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## BIOMARKERS OF OXIDATIVE STRESS AND INFLAMMATION IN RESEARCHERS EXPOSED TO NANOPARTICLES BY INHALATION DURING THE HANDLING OF NANOCOMPOSITES

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### Abstract

At present, little is known about the health effects in the workers processing nanocomposites. In our study, 20 researchers (41.8±11.4 y/o), handling nanocomposites for 17.8±10.0 years were examined pre-shift and post-shift, together with 21 controls (42.7±11.5 y/o). Biomarkers of oxidative stress derived from lipids, nucleic acids, proteins and markers of inflammation were analyzed in the exhaled breath condensate (EBC). Aerosol exposure was monitored during three nanoparticle generation operations: smelting, welding and nanocomposite machining. Mass concentrations during these operations ranged from 0.120 to 1.840 mg/m<sup>3</sup>, and median particle number concentrations from 4.8x10<sup>4</sup> to 5.4x10<sup>5</sup> particles/cm<sup>3</sup>. Nanoparticles accounted for 40 to 95% of particles, with Fe and Mn prevailing. Significant elevations were already seen in most oxidative stress markers and in several inflammation markers in the pre-shift samples relative to the controls. Significant associations were found between working in nanocomposite synthesis and the majority of EBC biomarkers. Chronic bronchitis was more frequent in researchers. A minor, but significant post-shift decrease of lung function parameters was found. We conclude that workers in nanocomposite synthesis may be at risk of developing airway disorders with time. From all the markers analyzed in EBC, the following markers were most robust and could be recommended for preventive examinations: 8-hydroxy-2-deoxyguanosine (8-OHdG) and 5-hydroxymethyl uracil (5-OHMeU) from nucleic acids; o-tyrosine (o-Tyr) and 3-nitrotyrosine (3-NOTyr) from proteins; and malondialdehyde and aldehydes C6-C13 from lipids. Among the markers of inflammation, tumor necrosis factor (TNF) and leukotriene B4 appeared to be the most useful.

**Keywords:** Nanocomposites, exhaled breath condensate, oxidative stress, inflammation, spirometry

### 1. INTRODUCTION

Thousands of workers are engaged in the research and commercial scale production of nano-enabled composites. However, among tens of thousands of papers focusing on the research of the toxicity of nanoparticles found in PubMed, those concerning workers, i.e. the most exposed subjects, is very limited

and will be mentioned in this study. The safety of nanoparticles for humans is the main goal of all *in vitro* and *in vivo* testing [1-6]. Experimental studies especially highlight respiratory health impairment. A common feature of many aerosols in the workplace is their ability to generate reactive oxygen species, which induce oxidative damage to biomolecules, leading to the activation of redox signaling pathways [7, 8]. Elevated markers of oxidative stress in the biological fluids pro-inflammatory markers, such as tumor necrosis factor (TNF), pro-inflammatory leukotrienes (LTs), antioxidant enzymes and cardiovascular markers could give a signal of impairment. Lipid mediators, known to be immediately generated in response to tissue injury, might be strong candidates for priming this particle-triggered change of the inflammatory balance [9-10]. The results from the few publications focusing on exposed humans agree with the experimental studies [11-19]. Accordingly, our studies in 2012 and 2013, showed an elevation of markers of oxidative stress and inflammation in the exhaled breath condensate (EBC) [20] both in the production workers, and office employees, visiting the workshops for about 15 min a day [21-24]. In this article we focus on workers producing and handling nanocomposites as new materials with better technical characteristics, especially hardness and resistance. This study started in September 2015 and continues with a yearly follow-up of the group of researchers and matched controls.

## 2. MATERIALS AND METHODS

### 2.1. Working processes

Researchers usually work in two workshops: welding and smelting of mixtures containing nano-additives in workshop 1, and machining of the finished nanocomposite in workshop 2. All tasks occur simultaneously in both workshops and last on average 2.5 hours. On the day of medical examination in September 2016, in workshop 1, 11 out of a total of 20 researchers performed welding on metal surfaces on mild steel (content in wt%): Fe, 97.39; Mn, 1.70; C, 0.24; Si, 0.6; P, 0.035; S, 0.035) and smelting at 760°C. In workshop 2, the remaining 9 researchers performed the machining of surfaces of nanocomposite blocks of geopolymers and epoxide resins with nanoSiO<sub>2</sub> fillers. No respiratory protection was used. A detailed description of the working process was given in our last article [25].

### 2.2. Subjects

EBC samples were collected in 20 nanocomposite researchers (15 men, 5 women, one smoker, 19 non-smokers, with mean age 41.8±11.4 years; exposure duration 17.8±10.0 years). For simplicity, we refer to examinations of the researchers as pre-shift and post-shift, even though they were in fact post-exposure task measurements. The remainder of their total 8-hr shift was spent in their offices. There were 21 controls (15 men, 6 women; two smokers, 19 non-smokers, mean age 42.7±11.5 years), working as office employees in the same town.

### 2.3. Aerosol measurement

Nano-aerosol monitoring was performed with several aerosol spectrometers, including a Scanning Mobility Particle Sizer (TSI SMPS 3936L, USA), and an Aerodynamic Particle Sizer (TSI APS 3321, USA), covering the size range of aerosol particles from 6 nm up to 20 µm. Additionally, an Ultrafine Condensation Particle Counter (TSI UCPC 3025, USA) was used to measure the total particle number concentration (3 nm --1 µm), as well as three Optical Particle Sizers (TSI OPS 3330, USA) used to measure the number size distribution in the range of 300 nm-10 µm. Area and time integrated samplings were conducted using a Berner Low Pressure Impactor (BLPI, HAUKE GmbH, Austria) to sample aerosol particles onto 10 stages corresponding to their aerodynamic diameter covering the 25 nm - 13.6 µm size range. These samples were then analyzed by gravimetry, ion chromatography and Scanning Electron Microscope (SEM) (Tescan Indusem, Czech Republic) equipped by Energy-Dispersive X-Ray Spectroscopy (EDS) (XFlash detector

5010, Bruker, Germany) to analyze the elemental composition of size-resolved aerosol fractions, as previously described in detail [25].

#### 2.4. Biological samples

EBC samples were collected using Ecoscreen Turbo (DECCS, Jaeger, Germany) All samples were immediately spiked with deuterium labelled standards, immediately frozen and stored at -80°C for subsequent processing [20]. TNF was analyzed by a matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) and analyses of low-molecular biomarkers LTs were performed using liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS), after solid-phase extraction (SPE), and MS/MS detection were used [22,25].

#### 2.5. Statistics

Basic descriptive statistics were calculated using Microsoft Excel 2013. Following tests were used for statistical comparisons of the pre-shift and post-shift markers (both within workers and between workers and controls): T-test, Mann-Whitney U test, paired sample t-test and the Wilcoxon signed-rank test. The bivariate relationship between the variables under study was assessed using the Spearman correlation coefficient. A multiple regression analysis was used to predict the markers in EBC by a set of predictors. All analyses were conducted using SPSS version 22.0 (SPSS, Inc., Chicago, Illinois, USA).

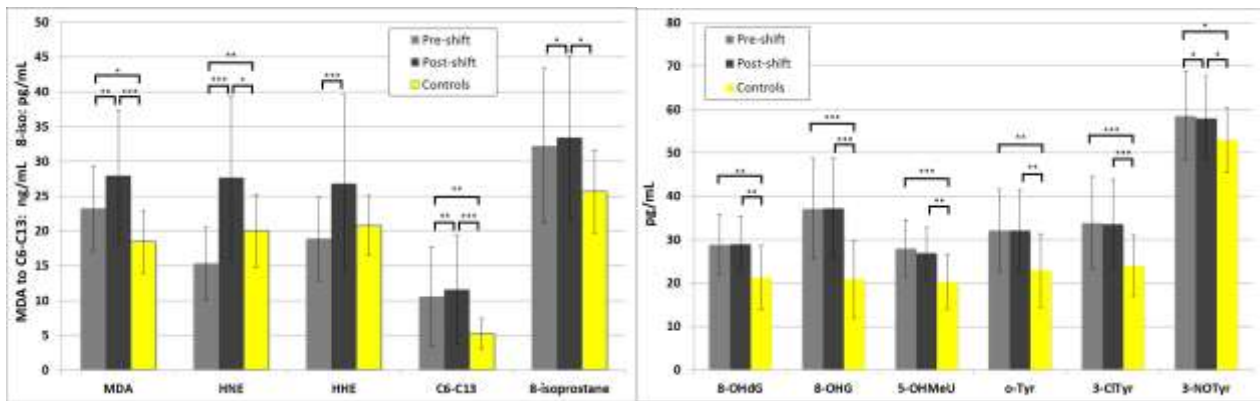
### 3 RESULTS AND DISCUSSION

Exposure data concerning aerosol measurements were provided in detail in our previous manuscript [25]. The average total mass concentration in the workshops was 0.120 mg/m<sup>3</sup> during smelting, 0.804 mg/m<sup>3</sup> during machining, and 1.840 mg/m<sup>3</sup> during welding. The total median particle number concentration was 1.3 x 10<sup>5</sup> particles/cm<sup>3</sup> (particles/cm<sup>3</sup>) during welding, 4.8 x 10<sup>4</sup> particles/cm<sup>3</sup> during smelting and 5.4 x 10<sup>5</sup> particles/cm<sup>3</sup> during machining. The highest proportion of particles smaller than 100 nm in diameter was found during smelting (95%), followed by machining (61%), and welding (40%).

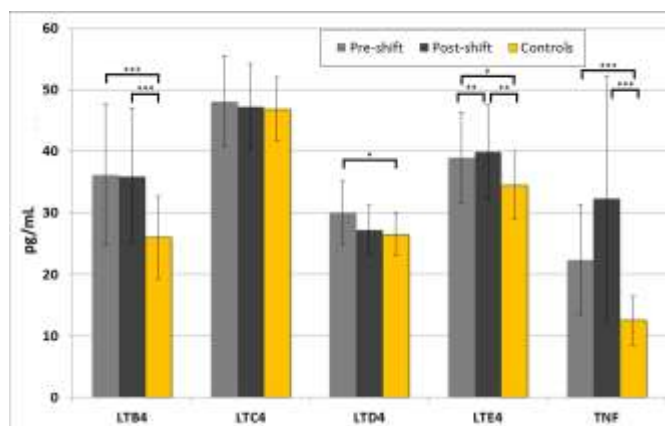
The group characteristics did not differ significantly, including smoking (5% smokers among workers with 10 pack-years; 9.5% in controls with average 14 pack-years). Chronic bronchitis and dyspnea, associated with minor limitations in ordinary physical activity, were only found in the exposed researchers (20.0% and 15%, respectively). A cough was 5 times more frequent than in the controls. A significant decrease in forced expiratory volume in 1 s (FEV1) and FEV1/ forced vital capacity (FVC) was found in the researchers post-shift ( $p < 0.05$ ).

The majority of the pre-shift and post-shift markers of oxidative stress and the three markers of inflammation were already elevated pre-shift, as shown in Figure 1 and Figure 2. Additionally, all markers of oxidation of lipids reacted by post-shift elevation (Figure 1, left). However, no difference was seen between the markers of inflammation and markers of oxidative stress in the subgroups of the 11 researchers working in workshop 1, and 9 researchers from workshop 2. The only exception was 8-isoProstaglandin F2 $\alpha$  (8-isoprostane), produced by free-radical lipid peroxidation of arachidonic acid, which was higher both pre-shift and post-shift in the researchers from workshop 1 ( $p < 0.05$ ) [25].

Both experimental and epidemiological studies have indicated that chronic inflammation is involved in, and plays a critical role in, several chronic diseases, including respiratory and cardiovascular diseases, and lung tumorigenesis.



**Figure 1** Markers of oxidation of lipids (left) and markers of oxidation of nucleic acids and proteins (right) MDA=malondialdehyde, HNE=4-hydroxy-trans-nonenal, HHE=4-hydroxy-trans-hexenal, C6-13=aldehydes C6-C13, 8-isoprostane=8-isoProstaglandin F2 $\alpha$ , 8-OHdG=8-hydroxy-2-deoxyguanosine, 8-OHG=8-hydroxyguanosine, 5-OHMeU=5-hydroxymethyl uracil, o-Tyr=o-tyrosine, 3-ClTyr=3-chlorotyrosine, 3-NOTyr=3-nitrotyrosine \*p<0.05, \*\*p<0.01, \*\*\*p<0.001



**Figure 2** Markers of inflammation TNF=tumor necrosis factor, LT=leukotriene B4, C4, D4, E4, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001

### 3. CONCLUSION

Handling nanocomposites is very complex and may lead to exposure nanoparticles in the workplace aerosol during welding, smelting of the metallic products at hot temperatures and machining and grinding of geopolymers epoxide resins with nanocomponents [26-27]. Since inhalation remains the major exposure pathway to nanoparticles during nanocomposite manufacturing, the respiratory system is the main portal of entry and the primary target organ of concern [28-30]. The initial minor effects on the obstructive parameters of lung function have been observed. EBC markers of oxidative stress and inflammation were already elevated in the pre-shift samples, therefore they may be useful in monitoring the chronic effect. The markers of oxidation of lipids may reflect the early effect of the shift. EBC is one of the few means of non-invasive monitoring of individuals exposed to nanoparticles. These markers could be further tested for their potential use in preventive examinations of the workers in parallel with spirometry.

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