Optimising preclinical studies for intranasal and pulmonary programmes

Julie D. Suman at Aptar Pharma and Conor A. Ruzycki at Lovelace Biomedical discuss the role of preclinical and animal studies when investigating nasal delivery systems, and how these can help overcome the challenges associated with pulmonary doses

PMPS: What are the objectives of preclinical studies?

Julie D. Suman (JS): When we consider the preclinical development of animal studies for intranasal and pulmonary programmes, we are generally interested in developing a proof of concept, estimating dosing, understanding toxicity and identifying any adverse effects of a new drug candidate. In using small animals (mice or rats) and larger species – (dogs or non-human primates (NHPs) – one of the biggest challenges is how to adapt device platforms intended for humans to function in animal studies, and to ensure that we select devices that will allow us to perform the preclinical studies necessary to establish safety and to bridge to humans.

There are always scenarios where certain animal models are not appropriate, which makes it critical to engage with experts and regulators early to ensure you choose a species most appropriate for the study.

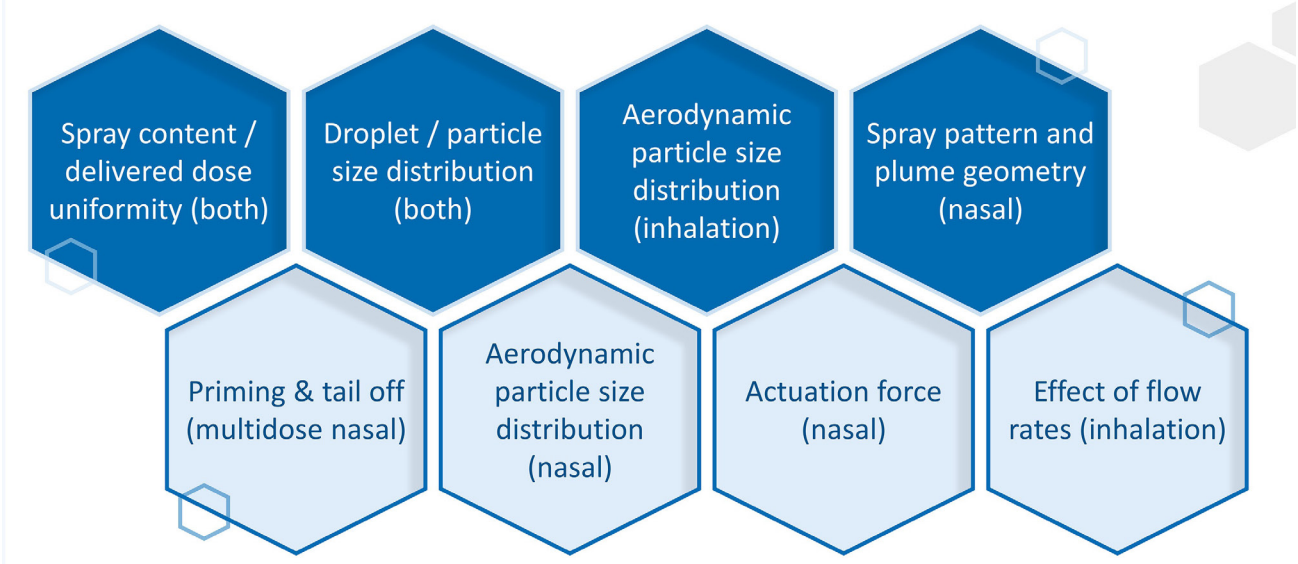


Figure 1: Proposed device characterisation parameters for nasal and inhalation devices for preclinical and early stage programmes

***PMPS*: What are the regulatory challenges with preclinical studies for intranasal and pulmonary programmes?**

JS: From a regulatory standpoint, the preclinical studies are broadly governed by 21 CFR Part 58.1: Good Laboratory Practice for Nonclinical Laboratory Studies, and similar guidelines from within the European Union, which are generally responsible for outlining the conduct, personnel, protocols' requirements and quality oversights.

However, considering the range of devices that humans use for inhalation and the challenges of adapting these device platforms to animal studies, specific guidelines don't exist for all use cases, so starting discussions early with experts is encouraged.

***PMPS*: What are the formulation approaches for liquid nasal sprays and powder formulations? What are the key characteristics to consider?**

JS: When developing aqueous nasal sprays, several key components must be considered: the active pharmaceutical ingredient (API), its solubility, the choice of excipients and the method of delivery. The pH and osmolarity also play crucial roles, as they not only affect the user's experience but are vital for the stability of the formulation.

Targeting specific areas within the nasal cavity, such as the olfactory region for central nervous system (CNS) delivery, is another important aspect. It is also important to evaluate whether the formulation requires enhancements such as the addition of mucoadhesives to increase retention in the nasal cavity or the use of permeation enhancers to improve absorption.

Recently, there has been an increasing interest in using powders for nasal delivery, especially for sensitive molecules that cannot be stored at room temperature. The development process for powders is similar to liquids. Start with the API to determine the physical and chemical characteristics such as density, morphology and crystallinity, before proceeding to particle engineering and processing as required to produce particles of an appropriate size for nasal delivery. These factors significantly influence formulation stability and delivery efficacy in animal or human studies. Depending on whether a nasal or inhalation product is being developed will also determine the characterisation needed to be considered from a drug product standpoint.

Determining the delivered dose and emitted particle size are important to ensure that the drug product is administered as intended. Additional characterisation studies may be needed to support an investigational new drug (IND) as shown in **Figure 1**.

***PMPS*: How do you choose an animal model and the delivery systems to be used?**

Conor A. Ruzycki (CR): Animals are typically subdivided into small animals, such as mice and rats, and larger animals including canines and NHPs. Additional animal models that we are getting more experience with include pig, mini pig, rabbit, ferret and guinea pigs, and these more novel models may be more appropriate for the specific aims of different programmes.

The selection of a model really depends on the specifics of a programme. For example, targeting CNS delivery could be considered, using a systemic delivery such as pulmonary or intranasal, or looking at more modern approaches like gene therapies or infectious disease. In each of the above cases, the interaction of device, formulation, mechanism of drug/vector action and characteristics of the model itself including – eg, study endpoints and desired sample types – all play a role in determining what model may satisfy a programme's needs.

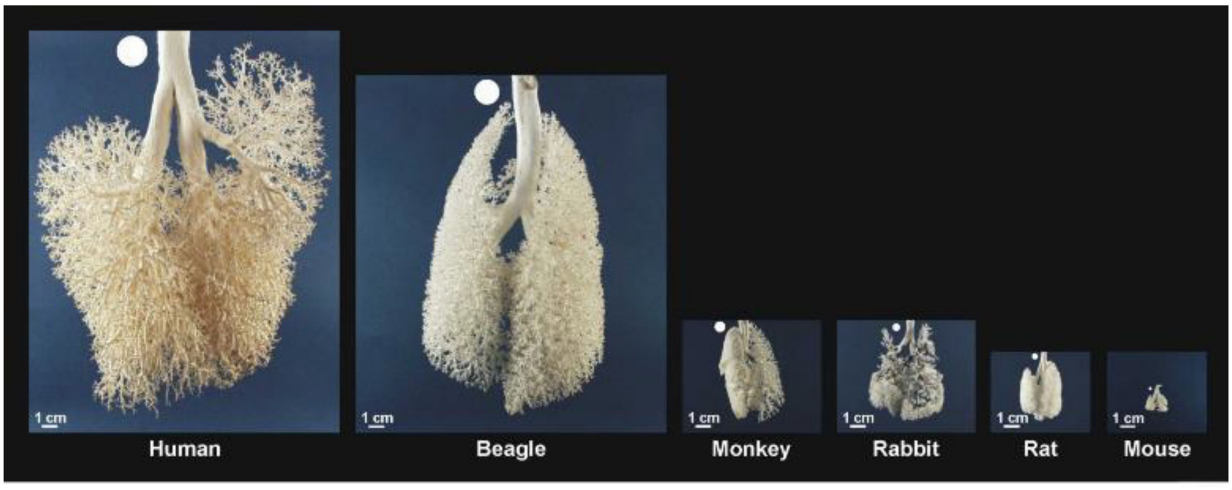


Figure 2: Comparison of mammalian lungs

The size of the molecule is also important. Traditionally, a rodent like a rat and a non-rodent like a dog species for small molecules would be used, but the appropriate species for a programme may very well be limited by pharmacological relevance.

When considering different delivery solutions, human delivery systems are the first consideration to prevent any bridging that may be required. However, for medium- and small-sized animals, it is possible to use an adapter or change the external diameter of the actuator to fit a smaller nostril. Alternatively, paediatric delivery systems can facilitate administration into smaller animals – minimising the need to bridge platforms as may be needed into humans.

***PMPS*: What considerations are needed for establishing pulmonary doses?**

CR: Knowing that pulmonary dosing is of interest for pharmaceutical aerosols, mammalian species are often selected for preclinical studies because of their anatomical similarities with humans.

When looking at mammalian lungs, very similar form and functions can be seen but there are very large differences in scales across our species of interest. Because of this, how aerosols behave in the pulmonary region need to be considered as well as the factors that influence how those aerosols are going to deposit out – such as particle and droplet size, airway dimensions and anatomy – as they will be influenced by different species and factors such as age and state of disease.

A general approach with these sorts of systems is to generate an atmosphere containing the test article as an aerosol, characterise the concentration within this atmosphere and estimate the dose that we're delivering to animals using generalised deposition fractions and allometric relations for respiratory rates. For larger species we may use something like a face mask or a head dome where animals are generally unanaesthetised, aware and awake.

***PMPS*: What are the challenges, and how can you maximise pulmonary doses?**

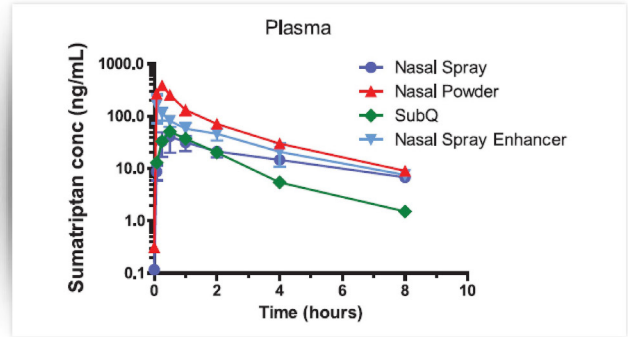
CR: Before reaching the lungs, nasal deposition occurs, which directly reduces the dose of drug that reaches the lungs. Nasal deposition is unavoidable because many animal models are obligate nasal breathers. To compound this issue, nasal airways show large differences in form, dimensions, surface area and volume of interest between different preclinical species.

There is a reasonable understanding of total deposition in the head airways for many of the common preclinical species. For some species there is a better understanding of local deposition within these airways. Our work with rodents, for example, tells us that we can expect a very high deposition of particles that are greater than about 3µm, particularly within the entrance region of the nose.

One technique to maximise pulmonary dose is to bypass the nasal airways through a method known as intratracheal instillation for liquids or insufflation for powders. However, there are disadvantages to keep in mind. The technique of the administrator plays an important role in determining delivery, potentially causing significant variation in where the drug is deposited in a manner that may not be clinically relevant. Any aesthesia can also affect certain endpoints that we are considering, including deposition, respiratory parameters and natural clearance processes in the lung.

***PMPS*: How are intranasal studies performed in animal models and what are the challenges?**

CR: Clinical devices designed for insertion into the human nostril can be used in larger animals, but there are challenges with smaller animals where human devices are not actually compatible and alternative approaches must be considered. With dogs, clinical devices can be used with unanaesthetised animals, where one technician holds the animal, and another performs the dose administration using clinical Aptar Pharma systems.



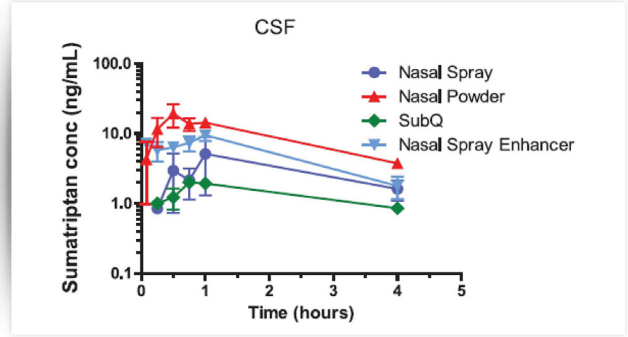


Figure 3: Plasma and CSF data vs sumatriptan concentration

Intranasal dosing in rodents is complicated due to the small dimensions of the nasal airways, in particular the nasal valve and vestibule region. To overcome this, pipetting the bulk liquid formulation directly into the nares is possible, but this does require the animal be under light anaesthesia and pilot studies have shown that there are limits in terms of how much volume can be pipetted. Alternative methods to deliver aerosols directly into the nasal cavity are in development. Challenges arise when considering the nasal anatomy of animal models as opposed to that of human models. Very large differences in the shape of the entrance region of that nasal region means that there's less of a clear path into the deeper nose that a lot of human devices are designed to take advantage of. Additional sources of errors can also be caused by how the animal is breathing, loss of aerosol, or animals sneezing or licking their noses. Because of this, when trying to interpret bioanalytical data down the line, this creates some ambiguity in the dose being achieved within the nasal cavity.

Exploratory *in vitro* benchtop models could work here to help improve the understanding of nasal deposition when looking towards using different devices within different animal models.

***PMPS*: Are intranasal powder and spray formulations comparable in animal models?**

CR: In a pilot study, we evaluated the central nervous system (CNS) delivery of sumatriptan for different administration routes, using a non-human primate (NHP) model with non-terminal crossover study design with clinical systems and devices. We performed serial sampling of plasma and cerebrospinal fluid (CSF) to understand if we could use this model for drug discovery of future compounds.

Using Aptar Pharma delivery systems, our study investigated exposure to a sumatriptan nasal spray, a dry powder formulation and a nasal spray incorporating a permeation enhancer to improve bioavailability. We collected plasma and CSF at different time points to perform compartmental pharmacokinetics (PK) analysis and compare plasma and CSF levels between these different formulations and systems. Our plasma data (**Figure 3**) showed key differences between our formulations. The powder formulation had the highest area under the curve indicating the highest bioavailability in the plasma. This was possibly caused due to longer retention of the powder within the nasal airways, but this could also be caused by differences in location of deposition between a powder and the spray, or by other factors that might be influencing this. Reviewing the CSF data, we again observed the highest area under the curve in the CSF, suggesting the same conclusion that there is longer retention of the powder within the nasal airways.

With that subcutaneous dose, it is only able to reach the CSF via systemic circulation absorption. By looking at the ratio of the area under the curve between the CSF and plasma for the subcutaneous route, this gives a baseline idea of how much sumatriptan we would expect to be absorbed again through systemic circulation, where any increase in this ratio above this baseline presumably represents absorption of the CSF by another route. Our model shows us that each nasal formulation provided a higher ratio of CSF and plasma to the subcutaneous route, suggesting some degree of nose-to-brain delivery of sumatriptan occurred with each means of nasal delivery.

In summary of the model, it allowed us to look at non-clinical evaluation of brain delivery using a clinical system directly. This is also a non-terminal study design, so it allows us to compare a formulation device and delivery route we know across the same animals at multiple timepoints. As we did not investigate the optimisation of formulations, systems or devices, or look at the effects of factors which might influence nasal deposition, it suggests providing additional scope that could be explored further with this kind of model.

***PMPS*: Are you able to compare the performance of fine mist spraying vs pipette?**

JS: In collaboration with our colleagues at Aptar China and Shanghai Jiao Tong University, we performed a study comparing the Aptar fine mist sprayer vs a pipette in delivering a nasal exosome formulation. A 25µL dose of the fluorescently labelled formulation exosomes was delivered with sampling performed at 30 minutes and two, four, six and 12 hours post-dosing.

Using whole body imaging, we saw no significant differences in the uptake of the exosome formulation into the brain based on the whole-body imaging, when comparing 30 minutes post-administration and then at the final time point of 12 hours (**Figure 4**).

When we looked at the distribution at an organ level at 30 minutes post-dosing, we saw no statistical differences between the distribution of the exosomes in the brain, heart, kidney, liver, lung and spleen in this mice model, concluding that the fine mist sprayer was successful in administering the dose to mice in the study, and being easier to use. One thing of note is that the fine mist sprayer did experience issues with the nano suspension exosome formulation, resulting in clogging of the cannula and limiting the ability to sequential dosing. Clogging was resolved by cleaning the canula between doses. Although this might cause issues for micro or nano suspensions, the same issue shouldn't be an issue for true solutions.

***PMPS*: How does this translate to humans?**

JS: Although challenging, a good starting point is the FDA guidance (2005 FDA Guidance: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in

Adult Healthy Volunteers) that talks about the key metrics that we want to think about in terms of establishing that first dose in humans. It starts with no-observed-adverse-effect level (NOAEL), with various factors that help scale the dose safely, including a scaling factor based on the species that we are going to choose and the species we have chosen. The key tenant is that there is no observed adverse effect, and that there are various factors that help – pharmacologic data, safety factors, scaling factors and species selection, for example.

Finally, we need to think about the indication where the target is in the nasal cavity because that then can influence the species we would use, and how we would calculate that first dose in man. For example, if we are thinking about CNS drug delivery and targeting the olfactory region, we know the NHPs have a nasal structure with relative proportions that are more similar to that of man than many other species, so it may be appropriate to use something like the surface area of the NHP nasal airways to consider those initial dose calculations.

***PMPS*: What advice would you give to anybody looking to perform intranasal or pulmonary studies?**

JS: The nasal and pulmonary drug delivery space is very much a specialised field, and it takes an understanding of the formulation and the device to really be able to know how to perform the administration of drugs. Because there is no standardisation in these processes, you will need to develop solutions that are either project specific or fit for purpose – requiring careful planning of your study, the formulation, where it is going to be deposited, what device or delivery system is going to be used and who is going to use it. You also need to understand how any data can be bridged to human models.

If you need help in planning or performing a preclinical study from a device standpoint, we encourage you to contact us at Aptar Pharma or Lovelace Biomedical.

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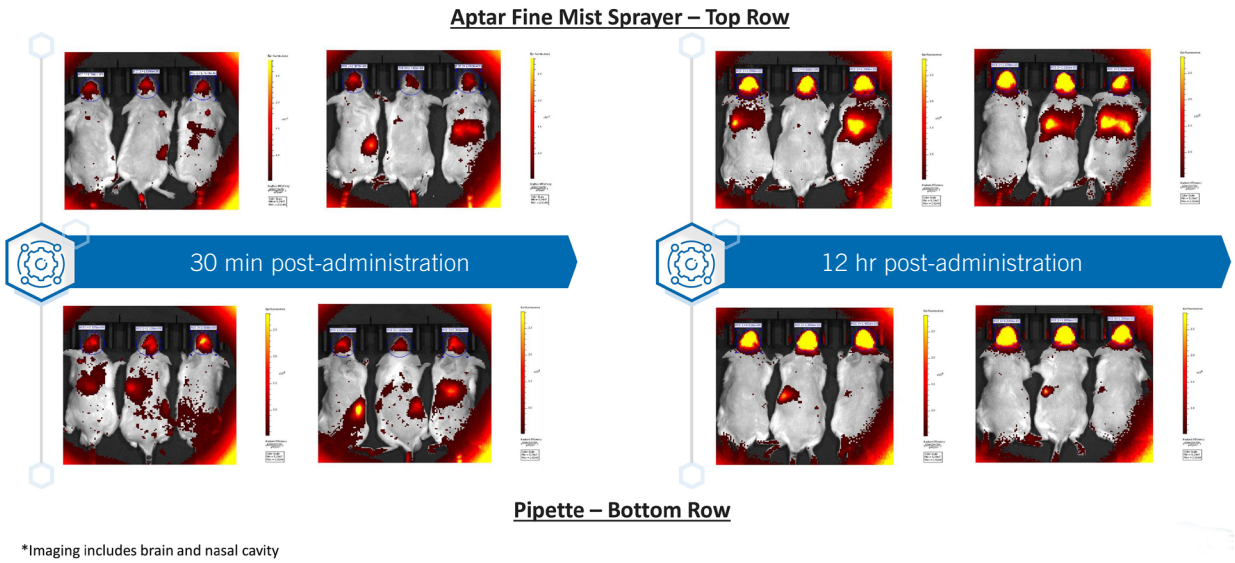

Figure 4: Whole body imaging for fine mist sprayer and pipette; 30 minutes vs 12 hours

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