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Effects of Breathing Parameters on Sidestream Cigarette Smoke Deposition in a Hollow Tracheobronchial Model

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The effects of variations in cyclic breathing parameters (i.e., tidal volume and breath frequency) have been the subject of few studies devoted to the deposition of submicrometer aerosols in the human respiratory tract. Therefore, a series of experiments was performed to investigate whether the deposition efficiency (DE) of sidestream cigarette smoke is altered by varying tidal volume and breath frequency in a child-size hollow tracheobronchial (TB) model while maintaining a fixed minute ventilation rate of 5 L/min. Under cyclic flow conditions with tidal volumes of 100 mL (50 breaths/min), 250 mL (20 breaths/min), 500 mL (10 breaths/min) and 750 mL (6.7 breaths/min), sidestream cigarette smoke was passed through replicas of an idealized hollow TB model. The smoke deposits were extracted and then quantitated spectrophotometrically. The experiments revealed a significant difference in DE between the 100-mL tidal volume (DE=6.0%) and the 750-mL tidal volume (DE=11.1%). Under equivalent steady flow conditions, the mean DE was 21.5%. A trend was evident in the data—DE increased as tidal volume increased (and breathing frequency decreased)—suggesting that the influence of diffusion and secondary flows on DE becomes greater as the air residence time increases and the degree of air turbulence decreases. The results provide evidence of the importance of breathing parameters when attempting to model in vivo deposition of environmental tobacco smoke and other similar-size respirable aerosols.

Keywords: breath frequency, environmental tobacco smoke, respirable aerosols, tidal volume

Various respiratory diseases, including lung cancer, bronchitis, and emphysema, have long been associated with the chronic inhalation of mainstream cigarette smoke (that drawn from a lit cigarette during a puff). In recent years concern has increased over the possible adverse health effects of environmental tobacco smoke (ETS)—an ambient aerosol that consists primarily of sidestream smoke (that emitted between puffs from a cigarette) and exhaled smoke. The degree to which ETS exposure represents a significant health hazard is still being debated, mainly because of limitations in epidemiological methods when they are applied to small relative risk ratios, and because it is difficult to quantitate the exposure to particulates due solely to smoking in environments typically encountered by the general population. Although the chemistry of cigarette smoke is complex, the main constituents of mainstream smoke and ETS have been identified.(1) Both mainstream and sidestream smoke have also been characterized with respect to their particle size distributions, and the results of many such studies were summarized in a review.(2) Briefly, mainstream smoke aerosols typically have mass median aerodynamic diameters (MMADs) on the order of 0.3–0.7 μm, and sidestream smoke aerosols typically have MMADs of about 0.4 μm. Both aerosols have geometric standard deviations (GSDs) on the order of 1.4. Similar sizing results have been

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obtained by others in more recent studies.[3–5] Inhaled particles in this size range would be expected to deposit mainly in the pulmonary (alveolar) region of the human lung.[6] However, clinical observations have revealed that malignant tumors have been more prevalent in the upper portion of the tracheobronchial (TB) tree of smokers.[7,8]

Theories to explain this apparent anomaly in cigarette smoke deposition have been explored in several studies. In extending a computer model of aerosol respiratory tract deposition to include particle sizes representative of mainstream and sidestream smoke, Muller et al.[9] proposed three mechanisms in an effort to account for the differences between expected smoke deposition sites and tumor loci in the lungs:

(1) Coagulation—the rapid agglomeration of freshly generated smoke particles produces an aerosol consisting of larger individual particles, which shifts the deposition toward larger airways

(2) Particle hygroscopicity—the adsorption onto smoke particles of water vapor present in the warm, humid conditions inside the respiratory tract significantly increases an inhaled smoke particle's aerodynamic diameter

(3) Breathing patterns—normal tidal volumes and breathing frequencies are altered during smoking, resulting in a deposition pattern different from that predicted by models using nonsmoking conditions

In addition to the three mechanisms proposed by Muller and associates, Phalen et al.[4] reviewed other phenomena that could enhance cigarette smoke deposition:

(1) colligative behavior, in which particles in a highly concentrated aerosol (such as cigarette smoke) exhibit physical behavior that is influenced by aerodynamic (hydrodynamic) interactions among nearby particles;

(2) electrostatic charge effects, which are caused by charged particles experiencing an electrostatic force when in close proximity to a respiratory tract surface or other charged particles; and

(3) vapor deposition on airway walls, which occurs due to the rapid diffusion of volatile vapor-phase constituents from cigarette smoke aerosol.

To varying degrees, all six of these mechanisms will influence smoke deposition. The first two mentioned by Muller et al. (particle coagulation and hygroscopicity) have been analyzed independently by both Martonen[8] and Ingebrethsen[10] and were found to be inadequate to explain the tumor locations by altered deposition efficiency. In fact, Martonen[8] proposed a mathematical model that explained how mainstream cigarette smoke deposition patterns, and the formation of deposition "hot spots" at airway bifurcations, were the consequences of two distinct effects: particle cloud motion and vapor-gas behavior, with the former effect being most prominent. Muller's third factor, breathing parameters, has received little attention in the past, although Hinds[11] demonstrated the significance of puff volume, duration, and frequency on the respiratory tract deposition of cigarette smoke. With this in mind, this study was specifically designed to further investigate the effects of breathing parameters on the deposition of cigarette smoke.

Although numerous studies have been performed to quantify the pattern of aerosol deposition in physical representations (i.e., replica casts and idealized surrogate models) of the adult TB tree, most of these have employed steady (constant) inspiratory flow conditions. Only a few have been devoted to characterizing deposition in bifurcating systems under more realistic cyclic inspiratory/expiratory flow conditions. Gurman et al.[12] and Schlesinger et al.[13] both used replica casts of an adult TB tree and exposed them to monodisperse ferric oxide aerosols, with MMADs of 3 μm and 8 μm, using a variety of steady and cyclic flow patterns having equivalent mean flow rates of 15–60 L/min. In both studies differences in deposition between cyclic and steady flow scenarios were found to be greater for the smaller (3 μm) aerosol. In a separate study Schlesinger et al.[14] utilized a dual-cast exposure system to investigate particle deposition during both inhalation and exhalation. At steady flow rates of 15 and 30 L/min, it was found that the deposition efficiency of monodisperse ferric oxide particles (3–6 μm MMAD) during exhalation was 50–80% of that during inhalation. Kim and Garcia[15] also performed particle deposition studies using a surrogate, single-bifurcation airway model made of glass tubes, and monodisperse oleic acid droplets (3, 5, and 7 μm MMAD) at breathing frequencies of 16, 30, and 50 cycles/min and mean flow rates of 2–20 L/min. In cyclic flow conditions the deposition efficiency (DE) was found to be 80–200% higher than with equivalent steady flows. The authors reported that their results were consistent with previous studies, indicating that inertial impaction was the dominant mechanism for particle deposition in the particle size range studied, and that deposition under steady and cyclic flow differed significantly.

However, because cigarette smoke consists of submicrometer-size particles, inertial impaction is probably not expected to be a primary deposition mechanism. Martin and Jacobi[16] studied the deposition of radioactive submicrometer aerosols in a plastic model of the upper bronchial tree. Their aerosols had activity median diameters (AMDs) between 0.2 and 0.4 μm, and their polyvinyl chloride airway model had dimensions conforming to the dichotomous lung model of Weibel.[17] Under steady flow conditions ranging from 0.1 to 50 L/min, their results indicated that deposition due to turbulent diffusion had an important role in the trachea and larger bronchi.

Deposition of ultrafine particles in cyclic flow conditions was studied by Cohen et al.[18] Using replicate hollow casts of the human upper TB tree, the deposition of 0.20, 0.15, and 0.04 μm diameter radiolabeled ferric oxide particles was quantitated at mean cyclic inspiratory flow rates of 18 and 34 L/min, and at frequencies of 18 and 20 cycles/min. Total deposition in the casts was found to be greater at low flow rates than at high flow rates for each of the particle sizes, an indication that diffusion was the dominant deposition mechanism. However, greater deposition for the 0.20 μm particles as compared with the 0.15 μm particles suggested additional deposition of the larger particles by impaction. In addition, increased deposition at airway bifurcations was attributed to secondary flows that result from the changes in flow direction at bifurcations and the cyclic changes in flow rate.

The studies above provide a framework for a further investigation of TB deposition in cyclic flow conditions, with the focus on the experimental exposure of a smaller, child-size TB tree to sidestream cigarette smoke, an environmental aerosol and indoor pollutant known to have an MMAD (approximately 0.4 μm) that is just above the size range of ultrafine particles (usually <0.1 μm diameter). The objective of this study was to quantitate the deposition of sidestream cigarette smoke in a hollow TB model under four different cyclic flow regimes that produced the same average flow rates as does a minute ventilation rate of 5 L/min. By introducing variations in tidal volume and breathing frequency, a better understanding can be obtained on how breathing parameters affect the respiratory tract deposition of a common indoor aerosol that poses a potential health risk to young children.

MATERIALS AND METHODS

Hollow Model Preparation

The simple, idealized hollow airway model used (Figure 1) was slightly different from Weibel's[17] Model A of the human

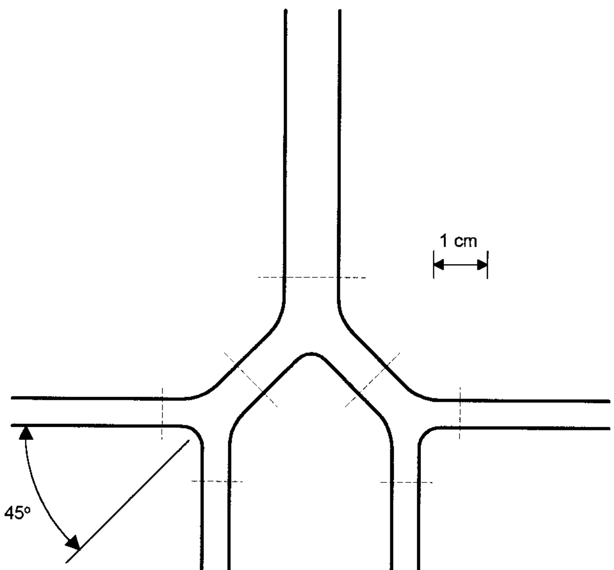

FIGURE 1. Hollow tracheobronchial model representing a four-year-old child. All branch angles are 45°. (Dashed lines indicate cuts to provide sections for analysis of deposits.)

bronchial tree. The dimensions of the model used in the current study were smaller than those of an adult, and the model included only three generations of airways. Specific lengths and diameters of each generation of the model used are shown in Table I. This model can be scaled using tracheal growth curves to obtain an approximate age equivalence of a four-year-old child.[19] This age was selected for study because young children are expected to have enhanced bronchial deposition in relation to adults, and at pre-kindergarten age they may be exposed more frequently to ETS in the home than older children who spend more time outside the home. A larynx was not included in the model due to a lack of accurate morphological data regarding the laryngeal dimensions for a four year-old child, and the effect of varying airflows on those dimensions (see Results and Discussion section).

Branch angles (angles of daughter branch deviation from the parent airway) for the two bronchial generations were set at 45°. Each of the six hollow models used was made of Silastic E (Dow Corning, Midland, Mich.) and RF-710 silicone rubber (Resin Formulators Company, Culver City, Calif.). Previous experiments with other models and casts made of these materials demonstrated that the use of coatings, such as silicone oil, to prevent particle bounce did not affect deposition, especially for aerosols composed of liquid droplets. Therefore, the interior of the model was left uncoated.

Following the deposition of cigarette smoke, the hollow model was dissected as shown in Figure 1. Deposits were removed and the model was reconstructed by carefully rejoining the cut segments with RTV silicone rubber adhesive (Dow Corning). Inspections involving visual checks (to prevent any misalignment of the cut edges) and water immersion to detect air leakage (by inserting plugs into the model's openings) were performed prior to reuse of the model. With this method, a hollow model could be used for more than one experiment.

TABLE I. Hollow TB Model Airway Dimensions

Generation	Airway Length (cm)	Airway Diameter (cm)
0 (trachea)	6.5	1.05
1	2.5	0.80
2	5.0	0.65

Sidestream Smoke Generation and Sizing

Because studies have shown that sidestream smoke comprises the majority of ETS,[20] it was decided that sidestream cigarette smoke would be used in this study. The smoke generation apparatus has been described previously.[4] Briefly, a cigarette was inserted lit end first into a vertical 5 cm diameter copper pipe and the smoke diluted with room air. The copper pipe was designed to allow sidestream smoke to rise 55 cm, a distance observed to be greater than that required for the smoke stream to lose its thermally driven vertical velocity, prior to it entering the TB model. The smoke was drawn by suction through a vertically oriented hollow TB model for deposition measurements. The smoke was supplied by smoldering unfiltered 1R3 research cigarettes (University of Kentucky, Tobacco and Health Research Institute, Lexington, Ky.), which were stored prior to use in a sealed glass container on a rack over a saturated solution of sodium bisulfate, which provided a constant relative humidity of 60±2% at about 24°C. The cigarettes were not puffed for this study.

A seven-stage, Mercer-type cascade impactor (Model MCR 02-140; In-Tox Products, Albuquerque, N.M.) was used to obtain an MMAD and the estimated GSD of sidestream cigarette smoke. This impactor has effective cutoff diameters ranging from 5.27 to 0.26 μm at the airflow used (1.8 L/min). The impactor was loaded with uncoated stainless steel substrates and a 25 mm glass-fiber backup filter (Type A/E; Gelman Sciences, Ann Arbor, Mich.). Sampling from the outlet of the smoke generation system was conducted for 3 minutes. The stainless steel substrates and filter were then agitated individually for 10 minutes in isopropyl alcohol using an ultrasonic bath. The filter extract was filtered using a 0.2 μm pore-size fluorocarbon syringe filter (Acrodisc CR, Fisher Scientific, Pittsburgh, Pa.) to remove loose filter fibers. All extracts were measured at a wavelength of 350 nm using an absorption spectrophotometer (Model 301, Spectronic; Milton Roy, Rochester, N.Y.). The wavelength of 350 nm was used because (a) cigarette smoke contains numerous aromatic compounds that absorb ultraviolet light,[1] and (b) the results of a previous study involving a comparison of impaction, centrifugal separation, and electron microscopy for sizing cigarette smoke indicated that the size characteristics obtained using a Mercer impactor, with spectrophotometric analysis at 350 nm, were similar to those obtained using the other two methods.[21] Duplicate absorbance readings from each extract were recorded. The spectrophotometer was periodically corrected for zero drift using an isopropyl alcohol blank.

Smoke Deposition: Cyclic Flow Conditions

Sidestream smoke from the cigarette smoke generator was "inhaled" and "exhaled" through the hollow model by connecting its tracheal opening to a two-way, directional-flow valve (Model 1400; Rudolph Valve Co., Kansas City, Mo.). The airway exits from the model were connected to 25 mm diameter glass-fiber filter containing cassettes with plastic "Y" connectors of a size that facilitated smooth transitions in flow. In addition, the plastic tubing used to connect the filter cassettes to the air pump was cut

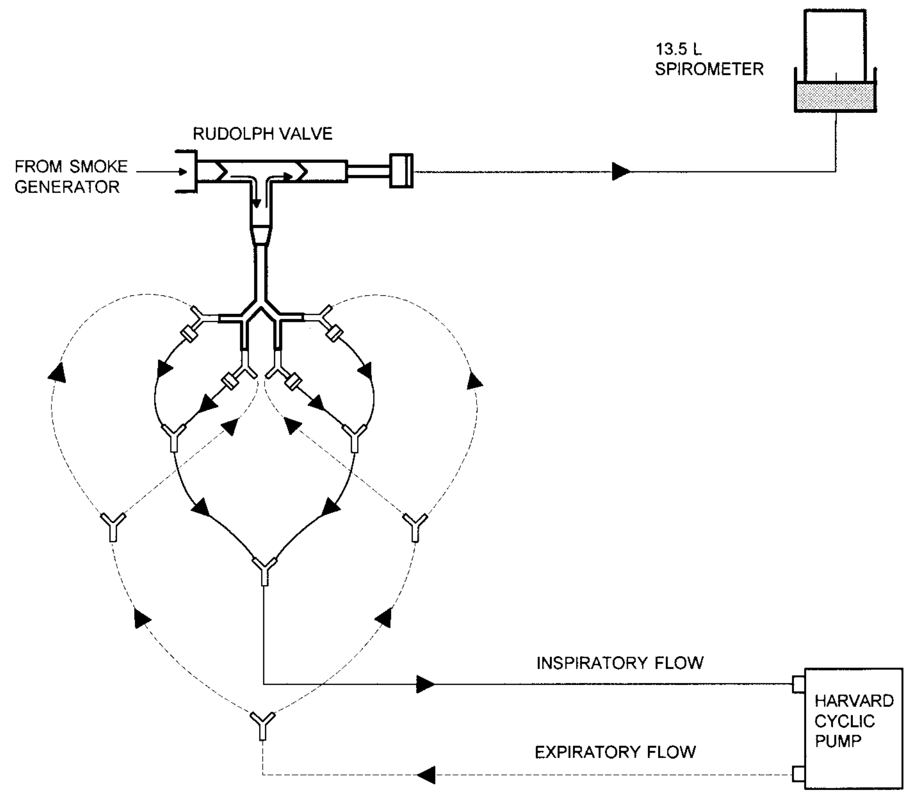

FIGURE 2. Schematic drawing of cyclic flow apparatus (not drawn to scale)

into equal lengths to avoid significant differences in flow resistance. As shown in Figure 2, the complete experimental apparatus was configured to pull sidestream cigarette smoke (during inhalation) from the generator and through the hollow model (which was mounted on a test stand to maintain the desired branch angles), and then to push residual smoke (in the airways, but not deposited in the model) up through the model during the exhalation phase. Additional air during exhalation was filtered, and was thus particle-free. Any smoke remaining in the airstream after passing through the model during inhalation was collected on 25 mm diameter glass-fiber filters (Type A/E; Gelman Sciences).

A variable-speed, variable-volume cyclic piston-type air pump (Harvard Apparatus Co., Millus, Mass.) was used at different settings of tidal volume and breathing frequency to simulate various respiratory patterns (from rapid, shallow breathing to slow, deep breathing) that shared the identical minute ventilation rate. During exhalation, air was pushed through the hollow model and Rudolph valve, then through a 47 mm diameter fluorocarbon-coated glass-fiber filter (Pallflex Filters, Putnam, Conn.), which collected any smoke that did not deposit in the hollow model during this phase.

The four cyclic flow regimes, each providing a minute ventilation of 5 L, were as follows:

(1) Tidal volume = 100 mL; breathing frequency = 50 cycles/min

(2) Tidal volume = 250 mL; breathing frequency = 20 cycles/min

(3) Tidal volume = 500 mL; breathing frequency = 10 cycles/min

(4) Tidal volume = 750 mL; breathing frequency = 6.7 cycles/min

A minute ventilation rate of 5 L/min was chosen because it best corresponds to an activity level midway between "low activity" and "light exertion" for a four year-old child.[22]

A 13.5-L spirometer (Collins, Inc., Braintree, Mass.) was employed for verification of the tidal volume. Checks of breathing frequency were conducted with an electronic stopwatch. For each of the cyclic flow regimes, the duration of the exposure to cigarette smoke was set at 15 minutes. At the 5- and 10-minute marks of each experiment, the pump was stopped so that the 47-mm filter could be changed. This was necessary to avoid any reduction in flow due to excessive filter loading by smoke deposits. After 15 minutes, the flow of smoke was terminated to allow smoke-free laboratory room air to cycle through the hollow model for at least 2 minutes.

At least two experiments were performed in order to obtain a measure of the variability in the DE for each cyclic flow regime. Following this, a comparison of the calculated means using an analysis of variance (ANOVA) was performed.

Smoke Deposition: Steady Flow Conditions

The apparatus for the steady flow experiments was nearly identical to that used for the cyclic flow experiments, except that a laboratory vacuum pump was utilized instead of the Harvard cyclic pump. Also, a calibrated rotameter was employed to set a steady flow rate of 10 L/min through the hollow TB model. This flow rate was selected to represent a volumetric equivalent; that is, the

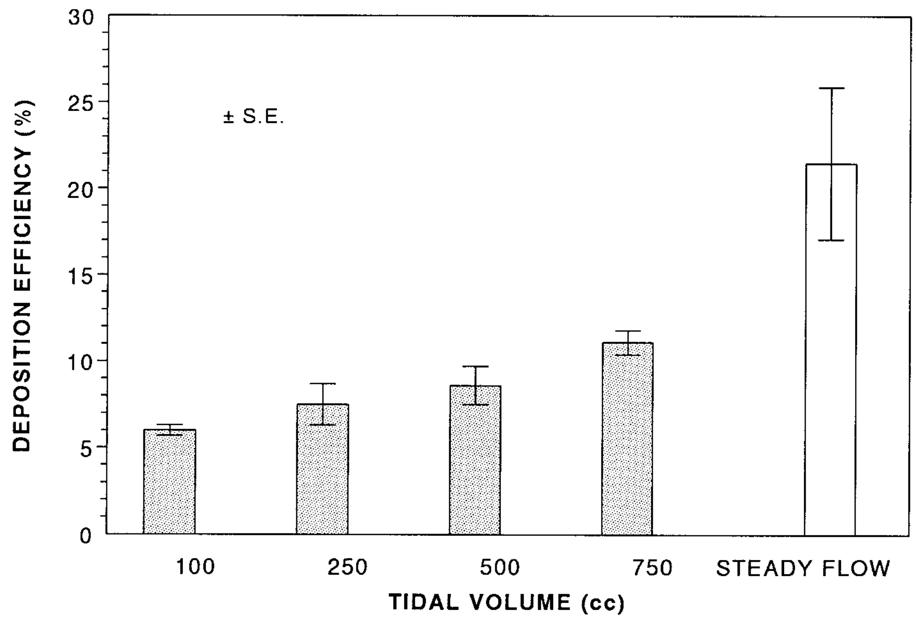

FIGURE 3. Mean deposition efficiencies (including standard errors) for four different cyclic flow conditions and for one steady flow condition

total volume of air that is inhaled and exhaled through the hollow model. This steady ventilation rate would be comparable to cyclic flow conditions where 5 L is drawn into the model during one-half of each minute, and 5 L of undeposited smoke and clean air is pushed out during the other one-half minute. Sampling through the hollow model was conducted for 5 minutes. Extraction and quantitation of deposited cigarette smoke was performed in the manner described later. Five experiments were performed, and a mean DE was calculated. Using an ANOVA, this value was compared with the cyclic flow DEs.

Analysis of Smoke Deposits

After sampling, the hollow model, directional-flow valve, exhalation-phase filters and exit filters, filter cassettes, and plastic "Y" connectors (with tubing) were placed into separate clean plastic bottles and were ultrasonically agitated for 10 minutes in isopropyl alcohol. As shown in Figure 1, the hollow TB model was dissected into pieces that represented the trachea, bifurcations 1–2, and the daughter tubes. The alcohol extracts from the glass-fiber filters were filtered to remove loose fibers. All extracts from the ultrasonification were then measured with the spectrophotometer at 350 nm. Corrections of absorbance readings for differences in the volume of alcohol added were made, when appropriate.

The DE of cigarette smoke in each of the experiments was determined by normalizing the total absorbance measured for the hollow model to the sum of absorbance values obtained for the hollow model, the directional-flow valve, the exhalation-phase and exit filters, the filter cassettes, and the associated "Y" connectors and tubing.

RESULTS AND DISCUSSION

The cascade impactor data from two separate particle-sizing runs were fit to a log-probability function, and the MMADs and GSDs were determined by using a weighted least-squares method, as described by Hinds.[23] The average MMAD of the sidestream cigarette smoke was 0.44 μm, and the calculated GSD was 1.5. These values are consistent with previously reported size measurements obtained by others.[2–5]

The mean DEs for each of the four cyclic flow regimes are shown in Figure 3. The correlation coefficient (r^2) for the plot of the four means provided in Figure 3 was 0.975. An ANOVA was performed to test the hypothesis that the mean DEs were not statistically significant from each other. Only in the comparison of results obtained at tidal volumes of 100 and 750 mL was there a significant difference ($p<0.05$).

For the five steady flow experiments the mean DE (and standard deviation) was 21.5% (9.9%). Comparison of this value with the four cyclic flow DEs was again performed by using an ANOVA. The DE under steady flow was significantly different ($p<0.05$) from the values reported for each of the cyclic flow regimes, except for the condition where the tidal volume was 750 mL (at a breathing frequency of 6.7/min).

Muller et al.[9] published a summary of previous deposition model predictions for monodisperse, nonhygroscopic aerosols that were in the size range of cigarette smoke particles. Reported DE values ranged from 5 to 50%, with most falling around 20%. In contrast, Hinds et al.[11] reported smoke DEs in human volunteers ranging from 22 to 75%, with an average of 47%. The discrepancy could be due in part to the nature of freshly generated cigarette smoke. In such a highly concentrated aerosol (up to 10^9 particles/cm^3), the potential exists for hydrodynamic interactions among particles, resulting in unusual colligative (bulk-type) behavior.[4,8,10] Therefore, as a collective entity, cigarette smoke may deposit in a manner quite different from that of a less dense aerosol of similarly sized monodisperse particles.

Inertial impaction was proposed as the dominant deposition mechanism in studies that utilized monodisperse particles of 3 μm and 8 μm MMAD.[12,13] It was argued that the observed increase in deposition for the 3-μm particles, compared with the 8-μm particles, was due to a reduction in the boundary layer during cyclic flow; this had a greater effect on the smaller particles, since the larger (8 μm) particles had sufficient inertia to reach the airway walls without facilitation by turbulent eddies. However, for small and ultrafine particles, diffusion has a predominant influence on deposition, particularly at lower flow rates.

Martin and Jacobi[16] reported that the average physiological flow rate is in the range from about 5–30 L/min. However, during each respiratory cycle, the flow rate will vary from zero, in the transitional phase between inhalation and exhalation, to the maximum value during the midpoint of each phase. According to Cohen et al.[18] it is not a good assumption that flow within human airways is laminar. The Reynolds numbers in the trachea and major bronchi range from about 600 to 2500, and flow dividers prevent fully developed flow. Therefore, flow is frequently neither fully laminar nor fully turbulent. However, turbulence increases at higher flow rates, which can disrupt secondary flows that tend to enhance the deposition of particles.

It has been demonstrated by Chan et al.[24] and Gurman et al.[25] that the presence of a larynx has a significant effect on airflow, turbulence, and particle deposition in hollow casts of the human TB tree. Using flow rates of 15, 30, and 60 L/min, the authors found a strong correlation (r = 0.88) between the deposition efficiency of particles entering the trachea and the Stokes number, for particles greater than 2 μm MMAD. In the present study, however, a larynx was not included in the experimental apparatus due to the uncertainty in producing a morphologically accurate larynx model for a four year-old child. Although Mostafa[26] studied the variation in subglottic size in children, the data were measurements of endotracheal tubes that were assumed to be the largest that would fit through the larynx. Additionally, the measurements were made while patients were under anesthesia (presumably at basal ventilation) and under the influence of a muscle relaxant. Therefore, it is uncertain how these values relate to normal laryngeal dimensions in children.

The observed weak dependence of DE on respiratory frequency is consistent with results from human studies. Heyder et al.[27] examined the total deposition of droplets in five human subjects using a variety of breathing patterns and reported a decrease in DE for 0.2–0.3 μm particles when the breathing frequency was doubled. Similar findings were reported by Giacomelli-Maltoni et al.[28] for particles below 0.7 μm in diameter. For small particles, where diffusion is a major deposition mechanism, higher frequencies decrease residence times in airways. Because it is likely that cigarette smoke deposition in this study was significantly influenced by diffusion and secondary flows, opportunities for both to occur would have been greatest at the lowest breathing frequency, under conditions that allowed the most time between the start of the inhalation phase and the start of the exhalation phase.

CONCLUSIONS

In previous studies, comparisons between steady and cyclic flows were made at various flow rates, and generally with monodisperse particles. In this study it has been demonstrated that altering the tidal volume and breathing frequency at a fixed inspiratory ventilation rate of 5 L/min has a significant effect on the deposition efficiency of sidestream cigarette smoke in a child-size hollow TB model. As in the case with ultrafine monodisperse particles, the deposition efficiency of the smoke is expected to be influenced primarily by diffusion and secondary flows within the hollow model. This was supported by the results, which showed that deposition was highest when the largest tidal volume (750 cc) was paired with the lowest frequency (6.7 breaths/min). Also, deposition measured using more realistic cyclic regimes was significantly lower than the 21.5% DE obtained under a comparable steady flow. The implication is that an overestimation of the TB dose for young children can occur if particle deposition is performed in models under experimental conditions based on adult-type patterns of slower, deeper inhalation and expiration. Therefore, it is prudent that future deposition studies and dose calculations should also consider the influence of breathing patterns when attempting to model *in vivo* respiratory deposition and dosimetry of submicrometer-size particles, whether they be pharmaceutical aerosols, atmospheric aerosols, or indoor air pollutants.

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