Breath-by-breath measurement of particle deposition in the lung of spontaneously breathing rats

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Institute of Lung Biology and Disease, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany; and Molecular and Integrative Physiological Sciences, Harvard School of Public Health, Boston, Massachusetts

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Karrasch S, Eder G, Bolle I, Tsuda A, Schulz H. Breath-by-breath measurement of particle deposition in the lung of spontaneously breathing rats. J Appl Physiol 107: 1293–1299, 2009. First published July 30, 2009; doi:10.1152/japplphysiol.00096.2009.—A number of deposition models for humans, as well as experimental animals, have been described. However, no breath-by-breath deposition measurement in rats has been reported to date. The objective of this study is to determine lung deposition of micrometer-sized particles as a function of breathing parameters in the adult rat lung. A new aerosol photometry system was designed to measure deposition of nonhygroscopic, 2-μm sebacate particles in anesthetized, intubated, and spontaneously breathing 90-day-old Wistar-Kyoto rats placed in a size-adjusted body plethysmograph box. Instrumental dead space of the system was minimized down to 310 μl (i.e., ~20% of respiratory dead space). The system allows continuous monitoring of particle concentration in the expired volume. Breathing parameters, such as respiratory rate (f), tidal volume (VT), as well as inspiration/expiration times, were also monitored at different levels of anesthesia. The results showed that VT typically varied between 1.5 and 4.0 ml for regular breathing and between 4.0 and 10.0 ml for single-sigh breaths; f ranged from 40 to 200 breaths/min. Corresponding deposition values varied between 5 and 50%, depending on breath-by-breath breathing patterns. The best fit of deposition (D) was achieved by a bilinear function of VT and f and found to be D = 11.0 • 0.09•f + 3.75•VT. We conclude that our approach provides more realistic conditions for the measurement of deposition than conventional models using ventilated animals and allows us to analyze the correlation between breath-specific deposition and spontaneous breathing patterns.

INHALATION AND DEPOSITION of particulate matter in the lung has long been known as an important issue, not only regarding hazardous effects of pollutants (17), but also as an elegant and effective way for application of pharmaceuticals (9, 12, 20). At the same time, deposited dose has been shown to vary substantially between individuals, as well as depending on breathing parameters (6).

As exposure of humans to potentially harmful substances is not ethically feasible, animals, typically small rodents like rats or mice, are widely used as a model. A thorough understanding of particle behavior and deposition in the lungs of these model animals is essential for a correct estimation and interpretation of dose response and the effects on health in humans.

By now, a number of exposure techniques and methodological approaches have been described to execute inhalation experiments on animals (16). However, in small rodents, these experiments do not resolve breath-specific deposition. The precision of their results is, consequently, limited to calculated means of breathing parameters, as well as mean deposition values. This problem also affects theoretical deposition models derived from these experiments or their empirical validation, respectively. Hence, the obvious desideratum is an approach to measure single-breath deposition, accounting for detailed information on flow and volume parameters, preferably during natural breathing.

The objective of this study is the determination of breath-specific deposition in adult rats. For this purpose, we introduce an experimental technology that allows online breath-by-breath measurement of total lung deposition of particles in anesthetized, intubated, and spontaneously breathing rats. To focus on the impact of physiological parameters, we used well-defined stable, monodisperse 2-μm particles. Our setting provides 1) intrindividual variability of deposition with regard to physiologically occurring fluctuations of breathing; 2) interindividual variation of deposition when breath-specific parameters, in particular flow and volumes, are virtually identical; 3) empirical deposition data for validation and development of breath-specific particle deposition predictions in adult rat lungs.

MATERIAL AND METHODS

Animals. Wistar Kyoto (WKY) rats were bred and raised at the facility of the Institute of Lung Biology and Disease, Helmholtz Zentrum München, under a 12:12-h light-dark cycle and had water and food ad libitum. Measurements were carried out in six male animals at the age of 90 days, according to German federal guidelines for the use and care of laboratory animals and approved by the Government of the District of Upper Bavaria, as well as by the animal care and use committee of Helmholtz Zentrum München. Weight of the animals was 467.3 ± 45.2 g (mean ± SD). Based on lung function measurements done in our institute in WKY rats at the same age, total lung capacity (TLC) can be estimated to be 16.7 ml, and functional residual capacity (FRC), defined as relaxation volume, to be 4.1 ml (4). These volumes were also applied to assess 2-μm particle deposition in theoretical prediction models (3, 21).

Anesthesia and intubation. Anesthesia was induced by inhalation of isoflurane (5%) in oxygen for 1 min in a whole body box, followed by subcutaneous (sc) injection of a mixture of 75 mg/kg ketamine and 0.5 mg/kg medetomidine. Rats were intubated, as described by Brown et al. (7), using a plastic catheter with a length of 80 mm and an inner diameter of 1.8 mm (Braun MT, Braun, Melsungen, Germany). After measurements, the animals were euthanized with an overdose of pentobarbital sodium.

Spontaneous fluctuations of breathing parameters during experiments, including substantial differences in tidal volume (VT) due to sporadic sigh breathing, allowed evaluation of breath-specific deposition in relation to varying respiratory patterns, including breathing frequency (f), VT, inspiratory time (Ti), peak inspiratory flow (PIF), etc.
peak expiratory flow (PEF), mean inspiratory flow (MIF), and mean expiratory flow (MEF).

**Technical setup.** The animals were placed on a heating plate adjusted to 37°C in a body plethysmograph box (Buxco model PLY4114) of 3.16-liter volume, and the endotracheal tube was connected to the photometer unit (dead space 160 μl) via a custom-made adapter (Fig. 1). At the opposite opening of the photometer, a metal tube with a length of 7 mm and an inner diameter of 2 mm protruded into a plastic tube with an inner diameter of 21 mm that provided a continuous stream of aerosol at ~170 times of rat minute ventilation during experiments to ensure a constant inhaled particle concentration. Total instrumental dead space between aerosol supply and endotracheal tube could be minimized to 310 μl [i.e., about one-fifth of adult rat pulmonary dead space (4)], while the instrumental resistance was kept considerably low at one-sixth of respiratory system resistance of adult rats (0.02 vs. 0.14 cmH2O·ml−1·s−1). In the photometer, a PM12 laser (Power Technology, Little Rock, AR) with a wavelength of 635 nm, as well as a R7400U subminiature photomultiplier (Hamamatsu) with a scattering volume of 3.1 μl and a scattering angle of 90°, were used. As aerosol, we used monodisperse nonhygroscopic 2-μm droplets of di-2-ethylhexyl sebacate generated by a system, as described by Stahlhofen et al. (23), at a concentration of 3.4·10^5 P/cm³. At this concentration, the noise, as expressed by coefficient of variation, was 3.7%, i.e., the signal-to-noise ratio was maximized to ~27. The inevitably remaining scatter is mainly caused by variations in particle number concentration in the aerosol stream. Only 0.002% of the noise was caused by electric circuits of measurement instruments. The photometer signal has been shown to be proportional to particle concentration within the concentration range of the conducted experiments for 2-μm particles, as shown in Fig. 2, as well as to be independent of flow direction and flow velocity.

**Data sampling.** Particle size was monitored via determination of settling velocity in a sedimentation cell (22) with a number of eight particles per sample using a custom-made PC program by Helmholtz Zentrum München. Pressure in the plethysmograph box and photometer signal were continuously monitored at a frequency of 500 Hz using Vision XP Portable Data Acquisition System (LDS Test and Measurement GmbH, Ismaning, Germany).

Typical primary experimental data, as gained from single-animal measurements, are shown in Fig. 3. Note that the pauses between breaths during slow breathing show minor oscillations due to cardiac action and are neglected for further calculation. We observed that, during spontaneous breathing, rats continuously exert unsystematic, temporary imbalances between inspired and expired volume (cf. Fig. 4), which is accounted for by a difference term in the deposition model, as described below.

**Data analysis.** Statistical analyses were performed using the commercial statistical package Statgraphics (Statistical Graphics, Rockville, MD). From the obtained data, the following breathing parameters were calculated per every breath: inspiratory (VTi) and expiratory VT (VTE), TI and expiratory time (TE), the relation of TI to breathing cycle time (TT), f, PIF, PEF, MIF, and MEF. Breath-specific deposition (D) was calculated by integrating particle concentration as measured by the photometer over respired volume (Eq. 1). The number of breaths analyzed per animal ranged from 930 to 1,443, and mean was 1,159.

$$ D = 1 - \int_{V_{in}}^{V_{ex}} \frac{CdV}{CdV} \left(1 \right) $$

where C is particle concentration, and V is respired volume minus instrumental dead space, ex is expired, and in is inspired (i.e., 150 μl; Fig. 4).

![Fig. 1. Schematic diagram of experimental setup. For details, see text.](image)

![Fig. 2. Plot of photometer signal against particle concentration (signal amplification different from animal exposure experiments). The latter was measured with a Condensation Particle Counter model 3022A (Thermo System, Shoreview, MN) at a flow of 0.3 l/min.](image)
RESULTS

Representative primary data: intra- and interindividual variability of deposition in relation to breathing patterns. Typically, f ranged between 40 and 200 breaths/min, and VT between 1.5 and 4 ml. Additionally, we observed casually occurring sigh breaths with a VT of 4 up to 10 ml and corresponding lower f. Examples of the relationship between f and deposition or VT and deposition, respectively, are given in Fig. 5, A and B. The graphs show that deposition primarily is determined by VT, while the relation to f is obviously weaker.

Fig. 3. Exemplary traces of photometer signal, airflow, and lung volume curve. Inspiration can be identified by a positive signal in the top graph. A sigh breath is shown in the right graph.

Fig. 4. Part of the aerosol concentration curve used for integration. The gap around the turning point corresponds to the excluded volume of instrumental dead space, which corresponds to 150 µl due to the instrumental dead space.

Fig. 5. A: primary deposition (D) data as a function of tidal volume (VT) for a single animal without adjustment for other breathing parameters. B: primary D data as a function of breathing frequency (f) for a single animal without adjustment for other breathing parameters.
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Breath-Specific Particle Deposition Measurement in Rats

Table 1. Breathing parameters during shallow anesthesia

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>f, breaths/min</th>
<th>Vt, ml</th>
<th>Ti, ms</th>
<th>Ti/TT</th>
<th>PIF, ml/s</th>
<th>PEF, ml/s</th>
<th>MIF, ml/s</th>
<th>MEF, ml/s</th>
<th>D, %</th>
<th>CV of D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>111±16</td>
<td>2.1±0.2</td>
<td>309±33</td>
<td>0.579±0.053</td>
<td>11.0±1.5</td>
<td>17.7±1.9</td>
<td>6.7±0.9</td>
<td>9.4±2.0</td>
<td>8.5±2.6</td>
<td>0.31</td>
</tr>
<tr>
<td>2</td>
<td>99±10</td>
<td>2.2±0.1</td>
<td>309±34</td>
<td>0.531±0.046</td>
<td>10.3±0.9</td>
<td>15.8±0.9</td>
<td>7.1±0.7</td>
<td>8.0±1.1</td>
<td>12.8±5.8</td>
<td>0.45</td>
</tr>
<tr>
<td>3</td>
<td>113±13</td>
<td>2.0±0.2</td>
<td>327±25</td>
<td>0.493±0.037</td>
<td>12.6±1.8</td>
<td>18.5±1.6</td>
<td>8.7±1.2</td>
<td>8.5±1.1</td>
<td>9.7±3.1</td>
<td>0.32</td>
</tr>
<tr>
<td>4</td>
<td>117±25</td>
<td>2.2±0.2</td>
<td>311±54</td>
<td>0.599±0.035</td>
<td>10.9±1.9</td>
<td>18.6±2.3</td>
<td>7.2±1.7</td>
<td>10.9±3.0</td>
<td>11.4±3.3</td>
<td>0.29</td>
</tr>
<tr>
<td>5</td>
<td>108±10</td>
<td>1.8±0.1</td>
<td>257±19</td>
<td>0.492±0.036</td>
<td>10.4±1.3</td>
<td>14.3±1.4</td>
<td>6.9±0.7</td>
<td>6.8±0.9</td>
<td>6.1±4.2</td>
<td>0.68</td>
</tr>
<tr>
<td>6</td>
<td>96±7</td>
<td>2.1±0.1</td>
<td>290±25</td>
<td>0.501±0.038</td>
<td>11.3±1.3</td>
<td>15.0±0.7</td>
<td>7.4±0.7</td>
<td>7.4±0.8</td>
<td>6.7±2.0</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Values are means ± SD. f, Breathing frequency; Vt, tidal volume; Ti, inspiratory time; Ti/TT, relation of Ti to breathing cycle time; PIF, peak inspiratory flow; PEF, peak expiratory flow; MIF, mean inspiratory flow; MEF, mean expiratory flow; D, deposition; CV, coefficient of variation.

To assess the impact of breathing patterns on deposition, three distinct breathing patterns were identified based on their occurrence: 1) breathing with higher Vt (2.5–4 ml) and lower f (40–80 breaths/min), corresponding to a state of deep anesthesia (18.3% of evaluated breathing parameters during shallow anesthesia) and very much comparable to physiological breathing of awake animals (62.2% of evaluated breathing conditions); 2) a pattern with lower Vt values (1.5–2.5 ml) and higher f (80–160 breaths/min), corresponding to a shallow anesthetized state and very much comparable to physiological breathing of awake animals (34.4% of evaluated breathing conditions); and 3) sighs (defined as single deep breaths with a large Vt and low f, 0.6% of evaluated breathing conditions).

Overall, 81.1% of total breaths were thus included in the analysis.

Deposition values obtained for the different breathing patterns are summarized in Tables 1–3. Average deposition values during shallow and deep anesthesia and during sigh breathing were 9.2 ± 0.3, 15.8 ± 1.1, and 43.4 ± 1.8%, respectively. Thus deposition increases by more than a factor of 4 with increasing Vt, from 2.1 to 7.1 ml, while f was decreasing from 107 to 34 breaths/min. Corresponding changes in mean minute ventilation are comparatively smaller, ranging between 221.7 ml/min (shallow anesthesia) and 173.7 ml/min (deep anesthesia) and thus changed by 22%. This finding suggests that it is more suitable to use Vt and f rather than minute ventilation to assess the impact of breathing on particle deposition.

A detailed overview of breathing parameters and corresponding deposition values is given in Tables 1–3. The intra-individual variability of breath-by-breath deposition, as expressed by the coefficient of variation (last column in Tables 1–3) was highest during rapid and shallow breathing (shallow anesthesia) and ranged between 30 and 45%, with an extreme value of 69% in one animal. The variability decreased with increasing deposition values. It ranged between 13 and 22% during slow and deep breathing (deep anesthesia) and 3 and 7% during sigh breathing.

The interindividual deposition variability (last row in Tables 1–3) was comparable among the three different breathing conditions. Lowest and highest average deposition values determined in the six rats differed by a factor of 2 for shallow and deep anesthesia and 1.6 for sigh breathing. Consistently, the coefficients of variation were found to be 0.28, 0.25, and 0.19, respectively. Since the breathing pattern was comparable between the six individuals for each of the three different breathing conditions depicted, the observed interindividual variability is considered to be primarily related to differences in structural and/or functional lung architecture.

Empirical model. Deposition was first described by a polynomial model, including all breathing parameters listed above. As slight unsystematic oscillations in the balance between Vti and Vte during spontaneous breathing were observed (Fig. 6), the difference between these volumes was included in the empirical model. Statistical analysis revealed that there was a close correlation within flow and volume parameters (Vt, PIF, PEF, MIF, MEF), as well as within timing parameters (f, Ti, Te, Ti/TT). Hence, starting with a comprehensive linear regression equation covering the full spectrum of breathing parameters, breathing parameters were excluded in a stepwise manner if they were shown to have no or only minor impact on the prediction quality, as defined by $r^2$, to achieve an applicable deposition model.

Table 2. Breathing parameters during deep anesthesia

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>f, breaths/min</th>
<th>Vt, ml</th>
<th>Ti, ms</th>
<th>Ti/TT</th>
<th>PIF, ml/s</th>
<th>PEF, ml/s</th>
<th>MIF, ml/s</th>
<th>MEF, ml/s</th>
<th>D, %</th>
<th>CV of D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53±12</td>
<td>3.4±0.3</td>
<td>298±41</td>
<td>0.495±0.042</td>
<td>16.9±2.4</td>
<td>25.2±1.3</td>
<td>11.5±1.9</td>
<td>11.0±1.2</td>
<td>12.3±2.4</td>
<td>0.20</td>
</tr>
<tr>
<td>2</td>
<td>62±10</td>
<td>3.1±0.2</td>
<td>304±39</td>
<td>0.475±0.030</td>
<td>15.7±1.2</td>
<td>21.0±1.3</td>
<td>10.3±1.3</td>
<td>9.2±1.0</td>
<td>24.5±5.3</td>
<td>0.22</td>
</tr>
<tr>
<td>3</td>
<td>73±4</td>
<td>2.9±0.2</td>
<td>240±27</td>
<td>0.471±0.025</td>
<td>18.3±1.1</td>
<td>25.7±1.4</td>
<td>12.2±1.1</td>
<td>10.7±0.8</td>
<td>21.5±2.7</td>
<td>0.13</td>
</tr>
<tr>
<td>4</td>
<td>56±5</td>
<td>2.8±0.2</td>
<td>279±22</td>
<td>0.505±0.032</td>
<td>14.9±1.5</td>
<td>22.7±1.7</td>
<td>9.9±1.1</td>
<td>9.9±1.2</td>
<td>17.5±2.3</td>
<td>0.13</td>
</tr>
<tr>
<td>5</td>
<td>64±4</td>
<td>2.6±0.1</td>
<td>259±25</td>
<td>0.460±0.026</td>
<td>14.3±1.0</td>
<td>20.7±1.0</td>
<td>10.3±1.0</td>
<td>8.6±0.7</td>
<td>18.7±3.2</td>
<td>0.17</td>
</tr>
<tr>
<td>6</td>
<td>45±4</td>
<td>3.0±0.3</td>
<td>282±20</td>
<td>0.470±0.026</td>
<td>16.0±1.7</td>
<td>21.0±1.8</td>
<td>10.8±1.2</td>
<td>9.4±1.2</td>
<td>13.8±2.5</td>
<td>0.18</td>
</tr>
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</table>

Values are means ± SD.
Finally, a linear model was found to describe deposition as a function of $V_T$, $f$, and volume balance by

$$D = a_0 + a_1 \cdot V_T + a_2 \cdot f + a_3 \cdot \Delta V_T$$  \hspace{1cm} (2)

for each of the six animals. The goodness of the fits was $r^2 = 0.81 \pm 0.15$ (mean $\pm$ SD).

An example of breath-specific deposition prediction gained by this calculation is given in Fig. 7 for a single animal. Provided that inspiration and expiration are balanced over a longer period of time, the last term of Eq. 2 approximates zero.

As each of the equations obtained for individual rats resolves predicted deposition in a breath-specific manner based on given $f$ and $V_T$, 25 defined combinations of these determinants have been chosen within the physiological range ($f$: 80–150 breaths/min, $V_T$: 2.0–3.5 ml). For each combination of $f$ and $V_T$, six deposition predictions were calculated according to the six prediction equations of the individual animals.

The average of the six predicted deposition values for each parameter combination is shown in Fig. 8. Based on these deposition values, a summary regression calculation was then applied to gain the final prediction equation incorporating breath-specific deposition of all six animals. It was calculated to:

$$D = 11.00 - 0.09 \cdot f + 3.75 \cdot V_T$$  \hspace{1cm} (3)

### DISCUSSION

An experimental setup has been developed that allows breath-by-breath measurement of lung particle deposition in adult rats using aerosol photometry. The technical challenge of precise measurement at this scale was achieved by miniaturizing the system down to an instrumental dead space of only 310 μl, corresponding to about one-fifth of the physiological dead space of adult rats (4). The system allows breath-specific deposition measurement in anesthetized, spontaneously breathing rats and thus the direct attribution of deposition values to defined characteristics of single breaths.

Our approach to estimate particle deposition in mammalian lungs provides more realistic conditions than conventional models using ventilated animals (25). It further enables us to specifically target deposition efficiency in the isolated lung (tracheobronchial plus alveolar fraction of particle deposition) compared with whole body (15) or nose-only (8) exposures. The chemically inert, nonhygroscopic properties of the di-2-ethylhexyl sebacate used here ensure the availability of well-defined monodisperse 2-μm particles. One limitation of our study is the necessity of anesthesia, causing slightly modified breathing patterns compared with awake animals. However, it showed that resulting alterations are comparable to the range of spontaneously occurring respiratory fluctuations (5).

For this study, ~7,000 breaths obtained in six rats were evaluated. The states of breathing were categorized into three groups, during deep anesthesia (high $V_T$ and low $f$), during

### Table 3. Breathing parameters for sigh breaths

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>$f$, breaths/min</th>
<th>$V_T$, ml</th>
<th>$T_i$, ms</th>
<th>$T_i/T_T$</th>
<th>PIF, ml/s</th>
<th>PEF, ml/s</th>
<th>MIF, ml/s</th>
<th>MEF, ml/s</th>
<th>$D$, %</th>
<th>CV of $D$</th>
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<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>6.5</td>
<td>548</td>
<td>0.563</td>
<td>18.8</td>
<td>33.9</td>
<td>11.9</td>
<td>14.9</td>
<td>33.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>38 ± 3</td>
<td>7.2 ± 0.9</td>
<td>502 ± 16</td>
<td>0.563 ± 0.030</td>
<td>26.7 ± 3.5</td>
<td>32.2 ± 1.4</td>
<td>14.4 ± 1.5</td>
<td>17.4 ± 0.3</td>
<td>53.6 ± 4.0</td>
<td>0.07</td>
</tr>
<tr>
<td>3</td>
<td>35 ± 1</td>
<td>7.5 ± 0.3</td>
<td>518 ± 14</td>
<td>0.619 ± 0.017</td>
<td>25.3 ± 1.5</td>
<td>41.9 ± 0.6</td>
<td>14.5 ± 0.6</td>
<td>22.3 ± 1.1</td>
<td>50.3 ± 1.8</td>
<td>0.04</td>
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<tr>
<td>4</td>
<td>26 ± 4</td>
<td>7.6 ± 0.6</td>
<td>536 ± 35</td>
<td>0.595 ± 0.041</td>
<td>23.5 ± 2.8</td>
<td>38.7 ± 1.0</td>
<td>14.2 ± 1.5</td>
<td>19.8 ± 1.9</td>
<td>45.6 ± 2.0</td>
<td>0.04</td>
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<tr>
<td>5</td>
<td>67 ± 4</td>
<td>4.3 ± 0.3</td>
<td>496 ± 31</td>
<td>0.581 ± 0.022</td>
<td>14.0 ± 0.2</td>
<td>23.5 ± 1.1</td>
<td>8.7 ± 0.6</td>
<td>12.0 ± 1.0</td>
<td>35.6 ± 1.0</td>
<td>0.03</td>
</tr>
<tr>
<td>6</td>
<td>15 ± 2</td>
<td>9.4 ± 0.6</td>
<td>583 ± 13</td>
<td>0.530 ± 0.010</td>
<td>27.9 ± 3.2</td>
<td>38.2 ± 1.0</td>
<td>16.1 ± 0.7</td>
<td>17.3 ± 0.4</td>
<td>42.2 ± 3.0</td>
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Values are means ± SD.

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<th>Intraindividual variability</th>
<th>Mean</th>
<th>$\pm$ SD</th>
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<tbody>
<tr>
<td></td>
<td>34 ± 19</td>
<td>7.1 ± 1.7</td>
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<table>
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<th>Interindivdual variability</th>
<th>Mean</th>
<th>$\pm$ SD</th>
</tr>
</thead>
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<tr>
<td></td>
<td>0.81 ± 0.15</td>
<td>(mean ± SD)</td>
</tr>
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</table>

**Fig. 6.** Particle D as a function of volume imbalance for shallow breathing pattern (90 < $f$ < 110 breaths/min; 2.8 < $V_T$ < 3.2 ml). Note that the volume difference ($\Delta V_T$) is balanced on the whole, while there are higher single values on the positive side, indicating stronger shifts to higher lung volumes that are then gradually returning to the baseline in an unsystematic manner.

**Fig. 7.** Observed D values vs. those predicted by the corresponding regression equation (%). Exclusion of sigh breaths has only negligible effect on the prediction equation due to their low number compared with breaths of other breathing patterns. $V_{Ti}$, inspiratory $V_T$; $V_{Te}$, expiratory $V_T$. 

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Fig. 8. Breath-specific calculated D values as a function of VT and f (n = 6) for physiological ranges of VT and f (mean ± SD). Predicted D was calculated with nine combinations of f and VT for each of the animals using their respective prediction equation. In this case, SD in an indicator of interindividual D variability.

shallow anesthesia (low VT and high f), and sigh, and lung deposition was measured during these three states of breathing. Average deposition in these animals was 18.1 ± 4.6% during deep anesthesia, 9.2 ± 2.6% during shallow anesthesia, and 43.4 ± 8.1% for sigh breaths. Whereas these values are in the range of published experimental lung deposition data in rats from analogous experimental conditions (e.g., similar particle size, comparable breathing patterns) (2, 18, 19), direct comparison is not possible, because most of the studies reported in the literature are nose-breathing exposures, which include the deposition in the nose.

Our deposition data are lower than the predicted values (ranging between 20 and 28%) by theoretical models for the shallow breathing pattern (3, 10, 21). This discrepancy might be related to certain assumptions that theoretical models rely on and that might not apply to our experimental setting, which is discussed below.

Most models idealistically assume isovolumetric relationships between alveolar space and conducting airway volume when scaling morphometric data from TLC to the actual level of lung inflation during breathing. This results in a disproportionate narrowing of conducting airways, which might result in an overestimation of particle impaction and/or gravitational deposition regarding larger particles at micrometer scale.

We often observed a defined pause after each breath, particularly during slower breathing, and, therefore, the actual TT is significantly shorter than the time modeled for the respective f. In this case, the theoretical model predictions would overestimate the residence time of particles for sedimentation and hence gravitational particle deposition in the lung.

Furthermore, in the theoretical models, the value of FRC is an important parameter for their calculations; the models usually set FRC at 25% of TLC. However, this assumption is only reasonable for slower breathing, corresponding to a pattern of deep anesthesia, where FRC actually equals relaxation volume (3, 19). For fast breathing, which corresponds to a pattern of shallow anesthesia, however, a shift upward may occur due to incomplete expiratory relaxation [described in mice (24) and most probably also occurring in rats (11)]. This would mean that, in the case of our “shallow anesthesia,” FRC exceeds relaxation volume; therefore, the predictions of theoretical models would overestimate the real lung deposition, which we measured.

An interesting finding of our breath-by-breath analysis is that minute ventilation alone, as often used in deposition studies, is not a sufficient parameter to estimate deposited particle fraction, as shown in Tables 1–3. At maximum, mean deposition increases by a factor of ~4 from shallow breathing to sigh breath (9.2 vs. 43.4%), despite the fact that mean minute ventilation is almost identical. Comparing shallow with deep anesthesia deposition doubles with a decrease of minute ventilation by 22%. Hence, we recommend defining breathing patterns by f and VT instead of minute ventilation in pharmacological or toxicological studies. These findings enrich recent recommendations for estimation of delivered dose, defined as the dose inhaled into the respiratory system (1), since our approach gives an estimate of the dose actually deposited in the lung.

A clear strength of the presented approach is the possibility to experimentally identify the most relevant breath-defining parameters for deposition of 2-μm particles. It reveals that sophisticated characterization of breathing by additional timing and flow parameters does not substantially improve estimates for particle deposition, a finding that might also enrich future deposition models. Moreover, our setting allows assessment of deposition variability between single breaths, even when breath-defining parameters, such as respiratory time and volume, are virtually identical. Such information is of particular significance for pulmonary drug delivery, as this is often done within one or only a few breaths.

With respect to the three different breathing types, it is especially striking that, in each individual rat, the coefficient of variation for deposition substantially decreased with increasing particle deposition due to transition of breathing from shallow anesthesia to sigh breathed, e.g., for rat 1 from 0.31 to 0.07. In contrast, interindividual variability, as expressed by coefficient of variation of mean deposition values obtained for each breathing condition, varied only little, i.e., it decreased from 0.28 (shallow anesthesia) to 0.25 (deep anesthesia) and to 0.19 (sighs). This suggests that individual morphological features of the lungs, either fixed or, probably more important, functional (13, 14), may contribute to deposition variability, which has so far not been addressed in theoretical prediction models.

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REFERENCES


