Original Article

VCAM-1 as an endothelial dysfunction biomarker in ambient nanoparticle-exposed workers

Safaa Elserougy*, Weam Shaheen, Eman M. Shahy, Safia Beshir and Mona Taha

Environmental and Occupational Medicine Department, National Research Centre, Giza, Egypt.

ABSTRACT

Nanoparticles present one of the biggest risks among the emerging occupational risks. Early health risk assessment studies on the effects of ambient nanoparticles are required to assure safety of exposed workers. The present study aimed at exploring the levels of biomarker of endothelial dysfunction (vascular cell adhesion molecule, VCAM-1) and high-sensitivity C-reactive protein, and spirometric functions among nanoparticle-exposed workers. The comparative cross-sectional study included 46 nanoparticle-exposed workers and 45 non-exposed workers. All participants filled an interview questionnaire and were submitted to measurement of ventilatory lung functions. Also, blood samples were taken from all the studied group to measure the former biomarkers. Diffusion size counter and electron microscopy were used to measure the emitted nanoparticles from research labs. Dimensions of ambient nanoparticles ranged from 31-81 nanometer. Mean serum VCAM-1 level was significantly higher in exposed group compared to non-exposed group ($p < 0.05$). Nanoparticle-exposed workers show significantly lower mean % predicted maximum expiratory flow (MEF_{50} and MEF_{75}) compared to non-exposed ones. In the exposed group, a significant negative correlation was found between biomarker of endothelial dysfunction and % predicted maximum expiratory flow (MEF_{50}). Elevated serum VCAM-1 among nanoparticle-exposed workers suggests presence of potential risk for endothelium of blood vessels.

*Corresponding author: safaaser@hotmail.com

1. INTRODUCTION

Nanoparticles (NP) present one of the biggest risks among the emerging occupational risks. NP have large surface-to-mass ratio, and therefore, reveal unique properties that can modify physical properties, chemical reactivity, and biological behavior, including toxicity. When the dimension of 50% or more of the particles range from 1 nm-100 nm, they are categorized as NP [1]. The small size of NP leads to high specific surface areas of these particles, which might affect NP toxicity compared to micrometer-sized respirable particlulate matter. Respirable particles depositing on the deep lung surfaces of the respiratory bronchioles or alveoli come into contact with pulmonary surfactants in the surface hypophase [2].

Respirable particulate matter is categorized into PM 2.5 and PM 10 μg/m^3. There is a scientific argument whether the respirable particles' morphology, size or chemical structure is the critical factor that affects human health. Several studies have been done to discover the impact of airborne particle inhalation on human health, principally respiratory diseases and inflammation [3, 4] and cardiovascular diseases [5].

The airborne particles' hazardous effects rise from their ability to penetrate human respiratory tract tissues, producing inflammation, and they may be translocated to blood, thereby transporting

carcinogenic and toxic compounds to other organs [6]. Therefore attention is focused on the respiratory system and the cardiovascular system [1]. Inhalation, skin contact or pharmacologic preparations are the probable routes of entry of NP. Inhaled NP may pass through alveoli to the blood stream and vascular endothelium. Vascular endothelium normally releases vasoactive factors to preserve the vascular homeostasis and control the blood vessel tone in response to stimulation of cell surface receptor or stress [7]. The balanced production of these vasoactive factors protects blood vessels, as injured endothelium disturbes the release of these factors. The resulting imbalance leads to endothelial dysfunction, which is an early indicator of atherosclerosis [8].

Vascular cell adhesion molecule (VCAM-1), a biomarker of endothelial dysfunction, is an endothelial adhesion receptor with a vital role for leukocyte enrollment in terms of cellular immunity [9]. It stimulates monocytes to adhere onto the endothelium, then differentiates into macrophages, which travel to the intima and change to foam cells [10]. On occurrence of leukocyte binding, these adhesion molecules stimulate endothelial cell signal transduction that change the shape of the cell to open pathway for leukocytes to pass. If the stimulation of this opening is blocked, inflammatory pathway is blocked. The endothelium is immunoregulatory as inhibiting the role of VCAM-1 prevents leukocyte enrollment and consequently tissue inflammation occurs. Expression of VCAM-1 and oxidative stress on vascular endothelial cells are early features in atherosclerosis pathogenesis and other inflammatory diseases [11]. Serum high-sensitivity C-reactive protein (hs-CRP) is a marker of low grade chronic inflammation and is used to evaluate the risk of cardiovascular disease [12].

Extrapolation of findings of toxicological animal studies to humans is not strong enough unless supported by human and epidemiological studies investigating health effects. The aim of the present study is to assess ambient NP concentrations in the workplace and find out their impact upon spirometric functions and biomarkers of endothelial dysfunction and low grade inflammation among workers occupationally exposed to NP.

2. MATERIALS AND METHODS

2.1. Assessment of ambient NP

Ambient NP were measured using portable diffusion size classifier/counter (DiSCmini V.2 - matter aerosol, a testo company, Switzerland). It directly measures particle number concentration (N) and average particle size (Dp) based on the electrical charging of aerosols. The counter-measured particle sizes were between 10-300 nm with 1 second sampling time. The counter also calculates the overall lung deposited surface area concentration (LDSA) through the alveolar- and tracheobronchial-deposited particle surface area concentration (SA_{alv} and SA_{tbr}, respectively) using the dosimetry model provided by the International Commission on Radiological Protection [13]:

$$LDSA = SA_{alv} + SA_{tbr} = (4.7 \times 10^{-5} + 0.95 \times 10^{-5}) \times N \times Dp \qquad \text{(equation 1).}$$

The counter was placed at the respirable zone in the nanomaterials' laboratory for successive 69 hours (4146 minutes).

2.2. Imaging ambient NP

Dust samples were collected using filter paper inserted in an ambient air sampler. The sampler was run over 24 hours of a working day. It was placed at the respirable zone in a nanomaterials' laboratory. Electron microscopy (Quanta FEG 250, USA) was used to detect the presence of NP accumulated on the filter paper.

2.3. Study design and population

The present study was designed as a comparative cross-sectional study including NP-exposed workers and non-exposed group. The study protocol was approved by the Medical Research Ethics Committee (National Research Centre, Egypt, registration number 15174) and signed written informed consents were obtained from all participants. The NP-exposed workers (n = 46) were researchers occupationally exposed to NP. The non-exposed group (n = 45) was administrative employees not occupationally exposed to NP. The NP-exposed group and non-exposed group were comparable (p > 0.05) with regard to median age (34 and 43 years respectively), percentage of males to females (58.7% and 64.4%), and percentage of smokers (10.9% and 22.2%). The median duration

of exposure in exposed workers was seven years. Workers with current respiratory infections were excluded.

2.4. Clinical findings

An interview questionnaire was filled by the participants and revised by the interviewer. The questionnaire enquires personal data, smoking habits, occupational and medical history. Clinical examination was done for all participants.

2.5. Spirometry

All participants were submitted to measurement of ventilatory lung functions (spirometry) using ZAN Messgerate GmbH spirometer (Oberthulba in Bad Kissingen, Bavaria, Germany). The participants were instructed to do forced expiratory maneuver with at least three acceptable, valid and reproducible (within 5%) spirograms. Spirometry prediction equations for Whites [14] automated in the spirometer were used. The predictive values of spirometric functions were interpreted according to Global Initiative for Chronic Obstructive Lung Disease [15]. The maximal expiratory flow (MEF_{25}, MEF_{50} and MEF_{75}) is the flow where 25%, 50% and 75% of forced vital capacity (FVC) remains to be exhaled [16].

2.6. Collection of blood samples and biochemical analysis

Five millilitres of blood samples were collected from all participants. The separated serum was stored at -20 ^{o}C till biochemical analysis of VCAM-1 (VCAM-1/CD106 ELISA Kit, Bio-Techne, Minneapolis, USA) [17] and hs-CRP (AccuBind ELISA Kit - 96 wells, Monobind, California, USA) [18].

2.7. Statistical analysis

Data entry and statistical analysis was done through SPSS 18.0. Time series graphs of NP were plotted to find out the periods with high emissions. Independent t-test was used for comparison between means of parametric data and Mann-Whitney for comparison of non-parametric data. Bivariate correlation was done to study the relation between V-CAM-1 with other variables. The significance level was set at p-value < 0.05. Dose index for exposed group = mean dose (NP/cm^3) × mean duration of exposure (years).

3. RESULTS

All the particles were found to be NP as they have at least one dimesion below 100 nm (13-81 nm) (Figure 1a). The sequential NP numbers showed almost cyclic (sinusoidal) pattern with three prominent spikes of high numbers at working hours (Figure 1b). The NP with the smallest size (13 nm) were found to appear simultaneously with the NP having the highest concentration (NP number/cm^3) (565186 pt/cm^3) (Figure 1b). The NP numbers were magnified to show the wavy pattern of the time series graph (Figure 1b^x). LDSA is shown in (Figure 1c) and follow the wavy pattern of NP numbers magnified (Figure 1b^x).

Median dose index for exposed group = median dose (27597.6 NP/cm^3) X lifetime workhours,

where lifetime workhours = median duration of exposure in years X workdays/year X workhours/ workday.

The electron microscopy image shows the presence of NP on the filter paper. Due to their small size, the NP can be seen precipitated over the net of strands (mesh/ trabeculae) of the filter paper. The NP are not seen as trapped at the pores of the filter paper as the diameter of the pores is larger than NP and allow the passage of the NP through them. The NP were almost rounded and agglomerated (Figure 2).

Mean serum V-CAM-1 concentration was found to be significantly higher in exposed group compared to non-exposed group (p = 0.016). HsCRP did not show significant difference between exposed and non-exposed group. NP-exposed group showed significantly lower mean % pred MEF_{75} and MEF_{50} compared to non-exposed group (p = 0.019 and p = 0.032 respectively). Mean principal spirometric parameters and % pred MEF_{25}, showed no significant difference between both groups. The NP-exposed group showed a significantly lower mean % pred PEF compared to the reference value of 80% but there was no significant difference between means of both groups (Table 1).

In the exposed group, a significant negative correlation was found between serum V-CAM-1 with FEV1/FVC, and % pred MEF_{50} (Table 2).
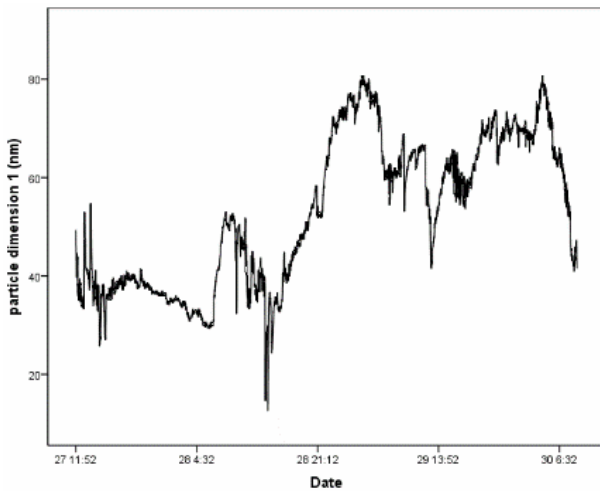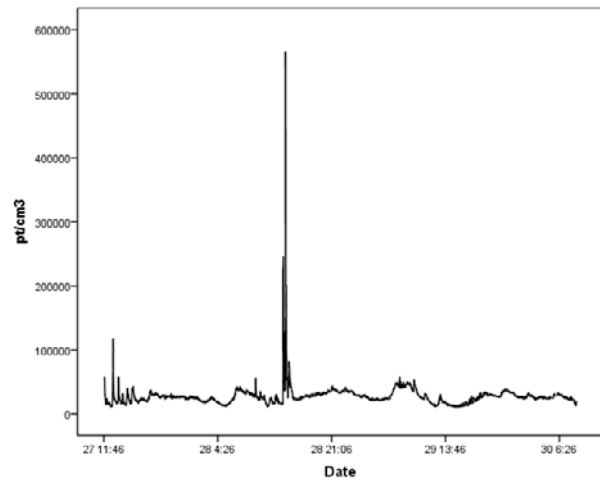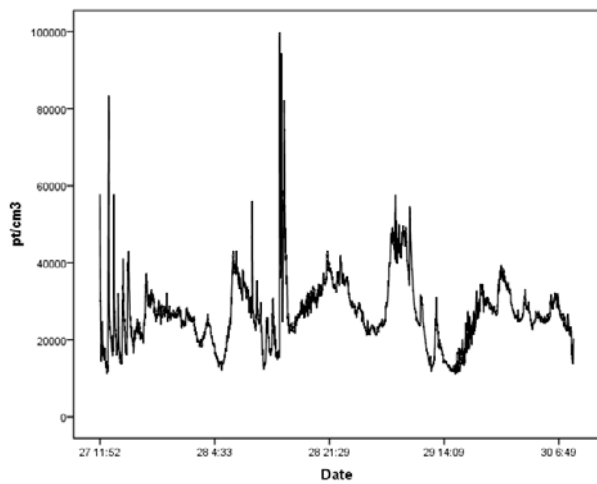
Figure 1a

Figure 1b

Figure 1b^{x}
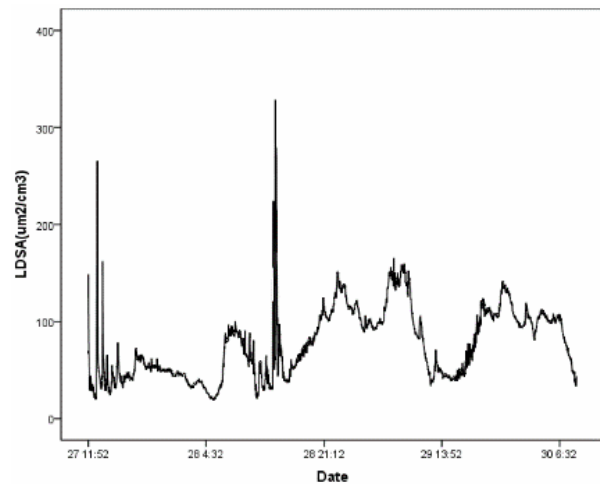
Figure 1c

NP	(Figure 1a) dimension 1 (nm)	(Figure 1b, 1b^{x}) conentration (number/cm^3)	(Figure 1c) LDSA
Mean	52.8	27597.6	76.6
SD	15.1	15467.7	35.7
Median	52.5	26242.5	71.5
Minimum	13	11034	18
Maximum	81	565186	329
Range	68	554152	310

Figure 1 continued..

NP	(Figure 1a) dimension 1 (nm)	(Figure 1b, 1b^x) conentration (number/cm^3)	(Figure 1c) LDSA
IQR range	28	9032	58
Skewness	0.06	15.4	0.6

Figure 1. Time series charts of (a) NP dimensions, (b, b^x) concentration (NP number/cm^3), magnified view of b and (c) Lung deposited surface area concentration (LDSA) every minute for 4146 sequential minutes (about 2.9 days) at a nano research lab, LDSA, lung deposited surface area concentration.
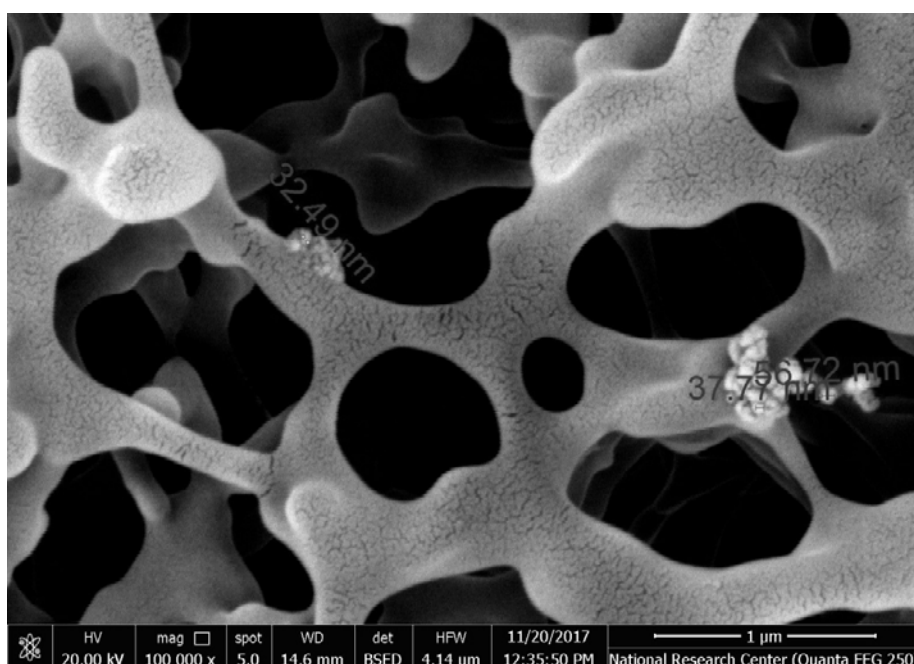
Figure 2. Electron microscopy image (magnified 100,000 X) showing a filter paper with NP dust precipitated on the mesh of the paper.

4. DISCUSSION

The physicochemical properties of nano-sized materials are different from those of ultrafine or bulk materials with the same chemical composition [19]. PM2.5 concentration (which is a subset of PM10) in the present study exceeded the Egyptian maximum allowable concentrations (MAC) for PM10 (150 μg/m^3/24 hours) documented in Egyptian Environment Protection Law no. 4, 2005.

All NP dimensions in the present study ranged from 13-81 nm despite the fact that the counter can measure particles up to 300 nm which suggests a gap in the spectrum of PM2.5 particle sizes (82-300 nm). Both NP number concentration and average diameter are considered in determining health risk. The NP with the smallest size (13 nm) were found to appear simultaneously with the NP having the highest concentration which suggest highest hazardous effect during this time. Previous studies have showed that particle surface area is a more suitable metric for NP-related health effects and that the biological reaction depends on the

Table 1. Serum V-CAM1 and Hs-CRP concentrations and spirometric measurements of the NP-exposed group and non-exposed group.

	Reference range (normal)	Exposed N = 46		Non-exposed group N = 45		Statistical test	p-value
		Median (IQR) [min-max]	Mean ± SD	Median (IQR) [min-max]	Mean ± SD		
% pred FVC	≥ 80%	89 (17.5)	91.7 ± 12.4	91 (26)	91.4 ± 19.5	0.1*	0.937
% pred FEV1	≥ 85%	94 (15)	94.0 ± 13.0	98 (9)	93.6 ± 24.0	0.1†	0.932
FEV1/FVC	≥ 0.8	87 (11.5)	86.1 ± 8.8	85 (16)	87.1 ± 10.4	0.4‡	0.680
% pred PEF	≥ 80%	75 (23.8)	74.0 ± 18.2	83 (37)	82.9 ± 30.8	1.5†	0.141
% pred MEF$_{75}$		70 (33.3)	71.7 ± 22.7	89 (39)	88.5 ± 34.6	2.4†	**0.019**
% pred MEF$_{50}$		90 (28)	80.6 ± 28.7	100 (36)	97.8 ± 33.7	2.2†	**0.032**
% pred MEF$_{25}$		93 (52.3)	95.9 ± 38.4	88 (59)	92.6 ± 38.7	0.3†	0.742
V-CAM-1 (ng/ml)	349-991	433.6 (102.5) [207.4-819.2]	441.5 ± 150.6	376.6 (262) [235.2-592.0]	376.8 ± 91.0	2.5*	**0.016**
Hs-CRP (mg/L)	0 -10	3.96 (6.5) [0.18-31.0]	6.1 ± 6.9	4.72 (7.4) [0.5-22.7]	5.8 ± 5.2	953.0†	0.515

% pred FVC, % predicted forced vital capacity; % pred FEV1, % predicted forced expiratory volume in one second; % pred PEF, % predicted peak expiratory flow; % predicted MEF$_{75}$, MEF$_{50}$, MEF$_{25}$, (maximal expiratory flow at 50%, 25%, and 25-75% of FVC respectively); p < 0.05 (GOLD, 2016). V-CAM1, vascular cell adhesion molecule; hsCRP, high sensitive C-reactive protein; NS, non-significant, p ≥ 0.05; *, significant, p < 0.05; †, independent t-test, ‡, Mann-Whitney U test.

Table 2. Correlation between VCAM1, hsCRP and spirometric functions in both exposed group and non-exposed group.

			hsCRP	% pred FVC	% pred FEV1	% pred PEF	% pred MEF$_{75}$	% pred MEF$_{50}$	% pred MEF$_{25}$	FEV1/FVC
V-CAM1	Exposed	R	0.037	0.124	-0.286	-0.256	-0.273	**-.398-****	-0.28	**-.530-****
		P	0.806	0.41	0.054	0.086	0.066	**0.006**	0.059	**0.000**
	Non-exposed	R	-0.219	0.221	0.363	0.222	0.239	0.346	0.383	0.345
		P	0.164	0.323	0.097	0.321	0.284	0.114	0.078	0.116

hsCRP, high sensitive C-reactive protein; V-CAM1, vascular cell adhesion molecule; *, significant, p < 0.05; **, highly significant, p < 0.01.

particles' surface area rather than other metrics [20, 21].

Larger surface area of the particle increases the possibility of carrying and transmitting toxic compounds [22]. NP-generating procedures differ in their emission of NP, and hence engineering and chemical control should be considered. NP release during nanotechnology procedures can be controlled in containment systems as in under-liquid seal systems.The present study shows that the emissions of NP were not constantly high. Awareness of the work staff about hazardous operations led them to minimize the duration of stay inside lab during operations with possible high emission. There are other sources of exposure to ambient NP including cooking and eating (e.g. time spent in cooking activities) that markedly increase the daily NP exposure of the population. On the contrary, a previous study recognized minor involvement of the outdoor microenvironments [23].

With the progress in nanotechnology the number of workers exposed to NP increases [1]. Normal principal spirometric functions in the present study might be explained by short duration, low dose or mild effect at the level of respiratory tract. This is probably because workers leave the lab during its operation in addition to their tendency to change to alternative safer techniques. In the present study, ventilatory parameters MEF_{75} and MEF_{50} which indicate obstruction of small airways [24] were affected more than the principal parameters, which might help in the early detection of small airways affection in NP-exposed workers. According to Tangour *et al.* [25] results, the average MEF_{50} and MEF_{75} for early stage of COPD patients (stage I) were 41% and 48% of the predicted values, respectively.

Though lungs are the chief target of NP, they might move into the circulation to reach other organs. Adverse health effects correlated more to penetration of NP into blood vessels to reach vascular endothelium. NP injure organs and cells, especially those vulnerable to oxidative stress [26]. The NP vascular effects include expression of endothelial cell adhesion molecules such as VCAM-1, in addition to monocyte adhesion onto the endothelial cells [27], vasomotor dysfunction [11], and lipid accumulation intracellularly [28] and hastened progress of atherosclerosis [11].

In an *in vitro* study, cobalt- or manganese-containing silica NP were found to be able to efficiently enter the cells by a Trojan-horse type mechanism. This mechanism triggered higher oxidative stress when compared to reference cultures exposed to aqueous solutions of the same metals [29]. In another study, using fetal bovine serum to prepare silver NP, cytotoxicity to cultured cells and cellular apoptosis, diminished intracellular glutathione, increased nitrogen oxide emission and increased gene expression of matrix metalloproteinases were found. It seemed also that silver NP caused cytotoxicity by a Trojan-horse type mechanism [30].

NP can pass to blood vessels and to the liver, generating oxidative stress and inflammation [31]. Liver is the main organ responsible for the accumulation of various materials, and detoxifyimg toxins, and is dependent on the oxidation ability of liver cells [32]. Consequently, elevation of serum VCAM-1 (biomarker of endothelial dysfunction) was found in NP-exposed workers in the present study. Endothelial dysfunction might have an etiological part in the progression of early atherosclerotic cardiovascular disease [33].

Serum hs-CRP did not show a role in the present study. A serum CRP threshold of less than 3-10 mg/L is considered to be normal when using immunoassays (e.g. ELISA) [34]. Due to its variability, measurement of hs-CRP more than one time is more suitable than the use of a single sample value [35]. In the present study, it was measured once for showing a possible role for inflammation. It seemed that inflammation played no role in the present study.

5. CONCLUSION AND RECOMMENDATIONS

Elevated serum VCAM-1 among NP-exposed workers suggests potential hazardous impact upon endothelium of blood vessels. MEF_{50} ventilatory parameters indicate small airways obstruction and may be used as a parameter helping early suspicion of small airways affection. Periodic assessment of workplace NP emissions and serum V-CAM-1 concentration as well as spirometry test for NP-exposed workers is recommended. The authors recommend that nanotoxicology studies should precede or go parallel with further applications of nanomaterials to avoid past errors e.g. as in asbestos or silica.

ACKNOWLEDGEMENT

CONFLICT OF INTEREST STATEMENT

No conflict of interest.

REFERENCES

1. EU-OSHA (European Agency for Safety and Health at Work). 2009, Workplace exposure to nanoparticles. https://osha.europa.eu/en/tools-and-publications/publications/literature_reviews/workplace_exposure_to_nanoparticles
2. Wallace, W. E., Keane, M. J., Murray, D. K., Chisholm, W. P., Maynard, A. and Ong, T. M. 2007, J. Nanoparticle Res., 9(1), 38.
3. Schmid, O., Moller, W., Semmler-Behnke, M. A., Ferron, G., Karg, E., Lipka, J., Schulz, H., Kreyling, W. G. and Stoeger, T. 2009, Biomarkers, 14, 73.
4. Buonanno, G., Marks, G. and Morawska, L. 2013, Environ. Pollution, 180, 250.
5. Buteau, S. and Goldberg, M. S. 2016, Environ. Res., 148, 247.
6. Weichenthal, S. 2012, Environ. Res., 115, 36.
7. Liu, X. and Sun, J. 2010, Biomaterials, 31(32), 8209.
8. Lerman, A. and Zeiher, A. M. 2005, Circ., 111(3), 368.
9. Schlesinger, M. and Bendas, G. 2015, Intern. J. Cancer, 136(11), 2514.
10. Packard, R. R. and Libby, P. 2008, Clin. Chem., 54(1), 38.
11. Møller, P., Mikkelsen, L., Vesterdal, L. K., Folkmann, J. K., Forchhammer, L., Roursgaard, M., Danielsen, P. H. and Loft, S. 2011, Crit. Rev. Toxicol., 41(4), 368.
12. Clyne, B. and Olshaker, J. S. 1999, J. Emerg. Med., 17, 1025.
13. International Commission on Radiological Protection. 1994, Annals of the ICRP, 24, 482.
14. Knudson, R. J., Lebowitz, M. D., Holberg, C. J. and Burrows, B. 1983, Am. Rev. Resp. Dis., 127(6), 734.
15. GOLD (Global Initiative for Chronic Obstructive Lung Disease). 2017, Pocket guide for the diagnosis, management and prevention of COPD.
16. Miller, M. R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A. and Crapo, A. 2005, The Europ. Resp. J., 26, 338.
17. Cybulsky, M. I., Fries, J. W., Williams, A. J., Sultan, P., Davis, V. M., Gimbrone, M. A. and Collins, T. 1991, Am. J. Path., 138(4), 820.
18. Kushner, I. and Rzewnicki, D. L. 1994, Baill. Clin. Rheum., 8(3), 530.
19. Willhite, C. C., Karyakina, N. A., Yokel, R. A., Yenugadhati, N., Wisniewski, I. M., Arnold, I. M., Momoli, F. and Krewski, D. 2014, Crit. Rev. Toxicol., 44(4), 80.
20. Cauda, E. G., Ku, B. K., Miller, A. L. and Barone, T. L. 2012, Aero. Sci. Tech., 46, 1381.
21. Schmid, O. and Stoeger, T. 2016, J. Aero. Sci., 99, 143.
22. Nygaar, U. C., Samuelsen, M., Aase, A. and Løvik, M. 2004, Toxicol. Sci., 82, 554.
23. Pacitto, A., Stabile, L., Moreno, T., Kumar, P., Wierzbicka, A., Morawska, L. and Buonanno, G. 2018, Environ. Poll., 232, 122.
24. McFadden, E. R. and Linden, D. A. 1972, Am. J. Med., 52, 737.
25. Tangour, E., Mhamdi, S., Moatameri, Z., Dabboussi, S. Sellami, A., Zaibi, H., Jary, A., Aichaouia, C., Khadhraoui, M. and Cheikh, R. 2013, Annual Congress of European Respiratory Society.
26. Ferreira, A. J., Cemlyn-Jones, J. and Robalo Cordeiro, C. 2013, Rev. Portuguesa de Pneumol., 19(1), 37.
27. Forchhammer, L., Loft, S., Roursgaard, M., Cao, Y., Riddervold, I. S., Sigsgaard, T. and Møller, P. 2012, Toxicol. Letters, 209(2), 128.
28. Cao, Y., Jacobsen, N. R., Danielsen, P. H., Lenz, A. G., Stoeger, T., Loft, S., Wallin, H., Roursgaard, M., Mikkelsen, L. and Møller, P. 2014, Toxicol. Sci., 138(1), 116.
29. Limbach, L. K., Wick, P., Manser, P., Grass, R. N., Bruinink, A. and Stark, W. J. 2007, Environ. Sci. and Tech., 41(11), 463.
30. Park, E. J., Yi, J., Kim, Y., Choi, K. and Park, K. 2010, Toxicol. In Vitro, 24(3), 878.
31. Buzea, C., Pacheco, I. I. and Robbie, K. 2007, Biointerphases, 2(4), 71.
32. Pessayre, D., Fromenty, B., Berson, A., Robin, M. A., Lettéron, P., Moreau, R. and Mansouri, A. 2012, Drug Metab. Rev., 44(1), 87.
33. Price, D. T. and Loscalzo, J. 1999, Am. J. Med., 107(1), 97.
34. Ridker, P. M., MacFadyen, J., Libby, P. and Glynn, R. J. 2010, Am. J. Cardiol., 106(2), 209.
35. Fonseca, F. A. and Izar, M. C. 2016, Clinics (Sao Paulo), 71(4), 242.