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Issues in the Development of Epidemiologic Studies of Workers Exposed to Engineered Nanoparticles

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Learning Objectives

- Demonstrate familiarity with the emerging industrial potential of nanotechnology, including industries likely to have the highest numbers of exposed workers.
- Discuss key issues to be addressed in the design and conduct of epidemiologic studies of nanoparticle exposure.
- Outline the authors' recommended steps to lay the groundwork for studying occupational exposure to nanoparticles and their potential health effects.

Abstract

Objective: *Capitalizing on phenomena at the nanoscale may present great benefits to society. Nevertheless, until the hazards and risks of engineered nanoparticles are determined, the technological products and advances of nanotechnology may be impeded by the societal concerns. Although animal data provide the necessary first step in hazard and risk assessment, ultimately epidemiological studies will be required, especially studies of workers exposed to engineered nanoparticles. It may be too soon to conduct informative epidemiological studies but it is now appropriate to identify issues that will be pertinent and prepare strategies to address them.* ***Methods:*** *The published scientific literature on incidental and engineered nanoparticles and air pollution were reviewed to identify issues in the conduct of epidemiological studies of workers exposed to engineered nanoparticles.* ***Results:*** *Twelve important issues were identified—the most critical pertaining to particle heterogeneity, temporal factors, exposure characterization, disease endpoints, and identification of the study population.* ***Conclusion:*** *Consideration of these issues provides the foundation for initiating epidemiologic research on workers exposed to engineered nanoparticles.* (J Occup Environ Med. *2009;51:323–335*)

Nanotechnology is permeating all economic sectors and one of the most pressing questions is the risk to workers exposed to engineered nanoparticles. Engineered nanoparticles are nanoscale particles intentionally produced according to explicit specifications to derive their unique characteristics. Some engineered nanoparticles have hazardous properties and precautionary workplace control measures have been widely recommended.[1–5] Generally, particles in the nanoscale range appear to be potentially more toxic than larger particles of the same composition.[6–11] Nevertheless, the evidence is not extensive or definitive, and there are no published studies of the risks of workers exposed to engineered nanoparticles. Such exposures are relatively new and generally occur in relatively controlled situations so studies of long-term exposures and chronic effects have not been possible to date. Nevertheless, the number of manufacturer-identified nanotechnology-based consumer products and commercial products containing engineered nanoparticles is over 800 (www.nanotechproject.org/inventories/consumer), and the number of workers exposed is increasing. The actual number of commercial products containing engineered nanoparticles is difficult to track. One estimate has identified $147 billion worth of products sold in 2007.[12] Therefore, given the growing use of engineered nanoparticles, epidemiologic studies of exposed workers will be needed in the near future to provide society

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with an assessment of any risks. This conclusion reflects one of the priorities in the U.S. National Nanotechnology Initiative strategy for environment, health, and safety which calls for efforts to characterize the health of exposed populations and environments.[13] Epidemiologic studies form an important link in understanding health outcomes associated with exposures to potentially hazardous materials. Well-conducted epidemiological studies of workers in other industries have provided evidence for and against various occupational chronic diseases resulting from workplace exposures. Such studies have formed the basis for quantitative risk estimations to establish levels protective of human health.[14–16]

Appreciation of the unusual behavior of materials at the nanoscale has been known for over a 100 years, but in the last 20 years, there have been tools to visualize and move matter at this scale.[17] Those capabilities and understanding have lead in the last 10 years to the development of a broad range of products and applications. Nevertheless, there is no nanotechnology industry or sector emerging rather nanotechnology spans many economic sectors, and characterizing the workplaces and workforce is a complex endeavor.[12,18]

Although the methodological issues involved in estimating risks of exposure to engineered nanoparticles epidemiologically are not intrinsically different from those involved in designing and carrying out long-term studies in other industries, there are significant issues that need consideration.[19] Inherent characteristics of nanoparticles and contemporary workplaces and the workforce may make the conduct of epidemiologic research difficult for various reasons. Consequently, it may be helpful to begin to comprehensively identify issues and barriers in conducting studies of workers exposed or who will be exposed to engineered nanoparticles so that efforts can be made to address these issues and overcome the barriers. In this article, those aspects of epidemiologic research that particularly pertain to engineered nanoparticles were identified and assessed.

Heterogeneity of Nanoparticles

Possibly, the most critical factor that will influence the conduct of epidemiological studies of workers exposed to engineered nanoparticles is the heterogeneity of nanoparticles. Figure 1 shows a conceptual depiction of a range of nanoparticles types. Various physicochemical features (size, shape, composition, charge, crystallinity, solubility, added functional groups, and impurities) can be combined in any particular nanoparticle leading to different toxic potential.[20–26] The variability in toxic potential can make it difficult to identify similarly exposed occupational groups. Failure to account for exposure heterogeneity can lead to misclassification on exposure and bias measures of association toward the null hypothesis. This is a problem that exists in every occupational epidemiological study but which is possibly more prevalent in studies of workers exposed to engineered nanoparticles. Because of the tremendous potential for variability of particle types it may be difficult to identify adequately large cohorts with exposure to the same materials. It is not known if particle size and hence, surface area, is the predominant causal factor, and whether characterization of exposure on that basis is adequate or will introduce significant differential classification of exposure which ultimately could lead to misclassification. Furthermore, the lack of a consistent industry-wide exposure assessment program across facilities producing or using nanoparticles will increase the challenge associated with epidemiologic research. It is likely that during the various time periods considered for study that exposure will be to more than one type of engineered nanoparticle as well as to incidental nanoparticles (often called ultrafines) and to other toxic substances making it difficult for the epidemiologist to differentiate risks associated with specific nanoparticles.

The issue is further complicated by the fact that there is still debate over what actual particle size threshold should be considered for assessing human health impact.[27] Generally, 100 nm has been the arbitrary demarcation point for nanoparticles; however, particles with a dimension greater than 100 nm also can have the same potential health effects as those less than 100 nm.[28] Moreover, agglomerates of nanoparticles in these larger size ranges can de-agglomerate, or even when whole, present large surface areas that can influence biological response. The perceived heterogeneity of nanoparticles is also influenced by the terminology to describe nanoparticles, particularly carbon nanotubes. The American National Standards Institute concluded that the lack of universal terminology was related to 1) commercial reasons; 2) patents and intellectual property protection; 3) regulatory impacts and labeling concerns.[29,30] Nevertheless, the International Organization for Standardization recently has developed "ISO/TS 27687:2008, Nanotechnologies—Terminology and definitions for nano-objects—nanoparticle, nanofibre, and nanoplate" which is the first in a projected series on terminology and definition documents covering the different aspects of nanotechnologies.[31] In addition to the question of terminology, there is still the reality that there could be large numbers of different engineered nanoparticles. For example, more than 50,000 different carbon nanotube types have been produced.[32] The basis for this estimate is a calculation of the combination of different production processes, purification methods, surface coatings, and structural types. Given the number of combinations of physicochemical and process factors, the estimated number may not be unreasonable and a similar estimation procedure might be applied to other

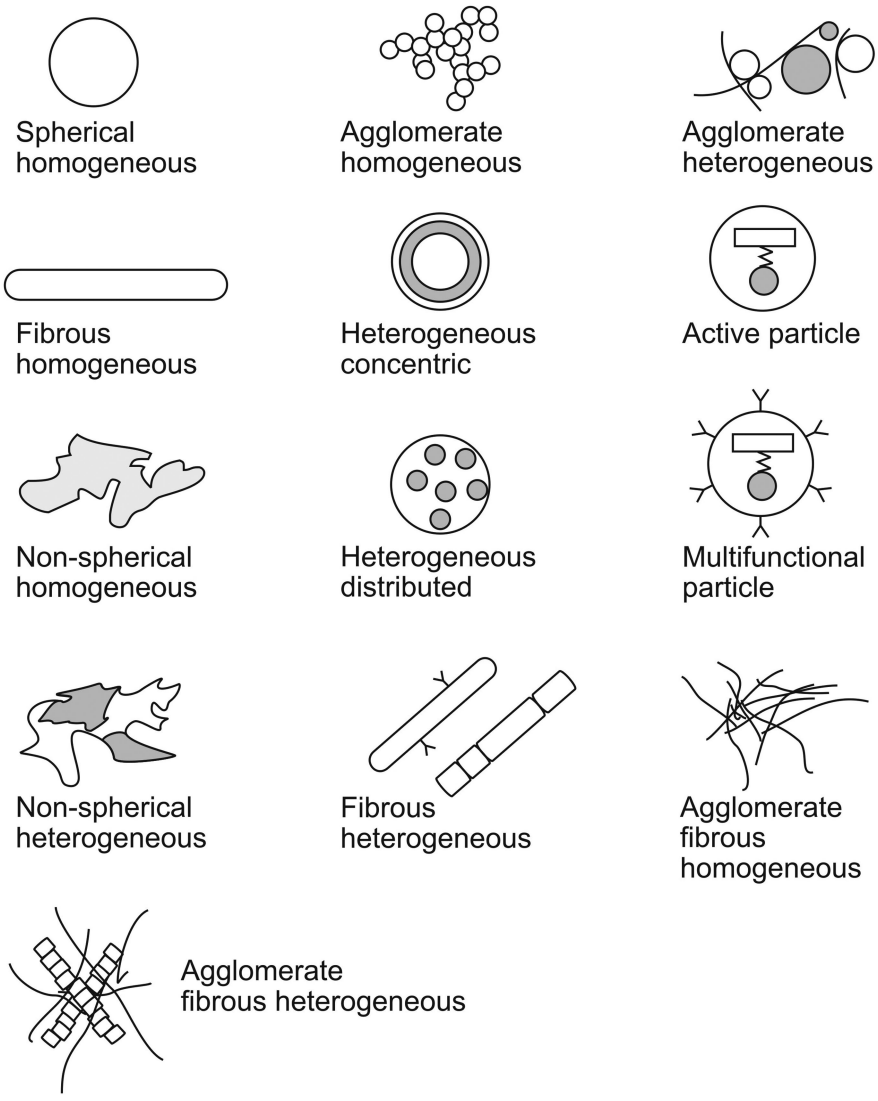

Fig. 1. Example of different types of airborne engineered nanoparticles (adapted from reference 20).

types of engineered nanoparticles. Whether this vast number of carbon nanotubes will be used in commerce is not known. It is known that the number of U.S.-based carbon nanotube-related patents issued between 1994 and 2006 is 1865, and as of 2008, there are 4500 patents pending.[30,33]

Disease Endpoints

Another important question is what disease endpoints to include in epidemiological studies of workers exposed to engineered nanoparticles. This is important because a priori hypotheses can influence study design and interpretation of results. Hypothesis generating studies are also useful but studies that test hypotheses involving specific disease endpoints ultimately are more informative. Initial information on the adverse effects of engineered nanoparticles comes from the studies of incidental nanoparticles, air pollution epidemiology, and studies of workers with various occupational exposures such as welding fumes, ultrafine carbon (eg, carbon black), or diesel fumes.[7,19,28,34–41] From these studies, malignant and non-malignant respiratory disease and cardiovascular diseases were most often found although not all nanoparticles give the same biological responses.[6,7,24,28] Moreover, many air pollution studies do not indicate specific size particles, only categorical ranges, and many studies are ecological with no individual measure of exposure. Nonetheless, there have been numerous studies that investigated health effects of ambient and occupational air pollution particles of various size ranges using personal monitoring.[27,42] Exposure to incidental nanoparticles (eg, generated from combustion or hot processes) has been associated with various adverse health effects in workers. For example, diesel exhaust has been associated with eye and respiratory irritation, endothelial dysfunction (impaired vasodilation) with mild systemic inflammation, and lung cancer, whereas welding fume exposure has been associated with metal fume fever, susceptibility to pulmonary infection, obstructive lung disease, and possible neurologic changes.[38,42–52] From the enormous body of epidemiology concerning health effects of exposure to particles in the workplace and outdoor air and indoor environments, health effects have been found to vary according to:

- intensity of exposure
- duration of exposure
- size distribution of particles in the inhalable size range
- composition of particles
- susceptibility, including preexisting health status of individuals
- possible interactions with other risk factors (socioeconomic, smoking habits, etc).[19]

Investigation of health effects from exposure to engineered nanoparticles generally has been limited to animal studies. Nevertheless, there have been epidemiological studies of carbon black and synthetic amorphous (fumed silica) which found respiratory effects including lung cancer for carbon black and pneumoconiotic effects for fumed silica.[53,54] Pulmonary exposure to carbon nanotubes has shown progressive fibrotic effects after single or short-term exposures as well as transient pulmonary and circulatory inflammatory

effects.[8,9,55–59] Studies of animals have shown that various engineered nanoparticles can translocate from the lungs to the circulatory system and to various organ systems including the brain either through translocation along the olfactory nerve or through the circulatory system.[60–62] Exposure of animals to some high aspect ratio engineered nanoparticles has resulted in increased susceptibility to infectious disease.[63] Some in vitro studies have shown cytogenetic effects of different types of engineered nanoparticles but only a very small number have been studied in animals. There are no published animal studies of the effects of long-term exposures. It is not clear the extent to which the findings of short-term animal studies can indicate what endpoints to include in epidemiological studies or medical surveillance. Nevertheless, inference from findings in studies of workers exposed to other high aspect ratio particles, such as asbestos, suggests that malignant and fibrotic respiratory diseases are likely outcomes of interest for workers exposed to high aspect ratio nanoparticles.[26,55,56,58,64–66] As long-term exposures occur and lifetime disease assessments are conducted in animals, more information of specific outcomes that should be able to be assessed in humans will become known. At this time, assessment of which effects to consider may be speculative but initially decreased lung function and pulmonary radiologic changes might be useful endpoint surrogates. Nevertheless, it is known from ambient air pollution studies that cardiovascular effects become evident at air concentrations where respiratory effects are very weak and difficult to detect.[67] Changes in cardiac function may also be useful endpoints. Nonetheless, a review of all the relevant health data concluded that medical testing of asymptomatic workers is not warranted at this time because a strong rationale for specific disease endpoints could not be found.[68]

Temporal Factors

Although there have been historical antecedents and long-term scientific awareness and utilization of some types of nanoparticles (eg, as catalysts and colloids), the major commercial emergence of nanotechnology is generally less than 10 years old. The initial steps in the emergence involved research and pilot facilities, rapidly changing nanoparticle products, and a relatively small number of workers. Consequently, currently there may not be a large population of workers with long-term exposure to engineered nanomaterials. Moreover, given the absence of extensive toxicological data, including data on acute effects from exposures, a discussion of temporal issues is problematic. Even as the technology permeates various economic sectors and is used to develop numerous products that result in various exposures, the number of people actually exposed for some period that could significantly put them at risk of chronic effects may not be large enough to form an adequate recruitment pool or sampling frame for conducting epidemiologic studies for many years. This means that obtaining results from sufficiently powered studies of chronic effects with adequate latency may not be feasible in the near future. Determination of when there would be adequate exposures and latency to begin to conduct epidemiological studies is compounded by issues of exposure such as heterogeneity of exposure, lack of contemporaneous exposure information that is consistently collected within and across industries, and extent/magnitude of exposure. Will there be enough workers with actual exposure to the same nanoparticle so that if these exposures cause disease cases can be found for a case-control or case cohort study? Similarly, will we need to wait at least 20 years before retrospective cohort studies have sufficient power to detect risks associated with disease with long latencies, like cancer? If prospective studies are used and similar issues arise, analysis decisions and research may possibly be made in a more timely fashion. In the meantime, it may be possible and desirable to conduct studies using biological markers of intermediate effects such as indicators of reactive oxygen species formation or inflammation (refer Intermediate Biomarkers section). These could be used in exposure-selective cross-sectional studies or prospective studies. Small scale studies of 50 or 100 workers could be conducted within the next 5 years, but at best will yield useful information if the biological markers included in them have been validated for disease risk prediction. It is not known whether there are other acute effects that could be studied in a shorter period.

Exposure Identification and Characterization

Critical in conducting epidemiologic studies is assurance of the sufficiency of exposure of study participants to engineered nanoparticles. If workers are minimally exposed, due to enclosed processes, studies may be uninformative. Nevertheless, if there is sufficient exposure to cause acute and chronic effects, studies may be able to be conducted. In order to select subjects for study, there will be a need to know the level of exposures by jobs and processes. This is necessary in design of studies as well as for data analysis. The choice of exposure metrics is important since it is likely that various metrics such as weight/unit volume, particle number, particle size distribution, surface area, and surface chemistry will be useful for characterizing risks. Moreover, nanoparticle aerosols are highly dynamic; nanoparticles in sufficient concentrations will agglomerate rapidly.[17,19,27] This can affect particle number concentrations, as well as physical and chemical characterizations.

There are currently no national or international consensus standards on

measurement techniques for engineered nanoparticles in the workplace, and there have been few published studies of exposure concentrations of workers to engineered nanoparticles. The National Institute for Occupational Safety and Health (NIOSH) has proposed a strategy for conducting exposure assessments that discusses the strengths and limitations of numerous nanoparticle measurement techniques.[5] Although it is not a national consensus standard, this document may be useful to researchers and practitioners in nanotechnology-related manufacturing. NIOSH has also conducted preliminary site surveys where a limited number of nanoparticle metrics were collected to assess potential worker exposure.[69–72] More exposure data will need to be collected if epidemiologic studies are to be designed properly and accurately represent exposures. Some of the most informative epidemiologic studies for hazard assessment and quantitative risk estimation (eg, studies of uranium miners on the Colorado Plateau) rely on exposure data that were collected and identified concomitantly with specific job tasks. Comprehensive exposure surveys can convey critical information about the size of the exposed population and the heterogeneity of exposure types, as well as provide exposure estimates by job or job tasks for use in epidemiologic studies. The assessment of exposure of engineered nanoparticles may need to include assessment and adjustment for incidental nanoparticles (eg, products of combustion from forklifts and heaters) that are often found in the same environments. In both the adjustment for incidental nanoparticles and in assessing of particle identity, there will be a need for electron microscopy analysis to characterize and classify particles.

The distribution of exposures and exposure variability are also critical issues especially in studies of exposure-response relationships. Such studies require sufficient variability in exposures and sufficient distribution of exposures to provide contrasts necessary to estimate relationships reliably.[19] It may be in nanotechnology-related industries that such variability will be difficult to identify in terms of duration or intensity of exposure reaffirming the need to document exposures by job and job tasks so that appropriate exposures can be assigned to individuals.

Exposure variability and characterization can also be assessed using mathematical models involving key elements such as the air dispersion characteristics, the engineered nanoparticle emission rate, the worker's distance from the emission source, and other factors.[73] In addition, environmental and occupational models involving data that utilize individual, indoor and outdoor background measurements can be analyzed using statistical methods to understand multifaceted covariates.[74] In another approach, NIOSH investigators utilized a semi-quantitative process, the Nanoparticle Emission Assessment Technique[5], to identify and understand engineered nanoparticle emissions and distinguish them from background emissions. In addition, a Nanoparticle Emission Simulator is currently being developed in Finland to quantify real-time measurement of nanoparticle properties for emission sources (http://www.uku.fi/laitokset/ift/projects.shtml). These and other methods and tools will be important for understanding and quantifying nanoparticle exposure.

Identification of the Study Population

A critical factor in developing epidemiological studies of workers exposed to engineered nanoparticles is identifying the workers in the source population and in the study population. Nanotechnology is not an industry, but a value chain (the chain of activities and companies that give products additional value) with various functional sectors and occupational groups.[12] Therefore, understanding the current and future nanotechnology market trends, along with business and research targeted surveys will be essential to identifying workers with potential exposure to engineered nanoparticles in the source population. The largest and earliest-developed occupational sector of engineered nanoparticles is the manufacturing and materials sector. By 2014, it is projected that about 4% of manufacturing and materials output will incorporate nanotechnology. Nevertheless, 16% of manufactured goods in health care and life sciences, and 50% of manufactured goods in electronics and information technology will involve nano-enabled materials and products.[12] At present, there is not a standardized nomenclature for nanomaterials, however, one is being developed (http://www.ansi.org/isotc229tag). Generally, the most common nanomaterial types are fullerenes, carbon nanotubes, quantum dots, metal nanoparticles, nanowires, nanoporous materials, metal oxide/ceramic nanoparticles, and nanofibers.[12] According to the Lux Research reference study, the Nanotech Report 5th Edition, ceramic nanoparticles (including metal oxides) are the most common engineered nanoparticle type (Fig. 2) with a $1.1 billion projected market size by 2011.[12] It is estimated that over 150 companies worldwide are manufacturing or incorporating metal oxide/ceramic nanoparticles into products, and nanotechnology workers in these companies could provide a good initial source population from which to begin selecting a cohort.[75] The next most prevalent engineered nanoparticles are carbon nanotubes, which have received considerable attention due to their unique properties and the potential to cause inflammatory effects in mice toxicology studies.[76,77] Carbon nanotubes are expected to comprise a $460 million market value by 2011, and will involve workers in electrical, medical, energy, manufacturing sectors and materials.[12] To identify potential study

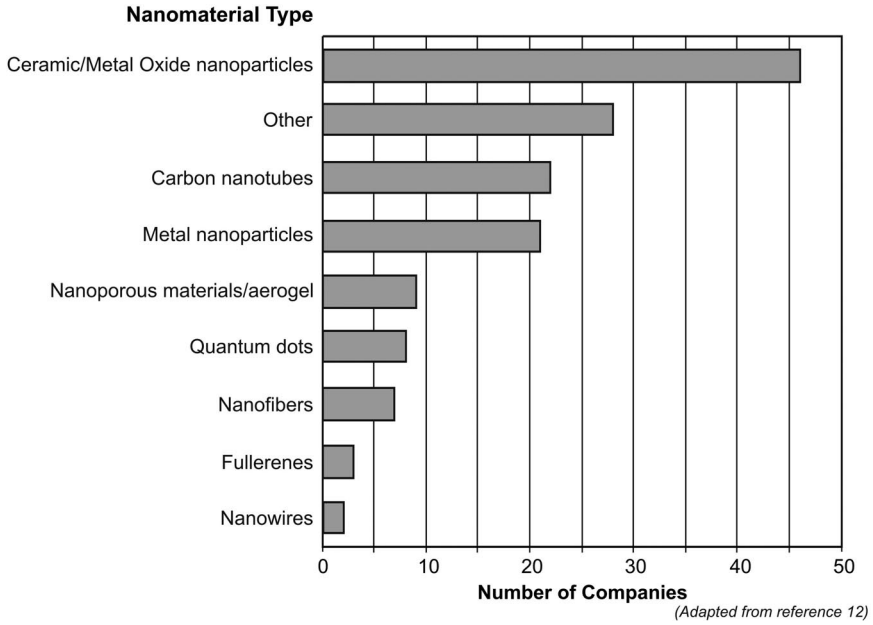

Fig. 2. Distribution of engineered nanoparticles in a survey of 121 companies.

populations, it would be useful to analyze the current and future landscape of nanotechnology occupational sectors and trends of prominent engineered nanoparticle types, and to monitor the research and overall employment trends of universities, companies, and government agencies conducting nanotechnology research.

A study population is a subset of the source population from which a cohort is developed. Three main factors influence the identification of the study population. First, the average size of the exposed workforce in any one location may be relatively small. The total number of nanotechnology workers has significantly increased over the past several years and is projected to continue to increase.[12] Nevertheless, the size of the workforce in a respective facility will depend on the economic sector. Although start-up businesses and university research facilities comprise a large sector of the nanotechnology workforce, small/midsize to large manufacturing companies may ultimately employ more nanotechnology workers (Fig. 3). Based on an analysis of materials and electronic firms, there was an average of 75 engineered nanoparticle-related workers per company in 2008, and that number is projected to rise in subsequent years.[12] The challenge is to identify a workforce group within a respective economic sector that is representative of the source population in that sector. Currently, the number of workers with potential exposure to engineered nanoparticles is not known. The National Science Foundation estimated (basis unknown) that by 2015 there will need to be at least 800,000 workers in the United States and 2 million workers globally to support a $1 trillion market.[78]

Boccuni et al,[18] reported on one of the initial efforts to identify more precisely the number of workers in Italy potentially exposed to engineered nanoparticles. They used an approach that involved identifying industrial activities likely to cause exposure to engineered nanoparticles. These activities included nanotechnology research and development, ultrafine particle manufacturing processes, and powder handling processes. Then, using the Statistical Classification of Economics Activities in the European Community, they identified the production/industrial processes that could involve exposure to engineered nanoparticles and where these processes occurred in each economic category.[79] They then used the 8th Census of Industry and Services in Italy to identify the number of employees in each industrial activity for each category and designated these as potentially exposed.[80] They concluded that up to 670,000 workers were potentially exposed to engineered nanoparticles; however, the authors indicated that this is an overestimate since they refer to all workers in each economic category potentially at risk but with no data on the percentage of companies that use manufactured nanoparticles in each sector. Nonetheless, this effort represents an early attempt to more precisely identify the size and location of source populations of workers potentially exposed to engineered nanoparticles.

Another factor contributing to the determination of the source population is the ability to identify workers in various locations exposed to the same engineered nanoparticle (or nanoparticles with the same toxic potential). This is a complex task because of the absence of standardized nomenclature of engineered nanoparticles which further complicates characterization of the types of particles to which workers are exposed. In addition, toxicological studies with engineered nanoparticles are just beginning, so it may be years before engineered nanoparticles can be grouped by their toxic potential. Tracking "nanodistricts," regional networks of public and private organizations and companies with nanotechnology-related interests, may present a reasonable method to identify the locations and characteristics of nanotechnology workers.[81,82] It is of particular interest to find out whether these nanodistricts are producing or using similar types of engineered nanoparticles. According to the Project on Emerging Nanotechnologies (www.nanotechproject.org), the top nano-

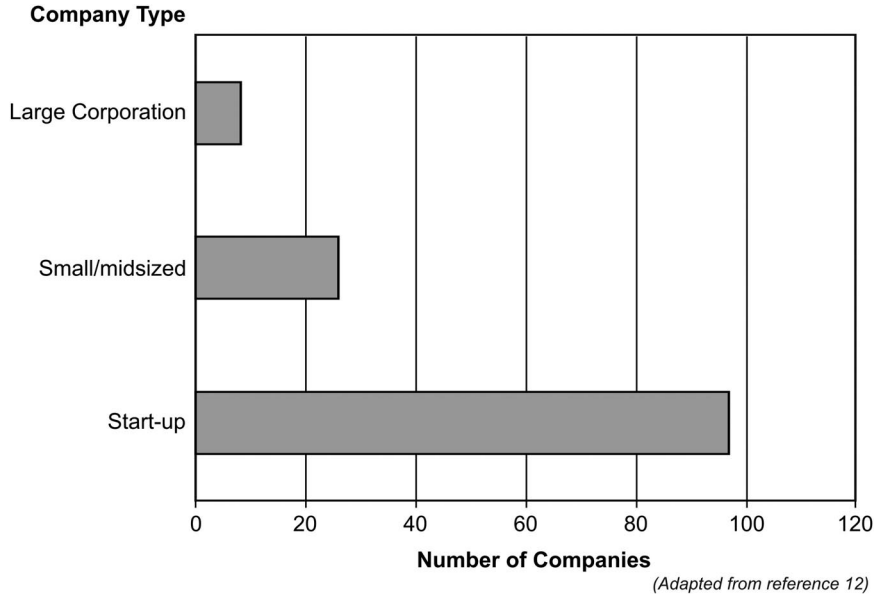

Fig. 3. Distribution of companies (n=121) involved with nanotechnology.

technology areas in the United States are California, Massachusetts, New York, and Texas. Nanotechnology is still in the early passive nanostructures phase, and it is challenging to fully predict the location and formation of all nanodistricts.[83,84] Currently, there is no extensive compilation of the level of demand or the number of jobs generated by the growth of nanotechnology.[85] A registry of nanotech job growth identified by job board indices, company and research facility job functions, and university alumni registries related to the nanotechnology occupational sectors could help epidemiologists identify and characterize a study population.

A third challenge to identifying a study population is finding workers with sufficient exposure concentrations and lengths of exposure (refer Temporal Factors section). Exposure to engineered nanoparticles can occur in various operations and job tasks across a range of economic sectors as shown in Fig. 4. These can be exposure in research laboratories, start up/pilot facilities, and in manufacturing, production, and disposal operations. Ideally, it would be best to select study populations from the same industry sector with similar operations and job tasks, but in order to identify sufficient numbers of workers with exposure to the same particles it may be necessary to draw samples from different industry sectors. This heterogeneity may also require that study populations be selected from various countries and combined into a multinational cohort to have similarly exposed groups. This will present additional logistic and methodological issues such as different job classifications, exposure misclassification, and other possible confounding variables.

Other Design Issues

Selecting the proper epidemiological study design will depend primarily on the goal of the study, and the quality, and type of available data. Factors that contribute to the type of study that is possible include the nature of exposure, the location of exposed populations, and various key temporal issues. If not enough time has elapsed for adequate exposure to occur and for disease to develop a study may not be feasible at all. Critical in the study design will be the need to account for particle heterogeneity.

Moreover, because many of the disease endpoints that could be expected in workers exposed to engineered nanoparticles may be the same as disease endpoints that may affect a significant fraction of the unexposed general population, it will be necessary to have sufficient size samples and statistical power to find differences between exposed and non-exposed groups. Prospective epidemiologic designs may prove useful for the study of selected nanomaterials (eg, carbon nanotubes). Such designs may reduce the impact of the limitations described above by permitting the contemporaneous characterization of exposure and early disease (or biomarkers of disease). Such an approach has precedent in occupational health and safety. With much foresight, a prospective cohort study initiated in 1950 of a new technology (uranium mining on the Colorado Plateau) permitted the quantitative assessment of lung cancer risk within 20 years of the initiation of the exposures.[86] By forming cohorts with potential nanoparticle exposure and following them forward, they would be available for study in the future. In addition, should a disease endpoint become clear, a nested case-control approach could be used. Nevertheless, all such studies should only be considered after primary prevention and prudent practices are put in place to control or reduce exposures.[87] Another critical feature in the design and analysis of epidemiologic studies of engineered nanoparticle exposures is the identification and measurement of confounding factors particularly those related to respiratory and cardiovascular disease. Since such studies will generally cover recent time periods, it may be relatively easy to collect data on various confounding factors.

Analysis Issues

Important in the analysis of data will be the need to adjust for misclassification of exposure (in terms of particle characterization) as well as for the appropriate confounding factors that will be involved with the

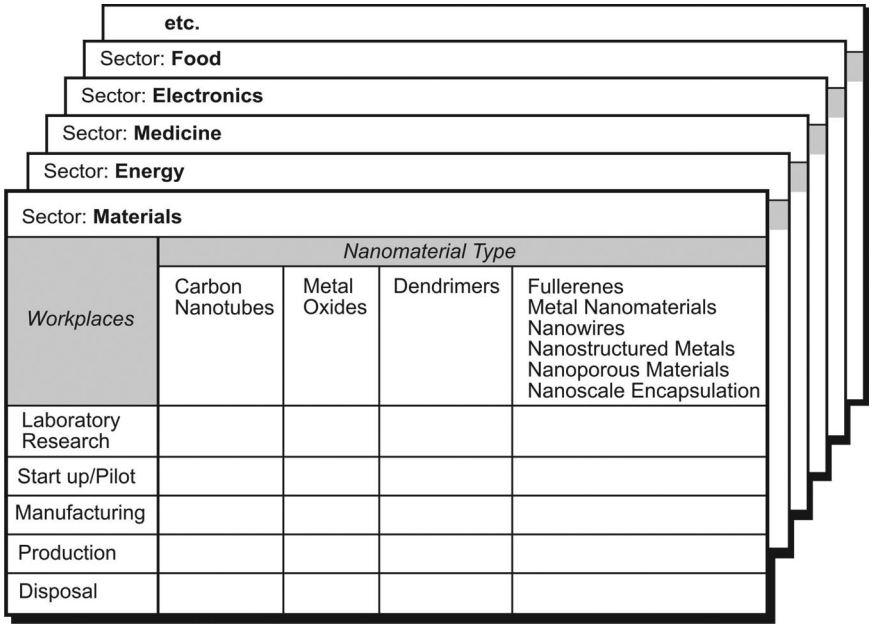

Fig. 4. Framework for identifying worksites with occupational exposure to engineered nanoparticles.

disease endpoints of interest. It will also be important to account for the types of workplace controls that could affect worker exposures. Although the research being discussed in this paper pertains to engineered nanoparticles, there may be other exposures (including incidental nanoparticles as well as various other respiratory or cardiovascular toxicants) in the workplace that will confound exposure disease associations and which should be addressed in the analysis. Analyses also will need to be conducted using different exposure metrics to determine which are most appropriate.

Intermediate Biomarkers

Because many study subpopulations will not have had long exposure or latency periods, frank disease may not have time to occur. Rather than waiting, it seems prudent to consider identifying and studying intermediate biological changes or effects that could be indicative or predictive of disease.[87–90] Nevertheless, before any use of biomarkers other than in research, there would need to be validation and consideration of their clinical utility.[91] Biomarkers indicative of the formation of reactive oxygen species or inflammation have been considered as possible early effect indicators for cancer, pulmonary fibrosis, and cardiovascular disease.[90,92–95] Utilizing such biomarkers in exposure-selective cross-sectional studies or in prospective studies could be useful in developing preliminary indications of risk. Ideally, markers sufficiently validated for disease endpoints would be used. Nevertheless, validation is not an all or nothing state, but rather a process requiring assessment of sensitivity, specificity, and a predictive value of the biomarkers.[91] Biomarkers found in animal studies after exposure to engineered nanoparticles may be then assessed in worker groups to determine their association with exposure and ultimately with disease. Biologic effect markers may also be useful in addressing the issue of particle heterogeneity and the inability to identify and recruit enough workers with the same exposure. It may be that exposure to the same type of engineered nanoparticles is not the critical feature but rather whether exposures to different engineered nanoparticles may lead to the same pathway for disease. For example, if formation of reactive oxygen species or inflammation is a key step in disease, then it may be possible to combine groups with different exposures if it is known that those exposures follow those same pathways.[90] Alternatively, workers could be monitored and tested for reactive oxygen species or inflammation markers and exposure assessment could be based on distribution of the magnitude of these biomarkers. Thus, exposure to different particles will be converted to categories of "units of inflammatory biomarkers." In addition, traditional exposure assessment might be supplemented with biological monitoring for biomarkers of exposure, which could be the actual particle in biological fluids. Such studies will require preliminary investigation in animals and greatly benefit from contemporaneous characterization of workplace exposures to nanomaterials of concern.

Informed Consent/ Privacy/Confidentiality

It is most likely that epidemiological studies will be initially focused on morbidity in workers. Recruiting workers will require development of informed consent procedures with a stipulation of individual privacy and confidentiality. This includes studies involving linkage to medical records, conducting medical tests, or a collection of biological specimens. It will be important to consider what message will be given to participants and employers about the findings of tests and studies. Most epidemiological studies will involve workers currently employed in companies involved with engineered nanoparticles. Thus, it may be necessary to secure cooperation and participation not only of the workers but also of the company as well. Many aspects of nanotechnology are considered proprietary and companies may be reticent to participate without assurances of confidentiality for information that pertains to both workers and

trade secrets and stipulations for interpreting and communicating results of tests and of studies. If individual medical tests or assays for biological markers are conducted, there needs to be planning for how results will be interpreted clinically and what procedures will be utilized for participants with test results in the extreme of the distribution of results. There will need to be a plan for how to maintain employee privacy and confidentiality of information when reporting group results that could be deciphered because of unique job-related circumstances or factors. Many workplaces may have relatively small numbers of workers. This means that variables that describe workers by exposure conditions could inadvertently identify the worker and health information linked to them. These considerations would need to be addressed in informed consent documents. In addition, if biological specimens are collected, workers will need to be informed of the purpose for which the specimen will be used and if specimens are stored, how future use will be determined, and whether further consents will be needed.

Exposure Registries

Exposure registries have been used in public health for over 50 years, and are especially useful when the risks to workers are not well-defined.[96] Because the formation of cohorts for an epidemiological study may involve combining workers from different companies and possibly different countries, there may be a need for a preparatory step such as the establishment of exposure registries.[97,98] Exposure registries may serve as sampling frames for epidemiological studies and provide for standardized approaches for exposure assessment. Many of the issues in conducting epidemiologic studies are foreshadowed in the establishment of exposure registries. This includes identification of target companies, obtaining participation of management and workers, collection of exposure data, and addressing issues of business and personal confidentiality. Exposure registries are not only useful as sampling frames for epidemiologic studies but also as tools for conducting surveillance and risk communication.[96]

Linkage With Medical Surveillance

Medical surveillance utilizes the findings of epidemiologic and medical research to indicate what disease endpoints should be monitored. These findings are the disease endpoints that are assessed as incidence data in medical surveillance at the population or group level, or on an individual level as the outcome that medical tests are administered to detect.[97] Medical surveillance of disease statistics on groups of workers should be analyzed on a group basis. This is an epidemiologic activity that is complicated by the non-specificity of most outcomes of concern (eg, malignant and non-malignant respiratory illness and cardiovascular disease). Ultimately, however, a designed analytic study is more likely to provide etiologic insight than a surveillance-based assessment. Medical surveillance is also useful to identify untoward or sentinel events in populations to indicate either the early occurrence of an "epidemic" which in a working population may be the increased incidence of some known outcome. Sentinel events may also be used to assess failure of control measures. Such use is complicated by the fact that many health outcomes of concern for nanomaterials exposure (eg, respiratory cancer, obstructive pulmonary disease, and cardiovascular disease) are relatively common and may have many other causes.

Utilization of Epidemiologic Data in Quantitative Risk Assessments

Quantitative risk assessment involves the assessment of dose (exposure)-response relationships to identify the shape of the curve and the levels of exposure that could result in different disease risks. Ideally, human data, obtained from epidemiological studies, would be used but often extrapolation from animal studies has been the basis for risk assessments. Extrapolation to humans requires various scaling factors to account for specimen differences (eg, in uptake, distribution, metabolisms, and excretion). Ideally, in conducting epidemiological studies, exposure will be assessed using metrics that have been identified in animal studies so that comparison with animal results can be readily accomplished and the choice of endpoints in epidemiologic studies can be supported.[64,99] Different metrics may be associated with different measures of risk. For example, lung cancer data from various poorly soluble, low-toxicity dusts have been shown to fit the same curve when plotted on a surface area basis.[64,99] Historically, epidemiologic studies sometimes have been difficult to use in risk assessment due to vague specification of exposure and dose.[100] Nevertheless, examples exist (eg, radon-exposed miners) in which prospective cohort studies have produced data that forms the basis of quantitative risk assessments.[14,101,102] This could be more problematic with risk assessments for engineered nanoparticles because of the different possible exposure metrics that could be used in epidemiologic studies. Ideally, toxicologists, epidemiologists, and risk assessors would collaborate and discuss these issues before research is conducted so that sufficient information, collected retrospectively and prospectively, will be available to characterize workforces and exposures.

Conclusions

At present, there have been no epidemiological studies of workers involved with engineered nanoparticles. It may be arguable that carbon black is intentionally produced to be a nanoparticle and there have been epidemiological studies of carbon

black, but this is not indicative or illustrative of nanotechnology or of the general classes of engineered nanoparticles. Nevertheless, there appears to be an increasing number of workers involved with engineered nanoparticles.[12,18,20,103] Unfortunately, there is a paucity of data on how many workers are potentially exposed, where, to what extent, and for how long. For epidemiologic research to proceed there is a need for this type of business intelligence to be collected. It is only when this information is obtained for epidemiologic research will investigators being able to begin to design studies in earnest. At present, as a first step, NIOSH has recommended that employers consider hazard surveillance as a basis for risk management programs.[68] Meanwhile, there is continued need for toxicological research to further identify adverse effects that might be seen in workers, the mechanisms of action, and to identify potential intermediate biological markers. In addition, there is a need for more extensive data on the nature, extent, and magnitude of exposure to engineered nanoparticles. To fully achieve this, there is a need for: 1) consensus on the exposure metrics that are correlated with the health outcomes of interest; 2) the development of practical and easy to use field instruments to allow for breathing zone assessments; and 3) the linking of nanoparticle exposure to jobs and tasks. Finally, the establishment of exposure registries may be warranted and would serve as a useful preparatory step in planning for epidemiologic studies. Questions that arise in considering exposure registries include:

- Who would manage them
- What data would be collected
- Who would have access to the data
- Could any investigator with an epidemiologic research proposal have access to registry data?
- Are there non-research implications and responsibilities for those who manage a registry and expectations by those who participate in them?

Clearly, exposure registries are not prerequisite for the conduct of informative epidemiologic studies, but if such studies are to be conducted, and they should be, the issues identified in this paper require consideration. In addition to the technical issues, it is also critical for the conduct of epidemiological studies to obtain buy-in from managements, workers, and others. This will require proactive efforts to engage employers, labor unions, trade associations, and government agencies to begin to consider epidemiologic studies.

Nanotechnology promises to contribute much to the betterment of humanity but without appropriate assessment of the risks, the technology will not be developed safely and with public confidence. The consideration of cross-sectional, prospective, or retrospective epidemiologic studies or exposure registries, should not preclude the application of prudent measures to minimize exposures among the nanomaterials workforce. Nevertheless, since epidemiologic studies are the epitome of safety and health assessments, it is not too soon to be anticipatory and to consider how effectively to conduct such studies.

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