Screening for Biological Markers of Exposure and Biomonitoring of Nanotechnology Workers: A Scoping Review

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**Abstract**

The ubiquitous use of nanomaterials (i.e., particulate materials measuring 1–100 nanometers (nm) in at least one dimension) poses potential issues regarding worker health and safety. Their unique properties have made nanomaterials useful in several industries. However, their production may compromise worker health, indicating emerging occupational health hazards that have not been fully assessed for their acute or chronic health effects. Previous study results regarding engineered nanomaterials and air pollution epidemiology underscore the importance of considering the risks of nanoparticle (NP) or engineered nanomaterial (ENM) exposure among workers. In this scoping review, we assessed the utility of biomonitoring to screen nanotechnology workers for nanoparticle exposure. PubMed and Web of Science databases were queried for publications within the last decade that possess cellular, animal, and human toxicological and epidemiological evidence regarding the use of biomarkers to estimate health outcomes, and laboratory and pathophysiological changes in workers exposed to nanomaterials. Inclusion criteria included articles assessing: (a) biological markers of occupational NP exposure, (b) health outcomes of exposure, and (c) laboratory test results from *in vitro*, *in vivo* (animals), and epidemiological studies. Of 286 articles initially retrieved, 31 were included after screening and eligibility. The reviewed articles indicate that sensitive, validated biomarkers in epidemiological studies, including those of potential disease progression and epigenetics, are useful for predicting toxicological effects and risks associated with NP exposure in the workplace. Our review could help policy decision-makers in the occupational health field promote regulations and define occupational exposure limits to contribute to worker health and well-being.

Keywords: biological exposure index; biomarkers; engineered nanomaterials; nanoparticle exposure; nanoparticle workers

**Introduction**

The utilization of nanotechnology and nanoscale materials is rapidly increasing in both scope and scale. As a result, the number of workers exposed to nanoparticles has grown significantly as nanoparticle (NP) use has extended to several different industries. Some industrial processes, such as cleaning, packaging, and recycling, may expose consumers to nanoparticles in addition to workers (Kuhlbusch et al. 2011). ENMs with new chemical and physical properties, which are valuable in technological applications, are being produced regularly. These properties, such as small dimensions, shape, large surface area, high reactivity, charge, crystal formation, and aggregation, create toxic effects for living organisms. The biological activity of ENMs and NPs depends on those physicochemical properties, which are crucial in understanding their toxic effects on worker health. According to Liao et al. (2014), these properties are not routinely considered in toxicity screening studies, and their adverse effects and toxicity remain mostly unknown. Exposure to nanomaterials may occur by various routes: oral, dermal, inhalation, and injection routes, depending on use patterns. Thus, the spectrum of NP biological effects is wide, and the potential for different biomarkers to be considered is also diverse. Biomarkers of exposure in industrial hygiene referred to measurable changes in a biological or biochemical response, ranging from molecular to physiological levels. Those changes can be measured in human fluids, tissues, or other noninvasive samples from exposed workers. Inhalation is the most biologically significant route of exposure, and NPs that accumulate in the lungs may contribute to the development of idiopathic respiratory pathologies. According to Rinaldo et al. (2015), approximately 10−20% of insoluble nanoparticles accumulate in the lungs. Pathological effects, including pulmonary fibrosis, granuloma, inflammation, cardiovascular effects, oxidative stress damage, pleural plaque formation, lung tumors, cytotoxicity, and genotoxicity, were found in animal inhalation studies (Oberdörster et al. 2005, Tkach et al. 2011, Oyabu et al. 2017). Epigenetic data regarding the effect of NP worker exposure on DNA alterations and related biomarkers is scarce. Rossnerova et al. (2020) and Yu et al. (2020) investigated global and gene-specific DNA methylation profiles among workers chronically exposed to NP and iron oxides. Both studies found DNA epigenetic alterations that could be considered to be biomarkers of chronic exposure at the molecular level. The present study undertakes a scoping review of research on the extent of knowledge and use of different biological markers of NP and their impact on workers’ health.

**Materials and Methods**

**Search Strategy and Inclusion Criteria**

We conducted a scoping review to identify studies addressing toxicology, industrial hygiene, and nanomaterial exposure epidemiology. Web of Science and PubMed search engines were queried utilizing an identification, screening, eligibility, and inclusion algorithm based on the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Tricco et al. 2018). Subject search terms included “nanoparticles” or “engineered nanomaterials,” “health effects,” “biological markers,” “biomarkers,” and “workers epidemiology.” Search restrictions included the English language, years of publication (2009–2020), and peer-reviewed studies. In order to expand the scope and reduce the probability of missing relevant literature, the term “nanoparticles” was used in addition to “nanomaterials.” Two hundred eighty-six unique search retrievals for each search term were obtained in both databases. Following the initial identification process, duplicate articles from different search terms were removed prior to starting the screening process.

**Table 1. Search terms and query results**.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  Search terms | PubMed | Web of Science |  |  |
| Nanoparticles AND workers AND biomarkers | 45 | 46 |  |  |
| Biomonitoring AND nanomaterials workers | 27 | 9 |  |  |
| Biomonitoring AND nanoparticles workers | 19 | 16 |  |  |
| Biomonitoring AND engineered nanomaterials workers | 14 | 3 |  |  |
| Biological markers AND engineered nanomaterials AND workers | 2725 | 22 |  |  |
| Biological markers AND exposure to engineered nanomaterials AND workers Biological markers AND exposure to nanoparticles AND workers |  42 | 9 |  |  |
| **Total** | **199** | **87** |  |  |

**Figure 1** outlines a flow chart of the selection process adapted from the PRISMA-ScR (PRISMA extension for Scoping Reviews) group statement (Tricco et al. 2018). Full texts of the papers considered eligible for this review were obtained, and reference lists were searched for articles that met the inclusion criteria. Only publications that measure biological markers of occupational exposure to NP were included.

**Figure 1**. Flow chart of study selection, screening, and eligibility

PRISMA

Eligibility

Included

Screening

Identification

PubMed: n = 199

Web of Science:n = 87

Total database searches:

n = 286

Records after duplicates removed:

n = 165

Papers included:

n = 31

Excluded: n = 134

1. Reviews: n = 44
2. Not Occupational: n = 67
3. Not Nanoparticles: n = 23

***Eligibility process***

After duplicates were removed from the original search (286 articles), the abstracts of 165 articles listed above were reviewed. We then narrowed the pool of relevant articles by excluding review articles as well as articles that were unrelated to occupational exposure (“not occupational”) or NPs (“not nanoparticles”). Following the screening for eligibility, 31 articles remained and were included in the review.

**Results**

The following paragraphs will show the most recent information from the literature to provide a comprehensive view of the available biomarkers and their applicability to monitor workers exposed to various nanoparticles.

The study population and type of exposure, methods used, health outcomes, and the expression of specific biological markers are summarized in **Table 2**.

The most used and studied Biological Exposure Markers—cardiovascular effects, lung fibrosis, lung inflammation and systemic inflammation markers, nucleic acids, lipid, and protein oxidative stress markers, antioxidant enzyme activity, and genotoxicity markers—in all studies reviewed are summarized in **Table 3**.

***The impact of exposure to different nanomaterials on worker’s health***

1. **Metal oxides NPs**. Printer toner is one of the largest consumables in daily office work. The nanoparticles released from printers and photocopiers, i.e., toner-based printing equipment, have been linked to genotoxicity and immunologic and respiratory diseases. Khatri et al. 2017 described the physicochemical and morphological properties of ENMs (e.g., titanium dioxide, iron oxide, fumed silica, and several other metals found in photocopier and printer toners) and their effects on human health in a follow-up study of photocopier operators. Khatri and colleagues found upper airway inflammation and systemic oxidative stress in photocopier operators chronically exposed to nanoparticles, in accordance with their previous study (Khatri et al. 2013) and Pirela et al. (2013, 2014), both of which included earlier results from cellular and animal toxicological studies. In humans, limited epidemiological studies report a 2-3 times higher prevalence of chronic cough, wheezing, nasal blockage, excessive sputum production, breathing difficulties, and shortness of breath in copier operators relative to controls. Respiratory symptoms were found to be exacerbated during chronic repeated exposures as well as in susceptible individuals. Thus, respiratory, immunological, cardiovascular, and other disorders may develop following such exposures.

George et al. (2010) studied the effect and resulting markers of metal oxides nanoparticles injury on pulmonary cells. They demonstrated the utility of using a rapid, throughput multiparameter cellular screening to evaluate toxic oxidative stress in bronchial epithelial and macrophage cell lines. **Titanium dioxide** (TiO2)—classified by IARC as a group 2B—cerium dioxide (CeO2), and **zinc oxide** (ZnO) NPs on those cell lines triggered a biological oxidative stress response. Among these materials, dissolution of ZnO nanoparticles and Zn2+ release were capable of reactive oxygen species (ROS) generation and activation of an integrated cytotoxic pathway that includes intracellular calcium flux, mitochondrial depolarization, and plasma membrane leakage, which could be used as parameters of exposure. While there is much debate on what constitutes appropriate safety screening methods, one approach is to assess cellular injury pathways to collect knowledge about hazardous material properties that could harm humans and the environment.

**Iron oxide** pigments are used in paints, ink, rubbers, plastics, cosmetics, and medical devices. Pelclova et al. (2016c, 2018) evaluated occupational exposure to iron oxide nanoparticles, and correlated the elevated levels of oxidative stress markers found in the exhaled breath condensate (EBC) of workers during pigment production. Similarly, Yu et al. (2020) recently evaluated the NP exposure of manufacturing/handling workers in a plant that manufactured iron oxide NPs. Yu and others found that significantly increased airborne particles at the worksite were associated with increased methylation of genomic DNA marker 5-hydroxymethylcytosine (5hmC) levels among occupationally exposed workers, suggesting this marker may be used to monitor epigenetic signatures and could possibly help predict clinically significant diseases.

**Zinc oxide** ENMs have also been widely used in cosmetics and sunscreens, advanced textiles, and self-charging and electronic devices. In addition to pulmonary damage, NP exposure is also strongly correlated with an increase in cardiovascular disease incidence; however, their toxic potential remains unclear (Chen et al. 2014).

The exposure of workers to nanoscale titanium dioxide has been extensively studied. Andujar et al. (2014), Liao et al. (2014), Pelclova et al. (2016a, 2017b), Liou et al. (2017), and Zhao et al. (2018) documented an increase in inflammation and lipid oxidation markers, as well as markers of lung and airway injury, and DNA and protein oxidative damage in EBC and, to a lesser extent, in bronchoalveolar lavage (BAL) of production and packaging workers exposed to nanoTiO2 relative to controls (Zhao et al. 2018). A significant dose-dependent association between TiO2 exposure and lipid oxidation markers in the EBC was established. Moreover, markers of cardiovascular disease and acute phase reactants were found in blood samples and some urine samples. These samples were considered to be sensitive and useful for noninvasive monitoring.

Two complementing studies performed by Pelclova et al. (2016a, 2017a) assessed the impact of short-term exposure to nanoTiO2 on physiological markers among office employees working in a building where TiO2 pigments were produced. Their findings strengthen previous results associating markers for systemic inflammation, oxidative stress, and pulmonary effect markers with occupational exposure to TiO2. Pelclova et al. (2018) examined EBC samples among three groups of workers exposed to varied NP exposure profiles and compared the results of the same markers of oxidation of lipids, nucleic acids, and proteins. They found elevated markers of oxidative stress in all workers, especially nanoTiO2 workers. Leukotriene B4 (LTB4) and cysteinyl LTE4 inflammation markers showed the highest association. Pelclova and colleagues considered EBC markers to be a sensitive technique for noninvasive monitoring of workers exposed to engineered nanoparticles. Their findings were also partly demonstrated *in vivo* by Oyabu et al. (2017), who examined the dose-response relationship of NiO and TiO2 nanoparticles—each having a different toxicity—by inhalation and intratracheal instillation studies. They also found pulmonary inflammation and oxidative stress markers alongside NP biopersistence in the lungs reflected by histopathological changes and other biomarkers in BAL fluid (BALF) samples. However, due to the ability of NPs to translocate to other organs, biopersistence—previously known as a useful toxicity indicator for micron-size particles—seems to be a useful indicator of hazards or biological effects in the lungs.

 Liou et al. (2017) described the effect of indium tin oxide (ITO) on NP handling workers, as ITO is increasingly used in liquid crystal display and semiconductor production processes; they examined the exposure of NP manufacturing and handling workers to TiO2, SiO2, and ITO NP granules or indium nano-sized fumes during different processes of splashing, pulverization, cutting, and grinding of the final ITO plates. Using noninvasive evaluation methods, they found the presence of NPs in EBC, blood, and urine, signaling that exposure to metal oxide NPs may lead to global methylation, DNA oxidative damage, and lipid peroxidation.

1. Several *in vivo* and *in vitro* mechanistic toxicology studies on a commonly used nanomaterial, **multi-wall carbon nanotubes** (MWCNT), have indicated that exposure to MWCNTs can potentially induce physiological effects in humans. These studies have shown considerable evidence of inflammation induction, oxidative stress, pulmonary fibrosis, mesothelioma-like effects, and cardiovascular effects (Kim et al. 2015; Kuempel et al. 2017; NIOSH, 2013). In an occupational health survey among MWCNT-exposed workers, the assessment of personal and area exposure levels to MWCNTs was performed using a walkthrough evaluation. Blood and EBCs from manufacturing and office workers were collected, and pulmonary function testing (PFT) was performed. Analysis of the EBCs revealed significantly higher levels of oxidative stress markers such as malondialdehyde (MDA), 4-hydroxy-2-hexenal, and 4-hydroxy-trans-nonenal in MWCNT manufacturing workers compared to those of office workers. MDA and n-hexanal levels were also found to be elevated in a similar study (J.S. Lee et al. 2015), suggesting that they could be considered useful biomarkers of MWCNT-exposure.

Vlaanderena et al. (2017) studied the effect of occupational exposure to **MWCNTs** through a molecular cross-sectional study. They evaluated the association between occupational exposure to MWCNTs and their effects on workers’ lung health and immune system at an MWCNT-producing facility. They observed significant upward trends for immune markers C-C motif ligand 20, basic fibroblast growth factor, soluble IL-1 receptor II, fractional exhaled nitric oxide (FENO), as well as differences in all measured hematological parameters between exposed and non-exposed workers. The results were found to be robust to sensitivity analyses, confirming the early effects of occupational exposure to MWCNTs on lung health and the immune system. The researchers concluded that some indications of early biological perturbations were associated with exposure to MWCNTs.

Studies performed on the blood of manufacturing workers exposed to MWCNT aerosols during fragmentation, packaging, and laboratory handling processes for at least 6 months were compared to blood samples of unexposed workers (Shvedova et al. 2016). The results of this study showed altered main regulators of biological processes, global mRNA (long non-coding RNAs, lncRNA and micro RNAs (miRNAs)) and non-coding RNA (ncRNA) expression profiles by interfering with gene expression. Several animal studies have shown pulmonary inflammation and fibrosis in rodents (Shvedova et al. 2005; Porter 2010, Mercer 2011, Poulsen 2015). The effects reported in animal studies have not yet been confirmed in humans. The potential markers of MWCNT-exposure must be further explored in humans.

A case report regarding unintended occupational exposure to dust-containing CNTs—among many other materials—was described by Wu et al. (2010). Wu et al. (2010) described the clinical and pathological findings in the lungs of first responders and rescue and recovery workers following the terrorist attack on World Trade Center (WTC), NYC, on 9/11/2001, who had been diagnosed with pulmonary fibrosis, chronic bronchiolitis, and granulomas resulting from CNT exposure. The findings were detected in biopsy specimens as well as in air samples collected at the crash site.

1. **Single-walled carbon nanotubes** (SWCNT), an important variety of carbon nanotubes, are the smallest possible crystalline wires with cross-sections as small as a single atom. This material is engineered to form seamless cylinders one nanometer in diameter, exhibiting unique electric properties that are widely used in microelectronics. Tkach et al. (2011) assessed pulmonary damage and cytokine release following exposure in an *in vivo* experiment by evaluating the activity of lactic dehydrogenase (LDH) and total protein in BAL specimens. Their results show that SWCNTs induced marked cell and tissue damage in the lungs of exposed mice with a significant dose-dependent release of LDH together with high protein and cytokines levels jointly with a significant increase of chemotactic monocytes signaling. The concentrations of TNF-α, IL-6, IFN-γ, IL-12p70, IL-10, and MCP-1 were determined, and researchers found that MCP-1 increased more than 60-fold after SWCNT-exposure and elevated IFN-γ levels.

**Welding fume NPs**. To date, there are no epidemiological studies that have specifically investigated the neurotoxic effects of manufactured NPs; however, studies of populations exposed to anthropic NPs provide an interesting perspective on concerns related to the possible effects of nanoparticles in humans. Studies of workers exposed to occupational pollutants released at the nanoscale (welding fumes and other non-intentional combustion-related mineral or metallic NPs) present the greatest interest for assessing this evidence. Andujar et al. (2014) provided the first confirmation of a link between human NP exposure and long-term pulmonary effects. They identified welding-related NPs such as Fe, Mn, Cr oxides in welders’ lung tissue sections, macrophages of the alveolar lumen, and in fibrous regions of the lungs. The investigators also performed *in vitro* analyses exposing macrophages to those NPs, and discovered an increase in the production of a pro-inflammatory secretome (inflammatory markers, chemokines CXCL-8, IL-1ß, TNF-α, CCL-2, −3, −4). The effect of nanoparticulate components of welding fumes on the human central nervous system was studied by Graczyk et al. (2016) in a cross-sectional study. They assessed oxidative stress biomarker concentrations (8-hydroxy-20 -deoxyguanosine, malondialdehyde, hydrogen peroxide, and total reducing capacity) in EBC, blood, and urine collected from non-smoking male welding trainees at different time points. Their findings indicate significant increases in the measured biomarkers 3 hours after exposure. Similar results were obtained by Brand et al. (2014), Jarvela et al. (2013), and Kauppi et al. (2015); after investigating the association between nanoparticle exposure and inflammation and oxidative stress in both subject groups, pulmonary and systemic levels in welders were significantly higher. Some of these results were also confirmed by Andujar et al. (2014), Song et al. (2016), Dierschke et al. (2017), and Rossnerova et al. (2020). The risk of cardiovascular events resulting from short exposures to ultrafine zinc- and copper-containing welding fumes was investigated in nasal secretions by Baumann et al. (2018). They found a significant increase in nasal inflammatory mediators IL-6, C-reactive protein, and serum amyloid A (SAA) in exposed workers; thus, they concluded that measuring nasal inflammatory mediators may provide a useful noninvasive method for occupational surveillance of workers exposed to ultrafine metal fume particles.

1. **Exposure to mixed NP types.** In a longitudinal study performed among nanomaterial-handling workers (recruited from 14 different factories) by Liou et al. (2012), the health hazards and possible exposure surveillance markers of nanomaterial workers were compared to those of unexposed workers being monitored six months later. The researchers investigated markers of pulmonary and cardiovascular disease, inflammation, oxidative stress, antioxidant enzymes, and genotoxicity. They found that antioxidant enzymes (superoxide dismutase, glutathione peroxidase) and cardiovascular markers (vascular cell adhesion molecule, paraoxonase) were significantly associated with nanomaterial-handling during the follow-up period. In a similar study, Liao et al. (2014) confirmed many of Liou’s findings. Also, they noticed that a small airway damage marker, Clara cell protein 16, and lung function test parameters were also significantly associated with handling nanomaterials, suggesting that the study markers and lung function tests could be useful for the surveillance of nanomaterial-handling workers. Kathria et al. 2017 studied the influence of exposure to a mixture of organic compounds on photocopiers workers; this NP mixture included metal ENMs in nasal lavage (NL) samples and urine. They found a significant increase in markers of systemic inflammation (IL-6, IL-8, TNFα, IL-1β, and eotaxin) in NL samples as well as oxidative stress markers in urine, showing a good correlation with previous results for the tested biomarkers.
2. **Silica nanoparticles (SiNPs).** According to the World Health Organization (WHO 2017), SiNPs are currently ranked as the second-largest produced NPs of all manufactured nanomaterials in the global market, implicating the potential release of SiNPs into the industrial environment and their impact on human health. The potential adverse effects of SiNPs on the cardiovascular system were described by Nemmar et al. (2014). Using an *in vivo* assay, they show that the intratracheal instillation of SiNPs could cross the alveolar-capillary barrier and impair vascular homeostasis, causing systemic inflammation and toxicological outcomes. Feng et al. (2019) investigated the effect of SiNPs on exposed animals and described similar effects such as hemodynamic changes, vascular endothelial damage, and prethrombotic state denoted by a rise in endothelial injury markers, vascular and lipids oxidative markers, among other findings.
3. **Polyacrylates.** Song et al. (2009) examined a group of workers presenting unusual symptomatic findings after being exposed to a mixture of polyacrylate and other nanoparticles (zinc oxide, titanium dioxide, nanoscale silver cluster, and other engineered nanomaterials) in the process of coating polystyrene boards with the aerosolized mixture in a printing and decorating factory. Pathological examinations of the workers’ lung tissue displayed nonspecific pulmonary inflammation, pulmonary fibrosis, and foreign-body granulomas of the pleura, raising concerns that long-term exposure to nanoparticles without protective measures could severely damage human lungs. Markers of pulmonary lesions, tissue damage, and inflammation after exposure to toner containing acrylates, among other nanomaterials, were also found by Bai and colleagues (2010) in *in vivo* experiments, correlating at least in part with Song’s findings.

**Table 2.** Summary of study population, exposure, and outcome metric.

| Author | Nanomaterial Exposure | Population Exposure/ Study/ Assay  | Health Outcomes/ Biological Markers  | Tested in human workers | Results | Confounding factors |
| --- | --- | --- | --- | --- | --- | --- |
| Y. Song et al.Eur Respir J2009 | Polyacrylate (polyacrylic ester)30 nm diameter | 7 females and 1 male (ages 18-47) working in print plant 5-13 months.Assays:1. Histopathology STEM
2. HE stain
3. Protein electrophoresis
4. Spirometry
5. Thoracentesis
 | * Shortness of breath, pleural and pericardial effusions.

Skin exposure, itching on faces and arms* Biomarkers:

Blood cells monocytes, lymphocytesBiochemical markersFibrosis, granuloma in lung tissue | Samples:Lung tissueThoracic exudate PleuralBALF effusionBloodUrineFunctional tests: liver, kidney, and lung  | **Increased:**Blood & serum: Monocytes, ESR, ALT, ASTExudate: monocytosisPleural effusion: glucoseBALF: lymphocytosis. **Decreased:**Blood & serum: neutrophils, albuminPleural effusion: chloride ion in all patients: very lowBALF: macrophagesPathological examinations: nonspecific pulmonary inflammation, fibrosis, and foreign-body granulomas of pleura | NonsmokersNot exposed to hazardous materials |
| M.WuEnviron. Health Perspect2010 | Case ReportCNTAluminum &magnesium silicates, chrysotile asbestos, calcium phosphate & sulfate | 7 previously healthy rescue and recovery workers exposed to WTC dust on 09/ 11/ 2001. Assays:Histopathology: mineralogic tissue analyses by STEM & EDS | * Severe respiratory impairment- interstitial lung disease, bronchio-parenchymal disease, non-necrotic granuloma, asthma, bronchitis, pneumonia
* Unexplained radiologic findings.
 | Samples:Lung tissue sections | **Increased:**Lung tissue: CNT and silicatesExtensive interstitial/ parenchymal abnormalities, Small airways disease | AgeGenderOccupationSmoking history ComorbiditiesLength of exposure |
| R. BaiTox. Letters2010 | Toner particles: metals, polymers acrylates, Carbon Black PM 2.5; PM 1050-200 nm | Animal experiments on exposed vs. non-exposed. | * Pulmonary lesions
* Biomarkers:

NOS, IL-6, and IL-1betaLdH, TPToner particles in the alveoli | \_ | Samples:**Increased:** significantlyBAL: (p<0.05; p<0.01) Total Protein, LdH.Lung tissue: alveolar macrophages Acid PhosphataseCell lysates:NOS, IL-6, and IL-1beta | \_ |
| S. GeorgeACS Nano2010 | TiO2CeO2ZnO | *In vitro*: bronchial epithelial and macrophage cell lines.Assays:Semi-automated epifluorescence | * Oxidative stress, plasma membrane leakage
* Biomarkers:

 ROS; Intracellular calcium flux, mitochondrial depolarization  | \_ | Samples:cell lines**Increased** Oxygen radicals; toxic metal ions**Decreased** mitochondrialmembrane potential (MMP) | \_ |
| Tkach et al.ACS Nano2011 | SWCNT | *In vivo*: mice exposed vs. non-exposed to SWCNTinhalation. Assays:1. Cytometric Bead Array
2. Spectrophotometry
 | * Pulmonary damage

Systemic inflammation* Biomarkers: LDH; Total Protein; TNF-α, IL-6, IFN-γ, IL-12p70, IL-10, and MCP-1
 | \_ | Samples: BALElevated LDH, Total Protein, IFN-γ, MCP-1 (60-fold increase) | \_ |
|  J. H LeeNanotoxicology 2012 | Silver NPs | Case study:Walkthrough evaluation of manufacturing process of 2 workers over 7 years. | No significant findingsSilver concentration | Samples:BloodUrine | Silver in urine: not detectedSilver in blood: low conc.  | \_ |
| S.H. LiouJ. Nanopart Res2012 | NPs:CNT, TiO2, SiO2, Silver, Gold,nanoresins, nanoclay, nanoalumina, and metal oxides20-100 nm | Cross-sectional study ofmanufacturing & application workers.227 exposed vs. 137 unexposed controls from 14 NP plants.Assays:Questionnaire | * Biomarkers:

Cardiovascular: fibrinogen, ICAM, interleukin-6Antioxidants: MPO, SOD, GPX | Samples:BloodUrineEBCPulmonary functions (FVC, FEV1, PEFR, MMF, FEF25 %, FEF50 %, FEF75),Heart rateNeurobehavioral function (correct rate of 7-digit backward memory)  | **Increased**: fibrinogen, ICAM, and interleukin 6Significantly higher in part of workers**Decreased**:SOD significantly (p < 0.05)GPX significantlyin part of workers.Neurobehavioral functions Significantly lower in part of workers.**No changes** in DNA damage, genotoxicity, and pulmonary markers | Exposure statusDemographicsGeographic and socioeconomic status Smoking and alcohol consumptionBetel nut chewing habitsHistory of respiratory diseaseDusty environment |
| Nemmar et al.Int’l. J. Nano- medicine 2014 | Amorphous Silica NPs (SiNPs)50 nm vs. 500 nm | Assays:1. *In vivo*: rats I.P injected
2. *In vitro*: plasma tests by ELISA, Coagulometer
3. HUVEC viability: Luminescent Assay
 | * Alteration of vascular homeostasis: systemic inflammation; thrombosis; fibrinolysis; alteration of vascular reactivity
* Biomarkers: von Willebrand factor; LDH, TNF α, interleukin 1β; thrombocytes
 | \_ | Samples: plasma**Increased**: In the 50 nm particles tests: von Willebrand factor, PAI-1 fibrinogen; LDH, TNF α, interleukin 1β**Decreased**:ThrombocytesIn vitro: platelet aggregationHUVEC viability | \_ |
| Andujar et al.Part. & Fib.Toxicol.2014 | Cross-sectional studyIron 20–25 nmchromium and /or manganese, titanium, aluminum, silica and nickel in lung tissue | 21 welders vs. 21 controls.Assays:a. Questionnairesb. *In vitro* tests on macrophages from BALc. Quantification of NPs in tissue: Imaging & material science techniques: STEM; μXRF; EDX.d. Immunohistochemistry: Lung tissue sections stained HES (hematoxylin-eosin-saffron) or Perls PrussianCD68 staining.e. Fibrosis evaluation: Roggli Semi-quantitative score | * Biomarkers

Pulmonary markers of inflammation: CXCL-8, IL-1ß, TNF-α, CCL-2−3, −4 | Samples:Lung tissue sections BAL macrophagesFibroblasts  | **Increased:**CXCL-8, IL-1ß, TNF-α, CCL-2. **Moderate increase**: IL-6, CCL-7, and −22 in macrophages in alveolar lumen and fibrous regions. No fibroblasts differentiation.CD68 staining: **High** number of macrophages in lung tissue; Perls stain: **high** iron load; **elevated** count of siderophages (iron-laden macrophages),**high** number of fibrotic lesions | GenderSmoking habitsOccupational seniority |
| H.Y. Liao et al.Nanotoxicology2014 | Nanosilver, Nanogold,Fe2O3, TiO2,CNT, SiO2Multiple exposures to mixed types of NPsSize < 100nm | Longitudinal study of workers from colors, LED, colorants, air cleaners, CNT, photocatalyst, and textile industries exposed vs. unexposed.Assays:1. Questionnaires
2. Outcome biomarkers first examined in a cross-sectional manner and then 6 months later.
 | * Biomarkers:

Pulmonary, cardiovascular disease, genotoxicity; inflammation and oxidative stress. Airway damage marker: Clara cell protein 16; lung function test Antioxidant enzymes;  | Samples:EBCBloodUrine | **Increased**:VCAM, IL-6 ICAM, LF, VLF.**Decreased**:SOD, GPX CC16, PON1, Pulmonary function (changes of maximal mid-expiratory flow, PEFR, and FEF 25%) in exposed group. | AgeGenderSmoking habitsHistory of respiratory diseaseDusty environment |
| Chen et al.ACS Nano2014  | Zinc oxide (ZnO) NPs28-150 nm diameter | *In vitro*: HUVECs. | * Biomarkers:

Cellular responsesand ER stress (sensitive marker for homeostasis interruption) | \_ | Samples:HUVECs**Increased:**Proteins BiP, Chop, GADD34, p-PERK,p-eIF2R,cleaved Caspase-12mRNA level: ER **higher** expression of spliced xbp-1, chop, and caspase-12 | \_ |
| Lee et al. Nanotoxicology 2015 | Health surveillance study Walkthrough personal and area exposure levels evaluationMWCNTs | 9 manufacturing workers and 4 office workers.  | * Biomarkers:

Oxidative stress  | Samples:Whole bloodEBCPulmonary function test  | **Increased:**EBC:MDA, 4-HHE, and n-hexanal in manufacturing workers significantly higher than in office workers.Blood:Normal hematology and biochemistry valuesLung function: normal | \_ |
| Shvedova et al. PLoS One2016 | Exposure to MWCNTs aerosols | Cross-sectional study.Exposed (n=8) vs. non-exposed (n=7) workers in a 6-month period.Assays:1. Spectrophotometer IPA global mRNAs, ncRNA expression profile blood
2. RT-PCR miRNA sequencing
3. TEM count of CNT from breathing zone.
 | * Lung inflammation and/or fibrosis; granuloma; lung different type tumors; systemic inflammation; cardiovascular injury
* Biomarkers:

IL6, EGFR, TGFβ; ERK, PDGFA, CASP8 KL-6 (MUC 1) | Samples:Whole bloodParticles in personal breathing zones | **Dysregulation:** mRNA, lncRNA, and miRNA expression profiles of target genes affecting cell cycle regulation IL6, EGFR, TGFβ; ERK, PDGFA, CASP8KL-6 (MUC 1) | AgeGenderPernicious habitsWork experience History of disease |
| Fatkhutdinova et al.Toxicol. & Applied Pharmacol.2016 | Exposure ofworkers to MWCNTs aerosols | Cross-sectional study.Exposed (n=10) vs. non-exposed (n=12).22 workers (18 males, 4 females) aged 19–63 working > 1 year. Assays:1. TEM count CNT particles
2. EC: elemental carbon analysis
3. ELISA (serum and sputum)
4. Flow cytometry (serum and sputum)
 | * Biomarkers:

Inflammatory and fibrotic markers | Samples:Nasal lavageInduced sputumBloodSerumAir samples from specific areas and personal breathing zones | **Increased:** significantlySputum: IL-1β, IL6, TNF-α, inflammatory cytokines, KL-6.Serum: TGF-β1(in young)  | AgeGenderSmoking habitsWork experience |
| Graczyk et al.Sci. Translat. Med.2016 | Welding fumes | Cross-sectional study.Non-smoking male welding trainees. | * Biomarkers:

Oxidative stress: 8 hydroxy-20 -deoxyguanosine, MDA, hydrogen peroxide, and total reducing capacity | Samples:Exhaled breath condensate (EBC) BloodUrine | **Increased:** significantlyPlasma H2O2 24%;14% 8-OHdGurinary H2O2 91%;45% urinary 8-OhdG | - |
| Pelclova et al. Occ. & Env. Med. 2016a | nanoTiO2 pigment | 36 male workersworking with TiO2 pigment for at least 6 months and 45 unexposed controls.Assays:1. Questionnaire
2. Ecoscreen Turbo DECCS Jaeger: EBC sampling
3. Crystallography: Gemini 4 circle CCD diffractometer: for Ti in EBC
4. LC-ESI-MS/MS: Markers of oxidative stress in EBC
 | * Biomarkers:

TitaniumOxidation of nucleic acids: (8-OhdG), (5-OHMeU)Proteins: o-tyrosine (o-Tyr), 3-chlorotyrosine (3-ClTyr) and 3-nitrotyrosine (3-NOTyr) | Samples:EBC | **Increased:**EBC: Titanium and most oxidative stress markers **significantly higher** in production workers (p<0.001) than in research workers and unexposed controls. | Occupational historyMedical treatments Lifestyle habits (e.g., diet, alcohol intake, smoking, physical activity) |
| Pelclova et al.J. Breath Research2016b | nanoTiO2 aerosol80% of particles <100 nm diameter | 30 workers exposed to TiO2 aerosol, 22 office employees, and 45 unexposed controls.Assays:1. LC-ESI-MS/MS
2. FENO
3. Spirometry
 | * Potential fibrotic changes in lungs, inflammation
* Biomarkers:

 EBC: LT B4, C4, E4, D4 Lungs: % VCIN; % PEF | Samples:EBCUrine | **Increased** in EBC: LT B4, C4, E4, D4 in workers relative to controls (p < 0.01).Cysteinyl LTsImpaired %VCIN and %PEF (both *p* < 0.01). | Allergic diseasesSmoking status |
| Pelclova et al.J. Breath Research2016c | Iron oxide aerosol80% of particles <100 nm diameter | 14 workers aged 43± 7 y. exposed 10±4 y. and 14 controls (aged 39±4 y.)Dose-dependent.Assays:1. LC-ESI-MS/MS
2. SMPS; APS; P-TRAK; DustTRAK DRX: for workplace aerosol
 | * Asymptomatic with Oxidative stress markers
* Biomarkers:

Oxidative stress, oxidation of nucleic acids- and inflammationProteins: o-tyrosine, 3-chlorotyrosine, and 3-nitrotyrosine | Samples:EBCUrine | **Increased**:EBC: MDA, HHEHNE, 8-isoprostane,aldehydes C6–C12, 8-OHdG,8-OHG, 5-OHMeU, 3-ClTyr,3-NOTyr, o-Tyr (all *p* < 0.001)in workers relative to controls (p < 0.01).Urine: no increase | AgeBMISmoking and alcohol consumption |
| Pelclova et al. Nanotoxicology 2017b | Cross-sectional studynanoTiO280% of particles <100 nm diameter | Production workers and Controls. | * Lung injury, inflammation,
* Biomarkers:

Lipid oxidation, oxidative stress, cytotoxicity, and genotoxicityMDA, 4-hydroxy-trans-hexenal, 4-hydroxy-trans-nonenal, 8-iso Prostaglandin F2α; aldehydes C6–C12 | Samples:EBC | **Increased**:11 markers of lipidoxidation in productionworkers relative tocontrols(p < 0.001)  | \_ |
| Pelclova et al. Rev. Environ. Health2017a | Nanoscale titanium dioxide (nanoTiO2)<100 nm diameter | Cross-sectional study.Short-term MVA. 22 office workers intermittently exposed to TiO2 vs. 14 unexposed. Assays:1. Questionnaires
2. Physical exam
3. Spirometry
4. TiO2
 | * Inflammation
* Biomarkers:Biomarkers of lipid oxidation MDA, HHE, HNE, 8-isoprostane, aldehydes C6−C12
 | Samples:EBCUrine | **Increased:**9 markers of lipidOxidation, DNA and proteinoxidative damage inproduction workers.EBC: highly significant difference between production and office workers. (p < 0.001) Urine: No increase | AgeSmoking and alcohol consumptionPhysical activity |
| Vlaanderenet al. Nanotoxicology 2017 | MWCNT | Exposed vs. non-exposed workers. Assay:Molecular cross-section | * Pulmonary and immune system damage.
* Biomarkers:

 Immune markers and pneumoproteins: C-C motif ligand 20, basic fibroblast growth factor, soluble IL1 receptor II | Samples:SerumWhole blood (CBC)FENOLung function | **Increased:**Significant upward trends for immune markers C-C motif ligand 20 (p= 0.005), basic fibroblast growth factor (p= 0.05), and soluble IL-1 receptor II (p= 0004) | AgeBMIGenderSmoking status |
| Liou et al.J. Hazardous Mat. 2017 | TiO2, SiO2, indium tin oxide (ITO) | Cross-sectional study.130 workers 70% males and 30% females; mean age 35 yr. handling NPs 3.4 ds. / wk. 4.4 h/ day Exposed vs. non-exposed workers.Assays:Questionnaires | * Biomarkers:

Global methylation, DNA oxidative damage, lipid peroxidation.Oxidative stressUrinary and WBC 8-OHdG, EBC 8-isoprostane | Samples:BloodUrineEBC  | **Increased:**WBC; 8-OHdG8-isoprostane8-OHdG (negatively correlated with global methylation)WBC and urinary 8-OHdG positively correlated.Lower global methylation in ITO handling workers. | DemographicSocioeconomic characteristicsLifestyle (or smoking and alcohol consumption) Occupational history Personal and family disease |
| Oyabu et al.Int’l. J. Mol. Sci.2017 | NiO (high tox. Size 60 nm) and TiO2 (low tox. Size 45 nm) | *In vivo*: rats exposed to NP by inhalation and intratracheal instillation.1. ICP-AES: quantitation of NPs in lung
2. MMPD: Calculation of BHTs
 | * Pulmonary inflammation
* Biomarkers:

PMN in BALF, cytokines; oxidative stress, Biopersistence of particles in the lung | \_ | 1. In both inhalation and instillation, NiO NPs persisted for longer in the lung compared with TiO2.2. Biological half times of NiO NPs longer than that of TiO2, correlates with histopathological changes, inflammatory response, cytokines elevation. | \_ |
| Dierschke et al. Int’l. Arc. Occ. & Env. Health2017 | Welding fumes -mild steel welding (Iron 70% and Mn 30%) | 11 welders with and 10 without work-related symptoms from lower airways and 11 asymptomatic non-welders. Assays:1. Questionnaires:

Exposed vs. non-exposed to fumes workers, random double-blind1. RTube for EBC sampling
2. Cytospin and May-Grüenwald Giemsa for NL
3. Luminex Immunoassay (cytokines)
4. Sysmex XE-5000/1800i counters (neutrophils)
5. Visual analog scale for eyes examining
 | * Biomarkers:

EBC: leukotrienes LT-B4NL: IL-6NeutrophilsIL-8 | Samples:EBCBloodSerumNasal lavage (NL)Lung function | 1. Lung function: No adverse effect.2. EBC: LT-B4 pre-exposure significantly **increased** (tenfold higher level) in symptomatic sensitive welders with work-related airway symptoms (chronic exposure to welding fumes).3. NL: IL-6 increased in non-symptomatic groups4. Blood: rapid elevation of IL-8 and neutrophils after exposure followed by a significant decrease (ongoing neutrophilic low-grade inflammation). | Non-current smokers (5 yr.)Total welding time AgeExposureAllergiesWork-related symptoms from eyes and airways |
| Khatri et al. NanoImpact2017 | Mixture of organic compounds with metal ENPs | 6 photocopiers workers vs. 11 controls 3 random weeks during 2 years.Assays:1. Quantitative airborne NPs
2. Chemistry
3. Lung burden estimates
 | * Chronic upper airway inflammation and systemic oxidative stress
* Biomarkers:

NL: 14 pro-inflammatory cytokines/ chemokines, inflammatory cells, and total protein8-OH-dG | Samples:Nasal lavageUrine: 8-OH-dG  | **Increased:**NL: IL-6, IL-8, TNFα, IL-1β and Eotaxin (significantly p˂0.0001)inflammatory cell infiltration 2.7-foldTotal Protein: 4-foldUrine: 8-OH-dG 4.3-fold  | \_ |
| Pelclova et al. Occ. & Env. Med. 2018 | TiO2Iron oxidesNanocompositesExposure to aerosols > 2 years | 3 groups of workers vs. comparable control groups. 34 nano TiO2 workers over 2 years.14 nano Iron oxides; and32 nanocomposites workers 2-year follow-up.Assays:1. Spirometry
2. FENO
3. LC-ESI-MS/MS
4. APS, SMPS, OPS, and CPC (Aerosol exposure)
 | * Biomarkers:

Lipids oxidation, nucleic acids and proteins: MDA, HHE, HNE, 8-isoProstaglandinF2α, C6-C13, 8-OHdG, 5-hydroxymethyl uracil, o-tyrosine, 3-ClTyr, 3- NOTyr; pro-inflammatory leukotrienes LTB4, LTC4, LTE4, LTD4 | Samples:EBC | **Increased:**Markers of oxidative stress: LTB4 and cysteinyl LTE4 most useful and elevated in nanoTiO2 workers. | \_ |
| Zhao et al. Nanotoxicology 2018 | Cross-sectional studyTiO2 | 83 exposed workers vs. 85 controls in packaging workshop.Assays:1. Questionnaire
2. Spirometer
3. ELISA
4. Cytometric Bead Array
5. BD FACSCalibur flow cytometer
 | * Significant changes in chest X-ray images
* Biomarkers: Pulmonary surfactant protein D (SP-D)

Cardiovascular disease: VCAM-1, ICAM-1, LDL, and TCInflammatory and acute phase reactants, oxidative stress | Samples:SerumChest radiographyPFT | **Increased**:SP-D; VCAM-1 and ICAM-1LDLIL-8, IL-6, and TNF-alfaIL-1beta, IL-10**Decreased** (significantly): creatinine, triglyceride, and total cholesterol | DemographicSmoking and alcohol consumptionOccupational and medical historyUse of personal protection equipment (PPE) |
| Baumann et al.Nanotoxicology 2018 | Zinc- and copper welding fumesControlled exposure to welding process (used for vehicle construction and interiors)50 - 300 nm different shapes | 15 healthy male volunteers (age 26, nonsmokers).Assays:1. Lavages nasal strips (Leuco- sorb)
2. Coomassie Plus (Pierce)
3. ECL
4. Spirometry
5. Plethysmography
6. Cycling ergometry
7. ROC curve analysis -Receiver operating characteristic to differentiate welding fume from control exposure
8. Field Emission SEM
9. EDX
 | * Inflammatory responses Cardiovascular events: elevated risk
* Biomarkers:

Systemic: IL-6, CRP, and SAAICAM-1, and VCAM-1Nasal interferon-c (IFN-c)Total protein | Samples:Nasal secretion (serial)Lung function | **Increased:**Nasal: (IFN-c)Nasal Total protein, CRP, and SAA: significantlyNo significant changes: IL-6, sVCAM-1, sICAM-1 | Smoking status |
| Feng et al.Part. & Fib.Toxicol.2019  | Silica NPs (SiNPs) | *In vivo*: rats exposed. Assays:1. Doppler ultrasound rats’ aortic arch
2. Immunohistochemistry
3. Microarray
4. qPCR
5. Western blot
6. Bioinformatics analysis
 | * Hemodynamic changes

Vascular endothelial damage and prethrombotic state.* Biomarkers: miR-451a

MDA; PECAM-1SOD, GSH-PxJak1, Stat3, TF, Il6rFib | \_ | **Increased:** Hypercoagulation; decreased blood flow velocityLipid perox. MDA; PECAM-1 positive cells;**Decreased**:Antioxidant SOD, GSH-Px.Downregulated miR-451a rises endothelial injury markers, TF, inflammatory cells, vascular oxidative damage: PECAM-1, SOD and GSH-Px**Upregulated** gene expression: Jak1, Stat3, Tf, Il6r, and Fib  | \_ |
| Yu et al.Toxicology & Industrial Health 2020 | Iron oxide NPs (IONP) | Cross-sectional study.23 workers aged 23 unexposed to metal.Assays:1. ELISA
2. PCR
 | * Biomarkers:

Iron status, oxidation markers, methylation of genomic DNA5-methylcytosine (5mC), hepcidin, iron, soluble transferrin receptor (sTfR), ferritin, 8-OHdG, and glutathione | Samples:Blood | **Increased:**5hmCNo change: the restPositive correlation: 5hmC and IONP | Demographics (e.g., gender)Occupational history |
| Rossnerova et al. Int’l. J. Mol. Sci. 2020 | Aerosolized welding fumes 2 fractions<25 nm25–100 nm | Cross-sectional study.20 exposed welding and machining vs. 20 unexposed. (both genders) 14.5± 9.2 yr exposure.No PPE used.Assays:1. Infinium Methylation Assay EPIC BeadChips microarray
2. ELISA
3. SMPA
4. APS
 | * Biomarkers:

Methylation pattern at CpG loci | Samples:Blood | **Increased:**Significant CpG in genes of lipid metabolism, immune system, lung functions, signaling pathways, cancer,xenobiotic detoxification. | AgeGenderBMIPPE |

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**Table 3. Summary of most studied Biological Exposure Markers**

| Genotoxicity markers (DNA damage) | Oxidative stress markers | Pulmonary effect markers (tissue damage) | Systemic inflammation markers | Antioxidant markers |
| --- | --- | --- | --- | --- |
| xbp-1  | 3-NOTyr (3-nitrotyrosine) | CC16 (Clara cell protein) | hsCRP (Highly sensitive C-reactive protein) | SOD (Superoxide dismutase) |
| caspase-12 | 5-OHMeU (5-Hydroxymethyl uracil) | FENO Fractional exhaled nitric oxide | IL1b (Interleukin1b) | GPX (Glutathione peroxidase) |
| chop | 8-isoprostane (8-Iso-prostaglandin F2α) | KL-6 (Krebs von den Lungen 6) | IL8 (Interleukin 8) | PON1 (Paraoxonase 1) |
| GADD34 | 8-OHG (8-Hydroxyguanosine/8 hydroxy-20-deoxy guanosine) | MIP-1beta (Macrophage inflammatory protein-1b) | IL6 (Interleukin 6) |  |
| miRNAs,mRNA | C6–C12 (n-alkanes) | PFT (Pulmonary function test) | IL6sR (Interleukin 6 soluble receptor) |  |
|  |  |  | IL4 (Interleukin 4) |  |
|  | HNE (4-Hydroxy-trans-nonenal) | FVC (Forced vital capacity) | NF-kb (Nuclear factor-kappa beta) |  |
|  | HHE (4-Hydroxy-trans-hexenal) | FEV1 (Forced expiratory volume at 1s) | TNF alpha (Tumor necrosis factor alfa) |  |
|  | LTs (Leukotrienes) | MMF (Maximal mid-expiratory flow) | Vascular endothelial function biomarkers: miR-451a |  |
|  | MDA (Malondialdehyde) | PEFR (Peak expiratory flow rate) | ICAM (Intercellular adhesion molecule) |  |
|  |  |  | VCAM-1 vascular cell adhesion molecule-1 |  |
|  | o-tyr (o-Tyrosine) | FEF 25 / 50 / 75% (Forced expiratory flow at 25/50/75% respectively) | MPO (Myeloperoxidase) |  |
|  | 3-Cl-Tyr (3-chloro-tyrosine) | TGF-b1 (Transforming growth factor beta1) | HRV (Heart rate variability) |  |
|  |  | LDH (Lactic dehydrogenase) | IFN-c (Nasal interferon-c)  |  |
|  |  | Total Protein | NOS (Nitric oxide synthase) |  |
|  |  | Acid Phosphatase | CCL-2, CCL-3, CXCL-8 |  |
|  |  | p-PERK | leukotrienes LTB4, LTC4, LTE4, LTD4 |  |
|  |  | IFN-γ (Interferon-gamma) | Cysteinyl LT |  |
|  |  | MCP-1 | SAA (Serum amyloid A) |  |

**Discussion**

This review attempts to draw on the most recent information from *in vitro,* animal, and epidemiological studies to identify the expression of biological markers resulting from occupational exposure to different nanomaterials and nanoparticles. The literature to date has assessed a variety of biological markers, many of them showing statistically significant changes in biomonitoring and respiratory functions in animal and human studies. Experiments on animals exposed to different NPs at varying doses and exposure routes allow for comparing doses related to relevant physiological human exposure. Increased or decreased markers of lipid oxidation and inflammatory cell activation, cardiovascular disease markers, markers of oxidative damage to DNA, antioxidant markers, serum pneumoproteins, acute phase proteins, clotting factors, and adhesion molecules were described. Markers showing statistically significant changes among exposed NP workers such as miRNAs, fibrogenic markers, micronuclei, and ICAM-1 in macrophages were also shown. *In vitro* studies were useful in showing significant changes in protein or gene expression and to get molecular insights into NP-induced toxicity and pathogenesis in humans. The majority of epidemiological studies involved NPs from the manufacturing and printing technologies, mainly single- and multi-walled carbon nanotubes, titanium dioxide, metal oxides, silicon dioxide, and other nanomaterials including nanoresins, nanosilver, nanogold, nanoclay, and nanoalumina; multiple exposures to mixed types of nanoparticles were very common, Titanium dioxide was the most frequently described, either separately or in combination with other NPs followed by mixtures of nanomaterials in welding fumes and carbon nanotubes in a variety of industries. From all human samples collected in the different epidemiology studies, EBC was found to be a sensitive technique for the noninvasive monitoring of workers exposed to NPs with biomarkers that reflect intrinsic changes in the airway lining fluid and lung inflammation. When released from metal NPs when dissolved in biological media, some ions were detectable with analytical methods and could serve as valuable markers of exposed workers. Although the results described in this scoping review demonstrate a good relationship between exposure of workers to NP and physiologically significant biomarkers, in order to use these biomarkers in routine occupational medical surveillance, large scale epidemiological studies among well-defined groups of workers will be required to confirm the utility of routine occupational biomonitoring.

**Conclusions**

The adverse physiological effects of occupational exposure to nanomaterials, demonstrated by the significant association with exposure biomarkers, were highlighted in this review. Validated biomarkers will enable the progression of knowledge about potential health effects associated with occupational NP exposure in general and will contribute to the implementation of reliable, noninvasive occupational medical surveillance. The development of biological exposure indices and occupational exposure limits will protect workers from emerging exposures. With further research, biomarkers could be recommended for preventive occupational medicine surveillance and to help monitor workers with occupational exposure to nanoparticles.

Based on the findings within the reviewed literature, workplaces with significant NP exposure should implement preventive measures such as the substitution of certain NPs, or administrative, engineering, or personal protective equipment in order to reduce exposure levels and protect workers from potential adverse health effects.

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**References**

Andujar, P., Simon-Deckers, A., Galateau-Sallé, F., Fayard, B., Beaune, G., Clin, B., Billon-Galland, M.A., et al., 2014. Role of Metal Oxide Nanoparticles in Histopathological Changes Observed in the Lung of Welders. *Particle and Fibre Toxicology* 11(1): 23. doi: 10.1186/1743-8977-11-23.

Bai, R., Zhang, L., Liu, Y., Meng, L., Wang, L., Wu, Y., Li, W., et al., 2010. Pulmonary Responses to Printer Toner Particles in Mice after Intratracheal Instillation. *Toxicology Letters* 199(3): 288-300. doi: 10.1016/j.toxlet.2010.09.011.

Baumann, R., Brand, P., Chaker, A., Markert, A., Rack, I., Davatgarbenam, S., Joraslafsky, S., et al., 2018. Human Nasal Mucosal C-reactive Protein Responses after Inhalation of Ultrafine Welding Fume Particles: Positive Correlation to Systemic C-reactive Protein Responses. *Nanotoxicology* 12(10): 1130-1147. doi: 10.1080/17435390.2018.1498930.

Chen, R., Huo, L., Shi, X., Bai, R., Zhang, Z., Zhao, Y., Chang, Y., et al., 2014. Endoplasmic Reticulum Stress Induced by Zinc Oxide Nanoparticles is an Earlier Biomarker for Nanotoxicological Evaluation. *ACS Nano* 8(3): 2562-2574. doi: 10.1021/nn406184r.

Dierschke, K., Isaxon, C., Andersson, U.B., Assarsson, E., Axmon, A., Stockfelt, L., Gudmundsson, A., et al., 2017. Acute Respiratory Effects and Biomarkers of Inflammation Due to Welding-derived Nanoparticle Aggregates. *International Archives of Occupational and Environmental Health* 90(5): 451-463. doi: 10.1007/s00420-017-1209-z.

Fatkhutdinova, L.M., Khaliullin, T.O., Vasil’yeva, O.L., Zalyalov, R.R., Mustafin, I.G., Kisin, E.R., Birch, M.E., et al., 2016. Fibrosis Biomarkers in Workers Exposed to MWCNTs. *Toxicology and Applied Pharmacology* 299: 125-131. doi: 10.1016/J.TAAP.2016.02.016.

Feng, L., Yang, X., Liang, S., Xu, Q., Miller, M.R., Duan, J. and Sun, Z., 2019. Silica Nanoparticles Trigger the Vascular Endothelial Dysfunction and Prethrombotic State via miR-451 Directly Regulating the IL6R Signaling Pathway. *Particle and Fibre Toxicology* 16(1): 1-13. doi: 10.1186/s12989-019-0300-x.

Forest, V., Vergnon, J.M. and Pourchez, J., 2017. Biological Monitoring of Inhaled Nanoparticles in Patients: an Appealing Approach to Study Causal Link between Human Respiratory Pathology and Exposure to Nanoparticles. *Chemical Research in Toxicology* 30(9): 1655-1660. doi: 10.1021/acs.chemrestox.7b00192.

George, S., Pokhrel, S., Xia, T., Gilbert, B., Ji, Z., Schowalter, M., Rosenauer, A., et al., 2010. Use of a Rapid Cytotoxicity Screening Approach to Engineer a Safer Zinc Oxide Nanoparticle through Iron Doping. *ACS Nano* 4(1): 15-29. doi: 10.1021/nn901503q.

Iavicoli, I., Leso, V. and Schulte, P.A., 2016. Biomarkers of Susceptibility: State of the Art and Implications for Occupational Exposure to Engineered Nanomaterials. *Toxicology and Applied Pharmacology* 299: 112-124. doi: 10.1016/j.taap.2015.12.018.

Khatri, M., Bello, D., Gaines, P., Martin, J., Pal, A.K., Gore, R. and Woskie, S., 2013a. Nanoparticles from Photocopiers Induce Oxidative Stress and Upper Respiratory Tract Inflammation in Healthy Volunteers. *Nanotoxicology* 7(5): 1014-1027. doi: 10.3109/17435390.2012.691998.

Khatri, M., Bello, D., Pal, A.K., Cohen, J.M., Woskie, S., Gassert, T., Lan, J., et al. 2013b. Evaluation of Cytotoxic, Genotoxic and Inflammatory Responses of Nanoparticles from Photocopiers in Three Human Cell Lines. *Particle and Fibre Toxicology* 10(1): 42. doi: 10.1186/1743-8977-10-42.

Khatri, M., Bello, D., Martin, J., Bello, A., Gore, R., Demokritou, P. and Gaines, P., 2017. Chronic Upper Airway Inflammation and Systemic Oxidative Stress from Nanoparticles in Photocopier Operators: Mechanistic insights. *NanoImpact* 5: 133-145. doi: 10.1080/10408444.2017.1318354.

Kuhlbusch, T.A., Asbach, C., Fissan, H., Göhler, D. and Stintz, M., 2011. Nanoparticle Exposure at Nanotechnology Workplaces: a Review. *Particle and Fibre Toxicology* 8(1): 1-18. doi: 10.1186/1743-8977-8-22.

Lee, J.H., Mun, J., Park, J.D. and Yu, I.J., 2012. A Health Surveillance Case Study on Workers who Manufacture Silver Nanomaterials. *Nanotoxicology* 6(6): 667-669. doi: 10.3109/17435390.2011.600840.

Lee, J.S., Choi, Y.C., Shin, J.H., Lee, J.H., Lee, Y., Park, S.Y., Baek, J.E., et al., 2015. Health Surveillance Study of Workers who Manufacture Multi-walled Carbon Nanotubes. *Nanotoxicology* 9(6): 802-811. doi: 10.3109/17435390.2014.978404.

Liao, H.Y., Chung, Y.T., Lai, C.H., Wang, S.L., Chiang, H.C., Li, L.A., Tsou, T.C., et al., 2014. Six-month Follow-up Study of Health Markers of Nanomaterials among Workers Handling Engineered Nanomaterials. *Nanotoxicology* 8(sup1): 100-110. doi: 10.3109/17435390.2013.858793.

Liou, S.H., Tsou, T.C., Wang, S.L., Li, L.A., Chiang, H.C., Li, W.F., Lin, P.P., et al., 2012. Epidemiological Study of Health Hazards among Workers Handling Engineered Nanomaterials. *Journal of Nanoparticle Research* 14(8): 878. doi: 10.1007/s11051-012-0878-5.

Liou, S.H., Tsai, C.S., Pelclova, D., Schubauer-Berigan, M.K. and Schulte, P.A., 2015. Assessing the First Wave of Epidemiological Studies of Nanomaterial Workers. *Journal of Nanoparticle Research* 17(10): 413. doi: 10.1007/s11051-015-3219-7.

Liou, S.H., Wu, W.T., Liao, H.Y., Chen, C.Y., Tsai, C.Y., Jung, W.T. and Lee, H.L., 2017. Global DNA Methylation and Oxidative Stress Biomarkers in Workers Exposed to Metal Oxide Nanoparticles. *Journal of Hazardous Materials* 331: 329-335. doi: 10.1016/j.jhazmat.2017.02.042.

Nemmar, A., Albarwani, S., Beegam, S., Yuvaraju, P., Yasin, J., Attoub, S. and Ali, B.H., 2014. Amorphous Silica Nanoparticles Impair Vascular Homeostasis and Induce Systemic Inflammation. *International Journal of Nanomedicine* 9: 2779-2789. doi: 10.2147/IJN.S52818.

Oberdörster, G., Maynard, A., Donaldson, K., Castranova, V., Fitzpatrick, J., Ausman, K., Carter, J., et al., 2005. Principles for Characterizing the Potential Human Health Effects from Exposure to Nanomaterials: Elements of a Screening Strategy. *Particle and Fibre Toxicology* 2(1): 8. doi: 10.1186/1743-8977-2-8.

Oyabu, T., Myojo, T., Lee, B.W., Okada, T., Izumi, H., Yoshiura, Y., Tomonaga, T, et al., 2017. Biopersistence of NiO and TiO2 Nanoparticles following Intratracheal Instillation and Inhalation. *International Journal of Molecular Sciences* 18(12): 2757. doi: 10.3390/ijms18122757.

Pelclova, D., Zdimal, V., Fenclova, Z., Vlckova, S., Turci, F., Corazzari, I., Kacer, P., et al., 2016a. Markers of Oxidative Damage of Nucleic Acids and Proteins among Workers Exposed to TiO2 (Nano) Particles. *Occupational and Environmental Medicine* 73(2): 110-118. doi: 10.1136/oemed-2015-103161.

Pelclova, D., Zdimal, V., Kacer, P., Fenclova, Z., Vlckova, S., Komarc, M., Navratil, T., et al., 2016b. Leukotrienes in Exhaled Breath Condensate and Fractional Exhaled Nitric Oxide in Workers Exposed to TiO2 Nanoparticles. *Journal of Breath Research* 10(3): 036004. doi: 10.1088/1752-7155/10/3/036004.

Pelclova, D., Zdimal, V., Kacer, P., Fenclova, Z., Vlckova, S., Syslova, K., Navratil, T., et al., 2016c. Oxidative Stress Markers are Elevated in Exhaled Breath Condensate of Workers Exposed to Nanoparticles during Iron Oxide Pigment Production. *Journal of Breath Research* 10(1): 016004. doi: 10.1088/1752-7155/10/1/016004.

Pelclova, D., Zdimal, V., Kacer, P., Komarc, M., Fenclova, Z., Vlckova, S., Zikova, N., et al., 2017a. Markers of Lipid Oxidative Damage among Office Workers Exposed Intermittently to Air Pollutants including NanoTiO2 Particles. *Reviews on Environmental Health* 32(1-2): 193-200. doi: 10.1515/reveh-2016-0030.

Pelclova, D., Zdimal, V., Kacer, P., Zikova, N., Komarc, M., Fenclova, Z., Vlckova, S., et al., 2017b. Markers of Lipid Oxidative Damage in the Exhaled Breath Condensate of Nano TiO2 Production Workers. *Nanotoxicology* 11(1): 52-63. doi: 10.1080/17435390.2016.1262921.

Pelclova, D., Zdimal, V., Dvorackova, S., Schwarz, J., Ondracek, J., Komarc, M., Vlckova, S., et al., 2018. 1601b Noninvasive Biomonitoring of 3 Groups of Nanomaterials Workers with Elevated Markers of Oxidative Stress and Inflammation. doi: 10.1136/oemed-2018-ICOHabstracts.799.

Rinaldo, M., Andujar, P., Lacourt, A., Martinon, L., Canal Raffin, M., Dumortier, P., Pairon, J.C., et al., 2015. Perspectives in Biological Monitoring of Inhaled Nanosized Particles. *Annals of Occupational Hygiene* 59(6): 669-680. doi: 10.1093/annhyg/mev015.

Roco, M.C., Mirkin, C.A. and Hersam, M.C., 2010. Nanotechnology Research Directions for Societal Needs in 2020: Retrospective and Outlook. *WTEC*. doi: 10.1007/978-94-007-1168-6.

Rossnerova, A., Honkova, K., Pelclova, D., Zdimal, V., Hubacek, J.A., Chvojkova, I., Vrbova, K., et al., 2020. DNA Methylation Profiles in a Group of Workers Occupationally Exposed to Nanoparticles. *International Journal of Molecular Sciences* 21(7): 2420. doi: 10.3390/ijms21072420.

Shvedova, A.A., Yanamala, N., Kisin, E.R., Khailullin, T.O., Birch, M.E. and Fatkhutdinova, L.M., 2016. Integrated Analysis of Dysregulated ncRNA and mRNA Expression Profiles in Humans Exposed to Carbon Nanotubes. *PloS One* 11(3): e0150628. doi: 10.1371/journal.pone.0150628.

Song, Y., Li, X. and Du, X., 2009. Exposure to Nanoparticles is Related to Pleural Effusion, Pulmonary Fibrosis and Granuloma. *European Respiratory Journal* 34(3): 559-567. doi: 10.1183/09031936.00178308.

Tkach, A.V., Shurin, G.V., Shurin, M.R., Kisin, E.R., Murray, A.R., Young, S.H., Star, A., et al., 2011. Direct Effects of Carbon Nanotubes on Dendritic Cells Induce Immune Suppression upon Pulmonary Exposure. *ACS Nano* 5(7): 5755-5762. doi: 10.1021/nn2014479.

Tricco, A.C., Lillie, E., Zarin, W., O’Brien, K.K., Colquhoun, H., Levac, D., Moher, D., et al., 2018. PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. *Annals of Internal Medicine* 169(7): 467-473. doi: 10.7326/M18-0850.

Vlaanderen, J., Pronk, A., Rothman, N., Hildesheim, A., Silverman, D., Hosgood, H.D., Spaan, S., et al., 2017. A Cross-sectional Study of Changes in Markers of Immunological Effects and Lung Health due to Exposure to Multi-walled Carbon Nanotubes. *Nanotoxicology* 11(3): 395-404. doi: 10.1080/17435390.2017.1308031.

Wang, J., Asbach, C., Fissan, H., Hülser, T., Kuhlbusch, T.A., Thompson, D., and Pui, D.Y., 2011. How Can Nanobiotechnology Oversight Advance Science and Industry: Examples from Environmental, Health, and Safety Studies of Nanoparticles (Nano-EHS). *Journal of Nanoparticle Research* 13(4): 1373-1387. doi: 10.1007/s11051-011-0236-z.

Wu, M., Gordon, R.E., Herbert, R., Padilla, M., Moline, J., Mendelson, D., Litle, V., et al., 2010. Case Report: Lung Disease in World Trade Center Responders Exposed to Dust and Smoke: Carbon Nanotubes Found in the Lungs of World Trade Center Patients and Dust Samples. *Environmental Health Perspectives* 118(4): 499-504. doi: 10.1289/ehp.0901159.

Xiaoli, F., and Longquan, S., 2018. Neurotoxicity of Nanomaterials. In *Emerging Nanotechnologies in Dentistry.* William Andrew Publishing, 421-444. doi: 10.1016/B978-0-12-812291-4.00020-0.

Yu, M., Zhou, X., Ju, L., Yu, M., Gao, X., Zhang, M., and Tang, S., 2020. Characteristics of Iron Status, Oxidation Response, and DNA Methylation Profile in Response to Occupational Iron Oxide Nanoparticles Exposure. *Toxicology and Industrial Health* 36(3): 170-180. doi: 10.1177/0748233720918683.

Zhao, L., Zhu, Y., Chen, Z., Xu, H., Zhou, J., Tang, S., Xu, Z., et al., 2018. Cardiopulmonary Effects Induced by Occupational Exposure to Titanium Dioxide Nanoparticles. *Nanotoxicology* 12(2): 169-184. doi: 10.1080/17435390.2018.1425502.