Size-dependent deposition of inhaled nanoparticles in the rat respiratory tract using a new nose-only exposure system

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ABSTRACT
Concerns about the potential health effects of exposure to nanomaterials have led to a growing number of in vivo inhalation toxicity studies using nanoparticle aerosols. Estimates of aerosol deposition within the respiratory tract are important for these studies to enable: (a) the interpretation of the results, in particular, the evaluation of dose-response relationships; (b) comparison with the results of other related studies; and (c) the extrapolation of results from animal models to human. Unfortunately, only a limited number of studies have been undertaken to investigate respiratory tract deposition efficiencies for nano-sized aerosol particles. This is of particular importance as deposition efficiencies are predicted to vary significantly over the nano-size range for some elements of the respiratory tract. In this study, female Wistar-Kyoto rats were exposed in a new design nose-only inhalation exposure system to spark generated radioactive iridium-192 nanoparticle aerosols of four particle sizes chosen to cover the majority of the nano-size range (nominal sizes: 10, 15, 35, and 75 nm). The content of iridium-192 in the lung, head, gastrointestinal tract, and various other organs and tissues was measured. Aerosol deposition efficiencies in the whole respiratory tract and components (head airways, lung, alveolar region, and tracheobronchial region) were estimated and compared with the predictions of the Multiple Path Particle Dosimetry (MPPD) model (v2.11). The experimentally derived deposition efficiencies were broadly consistent with, but typically higher than, model predictions and the results of comparable studies in the literature.

Introduction
Detailed evaluation of the response of animals to an inhaled toxic or therapeutic material aerosol requires knowledge of the dose of the material deposited in the respiratory tract, and quantification of deposited dose is essential for the comparison of results between different studies. Many factors influence the amount and the site of deposition. The characteristics of the aerosol, most importantly particle size, and animal specific factors, in particular breathing pattern and the geometry of the respiratory tract, all play a role in determining the deposition pattern.

Rapid developments in the field of nanotechnology and associated concerns about the potential health effects of exposure to nanomaterials have led to a growing number of in vivo inhalation toxicity studies using nanoparticle aerosols. Unfortunately to date, few experimental studies have investigated the respiratory tract deposition efficiencies of such particles. This is of particular importance as deposition efficiencies are predicted to vary significantly over the nano-size range for some elements of the respiratory tract (Cassee et al. 1999).

An increasing number of reported nanoparticle inhalation studies use the Multiple Path Particle Dosimetry (MPPD) model (MPPD v2.11 2009) to estimate deposited doses. The MPPD software includes models for aerosol particle deposition in the human and rat respiratory tract. The deposition model for the rat is based upon the Anjivel and Asgharian (1995) multiple path deposition model, with minor modifications. For each airway, theoretically derived formulae are used to determine deposition by diffusion, sedimentation, and impaction. Deposition within the head is determined using empirically derived functions. While the model is generally considered robust, providing a reasonable fit to available experimental data for aerosols in the micron and submicron range (e.g., Anjivel and Asgharian 1995), the limited database of experimental deposition...
measurements for nano-sized particles makes it difficult to fully validate model predictions at these small particle sizes. It was considered, therefore, that the field would benefit from additional experimental results in this area.

The objective of this study was to determine particle size-dependent aerosol deposition efficiencies for nanosized particles in components of the rat respiratory tract. Rats were exposed in a nose-only system to radioactive iridium-192 nanoparticle aerosols of four sizes chosen to cover the majority of the nano-size range (nominal sizes: 10, 15, 35, and 75 nm). The content of iridium-192 in the lung, head, gastrointestinal tract (GIT), and various other organs and tissues was measured following exposure. Aerosol deposition fractions in the head airways, total lung, alveolar region, and tracheobronchial region were derived from these data and compared with the predictions of the MPPD model (MPPD v2.11 2009).

Materials and methods

Experimental design

Details of the experimental plan are presented in Table 1. The intention was for the exposure duration to be the same (1 h) for all aerosols, however, the requirement to deliver a measureable quantity of radionuclide, and limitations imposed due to radioactive decay of the iridium-192 electrodes, resulted in longer exposure durations for some of the groups, although all were below 3 h.

| Nominal aerosol particle diameter (nm) | Exposure duration (min) | Total number of animals | Group size |
|---------------------------------------|-------------------------|-------------------------|------------|
| 10                                    | 120                     | 10                      | 6 4        |
| 15                                    | 75                      | 15                      | 10 5       |
| 35                                    | 156                     | 12                      | 6 6        |
| 75                                    | 60                      | 5                       | 5          |

Sacrificed within 15 min of end of exposure.

A schematic diagram of the inhalation exposure system used is shown in Figure 1. Aerosols of nano-sized radioactive iridium particles were generated using a spark generator (DNP 4000, Palas, Karlsruhe, Germany) with radioactive iridium electrodes held in custom made electrode holders. Spark generators have been used to successfully generate metallic nanoparticles from a wide range of conductive materials, including iridium (Sammeller-Behnke et al. 2012). Briefly, primary particles, which for iridium are a few nanometers in diameter, are made by the homogenous nucleation of vapor produced by arcing between two electrodes in an inert argon atmosphere. Inside the sparking chamber, the primary particles rapidly coagulate to form aggregates and agglomerates (from here on referred to simply as agglomerates). The rate of primary particle production and the initial agglomerate size distribution are dependent on the sparking frequency, with agglomerate size increasing with sparking frequency.

The aerosol from the generator passes into a stainless steel neutralizing and mixing chamber, which contains a krypon-85 source (NER8180 capsule, 740 MBq (26/10/2009), Eckert & Ziegler Isotope Products GmbH, Berlin, Germany), where it is mixed with oxygen and nitrogen. Before mixing the oxygen and nitrogen were humidified

![Figure 1. Schematic of exposure system (note: chamber used for 75 nm aerosol only and TEOM non-functioning for 15 nm aerosol).](image)

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using a Nafion® gas dryer (Perma Pure PD-Series™, Toms River, NJ, USA) to achieve a relative humidity after mixing with the aerosol of at least 30%. Due to aging effects, the agglomerate size after the mixing chamber was influenced by the total gas flow rate, with lower flows typically resulting in larger agglomerates. The flow rates were therefore set to produce the required agglomerate size while ensuring an oxygen concentration of at least 19%, in line with OECD guidelines (OECD 2009). To achieve the largest agglomerate particle size additional aerosol aging was required and a 3.8 L stainless steel aging chamber, with an aerosol residence time of about 45 s, was introduced after the neutralizing and mixing chamber.

The resulting aerosol enters a custom built nose-only exposure manifold (EMMS, Bordon, UK) developed in collaboration with the supplier specifically for use with nanomaterials; designed to deliver a homogenous aerosol to the breathing space of up to 36 animals with an aerosol size distribution variation of less than ±5% and a concentration variation of less than ±20% between animals. The complete exposure manifold consists of four nose-only exposure chambers, each with nine ports. Depending on the number of animals to be exposed between 1 and 4 chambers can be used, with those not in use isolated from the system. Rotating fittings are used at the aerosol inlet and outlets allowing the chambers to rotate for easy loading and monitoring of the animals. The chambers are made from stainless steel and anodized aluminium to minimize static losses and reduce the possibility of contamination. Inside each exposure chamber, the aerosol enters a central cylinder where it is directed to the animal ports by a flow splitting cone (Figure S1 in the online supplementary information [SI]). The rats are held in restraining tubes attached to the chamber and the aerosol flow is directed to the nose area via individual tubes at the bottom of the cone. Aerosol exhaust flow (exhaled and excess aerosol flow) is separated from the inlet flow to avoid re-circulation and drawn out through an outer cylinder to the exhaust. The aerosol exhaust is high-efficiency particulate air (HEPA) filtered and the exposure manifold is inside a glove box held at a negative pressure of −3 inches H₂O to provide secondary containment of the aerosol.

Flow rates were set to ensure delivery of at least two to three times the respiratory minute volume of the exposed animals (i.e., at least 0.5 L/min per exposure port) (OECD 2009). The oxygen concentration, temperature, and relative humidity of the gas delivered to the exposure chamber was continuously measured during exposures using a MX300 medical oxygen analyser (Teledyne Analytical Instruments, City of Industry, CA, USA) and a HMT333 temperature/relative humidity sensor (Vaisala, Boulder, CA, USA).

**Nanoparticle aerosol characterization**

Aerosol activity concentrations were determined using Pallflex® emfab™ filters (Pall Life Sciences, Ann Arbor, MI, USA) held in a 47 mm anodized aluminium filter holder (Pall Life Sciences, Ann Arbor, MI, USA). The aerosol was drawn through the filter at between 0.2 and 1.2 L/min for between 30 s and 2 min, depending upon the electrode specific activity and aerosol mass concentration. A TEOM™ ambient particulate monitor (Model 1400a, Thermo Scientific, Franklin, MA, USA) was used to continuously monitor the aerosol mass concentration delivered to the exposure manifold at a sampling flowrate between 0.5 and 1 L/min for all aerosols except the 15 nm, when it malfunctioned. The number concentration and particle size distribution of the aerosol delivered to the exposure manifold (diluted to prevent coagulation) were also continuously measured using a condensation particle counter (CPC model 3775, TSI Inc., Shoreview, MN, USA) and a scanning mobility particle sizer (SMPS model 3936N76, TSI Inc., Shoreview, MN, USA) with a differential mobility analyser (DMA) (model 3081, TSI Inc., Shoreview, MN, USA) for the 75 nm aerosol and a nano-DMA (N-DMA model 3085, TSI Inc., Shoreview, MN, USA) for the others. The morphology of the aerosol particles was determined with high-resolution transmission electron microscopy (TEM) (JEOL 3000F, JEOL Inc., Tokyo, Japan). Samples for TEM were taken directly onto 400 mesh copper TEM grids with lacey carbon film using an electrostatic precipitator (TSI 3089 nanometer aerosol sampler, TSI Inc., Shoreview, MN, USA).

Using the standard experimental configuration described above, the aerosol delivered to the manifold was continuously monitored during exposures; however, it was not possible to monitor the aerosol delivered to the exposure chamber ports (point of administration (POA)) during exposures. It was therefore necessary to undertake studies to compare the characteristics of the aerosol delivered to the exposure manifold with that delivered to each port, for each aerosol particle size, to identify any potential differences due to deposition losses and coagulation. The system was operated without animals and sampling was undertaken from both locations, using the characterization instrumentation as described above. Studies were also undertaken using this setup to demonstrate consistency in delivered aerosol (particle size distribution and number concentration) across exposure ports for each exposure chamber.

**Iridium electrodes**

Iridium electrodes comprising 5 mm lengths of 0.8 mm diameter iridium wire (99.9% purity, Goodfellow
Cambridge Ltd., Huntingdon, UK) were neutron activated at the Imperial College Reactor Centre, Ascot, UK. The primary radionuclide produced is iridium-192, which has a half-life of 74 days and decays principally by beta emission producing associated gamma rays of various energies (Browne and Firestone 1986). The iridium-192 content of each electrode was approximately 250 MBq at delivery.

**Animals**

All procedures involving the animals were performed in accordance with the Animals (Scientific Procedures) Act 1986. The animals used were female rats (Wistar-Kyoto (WKY/NHsd), Harlan, UK). At the time of exposure the animals were aged between 8 to 12 weeks, weighing between 130 and 180 g (average 174 ± 19 g). Food (Type RMI, Special Diet Services, Witham, Essex, UK) and water were freely available at all times pre- and post-exposure. On completion of the exposure those rats to be sacrificed at 24 h were removed from their restraining tubes and maintained in standard stock cages. The remaining rats were euthanized immediately (i.e., within 15 min) on completion of the exposure. Animals were euthanized by lethal injection of pentobarbital sodium administered intraperitoneally. Dissection followed an established procedure to reduce the possibility of cross contamination. The abdomen and thoracic cavity were opened to expose the larynx, the trachea was tied off close to the larynx, and then the peritoneal cavity was opened to expose the larynx, the trachea was tied off and the larynx was further dissected into lobes and for a number of animals samples of blood were taken. The organs, tissues, and remaining carcass were weighed and placed in sample containers and refrigerated prior to radioactive counting.

**Radioactive sample counting**

The iridium-192 content of each tissue sample was measured using either a 1480 Wizard™ 3” Automatic Gamma Counter using MultiCalc software (Perkin Elmer, MA, USA) for small tissue samples or a Scintiwell Counter (ADL, Cambridge Ltd., Huntingdon, UK) for larger samples. The iridium-192 content (Bq) of a sample was determined by subtracting the background count rate from the sample count rate for the detector and dividing by the appropriate (i.e., counter and sample type) detection efficiency (Table S1 in the SI). Sample activities were normalized to the day of the exposure by correcting for radioactive decay. Minimum detectable activity (MDA) levels were calculated for each sample using the method of Currie (1968) and only values above the MDA reported.

**Deposition efficiency calculations**

The respiratory tract is a dynamic system with clearance mechanisms active during and post-exposure, which complicates the derivation of deposition efficiencies. Typically for short exposures, deposition efficiency within the lung, $DE_L$, is derived by dividing the mass (or equivalent quantity such as radionuclide content) of material in the lung immediately post-exposure by the mass (or equivalent) of material "delivered" to the lung, as indicated in Equation (1) for radioactive materials,

$$DE_L = \frac{A_L}{AC \times BR \times T}, \quad [1]$$

where $A_L$ is the radionuclide content in the lung (Bq), immediately post-exposure, $AC$ is the radionuclide concentration of the aerosol (Bq/m³), $BR$ is the breathing rate (mL/min), and $T$ is the exposure duration (min). However, this approach ignores material that is cleared from the lung during the exposure and thus provides an underestimate of the deposition efficiency, the extent of which depends critically upon the exposure duration. One approach to address this issue is to use an estimate of the total quantity of material originally deposited in the lung, $AD_L$, in the above formula. $AD_L$ can be estimated in a number of ways. One of the simplest is to sum the radionuclide content of the lung and the GIT, as mucociliary clearance to the GIT is the primary clearance route for the ciliated airways of the lung (Oberdörster 1993). This approach can be expanded to address other clearance routes by including the radionuclide content of other organs and tissues. These approaches, however, overestimate the deposition efficiency, as some of the radionuclide present in the GIT and other tissues may be a result of clearance of material originally deposited in the head airways, rather than originating in the lung. In this study, lung deposition efficiencies have been estimated using both $A_L$ and $AD_L$ in Equation (1), where $AD_L$ is the sum of the radionuclide content of the lung, GIT, and other organs and tissues. Deposition efficiencies in the head airways, $DE_{HA}$, have been estimated in a similar manner using the radionuclide content of the head, $A_{HA}$, and an estimate of the radionuclide originally deposited in the head airways, $AD_{HA}$, derived by summing the content of the head and GIT. In a similar manner to the calculations for the lung, the first generates an underestimate and the second an overestimate of the deposition efficiency to the head. Deposition efficiencies for the...
entire respiratory tract, $DE_R$, were also determined using an estimate of the total radionuclide originally deposited in the respiratory tract, $AD_R$, derived by summing the radionuclide content of the lung, GIT, head (minus pelt), and remaining organs and tissues. The deposition efficiency in the alveolar region, $DE_A$, was determined using the radionuclide content of the lung at 24 h post-exposure, $A_{L(24h)}$. This approach is possible because clearance from the ciliated rat lung airways is rapid with the majority cleared by 8 h post-exposure (Hofmann and Asgharian 2003) whereas clearance from the alveolar region is significantly slower, typically represented by a half-life of circa 70 days (Oberdörster 1993). Values for deposition efficiency in the ciliated regions of the lung have also been estimated by subtracting alveolar deposition from that in the lung as a whole. To estimate deposition efficiencies, a tidal volume of $1.29 \pm 0.13$ mL and a breathing frequency of $127 \pm 12$ min$^{-1}$ were used. These values were the average of measurements of five female Wistar-Kyoto rats, of similar age and size to those used in this study, made using a head-out plethysmograph (EMMS, Bordon, UK) in the same exposure system during similar exposure experiments.

**Deposition modeling**

The software package MPPD v2.11 (MPPD 2009) was used to determine estimates of deposition efficiencies. The MPPD model has components to model both deposition and clearance, but only the deposition module was used in this study. The deposition model requires input data on aerosol particle characteristics and respiratory system parameters. The count median diameter (CMD), geometric standard deviation (GSD), and particle density were used to define the aerosol. The respiratory system parameters required are the functional residual capacity (FRC), upper respiratory tract (URT) volume, tidal volume, and breathing frequency. Both MPPD default values and values considered more appropriate for the applicable rat strain and size were used (Table S2 in the SI). The experiment-specific tidal volume and breathing rate are those discussed above derived from measured data, the MPPD defaults are, respectively, 2.1 mL and 102 min$^{-1}$. The FRC of Wistar rats has been measured and reported in a number of papers (Tazaki et al. 2006; Tajiri et al. 2006; Filho et al. 2014). The most relevant study is that of Filho et al. (2014), which measured body weight and lung volumes and capacities of over 240 Wistar rats from 2 to 24 months. At the 3 month measurement point, the average female weight was broadly consistent with the animals used in this study and the average FRC per unit body weight was $21.41 \pm 7.88$ mL/kg. Given the average rat weight of $174 \pm 19$ g an FRC value of $3.7 \pm 1.4$ mL was assumed; the MPPD default is 4.0 mL. No specific information on the URT volume of Wistar rats could be found in the literature, this was therefore determined using the allometric scaling equation devised by Ménache et al. (1997), as 0.29 mL (MPPD default 0.42 mL).

**Results**

**Aerosol characterization**

The variation in the iridium aerosol delivered to each port was investigated: aerosol number concentrations and size distributions (CMD and GSD) varied by less than ±4% across all ports (Figure S2 in the SI). The characteristics of the aerosols delivered to the animals are given in Table 2. The average particle size distribution for each experiment is shown in Figure 2. Representative TEM images of aerosol particles collected during exposures show that the spark-generated aerosols consist of chain agglomerates (Figure S3 in the SI) of primary particles with crystalline structure (Figure S4 in the SI). Energy dispersive X-ray analysis indicated the purity of the iridium particles (Figure S5 in the SI). Experimental and supporting theoretical analyses undertaken to determine the link between the aerosol delivered to the manifold and that delivered to each port (POA) indicated that for all but the smallest aerosol particle size the concentrations were approximately the same, but for the smallest aerosol particle size losses occurred resulting in the

| Nominal particle diameter (nm) | CMD (nm) | GSD | Average mass concentration (mg/m$^3$) | Activity concentration (kBq/m$^3$) | Average mass concentration (mg/m$^3$) | Effective density (g/cm$^3$) |
|-------------------------------|---------|-----|--------------------------------------|-------------------------------------|--------------------------------------|-----------------------------|
| 10                            | 9.8 ± 0.1 | 1.37 ± 0.01 | 2.85 ± 0.10 | 491 ± 90 | 0.17 ± 0.03 | 7.7 |
| 15                            | 15.6 ± 0.3 | 1.67 ± 0.01 | 9.09 ± 0.14 | 2376 ± 251 | 1.39 ± 0.15 | 2.8 |
| 35                            | 35.2 ± 1.6 | 1.68 ± 0.01 | 5.74 ± 0.07 | 587 ± 144 | 5.03 ± 0.63 | 1.7 |
| 75                            | 76.3 ± 1.9 | 1.62 ± 0.01 | 1.10 ± 0.03 | 604 ± 100 | 6.92 ± 1.38 | 1.1 |

*For chain agglomerates particle density is not equal to material density because of their complex shape, including voids. Particle effective density can be defined as the particle mass divided by the volume of a particle having the same mobility diameter (McMurry et al. 2002). The average effective density for the aerosol was derived by dividing the average mass concentration by the average aerosol particle volume concentration determined from the particle size distribution.*
requirement for a correction factor of 0.72 ± 0.04. The aerosol concentrations in Table 2 include this correction factor. Across all experiments, the delivered gas temperature, relative humidity, and oxygen concentration ranged between 19.0°C and 23.5°C, 30% and 55%, and 21.0% and 21.5%, respectively.

Sample radionuclide content
The iridium-192 content of the lung, GIT, head, and sum of the remaining organs and tissues (excluding pelt) from animals sacrificed within 15 min of the end of exposure and lung from animals at 24 h post-exposure are presented in Table 3. These indicate that the majority of the iridium-192 is in the lung and GIT immediately post-exposure. For the smallest particle size, the levels of iridium-192 are similar in both, but for the others the lung content dominates. The content of the head and other tissues is significantly lower. The animals sacrificed within 15 min of the end of exposure had very limited opportunities for grooming during and post-exposure so the vast majority of material in the GIT can be assumed to arise from respiratory tract clearance only. Some material may have been lost due to excretion during exposure, but this is expected to be minimal as the exposure duration was much shorter than typical gastrointestinal transit times for rats (Tuleu et al. 1999). The iridium-192 content of other organs and tissues from animals sacrificed at 24 h (Table S3 in the SI) indicates that the majority is in the lung. Analysis of the lung lobes for the 15 nm aerosol indicates uniform iridium-192 content per unit mass of lung lobe (Figure S6 in the SI).

Deposition efficiencies
The experimentally derived deposition efficiencies are presented in Table 4 and Figure 3. Over the size range considered the deposition efficiency within the entire respiratory tract decreases with increasing particle size. Two values of the lung deposition efficiency are given; the first derived using the iridium-192 content of the lung only and the second assuming that the iridium-192 in the GIT and remaining organs and tissues originated in the lung. The first is clearly an underestimate, as it ignores clearance, and the second an overestimate, as some of the iridium-192 in the GIT and possibly other organs and tissues may have originally been deposited in the head airways. These estimates vary by a factor of around two for the smallest particle size, but the difference is less marked for the other particle sizes. The actual deposition efficiency is expected to lie between the two. In both cases, the deposition efficiency shows a reduction with particle size. Deposition efficiency in the alveolar region was derived using the iridium-192 content of the lung at 24 h post-exposure, as at this stage it is assumed that the majority of the ciliated regions will have cleared. However, this analysis ignores the potential impact of any slow clearance of the ciliated regions, which may be of importance for nanoparticles (Patrick and Stirling 1997), and could thus represent an overestimate of alveolar deposition. Two estimates of deposition in the head airways are given, the first determined using the iridium-129 content of the head airways only (i.e., underestimate as ignores clearance) and the second assuming all the iridium-192 within the GIT was initially deposited in the head airways (i.e., overestimate as some will have been cleared from the lung). The two estimates vary significantly, indicating the importance of assumptions.

Table 3. Iridium-192 content of organs and tissues.

| Nominal aerosol particle diameter (nm) | Lung (0 h) | GIT (0 h) | Head (0 h) | Remainder (0 h) | Lung (24 h) |
|---------------------------------------|-----------|-----------|------------|----------------|------------|
| 10                                    | 4444 ± 729 (46%) | 4713 ± 894 (48%) | 466 ± 217 (5%) | 109 ± 101 (1%) | 4370 ± 550 |
| 15                                    | 10,238 ± 1223 (74%) | 2195 ± 526 (16%) | 934 ± 236 (7%) | 479 ± 276 (3%) | 8195 ± 1729 |
| 35                                    | 3968 ± 184 (65%) | 2020 ± 378 (33%) | 66.2 ± 34.8 (1%) | 7.3 ± 4.1 (< 1%) | 3932 ± 416 |
| 75                                    | 1361 ± 125 (67%) | 572 ± 86 (28%) | 87.4 ± 36.3 (4%) | 4.7 ± 1.3 (< 1%) | NA |
regarding clearance in their derivation. The actual deposition is expected to lie between the two. Values for the deposition efficiency in the tracheo-bronchiolar region are also included, derived by subtracting the alveolar deposition from the lung deposition, these generally indicate a reduction with particle size, but it must be recognized that given their derivation there is significant uncertainty surrounding these values.

**MPPD model predictions**

MPPD model predictions of deposition efficiencies are presented in Figure 3 (Table S4 in the SI). Those generated using default values for the respiratory tract parameters are similar to, but in general higher than, those derived using the experiment-specific parameter values, except for the head airways, for which the predictions are very similar but the experiment-specific parameters produced slightly higher estimates.

**Discussion**

Deposition efficiencies for four sizes of nanoparticle aerosol have been experimentally determined and compared with results from the MPPD model. The experimental values in general follow the same trend with particle size as the model predictions, but are typically higher (Figure 3). This is particularly the case for the smallest particle size, for which the experimentally derived value for the whole respiratory tract is 101 ± 26%, when clearly

| Region                     | Deposition efficiencies (%) aerosol particle size (nm) | Derivation                                      |
|----------------------------|-------------------------------------------------------|-------------------------------------------------|
| Respiratory tract          | 101 ± 26, 59 ± 12, 40 ± 12, 34 ± 8                   | Lung, GIT, head, and remainder at 0 h           |
| Lung                       | 46 ± 13, 44 ± 9, 26 ± 8, 23 ± 5                       | Lung only at 0 h                                |
|                            | 96 ± 25, 55 ± 11, 40 ± 12, 33 ± 7                     | Lung, GIT, and remainder at 0 h                  |
| Head airways               | 4.8 ± 2.5, 4.0 ± 1.2, 0.44 ± 0.26, 1.5 ± 0.7           | Head only at 0 h                                |
| Alveolar                   | 54 ± 15, 13 ± 3, 14 ± 5                                | Head and GIT at 0 h                             |
|                            | 45 ± 12, 35 ± 10, 26 ± 8                              | Lung at 24 h                                    |
| Tracheo-bronchiolar        | 0.8 ± 9.5, 8.8 ± 9.2, 0.2 ± 3.0, 0.44 ± 3.0            | Lung at 0 h minus lung at 24 h                  |
|                            | 51 ± 18, 20 ± 10, 14 ± 6                              | Lung, GIT, and remainder at 0 h minus lung at 24 h |

Figure 3. Experimentally derived deposition efficiencies in the respiratory tract (a), lung (b), head airways (c), and alveolar region (d) and MPPD v2.11 model predictions using default respiratory parameter values (solid line) and experiment specific values (dashed line). Note that for the lung and head airways two experimentally derived values are presented. For the lung: (●) values derived using iridium-192 content of lung only, and (●) values derived using the sum of the iridium-192 content of the lung, GIT, and other organs and tissues (i.e., assuming iridium-192 in the GIT and other organs and tissues was originally deposited in the lung). For the head airways: (●) values derived using iridium-192 content of head airways only, and (●) values derived using the sum of the iridium-192 content of the head airways and GIT (i.e., assuming iridium-192 in the GIT was originally deposited in the airways). In both cases (●) is an underestimate and (●) an overestimate with the expected value lying between.
the efficiency cannot exceed 100%. There are a number of uncertainties associated with this value, including the correction for the manifold versus POA concentration. Experiments to address this were undertaken without animals within the system, which may have resulted in some modification to the flows resulting in an overestimate of the losses. This illustrates the importance of undertaking direct measurements at the POA in such studies.

The breathing rate is a key input parameter for the experimental estimates. Measured values for five rats of the same strain and typical weight of those in this study were used. The resulting average breathing rate was 0.16 ± 0.02 L/min. This is consistent with some values in the literature, including 0.16 L/min for Wistar rats of average mass 217 g (Whalen et al. 2006) and a range of 0.15 to 0.17 L/min for Fischer rats of similar weight (Mauderley 1986), but is lower than other estimates, including 0.23 L/min for similar sized Wistar rats (Filho et al. 2014), and the experimentally derived allometric relationship in Semmler-Behnke et al. (2012), which predicts a value of 0.24 L/min. The design of the head-out plethysmograph used is such that the seal is close fitting around the neck area, which may affect breathing rates, and the operation is such that once calibrated it is extremely unlikely to overestimate the value. It is thus possible that our measured values are an underestimate of the actual breathing rates of the rats in the exposure tubes. It would be useful in future to compare with alternative approaches for the measurement of breathing rates. Using the breathing rate from Filho et al. (2014) (i.e., 0.23 L/min) rather than the measured values results, in general, in a better fit between measured and modeled deposition efficiencies, especially for the smallest aerosol size (Figure S7 in the SI); however this breathing rate is greater than the MPPD default value of 0.21 L/min, intended to represent a significantly larger rat (Long-Evans, 0.38 ± 0.10 kg; Mauderley et al. 1979). This analysis clearly indicates the importance of breathing parameters in the experimental determination of deposition efficiencies and the significant variation in values within the literature.

Airway geometry can affect particle deposition. For example, modeling studies have indicated that differences in lung geometries between Long-Evans and Sprague-Dawley rats could lead to differences in deposition patterns (Miller et al. 2014). The conducting airway geometry in the MPPD model is based on measurements from the Long-Evans rat. Differences between the experimental results obtained using Wistar-Kyoto rats and the model predictions may therefore be due, at least in part, to this airway geometry “mis-match.” Particle shape also influences deposition patterns. The MPPD model assumes particles are spherical, however, those used in this study are chain agglomerates. Hofmann et al. (2009) used a model that generated a good fit to experimentally derived deposition efficiencies for monodisperse spherical particles, to predict the deposition of chain agglomerate combustion aerosols. The predictions were consistently lower than the measured values but modifying the model to include the effect of a non-spherical particle shape improved the fit. Using a model that included consideration of particle shape effects may have produced a better fit to the experimentally derived data presented here, however, it is important to note that the difference would be expected to be greatest for the larger particle sizes, which comprise a greater number of primary particles, and thus could not explain the significant difference between model and experiment for the smallest particle size.

There are few experimentally measured nanoparticle deposition efficiencies for the total respiratory tract in the literature. Semmler-Behnke et al. (2012) exposed Wistar-Kyoto rats of various ages in a nose-only system for an hour to spark-generated iridium-192 nanoparticle aerosols of two sizes: 20 nm and 80 nm (CMD). Deposition efficiencies in the respiratory tract were estimated using the total iridium-192 content of the animal (minus pelt contamination) and measured breathing parameters. For both the 35 day (ca. 180 g) and 90 day (ca. 400 g) old rats the deposition efficiencies were approximately 30% for the 20 nm aerosol and 10 to 15% for the 80 nm aerosol. These values are significantly lower than those determined in this study for similar aerosols, i.e., 59 ± 12% for 15 nm and 34 ± 8% for 75 nm, and are also significantly lower than the MPPD model predictions. The breathing rate used to derive the deposition efficiencies for the 35 day old rats, which are similar in mass to those used here, was approximately 0.25 L/min, which is significantly higher than the value of 0.16 L/min used in this study. It is this difference in breathing rate that accounts for the majority of the difference between the deposition efficiencies in Semmler-Behnke et al. (2012) and this study. It is important to note in this context that in Semmler-Behnke et al. (2012) breathing rates were measured using an unrestrained whole-body plethysmograph not within the exposure system during actual exposures. Kanapilly and Diel (1980) exposed Fischer rats in a nose-only system to an aerosol of 239PuO2 nanoparticles with particle diffusion diameters in the range 10–30 nm for 47 min. Levels of plutonium-239 in the whole body, lung, gastrointestinal tract, and various other organs and tissues were determined. The animal breathing rates were not measured by the authors of the study and so deposition efficiencies were not presented; however, using the MPPD default breathing rates indicates deposition efficiencies in the respiratory tract in the region of 40% and in the lung of
23%. These values are similar to those estimated in this study for the 35 nm aerosol.

The majority of the reported deposition efficiencies for nano-sized aerosols are for the lung. Petitot et al. (2013) exposed Sprague-Dawley rats in a nose-only system to a spark-generated aerosol of uranium nanoparticles, 38 nm (CMD), for 1 h and derived a lung deposition efficiency of 26.2% using the uranium content of the lung only. This is very similar to the value of 26 ± 8% determined in this study for the 35 nm aerosol, ignoring lung clearance. Takenaka et al. (2001) exposed female Fischer rats (150–200 g) to a spark-generated aerosol of silver nanoparticles (17.1 ± 1.2 nm [CMD]) for 6 h in a whole-body exposure system and measured the silver content of a number of organs and tissues using ICP-MS. Using their estimate of delivered dose, 7.2 μg, and lung content, 1.72 ± 0.17 μg, indicates a lung deposition efficiency of approximately 24%. In a similar study, Takenaka et al. (2006) exposed male Wistar-Kyoto rats (290–315 g) to a spark-generated aerosol of gold nanoparticles (modal mobility diameter 16 nm) for 6 h and measured the gold content of the lung using ICP-MS and derived a lung deposition efficiency of 25%. It is interesting to note that these values for different rat strains, masses, and sexes are very similar, although significantly lower than the value estimated in this study for the, similar sized, 15 nm aerosol of 44 ± 9%, again ignoring clearance. Takenaka et al. (2004) also exposed female Fischer rats (150–200 g) for 6 h to spark-generated cadmium oxide nanoparticles of two sizes (CMD): 50 nm and 73 nm. Lung deposition efficiencies of 19% and 16% were derived using the lung content of Cd determined using ICP-MS. These values are again significantly lower than the values determined in this study for similar aerosols. Differences between our results and those of the three Takenaka studies may be a result of different breathing patterns for animals in whole-body compared to nose-only systems, and the longer exposure duration may also have resulted in greater clearance from the lung before the content was assessed. It is also important to note that in none of these studies were the rat breathing rates measured; values derived from allometric relationships were used to derive the deposition efficiencies. The actual breathing rates may therefore have differed from the values used.

There are few experimental data for the head airways. The predicted deposition in the extra-thoracic airways derived by Petitot et al. (2013) (38 nm CMD) was 0.56 ± 0.07%, this was derived ignoring clearance and is similar to the value of 0.44 ± 0.26% for the head airways for the 35 nm aerosol in this study when clearance is ignored. Gerde et al. (1991) determined deposition efficiencies in the rat nasal airways for male Fischer rats (298 ± 31 g) using anesthetized rats and a flow through system for different inspiratory flow rates and the following particle sizes: 5, 7, 10, 40, and 100 nm. Deposition efficiencies reduced with particle size. For particles between 5 and 10 nm, the deposition efficiency varied between 28% and 68%. This is broadly consistent with the value from this study of 54% for the 10 nm aerosol derived assuming all material cleared to the GIT originated in the head airways. The value for the 40 nm aerosol ranged from 6% to 10% and for the 100 nm aerosol from 6% to 7%, broadly consistent with the results from this study for the 35 nm and 75 nm aerosols of, respectively, 14% and 11%, again assuming all activity in GIT is cleared from head airways. It must be noted, however, that the approach used by Gerde et al. (1991) is essentially equivalent to inspiration only and does not fully account for deposition on exhalation.

It is interesting to note that for the lung the experimental deposition efficiencies derived ignoring clearance are a better fit to the MPPD model than those including clearance, whereas the opposite is the case for the head airways. This may possibly indicate a model bias toward fitting to measured lung deposition efficiencies derived ignoring clearance.

The deposition efficiencies determined in this study will potentially be of use in the development and validation of deposition models. The analysis serves to highlight the importance of (a) using appropriate breathing parameters, both when interpreting experimental results and in generating model predictions; and (b) assumptions made about particle clearance in deriving deposition efficiencies.

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