Review

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

**Diana Blank-Porat 1\* and Eric Amster1\***

**1**Department of Environmental and Occupational Health, University of Haifa School of Public Health, Haifa 31905, Israel; dporat@staff.haifa.ac.il

Correspondence\*…………………….

**Abstract**

**Keywords**

Nanoparticle workers; Biological Exposure Index (Markers); Biomarkers; Nanoparticle exposure; Engineered nanomaterials; Engineered nanoparticles; Health effects.

The ubiquitous use of nanomaterials - particulate materials measuring approximately 1–100 nanometers (nm) at least in one dimension, poses unique issues regarding worker’s health and safety. Use of new substances or substances in a new form and unique chemical and physical properties made them useful in many industries. Still, those valuable properties by production means may challenge workers’ health showing emerging occupational health hazards that have yet to be fully assessed for their acute or chronic health effects. Results of early animal studies of engineered nanomaterials and air pollution epidemiology underscore the importance of considering the health risk from nanoparticles (NPs) and/ or engineered nanomaterials (ENMs) exposure among workers. In this scoping review we aim to assess the utility of biomonitoring to screen nanotechnology workers for exposure to nanoparticles. PubMed and Web of Science databases were queried for the past 10 years for publications including cellular, animal, and human toxicological and epidemiological evidence regarding the use of biomarkers to estimate health outcomes as well as laboratory and pathophysiological changes in workers exposed to nanomaterials. Inclusion criteria comprised of original publications in which (a) biological markers of occupational exposure to NP, (b) health outcomes of exposure, and (c) laboratory test results from in vitro, in vivo (animals) and epidemiological studies were included. Of 286 articles primarily retrieved, 16 were included in the scope review after screening and eligibility. The articles reviewed indicate that sensitive validated biomarkers in epidemiological studies, including those of potential disease progression and epigenetics are useful tools to predict the toxicological effects and potential risks associated with NPs exposure at the workplace. The information herein could drive policy decision makers in the field of Occupational Health to promote regulations and define occupational exposure limits to contribute to workers health and wellbeing.

**Abbreviations** CRP: C-reactive protein; SAA: serum amyloid A ;SOD: superoxide dismutase; GPX: glutathione peroxidase; NO: nitric oxide; NL: Nasal lavage; VCAM: vascular cell adhesion molecule; ICAM: intercellular adhesion molecule; IL-6: interleukin-6; Clara cell protein 16: CC16, VLF: very low frequency; LF: low frequency; L/H: tail/head ratio; PFT: Pulmonary Function Test; PEFR: peak expiratory flow rate; FEF25%: forced expiratory flow at 25%; LT: leucotrienes; PMN: polymorphonuclear neutrophils; LDL: Low Density Lipoprotein; BHTs: biological half times; ELISA: Enzyme Linked Immunosorbent Assay; ECL: electrochemiluminescense; ICP-AES: Inductively Coupled Plasma-Atomic Emission Spectrometer ; MMPD: Multiple Path Particle Dosimetry; PCR: Polymerase Chain Reaction; FENO: fractional exhaled nitrogen oxide ; HUVEC: human umbilical vein endothelial cells; PAI-1: plasminogen activator inhibitor-1; TF: Tissue Factor; MDA: malondialdehyde; 8-OHdG: 8 hydroxydeoxyguanosine; 5-OHMeU: 5-hydroxymethyl uracil; 3-ClTyr: 3-chlorotyrosine; 3-nitrotyrosine: 3-NOTyr; Titanium dioxide: TiO2; Cerium dioxide: CeO2 , MVA: Multi variate analysis; CCL-2, CCL-3, CXCL-8: chemotactic cytokines; TEM/ STEM: Scanning Transmission Electron Microscopy; μXRF: X-ray microfluorescence; EDX: energy dispersive X-ray; EDS: energy dispersive spectroscopy; IPA: Ingenuity Pathway Analysis; APS: Aerodynamic Particle Sizer; SMPS: Scanning Mobility Particle Sizer; CPC: Condensation Particle Counter; OPS: Optical Particle Sizer; LC-ESI-MS/MS: liquid chromatography-electrospray ionization-tandem mass spectrometry; VCin: Inspiratory vital capacity; PEF: Peak Expiratory Flow; yr: years; PPE: Personal protective equipment

**Introduction**

Nanotechnology and nanoscale materials are rapidly increasing in both scope and scale.The number of workers exposed to nanoparticles has grown significantly as the use of NPs is extended to a wide range of industries. Some industrial processes, such as cleaning, packaging, and recycling, may probably expose not only workers, but consumers (Kuhlbusch et al. 2011). ENMs with new chemical and physical properties, 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 a toxic effect on living organisms. The biological activity of ENMs and NPs depends on those physicochemical properties which are crucial in understanding their toxic effects on worker’s 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 and thus, the spectrum of biological effects of NPs 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, range from molecular to physiological level. Those changes can be measured in human fluids, tissues, or other non-invasive samples from exposed workers. Inhalation is the most biologically significant route of exposure and NPs accumulated 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 and inflammation, cardiovascular effects, oxidative stress damage, pleural plaque formation, lung tumors, cytotoxicity and genotoxicity were found in animal inhalation studies.(Oberdörster et al. 2005, Tcach et al. 2011, Oyabu et al. 2017). Epigenetic data regarding the effect of NP workers 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 workers. Both studies found DNA epigenetic alterations that could be considered as 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

A scoping review of the scientific literature was performed to identify studies addressing toxicology, industrial hygiene and epidemiology of exposures to nanomaterials. Web of Science and PubMed search engines were queried utilizing an identification, screening, eligibility, and inclusion algorithm based on the PRISMA guidelines (Tricco et al. 2018). Subject search terms included “nanoparticles “or” engineered nanomaterials” and/or: “health effects”, “biological markers”, “biomarkers”, and “workers epidemiology “. Search restrictions included English language, years of publication 2009–2020, peer-reviewed studies published. In order to expand the scope and reduce the probability of missing relevant literature, the term “nanoparticles” was used in addition to “nanomaterials”. A total of 286 unique search retrievals for each search term for both databases were obtained. 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 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 (Ref. 43) Full texts of the papers considered eligible for this review were obtained and reference lists were searched for articles that meet the inclusion criteria. Only publications which measure some biological markers of occupational exposure to NP were included.

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

Eligibility

Included

Screening

Identification

PRISMA

Database searches:

n=286

PubMed: n=199

Web of Science:n=87

Records after duplicates removed:

n=150

Excluded: n=134

 Review: n=44

Not Occupational: n=67

Not Nanoparticles: n=23

Papers included:

n=16

Eligibility process

After duplicates were removed from the original search (286 articles) the abstracts of 150 articles listed above were reviewed and narrowed the pool of relevant articles by excluding review articles as well as not occupational and not NPs related articles. Following the screening for eligibility, 16 articles remained and were included in the l review

**Results**

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

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

The most used and studied Biological Exposure Markers - cardiovascular effect, 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 the 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- toner based printing equipment (TPE) have been linked to genotoxicity, immunologic and respiratory diseases. Physicochemical and morphological properties of ENPs such as titanium dioxide, iron oxide, fumed silica, and several metals found in toners from photocopiers and printers and their effect on human health were described by Bello et al. 2013; Khatria et al. 2017 and reviewed by Pirella et al. 2017, based on evidences from cellular, animal and human toxicological adverse effects of particles emitted from TPE. These nanoparticles were described as biologically active and capable of inducing oxidative stress in vitro and in vivo, respiratory tract inflammation in vivo, cellular injury in monocultures and co-cultures, and moderate epigenetic modifications in vitro. In humans, limited epidemiological studies report 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 be developed following such exposures.

George and colleagues 2010 studied the effect and resulting markers of injury of **metal oxides** nanoparticles 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 affected that cells triggering a biological oxidative stress. Among the materials, dissolution of ZnO nanoparticles and Zn+2 release were capable of 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 a lot of debate of what constitutes appropriate safety screening methods, one approach is to use the assessment of cellular injury pathways to collect knowledge about hazardous material properties that could lead to harm to humans and the environment.

**Iron oxide** pigments are used in paints, ink, rubbers, plastics, cosmetics, and in medical devices. Pelclova et al. (2016, 2018) evaluated occupational exposure to iron oxide nanoparticles during pigments’ production. They correlated the elevated levels of markers of oxidative stress found in the EBC of workers to exposure to Iron oxide during the pigment production. Recently, Yu et al 2020 in a similar work evaluated exposure of manufacturing/handling workers in an Iron Oxid NPs plant. Yu and others found that significantly increased airborne particles at the worksite is 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 signature and possibly applied to predict clinically significant disease.

**Zinc oxide** ENPs have been also widely used in cosmetics and sunscreens, advanced textiles, self-charging and electronic devices. In addition to pulmonary damage, NPs exposure is also strongly correlated with the increase in incidences of cardiovascular diseases; however, their toxic potential remains largely unclear (Chen et al. 2014).

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

Two complementing studies performed by Pelclova et al. (2016, 2017) studied the impact of short-term exposures to nanoTiO2 on the physiological markers among office employees working in a building where TiO2 pigment was produced. Their findings strengthen previous results associating markers for systemic inflammation, oxidative stress, and pulmonary effect markers with occupational exposures to TiO2. Peclova, Zakharov and others 2018, examined in EBC samples among three groups of workers exposed to varied NP exposure profile, 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 in nanoTiO2 workers. LTB4 and cysteinyl LTE4 inflammation markers showed the highest association. The authors consider markers in EBC as a sensitive technique for noninvasive monitoring of workers exposed to engineered nanoparticles. Part of their findings were also demonstrated *in vivo* by Oyabu and colleagues (2017) who examined the dose–response relationship of NiO and TiO2 nanoparticles- each having a different toxicity- by inhalation and intratracheal instillation studies. They likewise found pulmonary inflammation and oxidative stress markers alongside with bio persistence of the NPs in the lungs reflected by histopathological changes and other biomarkers in BALF samples. However, due to the ability of NPs to translocate to other organs, bio persistence- previously known as a useful toxicity indicator for micron-size particles- seems to be a useful indicator of the biological effects or a hazard indicator in the lungs.

 The effect Indium tin oxide (ITO), which is increasingly used in liquid crystal display and semiconductor production processes, on NM handling workers was described by Liou tl al. (2017). They examined the exposure of NM manufacturing and handling workers to TiO2, SiO2, and ITO nanoparticle granules or indium nano-sized fumes during different processes of splashing, pulverization, cutting, and grinding of the final ITO plates. Using non-invasive methods of evaluation, they found in EBC as well as in blood and urine that exposure to metal oxide NMs may lead to global methylation, DNA oxidative damage, and lipid peroxidation.

1. Many in vivo and in vitro mechanistic toxicology studies on a commonly used nanomaterial, **Multi Walled 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 surveillance among MWCNT exposed workers, the assessment of personal and area exposure levels to MWCNTs was performed using a walk-through evaluation. Blood and exhaled breath condensates (EBCs) from manufacturing and office workers were collected, in addition to pulmonary function testing . Analysis of EBCs revealed significantly higher levels of oxidative stress markers malondialdehyde (MDA), 4-hydroxy-2-hexenal and 4-Hydroxy-trans-nonenal in the MWCNT manufacturing workers compared to those of office workers. MDA and n-hexanal levels were also found to be elevated in a similar study (Lee 2014) suggesting them as useful biomarkers of MWCNT exposure.

J. Vlaanderen et al (2017) studied the effect of the occupational exposure to **MWCNTs** through a molecular cross-sectional study. They evaluated the association between occupational exposure to MWCNTs and effects on lung health and the immune system among workers of a 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 measuredhematological parameters between exposed and nonexposed workers. The results were found to be robust to sensitivity analyses proving the early effects of occupational exposure to MWCNTs on lung health and the immune system. The researchers conclude that some indications of early biological perturbations were associated with exposure to MWCNTs.

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

A case report regarding unintended occupational exposure to dust containing CNTs – among many other materials- was described by Wu (2010). THe author described the clinical and pathological findings in first response rescue and recovery workers’ lungs following the terrorist attack on World Trade Center (N.Y) on 9/11/2001, who had been diagnosed with pulmonary fibrosis, chronic bronchiolitis and granulomas resulting from the exposure to CNT. 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-section as small as a single atom. This material is engineered to form seamless cylinders one nanometer in diameter exhibiting unique electric properties are widely used in microelectronics. Tcach et al (2011) assessed the pulmonary damage and cytokine release following exposure in an *in vivo* experiment by evaluation of the activity of LDH and total protein in BAL specimens. They show that SWCNT induced marked cell and tissue damage in the lungs of exposed mice with significant dose-dependent release of LDH together with high protein and cytokines levels jointly with significant increase of chemotactic monocytes signaling. The concentrations of TNF-α, IL-6, IFN-γ, IL-12p70, IL-10 and MCP-1 were determined, founding MCP-1 increased more than 60-fold after SWCNT exposure and elevated IFN-γ.
2. **Welding fumes NPs**: To date there are no epidemiological studies which has specifically investigated the neurotoxic effects of manufactured NPs, however studies of populations exposed to anthropic NPs provide an interesting perspective on the concerns related to 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. The first evidence of a link between human exposure to NP and long-term pulmonary effects was provided by Andujar et al. 2014. 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* analysis exposing macrophages to those NPs, founding increased 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 human central nervous system was studied by Grazcyk 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 exhaled breath condensate, blood and urine collected from non-smoking male welding trainees at different time points. Their finding indicate significant increases in the measured biomarkers 3 hours after exposure. Similar results were obtained by Brand et al. (2014); Jarvela et al. (2013); Kauppi et al. (2015); after investigating the association between nanoparticle exposure and inflammation and oxidative stress at both, pulmonary and systemic levels in welders. Part of these results were also confirmed by Andujar et al. 2014; Song et al. 2016 and by K. Dierschke et al. 2017; lastly, by Rossnerova et al. 2020.
3. **Exposure to Mixed types of NPs.** 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 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 follow-up period. In a similar study Liao et al. 2014 confirmed many of Liou’s findings and in addition he noticed that 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 surveillance of nanomaterial-handling workers. Kathria et al. 2017 studied the effect of the exposure of photocopiers workers to a mixture of organic compounds including metal ENPs in nasal lavage samples and urine. He found significant increase in markers of systemic inflammation IL-6, IL-8, TNFα, IL-1β and Eotaxin in the NL samples as well as oxidative stress markers in urine showing good correlation with previous results for the tested biomarkers.
4. **Silica nanoparticles (SiNPs).** According to the World Health Organization (WHO 2017), SiNPs are currently ranked as the second largest production of all manufactured nanomaterials in the global market, implicating the potential release of SiNPs into the industrial environmental and impact on human health. The potential adverse effects of SiNPs on cardiovascular system was described by Nemmar and colleagues( 2014). They show in an *in vivo* assay that intratracheally 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 described similar effects 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.
5. **Polyacrylates.** Song et al. (2009) examined a group of workers presenting unusual symptomatic findings after beening exposed to a mixture of Polyacrylate and other nanoparticles (zinc oxide, titanium dioxide, nanoscale silver cluster and other engineered nano materials) in a 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, rising concern that long-term exposure to some nanoparticles without protective measures may be related to serious damage to human lungs. Markers of pulmonary lesions, tissue damage and inflammation after exposure to toner containing acrylates among other nanomaterials were also found by Bay 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 | Nano Material Exposure | Population Exposure/ Study/ Assay  | Health Outcomes Biological Marker  | Tested in humans-worker-  | Results | Confounding factors |
| --- | --- | --- | --- | --- | --- | --- |
| Y. Song et al. Eur Respir J 2009 | Polyacrylate (polyacrylic ester)30 nm diameter | 7 females and 1 man aged 18-47 working in print plant 5-13 monthsAssays: 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, lymhocytesBiochemical markersFibrosis, granuloma in lung tissue | Samples: Lung tissue, thoracic exudate, pleural, BALF effusion,BloodUrine Functional tests: liver, kidney and lung  | **Increased**Blood & serum: Monocytes, ESR, ALT, ASTExudate: monocytosisPleural effusion: glucoseBALF: lymphocytosis. **Decreased** Blood & serum: neutrophils, albumin Pleural effusion: chlorid ion in all patients: very lowBALF: macrophagesPathological examinations: nonspecific pulmonary inflammation, fibrosis, and foreign-body granulomas of pleura | NonsmokersNon exposed to hazardous materials |
| M.WuEnviron. Health Perspect. 2010 | 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: tissue mineralogic 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 silicates Extensive interstitial/ parenchymal abnormalities, Small airways disease | Age, sex, occupation, smoking history, comorbidities length of exposure |
| R. BayTox. Letters 2010 | Toner particles: metals, polymers acrylates, Carbon Black PM 2.5; PM 10; 50-200 nm | Animal experiment exposed vs. non exposed | * Pulmonary lesion
* 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 Nano 2010 | TiO2CeO2ZnO | *In vitro*: bronchial epithelial and macrophage cell lines.Assay: Semiautomated 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;  |  |
| Tcach et al. ACS Nano 2011 | SWCNT | *In vivo*: mice exposed vs. non exposed to SWCNT inhalation. 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 from 2 workers during 7 yrs. | No significant findingsSilver concentration | Samples: bloodurine | Silver in urine: not detectedSilver in blood: low conc.  |  |
| S.H. LiouJ Nanopart Res 2012 | NPs: CNT, TiO2, SiO2, Silver, Gold,nanoresins, nanoclay, nanoalumina, and metal oxides20-100 nm | Cross-sectional studymanufacturing & application workers227 exposed vs. 137 unexposed controls from 14 NM plantsAssays:Questionnaire | * BioMarkers:

Cardiovascular: fibrinogen, ICAM, interleukin-6 Antioxidants: 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 significantly in part of workers. Neurobehavioral functions Significantly lower in part of workers.**No changes** in DNA damage, genotoxicity and pulmonary markers  | Exposure status, demographics, geographic and socioeconomic status, smoking, drinking and betel nut chewing habits history of respiratory disease, and dusty 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  interleukin 1β; thrombocytes
 | \_ | Samples: plasma**Increased**: In the 50 nm particles tests: von Willebrand factor, PAI-1 fibrinogen; LDH, TNF  interleukin 1β;**Decreased**:Thrombocytes In vitro: platelet aggregation **Decreased:**HUVEC viability |  |
| Andujar et al. Part. and Fib. Toxicol.2014 | Cross sectional study. Iron 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 Prussian CD68 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 macrophages & fibroblasts  | **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 lessions | Gender, smoking habits, occupational seniority |
| H.Y. Liao et al. Nanotoxicol. 2014 | Nanosilver, Nanogold,Fe2O3, TiO2,CNT, SiO2Multiple exposures to mixed types of NPs Size < 100nm | Longitudinal study of workers from colors, LED, colorants, air cleaners, CNT, photocatalyst and textile industries exposed vs. unexposed. Assay: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:EBC, blood and urine specimens | **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. | Age, gender, smoking habit, history of respiratory disease, dusty environment |
| R. Chen. ACS NANO 2014  | Zinc oxide (ZnO) NPs, 28-150 nm diameter | *In vitro*: HUVECs | * BioMarkers:

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

Oxidative stress  | Samples:Whole blood EBCpulmonary function test  | **Increased:**EBC: MDA, 4-HHE and n-hexanal in manufacturing workers significantly higher than in office workersBlood: Normal hematology and biochemistry valuesLung function: normal |  |
| Shvedova et al. PLoS One 2016 | Exposure to MWCNTs aerosols  | Cross sectional studyExposed (n=8) vs. non exposed (n=7) workers in a 6 months 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 blood; particles in personal breathing zones | **Dysregulation:** mRNA, lncRNA and miRNA expression profiles of target genes affecting cell cycle regulation IL6, EGFR, TGFβ; ERK, PDGFA, CASP8 KL-6 (MUC 1) | Age, gender, pernicious habits, work experience, history of disease |
| Fatkhutdinova et al. Toxicol. and 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 > 1year 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 lavage, induced sputum,blood and serumair samples from specific areas and personal breathing zones | **Increased:**SignificantSputum: IL-1β, IL6, TNF-α, inflammatory cytokines, KL-6. Serum: TGF-β1(in young)  | Age, gender, smoking work experience,  |
| Graczyk et al., Sci. Translat. Med.2016 | Welding fumes | Cross sectional study.Non-smoking male welding trainees | * BioMarkers:

Oxidative stress: 8 hydroxy-20 -deoxy guanosine, MDA, hydrogen peroxide and total reducing capacity | Exhaled breath condensate (EBC), blood and urine | **Increased:**Significant: Plasma H2O2 24%; 14% 8-OHdG urinary H2O2 91%; 45% urinary 8-OHdG | - |
| D. Pelclova et al. Occ. Environ Med 2016 | nanoTiO2 pigment | 36 male workersworking with TiO2 pigment for at least 6 months and 45 unexposed controlsAssays: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) | Sample: 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 history, medical treatments and lifestyle habits (diet, alcohol intake, smoking, physical activity |
| D. Pelclova et al.J. Breath Research2016 | 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: EBC Urine | **Increased** in EBC: LT B4, C4, E4, D4 in workers relative to controls (p < 0.01)Cysteinyl LTs Impaired %VCIN and %PEF (both *p* < 0.01) | Allergic diseasessmoking |
| D Pelclova et al.J. Breath Research 2016 | 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 dependentAssays: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: EBC Urine | **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 | Age, BMI, smoking alcohol consumption |
| D Pelclova. J. Nanotoxicology 2017 | Cross sectional study.nanoTiO280% 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 | Sample: EBC | **Increased**: 11 markers of lipidoxidation in production workers relative to controls (p < 0.001)  |  |
| D. Pelclova. Rev. Environ. Health 2017 | Nanoscale titanium dioxide (nanoTiO2) <100 nm diameter | Cross sectional studyShort term MVA. 22 office workers intermittently exposed to TiO2 vs. 14 unexposed Assay: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 lipid Oxidation, DNA and protein oxidative damage in production workers.EBC: highly significant difference between production and office workers. (p < 0.001) Urine: No increase | Age, smoking, alcoholphysical activity |
| J.Vlaanderena et 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: Serum Whole blood (CBC) FENO Lung 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) | Age, BMI, smoking, and sex |
| S. 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 NMs 3.4 ds. / wk. 4.4 h/ day Exposed vs. non exposed workersAssay: Questionnaires | * BioMarkers:

 Global methylation, DNA oxidative damage, lipid peroxidation. Oxidative stressUrinary and WBC 8-OHdG, EBC 8-isoprostane  | Samples: BloodUrineEBC  | **Increased:** WBC; 8-OHdG  8-isoprostane 8-OHdG (negatively correlated with global methylation) WBC and urinary 8-OHdG positively correlated. Lower global methylation in ITO handling workers. | Demographic, socioeconomic characteristics, lifestyle: alcohol, smoking occupational history, personal and family disease. |
| T. 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. |  |
| K. Dierschke et al. Int’l. Arc.Occ. and Env. Health 2017 | 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  Assay: 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 analogue scale for eyes examining
 | * BioMarkers:

EBC: leukotrienes LT-B4N.L: IL-6NeutrophilsIL-8 | Samples: EBCBloodSerumNasal lavage (NL)Lung function | 1. Lung function: No adverse effect2. 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 groups 4. Blood: rapid elevation of IL-8 and neutrophils after exposure followed by significant decrease (ongoing neutrophilic low-grade inflammation) | Non currently smokers (5 yr.)Total welding time, age, exposure, allergies, work-related symptoms from eyes and airways |
| M. Khatria et al. NanoImpact 2017 | Mixture of organic compounds with metal ENPs | 6 photocopiers workers in 3 random weeksAssay:1. Quantitative airborne NPs
2. Chemistry
3. Lung burden estimates
 | * Upper airway inflammation
* 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-fold Urine: 8-OH-dG 4.3-fold  |  |
| D.Pelclova, S. Zakharov et al. Occ.& Env. Med. 2018 | TiO2Iron oxidesNanocompositesExposure to aerosols > 2 years | 3 groups of workers vs. comparable control groups. 34 nano TiO2 workers during 2 yr.14 nano Iron oxides; and 32 nanocomposites workers 2 years follow upAssays: 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 study  TiO2  | 83 exposed workers vs. 85 controls in packaging workshopAssay: 1. Questionnaire
2. Spirometer
3. ELISA
4. Cytometric Bead Array
5. BD FACSCalibur flow cytometer
 | * Significant changes in image of chest X-rays
* 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  | Demographic smoking and drinking status, occupational and medical history, use of personal protection equipment  |
| R. Baumanna et al.Nanotoxicology 2018 | Zinc- and copper welding fumes Controlled exposure to welding process (used for vehicle construction and interiors) 50 - 300 nm different shapes | 12 male healthy volunteers (aged 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: significantly No significant changes: IL-6, sVCAM-1, sICAM-1 | smoking |
| Feng et al. Particle and Fibre 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 changesVascular endothelial damage and prethrombotic state. Markers: miR-451a MDA; PECAM-1SOD, GSH-PxJak1, Stat3, TF, Il6r  Fib | \_ | **Increased:** Hypercoagulation; decreased blood flow velocity Lipid perox. MDA; PECAM-1 positive cells; **Decreased**:Antioxidant SOD, GSH-Px. Downregulated miR-451a rises endothelial injury markers T.F, inflammatory cells, vascular oxidative damage: PECAM-1, SODand GSH-Px**Upregulated** gene expression: Jak1, Stat3, Tf, Il6r and Fib  |  |
| M. Yu et al.Toxicology and Industrial Health 2020 | Iron oxide NPs (IONP) | Cross-sectional study 23 workers aged 23 unexposed to metal1. ELISA
2. PCR
 | BioMarkers:Iron status, oxidation markers, methylation of genomic DNA 5-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 (gender)Occupational history |
| A. Rossnerova et al. Int. J. Mol. Sci. 2020 | Aerosolized welding fumes 2 fractions<25 nm 25–100 nm | Cross-sectional study 20 exposed welding and machining vs. 20 unexposed. (both genders) 14.5± 9.2 yr exposureNo PPE usedAssays:Infinium Methylation Assay EPIC BeadChips microarray ELISASMPAAPS | 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 |

| 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 F2) | 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 |  |  |

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

**Discussion**

This review attempts to draw on 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 as well as in respiratory functions in animal and human studies as well. Experiments on animals exposed to different NMs at varying dose and exposure routes allowed comparison of 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 NM workers such as miRNAs, fibrogenic markers and micronucleus, ICAM-1 in macrophages were also shown. In vitro studies were useful in showing significant changes in proteins or genes’ expression and to get molecular insights into the NPs-induced toxicity and pathogenesis in humans. The majority of epidemiological studies involved NM 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, single or in combination with other NMs followed by mixture 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 noninvasive monitoring of workers exposed to NM with biomarkers that reflect intrinsic changes in the airway lining fluid and lung inflammation. Some ions released from metal NMs when dissolved in biological media were detectable with analytical methods and could serve as valuable markers of exposed workers. Although the results described in this scoping review demonstrate good relationship between exposure of workers to NM and physiologicaly significant biomarkers, in order to use these biomarkers in routine occupational medical surveillance, large scall epidemiological studies among well defined groups of workers will be needed to confirm the utility of routine occupational biomonitoring.

**Conclusions**

The adverse physiological effects of occupational exposure to nanomaterials demonstrated by the significant association with biomarkers of exposure were highlighted in this review. Validated biomarkers will enable the progression of knowledge about potential health effects associated with occupational NM exposure in general and will contribute to the implementation of reliable, non-invasive 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 surviellanceand monitoring of workers with occupational exposure to nanoparticles.

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

**Acknowledgements**

The authors thank Ms. Sahara Elfaks from the Department of Env. and Occ. Health, School of Public Health

at University of Haifa for her assistance in building the PRISMA flow chart

**References**

1. How can nanobiotechnology oversight advance science and industry: examples from environmental, health, and safety studies of nanoparticles (nano-EHS). J. Wang; C Asbach; H. Fissan; T Hulser; T. A. J. Kuhlbusch; D. Thompson; D. Y. H. Pu. J. Nanopart Res (2011) 13:1373–1387
2. Nanoparticle exposure at nanotechnology workplaces: A Review. Kuhlbusch et al. Particle and Fibre Toxicology 2011, 8:22
3. Markers of lipid oxidative damage in the exhaled breath condensate of nano TiO2 production workers. D Pelclova, V Zdimal, P Kacer, N Zikova, M Komarc, Z Fenclova. Journal Nanotoxicology Volume 11, 2017 - Issue 1
4. Markers of lipid oxidative damage among office workers exposed intermittently to air pollutants including nanoTiO2 particles. D. Pelclova. Rev Environ Health 2017; 32(1-2): 193–200
5. Health surveillance study of workers who manufacture multi-walled carbon nanotubes

J S Lee, et al. Journal Nanotoxicology V. 9, 2014 - Issue 6.

1. Six-month follow-up study of health markers of nanomaterials among workers handling engineered nanomaterials. HY Liao, YT Chung, Ch.H. Lai, S. L. Wang, H. Ch. Chiang, L. Ann Li. Journal Nanotoxicology V. 8, 2014 - Issue sup1
2. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. G. Oberdörster, A. Maynard, K. Donaldson, V. Castranova, J. Fitzpatrick, K. Ausman, J. Carter, B. Karn, W. Kreyling, D. S. Olin, N.M.Riviere, D.Warheit, H. Yang. A Report from the ILSI Research Foundation/Risk Science Institute Nanomaterial Toxicity Screening Working Group. Particle and Fibre Toxicology 2005 2:8
3. Physicochemical and morphological characterization of nanoparticles from photocopiers: implications for environmental health. D. Bello et al. J. Nanotoxicology 2013. V.7 P. 989-1003, - Issue 5
4. Nanoparticle exposures from nano-enabled toner-based printing equipment and human health: state of science and future research needs. S.V. Pirela, J. Martin, D. Bello & P. Demokritou. Journal Critical Reviews in Toxicology V. 47, 2017 - Issue 8 P. 683-709
5. Endoplasmic Reticulum Stress Induced by Zinc Oxide Nanoparticles Is an Earlier Biomarker for Nanotoxicological Evaluation. R. Chen et al. Am. Chemical Society Nano 2014. V.8 No. 3 P. 2562–2574.
6. Perspectives in Biological Monitoring of Inhaled Nanosized Particles. M. Rinaldo, P. Andujar, A. Lacourt, L. Martinon, M. Canal Raffin, P. Dumortier, J.C. Pairon and P. Brochard. Ann. Occup.Hyg. (2015) 59, 669−680
7. A cross-sectional study of changes in markers of immunological effects and lung health due to exposure to multi-walled carbon nanotubes. J. Vlaanderena, R. Vermeulena et al. Nanotechnology. 2017 V.11, NO. 3, 395–404
8. Direct Effects of Carbon Nanotubes on Dendritic Cells Induce Immune Suppression Upon Pulmonary Exposure. A. Tcach, G.V. Shurin et al. ACS Nano 2011.V.5, No.7, P.5755-5762
9. Use of a Rapid Cytotoxicity Screening Approach to Engineer a Safer Zinc Oxide Nanoparticle through Iron Doping. S. George, S. Pokhrel, et al. ACS Nano, 2010 V.4, No. 1, P. 15–29.
10. Biological Monitoring of Inhaled Nanoparticles in Patients: An appealing approach to study causal link between human respiratory pathology and exposure to nanoparticles. V. Forest, J.M. Vergnon, and J. Pourchez. Chem. Res. Toxicol. 2017, 30, 1655−1660
11. Biopersistence of NiO and TiO2 Nanoparticles Following Intratracheal Instillation and Inhalation. T. Oyabu et al. Int’l Journal of Molecular Sciences 2017, 18, 2757.
12. Amorphous silica nanoparticles impair vascular homeostasis and induce systemic inflammation. Nemmar A, Albarwani S, Beegam S, Yuvaraju P, Yasin J, Attoub S, Ali BH. Int’l. J. Nanomedicine 2014, 9:2779–89.
13. Silica nanoparticles trigger the vascular endothelial dysfunction and prethrombotic state via miR-451 directly regulating the IL6R signaling pathway. Feng et al. Particle and Fibre Toxicology 2019, 16:16.
14. Global DNA methylation and oxidative stress biomarkers in workers exposed to metal oxide nanoparticles. S. Liou et al. Journal of Hazardous Materials 331 (2017) 329–335
15. Roco, M.C., C.A. Mirkin, and M.C. Hersam: WTEC panel report on nanotechnology research directions for societal needs in 2020: retrospective and outlook. WTEC. (2010).
16. Assessing the first wave of epidemiological studies of nanomaterial workers. S.H. Liou, C.S.Tsai,

D. Pelclova, M. K. S.-Berigan and P.A. Schulte. J Nanoparticle Res. 2015, 17:413.

1. Neurotoxicity of nanomaterials. F. Xiaoli and S. Longquan. Emerging Nanotechnologies in Dentistry. A volume in Micro and Nano Technologies. Book. 2nd Ed.2018, 20, 421-444.
2. Role of metal oxide nanoparticles in histopathological changes observed in the lung of welders. P. Andujar et al. Part. Fibre Toxicol. 11, 23 (2014).
3. Integrated Analysis of Dysregulated ncRNA and mRNA Expression Profiles in Humans Exposed to Carbon Nanotubes. A. A. Shvedova et al. PLoS ONE, 11(3), 2016
4. Fibrosis biomarkers in workers exposed to MWCNTs. L. M. Fatkhutdinova, A. A. Shvedova et al. Toxicology and Applied Pharmacology 299 (2016) 125–131
5. Case Report: Lung Disease in World Trade Center Responders Exposed to Dust and Smoke: Carbon Nanotubes Found in the Lungs of WTC Patients and Dust Samples. M. Wu et al. Environmental Health Perspectives.V. 118 No. 4, 2010
6. Noninvasive Biomonitoring of 3 Groups of Nanomaterials Workers with Elevated Markers of Oxidative Stress and Inflammation. D. Pelclova, V. Zdimal, S. Dvorackova, J. Schwarz, J. Ondracek, M. Komarc, S. Vlckova, Z. Fenclova, O. Makes, S. Zakharov. Occupational and Environmental Medicine 2018; 75(Suppl 2): A1–A650.
7. Biomarkers of susceptibility: State of the art and implications for occupational exposure to engineered nanomaterials. I. Iavicoli et al. / Toxicology and Applied Pharmacology 299 (2016) 112–124
8. Acute respiratory effects and biomarkers of inflammation due to welding-derived nanoparticle aggregates. K. Dierschke et al. / Int’l. Archives of Occupational and Environmental Health 2017, 90, 451–463
9. Song Y, Li X, Du X. Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma. Eur Respir J 2009; 34:559–67.
10. Markers of oxidative damage of nucleic acids and proteins among workers exposed to TiO2 (nano) particles. Pelclova D, et al. Occup Environ Med 2016;73:110–118. doi:10.1136/oemed-2015-103161
11. Leukotrienes in exhaled breath condensate and fractional exhaled nitric oxide in workers exposed to TiO2 nanoparticles. D. Pelclova et al. J. of Breath Research 2016; V.10, No.3
12. Oxidative stress markers are elevated in exhaled breath condensate of workers exposed to nanoparticles during iron oxide pigment production. D. Pelclova et al. J. of Breath Research 10 (2016) 016004
13. Markers of lipid oxidative damage among office workers exposed intermittently to air pollutants including nanoTiO2 particles. D. Pelclova et al. Rev. Environmental Health 2017; 32(1–2), 193–200.
14. Cardiopulmonary effects induced by occupational exposure to titanium dioxide nanoparticles. L. Zhao et al. Nanotoxicology 2018; 12(2), 169–184.
15. Human nasal mucosal C-reactive protein responses after inhalation of ultrafine welding fume particles: positive correlation to systemic C-reactive protein responses. R. Baumanna, P. Branda, A. Chakerb, A. Markerta, I. Racka, S. Davatgarbenama, et al. Nanotoxicology 2018; 12(10), 1130–1147
16. Chronic upper airway inflammation and systemic oxidative stress from nanoparticles in photocopier operators: Mechanistic insights. M. Khatria, D. Bello, J. Martin, A. Bello, R. Gore, P. Demokritou, Peter Gaines. NanoImpact 2017; V.5, 133-145
17. A health surveillance case study on workers who manufacture silver nanomaterials. Lee, J. H., Mun, J., Park, J. D., & Yu, I. J. Nanotoxicology 2012; 6(6), 667–669. https://doi.org/10.3109/17435390.2011.600840
18. Epidemiological study of health hazards among workers handling engineered nanomaterials.

S.H. Liou , T.C. Tsou, S.L. Wang, L.A. Li, H.C. Chiang, W.F. Li et al. J Nanopart Res (2012) 14:878 DOI 10.1007/s11051-012-0878-5

1. Characteristics of iron status, oxidation response, and DNA methylation profile in response to occupational iron oxide nanoparticles exposure. Yu, M., Zhou, X., Ju, L., Yu, M., Gao, X., Zhang, M., & Tang, S. Toxicology and Industrial Health 2020; 36(3), 170–180. <https://doi.org/10.1177/0748233720918683>
2. Fibrosis biomarkers in workers exposed to MWCNTs. L.M. Fatkhutdinova, T.O. Khaliullin, O.L. Vasil'yeva, R.R. Zalyalov, I.G. Mustafin, E.R. Kisin, M.E. Birchc, N. Yanamala, A. A. Shvedova. Toxicology and Applied Pharmacology 299 (2016) 125–131
3. Health surveillance study of workers who manufacture multi-walled carbon nanotubes. [J. S. Lee](https://pubmed.ncbi.nlm.nih.gov/?term=Lee+JS&cauthor_id=25395166) , [Y.C. Choi](https://pubmed.ncbi.nlm.nih.gov/?term=Choi+YC&cauthor_id=25395166), [J.H. Shin](https://pubmed.ncbi.nlm.nih.gov/?term=Shin+JH&cauthor_id=25395166), [J.H. Lee](https://pubmed.ncbi.nlm.nih.gov/?term=Lee+JH&cauthor_id=25395166), [Y. Lee](https://pubmed.ncbi.nlm.nih.gov/?term=Lee+Y&cauthor_id=25395166), [S.Y. Park](https://pubmed.ncbi.nlm.nih.gov/?term=Park+SY&cauthor_id=25395166), et al. Nanotoxicology 2015 9(6) :802-11 doi: 10.3109/17435390.2014.978404
4. DNA Methylation Profiles in a Group of Workers Occupationally Exposed to Nanoparticles. A. Rossnerova, K. Honkova , D. Pelclova , V. Zdimal , J.A. Hubacek, I.Chvojkova et al.Int. J. Mol. Sci. 2020, 21, 2420; doi:10.3390/ijms21072420
5. PRISMA extension for scoping reviews (PRISMA-ScR): checklist and explanation. Tricco AC, Lillie E, Zarin W, *et al*. *Ann Intern Med* 2018;169:467.