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Review Article

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Practical Considerations in Dose Extrapolation from Animals to Humans

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Abstract

Animal studies are an important component of drug product development and the regulatory review process since modern practices have been in place, for almost a century. A variety of experimental systems are available to generate aerosols for delivery to animals in both liquid and solid forms. The extrapolation of deposited dose in the lungs from laboratory animals to humans is challenging because of genetic, anatomical, physiological, pharmacological, and other biological differences between species. Inhaled drug delivery extrapolation requires scrutiny as the aerodynamic behavior, and its role in lung deposition is influenced not only by the properties of the drug aerosol but also by the anatomy and pulmonary function of the species in which it is being evaluated. Sources of variability between species include the formulation, delivery system, and species-specific biological factors. It is important to acknowledge the underlying variables that contribute to estimates of dose scaling between species.

Keywords: aerosol delivery, allometric scaling, dose, lung anatomy, lung function, species differences

Introduction

The use of animals as surrogates for humans to address safety of exposure to inhaled xenobiotics is well established.[1–5] As animal models of disease emerged, their use for assessing safety of therapeutic agents was an extension of their application to prediction of behavior in humans.[6,7] Presently, the ability to create disease models through genetic intervention or by selecting a relevant host for a pathogen has further promoted the use of animals in drug discovery and development.[8–11] These models often have a strong mechanistic underpinning at the level of molecular and cellular biology.

Early experiments to demonstrate the efficacy, disposition, and safety of inhaled drugs are usually conducted in small animals. Frequently, these are disease models intended as initial screens. As product development proceeds, formal, regulated studies are usually conducted in both rodent and non-rodent species. The intention of these studies is to extrapolate to safe and efficacious doses in humans.[3,5,12,13]

However, to extrapolate from animals to humans, the species differences must be thoroughly understood to establish relevant deposited dose estimates. Species differences present challenges in direct extrapolation to humans, which has long been acknowledged.[14] It is important to recognize formulation and delivery differences that can also contribute to variations that may need consideration to facilitate the estimation of dose deposited in the lungs. Figure 1 illustrates the interface between the inhaled drug product, the drug delivery technology, and the lung biology that is ultimately responsible for drug efficacy.[15] The technical and biological factors that exist between the drug formulation and its local biopharmaceutical interactions and ultimately bioavailability are species dependent. Species

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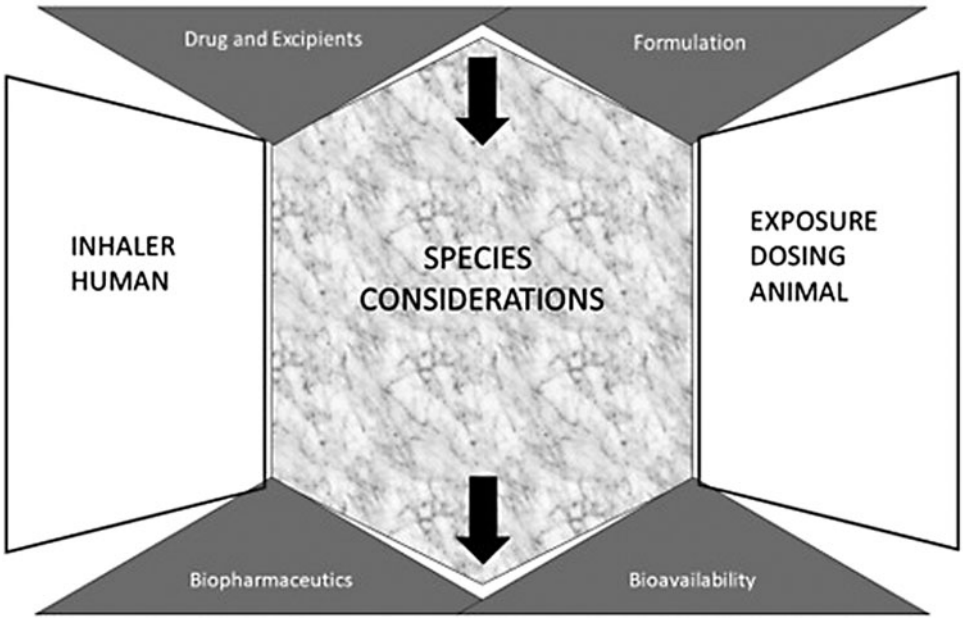

FIG. 1. Species considerations are required for both technical and biological factors influencing the dose to contrast human inhaler delivery of drug with that of animal exposure.

dependence results from a range of anatomical and physiological differences, including lung anatomy, clearance mechanisms, and receptor biology. These particular species differences will be described in detail below.

Figure 2 illustrates the elements of the technological approach that depend on the intended species in which the drug will be evaluated. The delivery device in combination with the drug formulation (i.e., drug, excipient, and processing) constitutes the drug product (or drug delivery system).[16] The drug product/delivery system needs to accommodate the requirements to deliver to the evaluating species, dispersing the aerosol under the influence of an airflow with a specific resistance to achieve the desired aerodynamic performance.[17] Ideally, the aerodynamic performance is equivalent or smaller to that when tested with human devices/delivery systems to support nonclinical testing in a clinically relevant manner. The drug delivery technology that requires consideration relates to differences in animal anatomy and physiology (i.e., pulmonary function). Strategies have been developed to address these issues, whether in early proof-of-concept efficacy and pharmacokinetic assessment or in formal nonclinical safety testing. Clearly, there are many sources of interspecies variation to be addressed in the experimental design and in subsequent data interpretation.

The intent of this review is to consider primary sources of variability, including formulation, device/delivery technology, lung anatomy, physiology (i.e., pulmonary function), lung deposition, clearance, and receptor/target location.

Formulation and Device/Delivery Technology

The drug formulation composition can readily be translated from one species to another and is central to the ability to establish tolerability. However, the physicochemical properties of the formulation, which are usually fixed to meet the needs of humans, impact the ability to effectively deliver drug to animals.[4,13,17] The device/delivery technology used for humans (e.g., inhaler) is rarely directly applicable to animals and requires either an alternative or modification to meet the needs of exposure.[3,13,17] There are clear biological differences between species self-evident at the level of size, but beyond that, the structure and function of the lungs differs to an extent that influences deposition. Consequently, extrapolation of dose from animals to humans is not simply a question of allometric scaling; it requires consideration of the purpose of the studies and the relevance of any animal model to the human situation.

Formulation options

The major formulation options are either liquids, molecular dispersions in aqueous or nonaqueous fluids, or solids, either as particles alone or suspended in liquids.[18] It is not within the scope of this article to thoroughly describe all possible formulations that might be delivered as aerosols to the lungs. However, it is important to acknowledge that the state in which the drug is presented defines the device requirements both for human and animal delivery.

Briefly, drugs in aqueous media include solutions, lipids (e.g., liposomes), and nanosuspensions.[19–23] The most prominent nonaqueous medium in which drug may be dispersed is volatile propellant used in metered dose inhalers.[24] The drug may be present in the nonaqueous medium as a solution or suspension. Dry powder formulations are used as prepared for the product for human use.[25] Both aqueous and nonaqueous drug preparations and dry particles are matched with inhaler and nebulizer devices to disperse their drug contents directly into air for delivery.

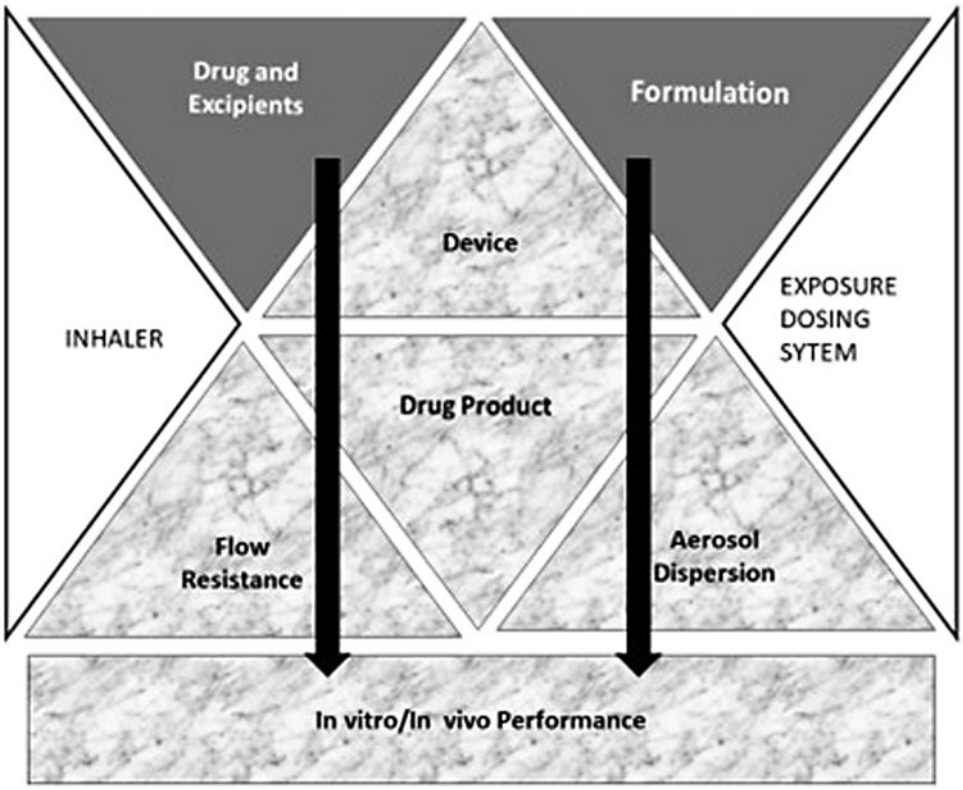

FIG. 2. Technical considerations that differ between inhalers for human use compared with exposure dosing systems for animals. *In vitro* performance refers to standard aerosol performance testing (e.g., delivered dose, APSD). APSD, aerodynamic particle size distribution.

Pulmonary delivery system

It is convenient when considering relevant methods of inhaled drug delivery to discriminate between liquid and solid aerosols. Liquid aerosols can be delivered by nebulizer, soft mist inhaler, or metered-dose inhaler.[21,24,25] Particulate aerosols are delivered by dry powder inhaler or metered-dose inhaler.[24–26] While these devices are valuable parts of the therapeutic regimen for a variety of human diseases, many of the devices are not easily translated for delivery to animals. The ability for humans to coordinate inhalation of airborne drug particles from a device with inspiratory flow through the oropharynx is difficult to mimic in most species. Consequently, the focus of aerosol delivery to animals is to achieve: (1) a defined dose and; (2) a representative particle size distribution that can be translated to humans. This focus addresses important and complex early development and preclinical testing requirements and the regulatory burden of relevance of the animal data to a human.

Target product performance

The key critical performance characteristics associated with inhalers have been defined by regulatory guidance and compendial standards with respect to dose and aerodynamic particle size characteristics.[27,28] The nominal dose, retained in the metering system, is depleted through each step of delivery. Initially, the emitted dose reflects that proportion of the nominal dose that exits the mouthpiece of the inhaler. The delivered dose is that entering the mouth. The respiratory or lung dose is that passing the oropharynx and entering the lungs. Note that lung deposition is throughout the entire lung below the larynx encompassing both tracheobronchial and pulmonary (alveolar deposition). Figure 3 illustrates the way in which the dose diminishes at each step by losses that occur in transit.[29,30]

The aerodynamic particle size distribution (APSD) is known to influence the proportion of the aerosol that can enter the lungs, since losses in the upper respiratory tract (mouth and oropharynx) are influenced by particle size.[31] For human lung deposition, 5 μm is considered as the cutoff above which most of the aerosol does not enter the lungs and below which most of the aerosol passes to the lungs.[31]

The methods most frequently employed to measure these properties are sampling tubes and filters for dose determination and inertial impactors, notably the Andersen Cascade Impactor and the Next Generation Impactor, to determine the APSD.[27]

Species Differences

At the fundamental level, it is important to recognize the similarity of mammals. In the era of genomics, this might best be addressed through sequence homology of the genomes. However, the data are not all available and the methods and completeness of the characterizations differ.

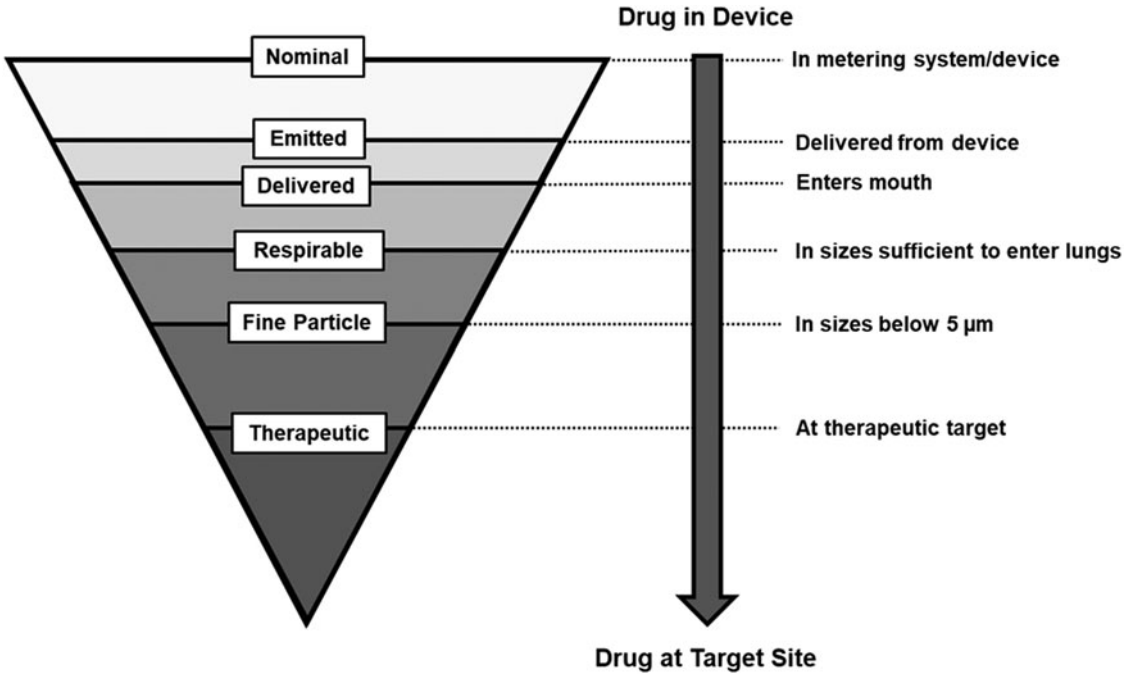

FIG. 3. Dose segmentation resulting from delivery efficiency and retention in the metering system/device and airways of humans.

Consequently, simply examining the evolutionary tree indicates the points at which divergence occurred, which presumably has underlying genetic origins. Figure 4 shows the phylogenic tree for several laboratory animals.[32,33] This would seem to indicate an expectation of genetic similarity in the following sequence: human, nonhuman primate (rhesus monkey), dog, rabbit, guinea pig, rodent (mouse/rat).

Anatomy

The structure of the lungs is an important consideration for aerosol delivery. The physical pathway and the fluid dynamics governed by breathing frequency and tidal volume (product = minute volume) influences the deposition of airborne particulates. The structure of the human lungs differs significantly from that of many frequently utilized species for aerosol-related research. The lungs of humans and guinea pigs have a bifurcating branching structure that is almost regularly dichotomous at all scales of scrutiny. Nonhuman primates (e.g., Rhesus monkeys) and rabbits exhibit irregularly dichotomous lung structure.[34] Rodent lungs are monopodial in that they exhibit a root and branch structure.[35,36] Combining multiple lung structures, dog airways are irregularly dichotomous in the upper airways but monopodial in the lower airways.[34] Features of bifurcating and monopodial lung structures are illustrated in Figure 5. The human lung cast, Figure 5A shows the bifurcating system, while the monopodial system of the dog is shown in Figure 5B.

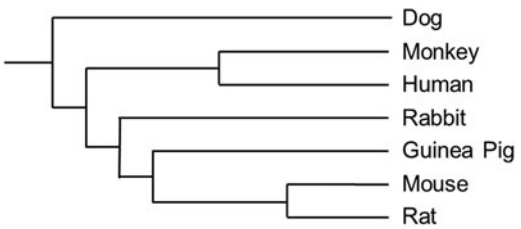

FIG. 4. Phylogenetic tree for laboratory animals utilized in inhaled therapeutic research. Genetic similarities based on data in the NCBI genomic database. NCBI, National Center for Biotechnology Information.

The overlaid schematic structures, at the right corner of each panel in Figure 5, illustrate the details of these lung anatomies.[37] It is evident that the fluid dynamics underlying the transport of aerosols on the inspiratory flow of these organisms will differ.

Beyond the anatomical features that will influence particle behavior in the lungs, there are species differences in the diameter of a particles that can enter the lungs following naso- or oropharyngeal inhalation.[38] Rodents, rabbits, and guinea pigs are obligate nose breathers, and therefore, nasal deposition automatically precedes lung deposition.[39,40] Humans are capable of inhaling through their mouth or nose at will, which allows differentiation of nasal from inhaled aerosol sampling and in turn nasal and inhaled products.

Nose/throat cutoff diameters

Once the critical performance characteristics of an inhaled drug product have been defined, an aerosol can be delivered to any species of animal for evaluation. At this point, the interaction of the aerosol is with the unique anatomy and physiology of the species employed. Figure 6 illustrates the way that particle size influences pulmonary deposition.[41] For illustration of the species differences, the diameter at which pulmonary penetration of aerosol occurs is not surprisingly at higher particle sizes for larger animals, human: 5.0 μm; dog/monkey: 3.0 μm; rat/mouse: 2.5 μm; and guinea pig: 1.8 μm.[42] Consequently, the proportion of

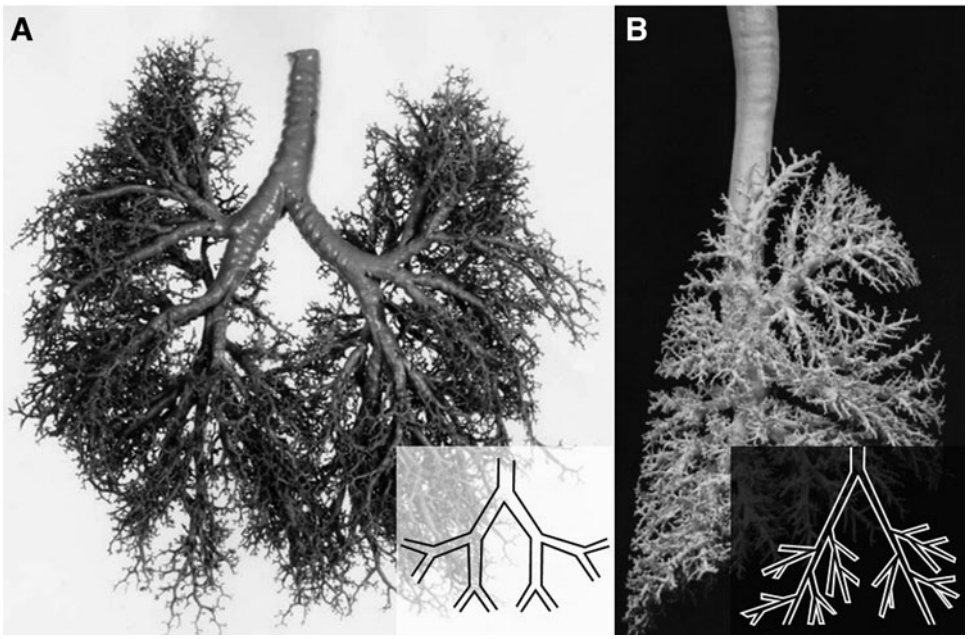

FIG. 5. **(A)** *In situ* cast of the tracheobronchial tree of a healthy 60-year-old man. **(B)** *In situ* cast of a healthy 10-kg laboratory beagle. The silicone rubber casts were prepared at the Inhalation Toxicology Research Institute (now Lovelace Respiratory Research Institute) in Albuquerque. Overlaid figure shows the branching structure of **(A)** bifurcating and **(B)** monopodial lungs. Figure reproduced with permission. Copyright 2008, Mary Ann Liebert, Inc.[35]

any aerosol that can enter the lungs varies from one species to another. The deposition curves in Figure 6 have been utilized to establish deposition fractions relative to humans. Moreover, regional deposition within the lungs differs from small animals to humans.[31,43]

Physiology (pulmonary function)

The unique anatomy of each species is accompanied by pulmonary function that impacts directly on the deposition of inhaled aerosols. The lungs expand and contract under the influence of intercostal and diaphragmatic muscles. The two parameters that direct the dose depositing in the lungs are the breathing frequency and tidal volume, that multiplied together, give the minute volume. Table 1 shows these parameters for various species of laboratory animals.[35,41,44–53] Not surprisingly, the minute volume is proportional to the size of the animal such that human > dog > guinea pig > rat > mouse. Of note, differences within a given species can be due to the breed, age, and size.[48] Further differences are demonstrated based on the measuring technique used (e.g., oscilloscopic respirograph, tracheal valve method) and subject conditions.[48] For example, Pleil et al. report that the respiratory minute volume of humans is 6 L/min at rest, 16 L/min during normal activity, and 40 L/min during moderate activity,[45] giving rise to a wide range of minute volume values dependent on the conditions during measurement. Consequently, the use of a single value is an approximation that is not intended to capture all sources of biological or physical variability.

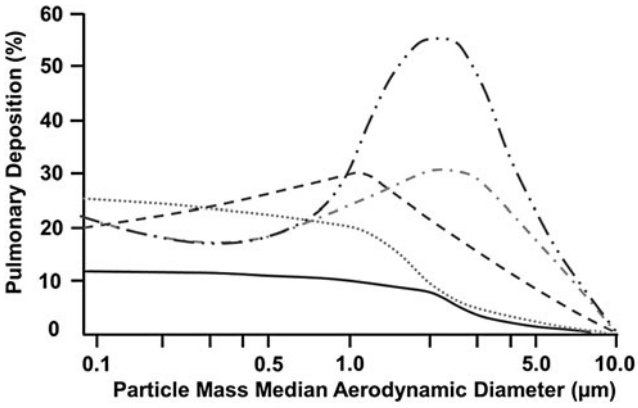

FIG. 6. Pulmonary deposition of particles inhaled by rats and mice (solid), guinea pigs (dot), dogs and monkeys (dash), and humans with nasal breathing (dot dash) or mouth breathing (dot dot dash). The pulmonary deposition represents the fraction of the total amount inhaled. Data based on that are presented by Snipes.[42]

Allometric scaling

The characteristics of the aerosol delivered and the anatomy and physiology of the species dictate the aerodynamic properties (or behavior) of the particles/droplets as they traverse the airways. Once the aerosol has deposited, the local concentration will drive pharmaco- and toxicokinetic and pharmaco- and toxicodynamic effects. The means of extrapolating to humans across species not only depends on the differences in anticipated deposition but also the allometric scaling across a variety of different

Table 1. Range of Reported Average Breathing Frequency, Tidal Volume, and Respiratory Minute Volume for Humans and Relevant Laboratory Animals

Species	*Breathing frequency (breaths/min)*	*Tidal volume (mL)*	*Respiratory minute volume (L/min)*	*Reference*
Mouse	109–163	0.15–0.18	0.02–0.04	38,44,46–48
Rat	85–200	0.87–1.5	0.07–0.26	38,44,46–48,53
Guinea Pig	42–90	1.7–3.7	0.13–0.46	38,44,46–49
Rabbit	39–85	16–24	0.62–1.6	38,46–48,52
Monkey	30–40	20–42	0.70–1.6	38,44,46–48,50,51
Dog	17–21	144–320	3.1–5.2	38,44,46,47,53
Human	12–16	400–616	6–20	38,44,45,47,48

dimensions, characteristic of the lungs.[14,54] These include surface area, volume, and weight. Figure 7 shows the relationship between these metrics and body weight.[54] There is clearly a relationship across species that can readily be accommodated in considerations that depend on these parameters. This relationship may be different for pharmacologic aerosols that need primarily central deposition in the lung (bronchodilators) versus aerosols that need peripheral deposition (anti-inflammatories/steroids).

Methods of Pulmonary Delivery

Intratracheal administration

Delivery of solutions or suspensions directly to the lungs by spray liquid instillation is an improvement on a method of administration of drugs particularly to rodents that has been employed for decades.[55] A tube is inserted into the trachea of an anesthetized animal and liquid is delivered from a glass syringe.[56] This method is a significant improvement on the

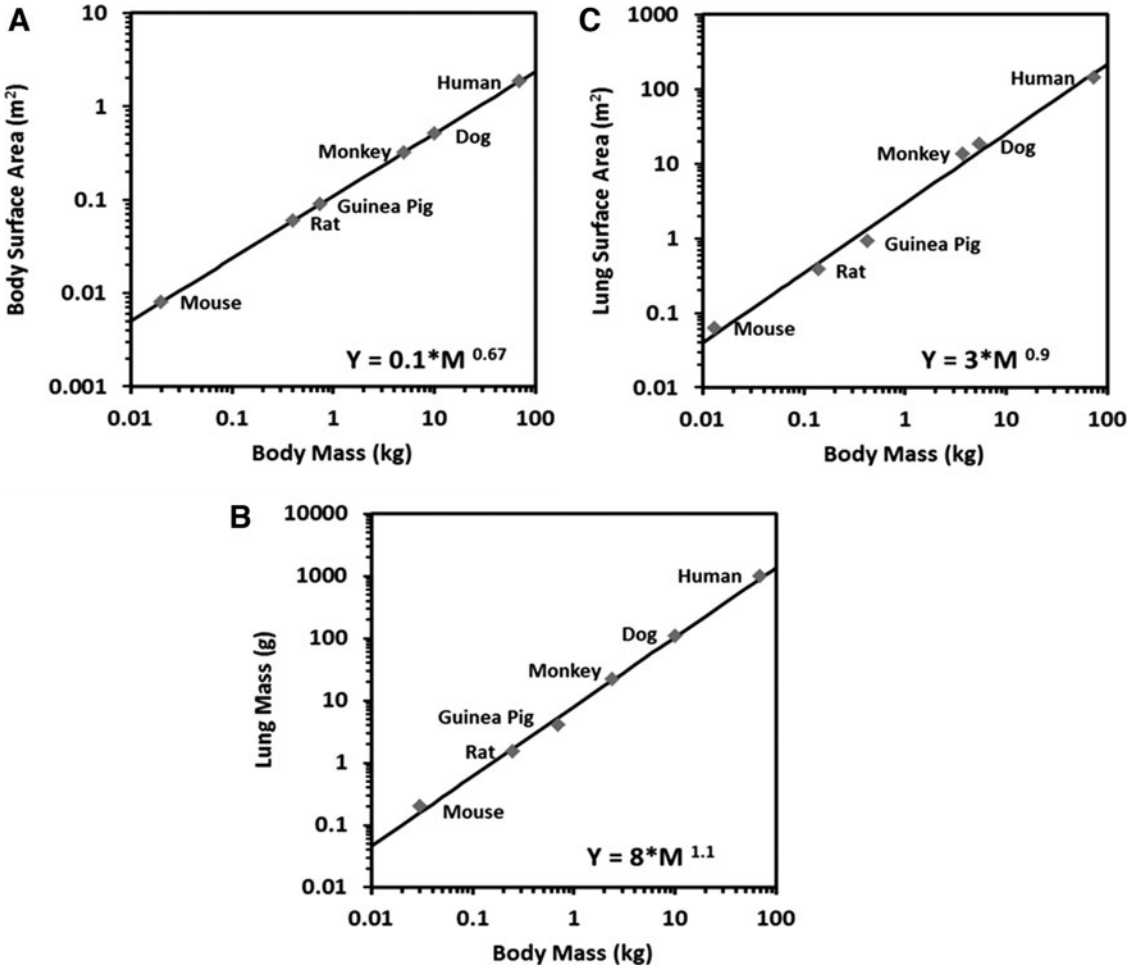

FIG. 7. Physiological parameters of **(A)** body surface area, **(B)** lung mass, and **(C)** lung surface area plotted log-linearly with body mass illustrating allometric scaling. Power function curve fits for each parameter in lower right-hand corner of each graph. Data from Snipes,[38] Chappell and Mordenti,[107] and Gehr et al.[108] Figure reproduced with permission. Copyright 2017, Elsevier.[54]

original liquid instillation method in that it achieves a more uniform regional distribution in the lungs of animals. A variation on this method was also developed in which dry particles maintained in a reservoir between the syringe and the administration tube were dispersed on an airflow from the syringe through the tube as airborne particles.[57] The most common examples of these systems were available commercially from Penn-Century (Fig. 8A), which has since discontinued their manufacture.[58] Similar to the Penn-Century device, Aptar has developed a commercially available Powder Administration Device for Animals system to support preclinical research in mice.[59] Furthermore, others have attempted to address the need for systems to deliver drugs directly to the lungs of small animals.[60–65]

Passive inhalation

Standard methods in inhalation toxicology for the delivery of drugs or xenobiotics involve whole body or more commonly nose-only exposure chambers (Fig. 8B).[66–69] Historically the quantity of drug required to conduct studies using these systems prohibited their use in early proof-of-concept studies. Wright's dust feed, rotating brush, jet mills, jet nebulizers, and fluidized bed powder generators were employed to disperse the aerosols.[6,63,70–73]

Custom-made systems were developed to allow for smaller quantities of drug aerosols to be employed, thereby increasing the feasibility of conducting early studies when very little drug was available.[74,75] It is also possible to measure a wide range of biometric parameters while conducting exposure studies, including pulmonary and cardiac function, thus maximizing the data collected from any experiment.[76] For example, the commercial system displayed in Figure 8B allows for plethysmography measurements during drug delivery. As a result, individual animals can be switched to fresh air when the desired drug dose is reached, supporting accurate and reproducible dosing.

Large animals require direct dosing, where the needs of which are served by connecting the aerosol generation system through tubing and a mask.[3,44,77,78] or less frequently to a head dome.[79] The animal is usually habituated to the presence of the mask before administration of the aerosol to minimize stress and support efficient and reproducible administration of the aerosol.

Delivery challenge

Delivery of liquids is usually achieved by a nebulizer, and the challenge is to assure the quality (dose and APSD) of the aerosol while minimizing the airflow required to deliver it to balance the dose delivered and the time required for delivery.[21,80] Generally, nebulizers produce airborne droplets effectively and reproducibly in relatively small volumes of air. The presence of suspended particles can influence the droplet size of the aerosol delivered.[81,82] Dry particles are historically administered by Wright dust feed, fluidized bed, or rotating brush high output delivery systems.[6,70,71] These dry powder systems require large quantities of drug to match the air required to disperse the particles into their primary sizes in concentrations sufficient to effectively and reproducibly support delivery to the animal and to provide sufficient airflow for adequate ventilation for the animals.

The challenges and opportunities of intratracheal (IT) administration of droplets or particles differ from those of passive inhalation from an aerosol of dispersed liquid or solid particulates. IT administration delivers the drug formulation directly to the lungs. The entire dose, with the exception of that retained in the device, which is usually very little, is deposited. However, spray instillation systems do not generate droplets <5 μm, and for powders, which may be in respirable size ranges, it is not clear that the plume can fully develop given the dimensions of the airways. Nevertheless, a known dose is delivered that has some capacity to penetrate into the lungs. Passive inhalation systems deliver aerosols in sizes that are biologically relevant to deposition in the species being employed. The efficiency of delivery is dependent on their anatomy and physiology. Again, it is worth noting that small animals are obligate nose breathers and, as such, deposition in the nasal cavity will occur in addition to lung deposition. A dose can be estimated based on knowledge of the biology as will be described below. However, the individual dose can only be estimated accurately following delivery by studying the pharmacokinetics of disposition.[83]

Inhaled Dose Considerations

Delivery and deposition

After considering all variables described above, a generally accepted expression for estimating the deposited dose

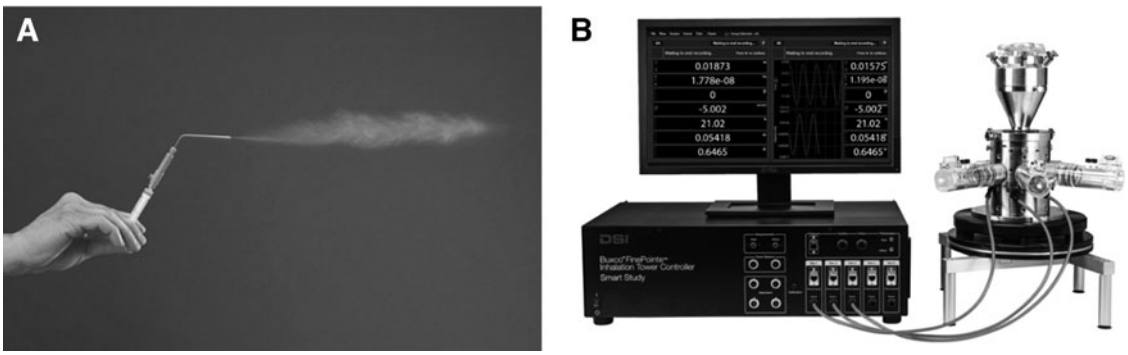

FIG. 8. **(A)** Penn-Century™ Dry Powder Insufflator™ for direct IT delivery. **(B)** DSI™ Buxco Inhalation Exposure System, a nose-only passive inhalation system. Dry Powder Insufflator and Buxco Inhalation Exposure System reproduced with permission of Penn-Century and DSI, respectively. IT, intratracheal.

into the lungs to any species is defined in terms of the aerosol concentration of exposure, the minute volume of the animal, the anticipated deposited dose, and the body weight (allometric scaling coefficient of 1) as the scaling factor.[44] This differs from the delivered dose only by the accounting for the deposition fraction.[84,85]

$$\text{Deposited Dose (mg/kg/d)} = (\text{C (mg/L)} \times \text{RMV (L/min)} \times \text{D (min)} \times \text{DF})/\text{BWT (kg)}$$

Where C is the aerosol concentration (mg/L), RMV is the respiratory minute volume (L/min), D is the duration of exposure (minutes), DF is the deposition fraction, and BWT is the body weight (kg).

The RMV is typically calculated as[84]:

$$\text{RMV (L/min)} = 0.608 \times \text{BWT (kg)}^{0.852}$$

Table 2 provides generalized values for terms in the equation, allowing dose estimation to be made. As described above (Table 1), the respiratory minute volume varies based on the species, breed, age, size, measurement technique, and measurement conditions. In Table 2, generalized respiratory minute volumes are provided for dose estimation. The rationale for the importance of these terms should be evident from the previous sections describing biological factors contributing to dose delivery and lung deposition.

Some outstanding publications have appeared that consider dosing of animals and extrapolation to humans.[30,35,86,87] Phillips has published an excellent general complement to the exposition above[54] and Wolff has considered the implications for dosing biologicals.[88]

Models of lung deposition based on experimental data have been cited elsewhere in the article.[31,80] Currently, efforts are being made to model lung deposition from first principles using computational simulations.[89–91] The use of these models may complement efforts to address human dose predictions from human cell and tissue systems currently under development as an alternative to animal studies.[92–94]

Clearance

The foregoing sections attempted to address considerations required to allow adequate prediction of the human inhaled dose from animal experiments whether for purposes of safety or efficacy assessment. However, considerations of dose cannot be complete without mentioning clearance. The local dose whether for safety or efficacy in the lungs or for availability for absorption to the systemic circulation is modulated by the combined effects of clearance mechanisms that remove particles or drug at rates that may differ between species.[95] The importance of kinetics in setting the dose cannot be understated.[96] Simulation of drug in the lung can be misleading without consideration of all aspects of its retention and clearance.[97]

Following lung deposition, which is influenced by all of the factors identified above, the residence time of the drug in the lungs is dependent upon the clearance mechanisms. Figure 9 illustrates the presentation of drug to each of the mechanisms of clearance that may also depend on the species to which the aerosol has been administered.[98]

Figure 10 shows the clearance of inert materials from the lungs of various species of animals.[80,87,98] The point on the y-axis at which each curve begins represents the quantity remaining following mucociliary transport. The curves themselves reflect the long-term clearance attributable to cell-mediated transport from the periphery. The importance of the differences shown in these plots relates to the likely exposure of the epithelium once the dose is deposited because extrapolation of dose is based on the concept that the pharmaco- or toxicodynamic effect is seen at a certain dose. It should be acknowledged that the local dose is modulated by species-dependent clearance mechanisms, in which case the approach to interpretation should be the best (safety longer residence time, efficacy shorter residence time) or worst-case (safety shorter residence time, efficacy longer residence time) scenarios.

Receptor/target biology

When considering animals as models for human disease, it is important to consider whether the target receptor or pathogen exists in the animal model as it does in human. The following examples are given to illustrate the cellular and molecular level differences that require attention. In some cases, the animal does not have the receptor that is present in human, as is the case for mice with ACE-2, the important binding site for SARS-CoV-2.[11] Consequently, genetic manipulation may be required to express the target moiety.[11] However, the question of the natural density of such receptors in the model versus humans must be considered.[99]

It was shown decades ago that paracellular clearance rates of a range of molecular weight dextrans across the pulmonary epithelium was linear, more rapid for small molecular weight than macromolecules across a number of mammalian species.[100] However, transcellular transport may occur for

Table 2. Standard Values for Inhalation Toxicology Calculations. Table Reproduced with Permission

Species	*Mouse*	*Rat*	*Guinea pig*	*Monkey*	*Dog*	*Human*
Body weight (kg)[a]	0.03	0.25	0.7	2.4	10	60
RMV (L/min)[b]	0.03	0.19	0.45	1.3	4.3	20
Lung weight (g)[a]	0.2	1.5	4	22	110	1000
Deposition factor[c]	0.1	0.1	0.2	0.25	0.25	1

[a]Actual values for lung and body weights can be used if available.
[b]RMV estimated using the formulation from Alexander et al.[84]
[c]For determination of lung-deposited dose, estimates were based on data from Wolff and Dorato.[87]
RMV, respiratory minute volume.

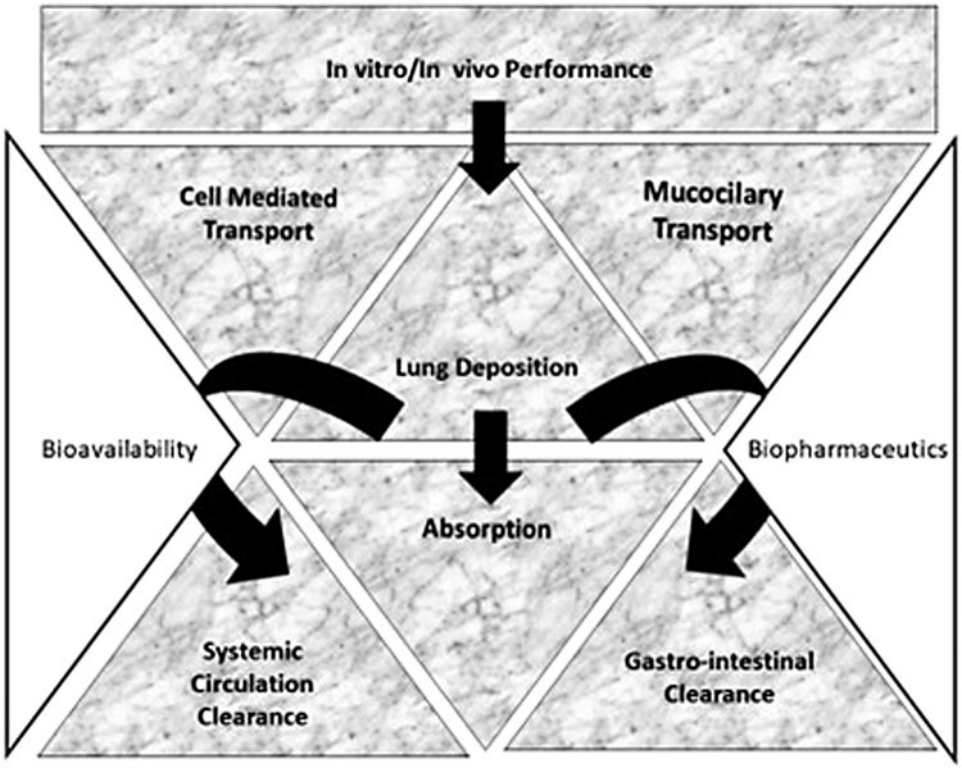

FIG. 9. Depicts the relationship between the three major mechanisms of clearance, including absorption, mucociliary, and cell-mediated transport, which result in presentation of drug to either the systemic circulation or the gastrointestinal tract.

less-soluble molecules or those with a propensity to interact with cells.[101]

Such interactions may depend on the expression of mucus, metabolizing enzymes and transporters in the lungs of different species, and the importance this may have in the disposition of drug.[102,103] Local binding or lysosomal storage as compartments that may influence uptake of drug may also vary between species, influencing the pharmacokinetics of systemic appearance of drugs and requiring interpretation to be used in predicting behavior in humans.[104–106]

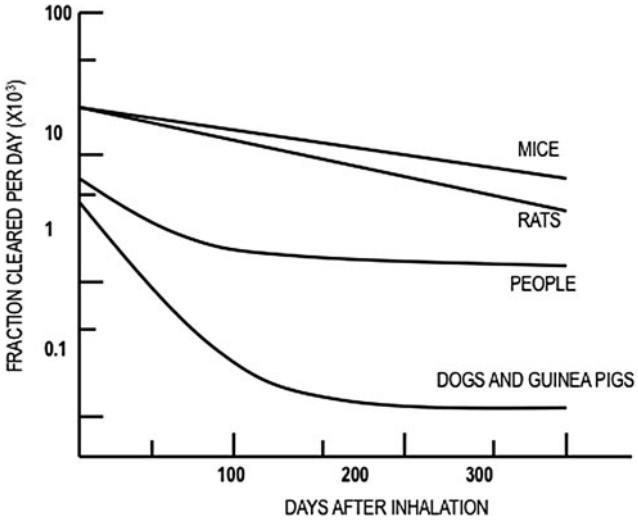

FIG. 10. Clearance curves for rodents (rats and mice), humans, dogs, and guinea pigs, illustrating the differences attributable primarily to mucociliary transport and cell-mediated transport.[15]

Conclusion

Given the significant differences in anatomy, physiology, and lung function between species, it is not surprising that there is no single aerosol delivery system that can readily be adapted across species.

Biological considerations with respect to similarities and differences between species that influence deposition are accompanied by differences in airway epithelial surface area, volume, and lung mass. Clearance rates also differ from one species to another, whether by absorption or mucociliary or cell-mediated transport. Dose prediction from animals to humans is often simplified to proportionality based on specific features of lung biology.

However, it is important to consider aspects of the formulation, method of delivery, and fundamentals of anatomy and physiology. The importance of these considerations is in interpreting deviations from simple allometric scaling. This is especially true when the target is located in specific cells or regions in the lungs where expression may differ between species.

Authors' Contributions

A.J.H.: conceptualization, writing—original draft, reviewing, editing, and visualization. S.E.M.: writing—reviewing, editing, and visualization. P.J.K.: writing—reviewing and

editing. J.E.P.: writing—reviewing and editing. R.K.W.: writing—reviewing and editing.

Author Disclosure Statement

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