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International Journal of Pharmaceutics 258 (2003) 227–240
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Erratum

# Planar gamma scintigraphy—points to consider when quantifying pulmonary dry powder aerosol deposition☆

Eva Bondesson[a,b,*], Thomas Bengtsson[a], Lars Borgström[a], Lars-Erik Nilsson[b], Kristina Norrgren[c], Eva Trofast[a], Per Wollmer[b]

[a] *AstraZeneca R&D Lund, SE-221 87 Lund, Sweden*
[b] *Department of Clinical Physiology, Lund University, Malmö, Sweden*
[c] *Department of Radiation Physics, Lund University, Malmö, Sweden*

**Abstract**

Methodological aspects of planar gamma scintigraphy used to quantify pulmonary aerosol deposition were investigated using an experimental dry powder formulation. Particles of micronized salbutamol sulphate were labelled with technetium-99m and admixed to an ordered mixture of unlabelled micronized salbutamol sulphate and larger carrier particles of lactose. The radioaerosol was administered to 24 healthy subjects, 12 in each of two consecutive, similarly designed studies. Pulmonary deposition was determined using two methods: repeated planar imaging, and pharmacokinetic assessments following charcoal co-administration to prevent gastrointestinal salbutamol absorption. After due consideration had been taken to ensure appropriate radiolabelling, image acquisition and processing procedures, a scintigraphic estimate of 26.2% (with 95% confidence interval of 24.2–28.4%) was obtained, which did not significantly differ from the pharmacokinetic estimate of 26.4% (24.4–28.7%). In summary, pre-study validation of the radiolabelling technique, quality control of radioaerosols produced during the study, correction for re-distribution of radiolabel from the lungs, selection of regions of interest, assessment of lung contours, correction for tissue attenuation of gamma rays and establishment of the actual recovery of radioactivity in the scintigraphic measurements could potentially affect the accuracy of the scintigraphic estimate of pulmonary deposition and, thus, should be carefully considered in the design or evaluation of any such study.


*Keywords:* Radionuclide imaging; Radioactive aerosol; Administration; Inhalation; Technetium-99m; Powder inhaler

## 1. Introduction

Pulmonary drug deposition is a critical determinant of the effectiveness of orally inhaled, locally acting pharmaceutical aerosols. Methods for estimating the total amount of drug aerosol reaching the lungs involve either gamma scintigraphic or pharmacokinetic measurements (Snell and Ganderton, 1999). In previous studies, a scintigraphic method (planar imaging) was compared with a pharmacokinetic method (measurements of urinary drug excretion after prevention of gastrointestinal drug absorption by the co-administration of activated charcoal) using terbutaline sulphate inhaled either via a dry powder inhaler, Turbuhaler® (AstraZeneca) or a pressurised metered dose inhaler, pMDI (Borgström et al., 1992; Newman

☆ doi of original article 10.1016/S0378-5173(02)00579-3.
* Corresponding author. Tel.: +46-46-33-60-00; fax: +46-46-33-66-66.
*E-mail address:* eva.bondesson@astrazeneca.com (E. Bondesson).

0378-5173/03/$ – see front matter 
doi:10.1016/S0378-5173(03)00148-0

et al., 1995). Currently marketed dry powder inhalers contain drug formulated either as an agglomerated micronized powder or as an ordered mixture. For Turbuhaler®, in which the drug is presented as an agglomerated micronized powder formulation, the two methods gave similar pulmonary deposition estimates. Knowing the precision of each of the two methods, it was concluded that either could be used, although they might reflect somewhat different aspects of aerosol deposition. Whereas the scintigraphic method gives an image of the initial deposition pattern, the pharmacokinetic method reflects drug absorbed from the tracheobronchial airways and alveolar tract. Thus, drug deposited in the central airways that would be detected by the scintigraphic method may not be captured by the pharmacokinetic method if the drug was removed by mucociliary clearance to the oral cavity before being absorbed into the blood.

The aim of our work was to investigate the relative influence of various aspects of the scintigraphic method on the quantification of pulmonary deposition of an ordered mixture dry powder radioaerosol and to compare scintigraphic measurements with pharmacokinetic measurements of pulmonary aerosol deposition. For that purpose, a formulation of technetium-99m ($^{99m}$Tc)-labelled particles of micronized salbutamol sulphate with a mass median diameter of 1.3 μm added to a commercially available salbutamol sulphate formulation, Ventolin® Diskhaler, 0.2 mg/dose, was developed, evaluated and administered to healthy subjects in two similarly designed, consecutive studies.

## 2. Materials and methods

### 2.1. Radiolabelling method

Micronized salbutamol sulphate was labelled with the gamma ray emitting radionuclide $^{99m}$Tc using a procedure originally developed for pMDIs modified for dry powder aerosols (Köhler et al., 1988; Newman et al., 1989). To avoid the toxic compound tetraphenylarsonium chloride, normally used to extract the radionuclide into chloroform, a more polar solvent, 2-butanone (methylethylketone, MEK), was chosen. About 7 GBq of $^{99m}$Tc-sodium pertechnetate was eluated in isotonic saline from a $^{99}$Mo/$^{99m}$Tc generator (Ultra-TechneKow® FM, Mallincrodt Medical BV, Petten, The Netherlands). The $^{99m}$Tc-sodium pertechnetate was extracted twice using extensively purified and dried MEK (i.e. MEK dried using a 4 Å molecular sieve, distilled and stored above the sieve). The extraction yield was about 90%. The extract was placed in a heating block and the organic phase evaporated under a stream of nitrogen at 45 °C until dryness. The residue was dissolved in 2-methyl-2-propanol (*tert*-butanol), the organic phase evaporated, and the ensuing residue dissolved in MEK. The organic phase was again evaporated, and the final residue dissolved in MEK. The MEK containing the $^{99m}$Tc-sodium pertechnetate was transferred dropwise to 4 mg (Study I) or 8 mg (Study II) of micronized salbutamol sulphate (AstraZeneca Liquid Production, Södertälje, Sweden) particles with a mass median diameter of 1.3 μm in an aluminium tube pre-treated with 65% nitric acid, washed and dried. In Study I, the salbutamol sulphate suspension was repeatedly vibrated (using an ultrasonic pin applied on the outside of the tube) and dried under a stream of nitrogen at 40 °C. In Study II, while adding the $^{99m}$Tc-sodium pertechnetate to the salbutamol sulphate particles, the tube was continuously vibrated using an ultrasonic bath at room temperature. In both studies, the salbutamol sulphate suspension was then stirred using a Vibra-mixer, gently vibrated in the ultrasonic bath and—while still being vibrated—dried under a stream of nitrogen. The resulting crust was dislodged by the use of a spatula. The tube was then placed in the heating block and kept under a slow stream of nitrogen at 45 °C for 45 min.

The aluminium foil covering each of the eight dose cavities in four discs of Ventolin® (salbutamol sulphate) Diskhaler, 0.2 mg/dose (GlaxoSmith-Kline), was meticulously cut off using a sharp scalpel. The Diskhaler powder was carefully collected and gradually added to the tube containing the micronized salbutamol sulphate particles radiolabelled with about 80% of the original amount of $^{99m}$Tc. In Study I, 100 mg of the Diskhaler powder was initially admixed to the radiolabelled salbutamol sulphate particles using a spatula and, following vibration of the tube using an externally applied ultrasonic pin, the remaining amount of the Diskhaler powder (∼700 mg) was admixed. In Study II, the Diskhaler powder was admixed in three portions of 25, 100 mg, and the remaining amount (∼675 mg).

The ensuing powder, radiolabelled to a specific activity of about 3.2 MBq ($^{99m}$Tc)/mg, was filled into thoroughly emptied, washed and ethanol-dried Diskhaler discs. Each disc was immediately sealed by a 20-μm Pharmaflex® aluminium foil (Lawson Mardon Pharmaflex Ltd., Cramlington, UK). One dose contained sulbutamol sulphate corresponding to about 250 μg (Study I) or 360 μg (Study II) of salbutamol, about 25 mg of lactose, and—at the time of administration—about 70 MBq of $^{99m}$Tc.

### 2.2. Validation and quality control of radiolabelling

The extent to which the radiolabel ($^{99m}$Tc) would act in vivo as a marker of the drug was evaluated pre-study using a multi-stage liquid impinger, MLI (European Pharmacopoeia, 1997). The MLI was used to fractionate the dose delivered as radiolabelled or unlabelled aerosol. The relative amounts of radioactivity and salbutamol in each of the different particle size fractions of the delivered dose were measured and compared. Radioactivity was measured using a gamma counter (Wallac Wizard, Wallac Oy, Åbo, Finland). Salbutamol was measured using high performance liquid chromatography with orciprenaline sulphate as an internal standard. In order to mimic in vivo relevant conditions, an airflow of 100 l/min through the MLI, which corresponds to a pressure drop over the inhaler of 3.1 kPa, was selected for the in vitro evaluation resulting in size bands of >10.1 μm (Stage 1); 5.3–10.1 μm (Stage 2); 2.4–5.3 μm (Stage 3); and <2.4 μm (Stage 4). For the radiolabel to perfectly match the aerodynamic behaviour of the marketed pharmaceutical aerosol, the relative amounts of radioactivity and unlabelled salbutamol on MLI stages 3 + 4 should have been identical, i.e. giving a size distribution ratio of 1.0.

The distribution of unlabelled salbutamol, delivered by (unprocessed) Ventolin® Diskhaler of the same batch as used for radiolabelling, was analysed on one (Study I) or three (Study II) occasions using three MLIs, each representing mean of four doses delivered at 100 l/min. For each radiolabelling (corresponding to a study inhaler), quality was controlled on the basis of size distributions of radioactivity and salbutamol in four doses of $^{99m}$Tc-labelled salbutamol sulphate analysed in duplicates using the MLI at 100 l/min, and specific activity measured in 21 doses (Study I) or 14–21 doses (Study II).

### 2.3. Subjects

A group of 12 healthy, non-smoking men, 10 of whom had never smoked and 2 of whom had not smoked within the past 12 months, with a mean age of 30 years (range: 18–65 years), body weight of 77 kg (64–90 kg) and height of 178 cm (170–194 cm) participated in Study I. At inclusion, their mean forced expiratory volume in one second ($FEV_1$) was 96% (81–116%) and vital capacity 98% (84–111%) of predicted normal values (Quanjer, 1983). Another group of 12 healthy, never-smoking men with a mean age of 27 years (range: 20–51 years), body weight 75 kg (64–92 kg) and height 178 cm (167–188 cm) participated in Study II. At inclusion, their mean $FEV_1$ was 111% (98–120%) and vital capacity 109% (99–127%) of predicted normal values (Quanjer, 1983). Subjects with a history of chronic respiratory disease or recent symptoms of an upper or lower respiratory tract infection were excluded. The studies were performed in accordance with the principles stated in the Declaration of Helsinki. Approvals were obtained from the Research Ethics Committee at the University of Lund/Malmö, Sweden, and the Radiation Protection Committee at Malmö University Hospital, Sweden.

### 2.4. Drug administrations

All subjects received three single doses of salbutamol sulphate (two inhaled and one intravenously injected) at about weekly intervals in an open, crossover and randomised fashion. On one occasion, radiolabelled salbutamol sulphate corresponding to a total dose of 500 μg (Study I) or 720 μg (Study II) of salbutamol was inhaled via Diskhaler. On another occasion, unlabelled salbutamol sulphate corresponding to a total dose of 600 μg of salbutamol was inhaled via Diskhaler to enable the pharmacokinetically determined pulmonary deposition of salbutamol inhaled as radiolabelled aerosol to be compared with that of the commercially available product. On yet another occasion, unlabelled salbutamol sulphate corresponding to a total dose of 200 μg (Study I) or 150 μg (Study II) of salbutamol was intravenously injected to allow the pharmacokinetically determined

pulmonary deposition to be expressed as percentage of dose. A total of 40 g of activated charcoal (Carbomix, Selena Fournier) was given orally before and over the 2 h after each administration of salbutamol sulphate (i.e. before and after inhalation of radiolabelled salbutamol sulphate, inhalation of unlabelled salbutamol sulphate and intravenous injection of unlabelled salbutamol sulphate) to prevent gastrointestinal absorption of salbutamol (~10 g of charcoal in 50 ml of water was swirled around in the mouth cavity and swallowed immediately pre- and post-dose; and at 1 and 2 h post-dose). In Study II, about 2.5 h prior to the radioaerosol inhalation, 130 mg of potassium iodide (Kaliumjodid Recip, Recip) was administered to prevent thyroid uptake of $^{99m}$Tc-pertechnetate.

### 2.5. Aerosol inhalation

Aerosol inhalation was always carried out under the supervision of a member of the study team. At inclusion and prior to first aerosol inhalation on a study day, each subject practised the inhalation technique using a placebo inhaler. Aerosol was inhaled by the subject in a sitting (Study I) or standing (Study II) upright position. Inspiratory flow and volume were measured using a Vitalograph MDI modified Compact spirometer (Study I) or a Vitalograph 2120 spirometer (Study II) calibrated on each study day according to the instructions provided by the manufacturer of the spirometers (Vitalograph Ltd., Buckingham, UK). Each subject was trained to take a full breath from residual volume to total lung capacity and to aim at a target peak inspiratory flow (PIF) of 100 l/min. After inhalation, the subject held his breath for at least 5 s and then slowly exhaled through his nose. Immediately following the subject's last aerosol inhalation on a study day, he rinsed his mouth with 250 ml of water. Residual salbutamol in the different parts of the study inhaler was measured using a high performance liquid chromatography method.

### 2.6. Scintigraphic method

Prior to inhalation of radioaerosol, a transmission image was obtained using a flood source of cobalt-57 to correct for tissue attenuation of gamma rays using a previously described method (Macey and Marshall, 1982) and to provide lung contours of each subject. Proper alignment of transmission and emission scintigrams was ensured by the use of individually adapted vacuum bags with Styrofoam beads supporting the head and neck of the subject while in the supine position in combination with laser beams running in the sagittal plane. After inhalation of radioaerosol, scanning of the upper part of the body was performed either twice (Study I) or an initial scanning of the upper part of the body was followed by a whole-body scanning (Study II). In preparation of Study II, procedures for inspiratory flow recording, mouth rinsing, intake of activated charcoal and gamma camera positioning were optimised (in order to minimise the time between dosing and imaging) and image acquisition protocols adjusted (scanning rate was increased from 15 to 20 cm/min and the length of first scanning decreased from 100 to 85 cm) to reduce the effects of the radiolabel transport post-deposition observed in Study I. Thus, mean mid-times of the two scannings were 13 and 29 min in Study I, but 9 and 25 min in Study II. A dual-headed gamma camera (Siemens Multispect II, Hoffman Estates, IL) with a field of view 53 cm × 39 cm and fitted with low-energy, high-resolution collimators was used. Quality control of the gamma camera was performed in accordance with the manufacturer's recommendations. A 15% energy window was centred over the 140 keV photo peak. Gamma camera sensitivity was calibrated using a thin $^{99m}$Tc source (NEMA, 1986). Anterior and posterior views of the lungs, oral cavity, larynx, oesophagus/trachea and stomach of the subject were acquired simultaneously through scanning in the caudocranial direction. Following each of the two scannings, lateral views of the subject's head were acquired with the subject still in the supine position. Following the second scanning, radioactivity in the mouth rinse and in the different parts of the inhaler was measured using the gamma camera. No attempts were made to measure radioactivity in exhaled air following aerosol inhalation, as that fraction was assumed to be negligible on the basis of published data on Diskhaler (Melchor et al., 1993) and the fact that, in Studies I and II, aerosol inhalation was followed by a 5-s breath holding pause and slow exhalation through nose. Radioactivity in each of the selected regions of interest in the body was calculated, after subtraction of background radioactivity and corrections for tissue attenuation

and radioactive decay to the time of administration, as the geometric mean of anterior and posterior counts. As tissue attenuation of gamma rays may differ quite markedly between organs and, in many cases, the upper part of the stomach is superimposed by structures of the basal left lung, it was decided to calculate the amount of radioactivity in the left lung and stomach on the basis of three regions of interest (the left lung, the stomach and the overlap of the two) and their respective attenuation factors. In the main analysis, total deposition in the left lung plus stomach was calculated as: (left lung + stomach) − overlap. In a separate analysis, carried out to examine the importance of adequate image processing, total deposition in the left lung plus stomach was also calculated on the basis of the unite region of interest with one common attenuation factor. Radioactivity in the mouth rinse and in the different parts of the inhaler was calculated on the basis of counts collected over 2 min in single views after subtraction of background radioactivity and correction for radioactive decay to the time of administration. Calibration factors were established for anterior and posterior views of the body scannings, the mouth rinse and the inhaler to enable deposition to be expressed as percentages of the metered dose of radioactivity (MBq) measured in the dosing disc prior to administration. The right lung only was used for quantification of total pulmonary deposition and regional distribution of radioactivity within the airways. Total pulmonary deposition was calculated as the percentage of radioactivity in the right lung multiplied by 1.9 as, in healthy subjects, aerosol deposition has been shown to be proportional to ventilation, and ventilation to the right lung has been found to be 52.5% of the total ventilation (Arborelius et al., 1970). More recently, a similar systematic difference in aerosol deposition between the left and right lungs has also been observed for $^{11}$C-labelled triamcinolone acetonide delivered by a pMDI (Berridge et al., 2000). Regional distribution of radioactivity within the airways was established as a penetration index (PI), i.e. a peripheral to central lung zone deposition ratio, calculated on the basis of two previously described methods dividing the right lung into either two lung zones (peripheral and central) or three lung zones (peripheral, intermediate and central) (Olséni et al., 1994; Newman et al., 1987).

### 2.7. Pharmacokinetic method

Urine was collected over 30 h in three separate fractions (0–6, 6–18 and 18–30 h post-dose) and 10-ml samples of those collections, together with a pre-dose sample, were analysed for unconjugated salbutamol using a gas-chromatography mass spectrometry method (Leferink et al., 1982). It was considered appropriate to quantify pulmonary deposition of salbutamol in the same way as for terbutaline (Borgström and Nilsson, 1990) as no metabolism of salbutamol appears to take place in the lungs (Evans et al., 1973), and systemic bioavailability of salbutamol administered as an oral solution of salbutamol sulphate with concomitant oral intake of activated charcoal, using the same dosing regimen as in Studies I and II, had previously demonstrated to be low, about 1%, in eight healthy subjects (unpublished observation).

### 2.8. Statistical considerations

In each of Studies I and II, scintigraphically and pharmacokinetically derived pulmonary deposition estimates for the radiolabelled aerosol were compared using a multiplicative analysis of variance (ANOVA) model in which method and subject were factors. Pharmacokinetically derived pulmonary deposition estimates for the radiolabelled and unlabelled aerosols were compared using a multiplicative ANOVA model in which subject, visit and treatment (i.e. administration of radiolabelled or unlabelled aerosol) were factors. A difference between treatments was expressed as a mean ratio of the deposition estimates with 95% confidence interval (CI) for the ratio. Possible correlations between depositions in various regions or between scannings were examined by graphical methods and coefficients of correlations between PIs calculated.

Pulmonary deposition estimates calculated for the two scannings were used to estimate pulmonary deposition at the time of aerosol inhalation. An exponential clearance of radioactivity from the lungs (by any or both of two mechanisms: mucociliary clearance and systemic absorption) was assumed, i.e. Deposition = $\text{Deposition}_0 \times e^{-\alpha(T-T_o)}$ where 'Deposition' is pulmonary deposition at the mid-time of scanning ('$T$'), '$\text{Deposition}_0$' is pulmonary deposition at the time of aerosol inhalation ('$T_o$') and '$\alpha$' is a measure of the clearance rate equal to ln 2 divided by the half-life.

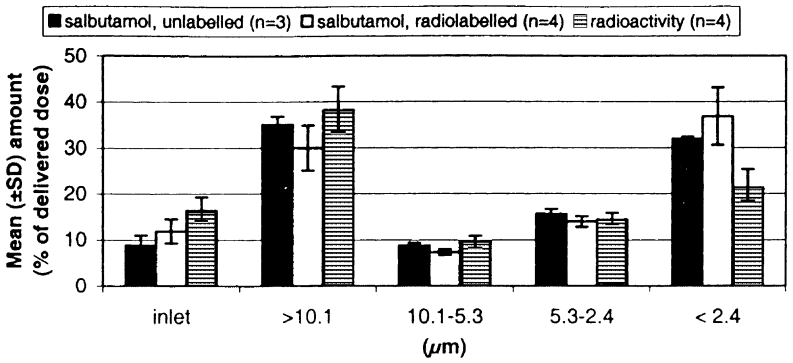


Fig. 1. Study I: Size distribution of salbutamol delivered as unlabelled salbutamol sulphate and size distributions of radioactivity and salbutamol delivered as $^{99m}$Tc-labelled salbutamol sulphate by Diskhaler at 100 l/min (% of delivered dose in the MLI inlet and on each of the four MLI stages) ($n$ = number of inhalers tested).

## 3. Results

### 3.1. Validation and quality control of radiolabelling

Size distributions of radioactivity and salbutamol, both delivered as $^{99m}$Tc-labelled salbutamol sulphate, and size distribution of salbutamol delivered as unlabelled salbutamol sulphate are presented in Figs. 1 and 2. Although validation of radioaerosol carried out prior to Study I was found to give a size distribution ratio of radioactivity to unlabelled salbutamol of 1.0 ($n = 5$), quality control of study inhalers showed a mean±standard deviation (S.D.) ratio of not more than 0.75 (±0.11). The amount of radioactivity, labelled salbutamol and unlabelled salbutamol on MLI stages 3 + 4 was 35.8, 50.8 and 47.5% of delivered dose, respectively. Thus, following Study I, the radiolabelling technique was re-evaluated and refined. In some separate experiments, a relationship was found between the size distribution ratio (radioactivity to unlabelled salbutamol) and the amount of micronized salbutamol sulphate used for radiolabelling (Fig. 3). Thus, the amount of micronized salbutamol sulphate was optimised accordingly. Furthermore, salbutamol sulphate particles were kept fluidized in air while the $^{99m}$Tc in MEK was added, the solvent slowly removed from the salbutamol sulphate particles during the initial part of evaporation and the Diskhaler powder gradually added to the $^{99m}$Tc-labelled salbutamol sulphate. In Study II, as compared with Study I, a size distribution

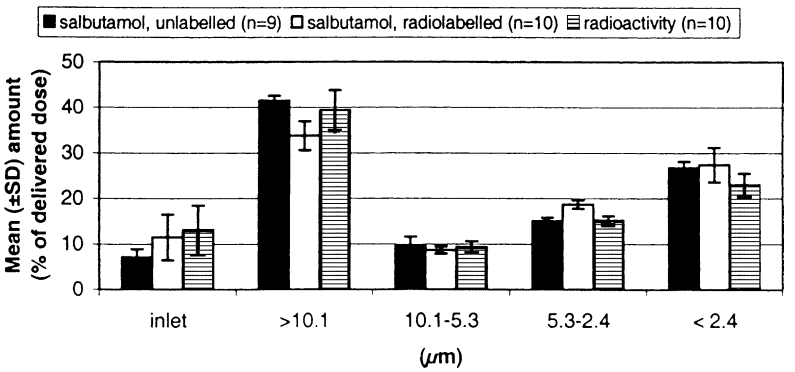


Fig. 2. Study II: Size distribution of salbutamol delivered as unlabelled salbutamol sulphate and size distributions of radioactivity and salbutamol delivered as $^{99m}$Tc-labelled salbutamol sulphate by Diskhaler at 100 l/min (% of delivered dose in the MLI inlet and on each of the four MLI stages) ($n$ = number of inhalers tested).

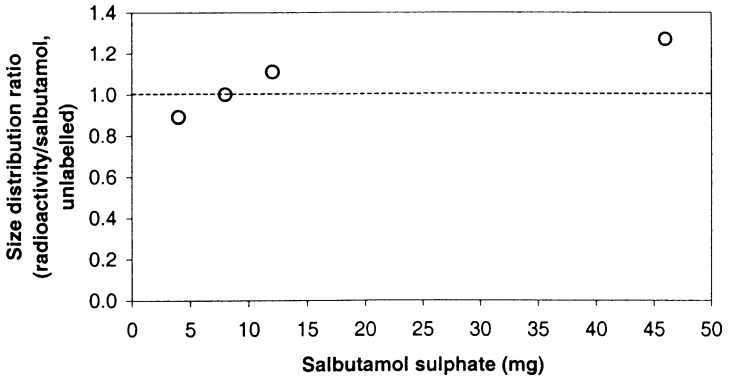


Fig. 3. Size distribution ratio of radioactivity to unlabelled salbutamol vs. the amount (mg) of micronized salbutamol sulphate used for radiolabelling. Each data-point represents one radiolabelling experiment analysed in duplicate for radioactivity and compared with unlabelled salbutamol sulphate analysed in triplicate for salbutamol. A size distribution ratio of 1.0 (dashed line) is indicated.

ratio closer to unity, 0.91 (±0.04), was obtained. The amount of radioactivity, labelled salbutamol and unlabelled salbutamol on MLI stages 3 + 4 was 38.2, 46.1 and 41.8% of delivered dose, respectively. The observed difference in fine particle fraction of unlabelled salbutamol between studies (47.5% versus 41.8%) was probably mainly due to differences between the two Ventolin® Diskhaler batches used for radiolabelling. The variation (relative standard deviation) in specific activity decreased numerically from 3.5% (Study I) to 0.7% (Study II).

### 3.2. Aerosol inhalation

Target PIF of 100 l/min was reached during aerosol inhalations in both studies (Table 1).

### 3.3. Scintigraphic results

At aerosol inhalation, 21.9% (Study I) or 17.6% (Study II) of the amount of radioactivity measured in the dosing disc prior to administration ('metered dose') was retained in the inhaler (Tables 2 and 3). Distribution of radioactivity among regions of interest in the initial scintigram of the upper part of the body following aerosol inhalation is illustrated in Fig. 4. The largest fraction of the metered dose, 60.1%, was deposited orally, i.e. found in the stomach, oral cavity and mouth rinse, in Study I (Table 2). Likewise, the largest fraction of the metered dose, 60.7%, was deposited orally in Study II (Table 3). Only some minor fractions, 1.6 and 2.1% (Study I) or 0.9 and 1.1% (Study II) were deposited in larynx and oesophagus/trachea, respectively. Visual inspection of lateral head images indicated that the oral cavity was lined by radioactivity.

Mean (95% CI) pulmonary deposition of radioactivity assessed by the first scanning post-dose was found to be 8.5% (7.9–9.1%) and 15.6% (14.5–16.7%) of the metered dose in Studies I and II, respectively (Table 4). However, the actual amount of radioactivity present in the lungs immediately following aerosol inhalation was bound to be greater than indicated by measurements as pertechnetate is rapidly absorbed over the lungs into the systemic blood circulation. In

Table 1
Mean ± S.D. peak inspiratory flow (PIF) and mean ± S.D. inhaled volume recorded during two consecutive inhalations of radiolabelled salbutamol sulphate or three consecutive inhalations of unlabelled salbutamol sulphate via Diskhaler

| | PIF (l/min) | Inhaled volume (l) |
|---|---|---|
| Study I | | |
| Radiolabelled | 91 ± 19 | 3.1 ± 1.1 |
| | 100 ± 20 | 3.1 ± 1.0 |
| Unlabelled | 102 ± 18 | 3.2 ± 0.7 |
| | 102 ± 16 | 3.0 ± 0.6 |
| | 107 ± 16 | 3.0 ± 0.7 |
| Study II | | |
| Radiolabelled | 105 ± 8 | 3.4 ± 1.1 |
| | 106 ± 6 | 3.2 ± 1.2 |
| Unlabelled | 111 ± 11 | 3.5 ± 0.9 |
| | 113 ± 12 | 3.5 ± 1.2 |
| | 112 ± 9 | 3.5 ± 1.1 |

Table 2
Study I: Distribution of radioactivity among regions of interest (ROIs) in body (measured at first and second scannings post-dose) and ex-body

| | First scanning (mean ± S.D.) | Second scanning (mean ± S.D.) |
|---|---|---|
| Right lung | 6.5 ± 2.2 | 3.7 ± 1.2 |
| Left lung[a] | 13.1 ± 5.2 | 9.3 ± 3.6 |
| Stomach[b] | 28.6 ± 11.0 | 30.1 ± 10.8 |
| Overlap[c] | 5.2 ± 4.6 | 5.3 ± 4.0 |
| Oral cavity | 2.9 ± 2.0 | 3.0 ± 1.9 |
| Larynx | 1.6 ± 1.8 | 2.1 ± 2.1 |
| Oesophagus/trachea | 2.1 ± 1.5 | 1.6 ± 1.4 |
| Mouth rinse | 28.6 ± 8.6 | |
| Inhaler: dosing disc | 8.6 ± 2.5 | |
| Inhaler: white part | 10.6 ± 4.3 | |
| Inhaler: blue part | 2.7 ± 0.7 | |

Values are arithmetic mean ± S.D. percentages of the total amount of radioactivity recovered at first scanning.
[a] Radioactivity in overlap of the stomach region with the left lung region included.
[b] Radioactivity in overlap of the left lung region with the stomach region included.
[c] Radioactivity in the (stomach and left lung) overlap region.

Study II, such re-distribution of radioactivity was also demonstrated to take place; the number of counts in the whole body was 20% larger than the number of counts in the upper part of the body collected at the same

Table 3
Study II: Distribution of radioactivity among regions of interest (ROIs) in body (measured at first and second scannings post-dose) and ex-body

| | First scanning (mean ± S.D.) | Second scanning (mean ± S.D.) |
|---|---|---|
| Right lung | 9.9 ± 1.8 | 3.8 ± 0.8 |
| Left lung[a] | 15.6 ± 7.0 | 9.8 ± 6.3 |
| Stomach[b] | 26.6 ± 10.7 | 28.3 ± 10.8 |
| Overlap[c] | 5.9 ± 7.5 | 6.0 ± 6.9 |
| Oral cavity | 1.8 ± 0.7 | 1.8 ± 0.7 |
| Larynx | 0.9 ± 0.6 | 1.6 ± 0.8 |
| Oesophagus/trachea | 1.1 ± 0.8 | 1.8 ± 1.3 |
| Mouth rinse | 32.3 ± 9.6 | |
| Inhaler: dosing disc | 4.1 ± 0.7 | |
| Inhaler: white part | 12.2 ± 2.7 | |
| Inhaler: blue part | 1.3 ± 0.8 | |

Values are arithmetic mean ± S.D. percentages of the total amount of radioactivity recovered at first scanning.
[a] Radioactivity in overlap of the stomach region with the left lung region included.
[b] Radioactivity in overlap of the left lung region with the stomach region included.
[c] Radioactivity in the (stomach and left lung) overlap region.

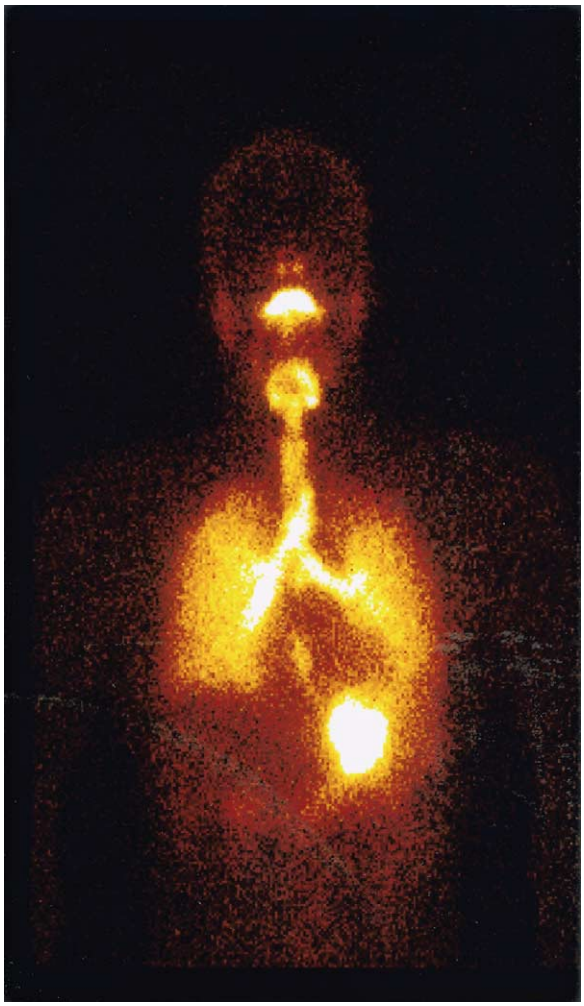

Fig. 4. Radioactivity distribution in the anterior view of one subject (Study II).

scanning. The repeated scintigraphic measurements in Studies I and II provided a possibility to extrapolate the pulmonary deposition of radioactivity to the time of aerosol inhalation assuming mono-exponential clearance of radioactivity from the lungs. Where a rather long mean ± S.D. clearance half-life of 21 min (±3 min) was found in Study I, a numerically much shorter half-life of 12 min (±2 min) was found in Study II. The latter half-life was well in agreement with published 10–11 min for inhaled pertechnegas (Monaghan et al., 1991; Fanti et al., 1996). After correction for radiolabelled transport post-deposition in

Table 4
Pulmonary deposition estimates (% of metered dose) of salbutamol inhaled as radiolabelled dry powder aerosol determined using scintigraphic and pharmacokinetic measurements, and contrasts between estimates

| | Study I | | Study II | |
|---|---|---|---|---|
| | Mean | 95% CI | Mean | 95% CI |
| Scintigraphy (first scanning) | 8.5 | 7.9–9.1 | 15.6 | 14.5–16.7 |
| Scintigraphy (extrapolated to time of aerosol inhalation) | 13.2[a] | 12.2[a]–14.2[a] | 26.2 | 24.2–28.4 |
| Pharmacokinetics | 25.1 | 23.3–27.1 | 26.4 | 24.4–28.7 |
| Pharmacokinetics/scintigraphy (extrapolated) | 190[a] | 171[a]–212[a] | 101 | 90–113 |

[a] Extrapolation not justified due to unreliable establishment of clearance half-life of pertechnetate from the lungs.

Study II, the scintigraphic pulmonary deposition was found to be 26.2% (24.2–28.4%), an estimate that did not significantly differ from the pharmacokinetic estimate of 26.4% (24.4–28.7%) (Table 4). Assessments of regional distribution of radioactivity within the airways resulted in mean ± S.D. PIs of 1.7 (±0.6) (Study I) and 1.4 (±0.5) (Study II) using the two-zone lung model, and 1.9 (±0.8) (Study I) and 1.5 (±0.6) (Study II) using the three-zone lung model. A coefficient of correlation of 0.98 ($P < 0.001$) between two- and three-zone based PIs was found in each of the two studies (Fig. 5).

Mean ± S.D. amount of radioactivity recovered in first and second scannings was 73% (±4.3%) and 69% (±4.5%) of the dosing disc radioactivity measured prior to administration in Study I, and 84% (±3.6%) and 77% (±4.5%) in Study II. Loss of radioactivity was partly due to rapid re-distribution of pertechnetate from defined regions of interest in body (Fig. 6). Whereas radioactivity deposited in the stomach seemed to remain unchanged between scannings, some radioactivity deposited in the lungs re-distributed to other compartments (Fig. 7). The apparent difference in the degree of re-distribution between the right and left lungs was most likely due to overlap of the upper stomach with the basal parts of the left lung.

The amount of radioactivity in the left lung and stomach could have been calculated relative to the total amount of radioactivity recovered in all regions of interest in the body and ex-body in either of two ways: on the basis of separate attenuation factors for the three regions of interest (the left lung, the stomach and the overlap between the two) or on the basis of one common attenuation factor for the unite region of interest. The latter approach would have underestimated the total amount of attenuation cor-

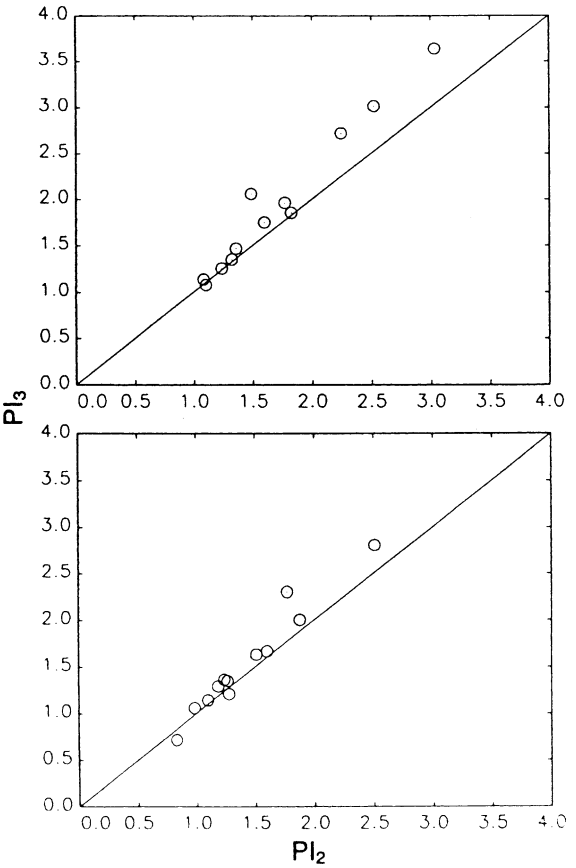


Fig. 5. Correlation between a three lung-zone based penetration index ($PI_3$) and a two lung-zone based penetration index ($PI_2$) in Study I (upper panel) and Study II (lower panel). Line of identity (solid line) is indicated. A coefficient of correlation of 0.98 was found in each of Studies I and II.

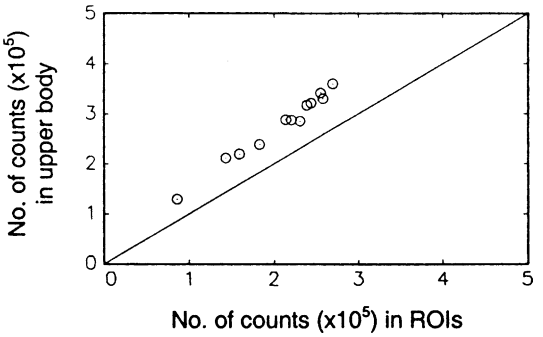


Fig. 6. Total number of counts in the entire upper part of the body vs. the total number of counts in the selected regions of interest (ROIs): the lungs, oral cavity, larynx, oesophagus/trachea and stomach in Study II. Line of identity (solid line) is indicated.

rected radioactivity in the left lung and stomach by about 12% (i.e. (32.1–36.4)/36.4) compared with the former, recommended approach (Table 5).

### 3.4. Pharmacokinetic results

At aerosol inhalation, 10–14% of the metered dose of salbutamol was retained in the inhaler (Table 6). Pulmonary deposition determined by pharmacokinetic measurements did not differ between radiolabelled and unlabelled aerosols in Study I, but a 16% difference was detected in Study II (Table 7). The relative amounts of unconjugated salbutamol excreted in the three consecutive urine fractions were similar after inhaled and intravenously injected administrations of salbutamol sulphate: out of the total amount of unconjugated salbutamol excreted over 30 h post-dose,

Table 5
Deposition of radioactivity in the left lung plus stomach calculated in either of two ways: on the basis of separate attenuation factors for the three ROIs (left lung, stomach and the overlap between the two) or on the basis of one common attenuation factor for the unite region (first scanning, Study II) (% of total radioactivity recovered in all ROIs in the body and ex-body)

| | Mean ± S.D. |
|---|---|
| Separate ROIs | |
| Left lung | 15.6 ± 7.0 |
| Stomach | 26.6 ± 10.7 |
| Overlap | 5.9 ± 7.5 |
| (Left lung + stomach) − overlap | 36.4 ± 10.1 |
| Unite ROI | 32.1 ± 8.1 |

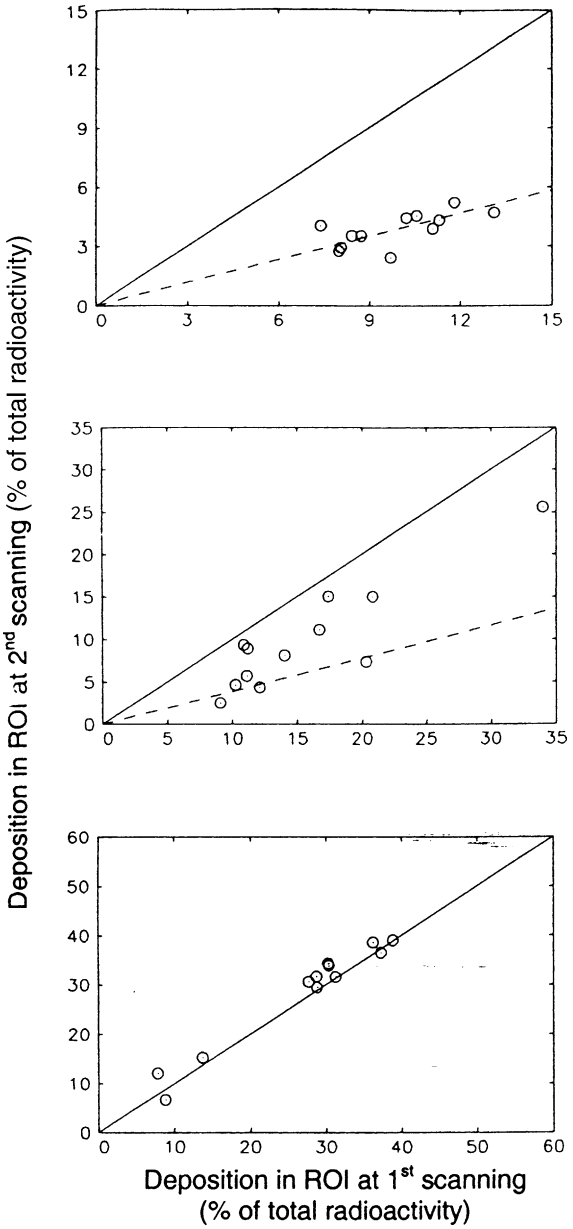


Fig. 7. Individual values of deposition (% of total amount of radioactivity recovered at first scanning) in region of interest (ROI)—the right lung (upper panel), the left lung (middle panel) and the stomach (lower panel)—measured at second vs. first scanning post-dose in Study II. Line of identity (solid line) and the regression line for the right lung deposition values (dashed line) are indicated.

Table 6
Metered dose (μg) and mean ± S.D. amounts (μg) of salbutamol retained in the dosing disc and in the white and blue parts of the inhaler

| | Metered dose (μg) | Inhaler retention (μg) | | |
|---|---|---|---|---|
| | | Dosing disc | White part | Blue part |
| Study I | | | | |
| Radiolabelled | 506.6 | 23.2 ± 6.9 | 36.3 ± 10.9 | 3.8 ± 0.5 |
| Unlabelled | 609.0 | 77.6 ± 17.9[a] | | 6.3 ± 0.9 |
| Study II | | | | |
| Radiolabelled | 718.4 | 20.0 ± 5.3 | 50.7 ± 10.3 | 5.3 ± 1.0 |
| Unlabelled | 633.0 | 65.7 ± 16.5[a] | | 6.3 ± 1.3 |

[a] Dosing disc and white part analysed together for salbutamol.

Table 7
Pulmonary deposition estimates (% of metered dose) determined using pharmacokinetic measurements, and contrasts between estimates for the radiolabelled and unlabelled dry powder aerosols

| | Study I | | Study II | |
|---|---|---|---|---|
| | Mean | 95% CI | Mean | 95% CI |
| Radiolabelled | 25.1 | 22.0–28.7 | 26.4 | 24.6–28.4 |
| Unlabelled | 25.3 | 22.2–28.9 | 22.7 | 21.1–24.4 |
| Radiolabelled/ Unlabelled | 99 | 82–120 | 116 | 105–130 |

between 68 and 76% was recovered in the initial 0–6 h fraction, between 20 and 26% in the 6–18 h fraction, and less than 7% in the last 18–30 h fraction.

## 4. Discussion

Scintigraphic quantification of pulmonary drug aerosol deposition rests on a number of assumptions. One is that the size distribution of the radiolabel closely resembles the size distribution of the pharmaceutical aerosol. Another is that the radiolabel remains associated with the drug particle for the duration of the measurement. Albeit a failure, Study I presented in this paper provides useful information in this context. Firstly, Study I showed that radiolabelling of dry particles is very sensitive to methodological detail and that, in addition to pre-study validation, quality control during a clinical study is important. Secondly, Study I showed how rapidly the radiolabel can get cleared from the lungs. Thirdly, a comparison between Studies I and II provided information about the differences to be expected from radioaerosols within and just below the acceptance range, 0.8–1.2, suggested for size distribution ratios of radioactivity to radiolabelled or unlabelled drug (Snell and Ganderton, 1999). Although there is consensus about that range, the scientific basis for it is rather weak.

The importance of using conditions relevant to the in vivo situation to measure adequately the in vitro characteristics of a dry powder radioaerosol and the corresponding unlabelled aerosol has previously been demonstrated (Bondesson et al., 2002). Thus, as a PIF of 100 l/min through Diskhaler was to be aimed at by subjects in Studies I and II, the same air flow was selected for the pre-study aerosol sizing measurements in vitro and for quality control of study inhalers. At 100 l/min, the radiolabelling technique used in Study I resulted in a size distribution ratio of radioactivity to unlabelled salbutamol of 0.75, i.e. slightly lower than the acceptance range. However, following re-evaluation and optimisation of the radiolabelling technique, a ratio closer to unity, 0.91, was obtained and the specific activity did not vary between study inhalers by more than 0.7% indicating that the radiolabelling technique used in Study II was robust and adequate for its purposes.

Pulmonary deposition was estimated at 8.5 and 15.6% of the metered dose as measured by the first scanning post-dose in Studies I and II, respectively. The numerical difference was probably attributable to improvements in radiolabelling technique and scanning protocols made for Study II. The time between aerosol inhalation and image acquisition was minimised in Study II, which—in combination with the repeated scintigraphic measurements—allowed pulmonary deposition at the time of aerosol inhalation to be estimated using mono-exponential extrapolation. The extrapolated scintigraphic estimate of 26.2%

(with 95% CI of 24.2–28.4%) did not significantly differ from the pharmacokinetic estimate of 26.4% (24.4–28.7%). In both studies, the count rate over the lungs decreased substantially from first to second scanning, corresponding to mucociliary clearance and systemic absorption of the radiolabel. Clearance of pertechnetate and other small hydrophilic solutes from the normal lung is generally considered to follow a mono-exponential course. In practice, however, strictly mono-exponential courses are not often found in biological systems and extrapolation can be expected to be less reliable late in the process. The clearance half-life of pertechnetate measured in the lungs in Study II was close to previously published values, wherefore we consider extrapolation relevant in that study. The extrapolated pulmonary deposition estimate of 26.2% deviated from previously published 12.4% for Ventolin® Diskhaler (Melchor et al., 1993), but was in agreement with what would have been predicted on the basis of in vitro data (obtained in Studies I and II) and taking the previously established relationship between fine particle dose and pharmacokinetically determined pulmonary deposition into account (Olsson et al., 1996). Thus, whenever pulmonary dry powder aerosol deposition is to be quantified using scintigraphy, re-distribution of the radiolabel from the lungs should be taken into account, as it may influence the pulmonary deposition estimate considerably. For instance, if mid-time of first scanning in Study II had been 2–3 min (which is probably relevant to most radioaerosol studies not including any administrations of activated charcoal or assessments of urinary drug excretion), measured pulmonary deposition would still have been underestimated by more than 10%.

Loss of radioactivity in scintigraphic measurements could be due to the inherent difficulties in correcting for attenuation in various tissues. It could also be due to any distribution of radioactivity into compartments located outside measured regions of interest. Thus, in order not to overestimate the pulmonary delivery from a given inhalation device, actual radioactivity recovery in the scintigraphic measurements should always be taken into account, but particularly when comparing pulmonary deposition estimates obtained in different studies. The accuracy of the effective attenuation coefficient determination has previously been demonstrated to influence quantitative organ radioactivity assessments in the thorax (Norrgren et al., 2003). With Study II, that observation (in torso phantoms) was supported by some in vivo data: quantification of left lung plus stomach deposition appeared to be quite dependent on the definition of regions of interest and their respective attenuation factors.

Transmission measurements were applied in Studies I and II due to their dual benefits of increasing the accuracy of the attenuation factors (Norrgren et al., 2003) and providing an adequate mean of outlining the lungs. There is some debate as to the assessment of lung contours in aerosol deposition studies. In healthy subjects, a transmission or a ventilation scan would be equally acceptable, but in patients with obstructive airways, a transmission scan should be used in order not to omit any poorly ventilated areas from the regions of interest.

Simultaneous measurements of aerosol deposition in the lungs, oral cavity, larynx, oesophagus/trachea and stomach would have required a gamma camera with a field of view larger than normally available in nuclear medicine practice. Thus, alternative approaches involving sequential image acquisitions of the head, thorax and upper abdomen, or continuous scanning imaging of the upper part of the body have to be considered for aerosol deposition studies. The latter approach was preferred in Studies I and II. Any increase in stomach deposition during the course of scanning, e.g. due to swallowing of aerosol originally deposited in the oral cavity, was prevented by careful mouth rinsing after aerosol inhalation and by scanning in the caudocranial direction.

Assessments of regional distribution of radioactivity within the lungs in Studies I and II showed strong correlation between the two PIs. Thus, the two-zone based PI appears to provide a measure of regional distribution of sufficient sensitivity for aerosol deposition patterns similar to the ones shown here. It could possibly be questioned whether or not deposition patterns generated instantaneously at aerosol inhalation in Studies I and II were truly reflected by the PIs. The clearance of radioactivity from various parts of the lungs might have differed, both in terms of mechanisms and rate, over the time that elapsed between aerosol inhalation and image acquisition; whereas clearance from the central lung zone may have occurred due to either or both of two mechanisms: mucociliary clearance and systemic absorption, clearance from the peripheral

lung zone was probably dominated by systemic absorption. To our knowledge, the relative influence of mucociliary clearance and systemic absorption on the overall lung clearance of hydrophilic substances, such as salbutamol sulphate, is yet to be quantified.

Pulmonary deposition of salbutamol delivered as a radiolabelled dry powder aerosol determined by pharmacokinetic assessments seemed to be consistent between studies: 25.1% (Study I) and 24.4% (Study II) of the metered dose. The somewhat larger (+16%) pulmonary deposition for the radiolabelled as compared with the unlabelled aerosol in Study II was most likely a consequence of the optimisation of the radiolabelling technique: in our hands a size distribution ratio of radioactivity to unlabelled salbutamol closer to unity was possible to achieve only by increasing the amount of labelled salbutamol on MLI stages 3 + 4 to a level exceeding that of unlabelled salbutamol. In conclusion, after due consideration had been taken to ensure appropriate radiolabelling, image acquisition and processing procedures, a scintigraphic estimate of 26.2% (with 95% CI of 24.2–28.4%) was obtained, which did not significantly differ from the pharmacokinetic estimate of 26.4% (24.4–28.7%). Pre-study validation of the radiolabelling technique, quality control of radioaerosols produced during the study, correction for re-distribution of radiolabel from the lungs, selection of regions of interest, assessment of lung contours, correction for tissue attenuation of gamma rays and establishment of the actual recovery of radioactivity in the scintigraphic measurements could potentially affect the accuracy of the scintigraphic estimate of pulmonary deposition and, thus, should be carefully considered in the design or evaluation of any such study.

## Acknowledgements

We would like to thank Birgit Andersson, Karin Frederick, Vivian Kristoffersson, Anders Luts, Anna Löfqvist, Edvard Sadlon, Magnus Simonsson, Yvonne Svensson and Sven Valind for skilful assistance.

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