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## The Relevance of Animal Models for Aerosol Studies

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### ABSTRACT

Animal models are essential for understanding the fates and effects of inhaled materials, because invasive methods are frequently necessary to provide the desired information. Because of the variability in humans of particle deposition, clearance, and effects, numerous animal models have been used in inhalation studies. Furthermore, humans are not typical mammals in some ways that affect inhalation phenomena. Humans have less fur, longer gestation and life times, simplified nasal structure, and symmetric bronchial branching in relation to other mammals. However, experience, plus the genetic similarity among mammals, underpins the use of animal models. Mammals are varied with respect to their inhaled particle deposition and clearance phenomena. Total inhaled aerosol deposition probability versus particle-size curves are qualitatively similar for various mammals of similar body mass, despite airway anatomy differences. However, more species variation is seen in regional particle deposition curves, complicating aerosol study design. The rates of clearance of deposited slowly dissolving particles are animal species dependent, apparently due to differences in gross, sub-gross, and cellular respiratory tract biology. Clearance rates for rapidly dissolving particles are not strongly species dependent. Inhalation toxicology studies require several animal species. Rodents are among the most frequently used, but for studies of lung development, diseases, exercise, etc., and for extrapolation to humans, larger mammals are also needed. Fortunately, the research database, and excellent monographs on inhalation phenomena provide ample guidance for study design.

### INTRODUCTION

**A**N ANIMAL MODEL IS an intact animal (typically a mammal) preparation having a defined scientific utility. The animal may be healthy, diseased, manipulated genetically, and/or altered by prior treatment. In the biomedical arena, animal models are used for basic and applied research, and for testing medications, other chemicals, devices, and procedures.

Today, rigorous ethical standards are routinely applied in animal studies. The three Rs, “replacement,” “reduction” and “refinement” as originally proposed by Russell and Burch<sup>(1)</sup> are central to humane experimental design. Such standards protect both the animal subjects from unnecessary pain and distress, and the validity of the studies through requiring proper study design. In addition, studies must be justified with respect to their importance to knowledge or to

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human/animal health and welfare to be approved by an Institutional Animal Care and Use Committee. Because animal research is currently essential for the prevention and treatment of disease and injury, one can argue that animal research is imperative, and that it is unethical to impede well-designed ethical animal studies.

The foundations for the validity of animal models with respect to application to humans are based on experience, and on genetic and physiological principles. Some examples from the literature support this claim.

“ . . . there is no more powerful tool [for protecting human health] than the combination of well conducted animal experiments and well conducted epidemiological experiments,” Rall.<sup>(2)</sup>

“ . . . a large number of values [based on 86 biological regression equations] for man coincided with remarkable accuracy with those calculated for the 70 kg animal,” Krasovskii.<sup>(3)</sup>

“ . . . these survey results [for 150 pharmaceutical compounds] support the value of *in vivo* toxicology studies to predict for many significant hts [human toxicities] associated with pharmaceuticals . . . ,” Olson et al.<sup>(4)</sup>

“ . . . the human genome is remarkably similar to that of evolutionarily ancient organisms. Thus, the various life forms on earth share much more in common than anyone had previously envisioned,” Ballatori and Villalobos.<sup>(5)</sup>

On the other hand, extrapolation across species, and even comparison of results within species can be difficult due to biological factors, and differences in techniques among investigators.

This paper reviews some of the important factors related to animal models used in inhalation studies.

## AEROSOL INHALATION STUDIES

Special considerations apply to animal models that are used in aerosol inhalation studies. Among these are respiratory tract anatomy, particle deposition and clearance characteristics, methods of exposure, and study designs for in-

haled agents. In addition, factors such as animal size, pre- and postnatal events, lifespan, ease of handling, tractable husbandry in the laboratory, and the availability of laboratory techniques are important.<sup>(6-8)</sup> For many purposes, similarity to humans with respect to many of these factors is also important.

A fundamental concern in aerosol inhalation studies is identification of the proper dose metric. A dose metric is a measurable property of the studied agent such that modulation of that property directly affects the studied response of the subjects. For inhaled substances, a large and growing number of dose metrics have been considered. For pharmaceuticals, the amount (specifically the mass) of active agent that deposits in a relevant region of the respiratory tract is an important dose metric. For rapidly dissolving particles, particle aerodynamic diameter and particle mass are common metrics. For slowly dissolving particles, deposited particle surface area, particle count, or any of several other physical properties may be more important than size and/or mass.<sup>(9-12)</sup> For simplicity in this paper, deposited particle count or mass will be taken as adequate metrics. For a collection of particles (having diameter larger than about 0.3  $\mu\text{m}$ ) the mass median aerodynamic diameter (MMAD) is usually used to represent the particle size that represents the total aerosol particle mass with respect to deposition in the respiratory tract. Variations on this metric, such as deposited particle mass per unit of respiratory tract surface, can be derived using anatomical and physiological data. Such derivations, which are important for extrapolation purposes, are represented in the literature.<sup>(13-15)</sup> For particles smaller than about 0.3  $\mu\text{m}$ , the physical diameter, rather than the MMAD, is used in particle deposition modeling.

## COMPARATIVE AIRWAY GROSS ANATOMY

There is significant variation in mammalian respiratory tract structure.<sup>(16,17)</sup> Although variations in nasal anatomy are the most obvious,<sup>(18,19)</sup> significant differences are also seen in the tracheobronchial tree and the deep lung region.<sup>(16,20-23)</sup>

Humans and other primates have a relatively simple nasal anatomy, with a few shelf-like nasal turbinates and a small olfactory region.<sup>(18,19)</sup> Most other mammals have a complex, often scroll-like

internal structure with a large olfactory apparatus.<sup>(18,19)</sup> In addition, rats, and possibly other rodents, are obligate nose breathers that are incapable of mouth breathing.<sup>(24)</sup> The major implication of this difference is that the probability of getting inhaled particles larger than a few micrometers through the nose of smaller mammals is low. On the other hand, at low ventilation rates (as in deep anesthesia) the complex olfactory region of common laboratory animals is largely bypassed, which increases particle passage through the nose.

In mammals, the tracheobronchial tree serves to distribute air to alveoli and to capture some inhaled particles. The tracheobronchial anatomy of mammals tends to fall into two categories; approximately dichotomous, and strongly monopodial<sup>(25)</sup> (Fig. 1). Humans have nearly dichotomous branching, which appears to relate to having a relatively spherical chest cavity; symmetric branching being an efficient way to fill such a

space. Other mammals have a more monopodial airway structure, which is more efficient for filling elongated chest cavities, and removing heat from the blood. It may be that a monopodial airway branching system can allow panting without hyperventilating, as a monopodial manifold appears to be inefficient for ventilating alveoli at high respiratory frequencies.

In several species, including humans (also dogs, ferrets, cats, and monkeys), the transition from fully ciliated tracheobronchial airways to fully alveolated alveolar ducts is gradual: several orders of respiratory bronchioles are present.<sup>(20-22)</sup> Respiratory bronchioles are absent in mice and rats, or very abbreviated in several other species such as hamsters, guinea pigs, oxen, sheep, and pigs.<sup>(20,22)</sup> The possible implications of having respiratory bronchioles include their being sites for deep lung disease (such as emphysema), and their possible suppression of the alveolar clearance of insoluble particles. Rats and

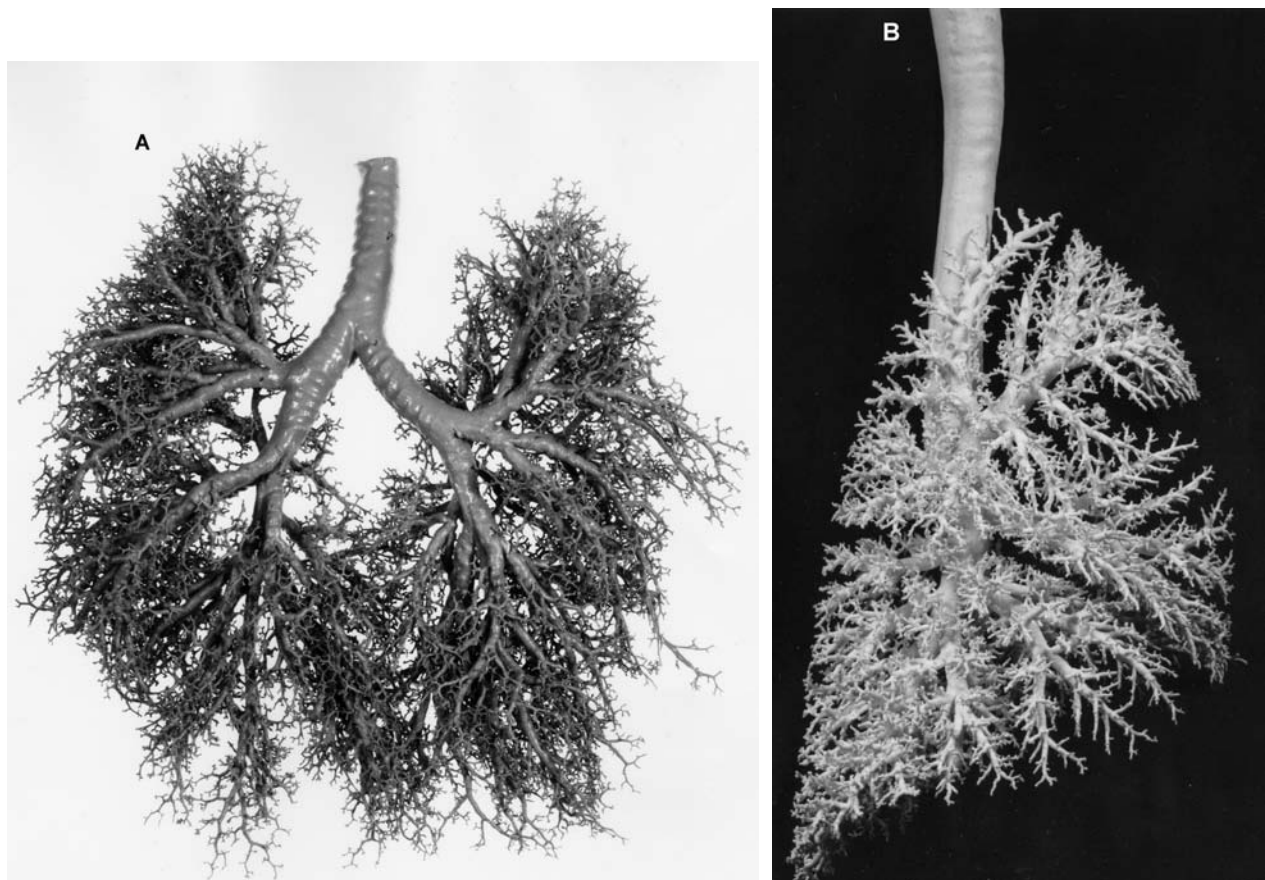


FIG. 1. (A) *In situ* cast of the tracheobronchial tree of a healthy 60-year-old man: (B) *In situ* cast of a healthy 10-kg laboratory beagle, which has a monopodial branching structure. The silicone rubber casts were prepared at the Inhalation Toxicology Research Institute (now Lovelace Respiratory Research Institute) in Albuquerque.

mice lack respiratory bronchioles, and appear to have a relatively fast early alveolar clearance of such particles, but monkeys, humans, guinea pigs, and dogs have much slower alveolar clearance.<sup>(26,27)</sup> Clearly, more information on comparative respiratory bronchiolar structure, and its implications for particle clearance and susceptibility to deep lung disease, is needed.

Further down the acinus (the structure that contains all of the gas exchange units, i.e., respiratory bronchioles, alveolar ducts and sacs, and alveoli), mammalian species differ with respect to the numbers and size distributions of alveoli.<sup>(21,28–30)</sup> As body size increases, so do alveolar size, alveolar number, and total lung surface area.<sup>(17)</sup> The extent to which differences in alveolar structure modifies aerosol deposition and clearance, and toxicological responses requires further study.

## INHALED AEROSOL DESPOSITION

An inhaled particle can be assumed to deposit in an airway where it first contacts a surface, whether the surface is that of a hair, the mucus, the surfactant, or an epithelial cell. In other words, the *sticking coefficient* is unity. Exceptions to this rule are probably very rare, if they ever occur. Because inhaled air is largely exhaled, inhaled particles must leave the air stream to touch a surface and deposit. The mechanisms that can cause particles to deposit are numerous, including: gravitational sedimentation, inertial impaction, Brownian diffusion, simple interception (for long fibers and large particles), electrostatic (e.g., image force attraction, and aerosol charge repulsion), thermal phoresis, vapor pressure, magnetic (when an external magnetic field is present), hydrodynamic (for highly concentrated aerosols), Raleigh-Taylor gas effects, and others. Most mathematical particle deposition models will consider the first three of these mechanisms, which are generally the dominant ones, and sophisticated models may include additional mechanisms.<sup>(31–36)</sup> Several reference books provide pertinent basic information on properties of aerosol particles and their behavior.<sup>(37–40)</sup> Traditional particle deposition models apply particle deposition mechanisms to simplified airway structures to predict deposition doses for the major regions of the respiratory tract.<sup>(32–34)</sup>

In addition to the traditional mechanistic mathematical models of particle deposition, sophisticated computational fluid dynamic (CFD) models have been recently explored.<sup>(41–45)</sup> In CFD modeling, the air flow structure in an airway-like cavity is first solved (using the Navier-Stokes equations) using iterative numerical techniques. Then, particles are introduced into the flow field and deposited at locations where they intersect the airway boundary. Although CFD models provide very detailed particle deposition patterns, the results are dependent on numerous input assumptions, including turbulence submodels and exactly where particles are introduced into the entrance of the airway.<sup>(46)</sup> Therefore, comparison of CFD simulations with bench-top studies are important for validation of the predictions.<sup>(46,47)</sup> Modern medical imaging methods (scanning techniques) can generate files describing airspace anatomy that can be input into CFD programs, and that can be used to fabricate hollow replicas.<sup>(47)</sup> Particle deposition studies in such airway replicas can be used to validate CFD particle deposition predictions. The potential value of CFD modeling is significant, in that particle deposition patterns in individual humans and other mammals can be obtained. Such information will allow more accurate extrapolations as well as better interpretations of individual responses to inhaled particles.

Semiempirical models of inhaled particle deposition have also been utilized.<sup>(32,33,48–52)</sup> Such models fit experimental deposition measurements to mathematical functions. The functions include dimensionless numbers (such as Reynolds and Stokes numbers) that relate to airflow and particle deposition phenomena. Semiempirical models fit experimental data well, but they have limited predictive value for unstudied cases that differ significantly from the original studied cases. The main value of such models has been their ability to provide dosimetry information for complex structures, such as the nose, that are difficult to describe anatomically.

As a result of the large number of inhaled particle deposition measurements in humans and hollow models, and extensive modeling, inhaled particle deposition as a function of particle characteristics is well described for simple particles (spherical shape, uncharged, nonhygroscopic, etc.) inhaled by humans.<sup>(32,33,53–58)</sup> The curves in Figure 2 summarize the total (anywhere in the respiratory tract) and regional (e.g., nose, mouth,



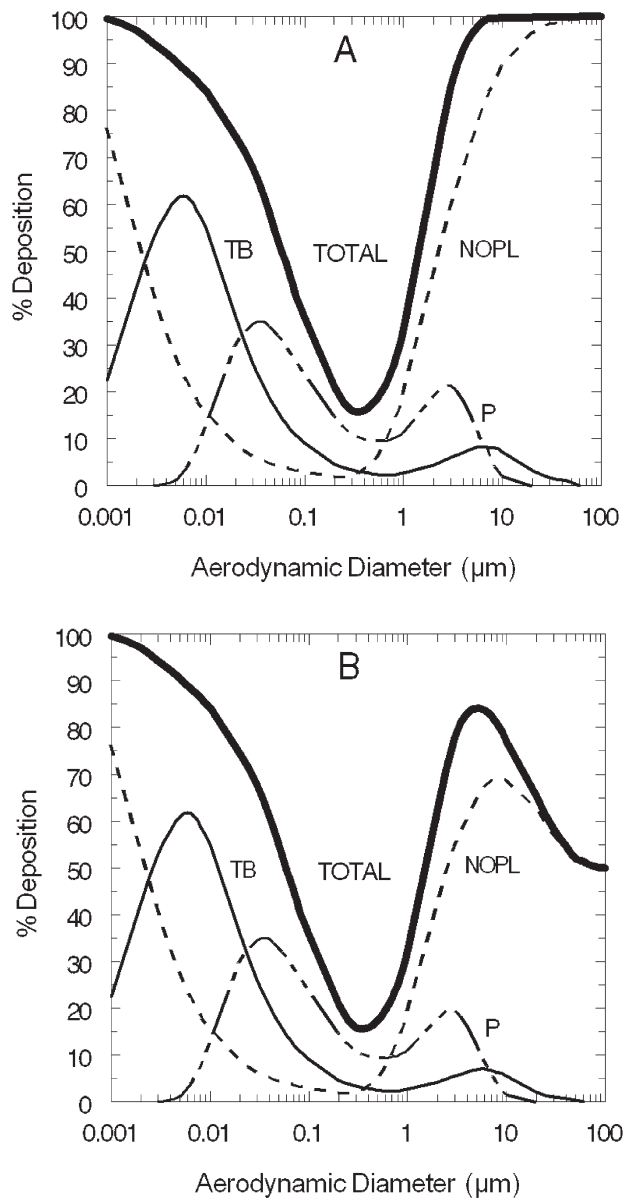


FIG. 2. (A) Particle deposition curves for typical adult human male normalized to particles that enter the nose; (B) Particle deposition curves for the same case normalized to particles in the breathing zone (i.e., corrected for particle-size dependent inhalability). Based on data from NCRP (1997).<sup>(33)</sup> NOPL, naso-oro-pharyngo-laryngeal region; TB, tracheobronchial region; P, pulmonary region.

tracheobronchial tree, and alveoli) deposition patterns for spherical unit density (1 g/cm<sup>3</sup>) particles in an average healthy resting man. Two sets of curves are presented in Figure 2 to represent deposition probability (expressed as a %) normalized to those particles that enter the nose (Fig. 2A), and normalized to those particles in the air outside the nose (Fig. 2B). The curves in Figure 2B are thus corrected for the inhalability, which

is dependent on particle aerodynamic diameter.<sup>(59)</sup> Inhalability, which is the efficiency at which particles in the immediate breathing zone are actually inspired into the nose or mouth, has been determined in wind tunnel experiments with head and head-plus-torso mannequins. The inhalability curve, as published by the American Conference of Governmental Hygienists<sup>(60)</sup> applies to a rotating adult-sized mannequin (i.e., orientation-averaged) at low wind speeds, inhaling particles up to 100-μm aerodynamic diameter. Therefore, this inhalability curve strictly applies only to this case. The inhalability of particles in laboratory animals is less well defined,<sup>(15,61,62)</sup> and is dependent on the specific aerosol exposure system used. Although the curves in Figure 2 are widely used, the variability in aerosol deposition in human subjects is often underappreciated. As an example, Figure 3 depicts clinical measurements of nasal deposition made by several investigators. For some particle sizes, deposition can vary by more than a factor of 10. Such variability in deposition not only limits the applicability of average deposition curves, but it may also partially explain the significant differences in individual responses to inhaled aerosols.

Inhaled particle deposition in common laboratory animals has been reviewed.<sup>(17,63,64)</sup> The general shapes of the total and regional particle deposition curves are qualitatively similar in laboratory animals and humans, but each species

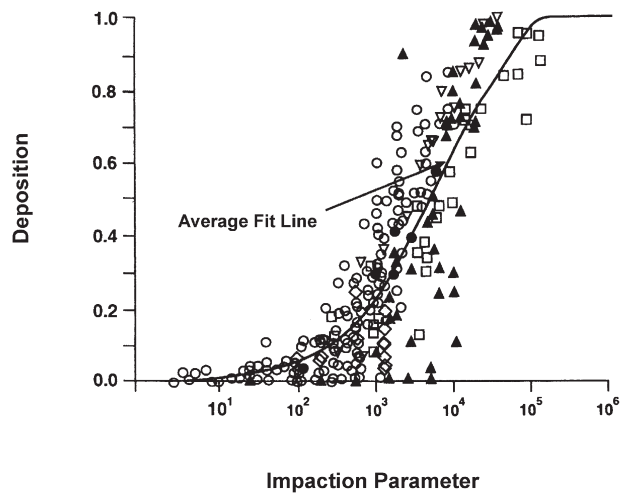


FIG. 3. Variability in aerosol deposition efficiency in the human nose, as measured by several investigators. The impaction index, related to a Stokes number, takes into account particle size and density, and inspiratory flow rate. Adapted from Figure 5.6 in NCRP (1997).<sup>(33)</sup>

has unique quantitative features. From the scant data available, for nasal breathing total deposition efficiency (for particles between 1 and about 5- $\mu\text{m}$  aerodynamic diameter) is roughly similar for humans, dogs, guinea pigs, and monkeys, but rats have lower total deposition than humans. Nasal breathing humans have greater total deposition than oral breathing humans. For upper respiratory tract deposition, nasal breathing humans have similar deposition to dogs, hamsters, and rabbits: tracheobronchial and pulmonary deposition is generally greater in humans than dogs, hamsters, and rats. However, for nasal breathing humans, pulmonary deposition is similar to that for dogs and monkeys, but greater than that for hamsters and rats. The peak particle size for pulmonary deposition is larger in humans than for dogs, guinea pigs, monkeys, and rats. These generalizations are crude, and based on very limited data, so Schlesinger's original papers<sup>(63,64)</sup> and the peer-reviewed literature should be consulted prior to designing any specific aerosol study that involves animal models.<sup>(24,65-67)</sup>

It is clear from experimental data and CFD modeling results that particle deposition within an airway is highly non-uniform.<sup>(41-43,68-71)</sup> Such hot spots of particle deposition probably occur in all airways in all mammals. However, the significance with respect to effects, beneficial or harmful, is still unexplored with the exception of bronchial cancer in cigarette smokers.<sup>(70,72)</sup> Deposition hot spots in the tracheobronchial region can deliver initial heavy particle doses to small groups of epithelial cells. Hot spots at bifurcations in the human tracheobronchial tree have been modeled in detail,<sup>(42)</sup> and deposition dose enhancement factors (EFs) calculated as functions of particle diameter, and size (patch diameter) of the local deposition site (Table 1). For the smallest patch (at the apex of a bifurcation) the calcu-

lated EFs range from 52 to 113 as particle diameter increases from 0.01 to 10  $\mu\text{m}$ . In other words, the cells in a patch size measuring 0.1 by 0.1 mm are predicted to receive about 50 to more than 100 times the particle deposition than the average cell in the airway. Carinal-to-tubular ratios of un-cleared particles of  $>100$  have been seen in the bronchi of nonsmokers at autopsy,<sup>(73)</sup> which appear to confirm the predictions. Hot spots of particle deposition have also been measured in pig lungs.<sup>(74)</sup> The implications of hot spot phenomena with respect to use of animal models in inhalation studies have yet to be well explored.

## DEPOSITED PARTICLE CLEARANCE

Before the clearance of deposited particles can be described it is necessary to classify the particles in question. Deposited particles can be classified as either rapidly dissolving or slowly dissolving. Sometimes particles are said to be either soluble or insoluble, but more accurately, the dissolution rate in airway fluids (or inside cells) is a more rigorous concept. When the dissolution rate is slow in comparison to physical transport rates of the deposited particles, the particles are in effect insoluble, poorly soluble, or slowly dissolving for the purpose of modeling particle translocation or clearance. The dissolution characteristics, and other properties of inhaled particles can strongly modify their toxicity.<sup>(12,75)</sup>

Rapidly dissolving particles, by definition, are not cleared intact by physical mechanisms. Soon after deposition their components are available to enter fluid and tissue compartments of the respiratory tract. In the case of deposition in the alveolar region, the particle components may rapidly be transported to nonpulmonary sites via the blood circulation.<sup>(32,33,76)</sup> In this case the pharmacokinetics of the particle's components will determine their distribution, sequestration, metabolism, and elimination from the body. Species differences in pharmacokinetics must be considered in selecting models.<sup>(76,77)</sup>

In the case of slowly dissolving particles their translocation and clearance is dependent on the physical particle size, the number of deposited particles, the site of deposition, the toxicity, and the animal species or health status (Fig. 4).<sup>(58,78-80)</sup> Particle size influences the deposition sites, which determines the mechanisms available for clearance, that is, removal from the site. Most slowly

TABLE 1. COMPUTED PARTICLE DEPOSITION EFFICIENCIES FACTORS (EFs) VS. SQUARE PATCH DIMENSION AND PARTICLE DIAMETER FOR UNIT SPECIFIC GRAVITY SPHERES

Particle Diam. ( $\mu\text{m}$ )	<i>Enhancement factors</i>		
	Patch size (mm)		3.0
	0.1	1.0	
0.01	52	18	6.7
0.1	58	18	7.1
1.0	81	20	6.9
10.0	113	42	22

Adapted from Balaszy et al., 1999.<sup>42</sup>

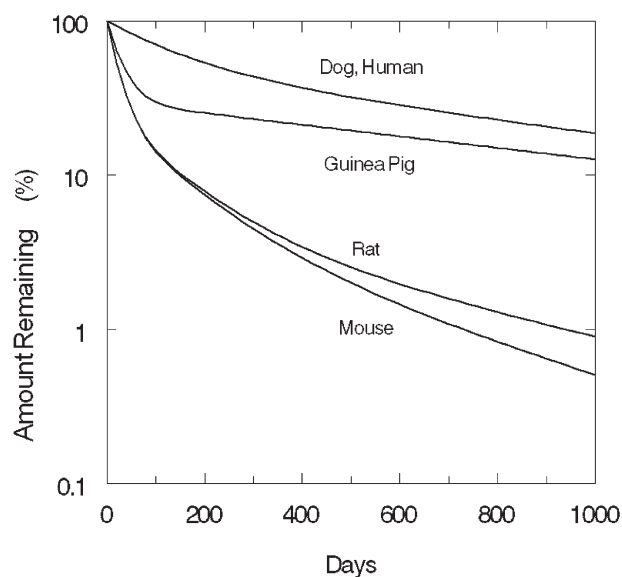


FIG. 4. Species differences in the clearance of slowly dissolving particles. Adapted from Wolff (1996).<sup>(26)</sup>

dissolving particles deposited in the anterior portion of the nose, the mouth, and to a lesser extent the posterior nose, the pharyngeal regions, larynx and trachea, are cleared by bulk mechanisms such as wiping, sneezing, blowing, spitting, and coughing: Nonbulk mechanisms include mucociliary movement followed by swallowing, and for some small insoluble particles (probably up to a few nanometers in diameter) movement to the brain via an olfactory nerve pathway<sup>(81,82)</sup> Although there are likely to be species differences in clearance from the head and neck airways, they have not been effectively reported. Because of significant species differences in relative size of the olfactory region, one expects major differences in the relative importance of clearance mechanisms in the nose.

The tracheobronchial region clears intact particles primarily by cilia-driven mucus movement.<sup>(32,33)</sup> The common assumption that this region is completely cleared of deposited particles within 24 h postdeposition, is certainly untrue. Incomplete clearance and uptake of particles by bronchial epithelial cells can lead to long-term retention in normal and smokers lungs.<sup>(73)</sup> Slow bronchial clearance has also been observed directly in rats<sup>(83)</sup> and by examining the clearance of bolus-inhaled particles in humans<sup>(32,58,77,84)</sup> Furthermore, lung disease states can also impair mucociliary clearance of particles.<sup>(78,85,86)</sup>

Evidence to date does not indicate major species differences in the mechanisms of tra-

cheobronchial clearance of particles. Smaller mammals have slower bronchial mucus velocities,<sup>(33,77)</sup> but they also have compensating shorter airways. With the exception of the question of how the presence of respiratory bronchioles affect mucociliary clearance, data in laboratory animal models should be applicable to some groups of humans, given the great variability in humans.<sup>(77,79)</sup>

Slowly dissolving particles clear from the alveolar region by a variety of mechanisms, not all of which are well understood. Cleared particles, either bare or in macrophages, may slowly dissolve, may move toward bronchial airways, into the blood, into the lymphatic system, or possibly move directly into the pleural region.<sup>(32,33,58,87)</sup> In the alveolar region, significant species differences in particle clearance kinetics have been observed (Fig. 4). The alveolar macrophages, a primary factor in particle clearance, differ significantly in number and size in mammals.<sup>(17,21,30)</sup> Thus, the capacity for particle engulfment is expected to vary considerably. In addition to alveolar macrophages, intravascular, interstitial and airway populations are known, but their interactions with particles are less well studied.<sup>88</sup> Also, the observance of clearance stasis, due to macrophage overloading, especially in rats, has significant implications for study design.<sup>(89-92)</sup> Short- and long-term clearance curves for slowly dissolving particles exhibit species differences.<sup>(17,26,27)</sup> There is a need for additional research on the use of animal models in alveolar clearance studies.

## INHALATION TOXICOLOGY MODELS

The enormous range of inhalation toxicology studies precludes any succinct review of the use of laboratory animal models. Such studies range from the most basic involving exploration of fundamental biologic, physiologic, and toxicologic principles, to the ranking and/or testing of devices, substances, and procedures regarding their safety and/or efficacy. Virtually all animal models that have been used have their strengths. For initial testing of tissue injury of inhaled substances, including ranking of substances and combinations, dose-response phenomena, and target tissues, rats, mice, and other small animals are desirable. These types of studies often require large group sizes and large numbers of groups to



provide statistically meaningful results.<sup>(93–96)</sup> Rodents, typically rats and mice, are also used for studies relating to lethality, reproduction, teratology, motor activity, metabolism, toxicokinetics, metaplastic events, etc.<sup>(94)</sup> More advanced, and confirmatory testing is generally conducted in dogs or nonhuman primates. A large variety of other species can be used for specific purposes. Hamsters, guinea pigs, rabbits, cats, ferrets, dogs, sheep, goats, pigs, horses, and cattle are among the more specialized animal models. Several monographs aid in selecting animal models for inhalation studies: *Comparative Biology of the Normal Lung*,<sup>(16)</sup> *CRC Handbook of Toxicology*,<sup>(97)</sup> *Inhalation Toxicology*, 2nd ed.,<sup>(98)</sup> *Particle Toxicology*,<sup>(99)</sup> *Inhalation Toxicology*,<sup>(100)</sup> *Handbook of Human Toxicology*,<sup>(101)</sup> *Animal Models in Toxicology*,<sup>(93)</sup> and *The Lung: Development, Aging and the Environment*.<sup>(7)</sup>

Inhalation studies present unique technical challenges that require expertise in creating and characterizing the atmospheres, and delivering aerosols to animal subjects. Among the helpful references on basic aerosol science and technology are: *Aerosols Handbook*,<sup>(102)</sup> *Aerosol Technology*, 2nd ed.,<sup>(37)</sup> *The Mechanics of Aerosols*,<sup>(38)</sup> *Aerosol Science for Industrial Hygienists*,<sup>(39)</sup> *Bioaerosols Handbook*,<sup>(103)</sup> and *Smoke, Dust and Haze*, 2nd ed.<sup>(40)</sup> For inhalation exposure techniques, the key monographs include: *Generation of Aerosols and Facilities for Exposure Experiments*,<sup>(104)</sup> *Concepts in Inhalation Toxicology*,<sup>(105)</sup> *Toxicology of the Lung*, 3rd and 4th eds.,<sup>(106,107)</sup> *Inhalation Studies: Foundations and Techniques*,<sup>(108)</sup> *Inhalation Toxicology and Technology*,<sup>(109)</sup> *Methods in Inhalation Toxicology*,<sup>(110)</sup> and *Inhalation Toxicology*.<sup>(100)</sup> In addition to the foregoing references, several recent monographs cover a variety of specialized topics of interest to investigators who design and conduct inhalation studies: *Particle Overload in the Rat Lung and Cancer*,<sup>(91)</sup> *Particle–Lung Interactions*,<sup>(111)</sup> *Particle Toxicology*,<sup>(99)</sup> *Drug Delivery to the Lung*,<sup>(112)</sup> *The Mechanics of Inhaled Pharmaceutical Aerosols: An Introduction*,<sup>(45)</sup> *Toxicology of the Lung*, 4th ed.,<sup>(107)</sup> *Optimization of Aerosol Drug Delivery*,<sup>(113)</sup> *Inhalation Aerosols: Physical and Biological Basis for Therapy*,<sup>(114)</sup> *Overcoming Challenges to Develop Countermeasures Against Aerosolized Bioterrorism Agents: Appropriate Use of Animal Models*,<sup>(115)</sup> *Medical Applications of Computer Modeling*,<sup>(44)</sup> *Inhaled Particles*,<sup>(116)</sup> and the remarkable continuing series on *Lung Biology in Health*

and *Disease*, with over 220 books in print, published by Marcel Dekker.

Engineered nanoparticles and environmental ultrafine particles (diameter <0.1  $\mu\text{m}$ ) have recently been emphasized in inhalation toxicology research. Such particles have dosimetric, biologic, and toxicologic properties that differ from larger particles. Such particles have been associated with adverse effects in epidemiologic air pollution studies,<sup>(82)</sup> appear to be more inflammatory than larger particles on an equivalent mass basis,<sup>(117)</sup> and are emerging in a large number of variations in aerosol medicine.<sup>(75)</sup> In addition to these cited references, *Particle Toxicology*,<sup>(99)</sup> *Ultrafine Particles in the Atmosphere*,<sup>(118)</sup> *Particle–Lung Interactions*,<sup>(111)</sup> and recent papers by Kreyling et al.,<sup>(80)</sup> Kuempel et al.,<sup>(14)</sup> and Moss and Wong,<sup>(11)</sup> provide additional useful information.

Clearly, the knowledge base on inhalation animal models, study designs, and study techniques is expanding at an astounding rate. Although the knowledge base in aerosol inhalation studies has matured significantly in the past few decades, major advances are still emerging.

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