



PARTICLE DEPOSITION IN THE CANINE RESPIRATORY TRACT*

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Abstract—Both total respiratory deposition of inhaled particles during breathing through the nose and thoracic deposition during breathing through an endotracheal tube were measured in beagle dogs using aerosol photometry and respiratory flow anemometry of individual breaths. Both sebacate and cobalt oxide (Co_3O_4) particles of densities of 0.9 and 3 g cm^{-3} were used in the aerodynamic domain from 0.4 to $5 \mu\text{m}$. For a given dog breathing through the nose, the deposition probability was similar for sebacate and Co_3O_4 particles of similar aerodynamic particle diameter. The results show that canine thoracic deposition for particles in the aerodynamic domain breathed at rest are not significantly different from those observed in the human respiratory tract rising from 0.12 to 0.35 of the inhaled particles of 0.7 to $3.7 \mu\text{m}$ aerodynamic diameter. When the fast cleared fraction of thoracic deposition was distinguished from the long-term retained fraction by means of lung clearance analysis of radiolabelled $^{57}\text{Co}_3\text{O}_4$ particles, it varied from 0.02 to 0.15 of thoracic deposition for particles of 0.7– $3.6 \mu\text{m}$ aerodynamic diameter. The intersubject variability of total deposition during nose breathing resulted from the variability of nasopharyngeal deposition similar to the human intersubject variability nasopharyngeal deposition.

INTRODUCTION

STUDIES designed to investigate the toxicity of an aerosol often have unique problems in controlling the administration of the specific initial quantities of the test material to the experimental animals. For most inhalation studies, only the exposure time and the air concentration of the test aerosol in the exposure chamber are normally controlled. One method used to attain a given initial lung burden in an experimental animal is to calculate the duration of exposure based upon the expected mean air concentration of the test material in the exposure chamber and to assume values for the animal's inspiratory minute volume and the percentage of the inhaled aerosol that will be deposited in the respiratory tract. However, many physical characteristics of aerosols and physiological characteristics of respiratory patterns significantly alter the quantitative deposition of inhaled particles in the respiratory tract.

Deposition of polydisperse aerosols was estimated in dogs from the mean inhaled aerosol concentration and the total deposited amount of aerosol mass at the end of an exposure lasting 10 min (CUDDIHY *et al.*, 1973). Deposition data were associated with mean tidal volumes and mean minute volumes. By means of radioactively labelled aerosols regional deposition of the extrathoracic region and the fast and slow cleared fraction of the thoracic region were estimated. The obtained canine data were in reasonable agreement with a human lung model for risk assessment available at that

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time (TASK GROUP OF LUNG DYNAMICS, 1966) although considerable scatter of the canine data was observed. The major deposition mechanisms in the human lungs are well described in dependence of the aerodynamic and thermodynamic properties of the particles and breathing patterns (LIPPMANN, 1977; HEYDER *et al.*, 1986; STAHLHOFEN *et al.*, 1989).

During recent years, numerous experiments have been conducted at this laboratory in which beagle dogs were exposed by inhalation to various aerosols to study particle clearance mechanisms and the functional response of the respiratory tract. In additional, experiments have been conducted to study the influence of aerosol size and breathing patterns upon total and regional deposition of particles in respiratory tracts of beagle dogs. The aim of this study was to derive deposition values for dogs and compare those with human data to prove the validity of the canine animal model with respect to risk assessment of inhaled toxicants.

MATERIALS AND METHODS

Eighteen male beagle dogs from the colony of the GSF were used in these studies. Ages ranged from 1 to 3 years and body weights from 11 to 17 kg. Monodisperse Co_3O_4 aerosols were produced within a range of 0.7–5 μm mass median aerodynamic diameter (MMAD) using a modified spinning top aerosol generator as described earlier (KREYLING and FERRON, 1984a,b). Monodisperse sebacate aerosols (di-2-ethylhexyl sebacate, DEHS) were produced within a range of 0.4–3 μm MMAD by a condensation aerosol generator (MAGE; Palas, Kartsruhe). The MMAD was measured by an Stober aerosol centrifuge (FERRON *et al.*, 1979). For inhalation the aerosol was warmed up to 32°C (which was found to be the mean temperature of exhaled air by dogs) and it was humidified to a relative humidity of 50–70%. The aerosol was continuously produced and the dog inspired and expired from this aerosol stream through a short duct containing the measuring devices.

Aerosol flow was measured using a hot-wire anemometer in a duct of block flow profile obtained by two screens in front and behind the probe. The block profile was proven by scanning the probe across the aerosol channel at various flow-in and expiratory rates. The pressure drop across these screens was recorded to identify the flow direction of the anemometer. The non-linear signal of the anemometer was calibrated immediately prior to the deposition study using a pneumotachograph in series when a cyclic flow pattern of conditioned air was maintained by a piston-type respiration pump (KREYLING, 1983). The aerosol concentration was measured by the 90° scattering light of the aerosol particles in a cylindrical volume perpendicular to the aerosol flow illuminated by an expanded laser beam (KREYLING, 1983).

Flow anemometry and aerosol photometry (dead space 7 cm^3) were carried out in front of the port to which either a nose mask or an endotracheal tube was connected. The nose mask consisted of a latex-rubber film with a hole for the nose which was pulled over the dog's snout and slightly greased. Then a flexible latex-rubber mask was pushed onto the rubber film on the snout. Both film and mask were molded from a cast of a canine snout resulting in an air-tight mask with minimal dead space (< 5 cm^3). The dog was supported in a cloth-type sling inside an air conditioned chamber such that its head was gently pressed into the nose mask. Prior to inhalation some of the dogs were slightly sedated (Rompun; Bayer AG, Leverkusen) and all dogs were allowed to adjust.

For bypassing the extrathoracic airways, the dogs were anaesthetized by a barbiturate and an endotracheal tube was placed into the trachea with the tip in the lower half of the trachea (dead space 12 cm³). The tube was sealed air-tight by inflation of a balloon around the tip of the tube. For inhalation the dog was placed on its right side inside the air-conditioned chamber.

The signals of the photometer, the anemometer and the flow direction were amplified and stored in a 10 ms cycle onto the disk of a computer (PDP-11/40) allowing for continuous measurement during 50 min. The data of the anemometer and flow direction were converted into inhalation and exhalation flow values, f , by using the calibration curve determined prior to the inhalation study (KREYLING, 1983). Single breaths longer than 80 ms were identified. Pauses in between breaths were not included into the tidal time. Data were rejected from analysis when several breaths did not balance volumetrically indicating leakage or inadequate breathing patterns. Each breath was categorized for inhalation time t_i , tidal volume V_i and mean and/or characteristic inhaled flow f_i ; the characteristic flow was defined as the average of all measured flow values of a given breath larger than 70% of the mean inhaled flow. Deposition of each breath was calculated from flow data $f(t)$ and photometer values $c(t)$ according to:

$$D = 1 - \frac{\int_{\text{ex}} c(t)f(t) dt}{\int_{\text{in}} |c(t)f(t) dt}, \quad (1)$$

where in and ex are the time of inspiration and expiration, respectively. Deposition data were corrected for dead space of the apparatus and the mask or the endotracheal tube.

Total deposition during breathing through the nose was studied on six dogs (group 1) using various particle sizes of two particle materials. Thoracic deposition of Co₃O₄ aerosols radioactively labelled with ⁵⁷Co was studied on 12 tracheally intubated dogs (group 2). In this case measurements of one particle size were carried out on each dog. These investigations were the initial part of long-term particle clearance studies (KREYLING *et al.*, 1986, 1988). When the dogs inhaled radioactively-labelled aerosols, subsequent gamma camera measurements indicated homogeneous deposition throughout the entire lungs. The fast cleared fraction eliminated within 1–2 days of inhalation was evaluated as a fraction of the total deposit by means of external gamma counting and excretion analysis (KREYLING *et al.*, 1986, 1988).

RESULTS AND DISCUSSION

The state of sedation or anaesthesia had effects on the breathing pattern. With and without sedation dogs showed a breathing pattern 'at rest' throughout the entire inhalation procedure with no pause between inhalation and exhalation and sometimes a small pause prior to the next breath. Although a large number of breaths were analysed for each particle size inhaled, each dog showed a characteristic spectrum of breathing patterns which did not necessarily overlap entirely between different dogs. The inhaled and exhaled flow patterns were fairly constant; the mean and the

characteristic inhaled flow were similar, but the mean exhaled flow was generally slower than the mean inhaled flow by a factor of up to 0.7. For all six dogs of group 1 the characteristic inhaled flow was within the range of $100\text{--}200\text{ cm}^3\text{ s}^{-1}$; for a given dog the mean characteristic inhaled flow varied usually very little within $30\text{ cm}^3\text{ s}^{-1}$ around the mean. The inhaled time varied from 1 to 2 s, i.e. tidal time was 2–5 s, and the tidal volume varied between 150 and 300 cm^3 .

Under anaesthesia, dogs of group 2 generally showed more shallow and rapid inhalation and exhalation patterns than under sedation, without any pause in between, but with a very long pause prior to the next inhalation. Both inhaled and exhaled flows were less constant but the inhaled and exhaled characteristic flows were usually similar for a given breath. As a result the inhalation times varied between 1 and 2 s but the tidal time was 4–6 s. The inhaled flow varied over a wide range, from 60 to $600\text{ cm}^3\text{ s}^{-1}$, but the tidal volume varied over a smaller range, from 50 to 250 cm^3 .

In Fig. 1 deposition is shown as the mean and standard deviation for breaths with a characteristic flow of $100\text{--}200\text{ cm}^3\text{ s}^{-1}$. On the upper panel, thoracic deposition obtained from 12 tracheally intubated dogs (group 1) is given for radioactively labelled $^{57}\text{Co}_3\text{O}_4$ aerosols. On the lower panel, total deposition obtained from six dogs (group 2) during breathing through the nose is given, including nasopharyngeal and thoracic deposition. The deposition data between 0.4 and $1\text{ }\mu\text{m}$ were very similar for all dogs of both groups breathing either through the nose or through an endotracheal tube. The deposited fraction below 0.2 was similar to human data for breathing patterns at rest (HEYDER *et al.*, 1986) since the particle fractions deposited by each of the three essential deposition mechanisms (diffusion, sedimentation and impaction) are low in this domain.

In order to study the effect of particle density, deposition of both sebacate and cobalt oxide (Co_3O_4) particles of densities of 0.9 and $2.8\text{--}3.3\text{ g cm}^{-3}$ in the aerodynamic diameter range of $0.5\text{--}3\text{ }\mu\text{m}$ were studied in dogs 3 and 4. For each dog no significant difference in total deposition was found for both particle materials when the aerodynamic diameter of the particles and the breathing pattern was similar (Fig. 1, lower panel).

Thoracic deposition during breathing through an endotracheal tube (Fig. 1, upper panel)

Although the dogs were anaesthetized and lying on their right side while breathing spontaneously, deposition was fairly uniform in the total lung as indicated by gamma camera images taken directly after the test inhalation. Moreover, a sensitive indicator of particle deposition in the peripheral lung was the visualization of the tip of the accessory lung lobe by gamma camera images (KREYLING *et al.*, 1986). These canine thoracic deposition data are in reasonable agreement with human data as shown in Fig. 1 (HEYDER *et al.*, 1986; STAHLHOFEN *et al.*, 1989).

Thoracic deposition increases with increasing aerodynamic diameter, due to the increasing particle fractions deposited by impaction and sedimentation. The analysis of the dependency of thoracic deposition on tidal time or characteristic inhaled flow was only possible for a few dogs, as a result of the range of breathing patterns. This analysis allowed us to distinguish between the effects of two deposition mechanisms at two different sites of the pulmonary region. Since impaction is proportional to flow and independent of time, it is a potential deposition mechanism in the airways but not in the

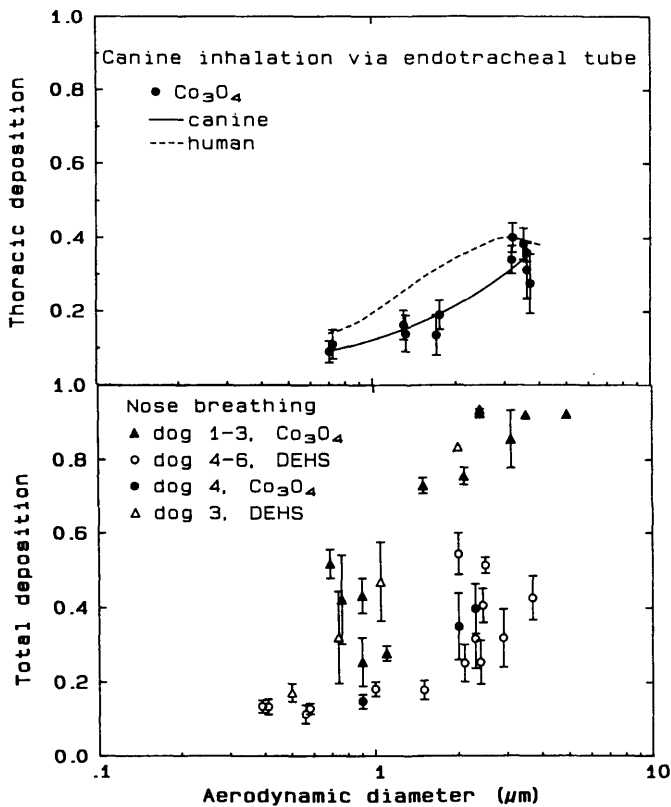


FIG. 1. Upper panel: canine thoracic deposition during breathing (at rest) for breaths of a mean inhaled flow of $100\text{--}200\text{ cm}^3\text{ s}^{-1}$ through an endotracheal tube is compared to the mean of human data (broken line) obtained under similar conditions (STAHLHOFEN *et al.*, 1989). Lower panel: total deposition during breathing through the nose obtained from six dogs for breaths of a mean inhaled flow of $100\text{--}200\text{ cm}^3\text{ s}^{-1}$ using monodisperse sebacate and Co_3O_4 particles.

alveolar region. While sedimentation is proportional to time but flow independent, it is the main deposition mechanism in small airways and the alveolar region. For particles of 1.3 and $3.5\text{ }\mu\text{m}$ aerodynamic diameter, thoracic deposition increased from 0.10 and 0.25 at 2 s tidal time, respectively, with increasing tidal time by about 0.02 and 0.10 s^{-1} (tidal time), respectively. The steeper increase of thoracic deposition of the large particles with increasing tidal time is reasonable, since the large particles settle faster by a factor of 7 than the small particles.

For $1.3\text{ }\mu\text{m}$ particles thoracic deposition did not change significantly with increasing characteristic inhaled flow from 200 to $600\text{ cm}^3\text{ s}^{-1}$. This is reasonable, since the inertia of these particles was so low that the impacted particle fraction in the airways was expected to be minute over the range of flow rates observed. However, for $3.5\text{ }\mu\text{m}$ particles thoracic deposition changed significantly from 0.31 at $100\text{ cm}^3\text{ s}^{-1}$ to 0.44 at $250\text{ cm}^3\text{ s}^{-1}$. This resulted from the increasing fraction of impacting particles in the airways with increasing flow. The monopodial branching of the canine airways suggested a more pronounced increase in deposition due to impaction with increasing inhaled flow when compared to the increase in deposition on the dichotomously

branching human airways. HEYDER *et al.* (1986) reported an increase of thoracic deposition of 3 μm particles from 0.36 to 0.55 for an increasing flow from 250 to 750 $\text{cm}^3 \text{s}^{-1}$. Considering the increasing deposition with the factor of increase in flow, the observed human and canine data are not significantly different.

From clearance analysis of the radioactively labelled $^{57}\text{Co}_3\text{O}_4$ particles the fast cleared fraction of the thoracic deposition was 0.02, 0.05 and 0.15 for particles of 0.7, 1.3 and 3.0–3.7 μm aerodynamic diameter, respectively. If we assume the fast cleared fraction to be the fraction deposited in the bronchial airways and the long-term retained fraction to be deposited in the alveolar region, then airways deposition relative to the inhaled aerosol would be 0.002, 0.008 and 0.05 for 0.7, 1.3 and 3.0–3.5 μm particles, respectively. These data are rather low but still in reasonable agreement with human data (STAHLHOFEN *et al.*, 1989). However, some concern has been expressed in the literature about the assumption that fast clearance might not represent the entire airways deposition. The dramatic increase of deposition of 3.5 μm particles with increasing flow rate we found suggests that the contribution of deposited particles by impaction resulting into airway deposition might be larger than 0.15 of the entire thoracic deposition determined by fast clearance.

Total deposition during breathing through the nose (Fig. 1. lower panel)

In the lower panel, total deposition increases faster with increasing aerodynamic diameter than in the upper panel, since nasopharyngeal deposition (mainly due to impaction) adds to the thoracic deposition. Deposition of particles larger than 1 μm obtained from the first three of the six dogs increased much faster than for the last three dogs. Interestingly, there was no significant difference in deposition within dogs 1–3 and within dogs 4–6. Taking into account that there was little variation of the thoracic deposition for a given aerodynamic size in 12 dogs—similar to human thoracic deposition (HEYDER *et al.*, 1986)—the differences observed between dogs 1–3 and dogs 4–6 were mainly due to differences of nasopharyngeal deposition. This was confirmed by the analysis of the expired aerosol, for particles larger than 2 μm the exhaled aerosol concentration dropped immediately after the beginning of exhalation in dogs 1–3 indicating high deposition in the nasopharyngeal region whereas in dogs 4–6 the aerosol concentration decreased more slowly in the initially expired air. In fact, from the 40 cm^3 of aerosol volume inspired at the end of inhalation and the 40 cm^3 expired first, the particle fraction penetrating the nasopharyngeal region could be estimated. When the thoracic deposition of the penetrating particles were calculated, the deposited fractions obtained from all six dogs were comparable to the measured thoracic deposition obtained from the dogs of group 2 and increased similarly with increasing aerodynamic diameter.

Endoscopical examination of the nasal cavity of dogs 3, 4 and 5 of group 1 indicated that dog 3 had narrower and more twisted turbinate airways than dogs 4 and 5. For the latter, the endoscope (5 mm diameter) could be penetrated into the larynx which was impossible for the former. These morphological observations support the differences in nasopharyngeal deposition we found in dog 3 vs dogs 4 and 5. The distinction between total deposition of dogs 1–3 vs dogs 4–6 was found by coincidence and indicates only the large intersubject variability; further investigations on other dogs applying the same aerosol photometric analysis confirmed the large variability of total deposition during nose breathing revealing fractions of total deposition of 0.12–0.95 for particles

of 2.8 μm aerodynamic diameter as a result of the large variability of nasopharyngeal deposition (HEYDER *et al.*, in press). The large variability of nasal deposition was also found in man (STAHLHOFEN *et al.*, 1989).

CONCLUSION

Based on a rigid analysis of individual breaths, this study shows that thoracic deposition in beagle dogs for inhaled particles in the aerodynamic domain is not significantly different from that observed in the human lungs. The large intersubject variability of total deposition during nose breathing was also similar in man and dog. The large intersubject variability was caused by the large intersubject variability of nasopharyngeal deposition in both man and dog.

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