also prefers data on size distributions in the workplace air to better characterize potential risks rather than relying upon assumptions as described in the exposure screening methods explanation below to assess potential exposures to nanomaterials.

EXPOSURE EVALUATION

The EPA, as part of the PMN review process, evaluates worker exposures and potential risks to workers. When appropriate, the EPA specifies personal protective equipment, engineering controls, and/or modifications to nanomaterials' manufacture to address these potential risks. Some understanding of potential exposures to workers, and others such as the general population can be gained by examining the types of industrial chemical uses seen to date in the TSCA new and existing chemicals' programs. Table 1 may be useful in identifying study populations for epidemiological studies of workers since current industries that produce and use nanomaterials, and market trends for future study populations, can be identified. A large proportion of the PMN nanomaterial applications received to date indicate that certain nanomaterial classes are represented in commercial products more than others. For example, at this time carbon-based nanomaterials are seen more than other material classes, followed closely by metal oxides/metals/other metal-containing nanomaterials. In the case of the metal-associated materials, approximately half of them are represented by modified silicas (Table 1).

Another level of understanding is gained by examining the production volumes, number of downstream users, and numbers of exposed workers for various nanomaterials reviewed to date. Looking across all PMN submissions, some have had very targeted uses and customers (eg, one customer and one use), while others have had very broad and diversified uses (eg, up to five uses) and numbers of downstream users (e.g., up to eight downstream users per use). Generally, the number of potentially exposed workers per site has not exceeded 10, although the EPA has estimated higher numbers per site in a case using literature data from the plastics industry. While production volumes and workers involved for specific materials cannot be released because of the confidentiality provisions under TSCA, general information about a class of materials, such as carbon nanotubes, can be helpful. For example, the EPA has received over 30 PMN submissions for carbon nanotubes, and production volumes are generally in the low tens of thousands of kilograms per year. The number of workers projected by industry to be involved in the manufacture of these CNTs is typically less than six per site, but can be as high as 100 workers for a single site where CNTs are incorporated into a final product.

Key information and data gathered for PMNs by the EPA includes locations of facilities, chemical identity information, environmental releases, manufacturing diagrams, throughput volumes of materials in kg/day, operating days in days/yr, physical states and concentrations of the nanomaterial of interest at key stages of handling, worker activities with exposure potential, personal protective equipment, engineering controls that limit exposures/releases, on-site waste treatment processes, and number of workers for each of these activities. This information is requested in the PMN Notice software for electronic submissions.[13] The information in the PMN Form could be useful in establishing standardized fields for exposure registries. To date, the EPA has estimated worker exposures to nanomaterials using the same methods that are applied to substances that are not nanosized. This is due at least in part to the metrics currently used for reporting toxicity, exposure, and risk data; and due to the limitations of current nanomaterial measurement

TABLE 1. TSCA Applications Reviewed to Date in the Premanufacture Notice (New Chemicals) Review Process

Nanomaterial Classes	General Uses	Approximate Range of Nanomaterials, per Class
Single-walled, and multiwalled carbon nanotubes; carbon nanofibers; and other carbon particles	Enhanced electrical conductivity, mechanical reinforcement, and/or color additives	Less than 50
Fullerenes with variable carbon number	Enhanced electrical conductivity, &/or mechanical strength; reduces friction	Less than 10
Other metal oxides (modified silica, titanium, and alumina), modified metals, and other metal-containing particles	Coating additives for scratch resistance, barrier films, self-cleaning surface; lighting applications; detection systems, additives in electrochemical systems	Less than 35
Other nanomaterials not listed above	Intentionally left blank due to confidentiality considerations	Less than 15

techniques. Current EPA risk evaluations use mass-based concentrations for inhalation estimates, where the units used are micrograms or milligrams per cubic meter ($\mu g/m^3$ or mg/m^3) as a time-weighted average (TWA) for the worker's shift, assumed normally to be 8 hours. The EPA recognizes that mass based metrics may not be the best approach for estimating exposures to nanomaterials and will continue to work with its researchers, other federal agencies, and other sources to identify and develop more appropriate methods.

The worker exposure evaluations of nanomaterials described in this article have been solely for Toxic Substances Control Act (TSCA) section 5 purposes, in which the EPA employs screening level approaches for estimating worker exposures to new chemical substances for which exposure monitoring data are unavailable. Information on these approaches, and the primary worker exposure estimation tool the Chemical Screening Tool for Exposures and Environmental Releases, which is a PC-based software program, are available from the EPA's public exposure Web site.[14] The EPA uses a hierarchy of preferred methods for estimating worker exposures to chemical substances, and the following three main tiers of this hierarchy apply equally to nanomaterials:

I. Personal monitoring data for the chemical of interest in the workplace of interest;

II. Personal monitoring data for the chemical of interest in a workplace situation that is similar to the workplace of interest (surrogate workplace situation) OR personal monitoring data for a chemical that is similar to the chemical of interest in the workplace of interest (surrogate chemical);

III. Modeled estimates or concentration assumptions based on regulatory limits.

Literature searches have found several studies that document personal monitoring data for specific workplace settings. Maynard et al[15] carried out a laboratory based study to evaluate the physical nature of the aerosol formed from single-walled carbon nanotube material during mechanical agitation, complemented with airborne and dermal exposure while handling unrefined material. Handling resulted in very low airborne concentrations (from 0.7–53$\mu g/m^3$), consistent with the tendency to aggregate into larger masses. The EPA has used these concentrations in several new chemical cases as tier II surrogate data where carbon nanotubes that were not identical to the single-walled carbon nanotube materials in the Maynard study were used, and workplace activities have seemed to match well to those documented. No other studies with mass concentration monitoring data found in the literature have yet matched new chemical case situations well enough to apply the data in these studies in any new chemicals cases involving nanomaterials. For example, for data from a particular study to be applicable to a given case, the nanomaterial's chemical structure and the workplace scenario and handling (eg, physical state during handling, specific worker activities that can result in dermal and inhalation exposures, and amounts of materials handled) must be adequately similar in both the study and the case.

The NIOSH (the National Institute for Occupational Safety and Health) has conducted site studies at several sites where carbon-based nanomaterials of several types (nanotubes and nanofibers) are manufactured. These studies have used several different methods for generating particle number concentrations. The EPA has used this information to indicate the potential presence of nanomaterials and nanoparticles in workplace air but has not changed its quantitative exposure assessments. Some of these studies have also documented mass concentrations using the NIOSH method 5040 for Diesel Particulate Matter (as elemental carbon).[16] In several new chemicals cases, submissions have included very limited amounts of data from this method, but the EPA has not found these data robust enough to consider them to be representative of worker exposures to the nanomaterials for these cases. Furthermore, the presence of more than one species of nanomaterial (eg, the manufactured nanomaterial, diesel exhaust, and others) can present additional challenges toward characterizing the exposure concentrations of the nanomaterial of interest.

In most new chemicals cases, the limited amount of applicable literature data leads EPA to employ standard screening methods for

estimating particulate exposures. Several of the primary screening methods for estimating dust exposures include the tier II "Small Volume Solids Handling Inhalation Model" and the tier III "OSHA PEL for Particulate, Not Otherwise Regulated, total and respirable particulate" models. Also, several primary screening methods for estimating aerosol exposures in "end-use" scenarios (eg, liquid spraying or roll coating mist generation) include the tier II "UV Roll Coating Inhalation Model (non-volatiles)" and the tier II "Automobile Spray Coating Inhalation Exposure Model (nonvolatile nonpolyisocyanates)" models. When airborne particle size distribution data are unavailable or potentially not representative, the EPA assumes 100% of particles may be respirable in exposure concentration estimates at or below the OSHA respirable Particulate, Not Otherwise Regulated PEL of 5 mg/m^3, with any remaining exposure concentration above 5 mg/m^3 assumed not to be respirable. The EPA also uses a suite of standard dermal exposure models to estimate dermal exposures (in mg/day) to nanomaterials. These inhalation and dermal models are documented in the Chemical Screening Tool for Exposures and Environmental Release help system.[13]

As part of EPA's analysis of potential human and environmental exposures to nanomaterials, under section 5, the EPA often found that, where there are exposures, they frequently involve workers who could be exposed to airborne nanoparticles during their manufacture. Other exposures later in the life cycle of the nanomaterial's production and use are also possible. For example, life cycle analyses of the use of CNTs in batteries, textiles, and epoxy resins have been examined.[17] In these cases, exposures are possible with shattering/manual recycling of batteries containing CNTs, shredding/recycling of fabrics with CNT external coatings, and sanding of epoxy resins containing CNTs. In the future, increased exposures to consumers are possible from spray-applied nanomaterials such as metal oxides used to treat hard surfaces.[18]

UNDERSTANDING POTENTIAL HAZARD AND SETTING OELs

Chronic or subchronic studies in animals are often used to estimate worker inhalation concentrations of concern, but only a few chronic studies are available at this time for traditional nanomaterials such as titania and carbon black.[19] To target nanomaterials, which are most likely to cause concerns for workers for future surveillance, exposure registries, and epidemiologic research, better understandings of their toxicity/carcinogenicity potential is necessary. For newer manufactured nanomaterials such as carbon nanotubes and fullerenes, no chronic (and few subchronic) studies are currently available. While the OECD's Working Party on Manufactured Nanomaterials is engaged in testing nanomaterials in subchronic inhalation studies, other Federal or independent industry testing have yielded data at this time using subchronic protocols accepted by regulatory bodies. A set of subchronic inhalation tests on 0.05 um and 1 um C60 fullerenes in rats has been completed by the National Toxicology Program, and results indicate that there were no biologically significant effects at the highest concentrations tested: 2.5 mg/m^3 for the nanosized fullerenes, and 30 mg/m^3 for the 1 um fullerenes.[20] These data indicate higher concern concentrations for some fullerenes via the inhalation route in subchronic studies, relative to another class of newer carbon-based nanomaterials MWCNTs. Data from subchronic inhalation studies on two different MWCNTs have indicated lower concentrations of concern, based on adverse lung effects in rats for MWCNTs which tend to agglomerate in air: Ma-Hock et al[21] identified a low observed adverse effect level of 0.1 mg/m^3; Paulhun[22] found a no observed adverse effect level of 0.1 mg/m^3. While data on MWCNTs are becoming available, it is unclear at this time how these data on two types of MWCNTs can be applied in a quantitative manner to estimate the adverse lung effects of MWCNTs with different physicochemical and agglomerate properties, and to other CNTs such as single-walled CNTs. This lack of data for different nanomaterials within a class of nanomaterials (such as the MWCNT class), as compared to the vast number of commercial CNTs coming into the marketplace, make other qualitative approaches to estimating adverse pulmonary effects for these materials necessary to complete premanufacture regulatory reviews in a timely manner under TSCA. Beyond adverse pulmonary effects, there are indications that other endpoints such as those associated with cardiovascular effects may need to be evaluated.[23]

The EPA has pursued a category approach for setting OELs, and identifying testing recommendations for certain categories of chemicals, when coupled with chemical-specific exposure information for 53 specific chemical categories applicable to the TSCA New Chemicals Program.[3] One of these categories is a health category that addresses certain respirable, poorly soluble particulates (RPSPs), including nanomaterials. For certain analogs of RPSPs (including crystalline silica, talc, titanium dioxide, lithium manganese oxide, and carbon black), if the particle size is less than 10 μm in diameter and their respective NIOSH Recommended Exposure Limit (REL) or OSHA PEL is exceeded then a subchronic inhalation toxicity test in rats is recommended. These tests may be required of the PMN submitter to better characterize potential risks. This approach can be expanded by considering new OELs such as the draft NIOSH RELs for titania: 0.1 mg/m^3 TWA for up to 10 hr/day during a 40 hour work week for ultrafine titania, and 1.5 mg/m^3 TWA for up to 10 hr/day during a 40-hour work week for fine size titania.[24] Thus, a range of OELs can be identified for particles of differing potencies, and analog nanoparticles with similar physicochemical properties from PMN submissions can be aligned with applicable OELs. For other unique nanomaterials which do not align with the particles that have established OELs, subchronic test data can be used to derive an occupational exposure limit for that material by the EPA, or the EPA can use OELs derived from the literature such as the 50 μg/m^3 TWA for a certain MWCNT as noted in Pauluhn.[25] Such a MWCNT-specific occupational exposure limit could be adjusted for other MWCNTs by taking factors such as the degree of agglomeration and/or differing catalyst effects into consideration.

RISK MANAGEMENT

While there is uncertainty associated with the risk assessment of nanomaterials, the EPA has identified potential hazards and exposures. To address potential environmental and health risks, the EPA has used its new chemical authority to prevent or limit human and environmental exposures. For example, the EPA limits use of the nanomaterial to the specific uses in the notice, does not allow spray applications of the nanomaterials, and controls any potential exposures to workers with protective equipment such as impervious gloves and the NIOSH-approved respirators when workers are reasonably likely to be exposed. For further details see the *Federal Register* 65751, 57430, and 5546.[26–28] The EPA requires that the nanomaterial be embedded in a polymer or metal matrix or other article before any consumer uses. In certain cases, the EPA has limited the amount of nanoparticles less than 100 nm for the as-manufactured nanomaterial. The EPA generally does not allow environmental releases directly to surface waters but in some cases has allowed limited releases resulting in stream concentrations of the nanomaterial less than one part per billion. Disposal of new chemical nanomaterials are usually via incineration or landfill. While some nanoparticles are released, most of the nanomaterials are disposed of after they have been embedded in a polymer or metal matrix or other article.

For nanomaterials subject to PMN review there are requirements in administrative orders agreed upon between the EPA and the PMN submitter to establish these restrictions limiting exposures and environmental releases while also requiring development of data such as subchronic inhalation toxicity studies (for examples, see *Federal Register* 65751 and 57430[26,27]), material characterization, particle size distribution and other physical chemical property. The

EPA also issues significant new use rules to establish these same requirements for all other manufacturers and processors of the same nanomaterial.

To better understand nanomaterials that are existing chemicals already in commerce,

The EPA is also developing a proposed rule under its TSCA section 8(a) information gathering authority to require the submission of additional information. This rule would propose that persons who manufacture these nanomaterials notify the EPA of certain information including production volume, methods of manufacture and processing, exposure and release information, and available health and safety data. The EPA also intends to propose a section 4 test rule for certain nanomaterials that are already in commerce. The proposed rule would require testing for health effects, ecological effects, and environmental fate as well as provide material characterization data. Finally, the EPA is developing a proposed significant new use rule to require reporting of new nanomaterials based on existing chemical substances. The significant new use rule would require persons who intend to manufacture, import, or process these new nanomaterials to submit a significant new use notice to the EPA before manufacturing the new nanomaterial. The EPA would review and manage any potential risks using the same process described for new chemicals. See http://www.epa.gov/oppt/nano/index.html for additional information.

The EPA will continue to gather data obtained through its new and existing chemical's authorities and other valid sources such as the peer-reviewed literature to improve its approaches to nanomaterial risk assessment and risk management. Such information may lead to new and/or improved approaches for nanomaterial worker surveillance, registries, and related epidemiological research.

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Development of a French Epidemiological Surveillance System of Workers Producing or Handling Engineered Nanomaterials in the Workplace

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Objective: Concern has been raised about the potential impact of nanomaterials exposure on human health, and France has decided to implement a timely epidemiological surveillance tool of workers likely to be exposed to engineered nanomaterials that could accompany the development of nanotechnologies. **Methods:** A comprehensive review of the toxicological and epidemiological literature has been conducted together with an exploratory study among French companies producing or handling nanoobjects. **Results:** A double surveillance system is proposed consisting of a prospective cohort survey and repeated cross-sectional studies. The aim of the cohort is (1) to monitor long-term health effects and (2) to allow of further research. Setting-up an exposure registry is the first planned step. **Conclusions:** The protocol is about to be submitted to the French Government for approval and funding.

Nanomaterials have unique physical and chemical properties, which make them highly attractive for industrial applications but also modify their interaction with biological systems, with the potential to generate toxicity.[1] Alerted by the possible impact of nanomaterials exposure on human health, the French Ministries of Health and of Labour have given the French Institute for Public Health Surveillance responsibility for designing the protocol of an epidemiological surveillance system of workers likely to be exposed to engineered nanomaterials. The Institute for Public Health Surveillance has benefited from the scientific support of a multidisciplinary working group held by the French Institute for Public Health Research. The protocol has been developed in close collaboration with the Institut National de Recherche et de Sécurité in particular for the inhalation exposure assessment.

Four major goals are commonly assigned to epidemiological surveillance: (1) to detect timely unusual health situations, (2) to assess the magnitude of a health problem to make decisions affecting public health policy and allocation of resources, (3) to contribute to further research, and (4) to evaluate the effects of prevention and intervention efforts.[2]

Designing the protocol of an epidemiological surveillance system in the field of occupational exposure to nanomaterials needs to face numerous issues such as the wide range of nanomaterials, the identification of health outcomes that need to be followed-up, the quantitative assessment of exposure, the identification and cooperation of companies involved in the manufacture and incorporation of nanomaterials, and the registration of workers producing or handling nanomaterials.

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THE WIDE RANGE OF NANOMATERIALS

The International Organization for Standardization defined nano-objects as materials with one, two, or three external dimensions in the nanoscale (from approximately 1 nm to 100 nm).[3] Engineered nanomaterials are commonly described as materials designed and produced to have structural features with at least one dimension of 100 nanometers or less.[4] This definition encompasses many forms of materials: nano-objects themselves (powder of nano-objects, aerosol of nano-objects), materials incorporating nano-objects (nano-objects in liquid suspension, nano-objects incorporated in solid materials, nano-objects linked to the surface of solid materials), mass or surface nanostructured materials. At this broad range of nanomaterials correspond different circumstances of exposure on the workplace. The exposure to aerosol of nano-objects occurring during the handling of powder is today the best documented situation.[5]

According to toxicological studies, chemical characteristics (such as composition, added functional group, surface coating, impurities), physical features (such as size, surface, shape, charge) as well as physicochemical properties (such as crystallinity and aggregation/agglomeration state) influence the toxicity of nano-objects. The combination of these different features adds to the great diversity of nanomaterials.[1] Therefore, it seems relevant to focus on epidemiological surveillance of workers likely to be exposed to a few nanomaterials of interest.

REGISTRATION AND COLLABORATION OF COMPANIES AND WORKERS PRODUCING OR HANDLING NANOMATERIALS

In France, companies producing the main sorts of nano-objects are well known; however, registration of companies incorporating nano-objects is not complete.[6] To collect critical information such as the number of workers likely to be exposed to nanomaterials, conditions of exposure, medical follow-up, and collaboration issues, an exploratory study was performed from 2008 to 2010 and several companies producing or incorporating carbon nanotubes, carbon black, titanium dioxide, or amorphous silica were contacted to be visited (Table 1). In each facility, the number of workers likely to be exposed to nanomaterials was quite low.

Three kinds of companies could be distinguished:

- Research and development facilities producing and incorporating emerging nano-objects like carbon nanotubes: three of them have been visited and are ready to collaborate in an epidemiological surveillance system. The probability of individual occupational exposure was low because of extensive engineering control measures implementation and the use of appropriate personal protective equipment. Nevertheless, accidental exposure could not be excluded.
- Chemical companies producing materials for decades such as amorphous silica, carbon black, or titanium dioxide: four companies were visited among seven existing in France. The workers were likely to be exposed to aggregated and agglomerated forms of nanometer-sized primary particles (existence of dust deposit on the work environment).

TABLE 1. Critical Data Collected From Companies Producing or Incorporating Nanoobjects, Exploratory Study for Implementation of a French Epidemiological Surveillance Design of Workers Likely to be Exposed to Engineered Nanomaterials, 2008–2010

	Industrial Sector	Activity	Beginning of the Activity	Nanomaterials	Probability of Exposure	Number of Workers Potentially Exposed
1	Public research and development	Production, incorporation, characterization	2000–2009	Various nanomaterials including CNT and TiO_2	Low	200–249
2	Commercial research and development	Production	2000–2009	Agglomerated bundles of MWCNT	Low	<50
3	Commercial research and development	Incorporation	2000–2009	Agglomerated bundles of MWCNT	Low	<50
4	Chemical industry	Production	1960–1969	Agglomerated and aggregated precipitated silica	High	<50
5	Chemical industry	Production	2000–2009	Agglomerated and aggregated pyrogenic silica	Low	<50
6	Chemical industry	Production	1960–1969	Agglomerated and aggregated carbon black	High	50–99
7	Chemical industry	Production	1980–1989	Agglomerated and aggregated TiO_2	High	100–149
8	Cosmetic industry	Incorporation in sunscreen	1980–1989	Agglomerated and aggregated TiO_2	Low	< 50

CNT, carbon nanotubes; MWCNT, multiwalls carbon nanotubes; TiO_2, titanium dioxide.

- Companies incorporating nanomaterials: the total number operating in France is not known at the moment. Three of the companies working in the fields of cosmetics and tire production were contacted, and one was visited. Cooperation issues could be anticipated from this experience. In this group of industries, the lack of standardized definition for nanomaterials was a matter of concern. Some of the companies refuted the word nanomaterial to describe agglomerated and aggregated forms of primary nanometer-sized particles.

In France, for all workers, occupational medical surveillance is mandatory by law (French labour code, articles R4624-10 to R4624-20). As all other workers, those producing or handling nanomaterials have a health follow-up, which is not specific to their exposure to nanomaterials but rather determined by their exposure to other nuisance materials. Visits to industrial sites showed that all workers concerned about nanomaterials had annual clinical examinations that in some cases included lung function tests, blood withdrawal (blood cell counts, creatinine, transaminase, C Reactive Protein), or chest radiography. This confirms that, for workers dealing with nanomaterials, health data already exists in companies through occupational medicine.

IDENTIFICATION OF HEALTH OUTCOMES THAT NEED TO BE FOLLOWED-UP

A comprehensive review of the scientific literature has been conducted. Toxicological studies gave some relevant information on toxicokinetic, short-term, and long-term effects on animal health and biological mechanism of action. These studies could be helpful to identify potential biological markers of effect. Human experimental studies provided an insight into toxicokinetic and short-term health effects. Epidemiological studies on the effects of particulate air pollution were also consulted as a parallel can be drawn between nano-objects and ultrafine particles. Epidemiological studies conducted among workers exposed to bulk materials produced for decades such as carbon black and amorphous silica constituted the last source of information.[7,8] Although none of them considered specifically the exposure to aerosol of nano-objects, some of the results seemed to be relevant.

An increased risk of adverse malignant and nonmalignant respiratory effects has been found in a number of toxicological studies and epidemiological studies on the effects of particulate air pollution and nanomaterials produced for a long time.[7–12] Different outcomes should then be monitored in an epidemiological surveillance system of workers likely to be exposed to nanomaterials such as pulmonary and systemic inflammation, occurrence and worsening of chronic respiratory illness (asthma, obstructive lung disease), increased susceptibility to infectious diseases, pulmonary fibrosis, and lung cancer. Concerning carbon nanotubes, toxicological studies drew special attention to the possible risk of fibrotic respiratory disease and mesothelioma.[13–15] Spirometry, chest radiography, exhaled nitric oxide, or exercise oxymetry could be implemented in the occupational medical surveillance. Although a promising tool for noninvasive assessment of lung inflammation, biomarkers analysis in exhaled breath condensate is still pending validation studies.[16]

Inference from findings in epidemiological studies of particulate air pollution suggests that cardiovascular effects should be a matter of concern for workers exposed to nanomaterials.[12] Ischemic heart disease especially myocardial infarction, ischemic strokes, thrombosis, arrhythmias, heart failure, and cardiac arrest are different health outcomes that should be followed-up.[12] Validated surrogate markers such as heart rate variability, measures of vascular function and atherosclerosis, or blood markers of cardiovascular risk might be candidate components of a medical surveillance collected by means of electrocardiogram, cardiac holter, cardiovascular imaging, exercise test, or phlebotomy.[17]

As described in experimental studies, inhaled nano-objects could cross the alveolar-capillar barrier into the bloodstream and gain access to various organs of the cardiovascular system and eventually to other organs.[18,19] Moreover, a direct access to the central nervous system via the olfactory pathway has been described for some nanoparticles.[20,21] Thus, the follow-up of workers likely to be exposed to nanomaterials needs to focus on health outcomes affecting respiratory and cardiovascular systems. Nevertheless, it should keep a nonspecific feature to be able to register health outcomes affecting other organs or systems.[22]

QUANTITATIVE ASSESSMENT OF EXPOSURE

Although current knowledge is far from conclusive, it is apparent that characterizing exposures to nanoaerosols in terms of mass concentration and chemical composition does not seem appropriate under all circumstances.[11,23] In addition to the two other major physical exposure metrics (ie, number and surface area concentrations), additional nano-object/nanoaerosol characteristics such as size fraction, shape, degree of agglomeration/aggregation, crystallinity, charge, surface chemistry, and solubility are thought to be relevant in determining the potential health impact. Such a full characterization cannot be carried out on a routine basis within an epidemiological study, and an adequate sampling strategy needs therefore to be developed.[24] This strategy could employ a combination of direct-reading instruments measuring different metrics coupled with specific aerosol samplers for subsequent characterization by chemical and/or electron microscopic analysis. The Nanoparticle Emission Assessment Technique approach, recently proposed by the National Institute for Occupational Safety and Health, could be used as a basis of this specific sampling strategy.[25]

A DOUBLE EPIDEMIOLOGICAL SURVEILLANCE DESIGN

A double epidemiological surveillance design is about to be proposed to the French ministries, consisting of a prospective cohort study and repeated cross-sectional studies (Figure 1). The two parts of the surveillance system should complement each other. Because of the costs of the prospective follow-up, the cohort will be limited to a few nanomaterials of interest, while all nanomaterials produced or handled in France will be in the scope of the repeated cross-sectional studies.

THE PROSPECTIVE COHORT STUDY

The objectives of the prospective cohort study will be to monitor medium- and long-term possible health effects of nanomaterials exposure and to allow for further research. It could also provide guidance for public health policy and be helpful to assess prevention efforts such as the control of exposure.

The protocol of the prospective cohort study needs to be simple and easy to implement, with a step-by-step approach and a nonspecific health follow-up but special focus on respiratory and cardiovascular conditions. The scope will be initially restricted to the production or incorporation of powder of nano-objects, including their aggregated or agglomerated forms.

Carbon nanotubes, titanium dioxide, carbon black, and amorphous silica are considered to be of high priority. Indeed, the greatest amount of available information related to hazards of nanomaterials includes titanium dioxide and carbon-based nanomaterials.[16] Moreover, titanium dioxide, carbon black, and amorphous silica are produced in large amounts in France, whereas carbon nanotubes production could increase in the coming years.[6] This selection of high-priority nanomaterials will be reexamined subsequently at regular time-intervals.

To deal with numerous scientific uncertainties inherent to the field of engineered nanomaterials, a step-by-step approach is necessary to implement an epidemiological surveillance system of workers likely to be exposed (Figure 2).

Initial Step

The first step will be to set up an exposure registry, which will keep record of workers using or handling powder of nano-objects on the workplace. The exposure registry is thought to be the initial step of the prospective cohort study. This step should be clearly identified in the protocol because of the small number of workers likely to be exposed in each single company and the need to incorporate workers from numerous industrial sites. In 2009, National Institute for Occupational Safety and Health has recommended consideration of the establishment of nanomaterials exposure registry as a preparatory step for epidemiological studies.[16,22] Critical data will arise from this first step like the description of geographical scattering of industrial sites and number of workers likely to be exposed to each nano-object. This information will be useful for finalizing the subsequent steps of the protocol such as the health follow-up and the exposure assessment strategy.

Developing an exposure registry requires identifying companies concerned about nano-objects, gaining management cooperation, defining inclusion criteria, addressing issues relating to the personal confidentiality, enrolling workers, and collecting exposure data.[16,22] Included workers will be those likely to be exposed to powder of nano-objects. In each site, a highly sensitive but nonspecific definition will be used for inclusion purposes. It will rely on job titles or work tasks but not on metrological data. It can be anticipated that inclusion criteria will be different in each plant although coherence between sites should be a matter of concern. In this first step, exposure will be assessed in a qualitative or semiquantitative way (job title, work tasks, duration of employment, etc). Inclusion and exposure data will be updated prospectively.

Data available in the registry will make it possible to design a mortality follow-up, through a linkage with French deaths and causes of deaths registries. Thus, this initial step will provide a first basic and nonspecific surveillance system.

Second Step

In a second step, an additional nonspecific health follow-up will be implemented for workers registered in the exposure registry and accepting to be included in the prospective cohort. Two different components could be identified with a passive health monitoring system using already existing medical data and an active health follow-up.

For passive health follow-up, medical records collected for administrative purposes will be gathered. These will include data from health insurance organizations (such as doctor's consultations, drug deliveries, and costly chronic diseases) and from hospitals (mainly medical diagnosis following hospital discharge). Medical data recorded on a regular basis by occupational health physicians will be collected as well. The active health follow-up will be based on annual self-administered questionnaire. Beyond the collection of health data, the annual self-administered questionnaire will be useful to update contact details of workers, to keep in touch with them and to forward feedback information.

Besides the health follow-up, a quantitative assessment of exposure will be conducted. It will combine epidemiological tools such as job or task-exposure matrix and measurement strategies of the ambient aerosol on the workplace.

Among numerous parameters, which are known to influence the biological toxicity of nanomaterials, six could be chosen for measurement strategies: chemical composition, size, shape, aggregated/agglomerated state, mass, and number (Table 2). Using simultaneously different sampling techniques could help to overcome nonspecificity of instrumentation. Among the available techniques, condensation and optical particle counters and size-distributive

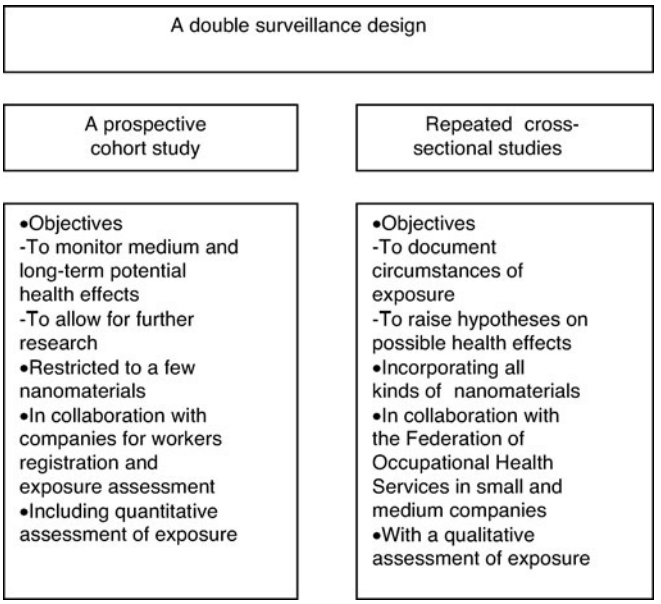

FIGURE 1. Schematic view of double surveillance design of workers likely to be exposed to engineered nanomaterials, French Institute for Public Health Surveillance, 2010.

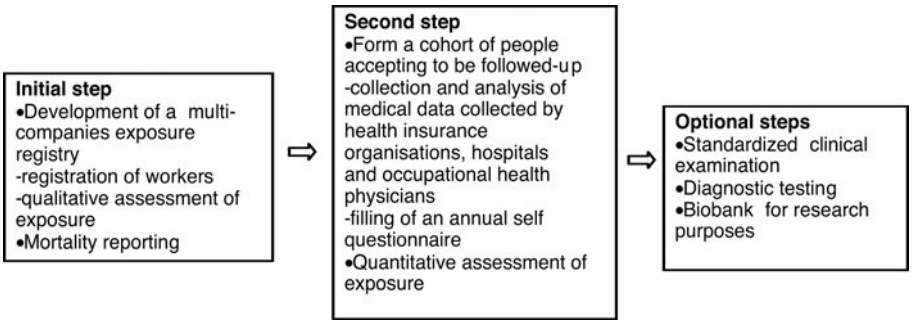

FIGURE 2. Schematic view of the step-by-step approach to implement a cohort of workers likely to be exposed to engineered nanomaterials, French Institute for Public Health Surveillance, 2010.

TABLE 2. Proposed Sampling Strategy for Measurement Protocols, Implementation of French Epidemiological Surveillance Design of Workers Likely to be Exposed by Inhalation to Engineered Nanomaterials, 2010

	Size, Shape, Aggregated/ Agglomerated State	Mass Concentration	Size Distributed Mass Concentration	Number Concentration	Size Distributive Particle Concentration
Sampled fraction	Submicronic to micronic fraction	Respirable fraction Filter-based personal sampler (ie, cyclone)	Submicronic to micronic fraction (ie, cascade impactor)	Submicronic fraction	Submicronic fraction
Methodology	Scanning and transmission electron microscopy (SEM/TEM)	Gravimetric and chemical analysis	Gravimetric and chemical analysis	Combination of condensation particle and optical particle counter	Low pressure impactor
Temporal pattern	Off-line analysis	Off-line analysis	Off-line analysis	Real-time measurement	Real-time measurement
Sensitivity to particle source or composition	Yes	Yes	Yes	No, but measurement before and after each task	No, but measurement before and after each task

particle concentrations devices should be part of the sampling strategy. Measurement campaigns could be repeated at regular time interval and after the introduction of significant improvements in the industrial process. Bulk samples of the nano-objects produced or handled on the workplace could be collected for future analysis as well. Among the possible analysis is the nanodustiness analysis, which is thought to be relevant for emission of particles from nano-objects in the form of powders.[5]

Optional Steps

Subsequently, optional modules could be implemented like standardized clinical examinations, diagnostic testing, and biobank for research purposes. Implementation of these modules will depend on serious health effects hypotheses identification, critical information arising from exposure registry, and the availability of economic resources.

Calendar

The exposure registry will be implemented within the next 3 years while finalizing the protocol of the health follow-up and the quantitative exposure assessment. It requires first to gain the authorization from the French authority in charge of privacy and personal data protection. Strong support from the government would be helpful to ensure companies collaboration.

REPEATED CROSS-SECTIONAL STUDIES

The objectives of the repeated cross-sectional studies will be to document the circumstances of exposure and to raise hypotheses on possible health effects. The protocol has not been finalized yet. Nevertheless, it will be implemented through a system of tracking workers exposed to nanomaterials, which is constituted at the moment by the Federation of Occupational Health Services in small and medium companies. Only qualitative assessment of the exposure will be available.

CONCLUSION

In response to concern about potential impact of nanomaterials exposure on human health, France has wished to develop in a timely manner an epidemiological surveillance tool that could accompany the development of nanotechnologies. In the meantime, there are not many workers producing or handling nanomaterials but the number could increase rapidly in near future.

Establishing exposure registries appears to be a valuable first step to prepare epidemiological surveillance and to implement further epidemiological research studies in this field of nanomaterials.[16,22] Working at an international scale with standardized protocols will increase the power of epidemiological studies when they are pooled. Finalizing the quantitative exposure assessment strategy will be a major issue in the coming years.

The general protocol of the health surveillance design is about to be submitted for approval and financing to the Ministries of Health and of Labour. Besides the protocol itself, the report provides some recommendations about further epidemiological research. Thus, cross-sectional studies using biological markers of effects (eg, biomarkers of pulmonary and systemic inflammation, response to oxidative stress, endothelial dysfunction, coagulation, blood viscosity, immunological effects) could be rapidly implemented on the workplace. In existing retrospective epidemiological studies of workers exposed to nanomaterials produced for decades, especially carbon black, the assessment of exposure should be reexamined in the light of what is known today about metrics likely to explain biological effects.

While scientific and social concerns have grown over the possible human health risks of nanomaterials, the development of an exposure registry of workers producing or handling nanomaterials will be a great advance for surveillance and research. Such a challenging project will require the support of all stakeholders.

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Engineered Nanomaterials

Learning from the Past, Planning for the Future

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Objective: The ongoing explosion in creation and use of engineered nanomaterials leaves stakeholders in government, industry, and labor uncertain of how best to proceed in protecting worker health. **Methods:** A synopsis is presented of the conference Nanomaterials and Worker Health, along with considerations of prior, analogous challenges in occupational health. **Results:** Progress has been made in defining and addressing the occupational threat of engineered nanomaterials, but future success demands coordinated effort. **Conclusions:** The conference Nanomaterials and Worker Health laid necessary groundwork for collaboration to proactively and preemptively address the occupational health effects of engineered nanomaterials.

Engineered nanomaterials (ENM) represent a potentially transformative challenge to public health, and the earliest health impacts of these tiny materials may be found among the workers involved in their manufacture, processing, and disposal. The National Institute for Occupational Safety and Health (NIOSH) and the Mountain and Plains Education and Research Center sponsored the conference "Nanomaterials and Worker Health" in Keystone, Colorado, on July 21 to 23, 2010, to address three key components of an occupational health response: medical surveillance; exposure registries; and epidemiologic research. The conference brought together various ENM stakeholders to share information, identify gaps in knowledge, examine successful approaches, and explore future strategies for each element of this three-part response, the importance of which has been previously articulated by conference cochairs Trout and Schulte.[1] The conference fostered thoughtful and spirited discussion toward a comprehensive plan for ENM worker safety as the exciting potential of these novel materials was realized.

An important lesson from the conference is that cooperation between stakeholders will be crucial to the success of occupational health efforts in the field of ENM. The breadth of the challenges will thwart any piecemeal approach, and unilateral regulation enforced by a single government would be insufficient to address what is already a global industry. Partnership is necessary for meaningful progress. An inspirational example was given of the Asphalt Paving Industry Partnership, a joint effort between labor, management, and government to prevent and control exposure-related illness.[2] Another example of a coordinated approach was the European response described in a report from the NanoImpactNet conference.[3] The ENM stakeholders must learn quickly from the successes and stumbles of such collaborations, as the diversity of exposures from ENM is growing at an exponential pace.[4] Also importantly, the "tripartite approach" of government, industry, and labor coordinating to address occupational health is built into NIOSH legislation. The conference was a milestone on the long journey of bringing together these stakeholders to develop a comprehensive ENM public health strategy based on shared concerns and common values.

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From diverse stakeholders came diverse perspectives, some reassuring about progress made so far and some unsettling regarding the challenges ahead (Table 1). Reports from toxicologists warned about the hazards of carbon nanotubes and nano-scale metal oxides in animal models, sometimes at far lower doses than comparable particles of larger size.[5,6] We learned that the measurement of ENM exposure lacks standard methods or validated metrics,[7] and further, we learned that we do not have information about how many and where workers are potentially exposed.[8] Risk assessment models using available animal and worker data paint a concerning picture of workplace exposures that may approach toxic levels despite being at the limit of detection with current technology.[9] More heartening, however, is research already investigating several candidate biochemical markers of ENM exposure.[10] In addition, novel assays like exhaled breath condensate and noninvasive cardiovascular monitoring may join existing screening tests to identify early disease.[11] And despite the dearth of easily accessible data, epidemiologic researchers have already made progress in identifying cohorts of workers with potential exposure for future studies.[12]

Coordination of efforts is facilitated by a systematic approach.[13] A useful systematic approach to such public health challenges is modeled by the cascade of occupational health prevention (Fig. 1). Each step along the cascade represents an opportunity for intervening to prevent exposure-associated illness. One feature of the cascade is that success at "higher" levels of prevention can reduce the burden "downstream." For example, substitution and elimination of toxic chemicals make personal protective equipment less important; early detection of pathologic changes by preclinical medical examination may prevent the need for rehabilitation. Another element of the cascade is surveillance, which provides feedback to "upstream" processes. Data from environmental monitoring guide engineering controls, and clinical diagnoses inform biological monitoring. Working from a framework like the cascade for occupational health prevention is valuable, because it brings together disparate efforts into a cohesive system greater than the sum of its parts.

The conference illuminated work being done at several steps along the prevention cascade, as well as opportunities for improvement. Premarket testing of ENM toxicology is ongoing,[5] although the pace of new materials is breathtaking.[4] The NIOSH nanotechnology field research team is defining the state and limitations of environmental monitoring of ENM exposure.[7] Presentations were given on the effectiveness of and progress in personal protective equipment,[14,15] biological monitoring,[6,10,11] and preclinical medical examination.[16,17] Lacking, yet, is the integration achieved by incorporating surveillance feedback along every step of the cascade. A collaborative conference, such as this one, lays good groundwork for such coordination.

EXPOSURE REGISTRIES AND MEDICAL SURVEILLANCE: THE CASE FOR STARTING NOW

Much of the conference dealt with the related questions of whether the time is right for instituting ENM exposure registries and regular medical surveillance of workers. Such registries would be a source of data and hypothesis generation for epidemiologic research as well as work in toxicology, biological monitoring, and exposure assessment and control. Appreciation of the value of starting to

TABLE 1. Impressions of Conference Presentations

Presenter	Topic	Impression	Presenter	Topic	Impression
Nasterlack	Surveillance	Challenges	Busnaina	Application	Motivation
Sng	Surveillance	Example	Castranova	Toxicology	Concerning
Lichty	Surveillance	Example	Kuempel	Toxicology	Concerning
Gause	Surveillance	Example	Ellenbecker	Exposure	Concerning
Kosnett	Surveillance	Promising	Geraci	Exposure	Concerning
Marchant	Registries	Challenges	Monteiro-Riviere	Exposure	Concerning
Cassidy	Registries	Example			
Wambach	Registries	Example	Erdely	Biomarkers	Concerning
Cone	Registries	Example	Li	Biomarkers	Promising
Kosnett	Registries	Example	Sayre	Regulation	Concerning
Bloch	Registries	Example		**Systematic Approach**	
Shulte (breakout)	Registries	Challenges	Trout	"Unique workplaces require unique applications of standard principles."	
Peters	Epidemiology	Concerning	Riediker	Illustrated Shewhart cycle	
Harthorn	Epidemiology	Challenges	Melius	Illustrated tripartite approach	
Eisen	Epidemiology	Challenges	Roisman	State government involvement	
Schubauer-Berigan	Epidemiology	Promising	Kreibel	"When do we know enough to act 'as if' an association is causal?"	

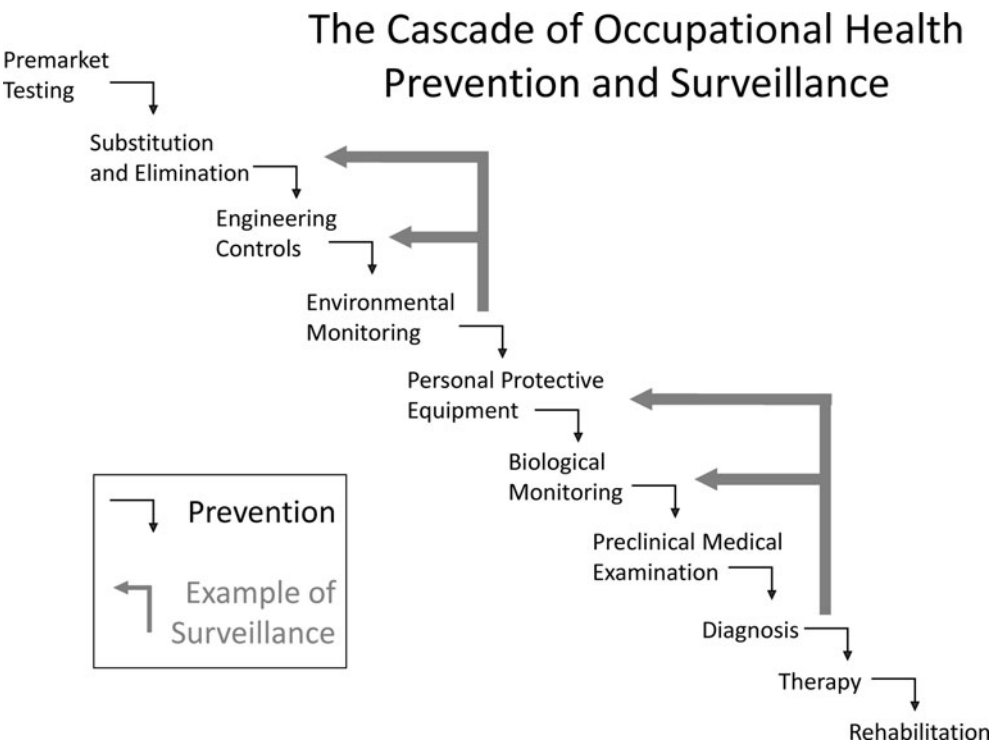

FIGURE 1. The cascade of occupational health is a model that illustrates the interacting roles of prevention and surveillance in keeping workers safe.

design and populate exposure registries as soon as possible requires consideration of three possible disaster scenarios for the burgeoning ENM industry.

As a "worst-case scenario" analogy for the risks of and for the ENM industry, consider the explosion of the Zeppelin Hindenburg in 1937. Not only was this incident a tragedy for the individuals who died in the crash, but it was likely disastrous for lighter-than-air travel, particularly hydrogen based. Although political factors and advances in airplanes certainly contributed to the decline of the industry, the dramatic footage of the burning Hindenburg ("Oh, the humanity!") signaled the end of an era of commercial dirigibles. The possibility of a similarly high-profile, "game-changing," preventable incident occurring to the nascent nanotechnology industry—or perhaps for a defined segment of it like, say, carbon nanotubes—does not far stretch the imagination, provided our imagination is primed by our prior experiences with other fibers such as asbestos.

The Hindenburg crash is an example of a "true positive" catastrophe, one in which the adverse effect is actually associated with the exposure. In contrast, we can describe a "false positive" event that could similarly cripple a growing industry. Call these possibilities type 1 and type 2 disasters, respectively. In a type 2 disaster, a cluster of adverse health outcomes is found in association with an exposure of interest, but the cluster is random and the association is just unlucky. Epidemiology and toxicology can reveal type 2 disasters to be nothing but statistical chance, but the research process can take time that may cost an industry and society dearly.

An illustration of a type 2 disaster is the case of video display terminals (VDT). Several clusters of spontaneous abortion were reported in 1980 in suspected connection with very- and extremely-low-frequency electromagnetic fields from cathode-ray tubes in VDT. Initial types of studies of occupational exposure to VDT yielded equivocal results, but many suffered from methodological flaws, such as using cohorts designed to answer a different question or assessing exposure by self-report. Radiation toxicology studies were inconclusive. Conduct of a proper epidemiologic study, with an appropriate control cohort defined and exposure to electromagnetic fields quantified in the field, took time. Fortunately for exposed women, the best-designed studies were clearly negative, showing no difference in abortion rates between cohorts and no dose–response effect with VDT exposure.[18] When a technology is as pervasive as VDT is (or ENM will be), such "false positive" clusters of adverse events are statistically inevitable. The interval between observation of chance clusters and definitive assessment will be a time of great uncertainty for industry, workers, and society that will inevitably impede the growth or unnecessarily increase the cost of this new and important technology.

A type 3 disaster is a public concern over an exposure due to fear of adverse events even in the absence of an outbreak. Consider the struggles of the genetically modified organisms industry. Promise for modern genetically modified organism technology has at times been comparable in breathlessness—a cure for world hunger and malnutrition—to that for ENM. Exciting products have been developed, such as "golden rice" containing β-carotene and "Bt-corn" that kills pests without synthetic insecticides. The most likely biological risks of the technology are similar to those for other agricultural techniques, such as cross-pollination or adverse effects on local ecosystems. Nevertheless, widespread public concerns have arisen about direct health effects on human consumers, concerns that, some say, are out of proportion to presently observed or reasonably expected consequences. The innovative and commercial promise of this technology has been retarded by bad public relations, even in the absence of a safety failure. Given the impressive novelty of ENM, similar scares are possible for its industry and should be preemptively addressed.

The public health challenge from ENM comes not only from its potential for diverse toxicologic effects[5,6] but also from its predicted ubiquity throughout industry and society. Given enough manufacturing plants, enough users along the value chain, enough consumers of ENM, eventually a high-profile cluster of health events will occur that can be plausibly linked in time and proximity to ENM exposure. This cluster may reveal an adverse effect of ENM exposure, previously unrecognized or underappreciated, a Hindenburg-like type 1 disaster that might have been prevented with more forethought and risk management. Alternatively, the cluster may be a "false positive" type 2 disaster like VDT, simply a chance grouping of unrelated health events that is statistically inevitable as the industry grows. Yet another possibility is that the cluster will happen in a movie or be a prediction of protesters, and the chilling effect of a type 3 disaster on industry may be no less severe for the outbreak being imagined. Whether the adverse effect is fact, fluke, or fiction, the human and commercial impact will grow until epidemiologists are able to precisely define the risk.

The considerable costs to establishing and maintaining exposure registries can be justified if the registries will protect workers and industry against type 1, 2, and 3 disasters. A specific example in occupational health that illustrates the value of registries is that of dioxin. In 1980, Centers for Disease Control and Prevention epidemiologists were asked by Department of Defense to study the alleged adverse effects of the herbicide "Agent Orange" used by military forces in Vietnam. To augment studies of solder exposure in the field, NIOSH proposed a study of civilian production workers exposed to a related industrial compound (2-,3-,7-,8-tetrachlorodibenzo-p-dioxin). The epidemiologists located chemical plants where exposure could be assessed, enrolled study cohorts of workers, and prospectively monitored exposure and health outcomes. Conclusions of the study were reported in 1991[19]; the time to mount the study and obtain an answer took a full decade. If preemptive registries can shorten the response time to event clusters that will inevitably affect the ENM industry and reduce the ensuing period of uncertainty, the cost of registries will be recouped many times over.

The usefulness of a registry depends on its comprehensiveness. One possible solution is to create a registry of companies: a list describing which companies are using which materials and processes. This kind of list that could be accessed to rapidly identify and assemble cohort groups of workers, saving the initial months to years of preparation necessary for finding appropriate industry cohorts to study an event cluster, is reported. The more detailed alternative proposed by many participants of this conference is a registry of workers: a list of individuals with their contact information, personal exposure history, and record of health outcomes obtained through regular surveillance. Examples were presented from the US Air Force and French Alternative Energies and Atomic Energy Commission experiences.[20,21] Such a registry would enable epidemiologists not only to design cohorts but also to immediately begin analyzing differences in exposure-controlled morbidity and mortality. After an event cluster, a preexisting worker registry would save the years to decades needed to prospectively follow individuals exposed to the suspected hazard. The savings in human health and public relations from quickly defining a "true positive," exonerating a "false positive," or assuaging nonspecific fear would easily justify the effort to build and maintain the exposure registry.

Although the future value of exposure registries is substantial, their implementation raises several nontrivial challenges, described in detail during this conference[22–24] that must be addressed. Issues of access, usability, and standardization of the registry database are crucial and will require coordination and cooperation among stakeholders. Agreements must satisfy industry needs regarding proprietary information and legal liability, needs that may otherwise be barriers to willing participation. In addition, a common understanding must be reached on the methods and appropriateness of medical surveillance of workers in the registry; such detailed consensus building might be the topic for a near-future workshop.

The contention surrounding medical surveillance centers on the clinical value to the worker of currently available screening tests. Concerns about the hazardous nature of ENM—supported to varying degrees by toxicology studies with animal models[5,6] and epidemiologic studies of ultrafine particles in diesel exhaust[25]—focus on diseases that are fairly common: restrictive pulmonary disease; cardiovascular disease and its diverse sequelae; malignancy of unspecified type; and systemic manifestations of chronic inflammation. These nonspecific endpoints, coupled with no biological markers of ENM burden, make individual screening a troublesome proposition from a clinical standpoint. Abnormal findings on pulmonary function tests, chest radiographs, or serum C-reactive protein may not be interpretable for the individual worker.[16] This uncertainty may be due to operating characteristics of the test (such as sensitivity and specificity) or due to potentially confounding risk factors (like smoking, diabetes, and other exposures). Clinically, uninterpretable test

results are particularly problematic as exposure-related pathology may impact employability or have legal repercussions. Nevertheless, even a test that is experimental, insensitive, or nonspecific can have occupational health value for comparing cohorts. Different average results in two comparable groups may suggest an engineering control flaw, resulting in higher than expected exposure, for example, or may reflect an unexpected biologic response at exposure levels previously thought safe. In addition, careful informed consent procedures can obviate the problems of test results that are not actionable at the individual level. A major precept of medical screening in nonoccupational settings is that test results must have therapeutic or prognostic value to the patient; occupational health screening in exposed workers, however, may be justified despite an indeterminate clinical value of the test. Occupational screening can detect early indicators of adverse effects and thereby lead to reduction of exposure for coworkers, a worthy goal even if the screened individual does not directly benefit from the procedure.[26]

Although existing methods and knowledge are incomplete, meaningful action is still possible. Examples of current medical surveillance were described in a variety of industrial and academic contexts.[27–29] The concerns raised at the conference about the limitations of medical surveillance with present technology, while important to address, do not outweigh the future benefits that detailed worker registries will accrue, namely aid to epidemiologists in rapidly addressing type 1, 2, and 3 disasters. Finally, we must keep in mind that populations other than workers may experience significant exposure in the future, so that proactive monitoring now may pay great dividends as ENM proliferate in society.

EPIDEMIOLOGIC RESEARCH: PREVENTIVE MEDICINE FOR A GROWING INDUSTRY

Epidemiologic research, in contrast to surveillance, is a preplanned study of limited duration that collects adequate—which often means detailed—information and samples regarding exposure to hazards, health outcomes, and other relevant variables defined in advance. The goals of analytic epidemiology are to characterize the relationship between exposure and outcome, often focusing on dose response. In contrast, exposure registries usually utilize data previously collected for administrative purposes and often have little information on confounding exposures. While registries are useful in recognizing hypothesized associations, they are of less value in assessing dose–response relationships.

Epidemiologic research is the means of confirming or refuting purported associations between hazards and health outcomes. Exposure registries of workers can provide data—the accumulation of which is the most time-intensive step in the research process—needed for epidemiologic analysis. Such research can save worker health by identifying true risk as well as reduce the commercially crippling uncertainty that accompanies suspected risk.

As described at this conference, exploratory epidemiologic studies of the ENM workforce have already begun,[12] and they should continue in earnest. All the necessary prerequisites of an epidemiologic study of ENM are present. Work in toxicology is prioritizing potential hazards. Surveys and registries are identifying vulnerable populations. Although agreement on standardized methods is yet needed, we can characterize exposure at least crudely. Through surveillance, we will assess morbidity and mortality. Perhaps, the most compelling reason to encourage and support epidemiology is that such an effort will push the development of novel methods and shape the careers of young researchers whose future expertise will be greatly needed as ENM become ubiquitous.

Several conference participants noted that we must be wary of "paralysis of analysis" or "letting the perfect be the enemy of the good." It is true that we do not yet know enough about ENM characteristics, measurement, toxicity, biological markers, and health outcomes to design precise surveillance, registries, or epidemiology. Given the scope of the challenge, however, we will likely never have as complete knowledge as we would wish. Like the clinician with a sick patient whose illness does not fit neatly into a diagnosis, we must act on imperfect information. We must be bold and wise, and importantly, we must be unafraid to change course as needed.

One answer to analysis paralysis is adoption of the well-known Shewhart cycle of iterative process improvement as a model of how ENM stakeholders should view exposure registries and medical surveillance. In the cycle of "Plan-Do-Study-Act," we are in the first stage, planning our objectives and what processes may achieve them. The necessary next stage is to do implementation of the proposed processes; only after trying them can we study their effectiveness in meeting our objectives. Study leads to action on the causes of differences between goals and current results. The cycle then repeats with new plans informed by previous attempts. Registries and surveillance should be started according to our best knowledge—best guesses when necessary—with the understanding that they will be continually refined by future conferences and workgroups in light of emerging evidence.

CONCLUSION

Someday, society may look back on the early stages of the ENM industry and ask whether appropriate caution was taken. Regret for insufficient prudence is certainly part of the history of other widespread occupational exposures in the twentieth century, such as asbestos. Nevertheless, the participants of this conference should be able to look back in pride at the proactive steps taken in Keystone. The broad array of presentations and discussions helped to define the state of the art and lay foundation for future consensus and standardization. Stakeholders in government and industry together are accepting their ethical obligation to share the costs of this wondrous new technology with the workers who will inevitably bear the brunt of exposure.

The challenge is where to go hence. Clearly, industrial innovation will be raging forward.[30] This conference presented clear messages that we must hasten our pace along numerous fronts, including toxicology, assessment of potential occupational exposure, and detection of biological effects. Although we yet need agreement on certain crucial details, we have from this conference, a path on which to proceed with epidemiologic studies and exposure registries. The efforts of this conference and the meetings to follow it reduce the chances of widespread adverse health effects and commercially crippling uncertainty as we enter the brave new world of ENM.

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