Assessment of nanoparticles and metal exposure of airport workers using exhaled breath condensate

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Assessment of nanoparticles and metal exposure of airport workers using exhaled breath condensate

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Keywords: exhaled breath condensate, nanoparticles, metals, airports

Abstract

Aircraft engine exhaust increases the number concentration of nanoparticles (NP) in the surrounding environment. Health concerns related to NP raise the question of the exposure and health monitoring of airport workers. No biological monitoring study on this profession has been reported to date. The aim was to evaluate the NP and metal exposure of airport workers using exhaled breath condensate (EBC) as a non-invasive biological matrix representative of the respiratory tract. EBC was collected from 458 French airport workers working either on the apron or in the offices. NP exposure was characterized using particle number concentration (PNC) and size distribution. EBC particles were analyzed using dynamic light scattering (DLS) and scanning electron microscopy coupled to x-ray spectroscopy (SEM-EDS). Multi-elemental analysis was performed for aluminum (Al), cadmium (Cd) and chromium (Cr) EBC contents. Apron workers were exposed to higher PNC than administrative workers ($p < 0.001$). Workers were exposed to very low particle sizes, the apron group being exposed to even smaller NP than the administrative group ($p < 0.001$). The particulate content of EBC was brought out by DLS and confirmed with SEM-EDS, although no difference was found between the two study groups. Cd concentrations were higher in the apron workers ($p < 0.001$), but still remained very low and close to the detection limit. Our study reported the particulate and metal content of airport workers airways. EBC is a potential useful tool for the non-invasive monitoring of workers exposed to NP and metals.

List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Al</td>
<td>aluminum</td>
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<tr>
<td>ANSM</td>
<td>Agence National de Sécurité du Médicament (French National Agency for Medicines and Health Products)</td>
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<tr>
<td>Cd</td>
<td>cadmium</td>
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<tr>
<td>Cr</td>
<td>chromium</td>
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<tr>
<td>DLS</td>
<td>dynamic light scattering</td>
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<td>EBC</td>
<td>exhaled breath condensate</td>
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<tr>
<td>EDS</td>
<td>energy dispersive x-ray spectroscopy</td>
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<td>ELPI</td>
<td>electrical low pressure impactor</td>
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<tr>
<td>GM</td>
<td>geometric mean</td>
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<tr>
<td>ICP-MS</td>
<td>inductively coupled plasma mass spectrometry</td>
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<tr>
<td>Na</td>
<td>sodium</td>
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<tr>
<td>NP</td>
<td>nanoparticle(s)</td>
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<tr>
<td>PNC</td>
<td>particle number concentration</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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<td>SEM</td>
<td>scanning electron microscopy</td>
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</table>
1. Introduction

Air pollution, including particulate matter, is associated with negative health effects in humans. For example, cardiac and respiratory symptoms have been associated with particle pollution as has increased mortality in these diseases [1–3]. The fine (0.1–2.5 µm) and ultrafine (<0.1 µm) particulate fractions are indeed associated with symptoms and diseases [4–6], and recently, the International Agency for Research on Cancer classified fine diesel particles as carcinogenic to humans.

Particles are also generated in significant volume by jet engines; it has been shown that airports greatly contribute to the atmospheric particulate matter pollution of large surrounding areas, up to several kilometers. In a Danish airport the particle number concentration (PNC) can reach 500,000 particles cm$^{-3}$ in comparison with peak concentrations in urban environment with heavy traffic around 40 with peak concentrations in urban environment with large surrounding areas, up to several kilometers. In

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sizers (detection range from 5–560 nm) and scanning mobility particle sizers (5–350 nm). Particles were collected (sampling range from 30 nm–10 μm) with an electrical low pressure impactor (ELPI) and characterized with scanning electron microscopy coupled to energy-dispersive x-ray spectroscopy (SEM-EDS). Measurements were conducted once at three workplaces representative of each group, either in Marseille or Paris, between March and April 2012. For the administrative group, the samplings took place in three different offices, and for the apron group, at a distance ranging from 3–10 m from airplane parking positions. The total sampling duration was 250 min and 374 min for the administrative and apron area, respectively, with an acquisition of data every second.

2.3. EBC collection and standardization
EBC collection was performed using the RTube™ device from Respiratory Research (USA) and following the American Thoracic Society/European Respiratory Society recommendations [26]. In addition, prior to sampling, each RTube was washed seven times with ultrapure water in order to minimize the analytical background for particulate and elemental analyses. During the preparation, an RTube was kept apart every 50 RTubes so as to constitute blank RTubes. Thereafter, RTubes were conditioned in clean individual hermetic plastic bags.

Before sampling, subjects were asked to wash their hands and rinse their mouth three times with tap water. The cooling sleeves were kept at −20 °C before collection. For the collection, subjects wore a nose clip and were asked to perform tidal breathing during 15 min. After sampling, EBC were immediately frozen at −20 °C in the collecting parts of the RTubes, sent frozen to the laboratory where they were frozen at −80 °C prior to analysis.

EBC were characterized by their volume, total protein and sodium (Na) concentrations. EBC volume was determined by weighing after thawing. Total protein concentration was measured using the MicroBCA Assay (Protein quantitation kit, Uptima Interchim) following the recommendations of the manufacturer, with a detection limit of 1 μg ml⁻¹ in EBC. Na concentration was measured by inductively coupled plasma mass spectrometry (ICP-MS) and included in the multi-elemental analysis described thereafter.

2.4. Determination of EBC particulate content
The particulate content was determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern Instruments). For each sample, 50 μl of EBC were analyzed in an automatic mode and scattered intensity and size distribution were recorded. The efficiency of the washing procedure and the potential residual background were assessed by the incubation of 1.5 ml of ultrapure water in 11 blank RTubes. Afterward, blank samples were processed as real EBC.

2.5. Observation of particles in EBC
In addition to the DLS analysis, SEM-EDS observations were performed on five EBC samples (two EBC from the administrative group and three EBC from the apron group). EBC was deposited on an aluminum membrane, and let to dry under a fume hood. Observations were performed on the same electron microscope than the one used for the aerosol characterization.

2.6. Multi-elemental analysis
A multi-elemental analysis was performed by inductively coupled mass spectrometry (ICP-MS) (Nexion 300 ×, Perkin Elmer) to measure sodium (23Na), aluminum (27Al), cadmium (111Cd), and chromium (52Cr). EBC samples were diluted in nitric acid and yttrium (89Y) was used as an internal standard. The settings of the ICP-MS apparatus were tuned before each run of analysis using a calibrated solution. Due to very low elemental concentrations expected in EBC, ICP-MS analysis was run without prior mineralization step. The multi-elemental technic was validated based on linearity, repeatability, reproducibility, accuracy, inter-sample contamination criterions, and the analytical limits of detection were determined as the sum of means and three standard deviations (SD) of 20 ultrapure water samples. Multi-elemental quality controls were used to validate each run of analysis. For each metal a low value, called positivity threshold, was validated in order to guarantee an accurate elemental quantification. The criterions used to validate the positivity thresholds were a repeatability lower than 15% and an accuracy deviation lower than 20%. In addition, the same 11 blank RTubes than previously described were used to determine the residual elemental background of the method. For Cd and Cr, no contamination was found and elemental concentrations were taken into account when superior or equal to the positivity thresholds. For Al, even after the extensive washing of the RTubes, a relatively constant contamination was detected so that measured concentrations were taken into account when superior or equal to the sum of the mean and three SD of the blanks.

2.7. Statistical analysis
Data analysis was carried out using a software from Statistical Package for the Social Science (SPSS) version 17.0 (USA). Chi² tests were used to study the characteristics of the population in terms of gender, age (transformed in two equally distributed classes) and smoking status. Based on the Kolmogorov–Smirnov test, none of the variables were normally distributed even after log₁₀ transformation attempts. Mann–Whitney tests were used to compare the different continuous variables between the different groups. Associations between variables were examined using the Kendall rank correlation coefficient τ. Left-censored values below detection limits (and positivity thresholds) were substituted by half the detection
limits, mainly due to the fact that non-parametric tests were used. A multivariable model was attempted to concurrently adjust for all covariates that may predict NP scattered intensity in EBC. Moreover it was verified that the same statistical results were found when using all left-censored data. Biomarker concentrations in EBC are presented with raw data but standardization attempts, dividing EBC concentrations by EBC volume, total protein or Na concentrations, were performed to study the differences between the groups. The $p$ value $p < 0.05$ was considered statistically significant.

3. Results

3.1. Description of the population and EBC characteristics

The description of the population study and EBC characteristics are summarized in table 1. Inside the buildings, female and older workers were significantly more represented, when the workers on the apron were mainly male and younger ($p < 0.001$). Active smoking was not different according to the site of work.

While the smoking status had no influence on EBC volumes, collected volumes were significantly dependent of gender and age ($p < 0.001$). Women and old subjects ($> 45$ years old) produced lower volumes than men and young subjects ($\leq 45$ years old).

Total proteins were detected in 64% of 436 EBC. Total proteins were neither dependent of gender nor age, but significantly elevated in active smokers in comparison to non-smokers ($p = 0.028$).

Na was detected in 100% of 435 EBC. No significant influence of gender, age and active smoking was found on Na concentration.

A negative correlation was found between EBC Na concentration and EBC volume ($r = -0.159, p < 0.01$) and a positive correlation was found between Na concentration and total protein concentration ($r = 0.072, p < 0.05$). EBC volume and protein concentration were not correlated.

3.2. Description of NP exposure

NP exposure is described in table 1 and figure 1. The PNC was significantly higher ($p < 0.001$) on the apron.
than inside the buildings. While the PNC was fairly constant for administrative workers, it showed frequent concentration peaks on the apron in connection with the activity of the aircrafts.

Both groups were exposed to small NP, and even smaller NP were found on the apron compared to the offices \( (p < 0.001) \).

SEM-EDS observations indicated that, whatever the site of sampling, NP were rather spherical and found as aggregated strings on ELPI membranes (figure 2). Only carbon was significantly detected in the NP composition, at the exception of few sulphur traces (data not shown).

### 3.3. Particulate content of EBC

The EBC particulate content description, including scattered intensity and size distribution, is presented in table 2.

There was neither an influence of age nor smoking status on the total scattered intensity of EBC, but a significant influence of gender was found, women having a higher total intensity measured in EBC than men \( (p = 0.027) \). A negative correlation was found between EBC scattered intensity and EBC volume \( (\tau = -0.252, p < 0.01) \). No correlation was found with total protein or Na concentrations.

No significant difference of scattered intensity was found between administrative and apron workers \( (p = 0.115) \), but the scattered intensity values indicated that a particulate content was brought out in EBC in comparison with blank RTubes. Indeed, for ten out of eleven blank RTubes, the DLS measurement was aborted indicating that the particle content was too low to allow proper measurement. In one blank, a scattered intensity of 236 Kcps was found which remains low in comparison with EBC values.

Based on the multivariate model performed on the \( \log_{10}(\text{scattered intensity}) \), a positive association was found with Al in EBC \( (p < 0.05) \) and Na in EBC \( (p < 0.001) \), and a negative association was found with EBC volume \( (p < 0.001) \).

Regarding EBC size distribution, no influence of gender, age, or smoking status was found. No influence of the group of exposure was found on the size distribution in EBC. The main peak of the size distribution was centered on 460 nm for all subjects. The second peak was characterized by a lower size than the main peak, nearer the nano-range, but very few subjects presented this peak.

### 3.4. Observations of particles in EBC

SEM-EDS explorations of EBC revealed a sparse population of particles around 500 nm that could correspond to the main peak of the DLS size distributions. Interestingly, similarly to what was observed in the air, sulphur was found on some particles in EBC (figure 3), as well as elements representative of a biological content such as Calcium and Potassium.
Metal content of EBC

Metal concentrations in EBC are presented in Table 3. Al, Cd, and Cr were detected in 19%, 22% and 79%, respectively, of all subjects. No significant influence of gender, age or smoking status was found for these metals. Al was correlated to Cd and Cr (τ = 0.093 and τ = 0.078, respectively, p < 0.05). No correlation was found between metals and DLS scattered intensity.

The comparison between administrative and apron workers showed no significant difference for Cr and Al concentrations, but a significantly higher concentration of Cd was found in apron workers in comparison with administrative workers (p < 0.001, figure 4).

4. Discussion

We have found that EBC allows the detection of a particulate content in airport workers airways, even if the particulate intensity measured in EBC could not be linked to air PNC. Among the three investigated metals, Cd was significantly elevated in workers of the apron. However the detection rate of this metal in EBC was globally very low.

The apron group was exposed to high concentrations, of the $10^4$–$10^5$ range with peak concentrations of the $10^7$ range, which is elevated compared to urban concentrations. The mean measured PNC at the roadside in European cities is $3.1 \pm 1.6 \times 10^4$ part cm$^{-3}$ [27]. On the opposite, the administrative group was exposed to lower PNC, with values of the $10^3$–$10^4$ range that are reported for city backgrounds or even rural zones [28]. However, peak concentrations of the $10^3$–$10^4$ range occurred episodically in the offices, when the doors of the building were open for instance (data not shown). Both groups were exposed to very small carbonaceous NP with few sulfur traces as a signature of the combustion origin [29]. Therefore, the two groups were rather defined as ‘highly

![Figure 3. SEM-EDS characterization of an EBC from an apron worker: SEM picture of a particle of interest at a magnification of 35,000 (A), and corresponding EDS spectrum (⋆1——) in comparison with the surrounding background (⋆2——) (B). The elements surrounded by a circle are found on the particle and not in the background.](image_url)
exposed’ and ‘less exposed’ than ‘exposed’ and ‘non-exposed’.

The analysis of NP requires the combination of different methods to obtain proper characterization. We have made the assumption that the scattered intensity measured in EBC was representative of its particulate burden, based on unpublished results from our group on standard NP. On the basis of the comparison with blank RTubes, a particulate content has been brought out in EBC, and the presence of particles potentially linked to occupational exposure was confirmed by electron microscopy. However, no difference between the two groups of exposure was found. EBC samplings were performed during the shift of each worker but the time lag between exposure and EBC collection was not standardized for all subjects which might be a first limitation. Also, the exposure was measured globally, not individually, mainly due to the technical limitation for measuring NP at the individual level with portable devices.

Moreover, it cannot be taken for certain that the origin of particles in EBC corresponds to inhaled NP during the shift, deposited in the airways and finally exhaled during EBC sampling. Indeed, the particles can also originate from inhalation and direct exhalation during the EBC sampling, or result from endogenous formation during breathing cycles [30]. The respective contribution of each source is still a major issue. As a first attempt, no filtration of inhaled air was performed in our study during EBC collection. The sampling sessions took place in dedicated rooms in the buildings of the airports. With the same sampling device than in our study, Sauvain et al also found that the total number of particles in EBC was not significantly changed by tobacco smoke particulate exposure [18]. On the contrary, Benor et al found that exhaled ultrafine particles in EBC, without filtration of inhaled air during the sampling, correlated with respiratory disorders in asthmatic children, which might reinforce the hypothesis of the airways origin of exhaled particles [19]. Such results indicate that the link between air particles and EBC particles is not trivial, and improvements in EBC collection should be investigated for future studies. Also, for the metal analysis in EBC, the potential influence of metal concentrations in the tap water used to rinse out the mouths of the subjects prior to collection might be verified in an ancillary study.

Regarding the size of particles found in EBC, it was higher than the size of particles measured in the air, and above the theoretical nano-limit of 100 nm. Our results are in agreement with the measurement of exhaled particles after HEPA-filtered inhalation at a median size of 320 nm in healthy volunteers [30]. If exogenous particles are, at least partly, found in EBC, they are most probably embedded in the biological matrix, with proteins also adsorbed at the surface, thus enlarging their hydrodynamic size which is measured with DLS.

As regards the metals, a significantly higher level of Cd was found in apron workers compared to administrative workers, which stresses out the fact that work shifts located in the proximity of airplanes might induce Cd exposure through emission in the air. However, even if a group effect was found, Cd concentrations were globally very low and near our positivity threshold. They were higher of almost one order of magnitude than those reported in 50 non-smokers [20] and in 28 patients with pulmonary disorders [31], but in the same range than those reported in 10 healthy volunteers [32]. That is why our findings should be considered with caution, and confirmed with further studies, along with urinary metal analysis as a reference method.

Due to a residual background brought by the device, our positivity threshold for Al was quite high with a low detection rate. However, even if this makes it difficult to compare with other published data, some particularly high Al values were found in our study with 25 subjects
above 10 µg l⁻¹ in EBC, which is high in comparison with concentrations found in volunteers or patients with respiratory disorders respectively [20, 31, 33]. EBC Cr concentrations found in our study were also a bit higher than those reported either for healthy controls [22, 32, 34, 35], or patients with different lung disorders [33, 34], or even welders [35, 36]. They were nevertheless much lower than those reported for chrome-plating workers [22, 23]. This indicates that the airport environment might induce a light exposure to Al and Cr, in a minor extent in comparison with chrome-plating industries for Cr.

Since standardization of EBC measurements is a main issue, we have investigated different ways to standardize our data. Different methods have been stressed out in the literature but with no consensual recommendations [37–39]. We have found that EBC volume was certainly representative of the pulmonary capacities on the basis of the gender and age effects. Accordingly, it has already been reported that EBC volume was directly dependent of the ventilation mode [39]. In contrast, total proteins and Na concentration seemed to be independent on such intrinsic parameters and found to be correlated to one another. On the contrary to EBC volume, the same statistical differences between the groups were found when these two standardizers were used (data not shown), indicating that they might be useful standardization tools. However, this study did not allow us to conclude firmly on specific indications for the use of one standardization strategy. Protein concentration is most certainly dependent of other factors than the dilution factor, as illustrated by the significant influence of the smoking status, also reported elsewhere [40].

5. Conclusion

To conclude, this study is the first to evaluate the particulate and metal content of EBC in a large population of airport workers of more than 450 subjects. Our study reported the airways particulate content using EBC in occupational exposure in the airport vicinity. The search for biomarkers of exposure in EBC is a potential useful approach for the non-invasive monitoring of workers exposed to NP. It offers the possibility to determine the respiratory particulate burden at the individual level in comparison with other monitoring methods. While atmospheric sampling does not take into account the use of individual protective devices, and several individual parameters, NP urinary excretion under their particulate form has still little evidence in humans. The next step will consist in the setting of a longitudinal study to follow EBC biomarkers in this group of workers, in association with pulmonary function tests to help determine the link between occupational exposure and the potential onset of respiratory disorders.

Acknowledgments

None of the authors have any competing interests in the manuscript. VCM, CM–D and MD take responsibility for the content of the manuscript, for the integrity of the data, and the accuracy of the data analysis, including and especially any adverse effects. LT, MK, PC, NM and IV contributed to the design of the study. MK was responsible for the acceptance of the study by the Air France company. LT contributed substantially to the inclusion of the workers. CM–D and VCM contributed to the data analysis and writing of the manuscript. MD, LT, LL, MK, NM, IV and PC contributed substantially to the revision of the manuscript. EZ and CD were responsible for the atmospheric measurements and SEM–EDS analyses. CM–D, MD and MG–M were responsible for the ICP–MS and DLS analyses and data analysis. LL performed the total protein measurements.

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