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Assessing the first wave of epidemiological studies of nanomaterial workers

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Abstract

The results of early animal studies of engineered nanomaterials (ENMs) and air pollution epidemiology suggest that it is important to assess the health of ENM workers. Initial epidemiological studies of workers' exposure to ENMs (<100 nm) are reviewed and characterized for their study designs, findings, and limitations. Of the 15 studies, 11 were cross-sectional, 4 were longitudinal (1 was both cross-sectional and longitudinal in design), and 1 was a descriptive pilot study. Generally, the studies used biologic markers as the dependent variables. All 11 cross-sectional studies showed a positive relationship between various biomarkers and ENM exposures. Three of the four longitudinal studies showed a negative relationship; the fourth showed positive

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Contributorship SHL and CSJT conceived the study. SHL, CSJT, DP, and MKSB searched and checked the databases according to the inclusion and exclusion criteria. PAS helped to develop search strategies. SHL, CSJT, DP, MKSB, and PAS extracted the data and assessed their quality. SHL wrote the draft of the paper. All authors contributed to writing, reviewing, or revising the paper and read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

findings after a 1-year follow-up. Each study considered exposure to ENMs as the independent variable. Exposure was assessed by mass concentration in 10 studies and by particle count in six studies. Six of them assessed both mass and particle concentrations. Some of the studies had limited exposure data because of inadequate exposure assessment. Generally, exposure levels were not very high in comparison to those in human inhalation chamber studies, but there were some exceptions. Most studies involved a small sample size, from 2 to 258 exposed workers. These studies represent the first wave of epidemiological studies of ENM workers. They are limited by small numbers of participants, inconsistent (and in some cases inadequate) exposure assessments, generally low exposures, and short intervals between exposure and effect. Still, these studies are a foundation for future work; they provide insight into where ENM workers are experiencing potentially adverse effects that might be related to ENM exposures.

Keywords

Epidemiological studies; Nanomaterial workers; Biological markers; Cross-sectional study; Longitudinal panel study; Sample size; Nanoparticle exposure

Introduction

Nanomaterials and advanced materials have been used in commerce since the early 2000s. Engineered nanomaterials (ENMs) are now being used in many different types of commercially available products, including electronics, medical and health care products, advanced polymers, food, textiles, athletic gear, and household products (Bekker et al. 2013; Borm et al. 2006). The use of nanomaterials in industrial and consumer applications continues to grow. As of April 2015, the publicly available online inventory of ENM-based consumer products (http://www.nanotechproject.org/cpi/) contained 1,800 + products. It is estimated that in 2015, the market for ENM-containing products will reach the $1 trillion milestone (Seal and Karn 2014). The use of nanomaterials in the development of novel materials for new purposes has led to an increase in the numbers of workers exposed to nano-objects, their aggregates and agglomerates (NOAA) (Bekker et al. 2013; Borm et al. 2006). There is increasing public, governmental, and scientific interest in the potential adverse health effects of ENM exposure.

Most available information about the toxicities of ENMs is from in vivo (animal) or in vitro studies. The health effects induced by ENMs in animal inhalation studies include pulmonary fibrosis, granuloma and inflammation, cardiovascular effects, oxidative stress damage, pleural plaque formation, and lung tumors (Oberdörster et al. 2005; Stern and McNeil 2008; Hesterberg et al. 2009, 2010). Most of the evidence for the health effects of nanoparticles on humans studied in the past was generated from unintentionally produced ultrafine particles. Evidence of human health hazards of ultrafine particles (lung inflammation, oxidative damage, worsening of heart disease, atherosclerosis, asthma, and possibly lung cancer) have come from air pollution epidemiological studies of ultrafine particles generated by traffic pollution and combustion processes, such as diesel exhaust and welding fumes (Hesterberg et al. 2009, 2010; International Agency for Research on Cancer 2012; Peters et al. 2004). In addition, needle-like fibrous carbon nanotubes (CNTs) induce asbestos-like granuloma

formation and increase the likelihood of mesothelioma in a tumor-prone mouse strain (National Institute for Occupational Safety and Health 2013). Recently, the International Agency for Research on Cancer (IARC 2014) categorized one type of multiwalled CNT (MWCNT-7) as possibly carcinogenic to humans (Group 2B) and single-walled CNTs (SWCNTs) and MWCNTs (excluding MWCNT-7) as not classifiable as to their carcinogenicity to humans (Group 3). Although NIOSH concludes that there is insufficient evidence to classify *fine* titanium dioxide (TiO_2) as a potential occupational carcinogen, it has determined that *ultrafine* TiO_2 should be considered a potential occupational carcinogen (NIOSH 2011).

Although toxicities of ENM have been identified in animal and in vitro studies, few epidemiologic study reports on human health effects have been published, in part because there is no single nanotechnology industry. Rather, nanotechnology generally permeates all industrial sectors, but the actual numbers of workers exposed to ENMs in any company can be quite small. A vast number of potential ENMs can be created from the combination of physical and chemical characteristics (Schulte et al. 2009). In addition, for many ENMs, their development and use are still limited (Schubauer-Berigan et al. 2011). As a result, it is technically difficult to identify and assess exposure in cohorts of a size appropriate for epidemiologic research (Schulte et al. 2009).

Although health hazards caused by ENMs have not been confirmed in humans, evidence accumulating from animal studies suggests that exposure to some nanomaterials could be harmful. There is a need to assess the risk of potentially adverse health effects among workers handling nanomaterials and to recommend biological markers, as well as preclinical and clinical outcomes that might be useful for their periodic examination to prevent late/delayed effects and identify failures of disease prevention (Bergamaschi et al. 2015; Schulte et al. 2008). This review aims to assess published and unpublished reports on epidemiologic studies of nanomaterial workers and studies in progress to provide perspective on their designs, findings, and limitations.

Materials and methods

The scientific literature was searched to identify completed and in-progress epidemiological studies of nanomaterial workers, with no limit on publication year. The keywords “nanomaterial,” “nanoparticles,” “nanotubes,” “health effects,” “biomarkers,” “fibrosis,” and “epidemiology” were used to search for related articles and/or abstracts from PubMed, Medline, websites, and the proceedings or abstract books for conferences or symposiums. Studies were included in this review only if human exposure or potential exposure to nanoparticles or nanoscaled particles was mentioned in the study design description. Those without such a focus on nanoparticles or nanoscaled materials were excluded, as were human experimental inhalation chamber studies, animal studies, and in vitro studies. Some authors were contacted to learn more about their nanoparticles-related studies.

This review is focused on newly engineered nanomaterials, which we defined as nanomaterials newly engineered to become nanosized particles for different or advanced applications (typically, not materials made in the past). Although some particles of carbon

black, which has been produced and used for a long time, are within the nanosize range, carbon black was not categorized as recently engineered material for advanced applications. In addition, some epidemiological studies of workers exposed to carbon black might not be found with the key word “nanoparticles.” Since we had no other criteria for distinguishing what to cite, we excluded carbon black from this review.

For the purposes of this review, we categorized the epidemiological studies as descriptive, cross-sectional, and cohort. Observational epidemiological study (in contrast to experimental study) includes a descriptive component (such as case report or case study) and an analytic component (cross-sectional, case–control, cohort, and panel or longitudinal study) (IARC 1999). Descriptive epidemiological study focuses on the distribution of disease in terms of various characteristics of person (race, age, or sex, for example), place (geographic location), and time (a specific year or span of time). A cross-sectional study examines the relationship between disease (or other health-related state) and other variables of interest as they exist in a defined population at a single point in time or over a short period (such as a calendar year). In a cross-sectional survey, the risk factors and outcomes are measured simultaneously, and therefore, it may be difficult to determine whether the exposure preceded or followed the disease. Cross-sectional study is limited by its ability to draw valid conclusions as to the association between a risk factor and health outcome. A cohort study focuses on following a group of people with defined characteristics to determine the incidence of or mortality due to a specific disease, all causes of death, or some other outcomes. The cohort is followed over time, and the occurrence of disease in the different exposure groups is measured and compared. A panel study or longitudinal study provides longitudinal data on a group of people who are interviewed and/or examined at regular intervals over a period of years. Because a longitudinal study tracks the same people, the differences observed among them are less likely to be the result of differences across populations. Therefore, longitudinal studies make observations of change more accurate, although these studies take a lot of time and are expensive.

Results

A total of 14 epidemiologic studies of nanomaterial workers and one descriptive pilot case study were identified (Tables 1, 2). These included six published peer-reviewed articles (Table 1) (Lee et al. 2012, 2015; Liou et al. 2012; Liao et al. 2014a, b; Wu et al. 2014) and nine unpublished articles (Table 2), comprising one conference paper (Pelclova et al. 2012), one doctoral dissertation (Cui 2013), and seven conference abstracts (Ichihara et al. 2013; Fatkhutdinova et al. 2013; Pelclova et al. 2013, 2014a, b; Vermeulen et al. 2014; Liou et al. 2013). In addition to the study reports published in journals and conference proceedings, we found three ongoing epidemiologic studies being conducted in the United States, France, and Australia (Schubauer-Berigan et al. 2013; Guseva Canu et al. 2013; Glass et al. 2013), but their findings have not been reported.

Nine different populations were evaluated in 15 studies; among them, the two most commonly studied were Taiwan populations (Liou et al. 2012; Liao et al. 2014a, b; Wu et al. 2014; Liou et al. 2013) and Czech Republic populations (Pelclova et al. 2012, 2014a, b). Of the 15 studies identified, 11 used a cross-sectional study design (Liou et al. 2012, 2014a, b;

Wu et al. 2014; Lee et al. 2015; Cui 2013; Ichihara et al. 2013; Fatkhutdinova et al. 2013; Pelclova et al. 2012, 2013, 2014a; Vermeulen et al. 2014), and 4 used a longitudinal study design with repeated measurements (panel study) (Liao et al. 2014b; Cui 2013; Liou et al. 2013; Pelclova et al. 2014b). One was a descriptive pilot study (Lee et al. 2012), and another used both cross-sectional and longitudinal study designs (Cui 2013).

The studies involved various types of nanomaterials. CNTs were studied in Korea, Russia, and the Netherlands (Lee et al. 2015; Fatkhutdinova et al. 2013; Vermeulen et al. 2014); others studied were nanosilver (Korea) (Lee et al. 2012), titanium dioxide (China and Czech Republic) (Ichihara et al. 2013; Pelclova et al. 2012, 2013, 2014b), iron oxides (Czech Republic) (Pelclova et al. 2014a), and calcium carbonate (China) (Cui 2013).

Biological markers used in these various studies included cardiovascular effect markers, lung fibrosis markers, lung inflammation and systemic inflammation markers, nucleic acids, lipid and protein oxidative stress markers, antioxidant enzyme activity, and genotoxicity markers.

The sample size for each study was small, varying from 2 to 258 ENM-exposed workers. Most studies selected nonexposed workers as the comparison group.

Published studies

Six published studies were identified (Table 1). These included a pilot case study (Lee et al. 2012), four cross-sectional studies (Liou et al. 2012, 2014a; Wu et al. 2014; Lee et al. 2015), and one longitudinal study (Liao et al. 2014b). Of the published studies, four involved a single group of workers in Taiwan and the other two involved workers in Korea.

All four cross-sectional studies (Liou et al. 2012; Liao et al. 2014a; Wu et al. 2014; Lee et al. 2015) and one longitudinal study (Liao et al. 2014b) (Table 1) indicated increased levels of various biological markers associated with exposure. The cross-sectional studies found reduced levels of antioxidant enzymes (Liou et al. 2012), increased cardiovascular effect markers (Liou et al. 2012), reduced memory function (Liou et al. 2012), increased levels of fractional exhaled nitric oxide (FENO) (Wu et al. 2014), increased symptoms of sneezing and allergic dermatitis (Liou et al. 2012), and increased lipid peroxidation products in nanomaterial workers (Lee et al. 2015) (Table 1).

The panel study researchers (Liao et al. 2014b), in comparing examination findings at 6 months' follow-up with those at baseline, also found significant decreasing slopes of antioxidants SOD and GPX, increasing slope of the cardiovascular effect marker VCAM, decreasing slope of the cardiovascular effect marker paraoxonase, and decreasing slopes of the small airway damage marker (CC16) and lung function.

Unpublished studies

Table 2 shows nine unpublished epidemiologic studies of nanomaterial workers. In general, the information on these studies was obtained from conference abstracts. Because these studies presented in conference abstracts or proceedings are otherwise unpublished (with the

exception of Cui 2013, a dissertation), it is difficult to provide a complete assessment of them. For the most part, seven of these studies were cross-sectional in design (Cui 2013; Ichihara et al. 2013; Fatkhutdinova et al. 2013; Pelclova et al. 2012, 2013, 2014a; Vermeulen et al. 2014); however, three were longitudinal (repeated panel designs) (Cui 2013; Liou et al. 2013; Pelclova et al. 2014b). One of them had both cross-sectional and panel study elements (Cui 2013) (Table 2).

Three cross-sectional studies occurred in the Czech Republic (Pelclova et al. 2012, 2013, 2014a), two in China (Cui 2013; Ichihara et al. 2013), and one each in Russia (Fatkhutdinova et al. 2013) and the Netherlands (Vermeulen et al. 2014). Longitudinal studies were conducted in Taiwan (Liou et al. 2013), China (Cui 2013), and the Czech Republic (Pelclova et al. 2014b). The studies were on titanium dioxide (Ichihara et al. 2013; Pelclova et al. 2012, 2013, 2014b), CNTs (Fatkhutdinova et al. 2013; Vermeulen et al. 2014), calcium carbonate (Cui 2013), and iron oxides (Pelclova et al. 2014a).

All seven cross-sectional studies (Table 2) identified increased markers of biological effects. The cross-sectional studies found variably increased ratios of low frequency to high frequency in heart rate variability (Ichihara et al. 2013), increased IL1β and IL8 levels in sputum (Cui 2013), increased TGF-β1 levels in serum and KL-6 levels in induced sputum (Fatkhutdinova et al. 2013), increased markers of oxidation of nucleic acids, lipid, and proteins (Pelclova et al. 2012, 2013, 2014a, b), and increased concentrations of immunological cytokines (Vermeulen et al. 2014).

Only one of the three longitudinal panel studies (Table 2) indicated increased markers of biological effects in follow-up study (Pelclova et al. 2014b). All exhaled breath condensate (EBC) markers of oxidation of lipids, nucleic acids, and proteins were elevated post-shift in 2013, in comparison with the unexposed control group (Pelclova et al. 2014b). In addition, post-shift elevation of several oxidative stress markers (C_9, C_{12}, 8-isoprostane, 8-OHdG, 5-OHMeU, and 3-NOTyr) was found in 2013 in comparison with post-shift EBC samples in 2012 (Pelclova et al. 2014b). Post-shift elevation of 8-OHdG, 5-OHMeU, and HNE, in comparison with pre-shift EBC samples, was also found in (Pelclova et al. 2012). However, no marker was increased in urine, and there was no difference in FENO and EBC pH levels (Pelclova et al. 2014b).

Analysis of 11 cross-sectional studies

The 11 cross-sectional studies (Liou et al. 2012; Liao et al. 2014a; Wu et al. 2014; Lee et al. 2015; Cui 2013; Ichihara et al. 2013; Fatkhutdinova et al. 2013; Pelclova et al. 2012, 2013, 2014a; Vermeulen et al. 2014) showed biological effects associated with exposure, indicative of early adverse health effects with exposure to NOAA; these are summarized in Tables 1 and 2. The biological effects were categorized into four organs/systems with their affecting markers:

1. Lung: increased lung fibrosis markers (serum TGF-β1 and sputum KL-6) (Fatkhutdinova et al. 2013), increased lung inflammation markers (sputum IL-1β and IL-8, FENO) (Wu et al. 2014; Cui 2013), and increased EBC LT B_4, C_4, and E_4 (Pelclova et al. 2012, 2013, 2014a);

2. Cardiovascular: increased cardiovascular effect markers (fibrinogen, intercellular adhesion molecule [ICAM], and interleukin 6) (Liou et al. 2012), increased LF/HF in HRV (Ichihara et al. 2013);
3. Immunological: increased immunological cytokines (Vermeulen et al. 2014);
4. Oxidative damage: increased EBC markers of lipid oxidation, including MDA, HNE, HHE, 8-isoprostane, n-hexanal, and C_6–C_{12}; markers of oxidation of nucleic acids and proteins, including 8-OHdG, 8-OHG, 5-OHMeU, 3-ClTyr, 3-NOTyr, and o-Tyr (Pelclova et al. 2012, 2013, 2014a); and urine C_6, C_7, C_{10}, C_{12}, HHE, 8-OHG, and 3-ClTyr (Pelclova et al. 2012, 2013, 2014a); and decreased antioxidant enzymes [superoxide dismutase (SOD)] and glutathione peroxidase (GPX) (Liou et al. 2012).

In addition, one study found excessive sneezing and allergic dermatitis related to work in nanomaterial areas (Liao et al. 2014a).

Analysis of four longitudinal studies

Two longitudinal panel studies (Cui 2013; Liou et al. 2013) yielded negative findings (no association between nanoparticle exposure and biomarker change), and two panel studies (Liao et al. 2014b; Pelclova et al. 2014b) showed positive findings, as summarized in Tables 1 and 2. The 6-month follow-up study in Taiwan (Liao et al. 2014b) found significant decreasing slopes of SOD and GPX, increasing slope of the cardiovascular marker VCAM, decreasing slope of paraoxonase, and decreasing slopes of the small airway damage marker (CC16) and lung function in follow-up versus baseline examinations. However, the extended 4-year follow-up study in Taiwan (Liou et al. 2013) showed no significant changes in slopes in the five examination periods for any markers, including lung injury markers, cardiovascular effect markers, heart rate variability (HRV), inflammation markers, oxidative stress and lipid peroxidation markers, comet assay, pulmonary function test, and neurobehavioral test.

So far, significantly elevated markers of oxidation of lipids, nucleic acids, and proteins in EBC in workers exposed to TiO_2 nanoparticles (Pelclova et al. 2014b) are the only known biological changes in nanomaterial workers from the 1-year follow-up study by Pelclova et al. The post-shift markers in 2013, including 8-isoprostane, 8-OHdG, 8-OHG, 5-OHMeU, 3-NOTyr, o-Tyr, C_7, C_9, and C_{12}, were elevated in comparison with post-shift in 2012. There was no difference in FENO, pH, and LTs, except for LTC_4 (Pelclova et al. 2014b); however, that finding may be explained by the exclusion of studied workers with a lower exposure risk (level 3), according to Paik (Paik et al. 2008), in the follow-up study. On the other hand, the aerosol TiO_2 mass concentrations (but not particle number concentrations) were lower in the follow-up study (Pelclova et al. 2014b).

Exposure assessment for manufacturing or handling engineered nanoparticles

Although each of the epidemiological studies involved exposure assessment of some type, most lacked 8-h time-weighted averages of specific nanomaterials from personal breathing zone sampling and lacked fully developed job-exposure matrices. Nonetheless, much of the exposure data is useful for making tentative assessments of the exposure-outcome

relationship. Exposure and physicochemical characteristics of nanomaterials identified in epidemiological studies are summarized in Table 3.

Most of the studied workplaces were manufacturing nanomaterials, and the nanomaterials handled were in powder form. The particle diameters reported in this review were less than 100 nm except for well-characterized CNTs, which have agglomerates up to 100 μm.

Personal and area samples were taken in three CNT studies and analyzed for elemental carbon. Researchers monitored respirable fractions in Russia (Fatkhutdinova et al. 2013), inhalable fractions in the Netherlands (Vermeulen et al. 2014), and total suspended particles in Korea (Lee et al. 2015). The mass concentration of respirable elemental carbon in Russia was from 6.2 to 9.3 $\mu g/m^3$ (in personal sampling) and from 5.5 to 7.3 $\mu g/m^3$ (in area sampling) (Fatkhutdinova et al. 2013). The geometric mean of inhalable CNTs in the Netherlands was 42.6 (1.4–1186.5) $\mu g/m^3$ for the production workers and 4.6 (0.2–59.5) $\mu g/m^3$ for research & development workers (Vermeulen et al. 2014). The mass concentrations of total suspended particle elemental carbon in Korea were from 0.54 to 6.11 $\mu g/m^3$ (in area sampling) (Lee et al. 2015).

Total mass concentration and particle count number concentrations were measured by direct-reading instruments (P-TRAK and DustTRAK DRX) in studies of titanium dioxide and iron oxides in the Czech Republic and China. The median total mass concentration of TiO_2 in the Czech Republic was 1.9 (0.1–27) mg/m^3 in 2012 and 0.9 (0.07–16) mg/m^3 in 2013 (Pelclova et al. 2012, 2013, 2014b). The median particle count number concentrations were 38×10^3 (3.6×10^3 to 2×10^5) particles/cm^3 in 2012 and 14.9×10^3 particles/cm^3 in 2013 (Pelclova et al. 2012, 2013, 2014b). The median total mass concentration of iron oxides (Fe_2O_3 and Fe_3O_4) in the Czech Republic was 0.13 (0.1–0.32) mg/m^3 (Pelclova et al. 2014a). The median particle count number concentration was 11.8×10^3 (10.2×10^3 to 30.8×10^3) particles/cm^3 (Pelclova et al. 2014a). The percentages of particles with diameter smaller than 100 nm were 70 to 90 % in TiO_2 exposure and 81 to 98 % in iron oxides production, as shown in Table 3 (Pelclova et al. 2012, 2013, 2014a, b). The total mass concentration by personal sampling of TiO_2 particles was from 10 to 30 mg/m^3 in a TiO_2-handling factory in China (Ichihara et al. 2013). Real time, size-dependent nanoscaled particle number concentrations were from 1×10^4 to 2×10^5 particles/cm^3 (Ichihara et al. 2013).

In some studies, mass concentrations were monitored for respirable particles in personal breathing zone and area samplings, whereas particle number concentration was measured by particle counters. Geometric mean mass concentrations of respirable particles were from 7.24 to 3195.86 $\mu g/m^3$ for area sampling, and from 44.69 to 10028.40 $\mu g/m^3$ for personal sampling in a calcium carbonate manufacturing factory in China (Cui 2013). Particles in the nanometer fraction (0.02–0.3 μm) dominate the number concentration in the size range of 0.02 to 5 μm. The particle number in nanometer fractions (0.02–0.3 μm) was 0.9 to $7.9 \times 10^{10}/m^3$ ($0.9–7.9 \times 10^4/cm^3$). Surface area concentration was 1 to 5×10^8 $\mu m^2/m^3$ ($1–5 \times 10^2$ $\mu m^2/cm^3$) (Cui 2013).

For other nanomaterial exposure, only total mass concentration was measured. Total mass concentrations of silver in total suspended particles were 0.35 and 1.35 μg/m^3 in a Korean nanosilver manufacturing company with a completely closed system (Lee et al. 2012).

Discussion

Exposure concentrations in the current epidemiological studies compared with the published literature

The selection of methodologies in the exposure assessments involved certain technical issues. Usually total mass concentration and particle count number concentrations were measured by direct-reading instruments such as P-TRAK and DustTRAK DRX, whereas aerosol spectrometers such as SMPS and APS were used for continuous monitoring of particle size distribution during an entire 8-h shift, with 5-min sampling intervals (Pelclova et al. 2012, 2013, 2014a, b). Random checks were made during the measurements to compare total number concentrations determined by SMPS with the P-TRAK values, and the differences between averaged values never exceeded 20 %. Similarly, the PM_{10} mass concentrations determined by the DustTRAK DRX were also comparable to the PM_{10} values integrated from the APS data (with an assumed particle density of 4 g/cm^3 for TiO_2) (Pelclova et al. 2012, 2013, 2014a, b). In addition, the total number and mass concentrations were usually determined as the sum of SMPS and APS integral concentrations without taking into account the overlap of the particle size distribution measured by SMPS (14–710 nm) and APS (0.5–10 μm). Therefore, depending on the particle size distribution, the real levels might be lowered by about 10 to 25 % (Pelclova et al. 2012, 2013, 2014a, b). Another issue is that direct-reading instruments appear to be less reliable indicators of exposure to specific ENM, since they are nonspecific and there are many other industrial sources of ultrafine particulates (Dahm et al. 2013). Finally, chemical analysis on workplace aerosols was not performed in most studies.

Mass concentrations in the studies in this review were much higher than those reported in various published exposures to the same materials (Erdely et al. 2013; Dahm et al. 2012, 2015; Lee et al. 2011; Huang et al. 2010; Koivisto et al. 2012; Tsai et al. 2011). The elemental carbon concentrations reported in this review by Vermeulen et al. (2014) were higher than those reported by Dahm et al. (Dahm et al. 2013; Erdely et al. 2013; Dahm et al. 2012, 2015). The mean concentration of inhalable CNTs reported by Vermeulen et al. (2014) in the Netherlands was 42.6 (1.4–1186.5) μg/m^3, whereas the average elemental carbon concentrations at the inhalable size fraction in eight MWCNT factories in the United States ranged from nondetectable to 79.6 μg/m^3 with an arithmetic mean of 10.6 μg/m^3 and a standard deviation of 17.2 or with a geometric mean of 4.21 μg/m^3 and a geometric standard deviation of 4.15 (Dahm et al. 2013; Erdely et al. 2013; Dahm et al. 2012, 2015).

The titanium dioxide total mass concentrations reported by Ichihara et al. (2013) and Pelclova et al. (2012, 2013, 2014b) were also much higher than concentrations reported in previous studies (Lee et al. 2011; Huang et al. 2010; Koivisto et al. 2012). The total mass concentration of TiO_2 particles was from 10 to 30 mg/m^3 in China (Ichihara et al. 2013). The median total mass concentration of TiO_2 in the Czech Republic was 1.9 (0.1–27) mg/m^3 in 2012 and 0.9 (0.07–16) mg/m^3 in 2013 (Pelclova et al. 2012, 2013, 2014b). The median

concentrations did not exceed the nationally allowed concentrations for inert dust (10 mg/m^3). In a Finnish TiO_2 factory, the workers' average exposure varied from 0.225 to 0.700 mg/m^3, and particle counts from 1.15×10^4 to $20.1 \times 10^4/cm^3$ were reported; more than 90 % of the particles were smaller than 100 nm (Koivisto et al. 2012). Total mass concentration of TiO_2 ranged from 100 to 4,990 $\mu g/m^3$ and particle number concentrations (particles 15–710 nm in size) ranged from 11,400 to 45,900/cm^3 during the reaction in a Korean factory that handled nano-TiO_2 (Lee et al. 2011). This concentration decreased to 14,000/cm^3 when the reaction was stopped (Lee et al. 2011). The respirable dust concentrations obtained by the cyclones in a Taiwan TiO_2 pigment factory were 0.487, 0.688, 0.268, and 0.314 mg/m^3 (Huang et al. 2010). The most common particle size in the nanoparticle number concentrations of the TiO_2 pigments was 30–50 nm, and concentrations ranged from 4.9×10^4 to 1.1×10^5 particles/cm^3 (Huang et al. 2010).

The mass concentrations of calcium carbonate reported by Cui (2013) were also much higher than concentrations reported previously (Tsai et al. 2011). The geometric mass concentrations of respirable particles were from 7.24 to 3195.86 $\mu g/m^3$ for area sampling and from 44.69 to 10,028 $\mu g/m^3$ for personal sampling in China (Cui 2013). The particle number in the nanometer fraction (0.02–0.3 μm) was 0.9 to $7.9 \times 10^4/cm^3$, and the surface area concentration was 1 to 5×10^2 $\mu m^2/cm^3$ (Cui 2013). The maximum concentration was reported to be 1.85 $\mu g/m^3$ in a Taiwan calcium carbonate plant (Tsai et al. 2011).

The data on exposure to nanoscaled iron oxides described in this review are apparently the first published data on such exposure. The nanosilver concentrations detailed in this review (Lee et al. 2012) are similar to the concentrations reported previously by Lee et al. (2011). The total mass concentrations of silver nanomaterials were 0.35 and 1.35 $\mu g/m^3$ in a Korean nanosilver manufacturing company with a completely closed system (Lee et al. 2012). The silver metal concentrations measured in another factory in Korea ranged from 0.02 to 1.18 $\mu g/m^3$ and the number of silver nanoparticles ranged from 57,789 to 2,373,309 particles/cm^3 inside the reactor. The average particle size was 20–30 nm, and the particle count ranged from 535 to 25,022 particles/cm^3; the wide range in particle sizes was due to agglomeration or aggregation after the release of nanoparticles into the workplace air (Lee et al. 2011). In contrast, the count of silver nanoparticles manufactured by the wet method ranged from 393 to 3,526 particles/cm^3 with an average size of 50 nm (Lee et al. 2011).

Comparison of cross-sectional and longitudinal panel studies

All 11 cross-sectional studies (Liou et al. 2012; Liao et al. 2014a; Wu et al. 2014; Lee et al. 2015; Cui 2013; Ichihara et al. 2013; Fatkhutdinova et al. 2013; Pelclova et al. 2012, 2013, 2014a; Vermeulen et al. 2014) found some biological changes that might be indicative of early adverse effects on nanomaterial-handling workers. However, assessing causality with lack of temporality is a major limitation to drawing conclusions from cross-sectional studies. A positive cross-sectional study might also be related to population-selection bias and reporting bias.

In the aforementioned exposure assessment data, both the mass concentrations of nanoparticles and the particle counts or surface area concentrations were relatively high compared to the data reported previously (Dahm et al. 2013; Erdely et al. 2013; Dahm et al.

2012, 2015; Lee et al. 2011; Huang et al. 2010; Koivisto et al. 2012; Tsai et al. 2011). These high mass concentrations and high particle counts of nanoscaled particles may contribute to the positive findings of all 11 cross-sectional studies. However, most of the exposure assessments were less than optimal because they did not show time-weighted assessments for each person for each nanomaterial. In addition, direct-reading instruments are nonspecific for the engineered nanoparticles of interest and may reflect other industrial sources within the workplace (Dahm et al. 2013). Fine and coarse particles have been shown to result in cardiopulmonary and genotoxic health effects, in air pollution epidemiological studies (McDonnell et al. 2000; Chang et al. 2013). Fine and coarse particles in nanotechnology factories might also contribute to positive findings in cross-sectional studies. However, no studies controlled for these ambient sources of ultrafine particulates or included these sources in their analyses of the effects of specific ENMs.

The longitudinal studies in Taiwan, China, and Czech Republic (Liou et al. 2013; Cui 2013; Liao et al. 2014b; Pelclova et al. 2014b) did not show significant biological changes after work (post-shift) (Cui 2013) or during longitudinal follow-up (Liou et al. 2013), except for the 6-month follow-up study in Taiwan (Liao et al. 2014b) and a 1-year follow-up study of workers exposed to TiO_2 in Czech Republic (Pelclova et al. 2014b). This discrepancy may be attributed to selection bias in the Taiwan cohort studies, where the selection of the study population for the cohort was somehow related to the probability of the outcome studied. In other words, the selected population might already have related health outcomes associated with other hazards or causes, which became confounding factors for investigating nanomaterial exposure. The appearance of biological changes in the time period after inclusion in the study may be due to the likelihood that the workers with the outcome studied were more prone to be recruited as members of the cohort (Törner et al. 2010, 2011). Selection bias of this type is likely to be more pronounced shortly after inclusion in the cohort. Cross-sectional studies and short-term (such as 6 months) follow-up studies would have a high probability of this type of selection bias, resulting in positive findings. After some time period, population characteristics leading to inclusion in the cohort could be resolved. Removal of observation time and events occurring shortly after inclusion in the cohort could reduce the impact of selection bias (Törner et al. 2010, 2011). In order to avoid such type of selection bias, the Taiwan panel study was extended to a long-term longitudinal study design with five repeated examinations in an interval of 4 years. Consequently, the extended 4-year follow-up study in Taiwan (Liou et al. 2013) showed no significant difference between exposed workers and controls in the changes of all markers. The relatively low fraction of nanoparticles might not have as strong an effect as those induced by fine and coarse particles. Therefore, negative findings in longitudinal studies may be due to the influence of fine and coarse particles overwhelming the difference between exposed and comparison workers for some biological markers.

Based on field studies conducted by the Taiwan Institute of Occupational Safety and Health (IOSH) that measured the emission of nanoparticles in different operations or processes in nanotechnology industry, as shown in Table 4 (Institute of Occupational Safety and Health 2012), nanoparticle emissions were found to be quite low in enclosed operations for coating nanomaterials, in enclosed operations for mixing or grinding nanopigments, and in wet processes for synthesis or centrifuge of nanomaterials. However, nanoparticle emissions

could be detected in spray drying of nanomaterials as well as dry processes for polishing, milling, and grinding. The Taiwan longitudinal panel study suggested that exposure to nanomaterials in study scenarios may not have an impact on cardiopulmonary and oxidative damage biomarkers (Liou et al. 2013).

Another possibility is that exposure at the workplaces decreased during longitudinal study because high awareness of the potential risk of exposure to nanoparticles led to more cautious workplace controls. Reduction of mass aerosol concentration in the second follow-up study by about 40 % was noted among the Czech TiO_2 workers (Pelclova et al. 2014b).

Strengths and weaknesses of current epidemiological studies of nanomaterial workers

The greatest weakness of the epidemiologic research reviewed in this paper is that most of the studies were cross-sectional. Thus, inherently they could not demonstrate causality. The few longitudinal studies conducted on nanomaterial workers were of a much stronger design, although most lacked rigorous exposure assessment.

Most of the studies lacked time-weighted averaged personal breathing zone exposure data. In the studies of the Taiwanese cohort where the control banding Nanotool risk-level matrix (Paik et al. 2008) was used to categorize the risk level of each participant as a surrogate of nanoparticle exposure (Table 3), lack of personal measured-exposure data was particularly evident. First, some studies measured total suspended particles (TSPs) or respirable mass concentration instead of nanoparticle mass concentration, because of either a shortage of equipment or lack of consensus of methodologies for environmental sampling and analysis of nanoparticles, especially for CNT fiber counts. Comparing studies is difficult; for example, a lack of harmonization was found in elemental carbon measurements for CNT exposure. Three CNT studies used different sampling strategies to measure elemental carbon. Total suspended particles, inhalable particles, and respirable particles, respectively, were used by Lee et al. (2015), Vermeulen et al. (2014), and Fatkhutdinova et al. (2013) in CNT studies. This illustrates that harmonization is a necessary element of epidemiological research.

Second, scientific evidence is still insufficient for determining which particle size range and exposure parameters of nanoparticles should be measured to characterize exposure or which instruments or methods are the most appropriate for measuring nanoparticle exposure. Most of studies measured nanoparticle mass concentrations instead of nanoparticle counts or surface area (Table 3). Although surface area has been found to be highly correlated with toxicities of nanoparticles, only one study in this review measured surface area (Cui 2013). Four studies (Ichihara et al. 2013; Cui 2013; Pelclova et al. 2012, 2013, 2014a, b) measured nanoparticle count in addition to mass concentration. It is also extremely difficult to measure the surface area attributable to specific ENMs in air samples. According to the currently available technology and instruments, measuring both mass and particle number concentrations of exposed particles and identifying particle size distribution are the practical approaches at this stage of development to provide appropriate information for exposure assessment. The respirable mass appears to be most relevant to present the particles containing individual and agglomerate nanoparticles, according to the particle size cut-off for the respirable mass. In addition, proper characterizations of the exposed particles

(including elemental composition, particle morphology, particle surface chemistry, and other particle physical and chemical properties) are important factors that need to be understood to analyze the exposure and potential human health effects.

Third, most studies used area sampling instead of personal sampling (which represents actual exposure of nanomaterial workers) to measure nanoparticle concentrations, due to a lack of consensus on equipment and methodology for personal sampling. A recent study of CNT-exposed workers in the U.S. has found that measurements from area samples of the same tasks are consistently lower than those from personal breathing zone samples (Dahm et al. 2012).

Fourth, it is possible that selection bias occurred in identification of the study population. Because some nanotechnology factories and some workers in the selected factory pool refused to participate, the study population could not be completely representative. Often, highly polluted industries or factories refuse to participate. Therefore, the early health effects may have been underestimated on account of the population selection. On the contrary, company workers who are concerned more about their health condition may be prone to participate in health examinations and report more negative health effects, which could lead to a biased evaluation. Another selection bias issue in studies was the selection of a nonexposed comparison group. Because of exposure to ultrafine particles in the background environment and in the workplace from other sources such as transportation, coexposure at work (such as to endotoxin), living close to traffic roads or factories (within 50 meters), and burning incense or anti-mosquito coils in the house, comparability of the workers studied could not be ensured. However, the most appropriate or best comparison group is difficult to identify and maintain.

Fifth, quality control and assurance of measurements for detecting biologic markers are important, especially for longitudinal repeated measurements. Comparability in precision or accuracy in different batches and different years was not reported to be evaluated or ensured. Data quality issues such as diurnal variations of some health effect markers were not identified. For example, diurnal and seasonal variation in fibrinogen, fibrin D-dimer, C-reactive protein (CRP), tissue plasminogen activator antigen (t-PA), von Willebrand factor, and H_2O_2 has been reported (Rudnicka et al. 2007; Nowak et al. 2001). Diurnal variation should be considered in the collection of biospecimens (blood, urine, and EBC) for studying cardiovascular markers and oxidative and lipid peroxidation biomarkers, such that the biospecimens are collected in the same time period during follow-up. Alternatively, adjustment for collection time should be considered in the data analysis.

Another limitation was the small size of the study populations, which hinders subgroup assessments and restricts generalizability of the results. The heterogeneous nanomaterial exposures made it difficult to find a sufficiently large group of workers exposed to the same nanomaterials and to present biomarker effects of a single nanomaterial. Most of the reviewed studies were based on small sample sizes, small factory sizes, and low exposure relative to effective doses in human inhalation chamber studies. However, further study is needed to investigate whether exposure to higher concentrations of ENMs or working longer in exposed jobs (other than operations or processes that were studied in this review) can

cause increases in biomarkers of adverse effects. There is a need for more cohort studies in countries with well-developed nanotechnology, such as the United States, Germany, United Kingdom, France, Belgium, and Switzerland (Bekker et al. 2013; Borm et al. 2006; Seal and Karn 2014), in order to reveal the biologic effects in larger samples with higher exposure. Epidemiologic studies are ongoing in the United States, France, and Australia (Schubauer-Berigan et al. 2013; Guseva Canu et al. 2013; Glass et al. 2013); thus, more information on biologic effects on nanomaterial workers is expected soon. Descriptions of the exposure assessment methods and exposure levels for the U.S. cross-sectional study of CNT-exposed workers have been published (Dahm et al. 2015). They include background-corrected personal breathing zone measurements of elemental carbon at the respirable and inhalable size fraction, as well as transmission electron microscope–based CNT structure counts by particle size class.

Another limitation was the use of markers of biologic changes instead of health hazards or adverse outcomes. These biomarkers are generally nonspecific with respect to ENM exposure and have not all been validated for health effects, and the methods may lack sensitivity or specificity. Validated health effect markers should be used to evaluate the chronic, long-term health hazards of nanomaterial workers. To date, only one study used symptoms and/or diseases as outcomes of health effect. This cross-sectional study found sneezing and allergic dermatitis (Liao et al. 2014a) increased in nanomaterial workers. In addition, lung function tests (Liou et al. 2012, 2013; Liao et al. 2014b; Cui 2013; Vermeulen et al. 2014), FENO (Liou et al. 2012, 2013; Wu et al. 2014; Liao et al. 2014b; Pelclova et al. 2012; Cui 2013), and heart rate variability (Liou et al. 2012, 2013, 2014b; Ichihara et al. 2013) were used in several studies.

Finally, in terms of process, the studies raise the issue of what employers and study participants are told of the findings. In addition to obtaining Internal Review Board approval and informed consent, an important issue is how the findings are interpreted and communicated. Generally, participants should be told of their individual and group findings (Schulte and Smith 2011). In terms of individual results, participants will want to know if the results pertain to their health. Employers need to be informed of group results so that they can consider changes in risk management.

Conclusion

According to all available studies, at this stage it is not possible to conclude whether there are health effects associated with ENM exposure in workers. That is not surprising, since in addition to all the methodological limitations identified above, we are still at an early stage of the commercial history of nanotechnology and of the history of studying its health effects. Many gaps still need investigation. The most critical part of an ideal epidemiological study is to appropriately characterize the exposure and distinguish workers with exposures to each specific ENM. There are also questions about what biomarkers will be useful to indicate potential hazards. The summary of reviewed studies in this article provides the first step in addressing the ultimate question of whether ENM workers are at risk of adverse health effects. As such, this review makes a contribution to answering that question. It appears that

various categories of biomarkers may be influenced by worker exposures to ENMs, and this finding serves to point to where and how future research might be conducted.

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Abbreviations

Antioxidant markers

SOD	Superoxide dismutase
GPX	Glutathione peroxidase

Oxidative stress markers

3-Cl-Tyr	3-chloro-tyrosine
3-NOTyr	3-nitrotyrosine
5-OHMeU	5-Hydroxymethyl uracil
8-isoprostane	8-Iso-prostaglandin F2α
8-OHG	8-Hydroxyguanosine
8-OHdG	8-Hydroxydeoxyguanosine
C_6–C_{12}	*n*-alkanes
HNE	4-Hydroxy-trans-nonenal
HHE	4-Hydroxy-trans-hexenal
LTs	Leukotrienes
MDA	Malondialdehyde
o-tyr	o-Tyrosine

Pulmonary effect markers

CC16	Clara cell protein
FENO	Fractional exhaled nitric oxide
KL-6	Krebs Von den Lungen 6
MIP-1beta	Macrophage inflammatory protein-1β

PFT Pulmonary function test

FVC	Forced vital capacity
FEV1	Forced expiratory volume at 1 s

MMF	Maximal mid-expiratory flow
PEFR	Peak expiratory flow rate
FEF25 %	Forced expiratory flow at 25 %
FEF50 %	Forced expiratory flow at 50 %
FEF75 %	Forced expiratory flow at 75 %
TGF-β1	Transforming growth factor beta-1

Systemic inflammation markers

hsCRP	Highly sensitive C-reactive protein
IL1β	Interleukin-1β
IL8	Interleukin-8
IL-6	Interleukin-6
IL-6sR	Interleukin-6 soluble receptor
NF-kβ	Nuclear factor-kappaβ
TNFalpha	Tumor necrosis factor α

Vascular or endothelial function biomarkers

hsCRP	Highly sensitive C-reactive protein
ICAM	Intercellular adhesion molecule
IL-6	Interleukin-6
IL-6sR	Interleukin-6 soluble receptor
MPO	Myeloperoxidase
VCAM	Vascular cell adhesion molecule

HRV Heart rate variability

SDNN	Standard deviation of all normal to normal R–R intervals
RMSSD	The root mean square of successive differences between adjacent normal cycles
LF/HF	Low frequency/high frequency ratio
LF	Low frequency
HF	High frequency
VLF	Very low frequency
CNT/CNF	Carbon nanotube/carbon nanofiber
EBC	Exhaled breath condensate

ENM	Engineered nanomaterials
ENP	Engineered nanoparticles
Mfg	Manufacturing
NAOO	Nano-objects their aggregates and agglomerates
UFP	Ultrafine particles

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Table 1

Characteristics and findings of six published epidemiological studies on nanomaterial-handling workers

Study type, authors, year	Country	Nanomaterials	Major findings	Sample size (no. exposed/no. controls)
Descriptive				
Lee et al. (2012)	Korea	Nanosilver	Blood and urine levels of silver were low in 2 workers. No abnormality was found in hematological data and blood chemistry values	2
Cross-sectional				
Liou et al. (2012)	Taiwan	Various[a]	Decreased antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPX) and increased cardiovascular markers, fibrinogen, intercellular adhesion molecule (ICAM), and interleukin 6 were noted in exposed workers	227/137
Liao et al. (2014a)	Taiwan	Various[a]	The only symptom identified as significantly work-related was sneezing. The only disease significantly worsened by work was allergic dermatitis	258/200
Wu et al. (2014)	Taiwan	Various[a]	A significant association between risk level 2 of NP exposure and FENO. When the multivariate logistic regression model was adjusted for confounders, nano-TiO_2 in all of the nanomaterial-exposed categories had a significantly increased risk in FENO > 35 ppb	241/196
Lee et al. (2015)	Korea	MWCNTs	The malondialdehyde (MDA), 4-hydroxy-2-hexenal (4-HHE), and n-hexanal levels in the EBC of MWCNT manufacturing workers were significantly higher than those in the office workers	9/4
Longitudinal				
Liao et al. (2014b)	Taiwan	Various[a]	Changes in the antioxidant enzymes (decreased SOD and GPX), cardiovascular markers (increased VCAM, decrease of paraoxonase), the small airway damage marker (decreased Clara cell protein 16), and lung function parameters (decreased MMF, PEFR, and FEF25 %) were significantly associated with nanomaterial-handling	124/77

[a] Carbon nanotubes, silica dioxide, titanium dioxide, nanosilver, and nanoresin

Table 2

Nine unpublished epidemiological studies on nanomaterial-handling workers

Study type, authors, year (presentation)	Country	Nanomaterials	Major findings	Sample size (no. exposed/no. controls)
Cross-sectional				
Pelclova et al. (2012) (2012 NANOCON Conference, Brno, Czech Republic)	Czech Republic	TiO_2 (70–90 % of particles <100 nm)	All pre-shift and post-shift EBC markers were higher in the workers, except pre-shift LT D4. Markers of lipid oxidation (MDA, HNE, HHE, and 8-isoprostane) were elevated ($p < 0.001$), as were markers of oxidation of nucleic acids and proteins: 8-OHdG, 8-OHG, 5-OHMeU, 3-Cl-Tyr, 3-NOTyr, o-Tyr; LT B4, C4, and E4 ($p < 0.001$). In the workers, EBC pH was lower and FENO was elevated in both samples. Pre-shift elevation of the markers suggests subacute/chronic effect. Spirometry (FEV1 % and FVC %) showed no impairment	20/19
Ichihara et al. (2013) (2013 NanOEH)	China	TiO_2 (primary particle diameters: 46–560 nm)	Nanoscaled particle number and age correlated significantly with the ratio of low-frequency to high-frequency heart rate variability, a parameter of sympathetic function	4/0
Cui (2013) (PhD thesis, University of Washington)	China	Calcium carbonate	The concentrations of IL 1β ($p = 0.043$) and IL8 ($p = 0.008$) in sputum were statistically greater in the high mass-exposure group than in the low mass-exposure group. The elevation of ILlβ and IL8 followed a dose–response pattern with increasing exposure	66–102/0
Fatkhutdinova et al. (2013) (2nd International School-Conference)	Russia	Multiwalled carbon nanotubes (MWCNT)	It was found that exposure to MWCNT aerosol at workplaces may alter the fibrosis biomarkers in blood serum and induced sputum. The levels of TGF-βl in serum were significantly dependent on exposure to MWCNTs ($\beta = 10.47$; 95 % BCa = 1.18–51.75); the KL-6 levels in induced sputum were significantly higher in the exposure group ($\beta = 235.9$; 95 % BCa = 21.2–482). Osteopontin proved to be an uninformative indicator	11/14
Pelclova et al. (2013) (2nd QNano Integrating Conference, Prague, Czech Republic)	Czech Republic	TiO_2 (70–90 % of particles <100 nm)	In the EBC, all C_6–C_{13} n-alkanes were elevated in both pre-shift and post-shift samples from workers ($p < 0.001$) and correlated with most markers of oxidation of lipids, proteins, and nucleic acids and with Ti levels in the EBC. In the urine, C_6, C_7, C_{10}, C_{12}, HHE, 8-OHG, and 3-Cl-Tyr were significantly elevated in pre-shift and post-shift samples in the workers versus controls ($p < 0.05$). In addition, they correlated with MDA, HNE, 8-isoprostane, 8-OHdG, 5-OHMeU, o-Tyr, and 3-Cl-Tyr of the same sample ($p < 0.05$). Again, pre-shift markers were already elevated.	20/19
Pelclova et al. (2014a) (TOXCON Conference, Stara Lesna, Slovakia)	Czech Republic	Fe oxides (81–98 % of particles <100 nm)	All markers of oxidation of lipids, nucleic acids, and proteins (MDA, HNE, HHE, C_6–C_{11}, 8-isoprostane, 8-OHdG, 8-OHG, 5-OHMeU, 3-Cl-Tyr, 3-NOTyr, o-Tyr), and LTB_4 examined in post-shift EBC were elevated ($p < 0.001$); C_{12} p value, < 0.05. There was no increase in cysteinyl LTs, pH, or FENO. No markers were increased in analyzed urine specimens	14/14
Vermeulen et al. (2014) (2014 EPICOH)	Netherlands	CNTs	Concentrations of cytokines were exposure-dependently upregulated, with higher levels among operators than R&D workers; levels for both were higher than unexposed workers (p for trend, <0.05)	8 operators +16 R&D workers/43
Longitudinal				
Liou et al. (2013) (2013 EPICOH)	Taiwan	Various (carbon nanotubes, silica dioxide, titanium dioxide, nanosilver, nanoresin)	No significant difference was revealed between exposed workers and controls in the changes of all markers, including lung injury markers, cardiovascular disease markers, heart rate variability (HRV), inflammation markers, oxidative stress and lipid peroxidation markers,	206/140 followed up no less than 2 times

Study type, authors, year (presentation)	Country	Nanomaterials	Major findings	Sample size (no. exposed/no. controls)
			comet assay, pulmonary function test, and neurobehavioral test, in this 4-year follow-up study	
Cui (2013) (PhD thesis)	China	Calcium carbonate	No significant cross-shift effect for FEV1, BP, and EENO	66–102/0
Pelclova et al. (2014b) (EUROTOX Conference, Edinburgh, UK)	Czech Republic	TiO_2 (70–90 % of particles <100 nm)	In 2013, all markers of oxidation of lipids, nucleic acids, and proteins (MDA, HNE, HHE, C_6–C_{11}, 8-isoprostane, 8-OHdG, 8-OHG, 5-OHMeU, 3-Cl-Tyr, 3-NOTyr, o-Tyr), and LTB_4 in EBC were elevated ($p < 0.001$); C_{12} p value was < 0.05 in 2013 versus unexposed controls. The following post-shift markers in 2013 were elevated versus post-shift markers in 2012: C_9, C_{12}, 8-isoprostane, 8-OHdG, 5-OHMeU, and 3-NOTyr. There was no difference in EENO, pH, and LTs, except for LTC_4. No markers were increased in analyzed urine specimens in 2013, when exposure to TiO_2 decreased	14/25

Table 3

Physicochemical characteristics and exposure assessment of nanomaterials in 15 epidemiologic studies

Authors, year	Nanomaterial	Workplace	Physical type (powder, liquid suspension, etc.)	Particle size	Mass concentration	Particle count/cm^3	Instrument and methods
Lee et al. (2012)	Nanosilver	Mfg (in completely closed system)	Powder, liquid suspension, wire	<100 nm, individuals and agglomerates (production rate, 5 kg/day)	Silver: 0.35 and 1.35 μg/m^3. The total suspended particle: 0.158 and 0.109 mg/m^3. Blood and urine levels of silver: 0.034 and 0.0135 μg/dl in blood; 0.043 μg/dl and nondetectable in urine	NA	Personal breathing zone samples collected with MSA pump. The total suspended particle concentration was determined gravimetrically with NIOSH method 0500
Liou et al. (2012), (2013), Liao et al. (2014a, 2014b)	Various NMs (carbon nanotubes, silica dioxide, titanium dioxide, nanosilver, nanoresin)	Mfg, handling	Powder, liquid suspension	<100 nm, raw material	NA	NA	NA
Lee et al. (2015)	MWCNTs	Mfg (continuous thermal CVD process)	Powder	>1 μm, agglomerates	TSP elemental carbon: 6.2–9.3 μg/m^3 (PBZ sampling), 5.5–7.3 μg/m^3 (area sampling)	NA	Elemental carbon concentration was analyzed on quartz filters of TSP (NIOSH Method 5040) Size distribution measured by SMPS, CPC, and a dust monitor (Model 1.109, Grimm) for 0.25 to 32 μm
Pelclova et al. (2012), (2013), (2014b)	TiO_2	Mfg	Powder	<100 nm (70–90 %)	1.9 (0.1–27) mg/m^3 in 2012; 0.9 (0.07–16) mg/m^3 in 2013	Median: 38 × 10^3 (3.6 × 10^3–2 × 10^5) in 2012; 14.9 × 10^3 in 2013	Number concentrations were monitored by P-TRAK and mass concentrations by DustTRAK DRX Size distributions in 15 nm to 10 μm were monitored by SMPS and APS
Ichihara et al. (2013)	TiO_2	Mfg	Powder	46 to 560 nm, individuals and agglomerates	10–30 mg/m^3, personal sampling	Nanoscaled particles: 1 × 10^4 to 2 × 10^5	Mass concentration was measured with personal cascade impactor. Real time size-dependent particle number concentration was monitored
Cui (2013)	Calcium carbonate	Mfg	Powder	30–100 nm, individuals; >10 μm, agglomerates	7.24–3195.86 μg/m^3, area sampling; 44.69–	0.02–0.3 μm particles	Personal and area samples of respirable particles

Authors, year	Nanomaterial	Workplace	Physical type (powder, liquid suspension, etc.)	Particle size	Mass concentration	Particle count/cm^3	Instrument and methods
					10028.4 μg/m^3, personal sampling, personal sampling	dominated; 0.02–0.3 μm count concentration, 0.9–7.9 × 10^4; surface area concentration, 1–5 × 10^2	were collected and analyzed by SEM. The elemental composition was analyzed with an energy-dispersive X-ray spectrometer (EDX) Airborne particle number concentrations were measured by Optical Particle Counter (OPC) and Ultrafine Particle Counter. Measurements of lung deposited surface area were performed with the online Nanoparticle Aerosol Monitor 9000
Fatkhutdinova et al. (2013)	MWCNTs	Mfg	Powder	0.5–10 μm, agglomerates	0.54–6.11 μg/m^3, elemental carbon	NA	Breathing zone samples were taken with and without cyclone for respirable fraction. Elemental carbon was evaluated in air samples and the CNT presence was confirmed by TEM analysis
Pelclova et al. (2014a)	Iron oxides (Fe_2O_3 and Fe_3O_4)	Mfg (pigment production)	Powder	<100 nm (81–98 %)	0.13 (0.1–0.32) mg/m^3	Median: 11.8 × 10^3 (10.2 × 10^3 to 30.8 × 10^3)	Number concentrations were monitored by P-TRAK and mass concentrations by DustTRAK DRX Size distributions in 15 nm to 10 μm were monitored by SMPS and APS
Vermeiden et al. (2014)	CNTs	Mfg	Powder	500 nm–100 μm, agglomerates (at production and R&D) 500 nm–5 μm, agglomerates (at office)	Quantitative analyses demonstrated geometric mean (GM) inhalable CNT levels of 42.6 μg/m^3 (min–max: 1.4–1186.5) and 4.6 μg/m^3 (min–max: 0.2–59.5) for the production and R&D workers, respectively	NA	Qualitative analysis: Personal 8-hr TWA inhalable dust samples (n = 5) were collected in all job categories and analyzed by SEM-EDX Quantitative analysis: 8-hr TWA samples (n = 30) were collected from the production and R&D workers, analyzed for elemental carbon, and corrected for soot with SEM/EDX

Table 4

Emission of nanomaterials in different operations or processes in the nanotechnology industry in Taiwan

Operation	Emission of nanomaterials
Coating of nanomaterials (enclosed)	Quite low
Mixing/grinding of nanopigments (enclosed)	Quite low
Synthesis of nanomaterials (wet process)	Quite low
Centrifuge of nanomaterials (wet process)	Quite low
Spray drying of nanomaterials	Yes
Welding	Yes
Polishing	Yes
Milling	Yes
Grinding	Yes
Foundry industry	Yes

Source Institute of Occupational Safety and Health (2012) Applications of the Newly Developed Nanoparticles Exposure Assessment Techniques to Workplaces in Nanoindustries (II). IOSH 101-H322