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RESEARCH ARTICLE

Development and set-up of a portable device to monitor airway exhalation and deposition of particulate matter

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Abstract

The aim of this study was to assess and monitor airway exhalation and deposition of particulate matter (PM). After standardizing inspiratory/expiratory flow and volumes, a novel device was tested on a group of 20 volunteers and in a field study on workers exposed to cristobalite. Both male and female subjects showed a higher percentage of deposition in the 0.5 µm channel than in the 0.3 µm channel on a laser particle counter, but it was higher in the males because of their higher exhaled lung volumes. The device was tested on a wider range of particles (0.3–0.5–1.0–2.5 µm) in the cristobalite productive division. The device has low intrasubject variability and good reproducibility, with geometric mean of %CV < 5%. Such a measure can be used to assess individual susceptibility to PM, making repeated measures in different environments, and examining the persistence of particles in the airways after a period in polluted environments.

Keywords: *Particulate matter; airway deposition; particle exhalation; susceptibility; vital capacity*

Introduction

Airborne particles are generated by primary emissions of anthropogenic sources (e.g. combustion and industrial processes) and natural sources (the erosion of soil and rock, natural disasters), but may also be secondary pollutants due to chemical reactions between primary or other secondary pollutants in the atmosphere (Franklin & Schwartz 2008, Harrison 2004). They can include carbon and silica compounds, metals, and other organic and inorganic compounds, some of which are known toxicants and carcinogens (Falta et al. 2008, Harrison 2004).

Experimental and epidemiological evidence shows that both chronic and acute exposure to particulate matter (PM) is associated with adverse health effects, particularly at the pulmonary level (lung function deterioration, chronic obstructive pulmonary disease, allergies and asthma) (Alfaro-Moreno et al. 2007, Churg & Wright 2003, Donaldson & MacNee 2001, Downs et al. 2007, Hart et al. 2006, Lippmann 2007, McCreanor et al. 2007), children being the most sensitive group of the general population (Delfino et al. 2008, Min et al. 2008, Oftedal et al. 2008, Salvi 2007). The pulmonary effects of acute exposure to very high PM concentration have been dramatically underlined by studies on the operators involved in the World Trade Center disaster (Feldman et al. 2004, Fireman et al. 2004, Herbert et al. 2006, Prezant et al. 2002). Exposure to PM has also been associated with cardiovascular effects and increased general morbidity and mortality (Eftim et al. 2008, Frampton 2001, 2007, Gordon 2007, Kappos

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et al. 2004, Lewtas 2007). Both the pulmonary and cardiovascular effects are at least partially mediated by inflammation (Frampton 2006, Scapellato & Lotti 2007).

PM mainly enters the body by means of inhalation and is deposited in different parts of the respiratory system depending on its chemical and physical properties: PM with an aerodynamic diameter < 10 μm (PM_{10}) can be inhaled and is retained in the airways, whereas those with an aerodynamic diameter < 2.5 μm ($PM_{2.5}$, fine particles) can penetrate deeper into the lower respiratory tract and reach the alveolar region (Churg & Brauer 2000). PM with an aerodynamic diameter between 2.5 and 10.0 μm are defined as coarse particles. A number of recent studies of ultrafine particles (UP) or nanoparticles, which have an aerodynamic diameter < 0.1 μm, have shown that they can efficiently deposit by diffusion throughout the airways, but do so particularly at the pulmonary level (Heyder 2004, Oberdorster et al. 2005b). Nanoparticle toxicity (inflammation, oxidative stress) has been related to their large active surface area (e.g. the surface at direct contact with cells and tissues) more than to their mass (Oberdorster et al. 2005a).

Various models have been designed to take into account bronchial bifurcations and pulmonary asymmetry using models of aerosols with spherical particles. Almost all authors agree that particles whose aerodynamic diameter is between 0.1 and 1 μm (which have low levels of diffusion and sedimentation efficiency) reach the lower respiratory tract but their deposition is low compared with particles with lower and higher aerodynamic diameter (Broday & Agnon 2007, Farkas & Balashazy 2008, Goo & Kim 2003, Heyder 2004, Li et al. 2007, Martonen et al. 2000, Mitsakou et al. 2005, Park & Wexler 2007, 2008, Zhang et al. 2001). Regional models have indicated that particles whose aerodynamic diameter is from 1 μm to 4 μm show relatively high levels of deposition in the lower respiratory tract due to impaction phenomena (Heyder 2004, Salma et al. 2002a), but regional pulmonary deposition is also determined by the biophysical parameters of the aero-dispersed particles (their active surface, density, hygroscopicity, shape, electrical charge, hydrophilicity, etc.), and individual differences in breathing cycle and respiratory morphology (Asgharian 2004, Bailey 1997, Crowder et al. 2002, Hofmann et al. 2002, Salma et al. 2002b, Scheuch & Siekmeier 2007). Therefore, theoretical models extrapolated using aerosol models do not always fit experimental data coming from real airborne PM, such as environmental tobacco smoke and vehicle emissions (Hofmann et al. 1999, Morawska et al. 1999, 2005). Furthermore, as it has been shown that the deposition of respirable particles is significantly higher in patients with pulmonary pathologies than in healthy non-smoking controls, such patients may be at greater risk after PM exposure (Kim 2000, Luo et al. 2007, Segal et al. 2002).

There are various experimental systems designed to measure the on-line concentration of exhaled particles during tidal breathing using aerosol models and airborne particles, and mathematical models designed to extrapolate the fraction of deposited particles in the airways while taking into account temporal variations in particle concentration due to changes in inspiratory/expiratory flow and volume (Kim 2000, Kim & Jaques 2000, Londahl et al. 2006, Montoya et al. 2004, Morawska et al. 2005). In an attempt to reduce intra- and intersubject variability, and to standardize the measure, Invernizzi et al. recently proposed a new method based on a single-breath exhalation at a relatively low and constant flow-rate (Invernizzi et al. 2006). In addition to fixed inhaled/exhaled air volume, we considered the effect of relatively high volumes approaching personal forced vital capacity (FVC).

In order to make the study feasible, we developed a transportable device for future field studies. In this article, we report the results of a calibration in controls inhaling environmental PM, measured using a commercially available particle counter with different size channels. A subsequent application in a small field study on workers occupationally exposed to cristobalite – used to decorate ceramic tiles at high temperatures – is also reported. Cristobalite is one of the three crystalline forms of silica (SiO_2) at atmospheric pressure, and its silicotigen properties are well known (Holland 1990, Saffiotti 1998). Furthermore, the International Agency for Research on Cancer (IARC) has concluded that crystalline silica, in the form of quartz or cristobalite, poses a carcinogenic risk to humans (Group 1) (Warheit 2001).

Methods

On-line data collection

The device developed in our laboratory is characterized by the use of a commercially available multichannel particle counter to monitor the time-course of particle concentration during a single expiration, with the possibility of using available mathematical models to extrapolate the percentage of particle deposition (%DEP) in the airways. It can be easily transported and maximizes the use of disposable components. The signal is highly stable and repeatable, as a result of simple standardization. Finally, it can operate under different respiratory conditions.

The device is illustrated in Figure 1. The subjects breath inside a pneumotachometer (Flowhandy ZAN100 USB, ZAN, Oberthulba, Germany) that can monitor both inhalation and exhalation: a one-way valve located immediately after the pneumotachometer permits the

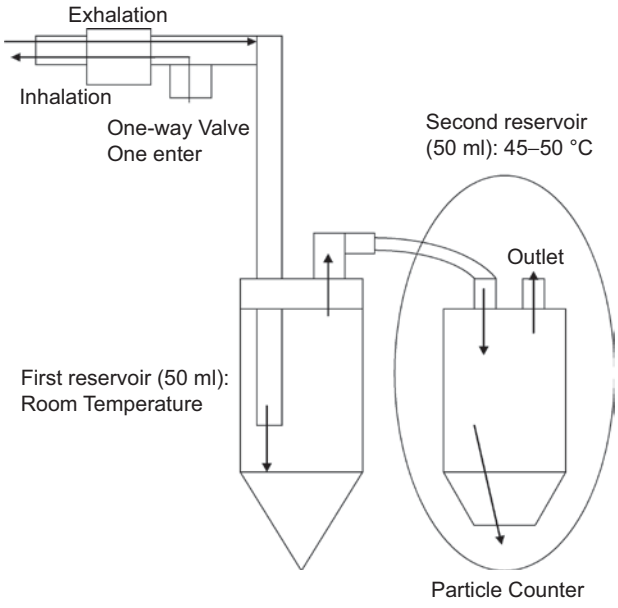

Figure 1. Schematic illustration of the device.

monitoring of the flow/volume of inhaled air and, in addition to providing the trend of inspiratory/expiratory flow as a function of total inhaled/exhaled volume, and the trend of total volume over time visualized as a default, the dedicated software automatically saves a .txt file with the trend of flow over time. The pneumotachometer is connected to a first reservoir of 50 ml that is kept at room temperature by means of a DECCS® tube system (Medivac, Parma, Italy). An L-shaped tube with an internal diameter of 0.8 cm and about 2–3 cm of flexible plastic tube (0.3–0.5 cm i.d.) connect the first reservoir to a second 50 ml reservoir which is kept at 50°C by means of a modified TURBO system (Medivac) that uses an inverted Peltier effect to heat the air (Caglieri et al. 2006). The second reservoir has a pressure outlet in the upper cap and the particle counter is connected to the reservoir below the TURBO system.

The laser particle counter used to set up the device (Handeld 3016; Lighthouse, Fremont, CA, USA) has six channels (0.3–0.5–1.0–2.5–5.0–10.0 μm), and detects the number and the concentration of particles over time at a flow-rate of 2.83 l min^{-1} (0.0472 l s^{-1}). The range of particles monitored by each of the channels are: (1) 0.3 μm: 0.3 μm ≤ aerodynamic diameter < 0.5 μm; (2) 0.5 μm: 0.5 μm ≤ aerodynamic diameter < 1.0 μm; (3) 1.0 μm: 1.0 μm ≤ aerodynamic diameter < 2.5 μm; (4) 2.5 μm: 2.5 μm ≤ aerodynamic diameter < 5.0 μm; (5) 5.0 μm: 5.0 μm ≤ aerodynamic diameter < 10.0 μm; and (6) 10.0 μm: aerodynamic diameter ≥ 10.0 μm.

Given the quality of air in the laboratory in which the device was set up, sampling points were recorded every 2 s (the lowest time limit is one every 1 s) as a compromise between the need to obtain a sufficiently stable and quantifiable signal for the 0.3 and 0.5 μm channels of the counter while still having multiple points during the kinetics of exhalation. The small number of detected particles meant that the other channels could not be used with sufficient stability, but it was not the case in the environment polluted by cristobalite dust (see below). The difference in the concentrations of environmental particles measured with and without the device was less than 10% in both channels (data not shown), indicating that the particles could freely flow inside it.

The relative humidity (RH) and temperature of the exhaled air measured at the entrance of particle counter were less than 90% and 32–35°C, respectively, and were therefore compatible with the counter's range.

Contamination of the system by exhaled breath

Except for the membrane inside the pneumotachometer, all parts of the device are disposable. Changing and sterilizing the pneumotachometer membrane is essential when monitoring both inhalation and exhalation, and highly recommended when monitoring only exhalation (see below). Commercially available spirometry filters could not be used because of their impact on the concentration of particles reaching the device. In fact, the proportions of PM passing the filter cut-off and reaching the counter were respectively 0% for 5 μm, 13% for 2.5 μm, 28.9% for 1.0 μm, 49.1% for 0.5 μm and 54.2% for 0.3 μm.

Mathematical models for calculating percentage deposition

Kim equations (Kim 2000) were used to calculate the percentage of inhaled particles that deposited in the airways (%DEP) (see Appendix).

Standardization of exhalation signal

In addition to standardizing inhalation (see Results), the exhalation signal had to be aligned with exhalation flow because particle concentrations vary over time. The expected time to fill up all the available 150 ml volume of the device is about 1 s at a flow-rate of 0.15 l s^{-1}. As a result, the flow signal and time-related variation in particle concentration are usually shifted by 0.5–1.5 s depending on the expiration flow and the delay in starting both the pneumotachometer and particle counter. The signals are realigned after the collection, bearing in mind that the lowest concentration of particles is measured almost at the end of the expiration.

As the device contains traces of the last portion of exhaled air (50–70 ml) with a lower particle concentration than that measured in the environment, the system needs to be normalized with environmental air at every measurement by starting the particle counter and

closing the pressure outlet for few seconds, until equilibrium in particle concentration is reached. This system (which can be automated) has an experimental error due to the phasing and collection procedure of less than 5% on the final deposition signal. It is possible to reduce the aligning error by collecting one point every second if the environmental PM concentration is sufficiently high.

Subjects

Standardization of inspiration and total inhaled/exhaled volume (part 1)

Ten healthy volunteers (five men, one current smoker, i.e. a subgroup of the subjects presented in Table 1) were asked to repeat three measures, after adequate training: (1) inhalation at relatively low peak flow (< 0.7 l s^{-1}) over a relatively long time, with total inhaled/exhaled volume of about 60–90% of their FVC and slow exhalation (0.13–0.25 l s^{-1}); (2) inhalation at relatively high peak flow (> 1.0 l s^{-1}) in a relatively short time, with similar total inhaled/exhaled volume and exhalation flow; (3) inhalation at intermediate flow (0.5–1.2 l s^{-1}), with a total exhaled volume of about 2 l (regardless of FVC) and similar exhalation flow. All the subjects were tested inside one of our Department's offices in Parma (Italy) on three consecutive days in winter during which the environmental particle concentrations were 85 000–125 000 p l^{-1} in the 0.3 µm channel and 8500–12 500 p l^{-1} in the 0.5 µm channel, the temperature was at 25 ± 1°C and RH 40–45%.

Standardization of exhalation flow and repeatability (part 2)

To study the main characteristics of the device, one subject was examined 15 times at different exhalation flow rates and three subjects were measured ten times at a constant flow rate. Given the results of part 1, inhalation was not monitored, but the subjects were asked to inhale deeply through the mouth for no more than 3 s outside the system, and then to exhale into the device in no more than 2 s because the study of Invernizzi et al. (2006) showed that the time of air retention can affect particle deposition. The measurements were made in the same way as those in part 1 over 2–3 h of the same day.

Validation of the signal and effect of inhaled/exhaled volume (part 3)

The characteristics of the 20 volunteers enrolled to validate the device are shown in Table 1: three subjects reported asthma episodes, despite their normal spirometry values; the other did not have any pulmonary symptoms or a history of lung disease. Given the results of part 1, inhalation was not monitored but subjects were asked to inhale deeply through the mouth for no more than 3 s outside the system, and to exhale inside the device at a relatively low flow-rate (0.13–0.25 l s^{-1}) in no more than 2 s. Preliminary measures showed that inspiration through the nose did not significantly change particle concentration over time (data not shown). Two repeated measurements were made for every subject in a period of 10 min in the same office as that used for parts 1 and 2 during one winter month in which environmental particle concentrations varied daily depending on the weather, the temperature was 24.5 ± 2°C and RH 40–55%.

Cristobalite workers (part 4)

The characteristics of the subjects enrolled are shown in Table 2. One control subject reported asthma episodes, despite normal spirometry values, and one test group worker was asthmatic (forced expiratory volume in 1 s, $FEV_1 = 59\%$), while another reported chronic rhinitis of uncertain origin, but had normal spirometry values. Each subject performed only one test as described above for the part 3 inside a polluted environment near the oven where tiles were baked (about 2 m). The worker test group consisted of workers who spent their working shift inside this environment tested in this experiment; the control group consisted of other workers from different departments (4/8 from the Administrative Division). A number of 3-min environmental samplings were made outdoors, in the administrative office, and near the site inside the Production Division, where the subjects were tested.

Table 1. Characteristics of the subjects enrolled for device validation. Mean (SD) is reported.

n	20
Sex (M/F)	10/10
Age (years)	28.2 (SD 3.3)
Smokers/ex-smokers/not smokers	6/0/14
Pack per year (smokers)	5.2 (SD 2.9)
FVC (l)	4.5 (SD 0.9)
FVC (%pred)	105.0 (SD 11.0)
FEV_1 (l)	3.8 (SD 0.7)
FEV_1 (%pred)	102.5 (SD 8.0)

FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s.

Table 2. Characteristics of the subjects enrolled in the industry. Mean (SD) is reported.

	Controls	Workers
n	8	8
Sex (M/F)	6/2	8/0
Age (years)	37.3 (SD 8.5)	35.1 (SD 7.4)
Smokers/ex-smokers/not-smokers	1/0/7	4/0/4
Pack per year (smokers)	2.5	9.2 (SD 6.0)
FVC (l)	4.7 (SD 0.9)	4.2 (SD 0.6)
FVC (%pred)	107.6 (SD 11.2)	95.1 (SD 9.9)
FEV_1 (l)	3.6 (SD 0.7)	3.5 (SD 0.7)
FEV_1 (%pred)	99.3 (SD 8.0)	90.6 (SD 15.9)

FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s.

None of the subjects enrolled in parts 1–4 smoked any cigarettes during the hour before measurement, and they were also asked to not eat or drink in the 20 min before measurement. All the subjects gave their informed written consent; the study was approved by local Ethics Committee and was conducted in conformity with the Declaration of Helsinki.

Statistical analysis

The percentage coefficient of variation (%CV) of deposition was calculated in a logarithmic scale (log-normal distribution) as a function of the average %DEP calculated using the two repeated measures in the 0.3 and 0.5 μm channels.

Owing to their non-significant deviation from normality (as assessed by the Shapiro–Wilk test), the variables are reported as mean values (SD) and were only analysed by means of parametric statistics. However, log(GSD) was also calculated for the variability of %DEP in order to allow comparisons with the literature. Between-group differences were tested using an independent or paired Student *t*-test. Mixed-effects ANOVA/ANCOVAs were used to test the differences between two or more repeated measures taking into account independent factors and covariates, and their interactions. When a factor had more than two groups, the Bonferroni's *post-hoc* test for multiple comparisons was applied. Pearson's squared correlation coefficient (R^2) was used to assess the correlations between variables. Generalized estimating equations taking into account repeated measures were used to test the effects of exhaled volume, expiratory flow rate and environmental particle concentration on %DEP. The statistical analyses were carried out using SPSS 16.0 (SPSS Inc., Chicago, IL, USA); $p < 0.05$ was considered significant.

Results

Standardization of inspiration and total inhaled/exhaled volume

The following parameters characterized three subsequent measurements (mean (SD)): (1) slow inhalation peak flow: 0.56 (0.08) l s^{-1}; inhalation time: 7.7 (2.0) s; total exhaled volume: 3.63 (0.68) l; average exhalation flow: 0.189 (0.029) l s^{-1}; (2) fast inhalation peak flow: 1.69 (0.52) l s^{-1}; inhalation time: 2.9 (0.7) s; total exhaled volume: 3.78 (0.72) l; average exhalation flow: 0.190 (0.028) l s^{-1}; (3) 2 l fixed volume inhalation/exhalation, peak flow: 0.82 (0.20) l s^{-1} for inhalation; inhalation time: 3.0 (0.7) s; total exhaled volume: 2.09 (0.10) l; average exhalation flow: 0.189 (0.028) l s^{-1}.

The differences between the total measured inhaled and exhaled volumes were < 0.2 l in the case of (1) and (2), and < 0.1 l in the case of (3). Total exhaled volume was not significantly different between (1) and (2), and average exhalation flow was not significantly different in all cases. Finally, (1) and (2) showed highly significant differences in inhalation peak flow and inhalation time ($p < 0.001$, repeated measures).

Figure 2 shows the trend of %DEP in the three experiments (1), (2) and (3), respectively. Results from both the 0.3 and the 0.5 μm channels are shown. All differences were significant. In particular, an average increase of 1.1 l s^{-1} in inhalation flow decreased %DEP by 3.4% (95% CI 2.0–4.8%) in the 0.3 μm channel ($p < 0.01$) and by 2.2% (0.6–5.0%) in the 0.5 μm channel ($p = 0.013$). Moreover, the sampling of 2 l greatly reduced %DEP in both channels. Comparing these values with those expected on the basis of the International Commission on Radiological Protection model (ICRP 2002), there was a moderately higher %DEP in comparison with mouth breath, but not nose breath: experimental 30.3% (SD 8.9%) vs 23–24% (0.2–0.5 μm, mouth) expected and 30–35% (nose) in the 0.3 μm channel; 44.7% (SD 10.1%) vs 24–28–34%, respectively, (for 0.5–0.7–1.0 μm, mouth) expected and 35–42–51% (nose) in the 0.5 μm channel. Reported and expected overall airway %DEP of 0.2, 0.5, 0.7, 1.0 μm diameter particles on the basis of the ICRP model, which relates to a reference nose and mouth breathing worker with a respiratory rate of 1.2 m^3 h^{-1} (heavy work gives similar results), assumes (1) log-normal particle size distribution, (2) density 3 g cm^{-3}, (3) shape factor $\chi = 1.5$, for either compact or irregular, but non-spherical particles (ICRP 2002).

Finally, log(GSD) for the 0.3 and 0.5 μm channels was, respectively: (1) 0.109–0.083, (2) 0.117–0.090, and (3) 0.139–0.104. On the basis of these results, we stopped monitoring inspiration flow and volume as a deep

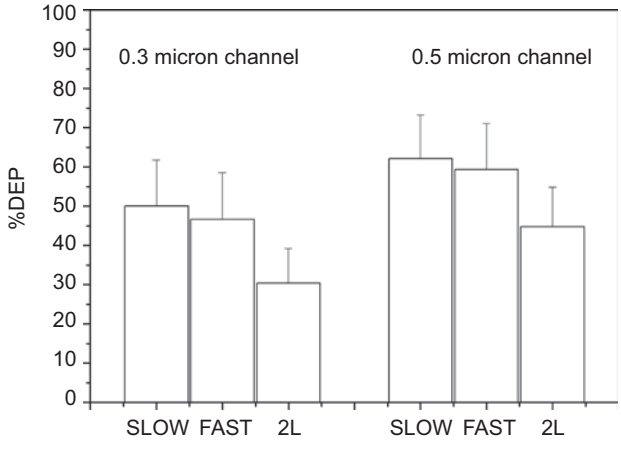

Figure 2. Percentage of particle deposition (%DEP) in the 0.3 μm channel and 0.5 μm channel in the three experiments. SLOW, slow inhalation flow and high volume (part 1); FAST, fast inhalation flow and high volume (part 2); 2L, moderate inhalation flow and about 2 l of volume (part 3). Mean and SD are reported.

inspiration of about 3 s outside the system should ensure similar conditions to those of (2) with minimal effects on %DEP.

Standardization of exhalation flow and repeatability

When the same subject repeated the measurements 15 times at different expiratory flow rates, %DEP was only slightly affected by flow rates between 0.13 and 0.25 l s^{-1} (Figure 3); the percentage decrease in the deposition fraction compared with the theoretical curve ranged from 59.0 to 55.5%, but the decrease showed a higher slope at flow rates > 0.25 l s^{-1}. Similar results were obtained from two additional subjects (data not shown).

The same subject was tested ten times using the 0.3–0.5 µm channels, variable flow rates between 0.15 and 0.19 l s^{-1} and a repeatable total exhaled volume of 4.2–4.6 l. In our office, the mean particle concentration (SD) was 75 000 (SD 5000) p l^{-1} in the 0.3 µm channel and 4500 (SD 500) p l^{-1} in the 0.5 µm channel; the PM concentrations in the other channels were too low to allow an estimate of %DEP. On the basis of the mathematical treatment of the signal, %DEP was 55.0% (SD 2.4%) in the 0.3 µm channel and 61.5% (SD 3.0%) in the 0.5 µm channel; the %CV was therefore, respectively, 4.4% and 5.0%.

Validation of the signal and effect of inhaled/exhaled volume

The mean (SD, min–max) concentration of airborne particles when the subjects did their test was 62 500 (38 000, 15 200–123 000) p l^{-1} in the 0.3 µm channel and 5600 (3500, 1700–13 800) p l^{-1} in the 0.5 µm channel, with an average environmental variability between the two repeated measures of, respectively, 1.7% (max 5.2%) and 2.4% (max 10.9%). For the 0.3 µm channel, the GM of %CV was 2.4% (GSD 2.6) with a maximum %CV of 11.6%; the corresponding figures for the 0.5 µm channel were 2.0% (GSD 3.1) and 9.8%.

The mean (SD) expiration flow rate was 0.200 (0.016) l s^{-1} for men and 0.172 (0.028) l s^{-1} for women, the difference due to gender being significant ($p = 0.02$). The average SD between the two repeated measures was 0.021 l s^{-1} with a maximum of 0.058 l s^{-1}; the overall range of flow rates was 0.137–0.218 l s^{-1}. The total exhaled volume was 4.20 (0.62) l for men and 3.03 (0.38) l for women, and the difference was significant ($p < 0.001$).

Figure 4 shows the trend of %DEP as a function of the channel (0.3–0.5 µm) stratified by gender. The overall difference between male and female subjects was significant (men higher than women, $p = 0.03$), and mixed-effects ANOVA revealed that the two measurements were significantly different ($p < 0.01$); however, the interaction between gender and channel was not significant ($p = 0.15$). The variation in %DEP was parallel in male and female subjects as shown in Figure 4. The subjects who reported asthma episodes (male and female) tended to have a high %DEP. When total exhaled volume was used as a covariate, the gender effect was no longer significant.

When the general estimating equation test was performed with the two repeated measures for every subject as subject variable, a linear scale response, and exhaled volume, environmental particle concentration and expiratory flow-rate as predictors, and maximum likelihood estimate as scale parameter method, Wald χ^2 was highly significant for exhaled volume ($\chi^2 = 11.0$, $p = 0.001$) but not for environmental particles ($\chi^2 = 1.7$, $p = 0.19$) and expiratory flow-rate ($\chi^2 = 1.9$, $p = 0.16$) for the 0.3 µm channel; moreover, a decrease of 1 l in exhaled volume

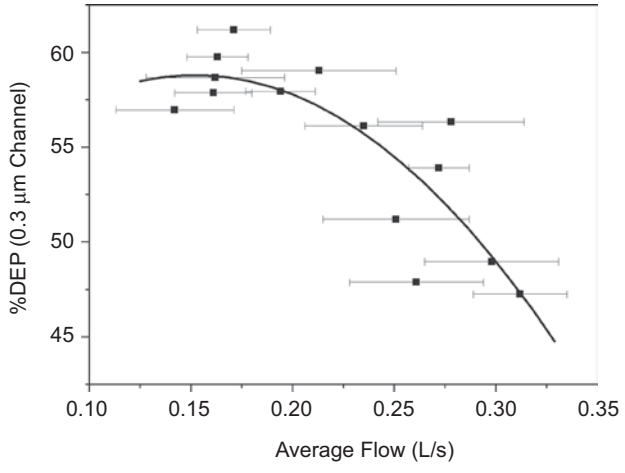

Figure 3. Percentage of particle deposition (%DEP) in the 0.3 µm channel as a function of exhalation flow (mean values and SD during the exhalation), with the best fitting curve.

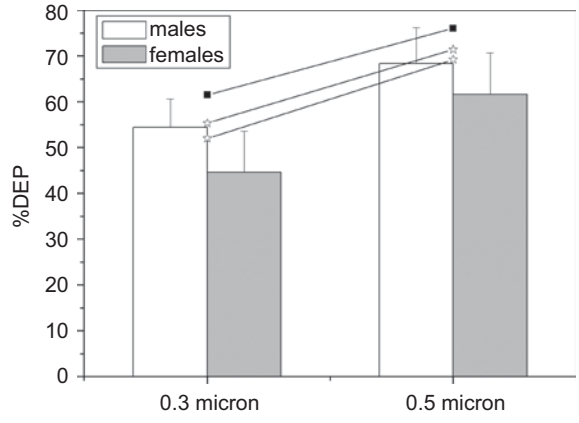

Figure 4. Percentage of particle deposition (%DEP) as a function of the considered channel in male and female subjects. Mean and SD are reported. ■, Male subjects with asthma episodes; ☆, female subjects with asthma episodes.

decreased average %DEP by 7.7% (95% CI 3.9–12.5%). With regard to the 0.5 µm channel, Wald χ^2 was again highly significant for exhaled volume ($\chi^2 = 5.1$, $p < 0.05$) but not for environmental particles ($\chi^2 = 2.2$, $p = 0.14$) and expiratory flow rate ($\chi^2 = 1.6$, $p = 0.21$); a decrease of 1 l in exhaled volume decreased average %DEP by 6.8% (95% CI 0.6–13.0%). Age did not correlate with %DEP in either channel, and was therefore not considered in the analysis.

Log(GSD) for the 0.3 and 0.5 µm channel was, respectively, 0.060–0.090 for men, 0.090–0.070 for women and 0.085–0.072 for the subjects as a whole.

Cristobalite workers

Figure 5a shows the PM concentrations in the 0.3–0.5–1.0–2.5–5.0 µm channels measured inside the production division at three different times: (1) at the beginning of sampling with the oven open; (2) after 1 h when the oven was definitively closed; and (3) at the end of the sampling, as well as those outdoors and in the office. The concentrations in the 1.0–2.5–5.0 µm channels were higher in the production area at all the sampling times, and also in the 0.5 µm channel at time (1). Indoor particle concentration in the 0.3 µm channel was always lower than the outdoor particle concentration, but at time (1) the difference was less than 10%. Figure 5b shows the concentrations of airborne particles in the 0.3–0.5–1.0–2.5–5.0 µm channels during sampling of the subjects: it can be seen that closing the oven greatly decreased the concentration of the smallest particles, whereas the decrease in all of the other channels was more gradual and reached a plateau after about 2 h.

Average expiratory flow was 0.207 (SD 0.029) l s^{-1} with no difference between the exposed workers and controls, the average expired volume being 3.50 (SD 0.40) l.

Table 3 shows the trend of %DEP as a function of the channel cut-off values in the subgroups of workers exposed to cristobalite and controls (only men). Mixed effects ANOVA indicated no significant differences between the two groups ($p = 0.69$), whereas the channels were always significantly different in pairs ($p < 0.001$ for all comparisons), %DEP increasing according to the aerodynamic diameter of PM. The work–channel interaction was also not significant ($p = 0.70$), thus indicating that the two groups had the same trend across the channels. However, the subjects who reported asthma, asthmatic episodes or rhinitis had higher %DEP values, particularly in the 0.3 and 0.5 µm channels. No data are given for the 5.0 µm channel because the concentration was too low and the signal was not reproducible. The use of exhaled volume as a covariate did not significantly change the results.

Exhaled volume and vital capacity

Considering all the study subjects as a whole (controls and cristobalite workers), total exhaled volume (mean volume in the case of repeated measures) closely

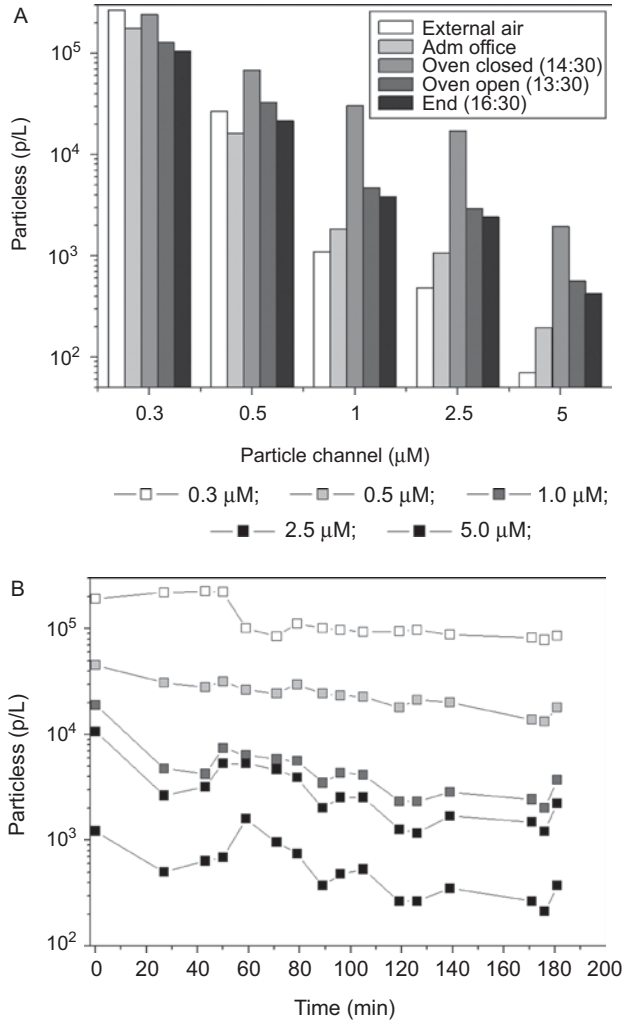

Figure 5. (A) Airborne outdoor particles in different divisions of the company in the 0.3–0.5–1.0–2.5–5.0 µm channels at the different time of sampling. Every concentration is the mean of two repeated measures of 3-min sampling each; (B) airborne particle concentration on the same channels during subject sampling. Every concentration is a 2 s punctual value immediately before each subject performed his exhalation inside the device.

Table 3. Percentage of particle deposition (%DEP) in the 0.3–0.5–1.0–2.5 µm channels for controls and workers. The subjects who reported asthma had %DEP of 65.6 and 67.2% in the 0.3 µm channel, while the subjects with rhinitis had %DEP 70.0% in the 0.3 µm channel and 84.6% in the 0.5 µm channel.

Channel	Controls (%DEP)	Workers (%DEP)
0.3 µm	57.8 (SD 7.2)	58.7 (SD 8.3)
0.5 µm	71.4 (SD 7.8)	68.9 (SD 11.0)
1.0 µm	78.8 (SD 6.0)	76.5 (SD 8.6)
2.5 µm	90.3 (SD 5.0)	88.2 (SD 4.8)

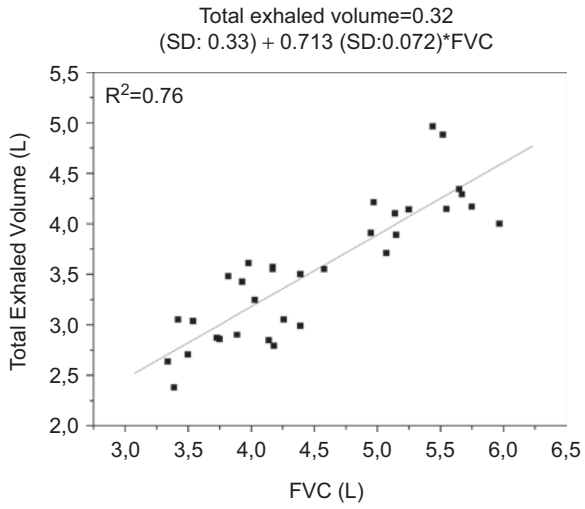

Figure 6. Regression model with forced vital capacity (FVC) as predictor and total exhaled volume as outcome, considering all the subjects enrolled in this study. Best fit straight line and best fitting function with the R^2 value are also reported.

correlated with FVC ($R^2 = 0.76$, EV = 0.32 (SD 0.33) + 0.71 (SD 0.07)*FVC, Figure 6), thus indicating that exhaled volume appropriately took into account the subjects' anthropometric differences.

Discussion

For the purposes of this study, the device described by Invernizzi et al. (2006) was modified in order to develop a system suitable for field studies, which we believe can add new information concerning the relationships between particle deposition, particle composition and airway susceptibility to airborne PM concentration under actual working conditions. The use of high inspiration/expiration volumes takes into account interindividual anthropometric differences. The device shown in Figure 1 is prevalently composed of disposable plastic parts, has a total volume of about 150 ml, and has been designed to reduce the RH of exhaled air (nearly 99% at the mouth) at the entrance to the particle counter. At room temperature, the first reservoir (50 ml) slightly condensates the water vapour upon collection, which means that a large fraction of non-volatile substances contained in exhaled breath should be entrapped. However, its structure is designed to keep exhaled particles in turbulent motion by the exhaled flow. As the total device had only a slight effect (< 10%) on total particles in all of the channels present in the atmosphere, PM could easily pass with minimal loss.

A narrow-calibre tube is used at the entrance to the second reservoir because it exerts a slight resistance, thus allowing a relatively slow expiration throughout exhalation. The subjects could therefore exhale air at a flow-rate of approximately 0.13–0.25 l s^{-1} without any effort or particular guidance. As the second reservoir (50 ml) is heated at 50°C, exhaled air enters the particle counter with RH < 90% and at 32–35°C, and does not interfere with the correct functioning of the particle counter itself. Although particle motion inside the second reservoir is not laminar, because of the asymmetry between the inlet to the second reservoir and its outlet to the particle counter (the calibre changes from 0.3–0. 5 cm to 3 cm), the flow of exhaled air entering the particle counter through the second reservoir is almost parallel to that of the particle counter itself. The main advantage of a parallel flow is that it allows the normalization of PM concentration inside the reservoir in about 0. 3 s at a flow of 0.15 l s^{-1}, whereas a normalization time of more than 1 s is necessary if the flow is perpendicular to the particle counter as it only depends on the pump flow-rate. In preliminary studies, the signals collected using parallel and perpendicular flows were slightly different, particularly at the start of the curve (a perpendicular flow was less sensitive to rapid changes in particle concentration), but the %DEP overlapped (data not shown). Given the greater velocity of the exhaled air in the small-calibre tube (7.5 km h^{-1}) and reservoir (< 1 km h^{-1}), the parallel flow did not alter the correct functioning of the particle counter itself.

Studies of inspiration and total inhaled/exhaled volumes confirmed a possible use at different inhalation flows and total exchanged volumes, thus including variable volumes approaching the FVC and fixed volume (2 l). Given the limited sensitivity of commercially available particle counters (one point every second is the shortest detection time) and the dead volume of the device, we suggest volumes of no less than 1.5–2 l. Tidal respiratory cycles require more sophisticated devices that work through several consecutive respiratory cycles and can also give information at fixed aerodynamic diameters (see, for example, Londahl et al. 2006).

The first important result was the finding that, at high volumes (> 3 l, near FVC), the %DEP of airborne particles with aerodynamic diameters of between 0.3 and 1.0 μm was greater than that predicted by the ICRP models (ICRP 2002), as was expected because when the airways are filled to near maximum vital capacity, particles penetration is greater. Inhalation flow had a modest but significant effect on final %DEP (0.6 vs 1.7 l s^{-1}). On the other hand, these results were consistent with those of the study of Invernizzi et al. (2006). However, at lower volumes (about 2 l), there was only a slight deviation between the experimental and expected values: 30% vs 23/24% for 0.3–0.5 μm particles, 44% vs ~30% for 0.5–1.0 μm particles, when breathing through the

mouth. These results are consistent with those of other published studies (Montoya et al. 2004, Morawska et al. 2005, Salma et al. 2002a).

The differences between the experimental and ICRP model data may be due to various reasons: (1) the ICRP model is based on one type of particle whose diameters are log-normally distributed, but real airborne particles are mixed and so their distribution is not log-normal (Ferron et al. 2000, Salma et al. 2005); (2) tidal inspired/exhaled volume is less than 2 l; (3) particles with aerodynamic diameters of 0.3–1.0 μm tend to deposit by both diffusion and sedimentation, thus the AMTD and AMAD models used to calculate %DEP are both valid and overlapping, whereas the determination of %DEP is more uncertain and critical (ICRP 2002); (4) the ICRP model does not consider a number of the chemical and physical properties of particles, such as their hygroscopicity and net charge (hydrophilicity), which tend to increase %DEP particularly in the case of particles with an aerodynamic diameter > 0.5 μm (Asgharian 2004, Cohen et al. 1998, Heyder 2004, Martonen & Schroeter 2003); moreover, shape factors other than 1.5 and a density > 3 g cm^{-3} could enhance %DEP (ICRP 2002); (5) some authors have shown that the ICRP model has a relatively high degree of uncertainty due to individual anatomical and physiological variability, particularly in the deep lung (Fritsch 2006, Hattis et al. 2001, Hofmann et al. 2002).

On the basis of our findings, we decided to continue our experiments at high volumes without monitoring inspiration because a 3-s inhalation is easier outside the device and does not require specific training. Moreover, inhalation flow should have a minimal effect on %DEP. Finally, particularly at high volumes, log(GSD) was comparable with, or lower than that observed in other studies (Hattis et al. 2001) even without correcting for exhaled volume.

The signals were highly stable in the range of 0.13–0.25 l s^{-1}, and multiple repeated measures showed that it was highly repeatable (%CV < 5%). If the signals of flow and particle concentration are smoothed and aligned, %DEP can be calculated – even if expiratory flow is not constant over time – and the convolution between the two signals can provide information concerning the influence of respiratory anatomy and expiration flow on the particle kinetics of exhalation.

The device was validated on a group of 20 young volunteers (10 women), who were recruited regardless of their smoking habits, although further studies will certainly include healthy non-smoking controls, asymptomatic smokers and subjects with different lung disorders (Kim 2000). In this group, %DEP was higher for 0.5–1.0 μm particles than for 0.3–0.5 μm particles. Interestingly, as previously reported (Kim 2000), subjects with asthma episodes tended to have relatively high %DEP values.

The stability of %DEP at a flow rate of 0.13–0.25 l s^{-1} was confirmed by the fact that there was no correlation between expiratory flow and %DEP in the two channels, which indicates that the variability due to expiratory flow was overwhelmed by intersubject variability. It is also very interesting to note that %DEP did not depend on environmental particle concentration in the two channels. The most important finding was that %DEP is mainly affected by total exhaled volume (6–7% l^{-1}). At similar flows, %DEP was significantly higher in men, but the difference was mainly due to differences in exhaled volumes. Kim and Hu have shown that, at constant tidal volume and expiratory flow, the %DEP of fine particles (1–3–5 μm) is comparable in the two sexes, although female subjects showed greater deposition in different regions of the respiratory tract depending on particle size (Kim & Hu 1998), particularly in the case of 3–5 μm particles (Kim & Hu 2006). The %DEP of ultrafine particles (> 0.04 μm) is also comparable in the two sexes (Jaques & Kim 2000, Kim & Jaques 2004), but that of smaller particles is higher in females under different flow and tidal volume conditions (Jaques & Kim 2000).

It has been found that exercise increases %DEP especially in men (Londahl et al. 2007). Women tend to have a higher degree of airway hyper-responsiveness (AHR), which has been related to greater susceptibility to environmental pollutants and PM (Boezen et al. 2004, Langhammer et al. 2003, Leynaert et al. 1997), whereas men more frequently report upper respiratory symptoms (URS) (Boezen et al. 2005). Lung size is comparable between the sexes, but it has been supposed that women have a smaller airway calibre associated with greater airway resistance and reduced anatomical dead space. It should imply a higher %DEP in the alveolar region than in males, who should have a higher %DEP at the beginning of the respiratory tract (particularly at the bronchial level) where PM has a direct irritant effect (Boezen et al. 2005). Log(GSD) tended to be lower than reported by others, also without considering the gender differences (Hattis et al. 2001).

The device was tested on a small group of ceramic tile workers exposed to cristobalite dust in order to test its applicability to field studies and define operating procedures. In fact, prior to proceeding with further occupational studies, the operating conditions of our device in a polluted environment were standardized, also in order to minimize workers' discomfort. The study was not primarily aimed at providing further information concerning cristobalite toxicity and therefore no differences between healthy workers and controls were necessarily expected, but gave us an opportunity to test the

device and to calculate %DEP using the 1.0 and 2.5 μm channels of the particle counter, whose use is limited by the relatively small number of particles in the range 1.0–5.0 μm aerodynamic diameter in environments that are not specifically polluted. Therefore, this study, despite the limited significance of differences between workers and controls, shows the great versatility of our device in studying %DEP also in a wider range of particles (e.g. aerodynamic diameter > 1 μm). In order to maximize the number of subjects tested in the limited time available, multiple testing of the same subject was not used. Indoor airborne particle concentration in the 0.3 μm channel was always less than outdoors and increased in the 0.5 μm channel only when the oven was open, but it was always higher in the other three channels (1.0–2.5–5.0 μm) even in the administrative office, which is not in direct contact with the Production Division.

Particle concentration was dependent on the current production activity. As expected, the %DEP of particles of 1.0–5.0 μm aerodynamic diameter was greater than that of smaller particles, and deposition increased with aerodynamic diameter (Heyder 2004). However, there were no differences between the production workers and controls, thus indicating that chronic exposure to cristobalite dust did not alter %DEP. As previously observed in the case of volunteers, the subjects who reported airway pathologies (asthma, rhinitis) tended to show high %DEP, particularly in the 0.3 and 0.5 μm channels, thus confirming that %DEP of particles with aerodynamic diameter of between 0.3 and 1.0 μm is influenced by the status of the respiratory system.

As shown in Figure 6, one of the advantages of working with high volumes is that it provides more information concerning airway anatomy and physiology than working with fixed inhaled/exhaled volumes; it is also very easy for subjects who usually perform the spirometry and does not require an external command to stop the inhalation at a fixed point. Furthermore, as our study clearly shows that %DEP is related to exchanged volume, we suggest making the measurements at high volume but then using volume itself as a covariate, particularly when differences in exhaled volume are present in different groups. This makes it possible to take differences in individual susceptibility into account by correcting it for pure effects of exhaled volume, where with the term 'susceptibility' we intend the ability of a subject to deposit PM based on his/her characteristics and particle properties.

Due to the limited variability of %DEP in healthy subjects, the influence of airway disease on %DEP and the versatility of our device, we think that %DEP could be used in different occupational and environmental field studies as a biomarker of individual susceptibility. Higher %DEP could indicate a higher dose of potentially toxic substances in PM deposited in the airways and a higher relative risk. It could be also relevant for workers with pulmonary diseases and could add information to spirometric measures traditionally performed during biomonitoring. Further studies are in progress on specific occupational activities, where exposure to PM of different aerodynamic diameter and composition is reported.

One of the most important points when calculating %DEP is the hygroscopic growth of particles once they have reached the airways. Models containing hygroscopic compounds have shown that, under physiological airway conditions, the growth factor of hygroscopic particles (Gf = diameter of humidified particle/diameter of dry particle) can increase 2–2.5 times in 2–3 s and up to > 3 in 10 s (Broday & Georgopoulos 2001). Hygroscopic growth is particularly important for particles with diameters of 0.1–1 μm (Asgharian 2004). Enlarged particles generally have higher %DEP values than their dried counterparts, particularly if their aerodynamic diameter is > 0.5 μm (Asgharian 2004, Martonen et al. 1985). This phenomenon is particularly critical in field studies because the distribution of exhaled particles may be right-shifted to higher aerodynamic diameters than that of inhaled particles. This is why some experimental systems use driers (Londahl et al. 2006).

Without a drier there is a risk that the hygroscopic particles initially counted in one channel, when exhaled, are subsequently counted in another channel with a higher aerodynamic diameter. It could affect the kinetics of the signals of a particle counter and thus the calculated %DEP. In the case of our device, the fact that particles enter the counter with less than physiological RH (85–90%) acts as a partial control.

Airborne particles are only in part hygroscopic (probably due to the presence of ammonium sulphate) and the percentage of non-hygroscopic particles is higher in urban locations (e.g. our office), probably because of the increased presence of non-hygroscopic particles deriving from car exhaust and combustion (Dua et al. 1999, Ferron et al. 1999). Furthermore, hygroscopic airborne particles have a Gf of about 1.1–1.2 at an RH of 40–60% (Swietlicki et al. 2008), 1.3–1.4 at an RH of about 85% (Ferron et al. 1999) and 1.3–1.6 at an RH of 90% (Swietlicki et al. 2008), which means that an increase of Gf of 1.1–1.4 can be expected in our device. Hygroscopicity could therefore slightly increase the concentration of exhaled particles in the 0.3 and 0.5 μm channels to above the level of measured environmental particle concentration, thus modifying the sigmoidal kinetic curve of exhalation, and leading to an underestimate of %DEP. This could be particularly critical in the case of the 0.5 μm channel, in which the concentration

of particles is generally lower that that measured in the 0.3 μm channel in non-specifically polluted environments. We found no evidence of it but, while contamination due to particles < 0.3 μm could be the same for all of the subjects tested in the same place, contamination in the 0.5 μm channel could be more critical. If this is the case, we suggest considering the two channels together, and the same thing should be considered for other channels with higher cut-off values. However, we do not exclude the possibility of incorporating a specific drier inside our device.

There are a number of limitations to the study. First, the concentration, composition, shape, size and chemical–physical properties of airborne PM may vary widely depending on the season, the weather and the sampling place. Therefore, the shape of the exhaled particle concentration curve and to a lesser extent %DEP may vary on the basis of the contingent conditions. Calculated %DEP should be considered a relative rather than an absolute value, and appropriated environmental monitoring should always be reported. Second, unless disposable flow meters are used, internal membranes of commercially available pneumotachometers are prone to contamination and it is not possible to use specific filters because they significantly alter exhaled particle concentration. Therefore, membranes should be frequently changed and sterilized. Third, the process of signal smoothing and alignment is not automatically controlled by specific software, and so some variability (< 5%) in the calculating of %DEP may depend on the operator. Further work is needed to completely standardize the signal analysis. Finally, we are currently undertaking further studies to extend the use of the device to nanoparticles.

In conclusion, our new device has been designed to monitor the on-line exhalation kinetics of particles in a single breath and has all the characteristics necessary for field studies: low intrasubject variability, high reproducibility of the signal, a limited size, versatility (it can be used for both fixed and variable volumes), and simplicity for operators and monitored subjects. We do not recommend it for measuring the dose of particles deposited in the airways over a long period (e.g. a working shift), but it is particularly promising for studying individual susceptibility to airborne or model particles, making repeated measures in the same subjects in different environments, and examining the persistence of particles in the airways after a period in a polluted environment. The use of inhaled/exhaled volumes that are near to FVC (60–90%) should reduce intersubject physiological and anatomical differences, and volume can be included as a covariate in statistical analysis in order to take into account the pure effect of exhaled volume on %DEP.

Appendix

Mathematical models for calculating %DEP

Kim equations (Kim 2000) were used to calculate the percentage of inhaled particles depositing in the airways (%DEP):

$$P_{in} = \int_{in,start}^{in,end} C_{in}(t) \times F_{in}(t)dt \tag{1}$$

$$P_{ex} = \int_{ex,start}^{ex,end} C_{ex}(t) \times F_{ex}(t)dt \tag{2}$$

$$\%DEP = 100 \times \left(1 - \frac{P_{ex}}{P_{in}}\right) \tag{3}$$

where 'in' = 'inspired', 'ex' = 'exhaled', start and end indicate the beginning and the ending of inspiration/exhalation; C is particle concentration and F the flow-rate of inspiration/exhalation.

With the approximations that $C_{in} = \text{constant} = C_{environment}$ and that

$$\int_{in,start}^{in,end} F_{in}(t)dt \approx \int_{ex,start}^{ex,end} F_{ex}(t)dt \approx V_{ex,TOT} \tag{4}$$

where $V_{ex,TOT}$ is the total exhaled volume calculated as the area below the F of exhalation over time,

$$\begin{aligned} P_{in} &\approx C_{environment} \times V_{ex,TOT} \\ &\approx C_{environment} \times F_{ex} \times \Delta t_{ex} \ \text{(at constant flow)} \end{aligned} \tag{5}$$

If F_{ex} is not constant, the convolution between $C_{ex}(t)$ and $F_{ex}(t)$ should be calculated by fitting both experimental data series with polynomials and multiplying the functions point by point. However, in various cases the approximation:

$$\begin{aligned} \int_{ex,start}^{ex,end} C_{ex}(t) \times F_{ex}(t)dt &\approx \overline{F_{ex}} \times \int_{ex,start}^{ex,end} C_{ex}(t)dt \\ &\approx F_{ex} \times \int_{ex,start}^{ex,end} C_{ex}(t)dt \\ &\text{(at constant flow)} \end{aligned} \tag{6}$$

is acceptable although not informative about the shape of the curve of particle exhalation (e.g. the convolution).

Being the integral of C(t) the area under curve (AUC) of the polynomial/sigmoidal curve, which fits the

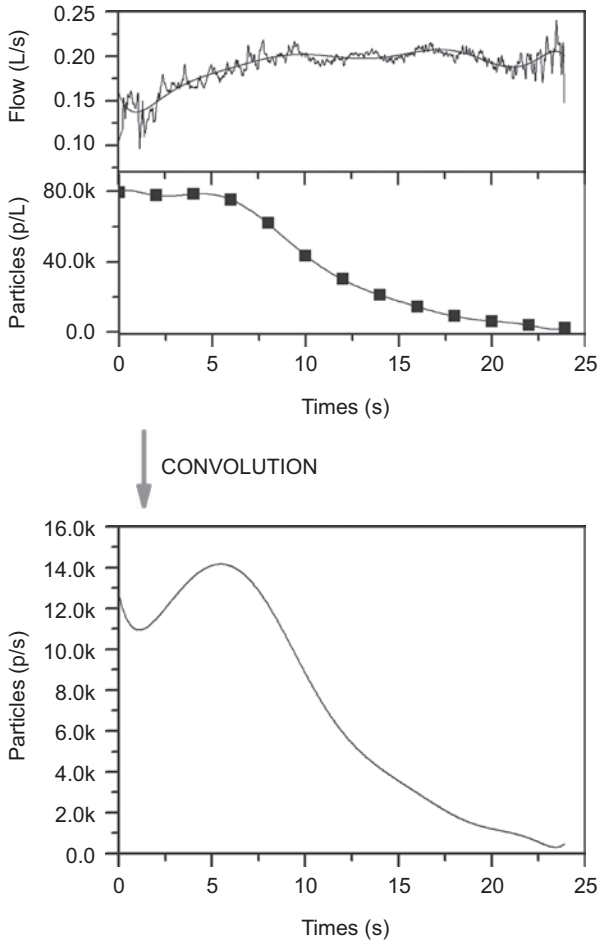

Figure 7. Exhalation flow and particle concentration as a function of time (0.3 μm channel), with their respective smoothing functions and convolution.

experimental data measured with particle counter, at constant flow-rate or using the average F_{ex} value:

$$\%DEP = 100 \times \left(1 - \frac{F_{ex} \times AUC}{C_{environment} \times F_{ex} \times \Delta t_{ex}}\right) = 100 \times \left(1 - \frac{AUC}{C_{environment} \times \Delta t_{ex}}\right)$$

as reported by Invernizzi et al. (2006).

The model does not take into account of the effect of inhalation flow on %DEP, and should therefore be tested before it is used. Figure 7 shows the signal and its convolution with exhalation flow.

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