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

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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

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

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 40 000 particles cm^{-3} [7]. Moreover, the emissions are mainly composed of nanoparticles (NP) between 6 and 40 nm. This has been reported in several airports [8, 9]. NP are able to reach the distal airways of the lung and to remain within the alveolar vicinity [10, 11]. In this context the potential effect of this exposure should be investigated in airport workers who are daily exposed for several hours. To date, only few studies have investigated the impact of NP emissions on the respiratory health of airport workers [12], and to the best of our knowledge no study of NP biological monitoring of airport workers has been reported.

Biological monitoring is challenging in the case of NP exposure. Biological monitoring typically uses conventional matrices such as urine and blood, but toxicokinetic data acquired in animals suggest that very few NP could be found in these biological fluids after inhalation, due to low translocation and rapid internalization in different organs [13]. Therefore, local sampling should be investigated, and in this regard, exhaled breath condensate (EBC) could be a useful matrix. EBC has been assessed as a research tool in respiratory disorders. It is a non-invasive respiratory sampling that can be used in the field of occupational health. It contains numerous compounds issued from the respiratory tract that potentially reflect pulmonary pathobiology [14]. Finding the primary origin of EBC content is yet to be clearly determined but both large and small airways are represented. The contribution of the lower airways has been demonstrated using proteomic approaches and following different collection procedures [15–17]. EBC is also a potential biological matrix to investigate NP in the respiratory tract following exposure to various atmospheres, but very few studies are available at present. Recently, Sauvain *et al* [18] measured a particulate content with a size distribution around 160 nm in EBC of volunteers exposed to side-stream tobacco smoke. In another study, the total ultrafine particle content in EBC correlated with wheezing, respiratory symptoms scores, and sputum eosinophilia in asthmatic children [19]. More data are available on metals measured in EBC in

relation to smoking [20] or in relation to occupational exposure [20–24], but the effect of airport exposure has not been studied so far. The airport workers are exposed to NP resulting from engine combustions. In addition to NP, some metals are ubiquitous in the aeronautical field. Aluminum (Al) is the most frequent metal that mainly constitutes airline cabins. Chromium (Cr) is a ubiquitous anti-corrosive constitutive of paints, varnishes and mastics, and cadmium (Cd) enters into the composition of engine parts. Due to the potential toxicity of these metals following inhalation, Air France physicians raised the question whether these metals could be released from materials during engine operations or after aging. This would lead to a global metal exposure in the airport environment, which would mean that not just workers working in maintenance workshops are concerned. That is why, a special attention was paid on Al, Cd, and Cr to clarify this suspicion of exposure.

Therefore, the aim of the present study was to determine whether EBC is a suitable matrix to assess the occupational exposure to NP and metals of airport workers. For that purpose, the atmospheric nanoparticulate matter was precisely characterized nearby emission sources and throughout the airports, and two groups of airport workers with different levels of exposure were compared.

2. Methods

2.1. Subjects

This study was approved by the Ethics Committee of Montpellier University and the French ANSM (Identification number 2011-A00646-35). All subjects were recruited by their occupational health doctor and gave written informed consent.

From October 2011–June 2012, 471 voluntary workers were recruited from Marseille Provence (Marseille) and Roissy Charles de Gaulle (Paris) airports, and divided in two exposure groups. The first group corresponded to workers assigned in the buildings of the airports, mainly in the offices for administrative tasks, and the second group was composed of workers operating directly on the apron nearby airplane parking positions (aircraft technicians, ramp agents, and storekeepers).

On each subject was performed an EBC collection during his shift. Each EBC sampling was associated to an information sheet detailing pre-analytical information, the time and duration of the collection, and the beginning and the end of the shift. On the 471 included workers, 458 EBC were obtained.

2.2. Exposure assessment

The exposure assessment was based on NP measurement in the air. The methodology for atmospheric measurements has been described in details elsewhere [25]. Briefly, the PNC was measured using condensation particle counters (detection range from 5 nm to 3 μm), and the particle size geometric mean (GM) using fast mobility particle

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 (^{23}Na), aluminum (^{27}Al), cadmium (^{111}Cd), and chromium (^{52}Cr). EBC samples were diluted in nitric acid and yttrium (^{89}Y) 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). Chi^2 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 log$_{10}$ 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

Table 1. Characteristics of subjects, EBC and air particles[a].

Characteristics	Administrative	Apron
Subjects, n	210	248
Male, n (%)	113 (54)	246 (99)[b]
Smokers/nonsmokers, n	49/122	68/156
Age, years	50 (26–67)	43 (23–64)[b]
EBC volume, μl	1302 (295–2075)	1436 (432–3114)[b]
EBC proteins, μg l^{-1}	1.3 (0.5–26.5)	1.3 (0.5–332)
EBC Na, μg l^{-1}	100 (21–1799)	109 (22–36124)
PNC, part cm^{-3}	8.3×10^3 (617–2.3×10^4)	1.5×10^5 (1.0×10^4–2.1×10^7)[b]
Particle size (GM), nm	23.7 (12.2–118.1)	17.7 (9.3–415.2)[b]

[a] Data are expressed as median (min–max).
[b] Significant difference between administrative and apron workers ($p < 0.001$).
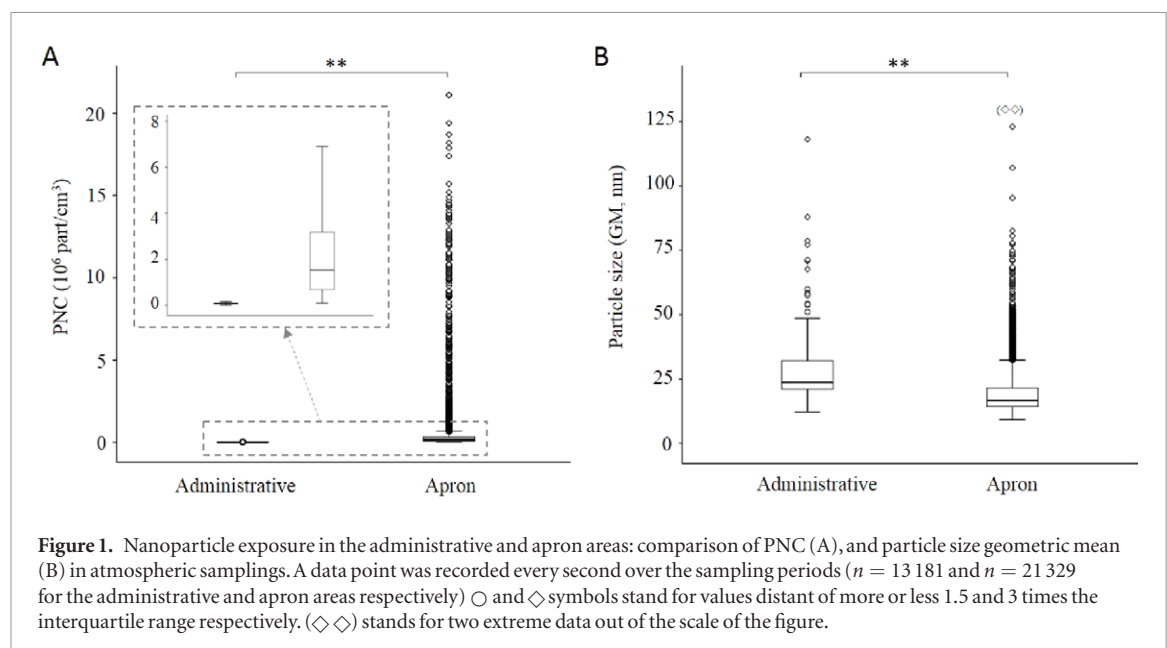
Figure 1. Nanoparticle exposure in the administrative and apron areas: comparison of PNC (A), and particle size geometric mean (B) in atmospheric samplings. A data point was recorded every second over the sampling periods ($n = 13\,181$ and $n = 21\,329$ for the administrative and apron areas respectively) ○ and ◇ symbols stand for values distant of more or less 1.5 and 3 times the interquartile range respectively. (◇ ◇) stands for two extreme data out of the scale of the figure.

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 (⩽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 ($\tau = -0.159, p < 0.01$) and a positive correlation was found between Na concentration and total protein concentration ($\tau = 0.072, p < 0.05$). 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
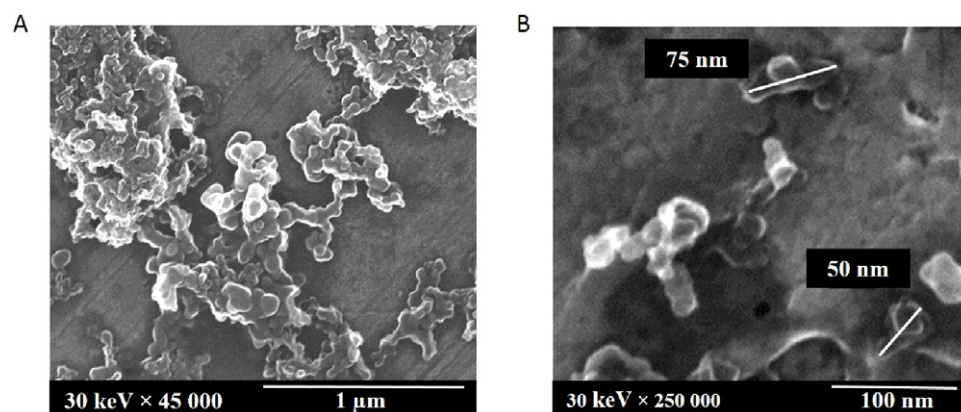
Figure 2. SEM pictures of atmospheric particles collected on the apron zone: an ELPI membrane of the collection stage 260–400 nm observed at a magnification of 45 000 (A), and an ELPI membrane of the collection stage 30–60 nm observed at a magnification of 250 000 (B).

Table 2. EBC particulate content[a].

Characteristics	Administrative	Apron
Subjects, n	209	244
Scattered intensity, Kcps	479 (117–7433)	441 (93–11093)
1st peak, nm	451 (98–1518)	468 (219–2888)
Subjects with 2nd peak, n (%)	25 (12)	18 (7)
2nd peak, nm	107 (41)	109 (29)

[a] Data are expressed as median (min–max).

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 $\log_{10}$(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.
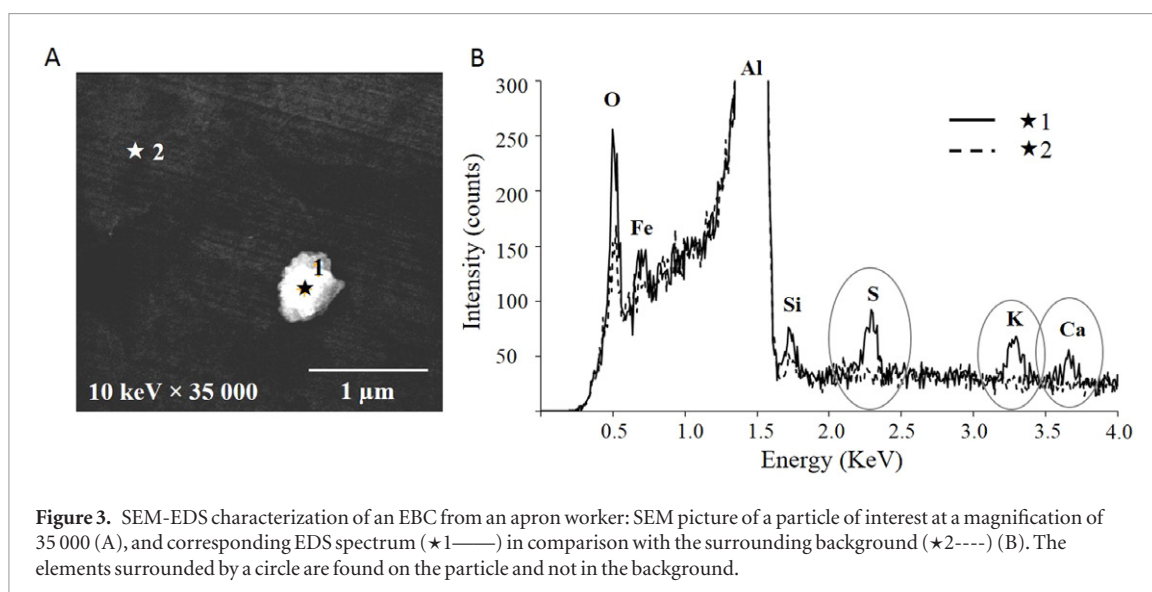
Figure 3. SEM-EDS characterization of an EBC from an apron worker: SEM picture of a particle of interest at a magnification of 35 000 (A), and corresponding EDS spectrum (★1——) in comparison with the surrounding background (★2----) (B). The elements surrounded by a circle are found on the particle and not in the background.

Table 3. Analytical limits of detection, positivity thresholds, and concentrations of Al, Cd, and Cr in EBC ($\mu g\, l^{-1}$).

Metals	LD[a]	PT[a]	Characteristics	Administrative	Apron
Al	0.09	6.0	Subjects, *n*	207	228
			Subjects $\geqslant$ PT, *n* (%)	47 (23)	38 (17)
			Median (min–max)	3.0 (3.0–131.7)	3.0 (3.0–34.9)
			Mean $\pm$ SD	5.9 $\pm$ 12.6	4.1 $\pm$ 3.3
Cd	0.003	0.060	Subjects, *n*	210	248
			Subjects $\geqslant$ PT, *n* (%)	27 (13)	75 (30)
			Median (min–max)	0.075 (0.075–0.720)	0.075 (0.075–4.52)[b]
			Mean $\pm$ SD	0.108 $\pm$ 0.106	0.174 $\pm$ 0.326
Cr	0.09	0.30	Subjects, *n*	210	248
			Subjects $\geqslant$ PT, *n* (%)	171 (81)	189 (76)
			Median (min–max)	0.57 (0.15–8.68)	0.52 (0.15–5.12)
			Mean $\pm$ SD	0.70 $\pm$ 0.76	0.64 0.61

[a] LD: analytical limit of detection, PT: positivity threshold.
[b] Significant elevation in apron workers in comparison with administrative workers ($p < 0.001$).

3.5. 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 ($\tau = 0.093$ and $\tau = 0.078$, respectively, $p < 0.05$). No correlation was found between metals and DLS scattered intensity.
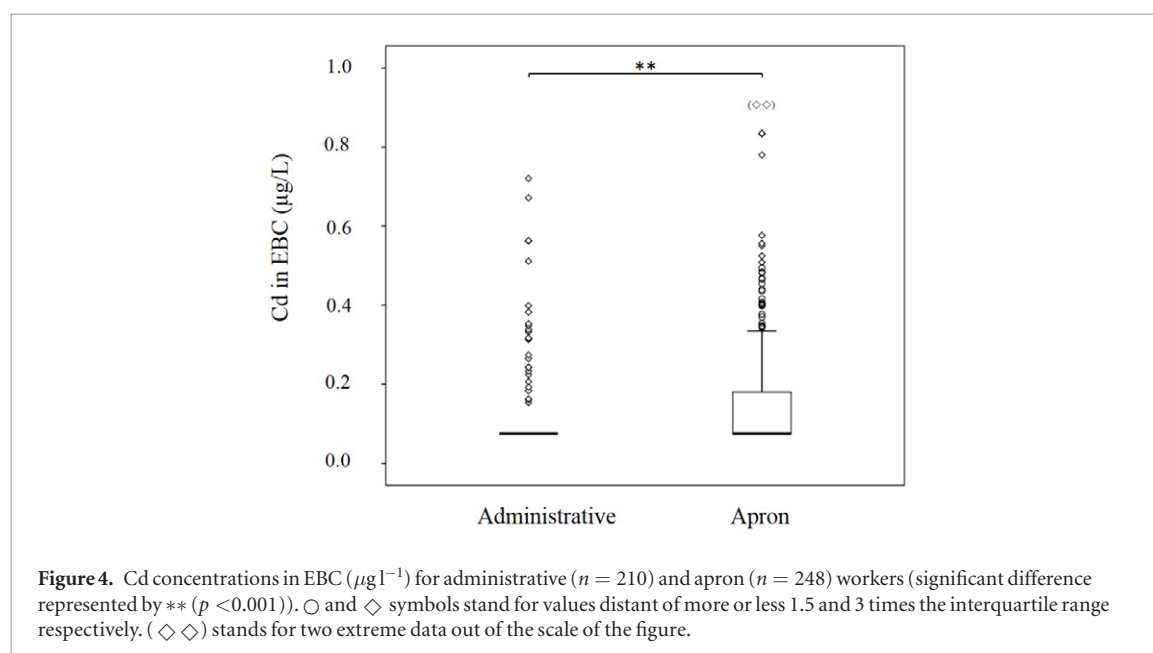
The comparison between administrative and apron workers showed no significant difference for Cr and Al concentrations, but a significantly higher concentration of Cd was found in apron workers in comparison with administrative workers ($p < 0.001$, figure 4).

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 10^4–10^5 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 4. Cd concentrations in EBC (μg l^{-1}) for administrative ($n = 210$) and apron ($n = 248$) workers (significant difference represented by ** ($p < 0.001$)). $\bigcirc$ and $\diamond$ symbols stand for values distant of more or less 1.5 and 3 times the interquartile range respectively. ($\diamond \diamond$) stands for two extreme data out of the scale of the figure.

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 asth-

matic 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$^-$ 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.

References

[1] Clancy L, Goodman P, Sinclair H and Dockery D W 2002 Effect of air-pollution control on death rates in Dublin, Ireland: an intervention study *Lancet* **360** 1210–4

[2] Kettunen J, Lanki T, Tiittanen P, Aalto P P, Koskentalo T, Kulmala M, Salomaa V and Pekkanen J 2007 Associations of fine and ultrafine particulate air pollution with stroke mortality in an area of low air pollution levels *Stroke* **38** 918–22

[3] Pope C A III, Thun M J, Namboodiri M M, Dockery D W, Evans J S, Speizer F E and Heath C W 1995 Particulate air pollution as a predictor of mortality in a prospective study of U.S. adults *Am. J. Respir. Crit. Care Med.* **151** 669–74

[4] Franck U, Odeh S, Wiedensohler A, Wehner B and Herbarth O 2011 The effect of particle size on cardiovascular disorders—the smaller the worse *Sci. Total Environ.* **409** 4217–21

[5] Schwartz J and Neas L M 2000 Fine particles are more strongly associated than coarse particles with acute respiratory health effects in schoolchildren *Epidemiology* **11** 6–10

[6] Stone V, Johnston H and Clift M J 2007 Air pollution, ultrafine and nanoparticle toxicology: cellular and molecular interactions *IEEE Trans. Nanobiosci.* **6** 331–40

[7] The Danish Ecocouncil 2012 Air pollution in airports *Publication No*. 990-120425 (available: http://ecocouncil.dk/documents/publikationer/990-120425-lufthavnshaefte/file (accessed: October 2015))

[8] Moller K L, Thygesen L C, Schipperijn J, Loft S, Bonde J P, Mikkelsen S and Brauer C 2014 Occupational exposure to ultrafine particles among airport employees—combining personal monitoring and global positioning system *PloS One* **9** e106671

[9] Touri L, Marchetti H, Sari-Minodier I, Molinari N and Chanez P 2013 The airport atmospheric environment: respiratory health at work *Eur. Respir. J.* **22** 124–30

[10] Nemmar A, Hoylaerts M F, Hoet P H, Vermylen J and Nemery B 2003 Size effect of intratracheally instilled particles on pulmonary inflammation and vascular thrombosis *Toxicol. Appl. Pharmacol.* **186** 38–45

[11] Oberdorster G, Oberdorster E and Oberdorster J 2005 Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles *Environ. Health Perspect.* **113** 823–39

[12] Tunnicliffe W S, O'Hickey S P, Fletcher T J, Miles J F, Burge P S and Ayres J G 1999 Pulmonary function and respiratory symptoms in a population of airport workers *Occup. Environ. Med.* **56** 118–23

[13] Mills N L *et al* 2006 Do inhaled carbon nanoparticles translocate directly into the circulation in humans? *Am. J. Respir. Crit. Care Med.* **173** 426–31

[14] Mutlu G M, Garey K W, Robbins R A, Danziger L H and Rubinstein I 2001 Collection and analysis of exhaled breath condensate in humans *Am. J. Respir. Crit. Care Med.* **164** 731–7

[15] Bredberg A, Gobom J, Almstrand A C, Larsson P, Blennow K, Olin A C and Mirgorodskaya E 2012 Exhaled endogenous particles contain lung proteins *Clin. Chem.* **58** 431–40

[16] Desvergne C, Dubosson M, Lacombe M, Brun V and Mossuz V 2015 Nanoparticle exposure biological monitoring: exposure/effect indicator development approaches *J. Phys.: Conf. Ser.* **617** 012005

[17] Fumagalli M *et al* 2012 Profiling the proteome of exhaled breath condensate in healthy smokers and COPD patients by LC-MS/MS *Int. J. Mol. Sci.* **13** 13894–910

[18] Sauvain J J, Hohl M S, Wild P, Pralong J A and Riediker M 2014 Exhaled breath condensate as a matrix for combustion-based nanoparticle exposure and health effect evaluation *J. Aerosol. Med. Pulm. Drug Deliv.* **27** 449–58

[19] Benor S, Alcalay Y, Domany K A, Gut G, Soferman R, Kivity S and Fireman E 2015 Ultrafine particle content in exhaled breath condensate in airways of asthmatic children *J. Breath Res.* **9** 026001

[20] Mutti A, Corradi M, Goldoni M, Vettori M V, Bernard A and Apostoli P 2006 Exhaled metallic elements and serum pneumoproteins in asymptomatic smokers and patients with COPD or asthma *Chest* **129** 1288–97

[21] Broding H C, Michalke B, Goen T and Drexler H 2009 Comparison between exhaled breath condensate analysis as a marker for cobalt and tungsten exposure and biological monitoring in workers of a hard metal alloy processing plant *Int. Arch. Occup. Environ. Health* **82** 565–73

[22] Caglieri A, Goldoni M, Acampa O, Andreoli R, Vettori M V, Corradi M, Apostoli P and Mutti A 2006 The effect of inhaled chromium on different exhaled breath condensate biomarkers among chrome-plating workers *Environ. Health Perspect.* **114** 542–6

[23] Goldoni M, Caglieri A, De Palma G, Acampa O, Gergelova P, Corradi M, Apostoli P and Mutti A 2010 Chromium in exhaled breath condensate (EBC), erythrocytes, plasam and urine in the biological monitoring of chrome-plating workers exposed to soluble Cr(VI) *J. Environ. Monit.* **12** 442–7

[24] Goldoni M, Catalani S, De Palma G, Manini P, Acampa O, Corradi M, Bergonzi R, Apostoli P and Mutti A 2004 Exhaled breath condensate as a suitable matrix to assess lung dose and effects in workers exposed to cobalt and tungsten *Environ. Health Perspect.* **112** 1293–8

[25] Zimmermann E, Derrough S, Locatelli D, Durand C, Fromaget J L, Lefranc E, Ravanel X and Garrione J 2012 Results of potential exposure assessments during the maintenance and cleanout of deposition equipment *J. Nanopart. Res.* **14** 1209

[26] Horvath I *et al* 2005 Exhaled breath condensate: methodological recommendations and unresolved questions *Eur. Respir. J.* **26** 523–48

[27] Kumar P, Morawska L, Birmili W, Paasonen P, Hu M, Kulmala M, Harrison R M, Norford L and Britter R 2014 Ultrafine particles in cities *Environ. Int.* **66** 1–10

[28] Kumar P, Robins A, Vardoulakis S and Britter R 2010 A review of the characteristics of nanoparticles in the urban atmosphere and the prospects for developing regulatory controls *Atmos. Environ.* **44** 5035–52

[29] Kumar P, Pirjola L, Ketzel M and Harrison R M 2013 Nanoparticle emissions from 11 non-vehicle exhaust sources—a review *Atmos. Environ.* **67** 252–77

[30] Edwards D A, Man J C, Brand P, Katstra J P, Sommerer K, Stone H A, Nardell E and Scheuch G 2004 Inhaling to mitigate exhaled bioaerosols *Proc. Natl Acad. Sci. USA* **101** 17383–8

[31] Corradi M, Acampa O, Goldoni M, Andreoli R, Milton D, Sama S R, Apostoli P and Mutti A 2009 Metallic elements in exhaled breath condensate and serum of patients with exacerbation of chronic obstructive pulmonary disease *Metallomics* **1** 339–45

[32] Fox J R, Spannhake E W, Macri K K, Torrey C M, Mihalic J N, Eftim S E, Lees P S and Geyh A S 2013 Characterization of a portable method for the collection of exhaled breath condensate and subsequent analysis of metal content *Environ. Sci. Process. Impacts* **15** 721–9

[33] Corradi M, Acampa O, Goldoni M, Adami E, Apostoli P, de Palma G, Pesci A and Mutti A 2009 Metallic elements in exhaled breath condensate of patients with interstitial lung diseases *J. Breath Res.* **3** 046003

[34] Goldoni M, Caglieri A, Corradi M, Poli D, Rusca M, Carbognani P and Mutti A 2008 Chromium in exhaled breath condensate and pulmonary tissue of non-small cell lung cancer patients *Int. Arch. Occup. Environ. Health* **81** 487–93

[35] Hoffmeyer F *et al* 2011 Increased metal concentrations in exhaled breath condensate of industrial welders *J. Envion. Monit.* **13** 212–8

[36] Hoffmeyer F *et al* 2012 Impact of different welding techniques on biological effect markers in exhaled breath condensate of 58 mild steel welders *J. Toxicol. Environ. Health* A **75** 525–32

[37] Edme J L *et al* 2008 Cytokine concentrations in exhaled breath condensates in systemic sclerosis *Inflammation Res.* **57** 151–6

[38] Effros R M, Biller J, Foss B, Hoagland K, Dunning M B, Castillo D, Bosbous M, Sun F and Shaker R 2003 A simple method for estimating respiratory solute dilution in exhaled breath condensates *Am. J. Respir. Crit. Care Med.* **168** 1500–5

[39] Gessner C, Kuhn H, Seyfarth H J, Pankau H, Winkler J, Schauer J and Wirtz H 2001 Factors influencing breath condensate volume *Pneumologie* **55** 414–9

[40] Garey K W, Neuhauser M M, Robbins R A, Danziger L H and Rubinstein I 2004 Markers of inflammation in exhaled breath condensate of young healthy smokers *Chest* **125** 22–6