Inhaled administration of drugs to animals in pharmaceutical research

An overview of industry practices for non-clinical studies performed in support of inhaled drug development

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Introduction

Studies characterizing the toxicity of drugs (and, ideally, non-clinical efficacy studies) dose animals via the most clinically relevant route. While oral or injected dosing of animals is straightforward, inhaled administration of drugs presents technical and logistical challenges. Short films describing inhaled administration of drugs to animals are available online.[1] This article outlines considerations and industry practices for performing non-clinical studies in support of inhaled drug development.

Selection of test species

The use of animals in scientific research should be ethically reviewed and performed in accordance with corporate policies and national legislation governing licensed scientific procedures and the care, welfare and treatment of animals. The selection of species in regulatory studies supporting a drug's development is often based upon guideline requirements for non-clinical safety, availability of historical baseline data, and the biological relevance of a species to humans. Animals must be small enough to permit handling and conduct of inhalation exposure procedures, be large enough to facilitate measurement of required parameters (within limits such as permitted blood sampling volume), and also be used in sufficient numbers to achieve experimental objectives using robust study designs.

The rat is commonly used as the rodent species for non-clinical general toxicology studies using inhaled materials. Mice are often considered as the second rodent species for evaluation of carcinogenicity or as an alternate rodent species if a drug's pharmacology or the etiology of toxicity observed in rats indicates poor translation to relevant mechanisms in humans. Historically, dogs and non-human primates (NHPs) were used as the non-rodent species for inhalation toxicology studies but these species are specially protected by European legislation and the United Kingdom's Animals (Scientific Procedures) Act.[2] Consequently, there has been increased discussion in recent years regarding the use of minipigs as the non-rodent species, in the absence of scientific evidence to support use of dogs or NHPs as the most relevant species for translation of drug-induced findings from animals to humans.

Inhalation exposure of animals

Exposure systems used in non-clinical studies consist of two key elements: apparatus to generate a test atmosphere containing a drug or formulation in air and apparatus to present this atmosphere to animals. In addition, the laboratory's infrastructure must be engineered to provide a clean supply of compressed air (contaminant particles may directly affect an animal or act as nuclei for absorption of test gases or liquids), a system of valves and flow meters to control and monitor the air supply and exhaust vacuum for atmosphere generation and animal exposure systems, and locally engineered ventilation to prevent exposure of staff to the airborne test material.

Generation of the test atmosphere

The potentially most harmful particle size or most responsive area of the respiratory tract for a species cannot be predicted for a given material. Therefore, non-clinical exposure systems for testing chemicals are normally designed to administer polydisperse aerosols (particles of varied sizes) to ensure exposure of the entire respiratory tract of animals in order to generate toxicology data suitable for hazard identification.[3] To achieve high doses in toxicology studies, animals are exposed to the airborne drug for a longer period than that intended to dose humans. A formulation's composition and/or clinical inhaler are often unknown in early drug development and custom or commercially available apparatuses or adapted clin-

ical devices are used to generate reproducible test atmospheres for sustained periods. More "industrial" apparatus, such as dust generators or atomizers, may be used to achieve a greater drug delivery rate than possible for clinical devices.

Test atmospheres can be classified according to the physical state of the constituent materials: solids (e.g., dusts, fibers and nanoparticles), fluid droplets (non-volatile liquids), vapor (generated from volatile liquids) or gases, and pyrolysis products (e.g., fumigants, tobacco or fire smoke). While inhaled anaesthetics are administered as gas or vapor mixtures with air or oxygen, most pharmaceuticals are solid at room temperature and are formulated as simple mixtures with excipients. Candidate drugs are thus typically presented to animals as an aerosol of particles or droplets generated from a formulation. Micronized drug crystals are generally robust particles that can be compressed and then dispersed in air without compromising dissociation of primary particles. However, with increased trends to develop inhaled biopharmaceuticals (biologically sourced drugs, e.g., oligonucleotides, antibodies or peptides) and/or use particle engineering to stabilize, target and/or modulate drug release,[4] care must be taken to ensure drug activity is not compromised during aerosol generation.

Various dust generators are available for dispersal of dry powder formulations into air. The Wright Dust Feed[5] introduces particles into an air stream by scraping powder from a column of compressed material. Other devices avoid the need for compression by using a venturi to draw free-flowing powder into a stream of air[6] or using compressed air to expel powder from capsules.[7] Selection of apparatus may be influenced by formulation properties. For example, particles of uniform size or those repelled electrostatically may be unsuitable for compression, or particle adhesion may impede free-flowing movement of powder.

Inhaled drugs are sometimes formulated as a solution or suspension in a pressurized metered dose inhaler (pMDI), the latter consisting of a relatively insoluble drug maintained as a slurry in liquefied propellant, solvent and surfactant. Dosing animals is straightforward but pMDIs must be agitated between actuations to ensure adequate mixing of propellant and formulation ingredients. Care must be taken to avoid frequent discharge excessively cooling or freezing the valve by evaporation of propellant and solvent. pMDIs may be accommodated in carousels for simultaneous actuation, increasing the "total dose" discharged into the exposure system with each actuation.

For dispersal of liquid formulations, many devices are available commercially. Air jet nebulizers or atomizers are probably most versatile, dispersing suspensions or solutions as droplets by directing compressed air through the liquid formulation (air jet nebulizers) or introducing the liquid into a stream of air (atomizers). This process is less likely to compromise heat-sensitive formulations than devices such as ultrasonic nebulizers that warm a liquid reservoir during use.[8] Clinical nebulizers holding a reservoir of liquid are easily adapted to facilitate maintenance of a constant fluid volume for sustained periods. Although technical guidance for testing inhaled materials in rodents do not state an acceptable pH range for aqueous formulations, it is common practice to ensure formulations are neither too acidic nor alkaline. While local practices vary for accepted limits of pH, a range of pH 3 to 9 is used by some laboratories and encompasses slightly narrower ranges applied by others (per personal communications).

Presentation of the test atmosphere to animals

Configurations of animal exposure systems are varied and best considered in relation to the size of the test species. An exposure system consists of apparatus for conveying a mixture of test material and air to a "breathing zone" for exposure of animals, and an exhaust system for safe removal of the spent test atmosphere. Inhalation exposure systems can be broadly categorized as whole-body chambers and snout-only systems. Snout-only (also described as "nose-only") inhalation exposure is most commonly used in pharmaceutical research. By presenting an aerosol to the snout of the animal, and targeting the nares in particular, the potential for dermal exposure and oral ingestion associated with grooming is reduced. In addition, the internal volume of a snout-only system is considerably smaller than that of a whole-body chamber used to accommodate a given number of animals, reducing the drug mass required to maintain the target concentration in air. Endotracheal tubes may also be used to expose larger species (non-rodents) individually for short periods; this technique is analogous to oral inhalation by humans.

Exposure systems commonly used for inhaled administration of drugs to animals are of a dynamic single-pass design, in which an aerosol is continuously delivered and exhausted from a "breathing zone" used to present a test atmosphere to the snouts of animals. Oxygen must be maintained at a minimum concentration of 19% (v/v) in air and CO_2 must not increase excessively, so as not to affect an animal's physiology. This is achieved for aerosols by applying sufficient airflow to refresh the test atmosphere in the "breathing zone." Additional factors for selecting airflow will depend upon the design of the apparatus and species used. For example, a minimum airflow may be required to efficiently disperse the test article in air to achieve a respirable droplet or particle size, which will depend upon the physical properties of the test article and the design of the aerosol generator used.

Alternatively, the airflow selected for larger "flow-through" chambers (a simple cylinder serving as a "common breathing zone" for small animals) may be

determined by the minimum number of air changes required to sustain oxygen and limit CO_2 concentrations in air. However, the total airflow selected for a mask-based exposure system and a "flow past" or "directed flow" chamber (a system of concentric chambers for delivery and removal of the test atmosphere at each animal port[9,10]) will be a function of the total number of animals attached to the exposure system. This requires a conservative estimate of the respiratory minute volume (RMV) of the species (i.e., considering the heaviest of the animals to be exposed) and any additional requirements for aerosol sampling. For instance, a total airflow of 25 L/min may be selected to sustain 4 dogs and an aerosol sampling port at 5 L/min per breathing circuit (slightly exceeding RMV for a body weight of 10 kg), or selecting 0.5 L/min/rat as twice the RMV for a body weight of 350 g.[11] Airflows selected for inhalation exposure of a given species are dependent upon the apparatus used and practices vary between laboratories.

For snout-only exposure, rodents are typically restrained in tubes attached to a chamber through which a test atmosphere is directed (Figures 1A and 2A). While the method of restraint varies for non-rodent species (e.g., dogs, pigs and NHPs), reflecting their size and manual dexterity, non-rodents are normally exposed to drugs using a mask attached to a breathing circuit that conducts the aerosol from a central aerosol generator and conditioning chamber (Figure 1B) and a rubber diaphragm or foam edging are used to achieve a seal around the snout. Provision (access ports and apparatus) is made for characterization of the concentration and particle size distribution of the aerosol. The duration of exposure varies for local practices and study objectives, but up to 60 minutes is common for inhaled administration of candidate drugs to animals.

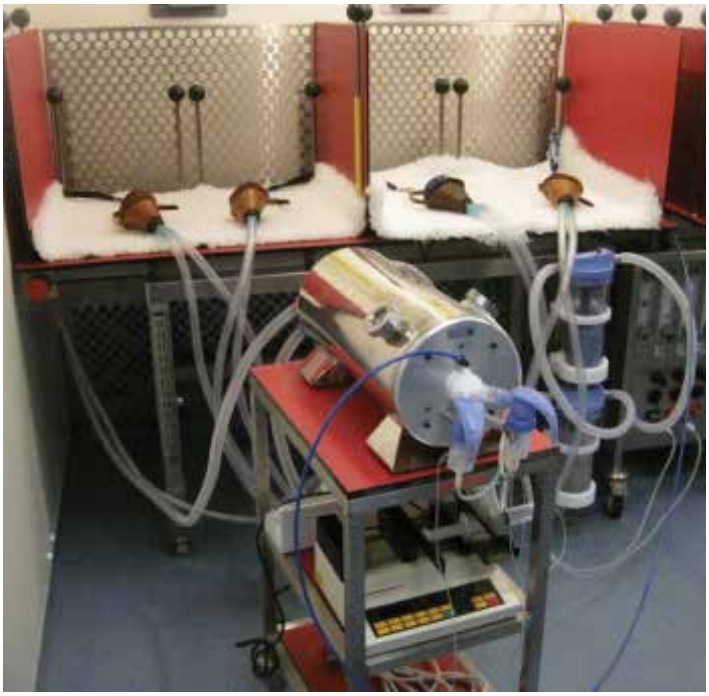
Before undertaking aerosol administration, animals are acclimatized to exposure procedures by exposing them to air only. Local practices vary but this training can take approximately two days for rodents and two weeks for non-rodents. Pauluhn and Mohr[12] described the need for tight-fitting masks and effective restraint of large species as a cause of stress that may alter breathing patterns and possibly make characterization of the test atmosphere difficult or even impossible. However, experience shows that, with sufficient pre-study training, dogs require minimal restraint during aerosol administration (Figure 2B). In fact, dogs have been observed sleeping during administration of drugs devoid of sedative activity. Nevertheless, it is important to frequently observe animals during snout-only exposure to ensure all test subjects inhale the aerosol and their welfare is not compromised. For example, a rat

Figure 1

Dynamic single-pass exposure systems for aerosol administration to animals. 1A: Snout-only chamber for exposure of rodents (rat restraint tubes shown), with a Wright dust feed[5] mounted on top of the chamber and vacuum venting atmosphere from the base. 1B: Mask-based exposure system for dogs, with two nebulizers attached to an expansion tank. The aerosol is conducted via tubing to masks or a sampling port and then an exhaust manifold. Films showing these procedures in rats and dogs are available online.[1]

A

B



may turn its head within a restraint tube presenting risk of asphyxiation without intervention. Tube restraint may also compromise a rat's condition with pharmacological inhibition of thermoregulation, when whole-body exposure would be more appropriate.[13]

Pharmaceutical regulatory agencies have not published explicit technical guidance for administration of aerosols to animals in non-clinical studies, perhaps because drug development is focused on optimizing dose delivery for humans and not laboratory species. Orally inhaled particles of ≤ 5 µm in diameter and nasally inhaled particles of ≤ 3 µm in diameter are thought to deposit in the lower airways of humans.[14] Drugs supplied for non-clinical safety studies are representative of those administered to humans. This presents toxicologists with a dilemma insofar as the clinical product should be assessed in animal studies[15] but the particle size distribution of an aerosol is driven by particle sizes in a formulation optimized for humans. Operational settings (to achieve the target aerosol concentration and particle size distribution) of an exposure system are confirmed during pre-study aerosol characterization work. Attempts are made to comply with guidance for testing chemicals in rodents,[16-18] for example, by reconfiguring an aerosol generation and exposure system to minimize particle aggregation and/or remove large particles by impaction or sedimentation. However, adherence to the particle size range cited in such guidance may not be technically feasible for the drug formulation supplied for evaluating efficacy or non-clinical safety in animals.

Aerosol characterization

When a test material is administered to animals via non-inhaled routes, the material is normally dissolved or dispersed in a vehicle and administered directly to the animals. Consequently, the concentration of "active drug" in the dosed formulation is the same as the nominal concentration prepared in the dispensary. This is not the case when formulations are dispersed as aerosols; a propellant (pMDI) and volatile solvent will vaporize and particles of different densities, size or shape have different aerodynamic properties and will sediment or impact to differing degrees within the apparatus, resulting in "enrichment" of the test aerosol for fine particulates including the drug.

While the concentration and size of particulates may be monitored continuously using optical spectrometers (e.g., dust meters or aerosol particle sizers, respectively), pharmaceutical aerosols are normally characterized using chemical analysis to quantify the mass of drug in the test atmosphere presented to the animals. For determination of aerosol concentration, a known volume of the aerosol is drawn through a sampling medium presented in a suitable device, such as a glass fiber filter mounted in an open-faced filter holder (Figure 3A);[19] the drug is extracted from the medium using an appropriate solvent for chemical analysis. For determination of particle size distribution, the aerosol is sampled using a cascade impactor, which consists of a series of cut-points of decreasing size that fractionates the aerosol into sub-samples of particles of decreasing aerodynamic diameter (Figure 3B). If the population of particulates is of a log-normal distribution, linear regression analysis of the probit (probability func-

Figure 2

Restraint of animals for snout-only inhalation exposure. 2A: Conscious rats restrained in tubes. 2B: With sufficient pre-study training, dogs remain calm throughout inhalation exposure and require minimal restraint. A harness is attached to a pole towards the rear of the "dosing table" but the dog is otherwise able to move freely while wearing the mask.

A

B

tion) of the cumulative proportion of aerosol mass for particles less than each cutpoint against Log_{10} of the cut-point can be used to extrapolate the mass median aerodynamic diameter (MMAD) and geometric standard deviation (σg or GSD) of the particle size distribution.[20] If the log-transformed data are non-linear, the MMAD may be determined using alternative methods such as interpolation of the two central data points encompassing the median value.[21]

The volume of aerosol sampled must be sufficient to permit quantification of the analyte, without overloading the media with particulate, and is thus dependent upon the anticipated (target) aerosol concentration and lower limit of quantification for the method of analysis. The air volume and therefore period of time sampled are thus shorter when sampling a high aerosol concentration than a low aerosol concentration at a given flow rate. Replicate samples taken during a single inhalation exposure, or on days of repeated exposure, represent a series of "snapshots" of aerosol concentration.

Samplers and airflows used for characterization of aerosol concentration and particle size distribution vary between laboratories but the flow rate used for a given design of cascade impactor must be consistent with that used to calibrate the cut-points of the device. Designs vary, with some operated at a low flow rate and others at a relatively high flow rate. Nevertheless, withdrawal of aerosol samples from an exposure system should not significantly affect the test atmosphere presented to the animals. Examples of methodology used for aerosol sampling and characterization are available online.[19]

Dosimetry and pulmonary deposition of inhaled test materials

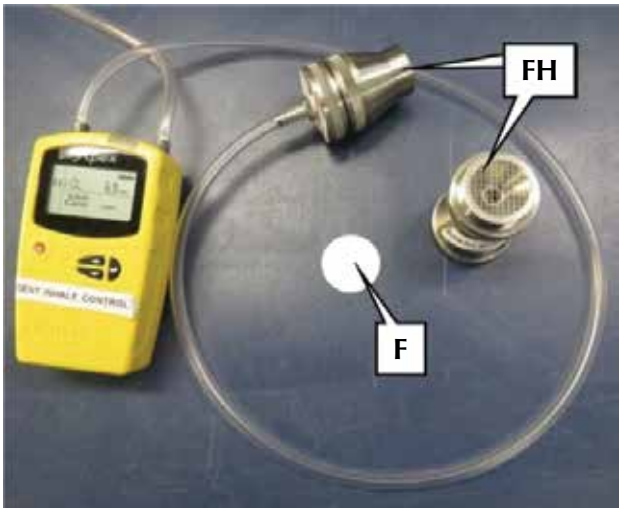
Laboratory species are not dosed *per se*, but exposed to an atmosphere containing test material. Animals breathe passively from the test atmosphere; some material is retained in the respiratory tract but most is not. Although a breath-activated device can be used to optimize dose delivery to larger species, this is impractical for rodents, given the small volume of air inhaled relative to the internal volume of the "dosing apparatus."

The administered dose is thus unknown but is typically calculated on a mass per unit body weight basis from the mean concentration of drug in air, inhalation exposure period, body weight and the volume of air inhaled. The inhaled air volume is often estimated from body weight using a published equation[11] but this overlooks the potential for physiological or toxicological effects on lung function induced by a test material.[22, 23] Furthermore, predicted lung function does not permit correlation of this variable and therefore dose with animal activity, toxicokinetics or biological responses (pharmacology or toxicity) for individual animals. Pharmaceutical doses administered to animals are limited by aerosol concentration. The upper limit

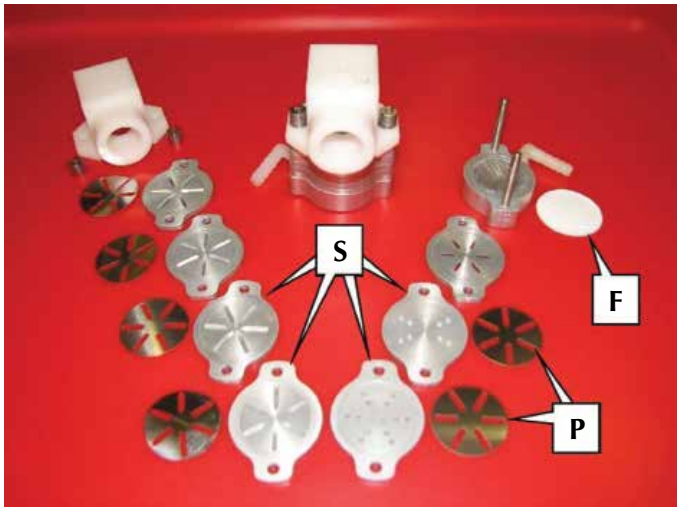
Figure 3

Aerosol sampling devices for determination of concentration and particle size distribution of drug analyte. 3A: Example of an open-faced filter holder (FH) connected to a vacuum (pump) used for drawing a known volume of air (sampling rate of 2 L/min) through a glass fiber filter (F).[19] 3B: Example of a cascade impactor (Marple 296, Westech Scientific Instruments, Bedfordshire, UK, sampling rate of 2 L/min); collection plates of stainless steel (for solids) or glass fiber (liquids) and a glass fiber filter (final stage) are used to collect aerosol sub-fractions for analysis.[19] Each collection plate (P) is supported by a stage (S) incorporating six slots or series of holes that determine the effective cut-point at a given airflow. The filter (F) is supported on a wire mesh incorporated into the base of the cascade impactor.

A



B



applied varies depending upon requirements of study sponsors, experimental aims and therapeutic indication. In the absence of technical guidance for administration of pharmaceutical aerosols to animals, a dose limiting aerosol concentration may be considered with reference to regulatory guidelines published for the testing of chemicals in rodents. While some study sponsors accept a dose-limiting aerosol concentration of 2 mg/L,[24] maxima of 1 mg/L[18] or 5 mg/L[16, 17] (total particulate in air) are sometimes accepted by others.

Deposition of inhaled droplets or dust within the respiratory tract is dependent upon the aerodynamic properties of the particulate, electrostatic charge, the anatomy of the respiratory tract and the breathing pattern of an animal.[25] Differences in the angle of bend in the pharynx of animals of pronograde (horizontal) and orthograde (upright) postures influence the proportion of particulate impacting against the wall of the airway. The nature of breathing also greatly influences the site of deposition; rodents are obligate nasal breathing animals, which results in humidification of aerosols and filters out larger particles. Human patients not only have larger airways but often inhale medicines orally, bypassing the nasal cavity and increasing lung dose.

During inhalation of a polydisperse aerosol, larger particles are predominantly deposited in the upper respiratory tract (nasal cavity and/or pharynx) and are typically swallowed or may be expectorated. Finer particles are drawn deeper into the lungs, a proportion of which are deposited in fluid or mucus lining the respiratory tract, and additional airborne particles may be deposited in the respiratory tract as the aerosol is exhaled. Approximately 85% of particles of 2 µm aerodynamic diameter are "inhalable" by rats.[26] Much of an aerosol is exhaled, with published values indicating approximately 30% of such particles are deposited in the upper respiratory tract[15] and just 7% of particles are deposited in the lungs[25] of rats. Analysis of non-clinical data must therefore consider the achieved particle size distribution and mass of drug likely to be retained by an animal. The United States Food and Drug Administration assumes lung deposition of 10% in rats, 25% in dogs and a conservative assumption of 100% in humans.[27-29]

Summary

Inhaled administration of drugs to animals presents technical and logistical challenges. While apparatus and laboratory practices vary, short films illustrating inhalation exposure procedures are available online[1] and considerations are summarized here.

Selection of the species for non-clinical toxicology studies is based upon guideline requirements for non-clinical safety, availability of reference (background) data and biological relevance for translation of findings to humans. The rat is generally selected as the rodent species of choice for inhalation toxicology studies. Historically, dogs or non-human primates were selected as the non-rodent species but there has been discussion in recent years regarding use of minipigs for inhalation toxicology studies.

To generate toxicology data suitable for hazard identification, inhaled drugs are generally administered to animals by snout-only inhalation exposure using apparatus designed to generate polydisperse aerosols of particles or droplets for exposure of the entire respiratory tract to the test article. Extremes of pH for aqueous formulations are avoided for animal welfare; accepted ranges vary within pH 3 to 9.

Rodents are typically restrained in tubes attached to a chamber. While methods of restraint for non-rodents are tailored to the size and dexterity of a species, snout-only exposure of non-rodents is generally achieved using a mask-based exposure system. The duration of inhalation exposure varies for local practices and study objectives but up to 60 minutes is common for the inhaled administration of pharmaceuticals. Animals are acclimatized to restraint procedures and operational settings of exposure systems are confirmed before the first administration of the test article to animals.

Animals are not dosed by inhalation *per se*, but exposed to an atmosphere containing a respirable drug that is inhaled passively; some material is retained in the respiratory tract but most is not. The inhaled dose of a drug is estimated from the mean aerosol concentration, duration of exposure, body weight and the inhaled volume (often derived from body weight). Doses are ultimately limited by time and aerosol concentration, with accepted maxima depending upon sponsor preferences, study aims, therapeutic indication and reference to regulatory guidelines for the testing of chemicals in rodents. While a dose-limiting aerosol concentration of 2 mg/L is accepted by some sponsors, a maximum of 1 mg/L or 5 mg/L (total particulate in air) is sometimes accepted by others. Lung deposition of the inhaled drug depends upon aerosol properties, anatomy and the breathing pattern of the test species. For non-clinical assessment of safety, the US FDA assumes lung deposition of 10% in rats, 25% in dogs and a conservative assumption of 100% in humans.

Finally, one could say, "The particle has landed, but the journey of the drug molecule has just begun."

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