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Comparative study of aerosol particles affecting rodents and humans

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Abstract

In-vivo studies with animal models have been crucial in understanding the prediction pathway and effects of inhaled aerosol particles. Rodents are used to represent the human respiratory tract, however, there are distinct differences in anatomy and physiology, which affect airflow and particle deposition. By studying anatomically realistic 3D models of both rat and human nasal cavities, physical parameters, such as internal dimensions can be measured, and the particle deposition of different-sized particles can be studied. Micron-sized particles are classified into low, medium, and high inertial particles based on the total deposition, sized 1, 2, and 3 µm for the rat and 2.5, 9, and 20 µm for the human model. The goal of our work is to compare the anatomical and functional features of the airways of the two species and to draw consequences on the extrapolation ability of rat, based inhalational experiments to humans. The results show high acceleration around the nasopharynx in humans, with particle deposition mainly in the main passage. For rodents, the highest flow acceleration is in the vestibule, close to the nostrils, where most of the particles deposit. Deposition of nanoparticles (1, 10, and 100 nm) showed the highest deposition in the main nasal passage in humans, the same results are valid for 10 and 100 nm particles in rats, but the 1nm particles had the highest deposition in the vestibule. According to the reviewed data, using rat models to reveal possible effects in humans, and using inhalation of test material is a valid and usable method, however, some consideration should be made. One of the main concerns is the particle size in the used aerosol, which is advised to be kept under a diameter of 2 µm to ensure that the defense mechanisms of the rat will not eliminate the particles from the inhaled air. Also, close monitoring of the breathing parameters is required, as these data should be evaluated together with the test material's possible biological effects, especially when a less-than-expected level of biological effect is experienced.

Abbreviations

Abbreviation	Meaning
NTs	Nasoturbinates
MTs	Maxilloturbinates
EMTs	Ethmoturbinates
NALT	Nasal associated lymphoid tissue
NPDs	Nasopalatine ducts
NLDs	Nasolacrimal ducts
VNO	Vomeronasal organ
NSB	Nasal swell bodies
MCC	Mucociliary clearance
NK	Natural killer cells
BMR	Basal metabolic rate
CT	Computer tomography
CMI	Cell-mediated immunity
Ig	Immunoglobulins

1. Introduction

Exposure to aerosol particles may cause health effects and impact worldwide health. The particle deposition probability in the respiratory tract is essential to study to gain knowledge of their toxic effect [1]. To gain a complete understanding of toxicology and the hazardous effects of aerosol particles, it is important to consider other factors affecting the deposition in different species, such as anatomy and physiology of the respiratory tract, as well as, airflow and particle deposition [2–4]. *In vivo* studies of animal models, especially rodents, are widely used tools for researchers to develop a better understanding of particle exposure in the nasal cavities of humans [3]. Prediction pathways for inhaled aerosol particles are important for evaluating health risks associated with respiratory diseases, toxicology and pharmacology studies [4, 5]. All these studies require information about particle deposition as well as particles escaping from the nasal cavity [4].

A direct comparison of particle deposition is difficult, due to the variable anatomical structures and characteristics in human and rodent nasal cavities [3]. The nasal cavity is an extremely complicated organ, both geometrically and functionally, therefore, it is difficult to visualize without a 3D viewer. Particle deposition is not attainable in some parts, due to the complexity of the turbinates [4, 6]. Particle deposition in local tissues is influenced by epithelial characteristics at the deposition site, whilst the regional deposition and uptake depend on inhalation and airflow patterns. Anatomical structures in the nasal cavity, such as turbinates and other accessory structures, affect the airflow pattern [6]. The respiratory conditions and the properties of the particles themselves may also affect respiration differently and can lead to different deposition regions, even for the same-sized particles [4, 6, 7]. An understanding of this is necessary for a proper evaluation of toxic effects concerning human risk assessment [6, 7].

2. Goal of the study

The goal of this study is to evaluate and consider whether rodents are adequate models in experiments to represent respiratory functions in humans. By using literature data, we intend to identify limitations of inhalation studies in rodents (e.g., particle size, particle concentration, etc.) due to anatomical and physiological differences in the upper respiratory tract of rodents and humans. Differences will be highlighted in the text, then the results will be discussed in the conclusion.

3. General review of the respiratory tracts

3.1. The respiratory tract

3.1.1. Structural components

The respiratory tract is a complex system consisting of multiple structural components and functional compartments. The structural parts consist of the nasal cavity, pharynx, larynx, trachea, bronchi, bronchioles, and lungs. The upper part of the respiratory system includes the organs outside the thorax e.g. nasal cavity, pharynx, and larynx, while the lower respiratory tract consists of the organs inside the thorax e.g. trachea, bronchi, bronchioles, and lungs [8, 9].

3.1.2. Functional components

Functionally, we can divide the respiratory tract into two parts; the proximal conducting zone and the distal respiratory zone. The conducting part extends from the nostrils until the terminal bronchioles. This part is known as the airways and works as a biological transport system. Gases are transported from outside, through the external nostrils and nasal passages, into the pharynx and trachea, and continuing to the lungs, however, no gas exchange takes place in this region [8–10].

The gas exchange takes place in the respiratory zone which includes the respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli. This region is known as the acinus, which is the functional tissue of the lungs and is responsible for the gas exchange in the lungs. During inhalation, the thorax increase in size, which decrease the pressure and air flows into the lungs. Expiration is a passive process caused by the relaxation of the intercostal muscles and the diaphragm. Air is forced out of the lungs and the thorax returns to normal size [8–11].

3.2. Anatomy of the respiratory tract

Even though the general layout of the respiratory tract in mammals is similar, there are significant differences in the structural and functional anatomy of the different species [6, 12, 13]. The species differences are distinct in the gross anatomy of the respiratory system. The differences may be due to compensation for species differences in body size and basal metabolic rate [8, 9].

3.2.1. The nasal cavity

The anatomical differences between rodents and humans in the nasal cavity, are affected by the primary function of the nose. Humans are microsmatic, since the main function is breathing, while rodents are macrosmatic since olfaction is their primary function. Resulting in a much more complex structure of the turbinates in the nasal chamber of rodents, also the olfactory region is larger. Humans are mouth-, and nose breathers, while rodents are obligate nasal breathers due to the close arrangement of the soft palate and epiglottis [6, 8, 9, 12, 14].

The macroscopic features of the rat and mouse nasal cavities are identical, while humans are very similar. The nostrils (nares) are two natural openings located in the rhinarium. Externally, the philtrum separates the nares, while internally the median septum separates the nasal cavity into two chambers, approximately equal in size. The septum is partly cartilaginous (cranially) and partly bony (caudally) [6, 12]. The nasal cavity extends from the nostrils to the nasopharynx. From rostral to caudal, the nasal cavity is divided into; the vestibule, the nasal chamber, and the nasopharynx [6, 8, 12].

3.2.1.1. Nasal vestibule

The vestibule forms a slight dilation just before the nasal chamber and is surrounded by flexible cartilage. The vestibule is smaller in rodents than in humans due to the atrioturbinates, which are believed to conduct airflow to the dorsal parts of the nose. In humans, the vestibule is spacious and empty [6, 8, 12]. Rodents have a sharp 180-degree turn right after the nostril entry, while humans have a 90-degree bend, these bends are important for airflow streamlines and particle deposition [4, 15]. Close to the nares, humans have hair follicles, which rodents do not. The function of these is to protect the airways from larger aerosol particles [13].

The nasolacrimal duct and the duct of the lateral nasal glands (Steno's glands) open into the vestibule. Steno's gland produces serous and seromucous secretion around the nasal airway entrance, and the vestibule works as a secretory reservoir. The Steno's gland is absent in humans, instead, they have a few rostral nasal glands for seromucous secretion into the vestibule. This is for humidifying and filtering, as well as heating and cooling the inspired air [6, 8, 12]. Due to the large number of autonomic nerves located in close contact with acinar cells, it is assumed that the gland can rapidly adjust the secretion based on the humidity of the inspired air and airborne irritants [8].

3.2.1.2. Nasal chamber

The nasal chamber is allegedly the main anatomical difference in the nasal cavity between laboratory animals and humans. The nasal chamber in both species contains turbinates. Which has a bony structure and is covered by well-vascularized mucosa and occupies most of the nasal cavity. The relative surface area of the nasal cavity is about five times greater in rodents than in humans due to the branching turbinates. The complexity and number of turbinates (conchae) vary according to the species and their olfactory capacity. Rodents have a far more complex system than humans, this is likely because rodents are more dependent on the olfactory function, and therefore require better humidification and conditioning of air [6, 12, 14].

The turbinates (conchae); nasoturbinates (NTs), maxilloturbinates (MTs), and ethmoturbinates (EMTs) occupy most of the nasal cavity. The NTs, or the dorsal nasal concha, project from the nasal bone and are located more cranially in the nasal cavity. MTs include the middle and ventral nasal concha and originate from the maxilla. This part is called the respiratory turbinates due to the large area surface of the branching concha, which is for heating and moisture of the air. Also, the branching helps clean the inspired air through the impaction of particles before directing air into the nasopharynx [6, 12, 14]. The simpler turbinates of humans, provide less protection than in rodents from aerosol particles in the respiratory tract. [13]

The EMTs, also called the ethmoid labyrinth are located more caudally. Rodents have 6-8 EMTs divided into endoturbinates and ectoturbinates. There are 3-4 inward turning endoturbinates, as well as 3-4 smaller and more external ectoturbinates. It consists of

extremely thin bony lamellae originating from the ethmoid bone. The lamella is covered by heavily vascularized mucosa and is designed for acute olfaction [12, 14].

3.2.1.3. Nasopharynx

The nasopharynx is defined as the airway posterior to the termination of the nasal septum and proximal to the termination of the soft palate. It has a tubular pathway and communicates with the oropharynx and the laryngopharynx in both rodents and man. The nasopharynx is the final anatomical portion of the nasal cavity, which makes it an important target for aerosol irritants. The toxicants reaching this structure have escaped the defense mechanisms of the anterior structures. The most sensitive region is the proximal region of the oropharynx and around the opening of the Eustachian tubes, which connects the middle ear to the nasal-sinus cavity. In this area, an epithelial transition from respiratory- to transitional- to squamous epithelium is observed [6, 8, 12].

In humans, Waldeyer's ring is located here. It is a structure constructed of lymphoid tissue and is strategically positioned where most of the nasal mucus secretion of the nose and inhaled air passes the entrance of the nasopharynx. Thereby, it can come in contact with inhaled particles before its swallowed into the digestive tract [6, 8, 12]. Rodents do not have the tonsils or Waldeyer's ring, instead, they have NALT (nasal-associated lymphoid tissue) around the nasopharyngeal entrance. NALT performs identical immune functions as the Waldeyer's ring in humans. The function of the NALT is not fully understood however, it is clear that it plays an important role in the immunity of the nasal cavity [12, 14].

3.2.1.4. Accessory structures

Olfactory organs, nasopalatine ducts (NPDs), nasolacrimal ducts (NLDs), and paranasal sinuses are additional structures in the nasal cavity of rodents. The olfactory organs are called the vomeronasal organ (VNO), the septal organ of Masera, and the septal organ of Grüeneberg. Unlike adult humans, rodents have the VNO which is believed to communicate with territorial marking and reproductive- and sexual behavior through the reception of pheromones. The masera organ can detect odor and is likely a part of alert functions, while the septal organ of Grüeneberg is a collection of neurons in the vestibule. It is believed that it is related to freezing behaviors in panic situations, however, the function is not fully understood [6, 12, 13].

Paranasal sinuses are empty air-filled bone structures with mucosal lining. Rodents have only one pair of paranasal sinuses, the maxillary sinuses, which are relatively small. While humans have four sinuses: maxillary, frontal, sphenoid, and ethmoid sinuses. Additionally, the maxillary sinus in humans is very large. The paranasal sinuses do not greatly affect the inhalation itself, but it affects the size of the nasal meatuses, which is important for inhalation [6, 12, 13].

3.2.2. Pharynx and larynx

The pharynx and larynx have clear differences in humans and rodents due to the position of the head and body sizes. The pharynx is a musculomembranous tube that connects the nasal, oral, and laryngopharynx. Since rodents are obligate nasal breathers, unlike humans, all the inhaled air flows through the nasopharynx. The main anatomical difference between humans and rodents in this area is the bend of the nasopharynx. Humans have an upright position causing a marked flexion of the pharynx at approximately 90 degrees, while rodents have a relatively straight nasopharynx due to their elongated heads, with only a slight bend at about 15 degrees. The degree of the nasopharyngeal bend can affect the airflow and particle deposition. Another interesting feature is the location of lymphoid tissue in this area, and mucus forming epithelia lining the nasal airway before being swallowed [13, 14].

The larynx is a symmetrical cartilaginous structure located between the pharynx and trachea. The epiglottis is located here, to protect the lower respiratory tract from water and food particles. At the base of the epiglottis, rodents gave a ventral pouch, which is a common site for inhaled irritants. Humans do not have this structure; however, the epiglottis is a common site of injury in both species. The larynx can expand and contract rapidly, hence it can create turbulence in the airflow causing particle deposition [13, 14].

3.2.3. Trachea, bronchi, and bronchioles

Trachea is continuous from the larynx and it has a conducting function for the airflow. The trachea consists of C-shaped cartilaginous rings varying from 15-25 incomplete rings in rodents and 15-20 incomplete rings in humans. It extends into the thoracic cavity where it bifurcates at the carina forming the left and right main bronchi. These further divide into smaller bronchi. The bronchi contain cartilage and are also a part of the conducting airways. For humans, there are seven generations of bronchi and the dichotomous branching is mostly

symmetrical, in opposition to the rodents, which are of unequal diameter and has monopodial branching pattern [9, 14].

Most distally, the terminal bronchioles are located. This is where the conducting airways and the respiratory/alveolar airways join. This junction is called the centriacinus, which is a common site for inhalational injury caused by hazardous particles reaching the lung. For humans, the terminal bronchioles end in multiple bronchioles, which contain alveoli and is referred to as alveolar outpocketings. In rodents, the terminal bronchioles end in one segment of respiratory bronchioles or into small airways lined by alveoli [9, 14].

3.2.4. Anatomical comparison and nasal cavity measurements

In studies performed by Shang et al. (2015) and Dong et al. (2016), nasal models of humans and rats are developed based on CT images constructing 3D models and 2D unfolded models (Figure 1). They divide the nasal surface into seven regions, according to anatomical and histological features. From cranial to caudal it can be divided into the 1. vestibule, 2. upper passage, 3. middle and 4. lower passage, 5. olfactory, 6. septum and 7. pharynx [3, 4].

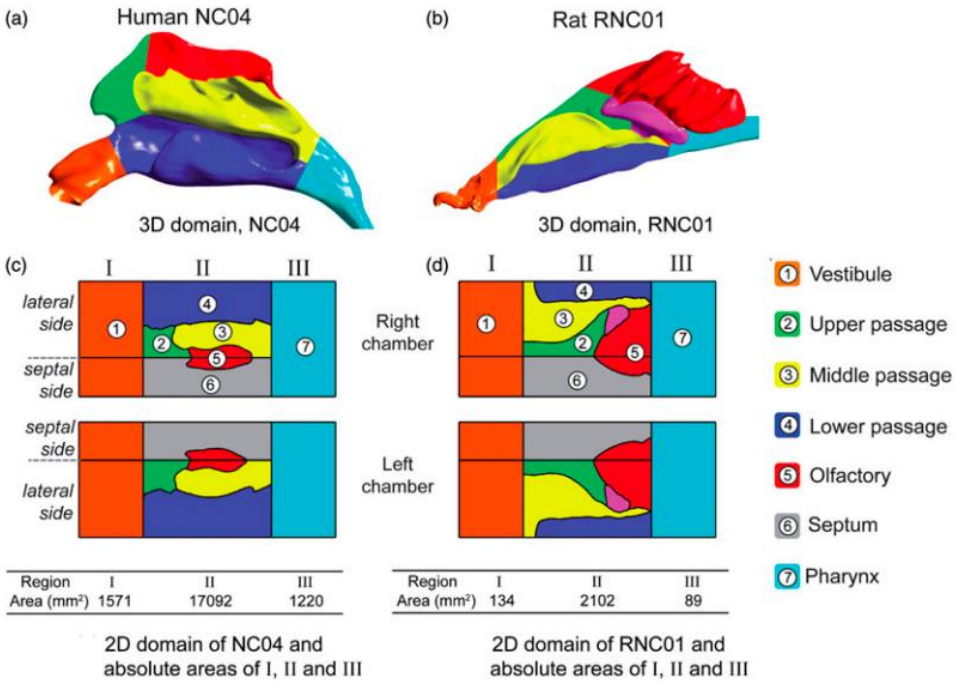

Figure 1

(a) and (b) presenting 3D models of the nasal cavity of humans and rats respectively. In (c) and (d) it shows planar 2D models of the nasal cavity of human and rat. The nasal cavity areas are divided into (I) the vestibule, (II) the main passage, and (III) the pharynx. The numbers 1-7 show various anatomical and histological compartments of the nasal cavity, indicated with color. The rectangular box representation shows the area-ratio of the different parts of the nasal cavity compared to the whole area of it. The figure is used with the approval of the original authors [4].

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The studies proved that the total surface area is approximately 10 times larger in humans than in rats. In humans, the middle (3) and lower (4) nasal passages has the largest surface area and together coverers about 52.8% of the total surface area. While in rats these passages only make up 16.3% of the total area. The olfactory region (5) in rats makes up 55.6% of the total surface area, compared to 10.5% of the same area in humans. The studies compared their findings in the vestibule and olfactory region with previous literature since these regions have especially important roles for basic nasal function. Epithelial mapping provides good agreements of results in multiple literatures, however the vestibule and olfactory shows slightly higher values in these studies [3, 4].

3.3. Physiology of respiratory tract

3.3.1. Basic physiologic respiratory functions

Particle deposition may be affected by many physiological factors such as breathing patterns, clearance mechanisms, and structure of the airways. Breathing frequency, tidal volume, total lung capacity, functional residual capacity, inhalation and exhalation breathing fractions play important roles in the particle deposition as well [16]. Increased inspiration will directly increase the inhaled particle dose. The frequency of inhalation and duration of pauses influence the habituation time of the particle in the respiratory tract and may therefore affect the deposition mechanisms. The tidal volume may affect the penetration depth of the inspired air [2]. There is an obvious size different between the humans and rodents, and the human body is 2 500 times larger than mouse, which influence the basal metabolic rate (BMR). Giving the mouse a BMR 7 times faster than the average human. Due to the size differences the airway diameter and alveolar size are much smaller in the mouse [17].

The characteristics, e.g., size, shape, and density, of the particles are also important for deposition. The particles deposited on the mucosa are either translocated or removed from the airway by mucociliary clearance, defeated by macrophages, or degraded [16]. The nose and its anatomy cause the largest differences in the respiratory tract between humans and rodents. The main function of the nose is to filter and clean the inhaled air, heat and humidify inhaled air, and olfactory function. The filtration and humidification of air is a part of the defense mechanisms, and cause protection of the lower respiratory tract [15]

3.3.2. Defense mechanisms of the respiratory tract

The respiratory tract is exposed to an enormous amount of aerosol particles every day. Inhaled air is essential for the uptake of oxygen, but the air may contain hazardous particles, toxic gases, and microorganisms that may cause illness or injury to the respiratory tract. To allow gas exchange without inappropriate inflammation, defense mechanisms are crucial. This includes physical and mechanical defense, innate and adaptive immunological defense [18–20].

3.3.2.1. Physical defense

Physical defense includes the airstream pattern, barriers i.e. anatomical structures and epithelial barriers, cough and sneezing reflexes, as well as mucociliary clearance. Overlapping of these mechanisms is important for the protection of the respiratory tract from injury and infections [20]. The first line of defense is anatomical barriers where particles may attach to the mucosa of the nasal structures due to impaction. This will prevent particles or microorganisms larger than 2-3 µm from reaching the lungs. Turbulent airflow increases the impaction of particles onto the mucosa in the nasal passage and nasopharynx. If particles are able to dodge the defense mechanisms and makes their way to the lower airways, the particles may deposit onto the mucosa of the branching airways. The anatomical barriers are associated with the cough reflex and mucociliary apparatus [18–20].

Cough is forced expiration towards the oropharynx, causing the expulsion of particles from the airways. Turbulence and force from the trachea and bronchi are intended to remove materials from the airways. The coughing reflex can be triggered by mechanical stimuli, chemical stimuli, or as a response to inflammatory mediators [18]. Mucociliary clearance (MCC) apparatus is found in the conducting airways. The airways are lined with ciliated mucous cells, forming a mucus gel that lines the airways. The mucus can trap particles and microorganisms, and thereafter transport them back to the oropharynx for elimination by swallowing or expectoration. In humans, MCC is present in the trachea and bronchi, while in rodents, this line of defense is only present in the trachea [21].

The epithelia are also considered a physical barrier due to the tight junction of cells forming a protective barrier against the entry of pathogens. In many parts of the airways, the luminal surface of the airways is covered by pseudostratified columnar cells covered with cilia. The cilia are connected to the MCC, where the job is to direct the contaminated mucous out of the airways by ciliary movements. Particles are pushed forward, but removed by swallowing or coughing/sneezing out of the respiratory tract [18, 20].

3.3.2.2. Immunological defense

The second line of defense is the innate immunological barrier. The innate defense does not need prior contact with the pathogen to be effective, hence they are not specific in their response. The innate response includes epithelial secretions, inflammatory and complement cascades, natural killer (NK) cells, and phagocytic cells. The respiratory epithelia work as a structural barrier, but can also produce antimicrobial chemicals i.e. lactoferrin, lysosome, defensins, and cathelicidins. These chemicals can be involved with the destruction of microorganisms, and they may be produced by phagocytic cells as well. The main phagocytic cells we see in innate defense are the neutrophils and the macrophages. Potential pathogens are destroyed by macrophages, then their remnants are transported out of the airways by MCC. NK cells are also a part of the innate immune system and can be programmed to induce apoptosis of targeted cells [19, 20, 22].

If a microorganism can escape the defense of the physical and innate immune system and reach into the regional lymphatic tissue, adaptive immunity is activated. The adaptive response needs a longer time to activate due to the maturation and differentiation of B- and T-lymphocytes for a pathogen-specific response. After an adaptive immune response, immune memory will be formed, so the response will be faster and more effective the second time the pathogen is induced. Dendritic cells will present the antigen to activate the adaptive immune response. The cell-mediated immunity (CMI) and humoral immunity are activated and cause the induction of B- and T-lymphocytes, macrophages, and cytokines. The first step of adaptive immunity is when the antigen is recognized, then the activation starts where lymphocytes proliferate and differentiate into immune effector cells. The last phase is when the specific effector lymphocytes elicit inflammatory responses to eliminate the antigen. This acquired immune response is one of the most complex inflammatory cascades in the defense system [20, 22].

Immunoglobulins are important for humoral immunity. IgA is the most important immunoglobulin for the upper airways and is released by the epithelial cells. IgA is important for the neutralization of microorganisms and blocks microbial-epithelial adhesion and uptake across the epithelial surface. IgG and IgM are more important for protection in the lung parenchyma, as they facilitate phagocytic function and activate the complement cascade [19, 20].

4. Functional differences between rodent and human upper airways

Anatomical features and structures have a huge impact on respiratory patterns and airflow. This information is important to be aware of when using animal models for toxicological studies, because the characteristics may affect the airflow pattern, the uptake and deposition of particles in the respiratory tract [6, 12, 14]. Deposition of harmful substances may cause lesions and infections in the respiratory tract, therefore, it is important to understand the airflow pattern and particle deposition [15]. As well as demonstrating the behavior differences of particles to see how accurate the rodent models can represent humans.

In rodents, the MTs lead inhaled air through the dorsal part of the main passage. The atrioturbinates and dilated nasal swell bodies lead air into the dorsal and lateral meatus, then it enters the olfactory region. The inhalation airflow in rodents is through the dorsal, middle, and lateral nasal meatuses, as well as the lateral recess of the nasal cavity. Only 20% of the inspired air reaches the olfactory region in rats. Most of the inhaled aerosol particles settle almost immediately after entering the nasal cavity and nostrils. Due to the turbinates and the presence of the transverse lamina, the olfactory structures are protected from direct airflow [6, 12].

4.1. Streamlines of airflow

Streamlines are used to trace and reveal the expected path of an inhaled particle (Figure 2). The streamlines are traced from the surrounding air as it enters the nostrils. Facial features are important to consider when portraying the flow passage to induce natural flow paths and to demonstrate a more realistic model. In the human model, the results showed that streamlines primarily flow through the center of the airway, rather than the top or bottom (Figure 2). Swirled flow can be observed around the olfactory region which allows gases to be taken up by olfactory neurons while preventing bigger-sized particles from reaching and harming the nerves. The single streamline in humans (Figure 2, left), presents an immediate increase in flow acceleration around the nostril inlet, due to narrowed nasal passage, as well as high acceleration in the nasopharynx [3, 4]. This will cause turbulent flow and increase the deposition to the pharyngeal area in humans.

In the rat model, the flow enters the nostrils and instantly make a steep curve, then a U-shaped turn, and a final 90-degree bend before reaching the main nasal tube (Figure 2, right). The sudden and sharp turn, serve as an important inertial impaction system. Due to the sudden changes in flow, the velocity increases from 0 m/s velocity to 10 m/s in the span of a few mm, while in humans the velocity increases more gradually from 0 m/s to 2.5 m/s after the nostrils (around the main passage) and 6.5 m/s in the nasopharynx (Figure 2). The rapid increase in velocity causes increased particle deposition [3, 4].

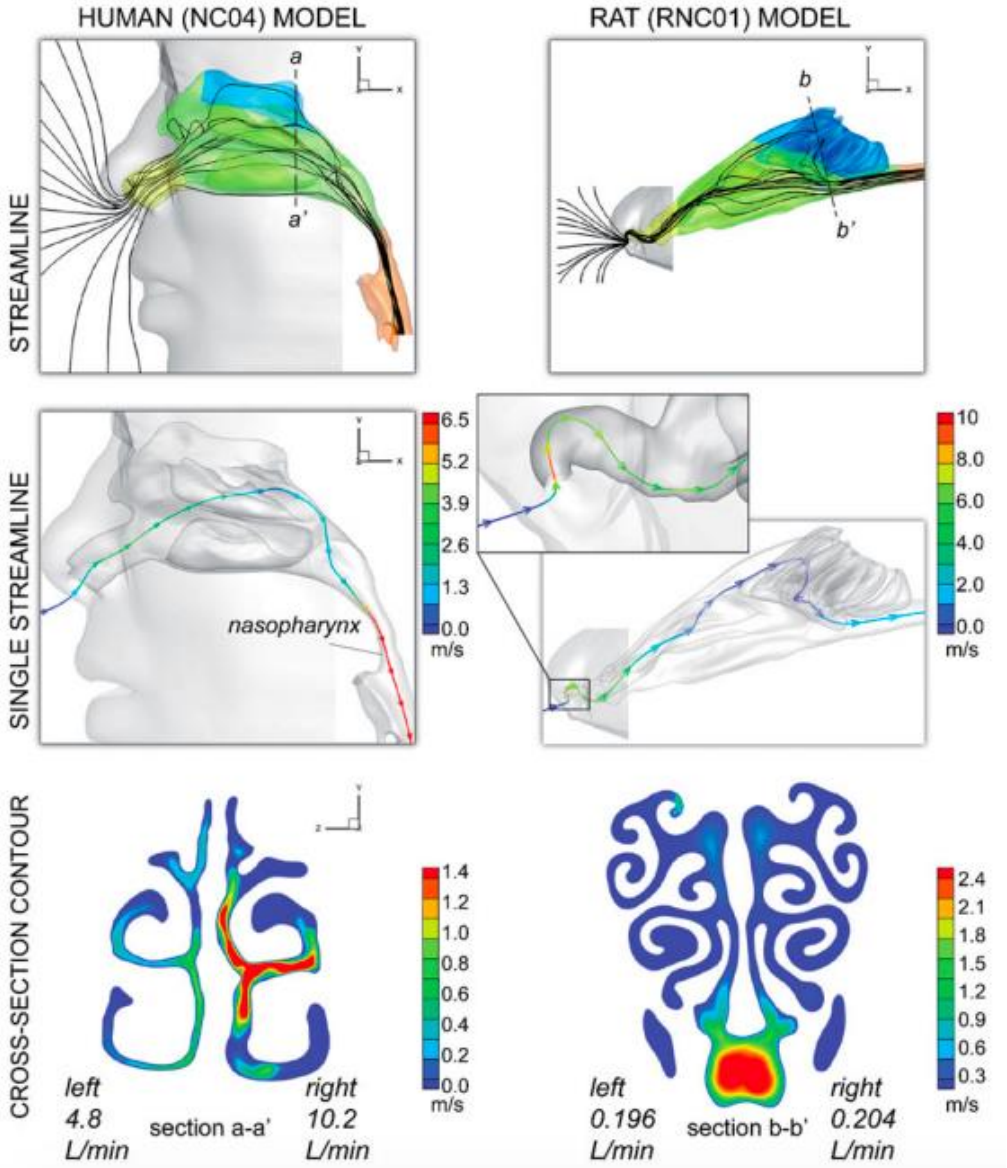

Figure 2

This presents streamlines of airflow in humans and rats, starting with outside air as it enters the nasal cavity. The single streamline use colors to show flow acceleration in the nasal cavity. It is clearly shown that the highest airspeed in human is reached in the nasopharyngeal area, whereas in rodents the highest airspeed is reached right at the beginning of the airways. The figure is used with the approval of the original authors [4].

The high velocity and the anatomy of the rat model make the deposition of particles extremely sensitive to particle size. For 3 µm particles in rats, the deposition is 100%, however, by decreasing particle size to 2 µm the deposition fraction decreases to 40%. For 1 µm sized particles only 3% deposits in the airways of rodents. The particle size influences the deposition behavior; For smaller-sized micron-particles, the deposition increases as the geometry of the airways decreases and the peak velocity increases, while particles in the larger range have higher deposition in airways with a larger diameter and the peak velocity is lower, as in humans [4].

The airflow in the airways may be laminar, transitional, and turbulent depending on the anatomical structures and the velocity of the air. Keyhani et al. (1995) concluded that the airflow in humans is laminar at normal rest breathing. When the inhalation increases and the speed of the airflow accelerates, the flow becomes unstable and turbulent [15]. This will increase the attachment of particles onto the mucosal wall. As the diameter of the airways decreases, the turbulent flow increases. This can be seen in e.g., the nasopharynx of humans [15, 23]. The flow can become unstable when increasing the speed of the flow during excessive breathing and hyperventilating, thus the flow becomes turbulent. The airspeed of humans is highest along the floor of the main passage and also the nasopharynx. The increase in airspeed causes increased particle deposition, especially in the main passage of humans and the vestibule of rodents [4, 15].

4.2 Particle filtration mechanisms

Particles may deposit onto the surface mucosa of the respiratory tract during inhalation, if they are not able to follow the streamlines [2]. The particles depositing on the mucosa can either cross the respiratory epithelium and reach the blood circulation or as mentioned in Oberdörster et al. (2004), deposit on the olfactory mucosa and translocate along the olfactory nerve, into the olfactory bulb and reach the brain [4, 24].

Many mechanisms that can affect particle deposition, but the main processes are shown in Figure 3. According to Méndez et al. (2010); inertial impaction, gravitational sedimentation, and Brownian diffusion are the three main particle motion mechanisms, and they are often included in mathematical dosimetry models. The particle deposition will depend on the particle characteristic, respiratory pattern, and anatomy of the airways. Factors affecting the

probability of particle deposition are the velocity of the airflow, the geometry, as well as the particle inertia [2].

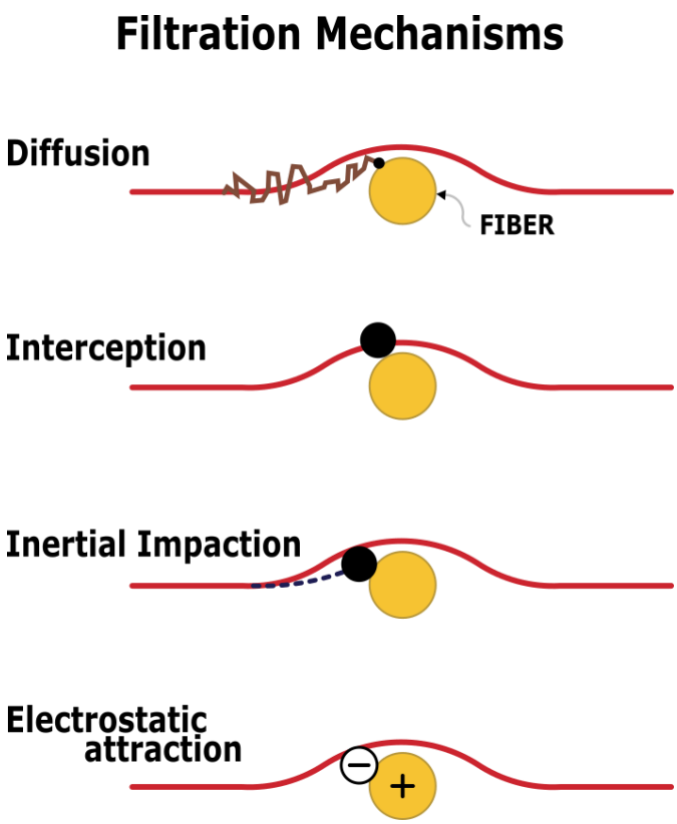

Figure 3

The main filtration processes in particle filtration in general. The yellow circle represents the filter material, the red line representing the streamline of the airstream, the black dot is representing the particle that should be filtered out [29].

Inertial impaction refers to the inability of a particle to follow the streamline of an airflow. The impaction is usually a result of a sudden and sharp change in direction of the flow, causing deposition on the walls, e.g., in the nasal vestibule of rodents, turbinates, and airway bifurcations. The probability of particle deposition due to inertial impaction depends on the anatomy, velocity, and the inertia/mass of the specific particle. Generally speaking, under the same airspeed and curvature radius, particles heavier than a certain threshold will leave the airflow and hit the wall of the respiratory tract. The higher the airspeed and/or the smaller the curvature radius the air should follow, the lower the threshold mass will be. Inertial impaction is the dominant deposition mechanism for particles with a diameter greater than 5 µm [2, 25, 26].

The velocity of air is an important factor for particle deposition. In Figure 2, we can see that the velocity increases in the vestibule and the nasopharynx due to the narrowing of the airways in the following areas. The increased velocity causes higher acceleration of particles, which increases particle inertia and increases the deposition [4]. Gravitational sedimentation refers to the settling of particles due to gravity and it is the major deposition of inhaled particles. This deposition mostly affects approximately 1-8 μm sized particles and is mainly observed in the small airways and the alveolar region. The probability of deposition due to sedimentation correlates with the amount of time in the airways, particle size, and density. However, it is contrary proportional to breathing frequency [2, 26, 27].

Lastly, Brownian diffusion is the deposition of particles caused by uncontrolled, random motions of particles caused by collision with gas molecules and may happen in all regions of the respiratory tract. Deposition caused by Brownian diffusion increases with decreasing particle size and affects particles smaller than 0.2 μm. The probability for deposition by diffusion is proportional to the amount of time in the airways and requires low airflow velocity and turbulent flow. Nanoparticles are often affected by this mechanism [2, 3, 25, 26].

Overall, particle motion mechanisms will affect the deposition of the micron- and nanoparticles. Nanoparticles are mostly affected by the Brownian diffusion rate, whilst micron-sized particles are affected by inertial impaction and gravitational sedimentation. The summarized filtration methods applied in the human airways are shown in Figure 4. A major difference in the deposition pattern of micron-sized and nano-sized particles is that the total deposition fraction in micron particles increases according to the increase in particle size (from 1 μm to 100 μm), whilst the total deposition fraction in nanoparticles decreases when the particle diameter increase (1 nm to 100 nm) [3].

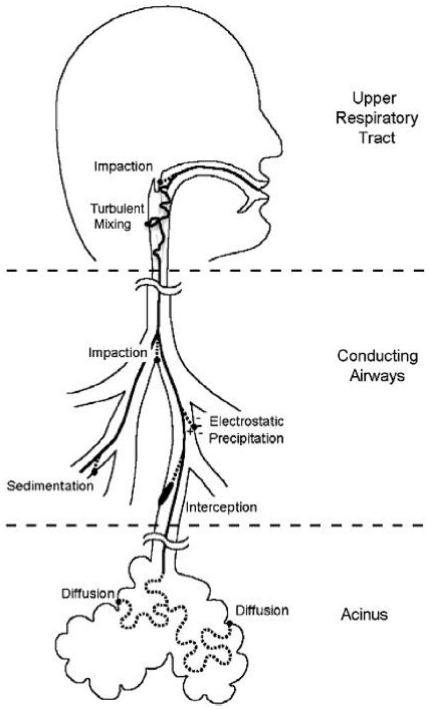

Figure 4

Filtration mechanisms applied in the human airways from the oronasal cavity to the acinus by Chantal Darquenne, 2020 [26]

4.3. Particle deposition in the respiratory tract

The comparative studies by Shang et al. (2015) and by Dong et al. (2016) are two similar studies comparing particle deposition in human and rat nasal cavities. Shang et al. (2015) focused on micron-sized particles, while Dong et al. (2016) focused on nanoparticles, but both studies used identical nasal models and inhalation rates [3, 4].

Both studies use computational models of humans and rats based on CT images to produce anatomically realistic 3D models of the nasal passage in both species. A surface-mapping technique, originally developed by Inthavong et al. (2014), was used to visualize the nasal cavity structures with a 3D model and unwrapping it onto a planar 2D domain (Figure 1). This makes it possible to get information and data on the entire wrapped surface of the nasal cavity walls, which allows direct comparison of the different species. Without a 3D viewer, it is extremely difficult to visualize the nasal cavity. This is due to the complicated anatomical geometry of the nasal cavity. Also, the characteristics of the nasal cavity may change from species to species, resulting in different deposition regions for the same-sized particles. The 2D structure makes it possible to make a direct comparison between species [3, 4].

4.3.1. Micron-sized particle distribution

4.3.1.1. Particle deposition

In the study of Shang et al. (2015) particles are divided into three categories based on their deposition fraction: low (approx. 3%), medium (40%), and high (100%) mass particles [4]. Particles larger than 3 µm cannot penetrate the upper respiratory tract in rodents, thus the particle size must be limited to 3 µm in an inhalational experiment [16]. The particle sizes used in the study are 1, 2, and 3 µm for the rat model and 2.5, 9, and 20 µm for the human model. The particle deposition visualization is performed by dividing the nasal cavity into three main compartments to evaluate and compare the filtering function, using the three different-sized particles. In Figure 1, area I. includes the vestibule, II. includes the nasal passage, and III includes the nasopharynx. The particle deposition fraction is defined as the ratio of the number of particles deposited on the mucosal walls, compared to the total amount of inhaled particles [4].

The results for the deposition fraction in humans (Figure 5), present that the deposition is the highest in the main nasal passage for all the different-sized particles. Deposition in this area occurs because of the opening of the airways from the foregoing nasal vestibule, allowing a higher number of particles to access the main passage. The vestibule (I) had the second highest deposition percentage, although the deposition for the low (0.95%) and medium (2.85%) particles are insignificant. For the larger-sized particles, on the other hand, the deposition fraction was as high as 20.9% in the vestibule. Due to the location, the pharynx showed the lowest deposition for particles of all sizes [4].

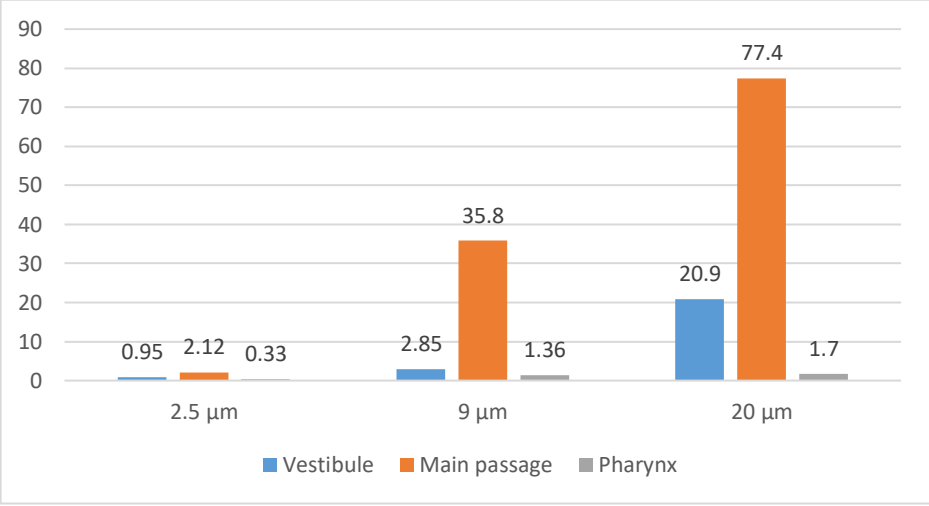

Figure 5
Deposition fraction in percent for microparticles in the nasal cavity of humans [4]

The deposition fractions in the rat model (Figure 6), showed very different results compared to the human model. The vestibule had the highest deposition fraction for all the particle sizes, respectively 2.4%, 38.9%, and 100% for low, medium, and high inertial particles. This proclaims a high-functioning particle filtering system in the vestibule, which correlates with the findings in Figure 2. Where we can see the U-shaped turn and the high acceleration of air in the vestibule. The largest particle of 3 µm shows a complete deposition fraction (100%) around the nostril bend, which shows great protection for the olfactory region and the lungs [4]. Technically, this area of the airways can be treated as an impactor- or cyclone filter unit, which has the threshold filtration mass of a normal dust particle equal to or larger than 3 µm, in the airspeed created by the animal in normal breathing conditions.

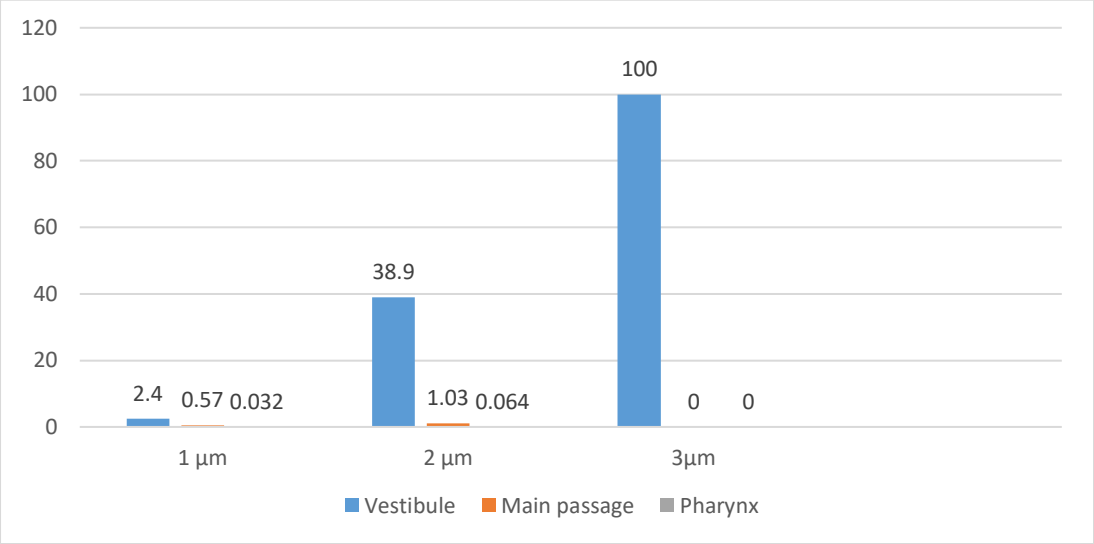

Figure 6
Deposition fraction in percent for microparticles in the nasal cavity of rat [4]

When the particle size is reduced, the deposition fraction will significantly decrease. If the peak velocity increases and the airway geometry is smaller, the deposition fraction will increase. Asgharian et al. (2014) stated that humans have a lower deposition fraction than rodents for all particle sizes, so more particles can enter the lower respiratory tract compared to rodents. This is due to the higher respiratory fraction in humans, thus more aerosol particles entering the respiratory tract, also due to the high filtration in the nasal cavity of rodents [16].

4.3.1.2. Deposition area of microparticles

In the human model, the smallest particles examined (2.5 µm) show a wide scattered deposition area in Shang et al. (2015), where the total deposition fraction is only 3.5%. This is typical in low inertial particles, as they are more likely to follow the airflow pathways and can reach further into the nasal cavity. The middle-sized particles (9 µm) have a total deposition fraction of 40% due to the increased particle inertia [4]. Fewer particles can follow the airflow, and will therefore attach to the mucosa, thus the deposition pattern regions are less than in the small-sized particles with wide distribution. The largest particles (20 µm) reach a deposition fraction of 100%, where most of the particles deposit in the main nasal passage (Figure 5). The deposition pattern is mainly near the entrance to the main nasal passage, on the septal side [4].

The rat model shows a remarkably different deposition pattern. The superiority of the high (3 µm) and medium (2 µm) inertia particles deposits in the nasal vestibule around the curvatures. Only the smallest and ultra-fine 1 µm particles with low particle inertia are able to penetrate the nasal passages. This is due to the anatomical characteristic of the rats' noses. This is demonstrated in the 3D model, showing the distribution pattern for the 3 µm particles at the upper wall of the first nostril bend, and the 2 µm particles infiltrate a bit further and deposit on the lateral wall between the two nostril bends. Lastly, the 1 µm particles were more scattered around the nasal cavity, although, the majority of the particles deposited around the second bend [4].

This study shows that the deposition fraction in rats is very sensitive to the size of the particles. The deposition fraction of 3 µm particles reaches 100%, while 1 µm particles only have 3%. Compared to humans, the same increase in deposition fraction is with particles of 2.5 µm to 20 µm in size [4].

4.3.1.3. Extrapolation of particle deposition from rat to human

A mathematical extrapolation method can be used to calculate the deposition for comparison in humans and rodents.

$$F = (N_{regional}/N_{total})/(A_{regional}/A_{total})$$

F value is the particle deposition flux, $N_{regional}$ is the number of deposited particles in a set area, N_{total} is the total amount of inhaled particles, $A_{regional}$ is the set area, A_{total} is the total surface area of the nose. The deposition of particles is not a linear pattern in regards to the human-to-rat model. Therefore, a scaling factor X is calculated to portray the deposition ratio between the rodent and human, and also to see how accurate the deposition comparison is. See Table 1 [4].

Table 1

Demonstrates the particle deposition flux with F value for low, medium, and high inertial particles in different regions of the nasal cavity. The scaling factor X shows the accuracy in ratio between human and rodent models [4].

	Low			***Medium***			***High***		
Region	F (rat)	F (human)	Scaling factor (X)	F (rat)	F (human)	Scaling factor (X)	F (rat)	F (human)	Scaling factor (X)
Vestibule	0.42	0.12	0.29	6.8	0.46	0.068	17	2.1	0.12
Upper passage	0.0035	0.0056	1.6	0.013	0.0024	0.18	0	0	N/A
Middle passage	0.012	0.039	3.3	0.016	0.9	56	0	0.32	N/A
Lower passage	0.009	0.01	1.1	0.0082	0.047	5.7	0	0.15	N/A
Olfactory	0.0045	0.004	0.89	0.011	0.035	3.2	0	0.13	N/A
Septum	0.0082	0.017	2.1	0.012	0.98	82	0	2.4	N/A
Pharynx	0.0073	0.053	7.3	0.015	0.26	17	0	0.25	N/A

Particle deposition flux f: Regional deposition fraction/regional area fraction

F value in rats has the highest values in the vestibule, for both low and medium inertial particles. This substantiates the results from other studies. The lowest particle deposition is in the upper passage for both species in low and medium inertia particles. For low inertial particles, the scaling factor shows that the upper-, and lower passage and the olfactory region show the closest 1:1 ratio in humans and rats [4]. Hence, the low inertial particles are the most accurate presentation for comparison between rodents and humans.

The largest scaling factors can be seen in medium inertial particles, located around the septum and middle passage in humans. These particles can reach further into the respiratory tract of humans, in rats, on the other hand, most of these particles are deposited in the vestibule of rats. It is not possible to make a scaling factor extrapolation with particles bigger

than 3 µm, since all the particles deposit in the vestibule of rats. The importance of this extrapolation is to highlight that there are great variations in the deposition between humans and rodents in different regions. There is no linear extrapolation between the two models, and the similarities are not significant [4]. In rodents, most particles attach in the vestibule, and less deposition in the rest of the nasal cavity. In humans, particles are more dispersed and have higher chances of entering the respiratory tract.

Respirable fraction is the fraction of particles penetrating the upper respiratory tract and reaching the lower respiratory tract. When using particles smaller than 100 nm, the respirable fraction shows relatively similar results in rodents and humans. However, when increasing the particle size, the differences increases. Mice have low respirable function due to the low inhalability and high deposition fraction in the upper respiratory tract. Particles larger than 3 µm will not reach the lungs in rodents, due to the mentioned effective filtration in the nasal cavity. Humans have high respirable function for all particle sizes, e.g. 0.2 µm particles have a 90% respirable fraction in humans, but only 30% in rodents. Therefore, when using rodent models for *in-vivo* studies, interspecies extrapolation should be based on particle size and target sites in the respiratory tract [16].

4.3.2. Nanoparticle distribution

The comparative study by Dong et al. (2016) studied the particle deposition of nanoparticles with the size of 1 nm, 10 nm, and 100 nm and compared the deposition in humans and rats. The deposition fraction is the ratio of particles depositing on the mucosa compared to the total number of inhaled particles. The results for deposition were compared with previous in-vivo and in-vitro articles. For the human model, the inhalation flow rate at 15 L/min was used. The results showed a drastic decrease in particle deposition as the particle size increased, which is equal to the data from previous literature. The rat model used a flow rate of 0.4 L/min, and showed a similar particle deposition pattern, however, they showed a more rapid deposition drop from 96.7% to 8.1% for particles from 1 nm to 100 nm [3].

4.3.2.1. Deposition fractions of nanoparticles

Similar to Shang et al. (2015) the nasal cavity is divided into three main regions: I. the vestibule, II. The nasal passage, and III. The nasopharynx. The particle size used is 1 nm, 10 nm, and 100 nm. Table 2 presents the total particle deposition in the nasal cavity in the human and rat model, enhancing that the deposition decreases as the particle size increases. The smallest particles (1 nm) are affected by Brownian diffusion and deposit greatly in the vestibule of rodents (59.89%) and the main passage of humans (52.26%). Whilst the largest nanoparticle (100 nm) has a much lower deposition on the mucosa, allowing more particles into the nasopharynx and lower respiratory tract [3].

Table 2
Shows the total particle deposition of nanoparticles in the nasal cavity

Total particle deposition in the nasal cavity demonstrated in percent		
Particle size	Human	Rats
1 nm	71%	97%
10 nm	17%	24%
100 nm	7%	8%

Comparisons between the deposition fraction in the vestibule, main passage, and pharynx of humans and rodents are shown in Figures 7 and 8. This makes the comparison between the different compartments of the species possible. In the human model (Figure 7), the main nasal passage was the preferred deposition site for all the particle sizes. The results were similar to the results presented in the study with micro-particles performed by Shang et al. (2015) [3].

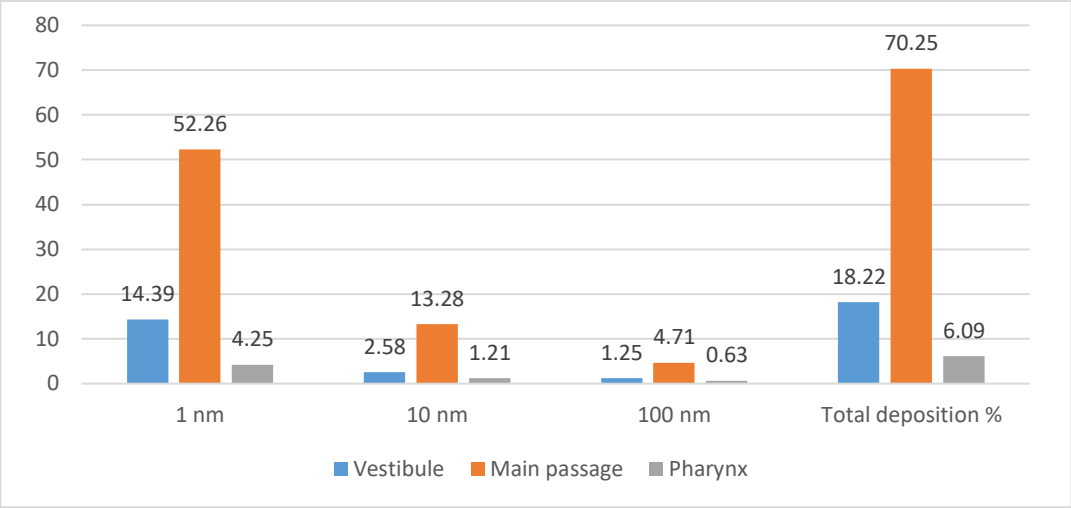

Figure 7

Particle deposition fraction of nanoparticles in the vestibule, main passage and pharynx of humans given in percent. The total particle deposition in the different regions are indicated as well [3]

Nevertheless, rodents showed a completely different pattern (Figure 8), where the 1-nm-sized particles mainly deposited in the vestibule, while the 10 nm and 100 nm particles predominantly deposited in the main passage. However, the total particle deposition for 10 nm and 100 nm sized particles, was quite low at only 24% and 8% respectively [3].

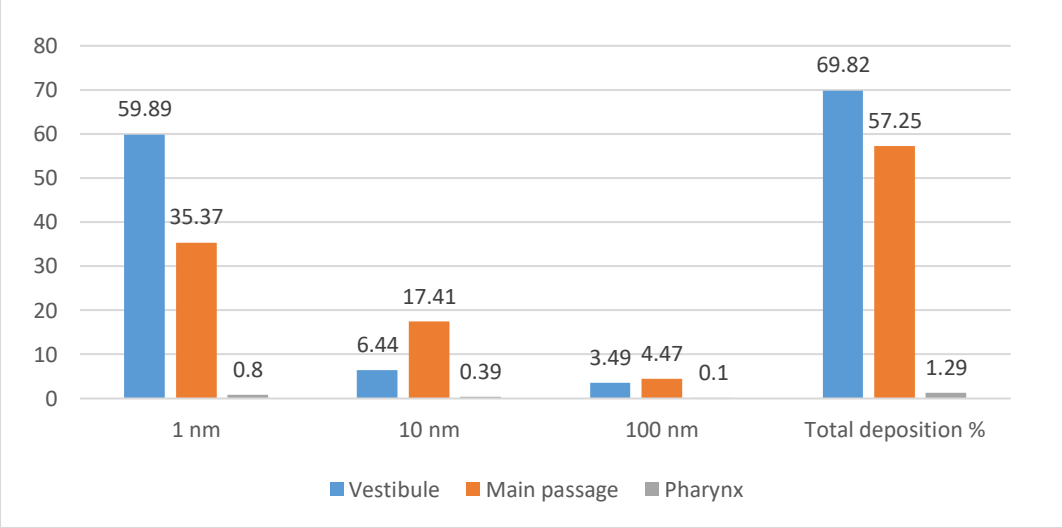

Figure 8

Particle deposition fraction of nanoparticles in the vestibule, main passage and pharynx of rats given in percent. The total particle deposition in the different regions are indicated as well [3]

To make a direct comparison of the particle exposure, the difference in the total surface area must be taken into account to make a deposition fraction per unit surface area. Hence, Dong

et al. (2016) calculated a deposition flux value (f = regional deposition fraction/regional area fraction) and plotted it into a 3D model presenting the particle deposition. The human model showed an extremely dispersed pattern for the deposition of 1 nm particles. It was distributed all over the nasal cavity, with some small areas having higher flux values e.g. in the upper part of the right chamber of the vestibule, near the septum in the left chamber, and in the middle passage in both chambers [3].

The rodent model showed a dispersed pattern as well, but the deposition regions have greater deposition flux and size, compared to the human. The deposition flux was highest in the vestibule, then the remaining particles deposited mainly in the middle nasal passage and septum. There was no visible deposition on the olfactory epithelium. The deposition pattern for the larger 100 nm particle, however, the total particle distribution was much lower (see Table 2), hence the distribution was much more limited. The same regions showed high deposition, but the deposition flux was reduced from 10 to 1.5 [3].

5. Conclusion

In this study, anatomy, physiology, and the regulation of rodents and human airways was compared. This was executed to gain knowledge of ways rodents are similar, and in ways they differ from humans. These factors influence and affect inhalation and particle deposition in the airways. Rodents are often used as *in-vivo* models in pharmacological and toxicological studies examining materials targeted for human use, therefore these are important questions.

The anatomical differences between rodents and humans in the nasal cavity are affected by the primary function of the nose. Human's main function is breathing, while olfaction is the primary function in rodents. Therefore, this results in a much more complex structure of the turbinates in the nasal chamber of rodents. Also, the olfactory region is larger and makes up more than half of the total surface area. Which permits high exposure to inhaled particles. The total surface area is approximately 10 times larger in humans than in rats, however the relative surface in rodents is about five times larger than in humans due to the branching turbinates [6, 8, 9, 12, 14]. The large surface area increases the filtration function and can cause the impaction of particles. This makes up an important difference in deposition comparison between human and rat models.

Another important divergence is the shape of the vestibule in rats, which influences the airflow streamlines and particle deposition. They have a 180-degree turn that accelerates the inhaled air to a velocity of 10 m/s and the inhalation rate is 0.4 L/min, compared to the 90-degree bend in the human vestibule, causing acceleration flow to 2.5 m/s and inhalation rate is 15 L/min [4]. The particle deposition for micron-sized particles is considerably increased by the velocity increase in the rats' noses. In comparison to the human nasal cavity; the airways have a more relaxed acceleration into the nasal vestibule [3, 4], causing less deposit onto the mucosa. The sharp curvature in the nasal cavity of the rat is important for particle filtration, it acts as an inertial impactor system. Increased velocity in the nasal chamber affects the flight path of the particles with different inertia. Due to high acceleration and anatomical differences, particle deposition will be variable in humans and rodents. As presented, most particles deposit in the vestibule of rodents due to the shape of the vestibule and the force of inertial impaction. Particles distribute more dispersed in the nasal cavity of humans.

Summing up the facts, the main particle-eliminating method in the nasal cavity of human, is the mechanical filtration by nose hairs and the mucosal surface binding of the particles in the turbinates' caused by turbulent airflow. In contrast, the main elimination method in rats is the effect of the inertial impaction in the vestibule, due to high-velocity airflow driven through a sharp curvature, causing particles (equal to or larger than 3 µm) to drop out from the airflow.

The deposition fraction for micro-sized particles showed that the deposition fraction in humans had the highest deposition in the main nasal passage, for all the different-micron-sized particles. Also, the total particle deposition increased (from approximately 3% to 100%) as the particle size increased (from 2.5 µm to 20 µm). The deposition in humans is very scattered and the deposition is much more regionalized in rats. The filter system in rodents is far more effective than in humans. In the rat, the vestibule has the highest deposition fraction for all the particle sizes (1 µm to 3 µm), due to the well-developed filtering system in this area [4]. The particle deposition pattern greatly varies between humans and rats. The particle deposition is important for the accuracy of rodents as animal models representing the human respiratory tract.

The total particle deposition in the vestibule, main passage, and pharynx show a remarkable difference in particle deposition. For the 1 nm-sized particles, as much as 97% of particles deposit in the upper respiratory tract in rodents, whilst humans have approximately 71% of total particle deposition. This makes up +30% deposition in rats for 1 nm sized particles, this is an important consideration for inhalation studies and models. Far more particles enter the lungs in humans than in rats [3].

The deposition fraction of nanoparticles proves that the preferred deposition in the human model is the main passage. The results show deposition in this area contributing to 74%, 78% and 71% for 1 nm, 10 nm, and 100 nm. The rat model showed unrelated results; 1 nm particles had the highest deposit in the vestibule with 62% of the total deposition, and the 10 nm and 100 nm particles had the maximal deposition in the main passage (72% and 55%) [3].

The use of animal models has been and most likely will continue to be important for medical research for a long time. However, no exact model to represent the human is available [28]. According to the mentioned findings, using a rat model in respiration experiments should be done with some considerations. The main thing to consider is the fact that the aerosol particles larger than 3 µm are promptly filtered out from the airstream and precipitated to the vestibular epithelia of the rat. The further destiny of this material is uncertain, some level of trans-epithelial absorption may be possible, but the main part of it will be eliminated via the nostril. However, in case of humans, particles between 3-5 µm in size are still able to penetrate the upper airways and reach the lungs. Considering the nanoparticles, the penetrating ability of these particles shows much less difference between the rodents and humans, so the experimental results gathered by treatment of rats can be extrapolated to possible human effects with higher certainty.

Another factor to consider when using rat models for inhalational tests, is that the airspeed in the airways of the rats can vary on a much larger scale than in case of humans. The airspeed is primarily influencing the effectivity of the inertial impaction filter area around the vestibule of the rats. With higher air velocity, particles even smaller than 3 µm would precipitate, resulting with an even smaller part of the experimental material to reach the deeper airways, in an experimental rat, than expected. This effect should be expected especially when the test material has irritating effects, which induces activation of self-protection measures in the experimental animals.

Due to the relatively straight and wide upper airways in humans, the changes in airspeed can influence the elimination of particular matter from the airstream at very low levels only. As stated in the literature, the highest airspeed in human can be measured at the nasopharyngeal region. The flow here may turn turbulent which helps to precipitate some of the particles to the mucosal membranes, however this filtration method is not nearly as effective as the inertial impaction found in rats. The result of the experiment under these circumstances may therefore show a less-than-real effect, or in a worst-case, even lack effect (false negative) of the test material.

In our opinion, to ensure the experimental conditions: the respirational pattern and values during a rat inhalational experiment should be closely monitored, and the aerosol particle size should be kept under 2 µm. If the biological effect of the test material is significantly

less than expected, during the evaluation test results, a repetition of the experiment should be considered using smaller sized aerosol particles or applying higher doses to the animal to balance the material loss due to the animal's defense mechanisms. Therefore, rodent models cannot fully represent the respiratory tract of humans, but by using extrapolation based on particle size and target sites, we can receive relatively accurate results for interspecies differences if the proper experimental conditions are set and the mentioned special properties are considered.

6. References

1. Rissler J, Gudmundsson A, Nicklasson H, Swietlicki E, Wollmer P, Löndahl J (2017) Deposition efficiency of inhaled particles (15-5000 nm) related to breathing pattern and lung function: an experimental study in healthy children and adults. Part Fibre Toxicol 14:10. https://doi.org/10.1186/s12989-017-0190-8

2. Méndez LB, Gookin G, Phalen RF (2010) Inhaled aerosol particle dosimetry in mice: A review. Inhal Toxicol 22:15–20. https://doi.org/10.3109/08958378.2010.541337

3. Dong J, Shang Y, Inthavong K, Tu J, Chen R, Bai R, Wang D, Chen C (2016) From the Cover: Comparative Numerical Modeling of Inhaled Nanoparticle Deposition in Human and Rat Nasal Cavities. Toxicol Sci 152:284–296. https://doi.org/10.1093/toxsci/kfw087

4. Shang Y, Dong J, Inthavong K, Tu J (2015) Comparative numerical modeling of inhaled micron-sized particle deposition in human and rat nasal cavities. Inhal Toxicol 27:694–705. https://doi.org/10.3109/08958378.2015.1088600

5. Kuehl PJ, Anderson TL, Candelaria G, Gershman B, Harlin K, Hesterman JY, Holmes T, Hoppin J, Lackas C, Norenberg JP, Yu H, McDonald JD (2012) Regional particle size dependent deposition of inhaled aerosols in rats and mice. Inhal Toxicol 24:27–35. https://doi.org/10.3109/08958378.2011.632787

6. Chamanza R, Wright JA (2015) A Review of the Comparative Anatomy, Histology, Physiology and Pathology of the Nasal Cavity of Rats, Mice, Dogs and Non-human Primates. Relevance to Inhalation Toxicology and Human Health Risk Assessment. J Comp Pathol 153:287–314. https://doi.org/10.1016/j.jcpa.2015.08.009

7. Ahmed A, Prime D, Burnell PKP, Högger P (2012) Development of an *In Vitro* Model to Assess Deposition of Aerosol Particles in a Representative Replica of the Rat's Respiratory Tract. J Aerosol Med Pulm Drug Deliv 25:169–178. https://doi.org/10.1089/jamp.2011.0902

8. Kling MA (2011) A Review of Respiratory System Anatomy, Physiology, and Disease in the Mouse, Rat, Hamster, and Gerbil. Veterinary Clin North Am Exot Anim Pract 14:287–337. https://doi.org/10.1016/j.cvex.2011.03.007

9. Meyerholz DK, Suarez CJ, Dintzis SM, Frevert CW (2018) Respiratory System. In: Comparative Anatomy and Histology. Elsevier, pp 147–162

10. Hallett S, Toro F, Ashurst JV (2022) Physiology, Tidal Volume. In: StatPearls. StatPearls Publishing, Treasure Island (FL)

11. Johnson GR, Morawska L (2009) The Mechanism of Breath Aerosol Formation. J Aerosol Med Pulm Drug Deliv 22:229–237. https://doi.org/10.1089/jamp.2008.0720
12. Alvites RD, Caseiro AR, Pedrosa SS, Branquinho ME, Varejão ASP, Maurício AC (2018) The Nasal Cavity of the Rat and Mouse-Source of Mesenchymal Stem Cells for Treatment of Peripheral Nerve Injury: THE NASAL CAVITY OF THE RAT AND MOUSE. Anat Rec 301:1678–1689. https://doi.org/10.1002/ar.23844
13. Harkema JR, Carey SA, Wagner JG, Dintzis SM, Liggitt D (2012) Nose, Sinus, Pharynx, and Larynx. In: Comparative Anatomy and Histology. Elsevier, pp 71–94
14. Harkema JR, Nikula KJ, Haschek WM (2013) Respiratory System. In: Haschek and Rousseaux's Handbook of Toxicologic Pathology. Elsevier, pp 1935–2003
15. Keyhani K, Scherer PW, Mozell MM (1995) Numerical Simulation of Airflow in the Human Nasal Cavity. J Biomech Eng 117:429–441. https://doi.org/10.1115/1.2794204
16. Asgharian B, Price OT, Oldham M, Chen L-C, Saunders EL, Gordon T, Mikheev VB, Minard KR, Teeguarden JG (2014) Computational modeling of nanoscale and microscale particle deposition, retention and dosimetry in the mouse respiratory tract. Inhal Toxicol 26:829–842. https://doi.org/10.3109/08958378.2014.935535
17. Rydell-Törmänen K, Johnson JR (2019) The Applicability of Mouse Models to the Study of Human Disease. In: Bertoncello I (ed) Mouse Cell Culture. Springer New York, New York, NY, pp 3–22
18. Nicod LaurentP (1999) Pulmonary Defence Mechanisms. Respiration 66:2–11. https://doi.org/10.1159/000029329
19. Nicod LP (2005) Lung defences: an overview. Eur Respir Rev 14:45–50. https://doi.org/10.1183/09059180.05.00009501
20. Cohn LA, Reinero CR (2007) Respiratory Defenses in Health and Disease. Vet Clin North Am Small Anim Pract 37:845–860. https://doi.org/10.1016/j.cvsm.2007.05.003
21. Symmes BA, Stefanski AL, Magin CM, Evans CM (2018) Role of mucins in lung homeostasis: regulated expression and biosynthesis in health and disease. Biochem Soc Trans 46:707–719. https://doi.org/10.1042/BST20170455
22. Welsh DA, Mason CM (2001) HOST DEFENSE IN RESPIRATORY INFECTIONS. Med Clin North Am 85:1329–1347. https://doi.org/10.1016/S0025-7125(05)70383-7
23. Issakhov A, Zhandaulet Y, Abylkassymova A, Issakhov A (2021) A numerical simulation of air flow in the human respiratory system for various environmental conditions. Theor Biol Med Model 18:2. https://doi.org/10.1186/s12976-020-00133-8

24. Oberdörster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, Cox C (2004) Translocation of Inhaled Ultrafine Particles to the Brain. Inhal Toxicol 16:437–445. https://doi.org/10.1080/08958370490439597
25. Cheng YS (2014) Mechanisms of Pharmaceutical Aerosol Deposition in the Respiratory Tract. AAPS PharmSciTech 15:630–640. https://doi.org/10.1208/s12249-014-0092-0
26. Darquenne C (2020) Deposition Mechanisms. J Aerosol Med Pulm Drug Deliv 33:181–185. https://doi.org/10.1089/jamp.2020.29029.cd
27. Darquenne C (2012) Aerosol Deposition in Health and Disease. J Aerosol Med Pulm Drug Deliv 25:140–147. https://doi.org/10.1089/jamp.2011.0916
28. Williams K, Roman J (2016) Studying human respiratory disease in animals - role of induced and naturally occurring models: Modelling respiratory disease in animals. J Pathol 238:220–232. https://doi.org/10.1002/path.4658
29. Wikiwand. “HEPA.”. wikiwand.com. Retrieved from https://www.wikiwand.com/en/HEPA (downloaded: 16/11/2022)

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Lastly, I would like to thank my friends and family for always supporting, encouraging, and believing in me.

8. Appendix

Re: Thesis literature review

Jiyuan Tu <jiyuan.tu@rmit.edu.au>
Mon 10/3/2022 9:00 AM
To: Vilde Amalie Mathisen <vilde-amalie@hotmail.com>

RMIT Classification: Trusted

Yes no problem

Regards
Jiyuan Tu
From my iPhone

From: Vilde Amalie Mathisen <vilde-amalie@hotmail.com>
Sent: Monday, October 3, 2022 12:56 am
To: Jiyuan Tu <jiyuan.tu@rmit.edu.au>
Subject: Thesis literature review

Dear Jiyuan Tu,

I am a last-year student at the University of Veterinary Medicine, Budapest, and I am writing a literature review on comparative studies of aerosol particles affecting rats and humans. I liked your illustrations in the article "Comparative numerical modeling of inhaled micron-sized particle deposition in human and rat nasal cavities" from 2015 and was wondering if I could use at least figures 2 and 3, and maybe more, with full reference to your article, for my review.

Best regards,
Vilde Amalie Mathisen

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I hereby confirm that I am familiar with the content of the thesis entitled ***Comparative study of aerosol particles affecting rodents and humans*** written by **Vilde Amalie Fon Mathisen** which I deem suitable for submission and defence.

Date: Budapest, 18.11.2022.

Kiss Dávid Sándor

.....

Kövágó Csaba
Gyógyszertani é
Méregtani Tanszék

Supervisor name and signature

BUDAPEST 1. 1787 ÁLLATORVOSTUDOMÁNYI EGYETEM Élettani és Biokémiai Tanszék

Thesis progress report for veterinary students

Name of student: Vilde Amalie Fon Mathisen

Neptun code of the student: FY6QSF

Name and title of the supervisor: Kiss Dávid Sándor, PhD. and Kővágó Csaba, PhD.

Department: Department of Physiology and Biochemistry

Thesis title: Comparative study of aerosol particles affecting mice and men

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022	2	21	Discussed thesis topic	
2.	2022	3	25	Changed assignment to literature review on the same topic	
3.	2022	4	17	First draft and literature discussion. Changed the focus of the review to a more comparative view	
4.	2022	5	6	Discussed articles and important parts to include. Made a list of content	

Grade achieved at the end of the first semester: 3 (satisfactory)

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022	09	10	Feedback on the second draft, was on a good path	
2.	2022	10	14	Discussed sources and articles	
3.	2022	10	20	Feedback for the third draft with notes	
4.	2022	10	27	Feedback on the research question and narrowed it down	

5.	2022	11	15	Finalization	

Grade achieved at the end of the second semester: 4 (good)

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

.....
signature of the supervisor

Signature of the student: Vilde Amalie F. Mathisen...

Signature of the secretary of the department: .....

Date of handing the thesis in.....18.11.2022.....