Report

Assessing lung deposition of inhaled medications

Consensus statement from a workshop of the British Association for Lung Research, held at the Institute of Biology, London, U.K. on 17 April 1998

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In vitro measurements of aerosol fine particle fraction (FPF) using particle-sizing apparatus (e.g. the twin impinger, multi-stage liquid impingers, cascade impactors) have a key role to play in the development of new pharmaceutical products and in quality control. However, use of in vitro methodology to attempt to predict lung deposition in vivo is of limited value due, in part, to the inability of current apparatus to mimic upper and lower airway anatomy satisfactorily. Estimates of FPF based on cut-off points ranging from 5–7 μm generally overestimate lung deposition as measured in vivo by gamma scintigraphy. We recommend that:

1. multistage apparatus (minimum five stages) be used to characterize particle size distribution adequately, over the range 0.5–5.0 μm;
2. where possible, measurements should be made at a range of rates and profiles of flow reflecting those likely to be generated using the inhalation device in clinical practice (including use by young and elderly patients with varying degrees of airflow obstruction);
3. encouragement should be given to the further development, standardization, and validation of apparatus with a ‘throat’ which more closely resembles the human oropharynx and larynx.

Pharmacokinetic methods can give a good estimate of total, but not regional, lung deposition, with drugs which are either not absorbed via the gastrointestinal tract, or whose absorption can be blocked by co-administration of charcoal, thus avoiding confounding by absorption of drug substance deposited in the oropharynx and subsequently swallowed. Techniques which rely on evaluation of a timed fractional output of drug substance in the urine are susceptible to the inherent variability of rate of absorption across the respiratory epithelium.

We recommend that consideration should be given to the further refinement and validation of PK methods which would more clearly identify the fractional dose deposited in the lung.

Lung-imaging methodology, e.g. gamma scintigraphy, employing formulations radiolabelled with gamma-ray-emitting radionuclides such as 99mTc, can measure total lung deposition and oropharyngeal deposition, provided that the radiolabelling process is appropriately validated and suitable corrections are made for attenuation of gamma rays by body tissues. An estimate of regional lung deposition can be made by drawing ‘regions of interest’ on the scintigraphic image; the precision of this measure is limited by the two-dimensional (2-D) nature of most images which mean that there is an overlay of structures of interest (alveoli, small and large airways), which is most marked centrally. Three-dimensional (3-D) imaging techniques (e.g. single photon emission computed tomography, SPECT, and positron emission tomography, PET) have the potential to give more detailed data on regional lung deposition, but are currently more expensive, employ higher radiation doses, and are less well validated than 2-D (planar) imaging.

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We consider that, of the available imaging modalities, planar gamma scintigraphy represents current best practice for the assessment of lung deposition from inhaler devices where regional differences may be important. The methodology should be optimized by the adoption of generally accepted standards for radiolabelling, imaging, attenuation correction, and interpretation. It is important that deposition in all sites (device, oropharynx, lungs, stomach) should be quantified. Consideration should be given to refining the concept of regions of interest to coincide more closely with anatomical lung structures. Statistical methods to compare the size distributions of drug and radiolabel in validation experiments should be developed. In the longer term it is envisaged that three-dimensional imaging may play a more important part in evaluating lung deposition; an optimal three-dimensional anatomical model of lung zones of interest needs to be developed.

**Introduction**

The pattern of intrapulmonary deposition of inhaled particles is of great interest to researchers in the fields of inhalation toxicology, air pollution, and pharmaceutical science. Knowledge of the probable regional distribution of inhaled particles in the lung can help predict the sites of pathological changes due to inhaled carcinogens or toxic pollutants, and may assist in predicting the pharmacodynamic effects (both beneficial and adverse) of inhaled medications. Of particular interest to pharmaceutical scientists is the possibility of utilizing assessments of lung deposition to predict therapeutic equivalence of different inhalation devices designed to deliver the same drug substance. Assessment of the pharmaceutical and therapeutic equivalence of inhaled medications was the subject of a British Association for Lung Research workshop held at the National Heart and Lung Institute, London, in 1994 (1). The topic of lung deposition measurement was one of particular interest and, 4 years on, with the availability of a substantial amount of new data utilizing both existing and novel techniques, it was felt appropriate to convene a further expert workshop concentrating specifically on this subject. To this end, a number of experts on inhalation therapy were invited to participate, together with experts from the fields of respiratory physiology, inhalation toxicology, air pollution, and lung imaging, with the aims of 1. reviewing current methodology for evaluating lung deposition, in order to make recommendations for current ‘best practice’ and for future research directions and 2. examining the relationship between deposition of drugs in the lungs and their clinical effects.

**Current Methodologies for Evaluating Particulate Lung Deposition**

Four types of methodology are currently in use for the evaluation of lung deposition of inhaled particles. The first encompasses *in vitro* systems which categorize the range of sizes of particles in an aerosol cloud, and which could be predictive of the probable total and regional lung deposition pattern *in vivo*. The other three all attempt to measure *in vivo* lung deposition, either directly or indirectly, and comprise 1. methods of direct assay of drug concentration in lung tissue, 2. classical pharmacokinetic studies, and 3. lung imaging methodology.

**IN VITRO PARTICLE-SIZING METHODS**

These all involve categorizing the particles in an aerosol cloud into ranges of size (2). A fairly arbitrary division is made between ‘respirable’ particles (with a mass median aerodynamic diameter, MMAD, below a certain cut-off size, typically between 5 and 7 µm), which are assumed preferentially to reach the lung (the ‘respirable fraction’, RF or more accurately the ‘fine particle fraction’, FPF), and larger ‘non-respirable’ particles which are assumed to a large extent to impact on the oropharynx (whence they can be swallowed, potentially leading to gastrointestinal absorption). The simplest apparatus in use is the Twin Impinger, which has an angled ‘throat’ followed by two collecting chambers, the first of which collects the larger particles (including those deposited on the ‘throat’), whilst the particles reaching the second chamber represent the FPF. More sophisticated samplers (multi-stage liquid impingers and cascade impactors) sub-divide the particles further by size range – the Andersen sampler (e.g. Copley Instruments Ltd., Nottingham, U.K.), for example, has eight stages preceded by a right-angled throat. Laser-based sizing devices can estimate the size range of an aerosol cloud and are particularly useful for measuring the output when solutions are nebulised; however, these detect aerosol, not drug, and will size particles and droplets whether they contain drug or not.

*In vitro* testing plays a key role in the development of novel inhaled products, and the demonstration of reproducible FPF data is central to quality-control procedures. However, these *in vitro* methods have a number of drawbacks if utilized to try to predict lung deposition: the anatomy of the human oropharynx, larynx, and airways is complex, and poorly simulated by standard techniques, and the inhalation phase of the human respiratory cycle is also poorly reproduced, although an apparatus which simulates this more realistically has now been developed and validated (3). Inertial deposition of aerosol particles is determined not only by particle size but also velocity, and the existing apparatus is typically calibrated to operate at either 28.3 or 60 l min⁻¹ (partly for historical reasons: Andersen cascade impactors were originally developed to sample
environmental air at a rate of 1 ft³ min⁻¹, equivalent to 28.31 min⁻¹). The velocity of particles released from an inhaler varies with the type of device (high, from a metered-dose inhaler, MDI; low, from a nebuliser), and in the case of a dry powder inhaler (DPI), is dependent on inspiratory effort and flow resistance of the device. Dry powder dispersion has also been found to vary with flow acceleration. Thus the flow-rate profile chosen for in vitro measurement may not be the most appropriate for the device under study, if it is to be used to predict clinical lung deposition. Lastly, the size of liquid aerosol particles can diminish during their passage from the inhalation device due to evaporation, and hygroscopic particles may subsequently enlarge in the humid environment of the lung (4), behaviour which cannot readily be simulated by in vitro testing. In practice, the FPF measured by in vitro methods generally over-estimates the lung deposition measured using in vivo systems (5). Recent developments in deposition modelling suggest that this problem can be redressed if a more realistic estimate of throat deposition is used (6, 7).

DIRECT ASSAY OF DRUG IN LUNG TISSUE

The pattern of lung deposition of inhaled particles can be determined directly in animals by utilizing aerosols labelled with a dye or with radioactivity, and examining the lungs directly or by autoradiography after sacrifice and dissection. However, the use of animal models for examining lung deposition of inhaled medications is generally of limited relevance to clinical practice due to significant interspecies differences in airway architecture (8); and patterns of respiration (frequency, tidal volume, and regional ventilation) (9).

Although clearly inappropriate for the study of inhaled medication in humans, post-mortem studies have been carried out on deceased young adults with no history of respiratory disease to determine sites of pathological change in the lungs associated with deposits of carbonaceous and crystalline atmospheric pollutants (10). However, analogous studies have been performed on patients undergoing lung resection surgery, who were given single doses of inhaled medication preoperatively. Drug concentrations were then assayed in peripheral and central lung tissue, and compared with the concentrations in blood taken during surgery (11).

PHARMACOKINETIC STUDIES

Classical pharmacokinetic (PK) methodology has been difficult to apply to most inhaled products as the doses delivered are generally small (often microgram quantities) and the resulting plasma levels are correspondingly low, often below the accurate detection limits of standard assays. Although the recent development of more sensitive assay systems has made it possible to determine the PK of inhaled medications more accurately (12), problems exist in trying to relate drug plasma levels to patterns of lung deposition. A sizeable proportion of the plasma content of the drug may be due to gastrointestinal absorption of the swallowed fraction resulting from oropharyngeal deposition (this is not a problem for some agents, e.g. sodium cromoglycate and fluticasone propionate, which are not absorbed to any extent via the gastrointestinal tract). For some other drugs gastrointestinal absorption can be blocked by co-administration of activated charcoal. Where gastrointestinal absorption is excluded, systemic levels will reflect total lung deposition and absorption provided that no drug metabolism occurs locally in the lung. The serum $T_{\text{max}}$ may vary for different drugs due to differences in the rate of absorption across the lung epithelium, or to prolonged binding to lung receptors (11) or airway mucus. The charcoal-block method has been used to quantify the percentage of inhaled dose deposited in the lungs for formoterol, ipratropium bromide, budesonide, salbutamol and terbutaline sulphate (13), and it has been shown that 48-h urinary recovery of inhaled terbutaline sulphate, measured using the charcoal-block method, correlates well with whole-lung deposition estimated by gamma scintigraphy (14). The contribution of swallowed drug to systemic levels can also be allowed for if the oral bioavailability of the drug under test is known (15). Lastly, it has recently been proposed that assay of drug levels in urine collected over the first 30 min after administration of inhaled medication predominantly represents lung deposition (16), absorption from the gastrointestinal tract being slower than from the lung; however, the rate of absorption across the lung epithelium is variable and may be affected by such factors as depth of inspiration and breath-holding (17), cigarette smoking, respiratory viral infections and other causes of epithelial inflammation, and this methodology is not yet fully accepted. Urine or plasma PK at best will reflect whole-lung drug deposition, and give no information on regional patterns of deposition.

LUNG IMAGING

Planar (2-D) gamma scintigraphy

This is the conventional method currently employed for radioaerosol lung deposition and mucociliary clearance studies (18). The test formulation is radiolabelled (usually with a gamma emitting isotope such as $^{99m}\text{Tc}$-technetium, $^{99m}\text{Tc}$) and after inhalation the thorax is scanned using a gamma-camera; the radioactive counts can be digitized to yield a two-dimensional (2-D) image encompassing the oropharynx, lung fields, and stomach in addition to the inhalation device and an exhalation filter. The lung outlines can be defined utilizing a $^{81\text{Kr}}$ Kr ventilation scan or a transmission scan and the images superimposed to quantify penetration and distribution of the gamma-labelled medication. Before this stage, the raw counts must be corrected to allow for a variety of potentially confounding factors including background radiation, image duration, depth of the source, and attenuation by body tissue (see Appendix). In some cases it is possible to incorporate a radiolabel directly into the drug molecule; usually this is impracticable and the radiolabel is incorporated in the formulation in association with the drug but not chemically bound to it. In order to validate this technique it must be shown that the
radiolabel is distributed in a similar fashion to the unlabelled drug, by in vitro particle distribution measurements.

The main drawback to this method is its 2-D nature. Conventionally, the planar image of the lungs is divided into ‘regions of interest’ (for instance central, intermediate, and peripheral), with the central zone predominantly encompassing the larger airways and the peripheral zone the smaller airways and alveoli. However, there will be considerable overlay of these anatomical structures, particularly centrally where alveoli, bronchioles, and bronchi will be superimposed. Nonetheless, a ‘penetration index’ (PI), calculated as the ratio of peripheral to central deposition, will give a measure of the degree to which the aerosol has reached the smaller airways in the lung periphery; this index has been shown to correlate with the split between tracheobronchial and alveolar deposition from model nebulised formulations (19).

Single photon emission computed tomography (SPECT)

In this technique, a radiolabelled aerosol is prepared in a similar manner to planar imaging, but the gamma-camera, instead of obtaining only anterior and/or posterior views of the thorax, rotates through 360° around the patient and can provide tomographic views in the coronal, sagittal, and transverse planes. Computer manipulation can provide a full 3-D reconstruction in a similar fashion to X-ray computed tomography (CT scanning) (20). SPECT was first applied to the quantification of regional lung aerosol deposition in 1989, when Phipps et al. (21) demonstrated that the 3-D method was more sensitive in discriminating between large and small airway deposition of an inhaled aerosol than conventional planar gamma scintigraphy. More recently, attempts have been made to relate these 3-D images directly to deposition within the morphology of the lungs (22). Thoracic CT scans can be used to provide better anatomical localization of radionuclide distribution, and to map the attenuation of gamma emission by the thoracic structures; further increasing the accuracy of the technique (23).

More recently, magnetic resonance imaging (MRI) has been utilized for the same purpose, instead of CT (24), since it does not give any additional radiation dose. Currently, there are several limitations to the use of SPECT (25). It is more costly and requires greater technical facility than planar imaging. It takes longer (up to 30 min), during which time redistribution of the radioaerosol by mucociliary transport, coughing, or absorption into the bloodstream may occur. To avoid extra tissue attenuation by the arms it is necessary to keep them raised during the procedure, which may cause discomfort. Lastly, the amount of radioactive material and hence the radiation dose involved is some 20-fold higher than for planar imaging, and this is increased further if combined with CT scanning. This may be of particular relevance if children are to be studied, since procedures involving irradiation pose special ethical problems (26).

Positron emission tomography (PET)

This method (27) involves labelling the inhaled drug with a radioactive isotope which emits positrons as it decays, rather than single gamma rays. These positrons annihilate with electrons to produce two high-energy photons which are emitted simultaneously at 180° to each other. These are detected by coincidence counting and recorded as a single event; this method of detection permits accurate correction for tissue attenuation. The resulting 3-D image can be co-registered with an MRI or spiral CT scan of the thorax to give accurate delineation of regions of interest, and the percentage of the inhaled drug dose delivered to these regions can thus be determined three-dimensionally.

The main advantage of PET is that some fundamental organic isotopes (e.g. 11C, 15O) are positron emitters, and these can be incorporated directly into the drug molecule by isotopic substitution, hence the drug itself becomes the radioactive tracer. However, the available range of positron-emitting radionuclides is limited; other disadvantages of the technique are the need for the study site to be located close to a cyclotron, since positron emitters generally have short half-lives, (e.g. 11C, 20 min), and the current high capital and running costs entailed. Also, current experience in using this methodology for the study of drug deposition patterns in the human lung is limited (28), and further validation is required.

Neither PET nor SPECT offers any advantage over planar gamma scintigraphy or optimal PK methods for the quantification of total (as opposed to regional) lung deposition of inhaled medications.

Discussion

It is tempting to assume that patterns of lung deposition can be directly correlated with the pharmacodynamic effects of inhaled medication. However, this assumption could be over-simplistic, as for many medicines the actual site of action within the Airways is poorly defined, and variations in initial regional lung deposition may be compensated for, to some extent, by redistribution mechanisms. Many inhaled drugs can be absorbed through the respiratory epithelium (or if swallowed, the gastrointestinal mucosa) and if not metabolized, reach the lung again via the systemic circulation, some may also be absorbed into the bronchial circulation and redistributed directly within the lung (29), avoiding the potential for metabolism in the liver. The local concentration (and hence potential activity) of an inhaled drug will be affected by the depth of the airway surface liquid (ASL) (30), and drug which persists in the ASL (perhaps due to drug-mucus interactions) (31) could be redistributed from the periphery to the central Airways by mucociliary transport.

β2-adrenergic receptors exist throughout the Airways (on epithelium, smooth muscle, alveoli and specialized cell types including Clara cells and mucus-secreting cells) (32). Although one might expect that the more peripherally a β-adrenergic aerosol penetrates, the more β-receptors should be occupied and stimulated, and hence the greater the clinical effect, in practice the total lung dose seems to be more important than the regional distribution within the lung (33). This partly reflects the fact that as one descends the bronchial tree, the exposed airway surface area
inhaled medications will require very specific targeting, e.g. may then be achieved provided the aerosol penetrates bronchial tree, and hence inhaled anti-inflammatory agents the alveoli of patients with nocturnal asthma) (37). Some effect of particle size (and hence regional deposition) on actuation into the nasal cavity (34). In order to demonstrate any change in lung deposition, drug potency it has been found necessary to conduct studies using monodisperse particles at doses sufficiently low to be on the steep part of the dose-response curve (35). Muscarinic receptors are situated predominantly in the central airways (36), so anticholinergic bronchodilators should also be clinically effective provided they can penetrate as far as the larger bronchi.

In contrast, in chronic inflammatory airway disorders the inflammatory process is probably present throughout the bronchial tree, and hence inhaled anti-inflammatory agents (corticosteroids and possibly cromones) presumably need to be distributed throughout the airways to bronchiolar level or beyond (there is evidence of inflammatory cells in the alveoli of patients with nocturnal asthma) (37). Some inhaled medications will require very specific targeting, e.g. the antiviral ribavirin, used to treat bronchiolitis due to respiratory syncytial virus, needs to attain inhibitory concentrations in the bronchioles, pentamidine (used for prophylaxis against Pneumocystis carinii pneumonia) and some therapeutic peptides, e.g. insulin, may need to penetrate consistently to alveolar level. Clearly for these agents there should be considerable predictive value for efficacy from lung deposition studies demonstrating good peripheral lung penetration. Sequential lung imaging can evaluate clearance of the radiolabelled drug from the lung by mucociliary transport (MCT); retention of insoluble radiotracer at 24 h has been considered to represent deposition distal to the ciliated airways, where MCT does not operate, i.e. in the alveoli (38). Although it is now clear that a considerable proportion of particles retained at 24 h are actually in the smaller ciliated airways rather than the alveoli (39), nonetheless the 24 h retention should give a good assessment of lung deposition in the bronchioles and distal air spaces and could clearly be valuable in demonstrating that agents required to access the distal airways actually do so. The data currently available in patients with lung disease are limited.

There have been surprisingly few studies published in which lung deposition and clinical effects of an inhaled medication have both been measured, however, there have been two recent comprehensive reviews of the relationship between in vivo measurements of airway deposition of inhaled bronchodilators and corticosteroids and their pharmacodynamic effect (40,41). These reviews related data from separate lung deposition and clinical efficacy studies with the same devices and formulations and showed that, significant correlations could in general be demonstrated. The reviews are based on published clinical studies, the majority performed to demonstrate equivalence between two inhalation devices or two drug formulations, and it is possible that the strength of the deposition/effect relationship is artificially enhanced as a result of publication bias (42). On the other hand this is probably compensated for by the fact that the methodology of many of the studies was not optimal for demonstrating such a relationship (e.g. bronchodilator doses may have been on the plateau of the dose-response curve; optimal disease control in the corticosteroid studies may not have been achieved). Two recent studies included measurements of both clinical effect and lung deposition [one using the charcoal-block PK method (43), the other gamma scintigraphy (44)]. In the first study PK measurements established that a DPI delivered approximately twice the dose of terbutaline to the lungs as an MDI, and that this observed difference in deposition was reflected in the bronchodilator response. In the second study, differences in the rate of inhalation of sodium cromoglycate were associated with differences in regional lung deposition and the decreased penetration seen with more rapid inspiration was associated with reduced protection against allergen challenge. This latter study correlates well with previous work using a PK technique, showing that rapid inhalation of sodium cromoglycate was associated with reduced total lung deposition (17).

Measurements of lung deposition of inhaled drugs may therefore be of value in two specific situations; 1. in demonstrating that the preparation under study reaches those parts of the respiratory tract where it is designed to exert its pharmacological effects, and 2. as part of the evaluation of the comparability of two inhaled products. If a study has sufficient statistical power, identical patterns of lung deposition should possess predictive value for therapeutic equivalence. However, it should be pointed out that the converse is not necessarily true; for some agents (e.g. β-agonists, anticholinergics) two different preparations might have identical pharmacological effects despite differences in regional lung deposition, for the reasons discussed above. Clinical trials designed to show equivalence between two treatments should be properly powered, and an appropriate range of doses should be studied whenever possible. In addition to measuring end-points reflecting efficacy, it is important to compare systemic activity and side effects, as equivalence in risks and benefits do not necessarily go hand-in-hand (1,45).

Which method of evaluating lung deposition is currently the one of choice? In vitro methods are the simplest and cheapest, but generally over-estimate lung deposition measured in vivo. In vitro apparatus that can more closely mimic the human upper and lower airway (46) and inhalation pattern (3) are under evaluation. Although it is now possible to use realistic patient flows in combination with mathematical models to obtain a reasonable approximation of lung deposition, it may be necessary to develop validated, anatomically realistic upper airway models before in vitro techniques are directly predictive of lung deposition. Some pharmacokinetic methods can measure total (but not regional) lung deposition; the charcoal-block method has been shown to give similar values to gamma scintigraphy for the estimation of whole-lung deposition of
terbutaline (47). Scintigraphic techniques give the most direct measure of total and regional lung deposition in vivo; 3-D methodology shows promise for the future, but 2-D gamma scintigraphy is currently the optimal technique, provided that it is rigorously standardized and validated (proposed guidelines for the performance of scintigraphic studies are included in the Appendix). It is possible that estimation of regional lung deposition and peripheral penetration could be improved by refining the conventional ‘regions of interest’ along more anatomical lines.

Most regulatory agencies currently have extensive clinical testing requirements for demonstrating equivalence of new inhaler devices or formulations with the original product. Acceptance of validated measurements of lung deposition as a surrogate for some or all of these clinical studies would greatly reduce the resources required for such developments (48). Although such acceptance will take time it might be possible for the concept to be tested by, for example, submitting a lung deposition study to ‘bridge’ between clinical trial data where several dose formulations of a particular product (e.g. an inhaled corticosteroid) are being developed.

Conclusions

In vitro measurements of aerosol particle size distribution are important during technical development of new inhaler devices, and in quality control, but give only imprecise estimates of potential in vivo lung deposition. Measurements should be made using multistage apparatus to give an adequate characterization of the distribution of particle sizes and should, when possible, be carried out at flow rates relevant to clinical practice. In order to improve ‘entry’ conditions into particle sizing equipment, more work is required on the standardization and validation of ‘throats’ intended to simulate the oropharynx and larynx.

PK methods have a role to play in assessing total lung deposition. Care has to be taken to exclude the potential component of plasma (or urine) drug concentration that can arise from gastrointestinal absorption following oropharyngeal deposition. The charcoal-block method works well with a number of drugs.

Planar gamma scintigraphy is a valuable tool for assessing total and regional lung deposition. Careful control is needed over radiolabelling validation, imaging, attenuation correction and interpretation. Three-dimensional lung imaging methods offer promise for specialized studies relating deposition sites more closely to anatomical locations.

Detailed information about drug deposition sites within the lung may greatly help in the design of future trials on clinical efficacy and particularly when this requires to be assessed for varying formulations or presentations of the same drug. Similarity of total and localized deposition between different products is likely to predict similarity of effect. The converse may not hold. For lung deposition data to attain their full potential value the interplay between the clinical efficacy and the systemic activity of the medication, and the deposited drug distribution within the lung, requires further exploration in order more clearly to define the relationships for each drug class and device.

References


**Appendix. In Vivo Lung Deposition: Standards for Scintigraphical Studies**

**INTRODUCTION**

In scintigraphical studies conducted to determine the in vivo deposition pattern of an inhaled drug, the accuracy of the lung deposition values reported depend upon two critical factors:

1. the quality of the radiolabelling method used;
2. how the raw counts are converted into percentage deposition values, and in particular how the attenuation of gamma rays by different body tissues is corrected for.

**VALIDATING RADIOLABELLING METHODS FOR PHARMACEUTICAL AEROSOLS**

Labelling organic molecules chemically with gamma-emitting radionuclides is impracticable for the majority of inhaled drugs due to the short half-lives of suitable radioisotopes, although there is some recent experience with $^{11}$C (half-life 20 min) (27). Consequently, radiolabelling methods involving the use of $^{99m}$Tc as a surrogate marker for the drug have been developed. As the labelling involves a physical association between the drug and radiolabel, and not a chemical bond, the method must be validated to prove that the radiolabel will match the distribution of the drug (49). The objectives of validating a radiolabelling are as follows:

1. To show that the radiolabel will act as a valid marker for the drug by demonstrating in vitro that the particle size distribution of the radiolabel matches the particle size distribution of the drug.
2. To demonstrate that the labelling process does not affect the size distribution of the cloud generated by the inhaler.

**Methodology**

This is accomplished by measuring the particle size distributions (PSD) in the delivered cloud of the following:

1. drug that has not been radiolabelled (designated 'unlabelled' drug);
2. drug that has been radiolabelled (designated 'labelled' drug);
3. the radiolabel itself.

The 'unlabelled' drug is measured by taking a commercial 'off the shelf' inhaler and firing a number of doses into an inertial cascade impactor. The PSD is calculated from the mass of drug (determined by chemical analysis) deposited on the mouthpiece, sampling port (throat) and each impaction stage. This process is then repeated using an inhaler that contains drug that has been radiolabelled; the PSD of the 'labelled' drug is calculated in the same manner as the 'unlabelled' drug. The PSD of the radiolabel is determined by quantifying the amount of radiolabel deposited on the mouthpiece, throat and each impaction stage using a gamma camera.

While validation experiments can generally be conducted using relatively low amounts of radioactivity, it is important that validation experiments are also conducted with the required in vivo levels of activity to ensure that scaling up the amount of activity used does not affect the outcome. A minimum of five unlabelled and five radiolabelled inhalers should be prepared and tested to ensure reproducibility of the labelling method. Validation experiments should be conducted for all dosage regimens and at all inhalation flow rates to be used in in vivo studies.

**Standards for validation of radiolabelling methods**

In attempting to standardize how radiolabelling methods are validated, there are three key issues that need to be addressed.

Investigators need to use an inertial impactor/impinger that separates the aerosol into a sufficiently large number of fractions so that a valid judgement can be made on the 'quality' of the labelling, i.e. how closely the radiolabel distribution matches that of the drug.

**Procedure for pressurized metered dose inhalers (pMDI)**

Use Method 2 of the European Pharmacopoeia (Andersen Cascade Impactor, ACI), operated at 28.3 l min$^{-1}$ (which is close to the optimal flow rate for MDIs). The ACI fractionates the aerosol cloud into 10 stages; the throat, eight impaction stages and a final filter. This level of detail may be required to establish that the radiolabel is associating with the drug and not with an excipient (50).
Procedure for dry powder inhalers (DPI)
Use Method 1 of the European Pharmacopoeia (five stage High Precision Multi-Stage Liquid Impinger, HPMLI) operated at a flow rate which corresponds to a pressure drop of 4 kPa through the device and pulling 4 l air through the device.

Presentation of data
The PSDs of unlabelled drug, labelled drug and radiolabel have been presented in different ways by different investigators. Examples of how the validation data have been expressed include:

1. % distribution of metered or delivered dose;
2. % distribution on the stages of the Impactor alone;
3. comparison of mass median aerodynamic diameters (MMADs);
4. cumulative size distributions;
5. comparison of fine particle fractions (FPFs) alone;
6. ratio of radiolabel to drug in fractions smaller and larger than FPF;
7. comparison of labelled drug and radiolabel only, using one of above methods.

The problem with many of these methods is that mismatches between the drug and radiolabel may be obscured. These mismatches may affect the accuracy of the in vivo data obtained.

The following criteria are proposed for judging the validity of labelling:

1. Express the PSDs of unlabelled drug, labelled drug and radiolabel as percentages of the delivered dose.
2. An indication of the amount of drug (both unlabelled and labelled) recovered is required. One of the following parameters should also be shown:
   (a) the fine particle dose (FPD, expressed as mass of drug);
   (b) the amount of drug recovered expressed as a percentage of the label claim;
   (c) the average mass of drug per shot (metered or delivered).
3. Summary descriptors such as FPFs or MMADs can be used in the text for the sake of brevity, but data must be provided for both drug and radiolabel on the impaction stages, the throat and device mouthpiece (where possible).

Methods may reveal a mismatch between the drug and the radiolabel. The ratio of the radiolabel FPF to the unlabelled drug FPF has hitherto been used to define acceptance limits for radiolabelling methods. If this ratio is within the range 0.8–1.2 (based on the means of FPFs for five inhalers), the labelling method has been considered satisfactory. Although this range is broad and focused on FPF alone, it is difficult, at present, to define an alternative which is realistically achievable. For the future, it is hoped that criteria for acceptance of the radiolabelling method which are based on the full particle size distribution will be developed. It is important to add that the suitability of the method should also be judged by reviewing the validation data set out above against the clinical objectives of the study in which they are to be used.

Pre-dose testing of study day inhalers
Study day inhalers should be tested prior to dosing volunteers to ensure that each inhaler prepared will produce a radiolabel PSD that is comparable to the validation data obtained. A simple impinger such as the Twin Stage Impinger (TSI) may be used for this QC check, provided that a correlation between the FPF measured by the TSI and the FPF measured during the validation experiments has been previously established.

STUDY SUBJECTS AND INHALATION TECHNIQUES
Lung deposition studies may be conducted either using healthy volunteers, or patients with lung disease relevant to the drug under evaluation. Provided the inhalation technique is standardized, there is little evidence that the total lung deposition of an inhaled drug will differ between healthy volunteers and patients although some studies have shown increased particle deposition in patients with airway obstruction (asthma and chronic obstructive pulmonary disease) (51). However, marked differences in regional lung deposition may occur, notably a more 'central' deposition in patients with airway obstruction. Where some airways are blocked by mucus (e.g. patients with cystic fibrosis) very abnormal regional distribution patterns may be observed. Smokers tend to have decreased 24 h retention of inhaled particles ('alveolar deposition') which may be due to a combination of airway obstruction and mucus hypersecretion.

Subjects of either sex are suitable for radioaerosol lung deposition studies, but women of childbearing potential should be pregnancy tested before receiving radiolabelled drugs, and excluded from the study if the test is positive. Although total lung deposition seems to be similar in men and women, there is some evidence that there is a greater deposition in the proximal airways in women compared with men (52).

Studies with radiolabelled agents in children are difficult to justify unless some therapeutic benefit is likely to accrue to the child.

Inhalation technique has a major influence on the deposition of inhaled particles and droplets, and hence should be carefully controlled in lung deposition studies. The subject should be instructed in the requisite technique, and the radiolabelled dose only administered once this has been mastered reproducibly. In order to ensure co-ordination of actuation and inhalation, one of the investigators (rather than the subject) should actuate pressurized MDIs. Of course, the study design may be such that a patient with asthma is required to use their normal inhaler technique, in which case instructions on technique are unnecessary. The inhalation manoeuvre should be recorded by connecting the inhaler device in series with a system which monitors inhaled flow and volume, such as a Vitalograph.
MDI - Compact (Vitalograph Ltd., Buckinghamshire, U.K.) spirometer, ideally providing the subject with visual feedback via a microprocessor screen; to achieve this the inhaler device may need to be enclosed in a container which maintains an airtight seal with the inhaler. Care must be taken to ensure that the flow rate of air through the inhaler has not been modified, and that the subject can seal his lips around the mouthpiece. Sometimes this type of arrangement is impracticable, and another type of monitoring system, such as a Respitrace respiratory inductance plethysmography system, will be preferable.

Because of the inherent variability between subjects (gender, lung pathology, inhalation technique) lung deposition studies should ideally utilize a randomized cross-over design, so that each subject acts as his own control. Study population sizes of 8-12 subjects are generally satisfactory, but statistical justification for the number of subjects included should be provided in the study protocol. Larger sample sizes may be required for studies designed to show mathematically defined equivalence between two inhaled products.

**QUANTIFYING AEROSOL DEPOSITION**

Following the administration of a radiolabelled aerosol, the following images are obtained using the gamma camera:

1. lungs;
2. oropharynx;
3. stomach;
4. inhalation device (e.g. mouthpiece, actuator, spacer);
5. exhalation filter.

It is essential that deposition at all of these sites is quantified accurately, to ensure that full allowance is made for deposition of the dose at all sites both inside and outside the body. In order to quantify regional lung deposition, the lung outlines may be defined using a $^{81m}$Kr ventilation scan or a transmission scan using a $^{99m}$Tc flood-field source. The methods of Phipps et al. (21) or Newman et al. (53) can be used to divide the lungs into a series of regions (e.g. central, intermediate, and peripheral), but other ways of subdividing the lungs are also acceptable. The penetration index (PI) can be calculated as the counts in the peripheral region divided by the counts in the central region, following subtraction of background counts. A ‘relative penetration index’ can be calculated as the ratio of the PI to that of the krypton ventilation image; this has been shown to reduce intersubject variability (54).

It is important to note that because $^{99m}$Tc is a surrogate marker for the drug and not chemically bound to the drug, investigators can only seek to quantify the initial deposition site. Once a radiolabelled drug particle impacts on the airway wall, it is likely that the radiolabel and drug particle will become physically dissociated, and the radiolabel cannot be used as a marker for clearance of the drug.

The time period over which images of the thorax are collected must be adjusted to take into account the nature of the radiopharmaceutical used. For example, $^{99m}$Tc-pertechnetate is rapidly cleared from the lungs into the systemic circulation and so imaging must be complete within the first few minutes following administration otherwise deposition will be underestimated.

Analysis of the images collected yields data in the form of radioactive counts, termed ‘primary’ counts. The primary counts are then corrected as follows, to form ‘secondary’ counts, involving the following:

1. subtraction of background radiation;
2. correction for radioactive decay, where appropriate;
3. correction for differences in image duration, where appropriate;
4. correction for the depth of the source (for lungs and stomach) by calculating the geometric mean of anterior and posterior counts.

Gamma rays are attenuated in an exponential fashion as they pass through body tissues and into the gamma camera. The secondary counts must, therefore, be corrected for tissue attenuation otherwise deposition cannot be accurately determined.

**Tissue attenuation corrections**

Tissue attenuation may be corrected for by utilizing tissue Attenuation Correction Factors (ACFs) as follows:

\[
\text{secondary counts} \times \text{ACFs} = \text{final corrected counts.}
\]

ACFs *must* be determined for the different anatomical areas imaged: the lungs, stomach and oropharynx.

ACFs may be determined using 'phantoms' (55) but this method has been criticized as it produces a single ACF for all the subjects and does not allow for the anatomical differences between volunteers. ACFs should be determined for each volunteer studied.

The following methods of determining ACFs were compared in a recent study (56).

1. **Body thickness technique**: based upon measurements of body thickness and a number of assumptions about how different tissues attenuate gamma rays.
2. **Transmission scanning**: involves measuring transmitted radiation through the area under investigation.
3. **Perfusion technique**: injection of a known amount of a radiopharmaceutical that becomes trapped in the lungs.
4. **Sealed source**: a source containing a known amount of activity held in the mouth.

The results showed that ACFs for the lungs and stomach, but not the oropharynx, were relatively independent of the method used. Different combinations of the ACFs were then used to calculate deposition values from source data (secondary counts) obtained in a previous scintigraphic study. In addition, deposition data were calculated without making any corrections for attenuation.

It was found that the deposition values obtained were broadly comparable, irrespective of which combination of ACFs were used. Failure to correct for attenuation resulted in lung deposition being significantly underestimated and device deposition being significantly overestimated.
In the light of these findings, it can be stated that:

1. it is essential to correct for tissue attenuation in the lungs, stomach and oropharynx;
2. the body thickness technique is the simplest of these methods and does not subject volunteers to any additional radiation exposure. Transmission scans are easy to perform but volunteers are exposed to a slightly higher radiation dose. Perfusion scintigraphy involves an intravenous injection and increases the radiation exposure of the study subjects;
3. any of the methods described can be used to correct for attenuation, as the ACFs they produce are broadly comparable in terms of the deposition data they produce. The method used to determine ACFs must be stated.

It is acknowledged that the use of a single ACF for a particular region (e.g. the lungs) may be an oversimplification and future work should investigate the possibility of defining attenuation maps for each region imaged, to increase the accuracy of the quantification.