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**Optimisation of inhaled corticosteroid delivery
to asthmatic children:
integration of *in vitro* and *in vivo* methods**

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DECLARATION

The research presented in this thesis was carried out under the supervision of Associate Professor Sunalene Devadason and Professor Peter Le Souëf, within the School of Paediatrics and Child Health, University of Western Australia. To the best of my knowledge, the work presented in this thesis is original, except as acknowledged in the text. Full acknowledgments have been made wherever the work of others has been used. This thesis has not been submitted in part or in whole for the award of a degree at any other University.

Research Article -Chapter 3

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Spacer inhalation technique and deposition of extrafine aerosol in asthmatic children.

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Contribution by candidate 75%

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LIST OF PUBLICATIONS

I have published two peer reviewed papers as first author, describing methodology and experimental results from this thesis. Many of the studies have been presented at scientific conferences and published as abstracts. The papers and abstracts are listed below.

PEER REVIEWED PAPERS ARISING FROM THESIS

Invited paper: **Roller CM**, Schaefer NC, Zhang G, Devadason SG. *In vitro* validation of ^{99m}Tc-HFA-FP delivered *via* pMDI-spacer. *Journal of Aerosol Medicine* 2006; 19(3): 254-260.

Roller CM, Zhang G, Troedson RG, Leach CL, Le Souëf PN and Devadason SG. Spacer inhalation technique and deposition of extrafine aerosol in asthmatic children. *Eur Respir J* 2007; 29: 299-306.

PEER REVIEWED PAPERS (not included in thesis)

Schuepp KG, Devadason SG, **Roller C**, Minocchieri S, Moeller A, Hamacher J, Wildhaber JH. Aerosol delivery of nebulised budesonide in young children with asthma. *Respiratory Medicine* 2009; 103(11): 1738-45.

Keller M, Jauernig J, Schuepp K, Stangl R, Ohl S, **Roller CM**, Devadason SG, Knoch M, Wildhaber J. Using Infant Deposition Models To Improve Inhaler System Design. *Respiratory Drug Delivery IX* 2008; 1: 221-231.

Wildhaber JH, Devadason SG, **Roller C**, Borgstrom L and Le Souëf PN. Lung deposition of budesonide from Turbuhaler in asthmatic children. *Eur J Pediatr* 1998; 157(12): 1017-22.

Devadason SG, Everard ML, **Roller C**, Summers QA, Swift P, Borgstrom L and Le Souëf PN. Lung deposition from the Turbuhaler in children with cystic fibrosis. *Eur Respir J* 1997; 10 (9): 2023-8.

ORAL PODIUM PRESENTATIONS

International Laboratory for Air Quality and Health, School of Physical and Chemical Science, Queensland University of Technology, Brisbane 2005:

Deposition of HFA-BDP inhaled *via* pMDI-spacer in asthmatic children.

Australia and New Zealand Society of Nuclear Medicine (ANZSNM) Conference,

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World Allergy Organization (WAO) Congress, Vancouver 2003: Deposition of HFA-BDP inhaled *via* pMDI-spacer in asthmatic children.

POSTER PRESENTATIONS

Roller CM, Devadason SG. Incentive for reduced variability in lung deposition of fluticasone propionate in young asthanatic children. Abstract P3952, **European Respiratory Society, 2008.**

Roller C, Schultz A, Le Souëf P and Devadason S. Comparison of ^{99m}Tc -HFA-FP deposition with Flow-Volume Simulation and Next Generation Impaction for drug delivery to asthmatic children. Poster P145, **International Society for Aerosols in Medicine, 2007.** Journal of Aerosol Medicine 2007; 20 (2): 164-209.

CM Roller, A Schultz, R. Thomas, PN Le Souëf, SG Devadason. Validation of Flow-Volume Simulation for estimation of fluticasone propionate delivery to asthmatic children. Poster P2755, **European Respiratory Society, 2006.**

Roller CM and Devadason SG. *In Vitro* Validation of ^{99m}Tc Radiolabelling Method for HFA-Fluticasone Propionate. Poster B19, **American Thoracic Society, 2005.**

Roller CM, Le Souëf PN and Devadason SG. Deposition of HFA-BDP inhaled *via* pMDI-spacer in asthmatic children. Poster 71, **International Society for Aerosols in Medicine (ISAM) 2005**. Journal of Aerosol Medicine 2005; 18(1): 83-136.

Roller CM and Devadason SG. *In vitro* validation of ^{99m}Tc -HFA-fluticasone propionate delivered *via* pMDI-spacer (Aerochamber plusTM). Poster 72, **International Society for Aerosols in Medicine (ISAM) 2005**. Journal of Aerosol Medicine 2005; 18(1): 83-136.

Schaefer NC, **Roller CM** and Devadason SG. An *in vitro* comparison of the Funhaler to commercial spacers. Poster 74, **International Society for Aerosols in Medicine (ISAM) 2005**. Journal of Aerosol Medicine 2005; 18(1): 83-136.

PAPERS IN PREPARATION (arising from thesis)

Roller CM, Zhang G and Devadason SG. Spacer inhalation technique and deposition of HFA-fluticasone propionate delivered *via* pMDI-spacer in asthmatic children: Part 1.

Roller CM, Zhang G, Schultz, A and Devadason SG. *In vitro/in vivo* comparison of inspiratory parameters, spacer device and deposition of HFA-fluticasone propionate delivered *via* pMDI-spacer in asthmatic children; gamma scintigraphy and breathing simulation in tandem with NGI: Part II.

ABSTRACT

Inhaled corticosteroids (ICS) are the treatment of choice for asthmatic children with persistent symptoms. The inter-relationships between the particle size of ICS, delivery device and inhalation profile can be used to determine the characteristics which optimise the delivery of ICS to children. Gamma scintigraphy has been widely used to assess the total body deposition and regional deposition of radiolabelled ICS from different delivery devices *in vivo*. Recorded breathing patterns can be transferred to a Flow-Volume Simulator connected to a Next Generation Impactor to measure drug output *in vitro*.

The main aims of this thesis are therefore:

- to use gamma scintigraphy to determine *in vivo* biodistribution of ICS with different particle sizes, delivered *via* pMDI-spacer, with different inhalation techniques.
- to determine the relationship between delivery device, inhalation profile and variability in lung deposition.
- to use Flow-Volume Simulation and Next Generation Impaction to predict lung deposition of inhaled corticosteroids from pre-recorded breathing patterns of asthmatic children *in vitro*.

The main hypotheses of this thesis are:

- that delivery of ICS *via* small volume pMDI-spacer can be improved by using formulations with a small particle size and a slow single maximal inhalation, followed by a ‘breath hold’.
- that variability in the delivery of ICS to young children can be reduced with a slow single maximal inhalation or with an incentive spacer device with tidal breathing.
- that inspiratory parameters of the first inhaled breath *via* small volume pMDI-spacer can be predictive of lung deposition and oropharyngeal and gastrointestinal.

- that *in vivo* lung deposition can be predicted *in vitro* from recorded breathing patterns and Flow-Volume Simulation in tandem with impaction.

High lung deposition of extrafine QVAR™ (MMAD 1.1 µm) (35-60%) was achieved in asthmatic children *via* pMDI-spacer (Aerochamber Plus™). The single maximal inhalation (SMI) technique significantly improved lung deposition of QVAR™ for children 5-7 years of age. Lung deposition of Flixotide® (HFA-FP, MMAD 2.8 µm) was markedly lower than QVAR™ (14-35%), regardless of inhalation technique.

Variability in drug delivery was decreased with a SMI followed by a ‘breath hold’, regardless of particle size. With tidal breathing, the incentive spacer device reduced variability of lung deposition of HFA-FP almost two-fold for children 5-7 years. Spacer retention of extrafine QVAR™ was less than half that of HFA-FP in the ‘breath hold’ group across all ages. Oropharyngeal and gastrointestinal deposition of QVAR™ (10-25%) was comparable to HFA-FP (17-27%) regardless of the breathing technique.

With recorded breathing patterns and simultaneous inhalation of radiolabelled FP, the % particles < 3.4 µm exiting spacer attached to FVS-NGI *in vitro* correlated significantly with % lung deposition *in vivo*. The time of inhalation of the first inhaled breath was a significant predictor of lung deposition. PIF was a significant predictor of OG deposition and volume of inhalation correlated significantly with PIF. Time of inhalation and breath type were significant predictors of spacer retention. By linking inspiratory parameters with the extrafine particle fraction (EFPF) and ultrafine particle fraction (UFPF) exiting spacer-FVS-NGI, a novel *in vitro* algorithm UF-EF was significantly predictive of lung deposition *in vivo*.

In conclusion, optimal lung deposition of ICS can be achieved in young asthmatic children with an extrafine particle size. This characteristic may be a critical consideration for optimising drug delivery to infants and children with moderate to severe asthma, who have smaller airway diameters and reduced lung function. The

slow single maximal inhalation followed by a 'breath hold' for 5-10 s can improve lung deposition of ICS from a pMDI-spacer and reduce variability. An incentive spacer device can reduce variability in drug delivery to young asthmatic children breathing tidally. With a small volume spacer, coordination of actuation with inhalation is more important with a coarser particle size. Furthermore, drug delivery of HFA-FP, measured by gamma scintigraphy can be predicted *in vitro* using pre-recorded breathing patterns and a Flow-Volume Simulator in tandem with a Next Generation Impactor.

ABBREVIATIONS

ACI	Andersen Cascade Impaction
AC+	Aerochamber Plus™
AF	attenuation factors
ARPANSA	Australian Radiation Protection and Nuclear Safety Agency
APSD	aerodynamic particle size distribution
BDP	beclomethasone dipropionate
CFC	chlorofluorocarbon
CFD	computational fluid dynamics
cps	counts per second
cps/cm²	counts per second per square centimeter
CT	computed tomography
CV%	% coefficient of variation
DPI	dry-powder inhaler
ECD	effective cut-off diameters
ED	emergency department
EFPF	extrafine particle fraction
FEV1	forced expiratory volume in one second
FH	Funhaler
FPF	fine particle fraction
FP	fluticasone propionate

FVS	Flow-Volume Simulation
GM	geometric mean
GSD	geometric standard deviation
HFA	hydrofluoroalkane-134a
ICRP	International Commission on Radiological Protection
ICS	inhaled corticosteroids
L/min	litres per minute
MBq	megabequerel
MMAD	mass median aerodynamic diameter
MRI	magnetic resonance imaging
µm	micron
mSv	millisievert
NGI	Next Generation Impaction
OG	oropharyngeal and gastrointestinal
P:C	peripheral to central ratio
PD	pharmacodynamics
PI	penetration index
PIF	peak inspiratory flow
PK	pharmacokinetics
pMDI	pressurised metered-dose inhaler
PSD	particle size distribution
ROI	region of interest

SMI	single maximal inhalation
SPECT	single photon emitted computed tomography
^{99m}Tc	technetium-99m
TBD	total body deposition
UF-EF	ultrafine-extrafine particle fractions
UFPF	ultrafine particle fraction
VHC	valved holding chamber
Yrs	age in years

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1 REVIEW OF LITERATURE

1.1 Introduction

Asthma is the most common chronic respiratory disease in children.[1, 2] Asthma is recognised as an inflammatory disease affecting the large and small airways of both adults and children.[3-5] Asthma can be associated with shortness of breath, persistent wheezing and reduced lung function. Symptoms of asthma often begin in infancy and can continue into adulthood.[6-9] Childhood asthma exacerbations present a significant burden in terms of hospitalisations and health care costs and quality of life.[10-12]

Chronic inflammation of the small airways as a result of poorly treated asthma may lead to a progression in severity of asthma with structural changes in lung tissue, remodeling, irreversible airflow limitation and the development of COPD in later life.[13, 14] Some asthma phenotypes may respond poorly to treatment and high doses of corticosteroids may be prescribed, with subsequent adverse side-effects.[15] Poorly managed, uncontrolled asthma can lead to acute symptoms of airflow obstruction that can sometimes be fatal. Optimal management of asthma symptoms may prevent the progression of childhood asthma to severe asthma with irreversible changes and reduce adverse side-effects.[16]

Early prophylactic treatment is recommended in order to maintain a child's best lung function, minimise their hospital admissions and improve their health related quality of life.[11, 17] During the 1990s, population studies have shown that the prevalence of childhood asthma had been increasing in many countries, including Australia, where more than one in four children (25-30%) children were diagnosed with asthma.[18, 19]

Over the last decade there has been a declining trend in the prevalence of wheeze in Australian children and now one in six children (14-16%) are diagnosed with asthma. However, other countries have shown an increasing trend and childhood asthma continues to represent a significant health care cost.[18, 20]

Inhaled drug delivery is the most common form of therapy for children with asthma. Optimal efficacy of inhaled medications is achieved by targeting the airways. Delivery of aerosolised medications for asthma therapy can be enhanced with specific inhaled formulations, specific devices and specific inhalation techniques. However, high doses of inhaled medications can lead to unwanted side-effects. Efficacy needs to be balanced with safety from adverse side-effects, especially in children.

High deposition of drug in the mouth and throat may be associated with local side-effects such as candidiasis and dysphonia. The absorption of swallowed drug across the gastric mucosa can contribute to systemic side-effects. Similarly high lung deposition may be associated with increased drug levels in the blood and this can contribute to systemic side-effects. Spacer devices attached to pressurized metered-dose inhalers (pMDI) are recommended for children using inhaled corticosteroids in order to reduce local and systemic side-effects and improve therapeutic efficacy.[21]

Optimising drug delivery systems is important in order to increase the efficacy of asthma therapy, decrease the cost and improve quality of life. Adherence to dosage regimens and compliance with the correct use of the drug delivery device are also essential for effective drug delivery in the paediatric age-group. Variability in dosing associated with different delivery devices is an ongoing clinical problem with children. Sub-optimal use of the delivery device could mean that the clinician increases the dosage regimen, because of the child's persistent symptoms. Increased dosages may increase the risk of both local and systemic side-effects, as well as increasing the

ongoing cost for the child's asthma therapy. Incentive devices and regular clinical review of symptoms can be used to improve the child's adherence and compliance with therapy. However, the current level of asthma control worldwide falls short of the goals for long-term management in international guidelines.[22] There are significant problems associated with effective drug delivery and the optimal management of childhood asthma, despite current 'best practice' guidelines.[17]

1.2 Definition of asthma

The currently accepted definition of asthma, as proposed in 1995 by the Global Initiative for Asthma (GINA) Committee, is:

“Asthma is a chronic inflammatory disorder of the airway in which many cells play a role, in particular mast cells, eosinophils and T-lymphocytes. In susceptible individuals this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness and cough particularly at night and/or in the early morning. These symptoms are usually associated with widespread but variable airflow limitation that is at least partly reversible either spontaneously or with treatment. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli”.[23]

1.3 Pathophysiology of paediatric asthma

Childhood asthma is associated with genetic predisposition, environmental triggers, allergen challenges, bronchial hyperresponsiveness, airway narrowing, inflammation, structural changes and adaptive changes in the developing immune system (Figure 1-1).

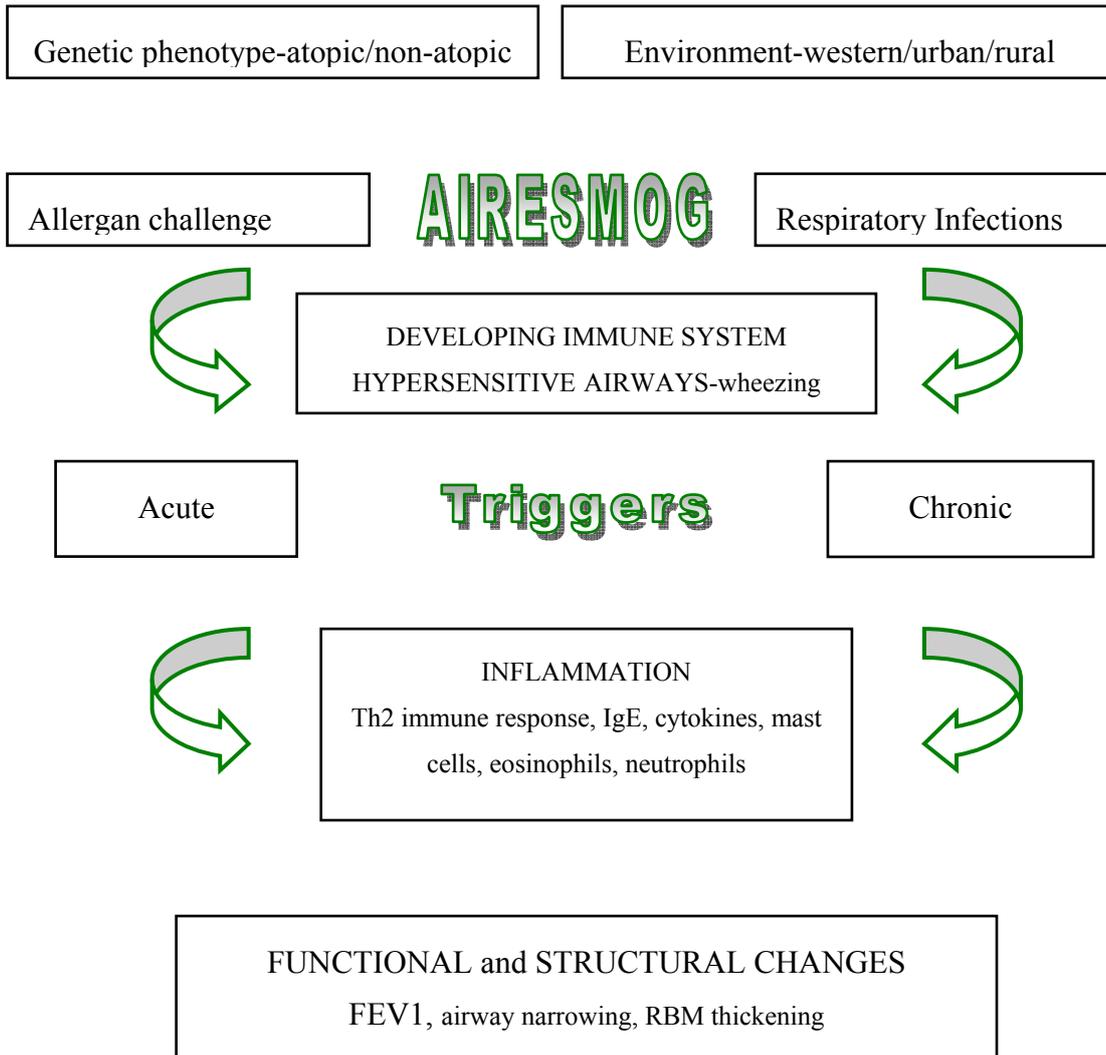


Figure 1-1: Development of functional and structural changes in asthma as described by Thorsteinsdottir* *et al* 2008.[24]

*Reproduced with permission; Mayo Clin Proceedings 2008; 83(7): 814-20. Copyright ©2008 Elsevier.

*AIRESMOG mnemonic for contributors to asthma [24]: Allergy and adherence; Infection and inflammation; Rhinitis; Exercise and error in diagnosis; Smoking and Psychogenic factors; Medications; Occupational exposures, obesity, obstructive sleep; Gastroesophageal reflux.

Longitudinal cohort studies have shown that the association between increased bronchial hyperresponsiveness and asthma are evident from the first year of life.[25-28] The persistence of childhood asthma has been found to be positively associated with specific variables including number of respiratory infections, inflammatory markers, cytokine production, activated eosinophils, mast cells, macrophages, elevated total immunoglobulin E (IgE) and T lymphocytes in the airway mucosa and lumen, which produce mediators of inflammation such as histamine, prostaglandins and leukotrienes. [6, 29, 30] [31-33]

In childhood asthma there is often an association with atopy (allergies) mediated by type 1 hypersensitivity, and asthmatic attacks may be precipitated by contact with inhaled allergens. Allergic symptoms are referred to as extrinsic asthma.

Exacerbations may also be precipitated by respiratory infections, exposure to cold, exercise, stress, inhaled irritants, and drugs such as aspirin. These symptoms are referred to as intrinsic asthma, which is seen more commonly in adults. Childhood asthma demonstrates T-helper (Th) cell type 2 mediated pulmonary inflammation in both atopic and non-atopic phenotypes.[34]

Pathological changes of the airways include edema of the airway mucosa, disruption of the airway epithelium with thickening of the reticular basement membrane, hypertrophy and hyperplasia of bronchial smooth muscle cells, goblet cell hyperplasia, mucus hypersecretion in the small airways and infiltration by inflammatory cells.[35-37]

Persistent inflammation may cause structural changes in the airways or ‘remodeling’ of the airway which may lead to reduced lung function.[38-40]

Physiological changes described in prospective, longitudinal cohort studies in childhood asthma [37], can be summarised as follows:

- Infants with transient wheeze (predominantly associated with viral colds before 3 years of age) are born with airflow obstruction and show decreased lung function at 16 years of age.[8, 9]
- Infants with persistent (usually atopic) wheeze are born with normal lung function, but by 6 years of age, have airflow obstruction, which persists into adolescence.[9] [41, 42]
- Children whose first episode of wheeze occurs after the age of 6 years, who show no evidence of airflow obstruction at 16 years of age (reversible).
- From the age of 7 years to the mid-40s, lung function in transient wheezers and atopic asthmatics follows exactly parallel tracks, with atopic asthmatics having a lower starting point.
- Lung aging, associated with increased airflow obstruction, is accelerated in children with transient wheeze.
- Chronic obstructive airways disease is associated with early life events.[37]

These pathophysiological changes indicate that it is important to manage childhood asthma symptoms appropriately from infancy. The future for the treatment of childhood asthma may lead to treatment options with a more individualized approach because future studies may further elucidate the pathophysiology of the disease, as well as individual genetic asthma susceptibilities.[24, 43]

1.4 Population studies

Childhood asthma may develop during the early years of life and symptoms may be intermittent and reversible.[44] Infants and young children often have episodic asthma related to viral infections.[45] However in some cases asthma progresses from mild intermittent to severe persistent during childhood and adolescence, with significant morbidity and with the need for long-term therapy and frequent use of healthcare resources.[44, 46]

Two large multi-centre international studies, the International Study of Asthma and Allergies in Childhood (ISAAC) and the European Community Respiratory Health Survey (ECRHS), have investigated the prevalence of asthma in both adults and children, through the use of standardized questionnaires. Both ISAAC (56 countries) and the ECRHS (22 countries) have shown worldwide variations in the prevalence of asthma (Figure 1-2).[47] [48]

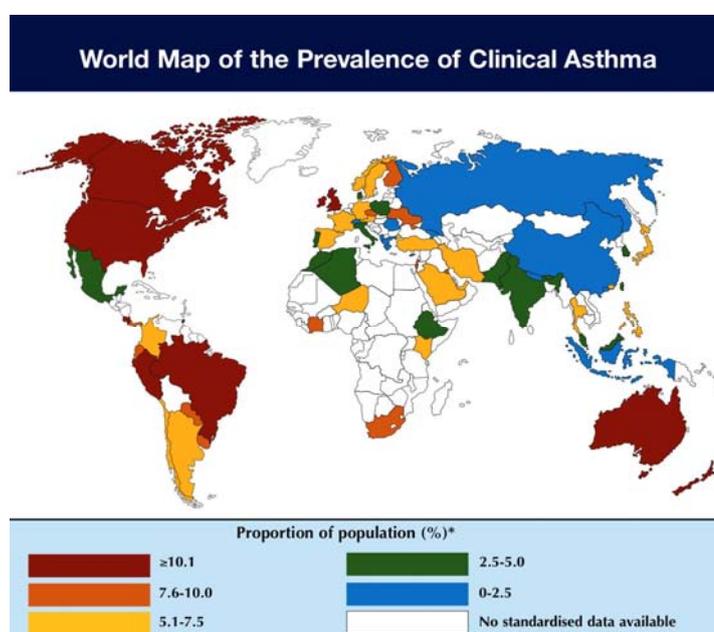


Figure 1-2: Global asthma rates throughout the world in 2004*.

*Source: From the Global Strategy for Asthma Management and Prevention 2012; used with permission from the Global Initiative for Asthma (GINA), Global Burden of Asthma Report 2004. www.ginasthma.org.

The ISAAC Phase I study (1992-1996) produced a standardised survey to compare the prevalence and severity of asthma and atopic diseases in children both within and between countries.[19] ISAAC I compared asthma and allergic disease prevalence in two age-groups (6-7 years and 13-14 years). The children 13-14 years of age (n =463,801) were studied in 155 centres (56 countries) and the children 6-7 years of age (n=257,800) were studied in 91 centres (38 countries). Marked variations in the prevalence of asthma symptoms were observed, with differences between countries up to fifteen-fold. The prevalence of wheeze ranged from 2-32% and was higher in English speaking countries and Latin America. Australia had one of the highest rates of childhood asthma with approximately one in four children being diagnosed with asthma.[49]

Environmental factors were considered to account for the major differences between global populations. ISAAC Phase II (1998-2004) was a multi-centre cross-sectional study of 840 children 9-12 years of age from six centres in the five countries of Albania, Italy, New Zealand, Sweden and the United Kingdom. ISAAC II investigated both the risk and protective factors that may contribute to the global variations observed in Phase I.[50] The occurrence, severity of symptoms and clinical management of symptoms were documented. Standardised protocols were performed to investigate flexural dermatitis, skin-prick testing, bronchial challenge with hypertonic saline, blood sampling for immunoglobulin E analyses and genotyping, as well as dust sampling for the assessment of indoor exposures to allergens and endotoxin.[50]

ISAAC Phase I was designed to allow worldwide comparisons of the prevalence of asthma symptoms. In phase III (2000-2003) the phase I survey was repeated after an interval of 5-10 years at 106 centres in 56 countries with children 13-14 years of age (n =304,679) and at 66 centres in 37 countries with children 6-7 years of age (n =193,404) in order to investigate time trends in the prevalence and severity of

symptoms of asthma and allergy.[51] [52] Although ISAAC I and II demonstrated that asthma symptoms in children tend to be more prevalent in more affluent countries, ISSAC III showed that asthma symptoms were more severe in less affluent countries.[53]

ISAAC phase III provided epidemiological evidence of the increasing prevalence of asthma and allergy in many Asian countries.[54] The increase in asthma symptom prevalence in Africa, Latin America and parts of Asia and the decrease in prevalence in Western countries shows that although the global burden of asthma is continuing to rise, the variations in global prevalence are changing.[52] This may reflect greater awareness of asthma symptoms and/or changes in the diagnosis of asthma.[18]

1.5 Asthma management

International guidelines for asthma management recommend that optimal control of asthma is the primary goal of treatment.[55] In 1993, the British National Heart, Lung, and Blood Institute collaborated with the World Health Organization and a Global Strategy for Asthma Management and Prevention, the Global Initiative for Asthma (GINA), was formed. The goal of the asthma management plan was to reduce asthma exacerbations and improve health-related quality of life. In 1999 the Childhood Asthma Management Program (CAMP) was the first randomized placebo-controlled prospective long-term clinical study, which followed 1,041 asthmatic children from 4-6 years of age.

The CAMP study demonstrated the importance of early diagnosis and ongoing treatment.[56, 57] In 2002, the GINA Report stated that “it is reasonable to expect that in most patients with asthma, control of the disease can, and should be achieved and maintained”. However, several studies have shown that childhood asthma continues to

be treated suboptimally in many countries, despite the availability of several effective therapeutic asthma drugs.[52, 58]

The World Allergy Organization recently issued the following information in its State of the World Allergy Report 2008:[59]

- 300 million people worldwide have asthma and 250,000 deaths per year worldwide are attributable to asthma and most largely preventable.[60]
- 5-15% of global pediatric population is thought to have asthma.
- Incidence of allergy and associated respiratory diseases has increased but the number of health care professionals for diagnosis and treatment has decreased.
- Asthma is underdiagnosed and undertreated, although the use of inhaled corticosteroids has had a positive impact on outcomes.
- Increasing number of hospital admissions for asthma, which are most pronounced in young children, reflects an increase in asthma severity in part because of poor disease management.

Although asthma medications are essential for the effective treatment of disease, patient education, written treatment plans and ongoing communication and review between patients and health care providers are key to effective management.[61] Many of the commonly used asthma medications are inhaled directly into the lungs as aerosols.

1.6 Airway targeting of inhaled medications

1.6.1 Inhaled therapeutic aerosols

An aerosol may be defined as any system of solid particles or liquid droplets of sufficiently small diameter to become suspended in air (about 0.001-100 μm).[62] [63, 64] [65] The deposition of inhaled therapeutic aerosol particles in the human

respiratory tract occurs by three principal mechanisms; inertial impaction in the oropharynx and large conducting airways, gravitational sedimentation and Brownian diffusion in the small conducting airways (less than 2 mm diameter) and alveoli.[63, 66, 67] [68] Gravitational sedimentation is time-dependent and enhanced with either breath holding or slow tidal breathing.[65, 69]

Aerosols have been used in a variety of diagnostic and research procedures, particularly for ventilation scanning, alveolar clearance, measurement of alveolar permeability, and for measuring the size of pulmonary air spaces.[67] The rate of removal of insoluble radioaerosols deposited in the lungs may be used as an index of mucociliary transport. The deposition of an aerosol will be influenced by the anatomy of the respiratory tract and the degree of airway obstruction; by physical and chemical properties of the aerosol or droplet, such as particle size or size distribution, density, shape, hygroscopic or hydrophobic character, chemical reactions of the particle and by physiological factors including airflow and breathing patterns.[63, 70]

1.6.2 Particle size

Inhaled aerosols are classified according to their particle size i.e. the aerodynamic diameter of the aerosolized droplets containing the drug particles. The aerodynamic diameter is defined as the diameter of the equivalent sphere of unit density which falls through air at the same terminal velocity as the particle under study. The volume of drug particles varies with the cubic radius of the equivalent sphere.[71] According to the International Commission for Radiological Protection (ICRP) model, particles in the size range from 0.01-10 μm deposit in the human respiratory tract.[72] Particle size is a key parameter in defining the deposition pattern and bioavailability of drug material delivered to the airways using inhalers.[73] [74] [75] [76] Aerosol particle size influences the extent, distribution, and site of inhaled drug deposition within the airways

and the aerodynamic diameter of a particle depends on both particle density and shape. Therefore, in order to deliver a drug to the lungs effectively, the optimal particle size for deposition into the airways should be between 1-5 μm . [77, 78] Particles between 2-5 μm in diameter have the greatest potential to be deposited throughout the lungs during inhalation, by inertial impaction and gravitational sedimentation (Figure 1-3). [79]

Particles less than 2 μm may deposit in the lower airways by sedimentation and diffusion (Brownian motion) and particles less than 1 μm may be exhaled. Studies have shown that the receptors for beta-agonists, inflammatory markers, and mast cells are distributed throughout the airways. [80] Hence airway targeting requires an even deposition of aerosol particles throughout the lungs and aerosol particle size can significantly change drug deposition within the airways and influence the efficacy of inhaled drug therapy. [81]

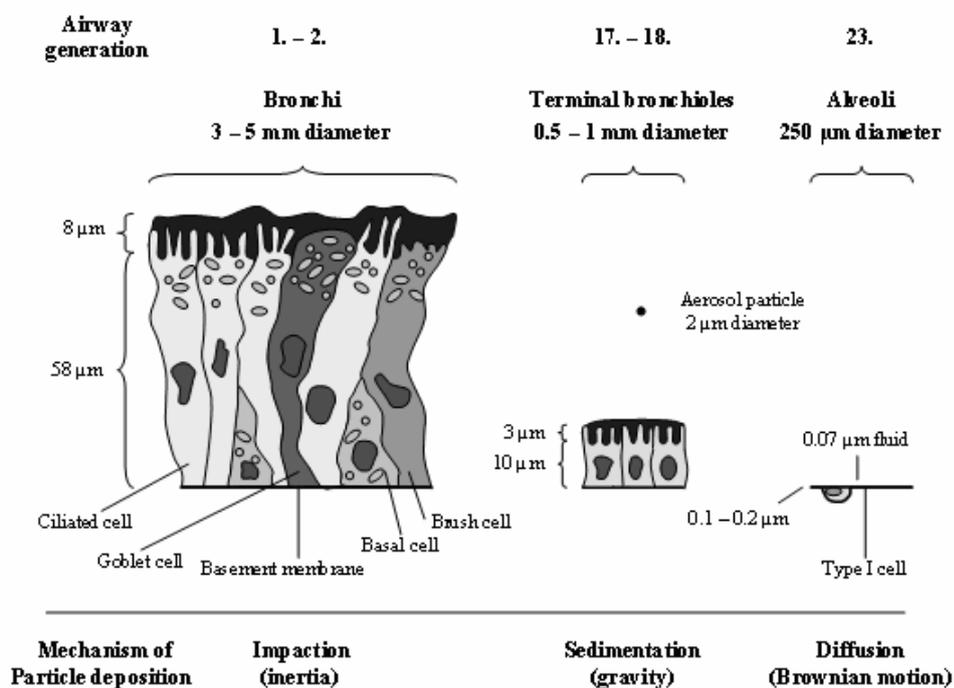


Figure 1-3: Lung epithelium and mechanisms of particle deposition at different sites within the bronchi, terminal bronchioles and alveoli*.[82]

*Reproduced with permission; Siekmeier *et al*, J Physiol Pharmacology 2008; 59(Supl 6):53-79.

1.6.3 *In vitro* particle size analysis

Impaction methods measure the size of aerosol particles in a drug formulation, based on their aerodynamic behaviour in an air-stream. Particle-sizing of inhaled drugs with cascade impactors is commonly used by pharmaceutical companies to characterise a drug formulation, by measuring both the particle size distribution and the mass distribution of drug in a specific size-fraction. In the cascade impactor larger particles with sufficient inertia are impacted on the upper stages whereas finer particles are penetrated to the lower stages of the separator.[83]

By analysing the amount of drug deposited on the various stages of the impactor, the fine particle mass (FPM) and the fine particle fraction (FPF) can be calculated. This *in vitro* technique provides a direct link between the mass of drug and the aerodynamic particle size, which is accepted as an indication of the likely deposition location within the respiratory tract (Figure 1-4).[84]

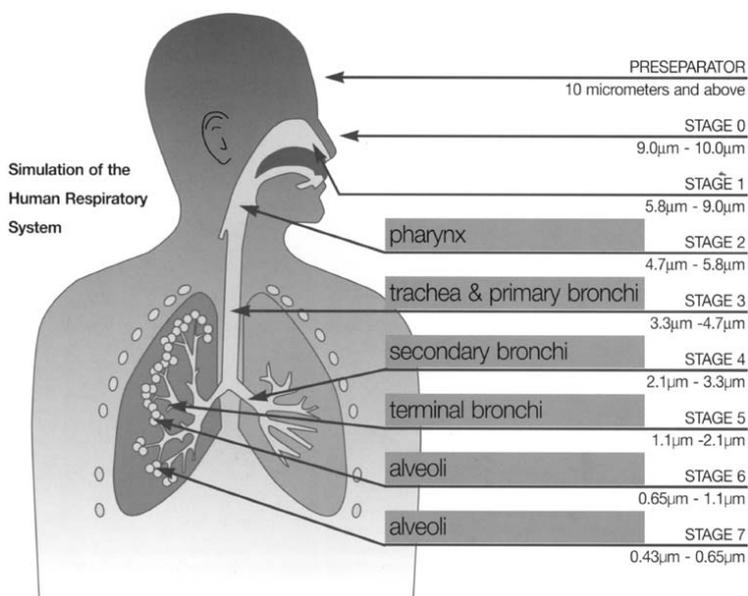


Figure 1-4: Analysis of Cascade Impactor Mass Distributions of aerosolized drug and the predicted location within the airways*, as described by Dunbar *et al.*[85]

*Reproduced with permission; J Aerosol Medicine 2005; 18(4): 439-51; Copyright© 2012 Mary Ann Liebert, Inc. publishers. All rights reserved.

Aerodynamic particle size analysis is usually carried out with a multi-stage impactor equipped with a United States Pharmacopeia/European Pharmacopeia (USP/EP) induction port. Several impactors are described in the USP/EP protocols for aerosol sampling.

1.6.3.1 Multi-stage Liquid Impinger

The multi-stage liquid impinger (MSLI) is a five stage cascade impactor which can be used for determining the aerodynamic particle size distribution of drugs delivered *via* dry-powder inhalers (DPIs) and pressurized metered-dose inhalers (pMDIs). The solvent is placed into each stage before fractionation of drug particles and the particles dissolve directly into the solvent. The MSLI is currently restricted to DPIs by USP and was not used in this thesis.

1.6.3.2 Andersen Cascade Impactor

Impactors are recommended by USP aerosol testing regulations as the reference instrument used to characterise aerosols from pMDIs. The current 'gold standard' in terms of *in vitro* testing of inhalation formulations is inertial impaction assessment using the Andersen Cascade Impactor, shown in Figure 1-5, as detailed in the European Pharmacopoeia. The Andersen Cascade Impactor has eight stages simulating the various parts of the human respiratory system.

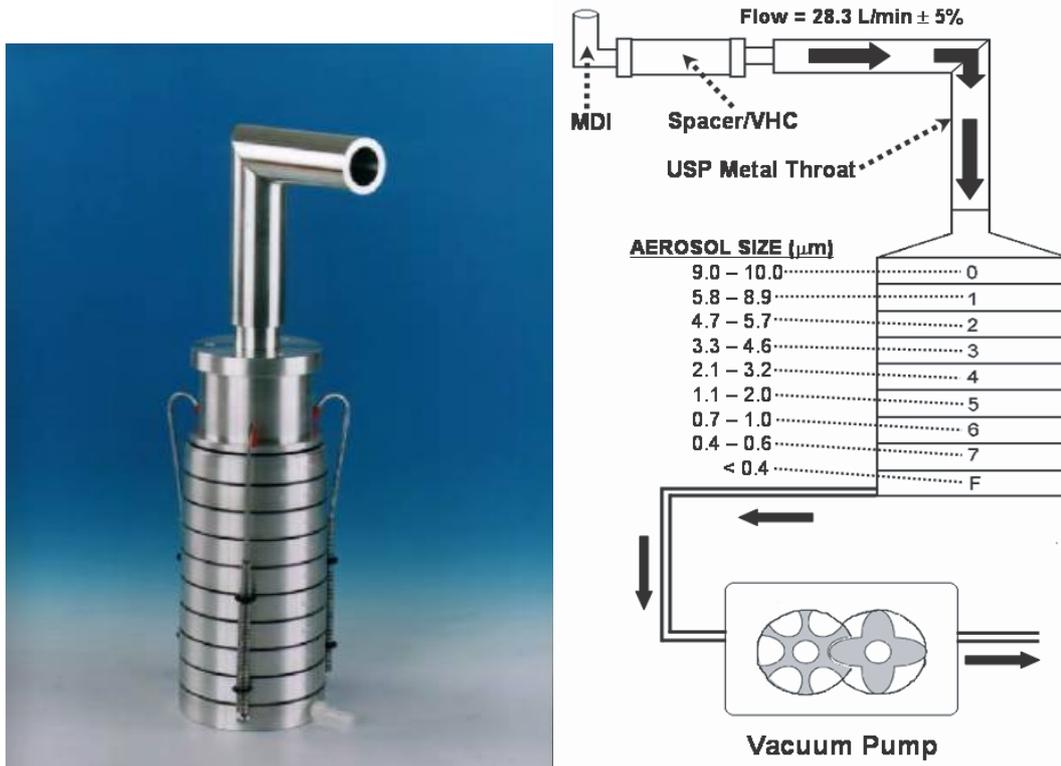


Figure 1-5: Schematic diagram of the cascade impactor with aerosol aerodynamic size range at each impactor stage 0-7 and at the final filter (F)*. MDI = metered-dose inhaler; VHC = valved holding chamber; USP = United States Pharmacopeia.

*Reproduced with permission; Asmus *et al* [456], *Pharmacotherapy* 2004; 24(2): 159-66. © 2004 Pharmacotherapy Publications Inc.; Wiley online library, John Wiley & Sons Ltd.

The aerosol stream passes through each stage at a constant airflow of 28.3 L/min.

Particles with the same inertia will impact upon a particular stage, whilst smaller particles will pass onto the next impaction stage (Figure 1-5).[86] The estimated cut-off diameters (ECD) for plates 0 to 7 are 9.0, 5.8, 4.7, 3.3, 2.1, 1.1, 0.65 and 0.43 microns respectively.

A disadvantage of impactors is that aerosol particles may undergo hygroscopic growth as a result of humidity and temperature changes in the respiratory tract, whereas *in vitro* cascade impaction is carried out under ambient conditions. Another disadvantage is that impactors are usually operated at a constant flow. Impactor airflow greater than 20 L/min are useful for estimating lung deposition for adults, however inspiratory flow can

be much lower for children and patients do not inhale at a constant flow, but at continually varying inspiratory flows.[87]

Several studies have used *in vitro* testing with ACI, using ‘throat’ models, infant models and breathing simulators, in order to investigate the effects of different airflows on drug output and fine particle fraction from different delivery devices.[88-91] Therefore in this thesis impaction will be used to assess drug delivery from pMDI-spacer using different airflows and breathing simulation.

1.6.3.3 Next Generation Impactor

The Next Generation Pharmaceutical Impactor (NGI) (MSP, St Paul, MN) has been designed to improve the assessment of the aerodynamic characteristics of inhaled drugs compared with the Andersen 8-Stage Cascade Impactor (ACI). The NGI is a high performance, precision, particle classifying cascade impactor used for testing pMDI, DPI and similar devices. The NGI has seven collection cups, instead of impaction plates and stages, plus a micro-orifice collector (MOC), as shown in Figure 1-6.



Figure 1-6: Next Generation Impactor opened with collection cups in the foreground and particle size cut-off stages in the background.

*Reproduced with permission; Dr Looi, Aerosol Research Group, School of Paediatrics and Child Health, UWA.

The NGI has been calibrated to document the stage cut-off aerodynamic diameters at 15, 30, 60 and 100 L/min, according to pharmaceutical standards. The particle sizes range from 0.23-14.1 μm . [92] The NGI collection cup cut-off size ranges from 0.98-14.1 μm aerodynamic diameter at 15 L/min flow; [93] 0.54-11.7 μm aerodynamic diameter at 30 L/min and 0.23-6.12 μm at 100 L/min. [94] The estimated cut-off diameters (ECD) for cups 0 to 7 are 6.12, 3.42, 2.18, 1.31, 0.72, 0.4 and 0.24 μm respectively. Particle reentrainment may lead to an overestimation of the fine particle fraction, particularly at high flows. [95]

1.6.3.4 Mass median aerodynamic diameter

Aerosols are said to be 'monodisperse' if each particle is approximately the same size. Therapeutic aerosols are 'heterodisperse' and may often be described by a Gaussian or normal distribution of sizes. [96] The mass distribution of particles is often used to reflect the particle size distribution. The mass distribution is characterised by the mass median aerodynamic diameter (MMAD) of the drug particle, whereby 50% mass of drug resides in particles with an aerodynamic diameter greater than the MMAD and 50% mass of drug resides in particles less than the MMAD. [85, 92, 97], [98]

The spread of particle sizes is shown by the geometric standard deviation (GSD). The GSD is a measure of dispersion of a log-normal particle size distribution and is greater than or equal to 1.0. The more uniform the particle size, the smaller the GSD. By definition, a GSD less than 1.22 is considered to represent a monodisperse aerosol. [99] Most therapeutic aerosols are polydisperse, and the larger the GSD, the smaller the proportion of particles within a specific size range. The MMAD, measured in microns (μm) and its GSD, estimated as the ratio of the 84.3% size to the 50% size in the cumulative mass distribution, is used to statistically describe the particle size

distribution of any aerosol (liquid or solid) based on the mass size of the particles
(Figure 1-7).[85]

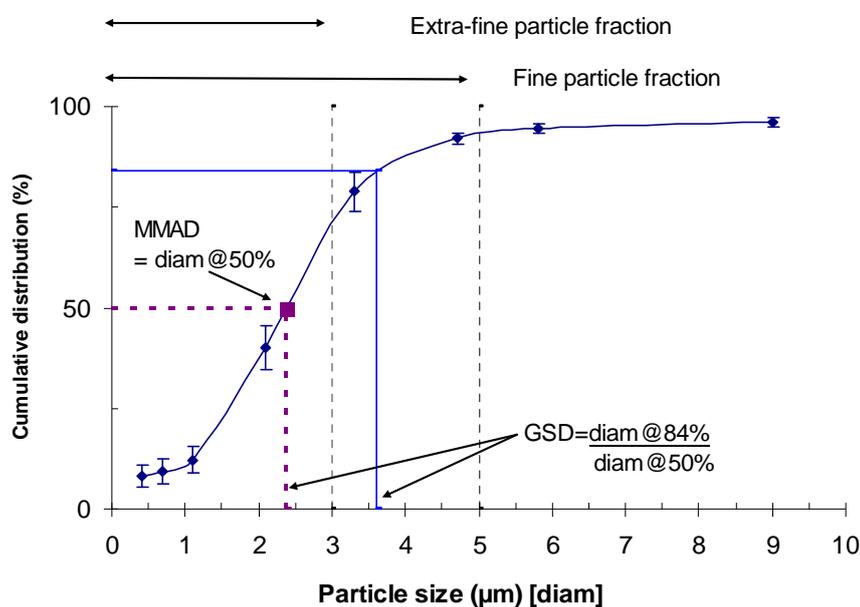


Figure 1-7: Cumulative mass distribution and the corresponding aerodynamic particle size*.

*Reproduced with permission; Dr Devadason, Aerosol Research Group, School of Paediatrics and Child Health, UWA.

1.6.3.5 Laser Diffraction

Laser diffraction techniques are widely used to measure the size distribution of aerosolized droplets, as this method is less time-consuming than cascade impaction methods. Light from a laser is shone into a cloud of particles and the scattered light can be measured by a series of photodetectors placed at different angles. The angle of diffraction increases as particle size decreases, so this method is particularly good for measuring sizes below 1 µm. Information from laser diffraction methods can be used in combination with the multi-stage liquid impinger for pre-formulation measures of drug output and particle size.

In vitro investigations with laser diffraction or cascade impaction can be used to determine the droplet size or particle size of inhaled formulations. The volume of a

particle containing drug is proportional to the cube of its radius.[71] The MMAD will be equivalent to the average volume density (Dv50) obtained from laser diffraction when spherical particles such as water droplets are measured.[100, 101] However for non-spherical particles or porous particles the Dv50 will be less than the MMAD.[102] Inertial impaction has an advantage over some light-scattering methods that assign particles or droplets to specific size bands irrespective of whether or not they contain drug substance.[103] Therefore for data consistency only cascade impaction assessments will be used for particles size analysis in this thesis.

1.6.4 Respirable fraction

The drug particles that are most likely to reach the airways are often referred to as the 'respirable' fraction. The percentage of particles that are within the 'respirable' range i.e. particles with MMAD between 0.5-5 μm are considered to be the fine particle fraction (FPF).[104] Particles between 2-5 μm in diameter have the highest probability of being deposited throughout the airways during inhalation, by impaction and sedimentation.[79] Particles less than 2 μm in diameter will be deposited in the lower airways by sedimentation, highlighting the importance of breath-holding after inhalation. Particles below 0.5 μm are thought to be exhaled, unless they come in contact with the airway lining and deposit by Brownian diffusion.[79]

The FPF provides a measure of the proportion of the inhaled mass of drug capable of reaching the airways of the lower respiratory tract and producing a beneficial therapeutic effect. The European Pharmacopeia specifies the upper size limit of 5 μm in aerodynamic diameter for FPF. The U.S. Pharmacopeia does not specify precise size limits for FPF and different range limits are chosen for the different inhalers being tested.[84]

The measure of FPF has been reported in several ways in the literature, with differences in particle sizing instruments, flow-rates and cut-off diameters.[105] Farr *et al* have suggested that the FPF should include particles $< 5.8\mu\text{m}$.[106] Chrystyn describes the fine particle dose as consisting of particles $< 5\ \mu\text{m}$.[107] Mitchell *et al* describe the FPF as consisting of particles $< 4.7\ \mu\text{m}$.[92] Finlay *et al* have suggested that particles in the range of $1.1\text{-}4.7\ \mu\text{m}$ are most likely to deposit in the lung.[108] The extrafine particle fraction (EFPF) refers to the percentage of particles $< 3.4\ \mu\text{m}$ in this thesis. These extrafine particles are more likely to be inhaled into the peripheral lung regions. Newman and Chan have suggested that particles $< 3\ \mu\text{m}$ are more likely to describe lung deposition.[109] Newhouse suggested that ultrafine particles $< 2\ \mu\text{m}$ are the ‘lung targetable fraction’ that is more relevant to describe lung deposition.[110]

Measurements of the FPF using laser diffraction and particle-sizing apparatus, such as multi-stage liquid impingers and cascade impactors, have a key role to play in the *in vitro* characterisation of inhaled aerosols and in the quality assurance and development of new aerosolised pharmaceuticals.[111] Lung deposition of ICS has been shown to increase when MMAD is decreased.[112] Systemic side-effects are thought to be associated with the fraction of drug deposited in the lung.[113] Therefore the FPF may provide information that is predictive of lung deposition, which might be helpful in estimating the clinical response in studies of the efficacy and safety of inhaled medications.[114][109]

1.7 Delivery devices

The choice of an optimal delivery device and drug formulation appropriate to the age of the child is of fundamental importance.[115, 116] British Asthma Management Guidelines state that “the most common reason for failure of inhaled drugs in children is

the inappropriate selection or incorrect use of an inhaler”.[117] Each inhaler type has advantages and disadvantages which must be considered for each patient.[118]

The delivery device influences the particle size characteristics of inhaled drugs and particle size is a key determinant of the efficacy of inhaled drug delivery.[119]

Aerosols with an MMAD less than 5 μm are recommended for optimal drug delivery to the airways of children. Advances in both drug formulation and inhalation device design have led to improvements in inhaled drug delivery. However, dose variability in inhaler therapy remains a common problem for children with asthma.

Drug delivery issues that are specific to children include age-group, use of mask versus mouth-piece, adherence to dosage regimens, compliance with the correct use of the delivery device, lower tidal volumes and inspiratory flows, determination of appropriate dosages and minimisation of adverse local and systemic side-effects.[120-122] Aerosol therapy in asthmatic children can be further complicated by recurrent and concurrent childhood infections.[123, 124]

Nebulizers, pMDIs, and DPIs can be used to as treatment options for different age-groups. New designs in jet or ultrasonic nebulizers have evolved for the efficient treatment of young children and the current generation of pMDIs and DPIs have been developed or tailored for the specific pharmaceutical being delivered, improving both treatment options and patient compliance.[125]

1.7.1 Nebulisers

Oxygen, compressed air, or ultrasonic power can be used to aerosolize drug solutions or suspensions into droplets for inhalation.[126] Nebuliser devices include conventional air-driven (jet) nebulisers, ultrasonic nebulisers, open-vent nebulisers, breath-activated open-vent nebulisers and adaptive aerosol delivery devices. Jet nebulisers play an

important role in the treatment of asthma and they are commonly used by infants and young children who are unable to use other delivery devices effectively.

Optimal use of jet nebulisers depends primarily on the choice of nebulisers with relatively small droplet size, the volume fill and compressed gas flow-rate.[127] Infants and toddlers (0-2 years) inhale the aerosolized drug using tidal breathing with a face mask. Blow-by methods (holding the mask about 2.5 cm from the child's face) have been used with uncooperative infants, however this method has been shown to reduce medication to lungs.[128-130] A mouth-piece can improve drug delivery when children are older and/or more cooperative.[131] These devices are inefficient in terms of time duration for drug delivery (10-15 minutes) and total drug output.[132] [133] Drug output from nebulisers is variable and less than 10% dose may be delivered to the airways.[134]

Drug delivery can be improved with breath-activated nebulisers (Venturi), because they deliver drug during inspiration only.[135] [136] Venturi nebulisers, such as Ventstream and Pari LC-Plus, enhance drug delivery during inhalation, reducing treatment times and drug wastage.[137] Pari LC-Plus, a breath-enhanced nebuliser, improves lung delivery compared to conventional jet nebulisers by entraining inspired air through a one-way valve into the nebuliser cup, which increases nebulizer output to the patient during inhalation.[138] Although there is less medication wasted, total treatment time can be extended with this type of nebuliser.

The goal of new generation drug-delivery devices is to optimise the effectiveness of treatments, decrease the treatment time and improve patient compliance and control symptoms.[139] Mesh nebuliser technology is more efficient, with more compact battery powered hand-held devices. Current new generation nebuliser devices use some of the following different methods to improve drug delivery:

- I-neb uses Adaptive Aerosol Delivery (AAD) technology which monitors a patient's breathing pattern throughout the treatment and aims to deliver the right amount of medication to the patient regardless of size or breathing pattern.[140]
- Aeroneb uses a high frequency, vibrating mesh nebuliser system for fast and efficient delivery of bronchodilators, inhaled corticosteroids, inhaled antibiotics, hypertonic saline and Pulmozyme®.[138]
- eFlow uses a vibrating, perforated membrane which can produce aerosols with a very high density of active drug, a precisely defined droplet size, and a high proportion of respirable droplets within minutes.[141-143]

Breath-enhanced nebulisers can measure inspiratory flow and volume over a series of breaths and then deliver more of the medication at the start of inhalation, therefore maximizing the amount of medication available to the patient.[99] These devices are more costly, but may lead to improved deposition of expensive medications in poorly ventilated lung regions, with shorter treatment times for children, resulting in improved quality of life.[144]

1.7.2 Pressurized metered-dose inhalers (pMDI)

The pressurized metered-dose inhaler (pMDI) is the most commonly used therapeutic inhaler for drug delivery to young children with asthma because of its practicality, ease of use and cost-effectiveness. The formulation in the pMDI is composed of drug, propellant and excipients, which act as suspension aids, cosolvents, and valve lubricants.[145] The drug can be present as a micronized crystalline suspension or in solution. Drug is dissolved in suspension or solution in chlorofluorocarbon (CFC) or hydrofluoroalkane (HFA) propellant under pressure. In the pMDI, the compressed propellant provides the energy needed to aerosolize the formulation and deliver the drug when actuated (Figure 1-8).

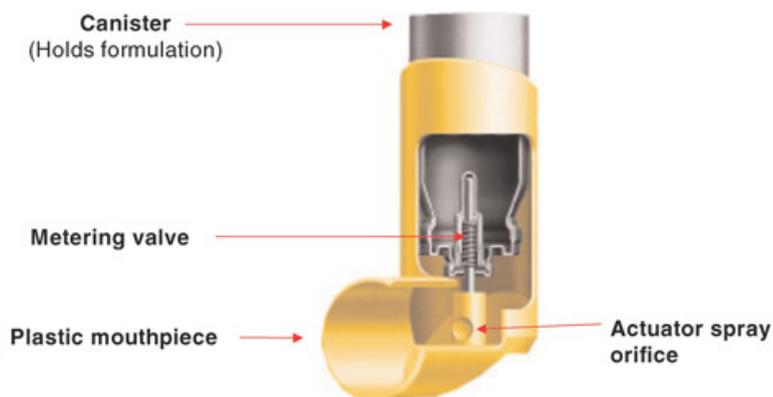


Figure 1-8: Cross-section of pMDI components with metering valve attached to actuator*.

*Reproduced with permission; Stefely J, Drug Development and Delivery; 2(6):62-69.[145] Copyright © 2012 Drug Development & Delivery. All rights reserved.

When activated, the valve system releases a metered-dose of drug and propellant.[126]

The formulation, in combination with the other components, determines the aerosol's fine particle mass, which in turn determines the respirability of the formulations.[145]

Aerosols should have MMADs less than 5 μm in order to be efficiently deposited into the upper airway and less than 3 μm for the peripheral airways. All of the components of the pMDI must work together, and a change in any of the components can result in a significant change in the product delivered to the patient.[145]

Several practical factors can affect drug delivery from pMDI, such as forgetting to shake or prime the canister before inhalation, storage of the canister in excessive hot or cold environments, rapid successive actuations, storing the canister stem down and using the canister beyond the labelled number of doses.[146, 147] Everard *et al* have shown that by storing a salbutamol pMDI inhaler stem down the total and 'respirable' dose delivered in the first actuation was reduced by 25.0% and 23.3% respectively, despite shaking the MDI before use.[147]

Deposition of metered-dose aerosols into the airways can be enhanced by using pMDIs correctly i.e. aerosol actuation is coordinated with a slow, deep inhalation, followed by

a period of breath-holding.[127] A major disadvantage of the pMDI is that many patients have poor inhaler technique and this means that there can be a lot of variability in the actual dose received. Mis-use of pressurized metered-dose inhalers is often due to poor coordination of actuation with inhalation, and can lead to decreased lung deposition of drug and decreased asthma stability.[148, 149]

Breath-actuated pMDIs such as the Autohaler™ and Easi-Breathe have been developed in order to counteract poor inhaler technique with pMDI and these devices can be used effectively by children older than 6 years of age. The advantage of these devices is that they only release drug when the child maintains a good inspiratory flow for 2-4 s. If the child is able to perform the specific inhalation technique required, the delivered dose is more consistent and therefore drug will not be wasted.[150] A disadvantage of the pMDI is that differences in the geometry of the throat exert a major influence on drug delivery variability.[151, 152] Spacer devices attached to the pMDI reduce throat deposition and are recommended for children.

1.7.3 Spacer devices

Spacers are aerosol-holding chambers into which the pMDI is fired and from which the patient inhales, usually *via* a one-way valve, so that the dose cannot be lost during exhalation.[153] All spacers used in this thesis had a one-way valve. The pMDI with an attached spacer has been recommended for infants and children under 6 years of age.[154, 155] The use of a pMDI-spacer allows young children to use pMDI more effectively, as the aerosol cloud is retained within the spacer for several seconds and the child does not have to coordinate actuation precisely with inhalation.[156]

Current recommendations suggest that children from 3-6 years of age can use the pMDI-spacer with a mouth-piece and with tidal breathing.[155] The pMDI-spacer can be used by young children with a low inspiratory flow and by children with symptoms

of acute asthma. Leversha *et al* demonstrated that the pMDI-spacer combination was a cost-effective alternative to a nebuliser in the delivery of albuterol to 30 young children from 1-4 years of age with moderate and severe acute asthma.[157]

The pMDI-spacer has been shown to be efficacious for delivering bronchodilators in children with acute asthma symptoms, in place of nebulisers.[158] The pMDI-spacer can improve the amount of drug delivered to the airways, improve clinical effect and decrease unnecessary mouth, throat and stomach deposition.[159-163] Therefore, spacers have been recommended for any inhaled asthma drug in young children, as a means of reducing local side-effects associated with oropharyngeal deposition, such as candidiasis and dysphonia, and also for reducing the systemic bioavailability of inhaled corticosteroids.[161]

The drug dose delivered to the airways from a particular device should be predictable for each drug/device combination, so that the dose can be adjusted to each patient's need. However there are problems associated with pMDIs and spacers which impact on drug delivery. Although spacer design allows for some delay, the aerosol should be inhaled very soon after the pMDI is discharged into the chamber, and only a single actuation should be discharged into the chamber for each inhalation.[146] A common mis-use of pMDI-spacer is the delivery of multiple actuations of drug at the same time and this can lead to reduced delivery efficiency.[118, 164] Spacer size and shape, electrostatic charge, volume, valve placement, and valve design all impact on drug delivery and the likelihood that the patient will use the device correctly.[146, 164]

Large volume spacers such as Volumatic® (Figure 1-9) have been recommended for delivering high doses of inhaled corticosteroids and their use may lead to a reduction in the dosage regimen.[162] However there is still considerable variability in drug delivery between different spacer devices.[165-167] *In vitro* measurements of particle

size and drug output and *in vivo* measurements have shown that reducing the level of static on the surface of plastic spacers results in an increase in drug delivery.[122, 153, 161, 168-171] This may be an important consideration for inhaled therapy with corticosteroids, although it has been shown to be of less significance for inhaled bronchodilators.[172]



Figure 1-9: Children inhaling *via* pMDI with: left, attached large volume spacer (Volumatic®) and right, small volume spacer (Aerochamber Plus™).

1.7.4 Inhalation techniques with pMDI-spacer

Meta-analyses have shown that when patients use the optimal inhalation technique recommended by the manufacturer, all inhalers can be effective, although different doses may be required.[79, 115, 126] However many patients do not use the correct inhalation technique.[173] Studies have shown that up to 40% children make inhalation errors with their pMDI-spacers.[174]

Inhalation technique depends on age and children less than 4 years of age may be too young to take a slow, deep inspiration, with their mouth sealed around a spacer mouth-piece, as shown in Figure 1-9.[175] [146] Young children may need to use a spacer with mask, breathing tidally and passively inhaling the aerosol from the spacer.[154] [175] Delivery of inhaled corticosteroids (ICS) such as fluticasone propionate and

budesonide, has been shown to depend on age, spacer device and whether the child uses a mouth-piece or a mask.[175]

Tidal breathing with pMDI-spacer is easy for younger children who cannot coordinate inspiration with actuation of the pMDI, however the inhalation technique performed by the child needs regular clinical review and the choice of inhaler should be matched to each individual child's age and ability.[58, 176]

Spacers can minimise problems associated with the coordination of inhalation with pMDI actuation,[162] however different inhalation techniques used with different spacer devices can effect drug delivery.[158, 174, 177-180] A number of studies in the literature support the use of extrafine aerosols, inhaled at low inspiratory flows to target peripheral airways, in order to improve the clinical effect.[75, 181] However a slow single maximal inhalation technique followed by a 'breath hold' has been shown to improve lung deposition due to enhanced gravitational sedimentation.[69, 182, 183] Therefore in this thesis two commonly used inhalation techniques recommended for children using pMDI-spacer, tidal breathing and a slow single maximal inhalation with 'breath hold' (5-10s), will be investigated with two formulations with different particle sizes and two different small volume spacers.

1.7.5 Dry-powder inhalers (DPI)

DPIs are breath-actuated devices and their effectiveness is related to the inspiratory flow-rate. Drug is released from a DPI only when the inspiratory flow-rate is sufficient to disaggregate the drug particles and children under 6-7 years of age tend to have problems with administration.[184] DPIs such as Turbuhaler, Diskhaler, Rotahaler and Accuhaler can deliver adequate medication to school-age children with regular instruction, although there can be considerable variability in drug delivery.[118, 120],

[126] DPIs are only recommended in Australia for older age-groups. Therefore DPIs were not investigated in this thesis, as the youngest age-group is 5-7 years.

1.7.6 Adherence and compliance

Effective treatment of asthma requires the correct dosage regimen and the correct use of the drug/device combination. Adherence and compliance refer to the agreement between ‘real-life’ practical experience and the clinician’s advice regarding the correct dosage regimen and the correct use of the delivery device. Compliance with aerosol therapy is complicated by issues of comprehension, competence, and contrivance.[185] Fink *et al* reported that approximately 30-70% of patients do not use their pMDI or DPI well enough to benefit from their prescribed medication.[164]

Education programs directed at children with asthma and their parents/care-givers, can significantly improve compliance and adherence to asthma therapy and this can lead to better control of symptoms.[186] However, adherence to ICS has been shown to be poor even among adults with asthma and is correlated with poor asthma control.[187]

Compliance with therapy can be improved if patients and care-givers understand how the devices work and what the drugs actually do.[118] Burgess *et al* have shown that compliance and adherence can be improved in the short-term when children use an age-appropriate incentive device.[188] However adherence to the novel incentive spacer device was shown to decrease within a three month period in children 2-6 years of age, even after education programs and monthly clinical review (Figure 1-10).[189]



Figure 1-10: Incentive spacer device (Funhaler*) showing ‘toy’ on expiratory arm.

*Reproduced with permission; Dr Sunalene Devadason, Aerosol Research Group, School of Paediatrics and Child Health, UWA.

Adherence to dosage regimens can have a significant influence on the efficacy of inhaled drug delivery.[120, 154, 190, 191] Patient adherence with prescribed inhaled therapy is related to morbidity and mortality and exacerbations are often related to medication non-compliance.[143, 192, 193] There are various measures of adherence, including biochemical monitoring, electronic monitors, medical/pharmacy records, counting remaining doses, clinician judgment, and patient self-report or diaries.[194] Strategies such as regular clinical review and monitoring adherence with electronic devices enables physicians to base clinical decisions on reliable and objective data.[195]

1.8 Therapeutic asthma drugs

Asthma management has evolved from treating clinical symptoms of bronchoconstriction to the management and long-term control of inflammation, using a range of asthma medications.[196] Different medications are available for treating childhood asthma, and most children can achieve good control of their asthma with regular clinical review.[24] However, not all asthmatics have the same response to

asthma medications and there are still many challenges regarding the optimal treatment of asthmatic children with different wheezing phenotypes.[197-199]

There is a wide variety of oral medications such as corticosteroids, leukotrienes and immunotherapies, however the focus of this thesis is to investigate factors which optimise the delivery of ICS.[200] International GINA treatment guidelines recommend inhaled therapy as the primary route to administer asthma medications to children with persistent symptoms.[44] Inhaled bronchodilators such as salbutamol and ICS, such as beclomethasone dipropionate (BDP) and fluticasone propionate (FP), play an important clinical role in the management of childhood asthma in Australia. The dose equivalence of inhaled extrafine BDP and FP has been reported in the Australian guidelines.[201]

1.8.1 Beta2 adrenergic agonists

Most young children have infrequent asthma episodes, which can be managed with bronchodilators. Beta2-agonists are a commonly prescribed bronchodilator. They are classified as ‘rescue’ medication and can be used for the initial relief of mild asthma symptoms, for the prevention of exercise induced bronchospasm and for acute symptoms of bronchoconstriction from the first year of life.[199] Beta2-agonists such as salbutamol, can stimulate the glucocorticoid receptor and promote its translocation to the nucleus, resulting in increased corticosteroid-mediated gene transcription.[202] However, beta2-agonists are not recommended for the long-term control of asthma.

Long-acting beta2-agonists, such as salmeterol and formoterol, may be added to one of the anti-inflammatory medications in order to improve the control of asthma symptoms and reduce the corticosteroid dose in children.[199, 203-205] [206] However interpatient variability in bronchodilation from inhaled beta2-agonists can be large and

clinically important.[207] Epidemiological evidence has suggested a link between sensitivity to beta2-agonists and increases in asthma mortality.[208]

1.8.2 Anticholinergics

Bronchodilators can be supplemented by anticholinergics such as ipratropium bromide, in more acute exacerbations.[209, 210] A systematic review of randomized controlled trials has shown that anticholinergics are beneficial in children and adolescents, particularly those with severe exacerbations of asthma.[209] Additional benefits have been shown with the addition of inhaled anticholinergic agents to beta2-agonists, such as improvements in lung function and reduction of the the risk of hospital admission by 25% for children from at least 18 months of age.[199, 209]

The additive benefits may be related to increased receptor targeting.[80] Because of its relatively slow onset of action, inhaled ipratropium is not recommended as monotherapy in the emergency department but can be added to a short-acting beta2-agonist for a greater and longer-lasting bronchodilator effect.[211] In some patients with asthma, bronchoconstriction can follow the use of ipratropium.[212]

1.8.3 Non-steroidal anti-inflammatory medications

Non-steroidal ‘preventer’ medications provide some benefit in early childhood asthma, but response is variable and depends on the severity of symptoms.[199] Several studies support the use of non-steroidal anti-inflammatory medications for either monotherapy or as add-on therapy.[213, 214]

- Cromones (eg Intal) and nedocromil (eg Tilade) are effective asthma-control medications for children with mild symptoms and there are no side-effects.[215] Cromolyn (disodium chromoglycate) has been used effectively prior to exercise

and allergen exposure and it can be administered *via* pMDI with an attached spacer device.[216]

- Leukotriene receptor antagonists (LTRA) are included in the GINA guidelines and they can be considered an alternative treatment for mild persistent asthma.[213] [217] Children with mild persistent or frequent intermittent asthma may begin with LTRAs as an alternative to low dose ICS, however their position in childhood asthma therapy is not yet fully established. [218, 219]
- Methylxanthines (theophylline) are mild to moderate bronchodilators. Data from randomized controlled trials suggest that xanthines are only suitable as first-line preventative asthma therapy in children when ICS are not available.[220] Theophylline has been used effectively for mild frequent wheezing and as an adjuvant to ICS for the prevention of nocturnal asthma.[215, 220-222]

1.8.4 Corticosteroids

Naturally-occurring glucocorticoid (cortisol or hydrocortisone) is produced in the adrenal glands and glucocorticoid steroids have potent anti-inflammatory actions. Oral systemic corticosteroids such as prednisone, prednisolone, methylprednisolone, and hydrocortisone prednisolone, are used for acute asthma exacerbations in the emergency department (ED), where they may be prescribed for 2-7 days at a dosage of 1-2 mg/kg for children.[223] A Cochrane review by Rowe *et al* concluded that treatment with corticosteroids within 1 hour of presentation to an emergency department, significantly reduced the need for hospital admission in patients with acute asthma, without significant side-effects.[223] However for severe chronic lung disease, daily doses may be prescribed for weeks or months, with dose-related side-effects and not all asthmatic inflammation responds to steroid therapy. [224] [200]

1.8.5 Inhaled corticosteroids (ICS)

ICS were approved for use in the treatment of chronic inflammatory airway diseases in the early 1970s. Current guidelines, including the GINA guidelines, recommend the use of ICS as prophylactic treatment of asthma in children with persistent asthma symptoms.[60, 199, 225-229] The Childhood Asthma Management and Prevention (CAMP) study of 1041 children from 5-12 years of age, with persistent asthma, was the first randomized placebo-controlled prospective long-term study designed to test for irreversible obstruction in childhood asthma.

The CAMP study highlighted the need for daily treatment with ICS to prevent disease progression, thus providing evidence for the focus of new asthma guidelines on improving asthma control versus altering natural history.[230-233] A key finding from the CAMP study was the absence of a continued effect of ICS on lung growth during long-term follow-up even as symptoms and airway responsiveness remained improved.[231]

Anti-inflammatory treatment is now commonly used to reduce inflammation, obstruction and hyper-responsiveness in airways. The Inhaled Steroid Treatment as Regular Therapy in Early Asthma (START) study enrolled 7241 patients from 5-66 years of age, with recent-onset, mild persistent asthma to assess the effect of early intervention with inhaled budesonide on long-term asthma control.[234] Over the five year study it was shown that patients with mild asthma had improved asthma control and had less additional asthma medication use.[234] Children with mild asthma who do not receive regular inhaled steroids may be at an increased risk for developing airway remodeling.[234, 235] Early intervention with ICS can reduce the loss in lung function (FEV1) over three years.[236, 237] However, uncontrolled asthma is still common and remodeling of the airways may be associated with long-term sub-optimal treatment.[34,

38, 45, 238] Anti-inflammatory treatment of asthmatic children with ICS can lead to a decreased production of pro-inflammatory mediators and a normal functional development of the central and intermediate airways.[38, 239] Long-acting beta2-agonists may be added to ICS to improve control of asthma symptoms and improve cost-effectiveness, however the dosage regimen needs to be monitored regularly in order to prevent long-term adverse effects.[240-242] Optimal ICS have a formulation with a small particle size, high lung deposition and increased lung residency time, as well as low systemic bioavailability and rapid systemic clearance.[243, 244]

1.8.5.1 Beclomethasone dipropionate (BDP)

The National Asthma Council of Australian Guidelines recommend BDP reformulated with hydrofluoroalkane (HFA) propellant for the treatment of children with asthma.[201] BDP is a synthetic glucocorticoid which produces anti-inflammatory activity and vasoconstrictor effects. Its chemical structural formula is seen in Figure 1-11. BDP delivered *via* pMDI or pMDI-spacer is commonly used to treat asthma in Australia.

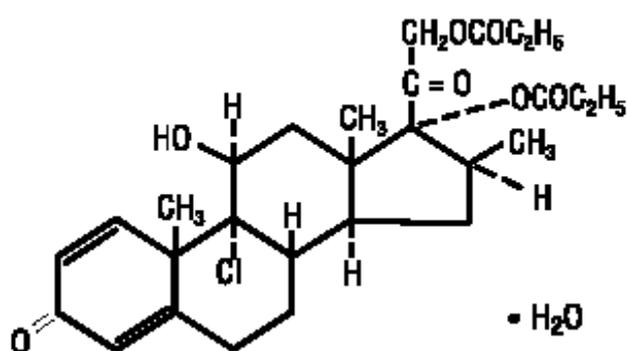


Figure 1-11: Chemical structural formula for beclomethasone dipropionate*.

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Beclomethasone 17, 21-dipropionate is a diester of beclomethasone, a synthetic halogenated corticosteroid. BDP, monohydrate is a white to creamy-white, odorless powder with a molecular weight of 539.06. It is very slightly soluble in water, very soluble in chloroform, and freely soluble in acetone and in ethanol.

The reformulation of BDP from chlorofluorocarbon (CFC) to HFA has changed its properties and the extrafine drug particles are dissolved in a solution, rather than suspension. HFA-BDP, with the trademark name, QVAR™, has an extrafine aerosol with a MMAD of approximately 1 µm. HFA-BDP has a softer, warmer spray with a lower spray force. HFA-BDP is metabolised in the lungs and liver to a number of active metabolites.

Leach *et al* have shown that, HFA-BDP, delivered *via* pMDI, is much higher than CFC-BDP, with lung deposition (% ex-actuator) of approximately 50-60% in adults (Figure 1-12).[112]

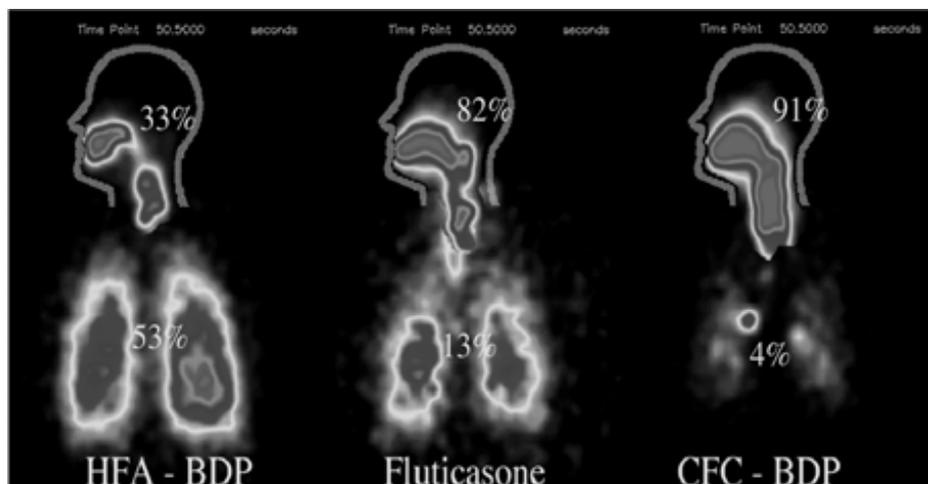


Figure 1-12: Comparison of gamma scintigraphic scans of HFA-BDP, CFC-BDP and fluticasone.

*Reproduced with permission; Dr Leach, Lovelace Respiratory Research Institute, USA.

Devadason *et al* have shown that HFA-BDP delivered *via* the breath-actuated Autohaler™, delivered 40-54% ex-actuator dose to children 5-14 years of age.[150]

As part of this thesis, I have reported high lung deposition of HFA-BDP delivered to children *via* pMDI-spacer, with less oropharyngeal deposition than Autohaler™.[245]

Extrafine HFA-BDP differs from other inhaled corticosteroids by its fine aerosol characteristics and this formulation may be particularly useful for treating peripheral airway inflammation.[246] Radiographic studies with high resolution computed tomography (HRCT) suggest that anti-inflammatory medication delivered as an extrafine aerosol produces beneficial changes in distal lung function and HFA-BDP has been shown to reduce air trapping compared with CFC-BDP.[247, 248] By delivering anti-inflammatory medication to the small airways more effectively, the new HFA-based corticosteroids have the potential to treat asthma at reduced steroid doses.[249]

1.8.5.2 Fluticasone Propionate (FP)

Flixotide®, fluticasone propionate (FP) reformulated with HFA, has been approved for use in the treatment of children with asthma in the National Asthma Council of Australian Guidelines.[201] FP is the most commonly prescribed inhaled corticosteroid for children in Australia. FP has a greater anti-inflammatory effects compared to BDP and has been shown to be effective in improving lung function in infants and children.[250] [251-253] Improvements following inhaled administration of FP can occur within 24 hours of beginning treatment, although maximum benefit may not be achieved for 1-2 weeks or longer after starting treatment.[254, 255]

FP is a synthetic, trifluorinated glucocorticoid with potent anti-inflammatory activity. *In vitro* assays using human lung cytosol preparations have established FP as a glucocorticoid receptor agonist with an affinity 18 times greater than dexamethasone, almost twice that of beclomethasone-17-monopropionate (BMP), the active metabolite of BDP, and over 3 times that of budesonide.

Fluticasone propionate is a white to off-white powder with a molecular weight of 500.6. It is practically insoluble in water, freely soluble in dimethyl sulfoxide and dimethylformamide, and slightly soluble in methanol and 95% ethanol. The chemical structural formula is seen in Figure 1-13.

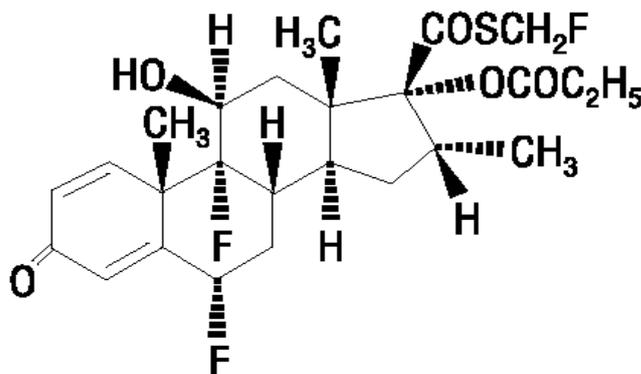


Figure 1-13: Chemical structural formula of fluticasone propionate*.

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Fluticasone propionate (FP) is more lipophilic than other corticosteroids and it has high glucocorticoid receptor affinity and specificity, high topical anti-inflammatory activity and low systemic bioavailability. The lipophilicity is proportional to the volume of distribution (Vd) and increasing the Vd also increases the elimination half-life. The Vd is 332 L for FP, compared to 183 L for budesonide.[244]

The oral systemic bioavailability of FP *via* the gastrointestinal tract is negligible because of high first pass metabolism in the liver to inactive metabolites. Therefore the systemic availability of FP depends on its absorption from the lungs.[255] A pharmacokinetic study and a deposition study have shown that fluticasone delivered *via* pMDI, has a systemic availability of 13-17% in adults.[256] Lung deposition was found to be less than 10% metered-dose in children 8-14 years of age using a Diskus DPI device.[257, 258]

Higher therapeutic efficacy of FP has been shown in a group of school-age children (8-15 years) after inhalation *via* pMDI-spacer (AIR) compared with DPI (Diskus), indicating that the spacer device enhances lung deposition of FP.[259] Delivery devices which increase lung deposition will increase systemic availability and side-effects. *In vitro* investigations have shown that the delivery of FP can be variable depending on the spacer device used, therefore lung deposition of the potent inhaled corticosteroid, FP, delivered *via* pMDI-spacer, will be investigated in this thesis in a population of children with mild asthma.[260]

1.8.5.3 Ciclesonide

Ciclesonide is a pro-drug and is converted to the active drug form in the lungs to provide potent anti-inflammatory activity. Ciclesonide has a small particle size of approximately 1 μm , similar to the particle size of HFA-BDP.[261] Ciclesonide is indicated for the prophylactic management of asthma in adults and in children over 12 years of age by the National Asthma Council of Australia.[201] Ciclesonide has been shown to offer significantly improved safety profile compared with fluticasone propionate and therefore should be ideal for use in children.[262] Ciclesonide is delivered *via* pMDI or pMDI-spacer, however ciclesonide is not currently used in Australia for younger children and will not be investigated in this thesis.

1.8.5.4 Budesonide

Budesonide has been shown to improve asthma symptoms in children.[237, 263] Nebulised budesonide can be used as an alternative to systemic corticosteroid in the treatment of acute asthma and nebulised budesonide has been shown to be safe for the treatment for asthma and wheeze in infants.[264, 265] The bioavailability of budesonide is low due to extensive first pass metabolism in the liver. Some children in the 5-7 year age-group are able to use DPI devices, however there is considerable

variability in the correct use of this device by young children, even after instruction in the correct inhalation technique.[154, 174] Budesonide is only available in Australia in a Turbuhaler DPI device and a nebulised suspension. Therefore this drug/device combination will not be investigated in this thesis.

1.8.5.5 Mometasone

Mometasone furoate has a high ratio of topical to systemic activity.[256] Inhaled mometasone furoate is delivered *via* DPI. Among the ICS, fluticasone and mometasone have the highest receptor binding affinity (1800 and 2200, respectively), followed by budesonide at 935 (relative to 100 for dexamethasone). Both des-ciclesonide and mometasone have a high protein binding fraction (98-99%).[244]

A pharmacokinetic study has shown that fluticasone and mometasone have similar pulmonary deposition (13-17%) and side-effect profile.[256] Mometasone therapy can lead to a decreased use of oral corticosteroids in patients with oral corticosteroid-dependent asthma and it has been shown to be as effective as other inhaled corticosteroids currently used in the treatment of mild to moderate persistent asthma in adults and adolescents.[266, 267] However, mometasone is not currently recommended for children in Australia, and therefore mometasone will not be investigated in this thesis.

1.8.6 Side-effects of ICS

ICS have proven superiority in improving lung function, symptom-free days, and inflammatory markers.[244, 265] A systematic review and meta-analysis has shown that early intervention with ICS over 3 years reduces loss in lung function (FEV1).[244] [265] However, there are potential side-effects from long-term use of ICS.[268, 269] [200, 270, 271] Greater mouth and throat deposition of ICS may lead to local side-

effects such as candidiasis (oral thrush) and dysphonia (throat hoarseness). Dental erosion may occur with powder forms of ICS because of their low pH and this would be undesirable in children.[268]

Hypothalamic-pituitary-adrenal (HPA)-axis suppression exists in normal individuals and asthmatic patients treated with high doses of ICS, but the extent of HPA-axis suppression is likely to be dependent on the dose, duration, and timing of corticosteroid administration.[272] Normal dosages for children are < 400 µg BDP per day or 200 µg FP per day.[228, 273] However, these dosages are frequently exceeded, and children with severe asthma are often prescribed adult doses of corticosteroids.

Systemic side-effects from ICS include bruising, dermal thinning, adrenal suppression, cataracts, glaucoma, growth suppression and altered bone metabolism, which can lead to a greater risk of osteoporosis in later life.[269, 270, 274-276] Growth retardation commonly occurs in children using high levels of glucocorticoids (>1000 µg/day) for more than 6 months.[272, 277, 278] Although the risk of side-effects is dose related, it has been shown that there is some variability in individual sensitivity to the growth effects of corticosteroids.[279]

Several longitudinal studies evaluating the effect of ICS on growth have shown a small decrement in growth velocity (approximate 1-2 cm) during the first year of treatment.[280] However, children treated with budesonide, who were followed for up to 10 years, showed no significant change in target adult height.[244] A review of randomized controlled clinical trials with prepubertal children taking low dose corticosteroids found no differences in height velocity for children given placebo, cromolyn sodium or fluticasone, 50-100 µg twice daily *via* dry-powder inhaler.[281]

The systemic bioavailability of ICSs depends on the oral bioavailable fraction and the amount absorbed directly from the lungs, thereby bypassing hepatic metabolism.[244]

[282] Fluticasone propionate (FP) has negligible oral bioavailability and the systemic exposure is related to lung deposition. Low to medium doses of beclomethasone, fluticasone, and budesonide have shown little or no effect on bone mineral density and bone metabolism and have not been shown to cause cataracts or glaucoma.[281]

Delivery devices that increase lung deposition may lead to an increased side-effect profile with FP. Several cases of adrenal insufficiency that may have led to acute adrenal crisis have been reported in children 4-10 years of age, receiving FP in doses of 500-2000 µg/day.[283] Adrenocortical suppression has been demonstrated, particularly with doses of FP (> 1000 µg/ day), due to its high potency and extended lung residence time.[277] There have been more than 50 reported cases of acute adrenal crises in children receiving ICS, but more particularly with FP, compared to beclomethasone and budesonide (Figure 1-14).[283-285]

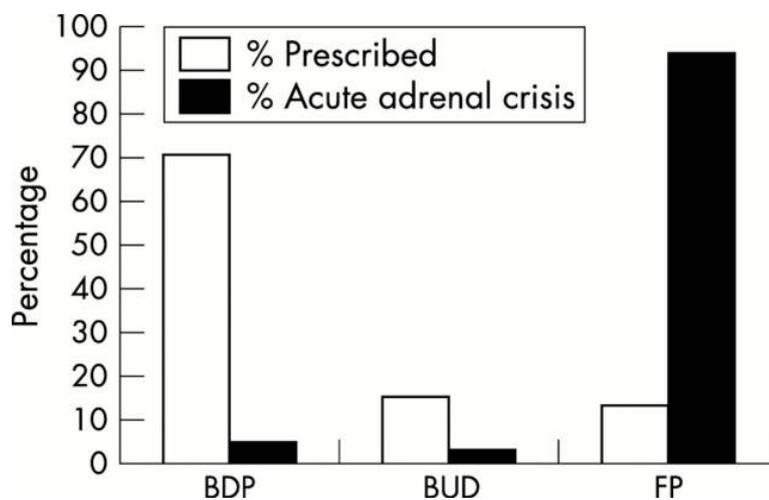


Figure 1-14: Percentage of prescriptions in the UK compared with percentage of cases of acute adrenal crisis reported in a UK survey*.

*Reproduced with permission; Todd *et al*, [284] Arch Dis Child 2002; 87: 457-461; BMJ Publishing Group Ltd., Copyright © 2013.

A two year study by Eid *et al* found that 36% of children taking FP (>176 µg/day) via pMDI-spacer had abnormally low morning cortisol concentrations.[286] Therefore

even at recommended dosages, FP may effect adrenal function in some children.[286] [287] This highlights the importance of knowing how much Flixotide® (HFA-FP) gets into the lungs, when FP is delivered *via* pMDI with different spacer devices and different inhalation techniques.

In order to minimize the risk of symptomatic adrenal suppression, GINA guidelines recommend a management approach based on symptom control, with back-titration of the ICS dose when control is achieved and education of the parent/carer of children about potential adverse effects (Table 1-1).[268] Sub-optimal use of the delivery device may lead to an increased dosage regimen, because of the child's persistent symptoms. The increased dosage regimen may increase the risk of both local and systemic effects, as well as increasing the ongoing cost for the child's asthma therapy.

Therefore it is essential to optimise the delivery of ICS to children by investigating drug delivery devices and lung deposition, in order to ensure that the lowest possible doses are used to achieve symptom control, thereby minimizing the risk of serious adverse effects. A summary of the medications available for the effective treatment of childhood asthma is shown in Table 1-1 and Table 1-2.[288]

Table 1-1: Global Strategy for Asthma Management*. Pocket Guide for Asthma Management and Prevention: A Pocket Guide for Physicians and Nurses (Revised 2010, page 14, Figure 5).

*Reproduced with permission from the Global Initiative for Asthma (GINA),[289] www.ginasthma.org.

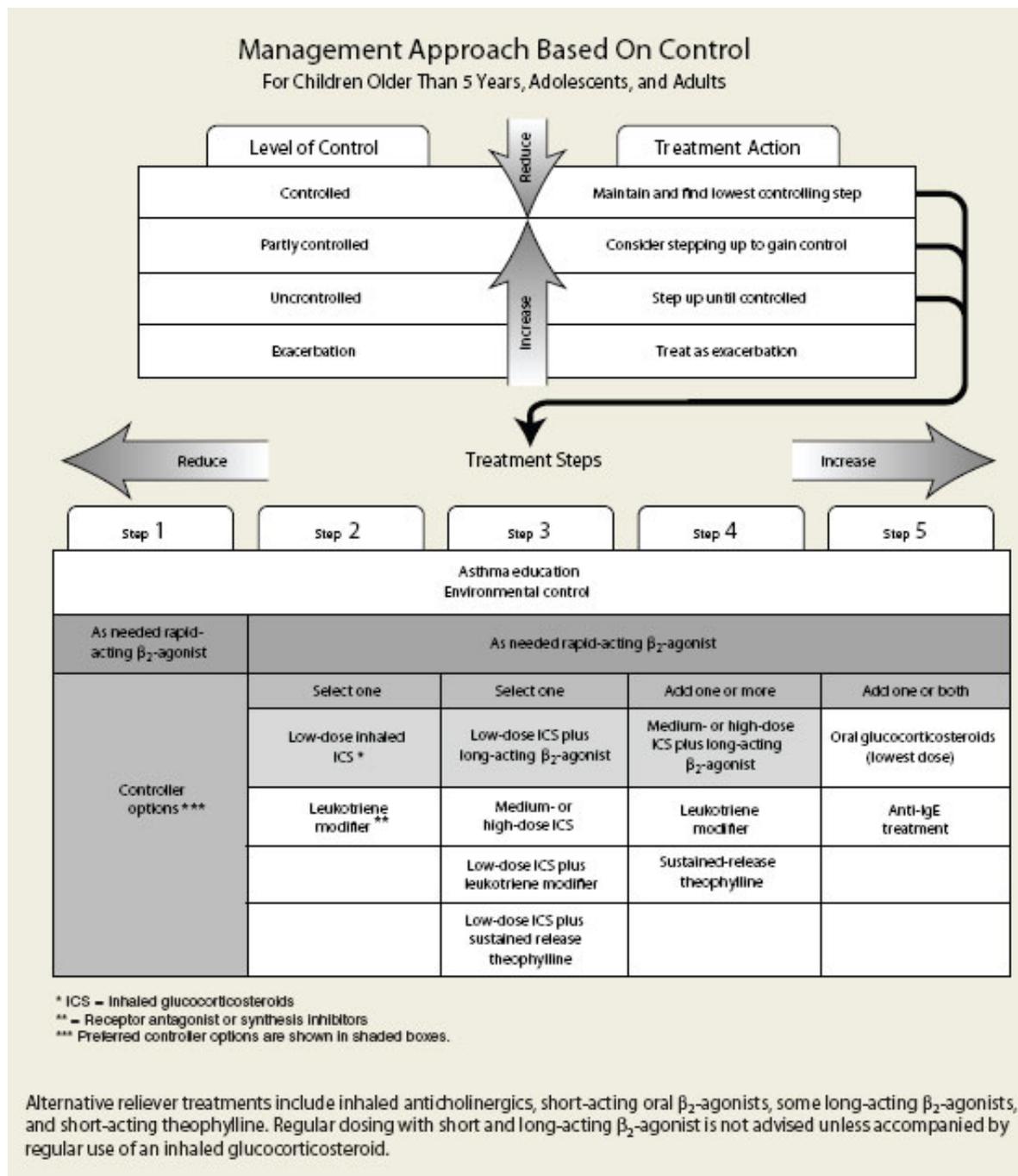


Table 1-2: Asthma medications used for prophylactic therapy with children.

Medications	When to use	Effect
Short acting β agonists (Ventolin, salbutamol), Long acting β agonists[204](Salmeterol, formoterol)	Intermittant mild symptoms; add on therapy	Bronchodilators
Cholinergics[209]Ipratropium bromide;	Mild symptoms; add on therapy with β agonists	Slow acting bronchodilator
Nedocromil[215](Tilade); Disodium chromoglycate(Intal)	Mild symptoms	Anti-inflammatory
Leukotrienes[290](Montelukast)	Mild persistent to moderate; use as add-on therapy	Anti-inflammatory Reduce airway eosinophilic inflammation Alleviate symptoms of airway obstruction
Xanthines[220] Theophylline	Mild persistent to moderate; use when ICS unavailable	Bronchodilator
Inhaled corticosteroids[291] Budesonide,Qvar™,Flixotide®,Ciclesonide,Mometasone Oral corticosteroids (Prednisolone)	Persistent mild to severe	Anti-inflammatory
Immunotherapy[292] Anti-IgE monoclonal Ab (Omalizumab)	Moderate to severe asthma children >12 years; steroid intolerant	Anti-IgE Reduce eosinophils
Biologic therapy: anti-interleukin 5[200, 293]	Severe asthma	Anti-cytokine/Th2 blockade

1.9 Assessment of *in vivo* drug delivery

1.9.1 Inspiratory filter studies

Inspiratory filter collection studies have been widely used to measure drug output from pMDI, DPI and nebulisers.[137, 294] [167] Drug is collected onto a filter positioned between the subject's mouth and the mouth-piece of the inhalation device (Figure 1-15). The subject 'inhales' the drug with the device and drug that would have been delivered to the subject, is collected onto the filter.[294] Therefore the drug level collected on the filter provides an estimate of *in vivo* drug deposition and this measure of drug output may be used to measure the efficiency of different devices and predict clinical effect.[111, 165, 175, 295] However, filter studies can only measure the total drug deposition from the device and give no information about lung deposition or oropharyngeal and gastrointestinal deposition.[296, 297]



Figure 1-15: Inspiratory filter inserted between child's mouth and Aerochamber Plus™.

1.9.2 Pharmacokinetic studies

Factors such as airway geometry, age, inhaler type, inhaler technique and drug particle size affect the deposition of inhaled medications within the airways, however drug pharmacokinetics (PK) and pharmacodynamics (PD) affect the biodistribution of

inhaled drugs. Therefore PK and PD are important predictors of the therapeutic effect. [104] [282] The development of optimal delivery devices, as well as optimal properties of inhaled drugs should be priorities for childhood asthma management.[244]

Pharmacokinetic (PK) studies measure blood levels of drug after inhalation or measure urinary levels of drug metabolites or cortisol, to show the relative lung and systemic bioavailability. Urinary metabolites of bronchodilators or urinary cortisol measures of corticosteroid effect following inhalation have been found to correlate with *in vitro* deposition data from DPIs.[298, 299] PK analyses cannot differentiate between drug that reaches the systemic circulation *via* lung absorption from drug swallowed into the stomach, unless the gastrointestinal path is blocked with charcoal.[300] However for inhaled drugs with high first pass inactivation of the swallowed fraction and negligible gastrointestinal absorption, such as fluticasone propionate (FP), lung deposition can be used as a measure of systemic exposure.

PK methods have been used to measure systemic exposure from FP and BDP in children with asthma.[73, 301] However multiple blood sampling was carried over 21 hours and 24 hour urinary cortisol excretion was measured. [73, 301] There is variability in the rate of absorption across the respiratory epithelium and it has been recommended that further refinement and validation of PK methods are required.[302] PK methods do not provide information on regional deposition, however they do generate data on systemic and pulmonary exposure, and so play an important role in the development and assessment of drug delivery systems.[300]

1.9.3 Gamma scintigraphy

Gamma scintigraphy has been widely used to assess the total body deposition and regional deposition of radiolabelled aerosols and dry-powders, delivered by different devices.[303] The radionuclide technetium-99m (^{99m}Tc), gamma ray energy 140 keV

has been used to radiolabel bronchodilators and ICS. The assessment of the drug deposition of a radiolabelled aerosol is considered to be the ‘gold standard’ for measuring pulmonary deposition.[304] Gamma scintigraphy shows ‘proof of concept’ *in vivo* for the regional deposition of inhaled drugs, and it links *in vitro* particle size analysis and device performance with *in vivo* outcomes.[305, 306] Lung deposition and gastrointestinal deposition ICS provide information on total systemic exposure and therefore can be used as a guide to safety and efficacy.

Pharmacokinetic (PK) data generally agree well with lung deposition data measured by gamma scintigraphy, especially when gastrointestinal deposition has been blocked with charcoal ingestion.[302, 307, 308] Newman *et al* compared gamma deposition of radiolabelled terbutaline sulphate in eight healthy adults to drug recovery in urine using a PK charcoal-block method. Drug was inhaled *via* pMDI at slow (27 L/min) and fast (151 L/min) inhaled flows. The two methods did not differ significantly in their estimates of whole lung deposition, however with fast inhalation, gamma scintigraphy measurements exceeded those obtained from the charcoal-block method. The regional distribution of drug within the lungs and deposition in the oropharynx could be assessed by gamma scintigraphy, but not by the charcoal-block method.[307]

Lung deposition of ^{99m}Tc radiolabelled beta2-agonists and corticosteroids have been assessed in both adults and children.[171, 302, 309-312] Inhalation studies using gamma scintigraphy can demonstrate body deposition,[111, 163, 303, 313, 314] providing there has been a rigorous validation of a match between drug and radiolabel across a range of particle sizes and corrections are made for the attenuation of gamma rays by body tissues using a transmission scan to determine individual attenuation factors.[160, 300, 307, 309, 314-316]

Gamma deposition data has been used to predict drug doses from new drug delivery devices used in subsequent clinical trials.[317] Measurements of drug delivery by gamma scintigraphy have shown that spacer devices can reduce oropharyngeal and gastrointestinal deposition and improve lung deposition in adults and children.[245] [316] Devadason and Wildhaber *et al* found that lung deposition of ^{99m}Tc-budesonide from Turbuhaler DPI was age-dependent but adequate, even to young children with low inspiratory flows.[132, 311] Lung deposition of ^{99m}Tc- HFA-BDP has been shown to be increased to approximately 50% compared to CFC formulations (10%), with a breath-actuated device.[150, 318, 319]

1.9.3.1 Planar imaging

Planar gamma camera scintigraphy is well established for measuring the deposition and clearance of radioaerosols.[320] Planar imaging refers to the two-dimensional (2D) image display of a three-dimensional gamma photon activity distribution detected by photomultiplier tubes under the collimators attached to the detectors of a gamma camera.[303] Lung deposition and regional distribution of radioactivity can be quantified using a region of interest (ROI) image analysis and the activity distribution in the delivery device, oropharynx, lungs and stomach can be assessed.

Planar imaging has a 2D display of the distribution of the radiolabelled drug, therefore there is an overlay of anatomical structures (alveoli, small and large airways), and this is most marked centrally.[302] Precise quantitative measurements with planar scintigraphy are difficult to obtain because of the scatter and attenuation of gamma photons, penetrating the sodium iodide (NaI) crystal and photomultiplier tubes beneath the collimator of the gamma camera, as illustrated in Figure 1-16.

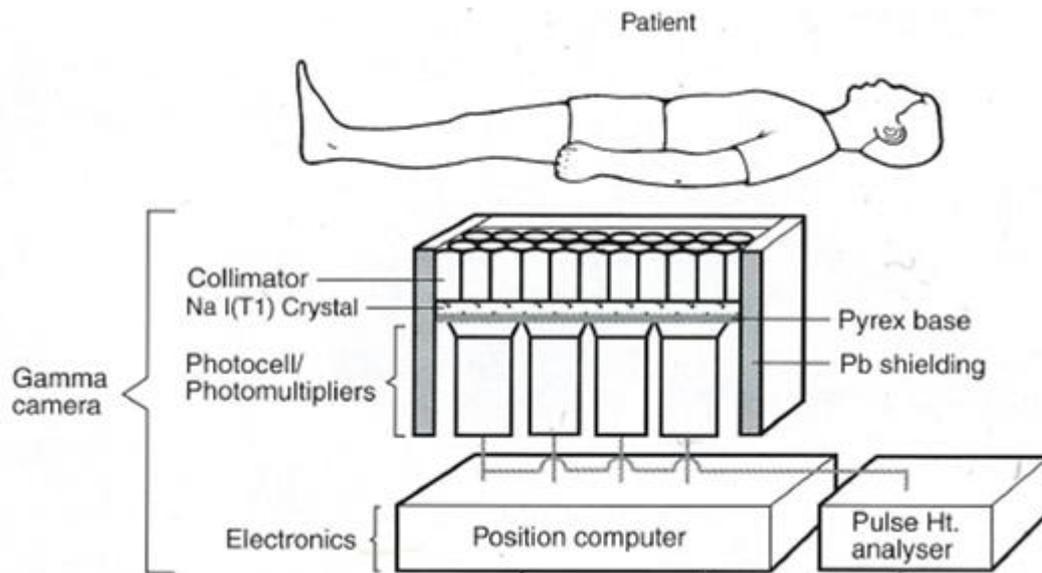


Figure 1-16: Schematic diagram of a gamma camera showing NaI crystal and photomultiplier tubes under the collimator of the detector*.

*Reproduced with permission; Nuclear Medicine: Science and Safety. Ed. A.C. Perkins 1995. John Libbey & Company Ltd., publishers: London.[321]

Imaging must be carried out as soon as possible after dosing, in order to minimize errors arising from clearance of the radiotracer out of the lungs.[303] The amount of scatter and tissue attenuation in the thorax region depends on the activity distribution and body attenuation.[322] The smaller body size of children involved in deposition studies means there is less distance between the camera detectors and the lungs and less body mass to attenuate and scatter the emitted gamma photons. However, a transmission scan of a uniform source of technetium-99m (^{99m}Tc -flood source) can be used to determine individual attenuation correction factors for a subject, Figure 1-17 and Figure 1-18.[309, 323]

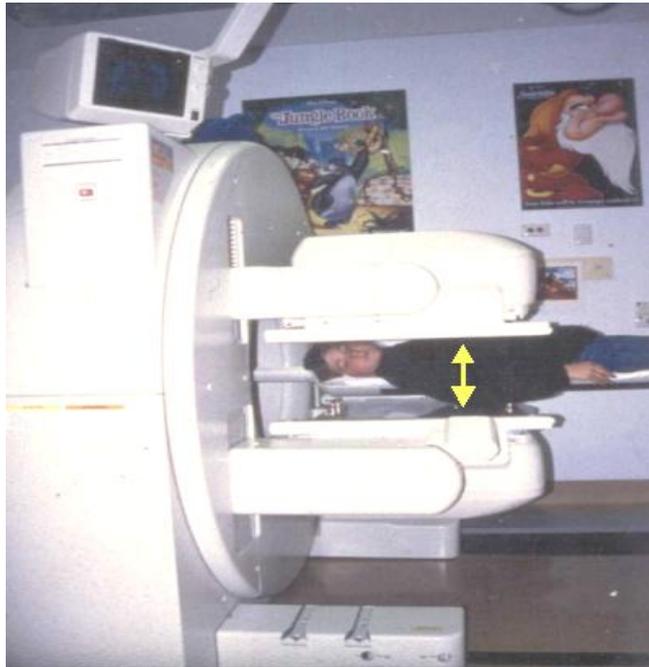


Figure 1-17: Dual detector gamma camera with uniform flood source positioned under the subject*.

*Reproduced with permission; Dr Wildhaber, Aerosol Research Group, School of Paediatrics and Child Health, UWA.

The geometric mean of the anterior and posterior images can be used to adjust for body depth. Most of the scattered photons come from the structures surrounding the radioactive source, including the camera bed.[324] The sensitivity of the gamma camera, the counts per second (cps) detected by the camera/collimator system for ^{99m}Tc activity in megabequerels (MBq), can be used to adjust for scatter error, however most planar scintigraphic studies do not use dedicated scatter correction methods.[323] The effects of scatter can be reduced by improving the energy resolution of the imaging system with a high resolution collimator. Regional distribution can be assessed by the penetration index, the ratio of mean counts per pixel in the peripheral region to the mean counts per pixel in the central region.[325]

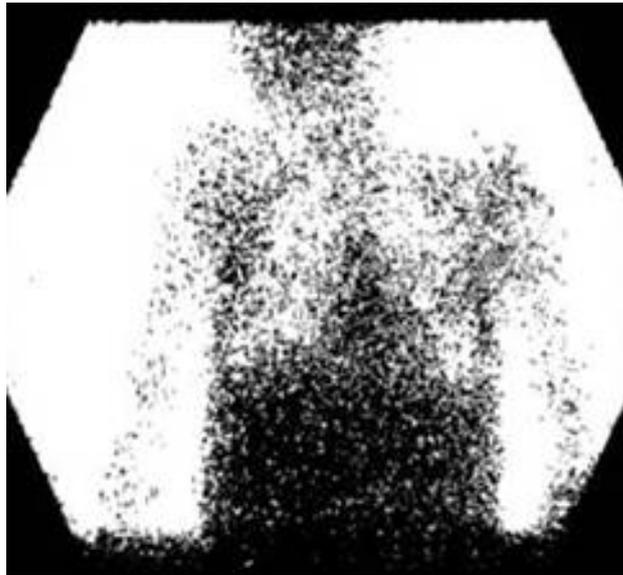


Figure 1-18: Transmission scan showing uniform flood source of technetium-99m positioned on the collimator of the gamma detector underneath the subject (white) and attenuation of gamma photons by different body tissues (black).

Reliable scintigraphic quantification of lung deposition depends on a number of assumptions. One assumption is that the particles size distribution of the radiolabel closely resembles the particle size distribution of the pharmaceutical aerosol. Another assumption is that the radiolabel remains associated with the drug particle for the duration of the measurement.[326]

Accuracy of quantitation is affected by the variation in the activity distribution and attenuation across an image of the thorax.[327] Although there is an assumption that there is some compensation for nonuniformity in the distribution in depth of activity in the lungs when the geometric mean of anterior and posterior count rate is used, the quantitative accuracy of planar imaging has been shown to be up to 10%.[328-330]

1.9.3.2 Single photon emission computed tomography (SPECT)

SPECT can be performed with multi-detector gamma cameras which rotate around a distribution of radioactivity. SPECT provides three-dimensional (3D) reconstructions of radioactivity distributions, and the 3D data is not compressed like the two-dimensional

(2D) planar images. Therefore SPECT can differentiate between large and small airways better than planar imaging, and can provide a more sensitive measure of the penetration of drug into distal airways. The penetration index (PI) can be estimated for detailed regional analysis.[111, 320]

Simultaneous transmission measurement during SPECT with an external activity source provides attenuation correction to enable absolute activity quantification and definition of lung volume.[331] Scatter correction applied prior to attenuation correction is more important for quantitative analysis with SPECT.[323] Dynamic SPECT imaging is technically and computationally more difficult and it requires higher doses of radioactivity, as well as longer imaging times in order to obtain adequate counting statistics and image resolution, therefore it is not generally used for measuring the deposition of radioaerosols in children and was not used in this thesis.[331]

1.9.3.3 Positron emission tomography (PET)

Three-dimensional PET imaging can be used to validate drug delivery to the site of action in the lung, measure the subsequent pharmacokinetics (PK) of the drug and link the regional distribution and PK of a specific drug to clinical efficacy.[332] Fluticasone propionate (FP) and triamcinolone acetonide (TAA), have been directly radiolabelled with fluorine-18(18F-FP) and carbon-11 respectively and inhalation studies with radiolabelled drugs, 18F-FP and 11C-TAA, delivered *via* pMDI have demonstrated the precise drug distribution within the lungs.[303, 332-334] PET imaging has been used to demonstrate alveolar and airway beta-adrenoceptors with carbon-11 tagged molecular markers.[335, 336]

PET imaging is increasingly being used in drug development as a means of establishing 'proof of concept'.[336] Quantitative measurements with PET have been used to assess different drug formulations and propellants and compare different delivery devices such

as spacers and nebulisers.[336] However PET requires high doses of high-energy positron emitting radioisotopes and is less well validated than 2D planar imaging and therefore PET has not been used for aerosol studies in children.[302]

1.9.4 Validation of radiolabelling method

Technetium-99m (^{99m}Tc) is not incorporated directly into the drug molecule and is thought to bind to the drug by inter-molecular forces of adhesion. The $^{99m}\text{TcO}_4^-$ ion is hydrophilic and is likely to associate with hydrophilic domains within the formulation.[337] There is a physical binding of ^{99m}Tc to the drug particles and the ^{99m}Tc matches the drug in its aerodynamic behaviour and therefore ^{99m}Tc can be used as a surrogate for drug level.

Gamma scintigraphy can only be performed after the rigorous *in vitro* validation of the radiolabelling method used for a specific formulation. The particle size distribution and mass distribution of drug delivered from the pMDI canister used for patient inhalation studies is measured both before and after radiolabelling, using cascade impaction methods, in order to validate that the delivered drug from the commercial canister is not significantly altered by the radiolabelling procedure and that the drug distribution matches the distribution of the radiolabel, ^{99m}Tc , across a range of particle sizes, as shown in Figure 1-19.

Several different validation methods have been employed, however the current standard used for quality assurance of an acceptable validation of a match between ^{99m}Tc radiolabel and commercial drug, is to measure the particle size distribution of the radiolabelled drug with the Andersen Cascade Impactor (ACI) and compare the proportion of the ^{99m}Tc radiolabel in the fine particle fraction to the proportion of commercial drug measured in the fine particle fraction of the delivered dose before radiolabelling (Table 1-3).[314]

Particle size distribution

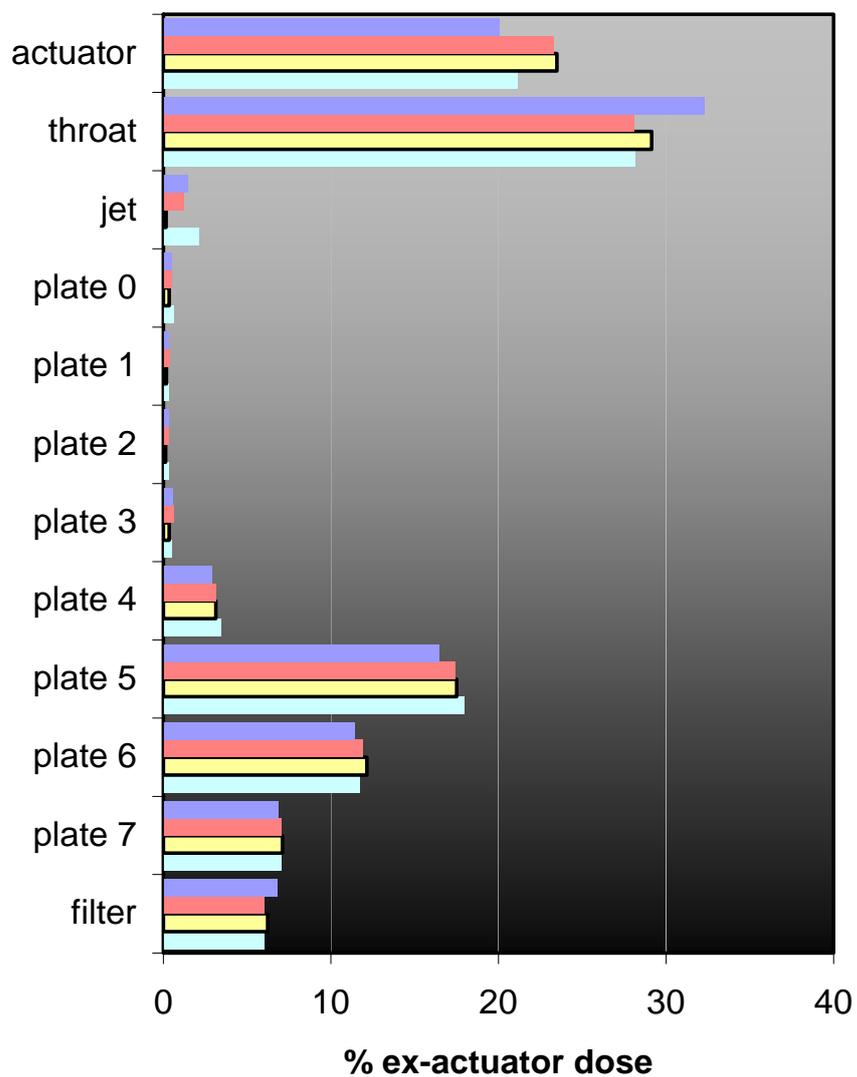


Figure 1-19: Particle size distribution showing drug (BDP) before radiolabelling (blue bar) after radiolabelling (red bar) and after decay of radiolabel (aqua bar) compared with the ^{99m}Tc radiolabel (yellow bar).

Table 1-3: Key papers showing different validation methods with Andersen Cascade Impactor (ACI) and Multi-Stage Liquid Impinger (MSLI) for deposition studies. *Fine particle fraction (FPF).

Author	Year	Device	Validation Method
Newman[183]	1989	pMDI	MSLI @ 60 L/min
Clarke[338]	1992	pMDI	ACI @ 28.3 L/min
Melchor[339]	1993	pMDI, DPI	ACI @ 28.3 L/min and 60 L/min
Pitcairn[340]	1994	DPI	MSLI 60 L/min
Borgström[341]	1994	DPI	MSLI @ 60 L/min
Newman[342]	1996	pMDI-spacer, nebuliser	MSLI @ 60 L/min
Devadason[132]	1997	DPI	MSLI @ 60 L/min
Laube[343]	1998	pMDI-large volume spacer	ACI @ 28.3 L/min; single stage liquid impinger @ 60 L/min
Wildhaber[344]	1999	Nebuliser, pMDI	MSLI @ 60 L/min
Snell[314]	1999	pMDI + DPI	ACI @ 28.3 L/min; FPF* ratio 0.8-1.2
Bondessen[345]	2002	DPI	MSLI @ 30, 40, 60, 80 L/min
Bondessen[326]	2003	DPI	MSLI @ 100 L/min, FPF* ratio 0.8-1.2
Newman[303]	2003	DPI	ACI @28.3 L/min; fast & slow flow-rates
Roller[346]	2006	pMDI-small volume spacer	ACI @ 28.3 L/min; FPF* ratio 0.8-1.2
Schuepp[347]	2010	Nebuliser	ACI @ 28.3 L/min
Laube[348]	2010	Nebuliser	No validation with infant model

1.9.5 Radiolabelled aerosols in children

1.9.5.1 Effective dose

Effective dose plays a crucial role in providing a risk-related dose that must be considered as part of the justification process in the planning of medical exposures.[349] Effective dose was developed by the International Commission on Radiological Protection (ICRP) as a radiation dose quantity linked to risk. The risk from exposure to radiation is believed to be negligible if the dose < 0.2 mSv. As part of everyday living, Australians are exposed to the naturally occurring background radiation dose of about 2 mSv each year.[350]

The National Health and Medical Research Council of Australia (NHMRC) has recommended dose constraints for children volunteers in medical research, based on the Code of Practice for the exposure of humans to ionizing radiation for research purposes (2005) set by the Australian Radiation Protection and Nuclear Safety Agency (ARPANSA).[350] This code takes into account the recommendations of the ICRP, Table 1-4. The total cumulative effective dose for children to age 18 years is recommended to be restricted to a total of 5 millisieverts (mSv).[350]

At this dose level, no harmful effects of radiation have been demonstrated as any effect is too small to measure.[351] For the studies in this thesis, the children's parents and/or guardians were given information about the research project, with the proposed 'research effective dose' compared with natural background effective dose per year and effective dose of air travel within Australia. The studies included in this thesis were carried out with children from 5-17 years of age and the dose received by each participant was equivalent to a single effective dose of approximately 0.1 mSv.

Table 1-4: Comparison of the effective radiation dose (mSv) received from a deposition study compared with the ICRP occupational limit and other common exposures.

Effective dose in millisieverts (mSv)	
Permissible occupational exposure (ICRP)	20 mSv / year
Whole body CT scan	8 mSv / year
Background radiation (Australia)	2 mSv / year
Diagnostic lung scan ^{99m} Tc-DTPA	1 mSv
Air travel 20 hours (Australia)	0.1 mSv
^{99m} Tc deposition study	0.1 mSv

1.9.5.2 Ethical Considerations

A review of radiolabelled studies by Everard, concluded that deposition studies may be justified in children when the effective dose carries a negligible risk and the information obtained from the study leads to improvements in the effectiveness or safety of the child's asthma treatment.[351, 352] Approval for the deposition studies performed in this thesis was granted by the Princess Margaret Hospital Ethics Committee, based on advice from the Radiation Safety Physicist at Royal Perth hospital, that the dose to children involved in deposition studies, carries a negligible risk. The effective dose delivered to children will be discussed in detail in the Methods section, Chapter 2 section 2.2.7.

1.9.5.3 Deposition studies in infants

Randomized controlled trials have shown that infants with asthma have improved lung function and clinical results during treatment with ICS.[265] ICS can be delivered to infants *via* nebulisers and pMDI-spacers with attached face-masks, however drug

delivery varies considerably and factors such as leakage around face-mask, lack of co-operation and crying can influence the lung dose significantly.[123, 175, 353]

A small number of radiolabelled deposition studies have been performed in infants in order to assess lung deposition, and it has been shown that the amount of drug that reaches the lungs of young children is generally less than 10%.[123, 309, 354] The choice of an optimal combination of delivery device and drug formulation is essential for adequate drug delivery to infants. Schueepp *et al* have reported that aerosols with a small MMAD and a narrow GSD can improve lung deposition in infants.[116, 120, 309, 347, 351, 355]

1.9.5.4 Deposition studies in children

Deposition studies with children have been used to demonstrate the effect of the drug delivery device, the breathing pattern and the formulation on regional deposition and lung deposition. These gamma scintigraphic studies provide valuable information for predicting dosage regimens and clinical effect for children with respiratory diseases such as asthma and cystic fibrosis. Deposition studies using ^{99m}Tc-labelled to inhaled saline, bronchodilators, corticosteroids, antibiotics and biological enzymes have been carried out.[122, 132, 150, 171, 245, 309, 311, 356, 357]

Several radiolabelled deposition studies have shown that nebulisers, DPI and pMDI show great variability in aerosol delivery. Studies with children, before 1997, have shown that between 0.5% to 15% of the total nebulised or actuated dose from a nebuliser or pMDI actually reached the lungs.[358] Deposition studies with budesonide delivered by DPI have shown that 10-30% of the dose was delivered to children.[132, 311] Deposition studies with new HFA formulations and pMDI have shown improved efficiency in aerosol delivery, with lung deposition up to 50% with HFA propellant (Figure 1-20).[150, 245]

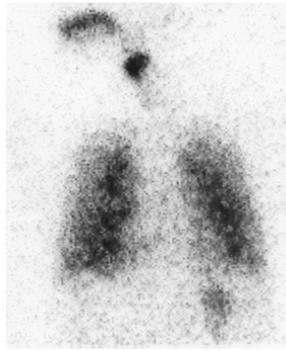


Figure 1-20: Anterior gamma scintigraphic image of subject immediately after inhalation of radiolabelled BDP, showing even distribution of the ^{99m}Tc radiolabel within the airways.

Several studies comparing the clinical efficiency of different inhalation devices have shown that the choice of an optimal inhalation device is particularly important for children.[358] Methods for determining total body deposition and regional deposition vary widely and it is difficult to compare deposition results from scintigraphic studies carried out in different centres. This may account for differences in results from different laboratories. Different validation methods may lead to errors in quantitation of gamma scans (Table 1-3). However the UWA Aerosol Research Group have carried out most of the reported deposition studies using the same methods to assess total body deposition and regional drug distribution (Table 1-5). Therefore the drug/device combinations in these studies may be compared.

Table 1-5: Literature search of paediatric deposition studies showing which studies performed validation and attenuation correction (AC) measurements.

Study	Year	Device	Formulation	Age	Disease	Validation/AC
O'DOHERTY <i>et al</i> [349]	1993	Nebuliser(Respirgard II)	99mTc-HSA	12 (8-15 years)	HIV infection	Attenuation Correction (AC)
CHUA <i>et al</i> [359]	1994	Nebuliser (Turret)	99mTc-saline	20 (8mths-18 years)	Cystic fibrosis	AC
MALLOL <i>et al</i> [360]	1996	Nebuliser: Bennet-twin jet; Hudson updraft II	99mTc-DTPA	20 (3-24months)	Cystic fibrosis	–
TAL <i>et al</i> [361]	1996	pMDI-spacer with mask	99mTc-salbutamol	15(3mths-5years)	CF, Asthma, BPD	–
DEVADASON <i>et al</i> [132]	1997	Turbuhaler DPI	99mTc-Budesonide	21(4-16years)	Cystic fibrosis	Validation / AC
ZAR <i>et al</i> [362]	1998	Spacer-Babyhaler, AC+, bottle	99mTc-DTPA	40 (3-7years)	Asthma	–
WILDHABER <i>et al</i> [311]	1998	Turbuhaler DPI	99mTc-Budesonide	23 (6-16 years)	Asthma	Validation / AC
WILDHABER <i>et al</i> [171]	2000	pMDI-spacer	99mTc-Salbutamol	18 (1-12 years)	Asthma	Validation / AC
FAUROUX <i>et al</i> [363]	2000	Nebuliser-Optineb, Optiplus	99mTc-Phytate	18 (6-21 years)	Cystic fibrosis	AC
DEVADASON <i>et al</i> [150]	2003	Autohlaer-pMDI	99mTc-HFA-BDP (QVAR)	16 (5-14 years)	Asthma	Validation / AC
BYRNE <i>et al</i> [357]	2003	Nebulisers: Halolite, Parii LC plus	Colomycin-99mTc-DTPA	15 (7-23 years)	Cystic fibrosis	–
ROCHA FILO <i>et al</i> [364]	2004	Spacer-Aerochamber, Inal Air, Flumax	99mTc-Phytate	6 (3-7 years)	Healthy volunteers	–
ROLLER <i>et al</i> [245]	2007	pMDI-spacer (AC+)	99mTc-HFA-BDP (QVAR)	24 (5-17 years)	Asthma	Validation / AC
SCHUEEPP <i>et al</i> [347]	2009	E-flow nebuliser	99mTc-Budesonide	10 (6mths – 4 years)	Asthma	Validation / AC

The delivery of ^{99m}Tc -labelled budesonide *via* DPI and nebuliser,[132] [311, 347] ^{99m}Tc -salbutamol *via* pMDI-spacer, ^{99m}Tc -QVARTM *via* pMDI-spacer and ^{99m}Tc -QVARTM *via* a breath-activated pMDI (AutohalerTM) have been assessed by the UWA Aerosol Research Group and these studies have shown the effects of age, delivery device, formulation and breathing pattern on lung deposition and oropharyngeal and gastrointestinal deposition.[150, 171, 245] Therefore in this thesis results from previous deposition studies performed by the UWA Aerosol Research Group, such as delivery of QVARTM by the AutohalerTM device and delivery of salbutamol (MMAD 2.9 μm) by pMDI-spacer, could be directly compared to delivery of QVARTM by pMDI-spacer and delivery of Flixotide® (MMAD 2.8 μm) by pMDI-spacer.

In vivo methods of assessing drug delivery to children use either pharmacokinetic studies with multiple blood sampling and urine collection or gamma deposition studies using ionising radiation. These studies are time-consuming and require the cooperation of children and parents as well as specialised equipment. Study numbers are generally small due to the problems associated with recruiting children for these research protocols. Therefore many studies have focused on the development of *in vitro* methods to predict drug deposition.

1.10 Comparison of *in vitro* and *in vivo* drug deposition

Aerosols are often characterised by *in vitro* bench-testing using constant flow cascade impactors to measure the aerodynamic particle size distributions (APSD). *In vitro* measures of the fine particle fraction (FPF) of inhaled drugs using particle-sizing equipment (e.g. the twin impinger, multi-stage liquid impingers, cascade impactors) are useful for the characterisation of new pharmaceutical aerosols, for comparison of new devices and for quality control. The FPF is expected to have some predictive power for lung deposition, and therefore for clinical effects.[109] However, bench-testing with cascade impactors has several limitations.[114, 365]

The clinical use of inhalers is not performed at constant flow and the site of deposition of an aerosol within the lung, as well as the amount of dose delivered to the lung, is known to be influenced by the breathing pattern.[75, 109, 366-369] *In vitro* methods do not predict the variability of lung deposition *in vivo* because particle-sizing equipment does not adequately reflect airway anatomy.[111] An optimal three-dimensional anatomical model of lung zones of interest needs to be developed.[314] Several respiratory tract models have been used to predict particle deposition *in vitro*. [370] [371]

1.10.1 Respiratory tract geometry models

The earliest lung morphometry model widely used was proposed by Weibel (1963). According to Weibel's symmetric model, airways branch 23 times, starting from trachea (generation 0), ending with alveolar sacs (generation 23).[372] In 1966, the ICRP Task Group on Lung Dynamics published a lung model for estimating dust deposition in and clearance from the respiratory tract. In 1994 the ICRP published an aerosol dosimetry model in publication 66.[373] The purpose of the ICRP model was to calculate

radiation doses to respiratory tract of workers, resulting from the intake of airborne radionuclides. The ICRP dosimetry model is the most widely referenced whole-lung model, however there are uncertainties in aerosol deposition and clearance mechanisms.[374]

A physiologically realistic model of the lung was developed by Martonen *et al* from planar gamma camera images and SPECT images.[375] This model was used to simulate the effects of aerosol polydispersity, hygroscopicity, patient ventilation, patient lung morphology, patient age, and patient airway disease.[376] Gravitational deposition of monodisperse particles was used by Brand *et al* and Lehnigk *et al* to determine effective airway diameters using aerosol-derived airway morphometry (ADAM) modelling.[377, 378]

Whole-lung models have been designed to provide deposition prediction for the whole lung, from the oronasal cavities to the pulmonary region and many of these models use simple lung geometry and one-dimensional transport equations to estimate total lung and regional airways deposition of inhaled aerosols.[372, 379] The whole-lung models provide both the whole-lung and the regional (lobar, tracheobronchial, pulmonary, alveolar) particle deposition fractions. Optimal parameters for targeting predetermined lung areas can be estimated, given representative pulmonary airways.[77, 380]

1.10.2 Mathematical modeling

Mathematical modeling of aerosol deposition in the human lung has been based on idealized assumptions regarding the morphometry of the lung, the fluid dynamics behavior of the inspired air under defined breathing conditions, the transport of particles through the branching airway system, the physical mechanisms acting upon inhaled particles, and the deposition of particles within airways, airway bifurcations, and alveoli.[381]

Theoretical models of inhaled particle deposition make use of approximations of the respiratory tract to predict deposition according to fundamental physical processes of impaction, sedimentation, and diffusion. Total deposition and regional (nasopharyngeal, tracheobronchial, and pulmonary) deposition with these models have been compared with experimental studies of inhaled dusts in humans or experimental animals.[63, 382]

A dosimetric model for adults was developed by Martonen *et al* to improve the efficacy of aerosol therapy by using selective deposition of inhaled pharmaceuticals at prescribed lung locations.[383, 384] Martonen's model described the behavior and fate of particles in the lungs under different breathing conditions.[383] A mathematical model of inhaled aerosol particle deposition for children was validated by Isaacs and Martonen, with data from two published experimental studies.[385] This model accurately predicted deposition fraction in children as a function of particle size for particles in the size range 1-3 μm for rest and exercise breathing conditions. The model has been used to predict age-dependent trends in deposition at the different particle sizes.[385] Robinson *et al* used a Eulerian model, with symmetric morphology and even ventilation, to predict aerosol deposition using pulmonary function data and different breathing parameters.[386]

Several studies have linked *in vitro* analysis of drug delivery with computational fluid dynamics (CFD).[108, 387, 388] Chan *et al* used CPD to investigate the effects of inhaler design (such as mouth-piece, grid structure, air inlet) on powder dispersal in DPIs.[388] CFD has also been used to study the effects of airway geometry, flow-rates, particle sizes, and different outlet conditions with pMDIs.[372, 389-392]

Isaacs *et al* used CFD to predict deposition patterns for high and low flow-rates for particles 8 μm in diameter.[390] Longest *et al* used a combination of CFD modeling

and *in vitro* experiments to characterize the transport and deposition of an aerosol emitted from the Respimat inhaler.[391] More recently Longest *et al* have investigated CFD model to look at the effects of inhaler design variables.[387]

Current CFD models of particle deposition can be used to predict particle deposition in the whole lung or within single airway bifurcations.[380, 381] However, CFD-based models are often limited to upper respiratory tracts and there can be numerical errors and artifacts that can lead to nonphysical CFD results.[389] De Matas *et al* evaluated correlations between *in vitro* data and *in vivo* data using artificial neural networks. [393] This model linked the *in vitro* aerodynamic characteristics of the emitted dose and body surface area with the urinary excretion of drug and its metabolite in the 24 hour period after inhalation. A predictive model correlating *in vitro* data, baseline lung function, body surface area and age with post-treatment improvements in lung function (FEV1) was also generated.[393] One of the difficulties with modeling aerosol deposition in the airways is related to the complexity of the airways geometry and the limited morphometric data available. Another difficulty is the simulation of the realistic physiological conditions of lung environment.[371, 372]

1.10.3 Modifications to *in vitro* methods

Particle-sizing measures of fine particle fraction (FPF) have shown that the measured fine particle dose is highly dependent on the geometry of the inlet to the impactor.[394] Studies in children using the cast of a human throat inlet, in place of a USP ‘throat’ attached to an impactor, have shown that lung deposition of budesonide, delivered *via* Turbuhaler, can be comparable to the *in vitro* FPF.[392, 394] Laboratory assessment of fine particle dose has been shown to correlate with lung deposition if the assessments are performed with a more *in vivo*-like set-up.[302, 395]

Modifications to particle-sizing methods have been used so that the *in vitro* measures are more consistent with *in vivo* measures.[392] The USP throat has been shown to underrepresent the oropharyngeal deposition of aerosols.[392] Studies using computed tomographic (CT) images and magnetic resonance imaging (MRI) models have been performed to explain the inter- and intra- subject variation in oropharyngeal deposition.[392] [396] Chan *et al* demonstrated a good correlation between fine particle dose from a DPI measured with *in vivo* lung deposition, when an anatomically correct throat inlet was used for the *in vitro* analysis.[392] [109]

Studies using anatomic models can provide a valuable complement to *in vivo* studies, however a constant inspiratory airflow is often used, while the airflow varies in both rate and direction during the breathing cycle.[397] The influence of breathing patterns on lung deposition and particle size distribution have been investigated using breathing simulators in combination with impactor inlets attached to CT generated upper airway models in order to study drug delivery *via* pMDI-spacer, nebuliser in infants and DPI in adults.[109, 142, 398] A database of lung deposition values based on breathing parameters, inhaler design, and formulation properties would be a useful aid for *in vitro* comparisons of drug delivery.[392] [398]

1.10.4 Breathing simulation

Describing a clear relationship between *in vitro* and *in vivo* measures of inhaled drug deposition is an important goal because it allows *in vitro* data to be used to predict the clinical effect of different drug/device combinations.[109] Newman and Chan compared *in vitro* assessment of the FPF with different delivery devices with lung deposition obtained from gamma scintigraphy.[109] Their study found significant correlations between *in vivo* lung deposition and *in vitro* FPF, however the FPF overestimated lung deposition.[109] Individual variability in breathing patterns in both

adults and children (tidal volume, inhalation volume, peak inspiratory flow and respiratory frequency) contributes to the variability in the emitted FPF and lung deposition of inhaled particles.[399] [400]

The match between *in vitro* and *in vivo* data may be improved by measuring the particle size distributions in ways that reflect clinical use. Cascade impaction cannot simulate the respiratory tract, since the impactor operates at a constant flow, while the respiratory cycle has a variable flow-time profile.[114] Lung deposition may correlate with the FPF across a range of inhaler devices, however lung deposition is strongly influenced by the patient's breathing pattern.[89] [109, 401, 402]

Breathing patterns can dramatically alter the measured drug output from different devices such as nebulisers, pMDI and DPI.[136] The aerodynamic particle size is the most relevant parameter to describe particle transport within the respiratory tract.[84] Variability in children's breathing patterns affects the mass of delivered drug dose in the FPF and therefore the breathing pattern affects lung deposition.[400] Several filter studies in adults and children have measured total drug output from different delivery devices after simulated breathing patterns.[136, 156, 403-406]

Nikander and Bisgaard demonstrated improved efficiency of drug output to infants and children, when drug aerosolization was synchronized with inhalation.[297] Adaptive aerosol delivery devices have been developed to release a precise dose that is tailored to the individual patient's breathing pattern.[121] Shrewsbury *et al* have shown that the variability of lung deposition of fluticasone was reduced in adults with a breath-synchronized device.[407]

'Real life' breathing patterns recorded during patient use of a device may help to improve the accuracy of *in vivo* dose estimations of drug suspensions for nebulisation and drug output from pMDI and DPI.[136, 408-411] Breathing simulators have been

designed to replicate breathing patterns *in vitro*. Breathing simulators can generate all of the waveforms specified by the American Thoracic Society (ATS) for testing spirometers, commonly used to test lung function in children and breathing simulation studies have been recommended for the assessment of nebulisers.[136, 405, 412, 413]

The use of breathing simulators is based on the assumption that there is a correlation between the *in vitro* and *in vivo* inhaled mass of drug. However, several studies with breathing simulators have used computer generated sinusoidal or square waveforms based on the average tidal volume, inspiratory flow and breathing frequency to measure the influence of breathing parameters on drug output (Table 1-6).[408, 414, 415] These computer-generated breathing patterns may show comparable dose output *in vitro*, however there is reduced variability compared with human breathing patterns and they do not accurately replicate the actual breathing pattern. [405]

Foss and Keppel designed a variable flow technique for measuring particle sizes and the respirable dose output from a cascade impactor set at a constant flow, while simultaneously using a breathing simulator to regulate the flow of aerosol through a pMDI attached to a throat model.[409, 416] Janssens *et al* used a similar set-up with an upper airway model of an infant attached to an Andersen Cascade Impactor (ACI) and a breathing simulator (Figure 1-21).[90, 398, 417] A modification of the variable flow technique described by Foss and Keppel and Janssens has been reported by Schultz *et al* and is described in this thesis, using children's breathing patterns to measure *in vitro* assessments of drug output and fine particle fraction from pMDI-spacer (Figure 1-22).

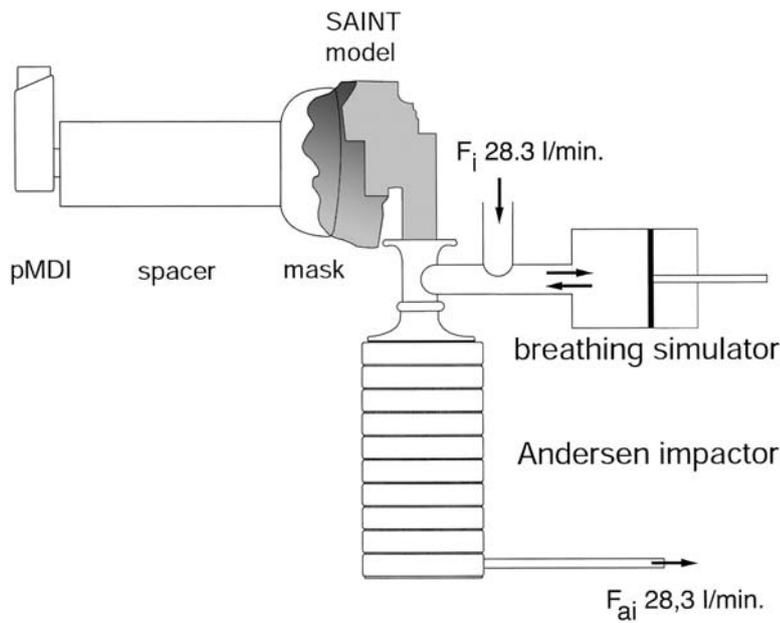


Figure 1-21: Experimental set-up* used to measure lung dose and particle size from pMDI-spacer during simulated tidal breathing. The breathing simulator supplied tidal breathing through the SAINT model and pMDI-spacer with a constant flow of 28.3 L/min through the impactor, balanced by an inflow (F_i) of 28.3 L/min.

*Reproduced with permission; Janssens *et al*, J Aerosol Med 2001; 14(4):433-41.[398] Copyright ©2012 Mary Ann Liebert, Inc., publishers. All rights reserved.

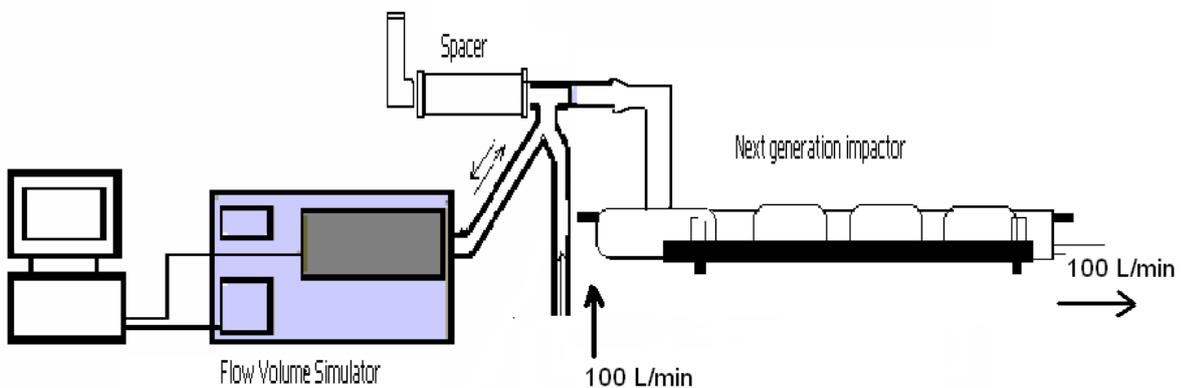


Figure 1-22: Experimental set-up* of Flow-Volume Simulator connected with T-piece to Next generation impactor.[418] The breathing simulator supplied breathing patterns through the Y-piece and pMDI-spacer with the impactor attached to a constant suction @ 100 L/min and balanced by a pressurized air inflow @ 100 L/min into the Y-piece. Airflow at the T-piece was zero.

*Reproduced with permission; Dr Schultz, Aerosol Research Group, School of Paediatrics and Child Health, UWA.

Table 1-6: Literature search for *in vitro* studies using a fixed ‘set’ or ‘real’ breathing pattern.

Study	Year	Formulation	Device	<i>In vitro</i> set-up
Everard [403]	1992	Sodium chromoglycate	Jet nebulizer	Starling ventilator, filter study, ‘set’ children’s breathing patterns
Barry and O’Callaghan	1998	Budesonide	Jet nebulizer	Sinusoidal pump, filter study, ‘set’ children, adult breathing patterns
Finlay [419]	1998	Beclomethasone Salbutamol	pMDI-spacers	Simulator ‘set’ children’s breathing patterns
Burnell [420]	1998	Salmeterol	DPI	Electronic lung, ‘set’ breathing patterns
Berg [156]	1998	Budesonide Fluticasone	AC+ Babyhaler	Ventilator, simulator ‘set’ children’s breathing patterns
Barry and O’Callaghan	1999	Budesonide	Nebulizer pMDI-	Sinus flow pump, filter study, ‘set’ children’s breathing patterns
Nikander [297]	1999	Budesonide	Jet nebulizer	Piston pump, filter study, ‘set’ children’s breathing patterns
Coates [415]	1999		Nebulizer	Simulator, ‘real’ children’s breathing patterns v ‘set’
Foss and Keppel [416]	1999	Albuterol	pMDI-spacer	Simulator, Cascade impaction, ‘set’ sinusoidal breathing pattern
Finlay and Gehmlich	2000		DPIs	Simulator, throat model, virtual impaction,
Nikander [408]	2000	Budesonide	Jet nebulizer	MIMIC breathing emulator, filter study, ‘real’ adult breathing patterns
Nikander [421]	2001	99mTc-DTPA	Jet nebulizer	Simulator, comparison ‘real’, sine or square waveforms
Janssens [398]	2001	Budesonide	pMDI-spacer	Simulator, upper airway cast attached to ACI
O’Callaghan [89]	2002	Flunisolide	Nebulizer	Sinus flow pump, ‘set’ adult and children breathing patterns
Corcoran [414]	2003	99mTc-DTPA	Nebulizer	Simulator, Doppler particle analyser, ‘set’ breathing pattern
Roth [410]	2003		Jet nebulizer	Simulator, comparison ‘real’, square waveforms, filter study
Janssens [417]	2003	HFA-BDP, CFC-BDP	pMDI-spacer	Simulator, upper infant model +ACI, ‘set’ sinusoidal breathing patterns
Janssens [90]	2004		pMDI-spacer	Simulator, upper infant model +ACI, ‘set’ sinusoidal breathing patterns
O’Callaghan[413]	2005	Budesonide	Nebulizer	Sinus flow pump, ‘set’ adult and children breathing patterns
Bosco [405]	2005	Levalbuterol	Breath actuated	Simulator, ‘real’ adult breathing patterns, filter study
Schuepp [142]	2005	Budesonide	Nebulizer	Simulator, ‘set’ infant breathing patterns, upper airway cast
Kamin [422]	2006	Budesonide	pMDI-spacer	Simulator, ‘set’ breathing patterns for toddlers and children
O’Callaghan [423]	2008	Salbutamol, Flunisolide,		Sinus flow pump, ‘set’ adult and children breathing patterns
Bauer [296]	2009	Arformoterol	Nebulizer	Simulator, ‘set’ adult breathing pattern
Schultz [418]	2010	Salbutamol	pMDI-spacer	Simulator, ‘real’ adult breathing patterns, filter study

1.11 Summary and thesis aims

This review has highlighted the global prevalence of asthma and the importance of the drug formulation, inhalation technique and device selection for asthma management in children. Asthma is recognised as an inflammatory condition and current evidence supports the key role of ICS as preventative therapy for children with persistent asthma. However despite international guidelines for asthma therapy and multiple choices of ICS and delivery devices, there are still problems associated with the optimal management of childhood asthma.

Variability in drug delivery is common and poor inhaler technique with pMDI-spacer is often used by children with asthma. Therefore it is crucial to evaluate total body deposition and regional deposition of different ICS delivered *via* different pMDI-spacer with different inhalation techniques to asthmatic children. *In vitro* studies with cascade impaction, using a constant airflow, have been widely used to measure drug output and particle size distribution of ICS, however predictions of *in vivo* drug delivery for different drug/device combinations with cascade impaction methods overestimate lung deposition and underestimate the variability in drug delivery to children.

Filter studies have been used to assess the total drug output from different drug delivery devices, but these studies do not give information about regional drug deposition which is important with regard to predicting systemic and local side-effects. Similarly, PK methods do not provide information on regional deposition and they are time-consuming and difficult to achieve with children. Theoretical models of aerosol deposition have been used to predict drug deposition. However these models cannot simulate realistic physiological conditions.

Gamma scintigraphic studies have been widely used to measure lung deposition of radiolabelled drugs from several different inhaler devices. Measures of lung deposition

are important because there is evidence to support the view that lung deposition data may be used to predict the clinical response to inhaled asthma drugs.[114] Devadason *et al* used a scintigraphic study to show that extrafine formulation, QVAR™ enhances lung delivery to asthmatic children, however they found that oropharyngeal deposition was 40-60% with the breath-actuated Autohaler™ device and this unnecessary dose may contribute to local and systemic side-effects.[150] Prior to the study published from work carried out during this thesis, there had not been a scintigraphic study with the new extrafine formulation, QVAR™, delivered *via* pMDI-spacer, which is the most common delivery device used by young children.

Berg *et al* and Asmus *et al* used *in vitro* studies to show that there are significant variations in the delivery of fluticasone propionate (FP) from different spacer devices.[260] Therefore different spacer devices may affect the variability in lung deposition of Flixotide® (HFA-FP). Flixotide® has high potency and has been associated with systemic side-effects, even when given at recommended dosages.

Different inhalation techniques with pMDI-spacer may affect lung deposition of Flixotide® and therefore systemic exposure. However, prior to the studies in this thesis, gamma scintigraphy had not been previously carried out in a pediatric study with radiolabelled Flixotide®, the most widely used ICS for children. A radiolabelling technique had not been previously developed for Flixotide® and the method developed during this thesis was published in the Journal of Aerosol Medicine as an invited paper.

The measurement of airflow is often omitted from asthma management strategies.[424] However, studies with breathing simulators indicate that there are significant variations in particle deposition patterns within the lungs for different respiratory parameters including tidal volumes, inspiratory flows, and ‘breath hold’ times.[425] [386] [423] Several *in vitro* and *in vivo* methods have been used to evaluate drug delivery to

children, however there are unresolved issues regarding the exact measure of the fine particle fraction with *in vitro* methods used for *in vivo* comparisons.[109, 114]

Janssens *et al* used an upper airway infant model with *in vitro* cascade impaction and breathing simulation to show lung deposition of extrafine QVAR™ from pMDI-spacer, however the breathing patterns were simulated for infants.[417] Schultz *et al* used breathing simulation with an inspiratory filter study to show that most of the Flixotide® inhaled *via* pMDI-spacer to children 2-7 years of age was delivered with the first two tidal breaths.[426]

Kamin *et al* used tidal breathing patterns of young children to show that 60-80% budesonide inhaled *via* pMDI-spacer is delivered in the first inhaled breath.[422]

However the effects of individual inspiratory parameters of the first inhaled breath on lung deposition of ICS *via* pMDI with an attached small volume spacer, has not been previously addressed in children using gamma scintigraphy. Furthermore drug delivery of ICS *via* pMDI-spacer using gamma scintigraphy has not been directly compared with *in vitro* analysis of drug delivery with a breathing simulator using ‘real-life’ breathing patterns and Next Generation Impaction.[61]

Therefore the primary aim of the experimental studies described in this thesis was to use gamma scintigraphy to investigate the effects of the inhalation profile on the drug delivery of two commonly prescribed ICS with different particle sizes, QVAR™ and Flixotide®, delivered *via* pMDI-spacer, to children with asthma. Chapter 2 outlines the methodology used in the studies in this thesis. Chapters 3-5 summarise the gamma scintigraphic studies for QVAR™ and Flixotide®. After the QVAR™ deposition study was completed (Chapter 3), a breathing simulator was acquired by the UWA Aerosol Research Group. The breathing simulator provided the opportunity for the secondary aim of this thesis, namely to investigate whether *in vitro* methods of breathing

simulation in tandem with cascade impaction could be used to reflect the gamma scintigraphic data for radiolabelled Flixotide® (Chapter 4-6).

Sub-optimal treatment with ICS delivered *via* pMDI-spacer may lead to airway structural changes (remodeling) and a progressive loss of lung function.[427]

Predictions of lung deposition may be used to balance safety and efficacy, guide dosage regimens, and predict clinical effect. Clear dosing protocol recommendations for different pMDI-spacer devices, different inhalation techniques and different ICS formulations are important as they may be used to effectively guide clinical protocols.

The following hypotheses have not been previously addressed in the literature with asthmatic children, using gamma scintigraphy, prior to the studies performed in this thesis:

- An extrafine particle size, in combination with the small volume Aerochamber Plus™ spacer device, will improve lung deposition to asthmatic children and reduce oropharyngeal and gastrointestinal deposition.
- The ‘breath hold’ technique, compared with tidal breathing, used with pMDI-spacer device, will significantly improve lung deposition of QVAR™ and Flixotide® in children.
- Different spacer devices, Aerochamber Plus™ and Funhaler, will alter the variability in the delivery of QVAR™ and Flixotide® to young asthmatic children using tidal breathing with pMDI-spacer.
- Inspiratory parameters associated with the first inhaled breath will have an effect on the regional delivery of Flixotide® *via* pMDI-spacer.

- Predictions of lung deposition based on *in vitro* breathing simulation, used in combination with cascade impaction, may be used to reflect lung deposition of Flixotide® obtained *in vivo* with gamma scintigraphy.

The information obtained from studies in this thesis is important because gamma scintigraphic studies are currently the ‘gold standard’ used for assessing *in vivo* lung deposition. However, scintigraphic studies involve the rigorous validation of a match between radiotracer and drug, as well as a radiation dose to the subject. Therefore an *in vitro* method that can be used as a surrogate for *in vivo* gamma scintigraphy would provide a valuable strategy to assess new formulations, inhalation techniques and delivery devices used in different age-groups. Furthermore reliable *in vitro/in vivo* comparisons could be used before future deposition studies to ascertain optimal drug/device combinations, best suited for gamma scintigraphic studies, and ultimately to predict and optimise treatment with ICS delivered *via* pMDI-spacer to asthmatic children.

2 MATERIALS AND METHODS

The materials and methods used for the experimental work carried out in this thesis include chemicals, reagents, pharmaceuticals, radioisotopes and equipment for the *in vitro* experiments and *in vivo* clinical studies. Listed with the materials, is the supplier and the supplier's location, city/town, country. The gamma cameras required for the clinical studies were made available by the Department of Nuclear Medicine at Princess Margaret Hospital, Perth, Western Australia.

2.1 MATERIALS

2.1.1 Chemicals and reagents

The chemicals and reagents required for the methods used in these studies are shown in Table 2-1:

2.1.2 Pharmaceuticals

The analytical standard solutions of beclomethasone dipropionate (BDP) and fluticasone propionate (FP, British Pharmacopoeia Chemical Reference substance, British Commission Laboratory, Teddington UK) were made up in absolute ethanol or methanol (HPLC grade) respectively and prepared as stock solutions for UV-VIS spectrophotometry (Shimadzu UV-1601, Kyoto, Japan). BDP and FP were purchased from Sigma Chemicals Co., (St. Louise, MO, USA).

Table 2-1: A list of chemicals and reagents used in the methods for validation of radiolabelling.

Chemical/Reagent	Source
Pyroneg detergent	Diversey Lever Pty. Ltd., Smithfield, Australia
Methanol (CH ₃ OH) HPLC grade, Purity 99.9%	Biolab Scientific, Clayton, Australia
Ethanol (C ₂ H ₅ OH) Absolute 100%	Scot Scientific, Perth, Australia
Chloroform BP (CHCl ₃)	Sigma-Aldrich Chemicals, Perth, Australia
Tetraphenylarsonium chloride (AsPh ₄ Cl ₄)	Sigma-Aldrich Chemicals, Perth, Australia
Ammonium hydroxide (NH ₄ OH) with NH ₃ content (28%-30%)	Sigma-Aldrich Chemicals, Perth, Australia
Butanone (C ₄ H ₈ O) BDH, AnalaR, Purity 99.5%	BDH Chemicals Australia Pty.Ltd., Kilsyth, Victoria, Australia
Ethyl methyl ketone (C ₄ H ₈ O) with Purity ≥ 99.5%	MERCK, Darmstadt, Germany
Liquid nitrogen	BOC, Welshpool, Australia
Decon 90	Decon Laboratories Ltd., Sussex, England
Sodium pertechnetate (Na ^{99m} TcO ₄)	⁹⁹ Mo/ ^{99m} Tc generator, Australian Radioisotopes (ARI), Sydney, Australia

2.1.3 Pressurised metered-dose inhalers (pMDI)

The pMDI required for the experimental methods used in the studies in this thesis are listed in Table 2-2. Commercial QVAR™ (HFA-BDP) pMDI canisters were purchased from 3M Health Care Ltd. Commercial Flixotide® (HFA-FP) pMDI canisters were purchased from Allen and Hanburys, GSK, Boronia, Australia. Empty pMDI canisters for the QVAR™ and Flixotide® studies were kindly donated by 3M Pharmaceuticals and Allen and Hanburys respectively.

Table 2-2: Inhaled drug formulations delivered via pMDI canisters.

Generic name	Brand name	Micrograms per actuation	MMAD μm	Manufacturer	Formulation Type
Beclomethasone dipropionate	QVAR™	100	1.0	3M Health Care Limited, Canada	HFA-134a propellant Ethanol cosolvent
Fluticasone propionate	Flixotide®	250	3.0	Allen & Hanburys, GlaxoSmith Klein Wellcome Australia Ltd, Boronia, Victoria	HFA-134a propellant
Salbutamol	Ventolin®	100	3.5	GlaxoSmithKlein PtyLtd, Australia	HFA-134a propellant

2.1.4 Radioisotopes

Sodium pertechnetate ($\text{Na}^{99\text{m}}\text{TcO}_4$) was eluted from a molybdenum/technetium ($^{99}\text{Mo}/^{99\text{m}}\text{Tc}$) generator (Australian Radioisotopes, Lucas Heights, Australia) in a volume of 10mL, into a sterile evacuated vial, contained within a lead shield. From the stock solution, 1-2 mL of sodium pertechnetate was withdrawn into a 2 mL syringe, shielded within a tungsten syringe shield, for the radiolabelling techniques.

2.1.5 Equipment

The equipment required for the *in vitro* drug analyses used in these studies is listed in Table 2-3.

Table 2-3: A list of the equipment used for *in vitro* experiments and the company from which they were obtained.

Equipment	Source
Pyrex Glassware	Crown-Scientific, Kewdale, WA, Australia
Micropipettes Gilsons Inc., Pipetman®P	John Morris Scientific, Willoughby, NSW, Australia
Disposable inspiratory filters	Curity®Anesthesia Filter, Kendall, MA
Whatman™ filter paper 934-AH	Crown-Scientific, Perth, Australia
Whatman™ filter paper 1PS	Crown-Scientific, Perth, Australia
Funhaler spacer with valve	Infamed Limited, Australia
Aerochamber Plus™ spacer with valve	Trudell Medical International, Canada
Andersen Cascade Impactor Mark II	Copley Scientific, Nottingham, UK
Mouthpiece adapter	Copley Scientific, Nottingham, UK
Vacuum Pump	Copley Scientific, Nottingham, UK
Next Generation Impactor	Copley Scientific, Nottingham, UK
Pressure Pump with digital flowmeter PHCP4	Copley Scientific, Nottingham, UK
High Capacity Vacuum Pump, HCP4	Copley Scientific, Nottingham, UK
Digital flowmeter model DFM2	Copley Scientific, Nottingham, UK
Ultraviolet-Visible Spectrophotometer 1601	Shimadzu Corporation, Kyoto, Japan
Quartz cuvette	Starna Pty. Ltd., Baulkham Hills, Australia
Vitalograph® MDI Compact Spirometry	Vitalograph® International, Buckingham, UK

Before each study day, actuator and spacer devices were soaked in a dilute solution (1:5000) of detergent (Pyroneg, DiverseyLever, Australia Pty Ltd) for a minimum of 10 minutes in order to reduce static. The spacers were drip-dried overnight and air-dried before use. The equipment required for gamma scintigraphy and Flow-Volume Simulation methods used in these studies are listed in Table 2-4.

Table 2-4: A list of equipment required for radiolabelling, gamma scintigraphy and Flow-Volume Simulation.

Equipment	Source
Pipecutter, TC 1000, Imperial Eastman	Newman Tools Inc. Hartford CT, USA
Crimper Type 555G, Pamasol	Willi Mäder AG, Pfäffikon, Switzerland
Atomlab 200 dose calibrator	Gammasonics, Sydney, Australia
Gamma camera 1, GCA 7200DI	Toshiba Australia; Perth, Australia
Gamma camera 2, ECAM	Siemens, GE Healthcare, USA
Perspex collimator flood source	Toshiba, Perth, Australia
High Resolution Collimators 1	Toshiba, Perth, Australia
High Resolution Collimators 2	Siemens, GE Healthcare, USA
Lead, tungsten and lead-glass shielding	Global Medical Solutions, Osborne Park, Australia
Wheaton glass scintillation vial	Thomas Scientific, Swedesboro, NJ, USA
Stainless steel tongs	Global Medical Solutions, Osborne Park, Australia
Whatman 1PS for solvent extraction	Crown-Scientific, Perth, Australia
Heating block	Thermolyne Barnstead Int, Dubuque, USA
Shaker	John Morris Scientific Pty. Ltd., Perth, Australia
Stainless steel liquid nitrogen Dewar	Cole Parmer, John Morris Scientific Pty. Ltd., Perth, Australia
Koko® spirometer with 3L syringe	Koko®, Pulmonary Data Service Instrumentation Inc., Louisville, USA
Linear Pneumotachograph RSS 100	Hans Rudolph Incorporation, Kansas USA
RSS Convert Software Version 1.04	Integrated Control Systems Incorporation, Phoenix, USA
Flow-Volume Simulator Series 1120	Hans Rudolph Incorporation, Kansas, USA
Flow-Volume Simulator Software Version 4.03	Integrated Control Systems Incorporation, Phoenix, USA
Breathing Simulation Calculation Program	Programmer Dr. Brad Zhang, UWA, Perth, Australia
Perspex flow chamber	Custom-built design Dr Andre Schultz, UWA, Perth, Australia

2.2 METHODS

2.2.1 Study population

The studies included in this thesis were carried out with children between the ages of 5 and 17 years, who were classified as having mild, stable asthma. Children were recruited from the outpatient clinics at Princess Margaret Hospital for Children. Children were required to complete a preliminary screening questionnaire, demonstrate adequate inhalation technique during an inspiratory filter study and have FEV1 > 80% after lung function spirometry on the study day. On the study day, each child had weight, height and lung function measured.

2.2.2 Exclusion criteria

Exclusion criteria were past or present diagnoses of cardiovascular, renal or liver disease, known hypersensitivity to beclomethasone dipropionate or fluticasone propionate, previous inclusion in a radiolabel deposition study for research purposes, exacerbation of asthma symptoms within the previous 4 weeks, inability to perform the required breathing technique or FEV1 < 80% on the study day.

2.2.3 Lung function testing

On the study day, each child had weight and height measurements and lung function measurements using a portable PC-based incentive spirometer (Koko®, Pulmonary Data Service Instrumentation Inc., Louisville, USA). Children withheld any bronchodilator medication for 4 hours prior to spirometry. The children were instructed to use the incentive Koko® spirometry program in order to optimise inhalation and exhalation. Three measures of lung function were made before children inhaled 2 doses of Ventolin® (GlaxoSmithKline PtyLtd, Australia), 100 µg/dose. This was followed by a further lung function measurement conducted in triplicate after 15 minutes.

Pulmonary function was expressed as a percentage of the value predicted based on age, gender, and height according to the methods of Knudson *et al.*[428] Only those children with mild, stable asthma and FEV1 > 80% predicted values were enrolled in the deposition study.[429] Ventolin® was given 30-45 minutes prior to gamma scintigraphy.

2.2.4 Drug assays

Solutions of BDP and FP (Sigma Chemical Co., St. Louis, MO) were prepared by dissolving 0.025 g BDP in 20 mL ethanol or 0.025 g FP in 20 mL methanol and this solution was subsequently made up to 50 ml with solvent in a volumetric flask so that the concentration of the stock solution was 500 µg/mL. The stock solution was further diluted with solvent to prepare standard concentrations ranging from 2-20 µg/mL. Standard solutions were prepared on each day of testing.

Standards were prepared from the known concentration (C) of the stock solution and made up to a volume of 10 mL (V) in volumetric flasks ($C_1V_1=C_2V_2$). The absorbance of drug at a specified wavelength was compared to the standard curve generated by measuring the absorbances of a series of solutions containing known concentrations of drug (C, Table 2-5).

Table 2-5: Representative absorbances at wavelength 238 nm (BDP) and wavelength 235 nm (FP) for standard prepared from stock solution concentration (C2) drug: 500 µg/mL.

C 1	Volume 1	C 2	Volume 2	Abs. BDP @ 238 nm	Abs. FP @ 235 nm
2 µg/mL	10 mL	500 µg/mL	40 µL stock	0.060	0.080
5			100	0.150	0.200
10			200	0.300	0.400
15			300	0.450	0.600
20			400	0.600	0.800

The absorbances of BDP (wavelength 238 nm) and FP (wavelength 235 nm) were measured in each wash by UV-VIS spectrophotometry (Shimadzu UV-1601, Kyoto, Japan), as shown in Table 2-5.

The standard curve showed a linear relationship ($r^2=1.0$) between absorbance and concentration for concentrations of drug between 0 $\mu\text{g/mL}$ and 20 $\mu\text{g/mL}$ where concentration = (slope) * (absorbance) + intercept (Figure 2-1 and Figure 2-2). All glassware was washed in detergent, rinsed 3 times in tap water and then rinsed in deionised water and dried.

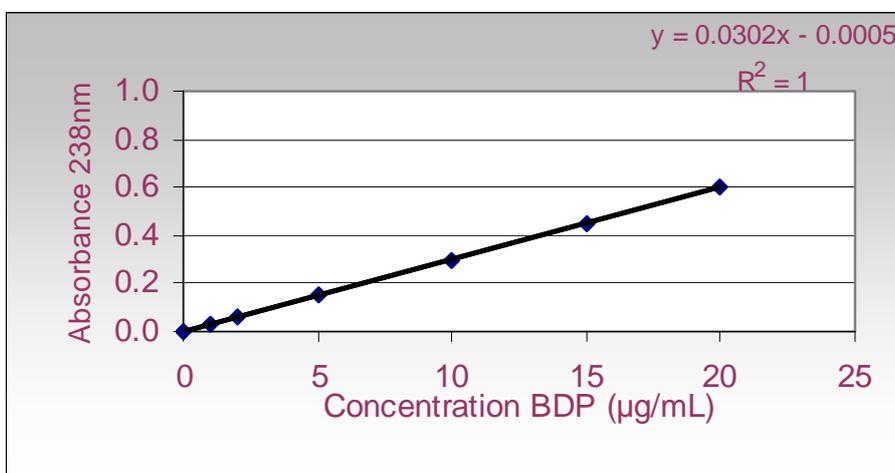


Figure 2-1: Standard graph for beclomethasone dipropionate (BDP) showing the relationship between UV-VIS absorbance at wavelength 238 nm and concentration of BDP.

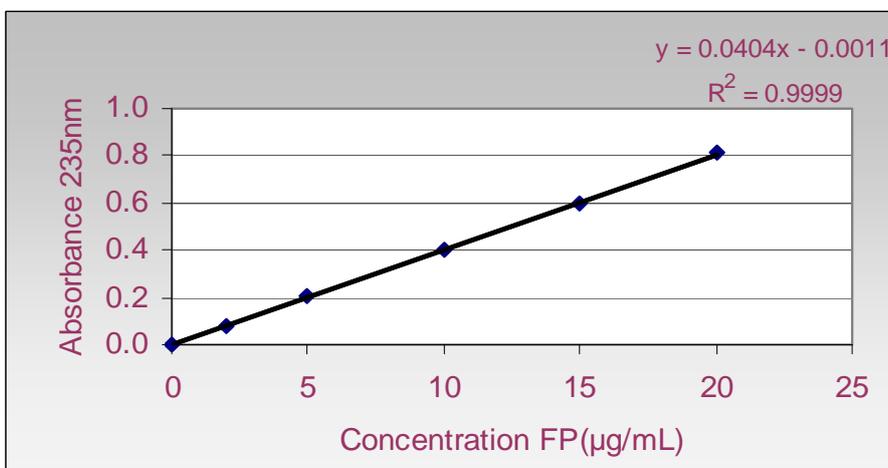


Figure 2-2: Standard graph for fluticasone propionate (FP) showing relationship between UV-VIS absorbance at wavelength 235 nm and concentration of FP.

2.2.5 Inhalation technique training

Each child was trained to inhale *via* pMDI-spacer, with either tidal breathing with Aerochamber Plus™ (AC+) and Funhaler (FH), (n=22) or a slow single maximal inhalation followed by a 5-10 s ‘breath hold’ with Aerochamber Plus™ (n=14). A low resistance inspiratory filter (Curity®Anesthesia Filter, Kendall, MA) was attached to the mouth-piece of the AC+ spacer or a FH spacer so that the child could rehearse the tidal breathing technique with 5 tidal breaths from the spacer device after firing 100 µg BDP or 250 µg FP.

Alternatively the child rehearsed with a slow single maximal inhalation, followed by a 5-10 s ‘breath hold’. This was repeated 3 times, so that the child understood the correct inhalation technique, prior to inhaling the radiolabelled drug (BDP or FP). These groups are referred to as ‘tidal’ and ‘breath hold’ respectively. Children were encouraged to inhale slowly, however children often exceeded inspiratory flows of 45 L/min, at which the audible flow signal of the Aerochamber Plus™ was heard.

Children in the BDP deposition study were divided into three subgroups according to age: 5-7 years (n=4), 8-10 years (n=4) and 11-17 years (n=4), in order to compare the results with the previous paediatric deposition study which used the Autohaler™ device to deliver the same BDP formulation. Comparative subgroups were made for analysis in the Flixotide® (HFA-FP) study. Children in the FP deposition study were divided into two subgroups according to age: 5-8 years (n=20), 9-17 years (n=16) in order to improve statistical analyses, as only two children were recruited for the ‘tidal’ group aged 11-17 years. Children aged less than ten years, using tidal breathing formed another subgroup (n=10), in order to compare drug output and lung deposition from the two different spacer devices, Aerochamber Plus™ and Funhaler.

2.2.6 Inspiratory filter study

After the inhalation training each child inhaled drug *via* pMDI-spacer, with a low resistance inspiratory filter inserted between the mouth-piece and the spacer, as shown in Chapter 1, section 1.9.1, Figure 1-15. QVAR™ (HFA-BDP) was inhaled *via* pMDI-spacer (AC+) with ‘tidal’ or ‘breath hold’ (n=24). Flixotide® (HFA-FP) was inhaled *via* pMDI-spacer (AC+ or FH) with tidal breathing (n=22) and pMDI-spacer (AC+) with ‘breath hold’ (n=14).

Each filter was wrapped in aluminium foil and stored in the laboratory, then washed with absolute ethanol (for BDP) or methanol (for FP) and the solution of drug and solvent was collected into a 50 mL volumetric flask. The concentration of drug in each wash was determined using a UV-VIS spectrophotometer, as described in section 2.2.4, Table 2-5, Figure 2-1 and Figure 2-2.[297]

2.2.7 Ethical considerations

2.2.7.1 Protocol

The protocol for the research studies carried out in this thesis and the informed consent form were reviewed and approved by Research and Ethics Committee, Princess Margaret Hospital for children (Subiaco, Western Australia). Written, informed consent was provided by parents or a legally acceptable representative. Approval for the research studies, involving the use of ionizing radiation, was obtained from the Research and Ethics Committee, in collaboration with the Radiation Safety Physicist at Royal Perth Hospital, Western Australia.

2.2.7.2 Effective dose

The effective dose, based on ICRP 53, was calculated by multiplying actual organ doses by the risk weighting factors (related to each organ's relative radiosensitivity to

developing cancer) and adding up the total to give the effective whole-body dose i.e. the effective dose, according to age (Figure 2-3).

The maximum level of radiation dispensed to each patient (2-4 MBq, depending on age) was approved by the Radiation Safety Officer at Royal Perth Hospital and was equivalent to a single effective dose of 0.1 mSv. This dose is associated with a negligible risk and is well below the limit set by the ARPANSA guidelines.[351, 430]

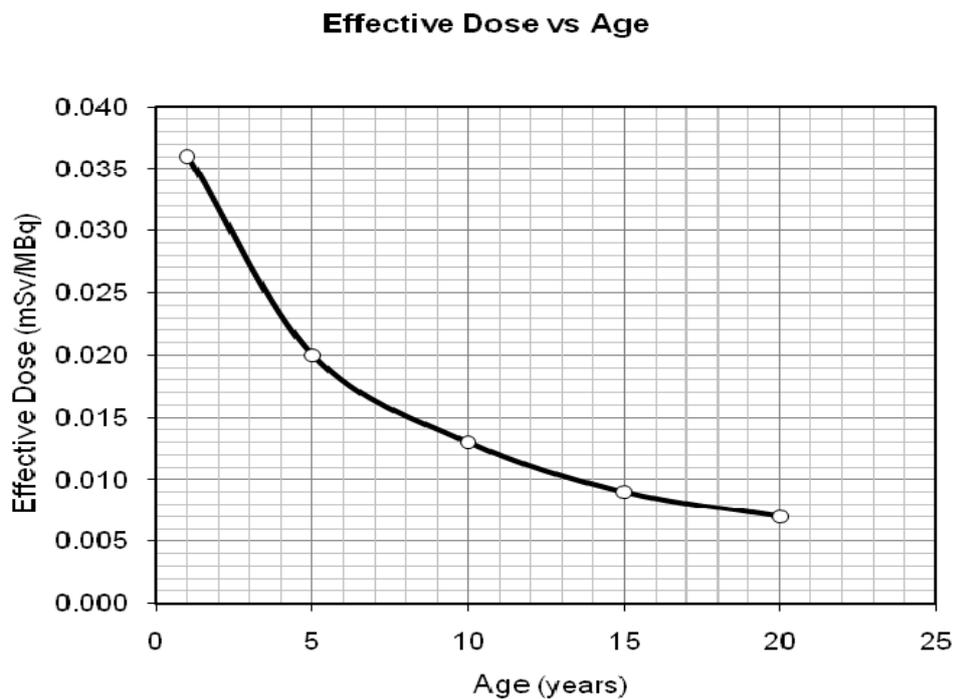


Figure 2-3: Relationship between effective dose (mSv/MBq) from deposition studies and age in years based on ICRP 53.

The dose from the technetium-99m uniform flood source was 0.003 μ Sv/MBq.[327] At this dose level, no harmful effects of radiation have been demonstrated as any effect is too small to measure. The risk is believed to be minimal (if the effective dose is less than 0.2 mSv).[431] A comparison of the effective dose (mSv) received from deposition studies carried out in this thesis to the radiation dose from other sources was shown in Chapter 1, section 1.9.5, Table 1-4.

2.2.7.3 Volunteers

Participation in the deposition studies was performed on a voluntary basis. The children's parents and/or guardians were given information about the research project and any questions were answered prior to the study. The parent/guardian could withdraw the child at any stage during the study. The proposed effective dose was compared with the effective dose from background radiation and air travel within Australia.

2.2.8 Inertial Impaction

2.2.8.1 Andersen Cascade Impaction (ACI)

The 8 stage non-viable Andersen Cascade Impactor (ACI, Copley Scientific, Nottingham, UK) was used for the *in vitro* characterisation of the aerodynamic particle size distribution and drug output from a series of commercial pMDI canisters before the radiolabelling procedure. Each canister was shaken for 10 s and then primed by actuating 3 times to waste; then each of 5 actuations was delivered at 10 s intervals, with the mouth-piece of the inhaler firmly attached to the induction port with an actuator adaptor (coupler) (Figure 2-4).

The impactor was fitted with a standard USP metal 'throat' and operated at a continuous flow of 28.3 L/min. Drug was deposited on the impaction plates according to size (Chapter 1, section 1.6.3.2, Figure 1-5). Flow through the impactor was maintained until approximately 30 seconds following the last actuation. The actuator, spacer, 'throat', jet stage and 8 impaction plates were washed with 25 mL solvent and aliquoted into 25 mL volumetric flasks.

The particle size distribution from each of the commercial canisters was determined using the stage cut-off sizes supplied by the manufacturer, in accordance with

compendial practice.[84] The estimated cut-off diameters (ECD) for plates 0-7 are 9.0, 5.8, 4.7, 3.3, 2.1, 1.1, 0.65 and 0.43 μm respectively, Figure 2-4 (refer also to Chapter 1 section 1.6.3, Figure 1-5 and Figure 1-7). The smallest particles, with an aerodynamic diameter less than 0.43 μm were retained by a glass fibre collection filter (absolute filter, Whatman™ 934-AH, Crown-Scientific, Perth, WA), which has a high collection efficiency for submicrometre particles.

The portion of the mass of drug measured with the impactor, contained in particles smaller than 5 μm aerodynamic diameter (plates 3-7 + absolute filter) was defined as the fine particle or ‘respirable’ fraction (FPF < 4.7 μm). With ACI, drug particles less than 3.3 μm (plates 4-7 + absolute filter) were referred to as the extrafine particle fraction (EFPF). The concentration of drug in each wash was determined using a UV-VIS spectrophotometer, as described in section 2.2.4.

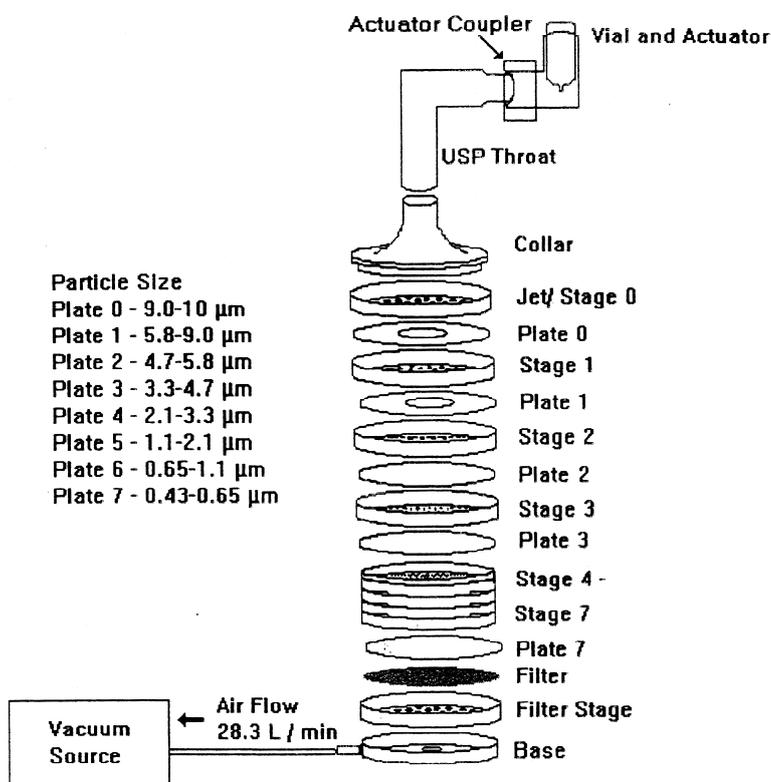


Figure 2-4: Schematic of stages and impaction plates of the Andersen Cascade Impactor*.

*Reproduced with permission; Dr Devadason, Aerosol Research Group, School of Paediatrics and Child Health, UWA.

The total drug output, fine particle fraction and extrafine particle fraction of drug were assayed for each drug (BDP and FP). From the particle size distribution, a cumulative mass distribution was obtained and the MMAD and GSD of each drug were determined. The MMAD and GSD, estimated as the ratio of the 84.3% size to the 50% size in the cumulative mass distribution, were used to statistically describe the particle size distribution of drug based on the mass size of the particles (Table 2-6).[85]

Table 2-6: Representative cumulative mass distribution drug (FP) collected on impaction plates and the corresponding aerodynamic particle size, MMAD and GSD calculations with Andersen Cascade Impactor at 28.3 L/min.

Impaction plates	Mass of drug μg	ECD μm	Log ECD	%	Cumulative %	Calculations	
Total	98.003						
Plate 0	7.08	9	1.0	8.9	92.8		
Plate 1	8.72	5.8	0.8	10.3	83.9		
Plate 2	10.12	4.7	0.7	29.8	73.6		
Plate 3	29.20	3.3	0.5	29.2	43.8		
Plate 4	28.65	2.1	0.3	12.8	14.5	slope	194.0
Plate 5	12.55	1.1	0.0	1.1	1.7	intercept	-56.8
Plate 6	1.09	0.65	-0.2	0.3	0.6	log D50	0.6
Plate 7	0.27	0.43	-0.4	0.3	0.3	MMAD (D50) FP	3.6
Filter	0.33					GSD	1.6

Farr *et al* reported a predicted lung dose, based on the product of the emitted dose and the fine particle fraction (particles < 5.8 μm). Mitchell *et al* reported the fine particle fraction as particles < 4.7 μm with ACI. As larger particles are more likely to be retained in the spacer, I have used a modified Farr *et al* formula to derive the predicted lung dose *in vitro*, using the fine particle fraction, modified from the particles with ECD < 5.8 μm to ECD < 4.7 μm , as a proportion of the emitted dose.[106] [84]

$$\text{Predicted lung deposition} = \% \text{ emitted} \times \text{FPF}/100$$

The predicted lung dose based on Newman and Chan was defined as the extrafine particle fraction (EFPF), particles < 3.3 μm measured with ACI and particles < 3.4 μm measured with NGI.[109] The predicted lung dose based on Finlay *et al* was defined as the particles within the ECD range 1.1-4.7 μm with ACI and 1.3-6.1 μm with NGI.[105] The predicted lung dose, based on Newhouse *et al* used the ultrafine particle fraction (UFPF), particles < 2.2 μm , exiting the spacer.[110]

2.2.8.2 Next Generation Impaction (NGI)

The NGI was used as supplied for measurements with Flixotide®. The micro-orifice collector (MOC) acted as a substitute for an absolute filter.[84, 92] The impactor was fitted with a standard USP metal ‘throat’ and the aerosol was entrained at a continuous flow of 100 L/min and deposited on the impaction cups according to size. The actuator, spacer, ‘throat’ and 7 impaction cups and the MOC were washed with solvent and collected into 25 mL volumetric flasks. The size distribution measured by the Next Generation Impactor (NGI) having the larger internal volume (1000 mL) might be expected to be finer than ACI (450 mL), as a result of more complete evaporation having taken place by the time that the particles were collected.[92] The extrafine particle fraction (EFPF), defined as particles < 3.4 μm , was also determined from the size distribution. The effective cut-off diameters (ECD) for the collection cups 0-7 are 6.12, 3.42, 2.18, 1.31, 0.72, 0.4 and 0.24 μm respectively (Table 2-7).

Table 2-7: Representative cumulative mass distribution drug (FP) and the corresponding aerodynamic particle size, MMAD and GSD calculations with Next Generation Impactor at 100 L/min.

Impaction cups	Mass of drug μg	ECD μm	Log ECD	%	Cumulative %	Calculations	
Total	115.86						
0	8.89	6.1	0.8	19.5	92.3		
1	22.57	3.4	0.5	23.2	72.8		
2	26.92	2.2	0.3	25.1	49.6		
3	29.09	1.3	0.1	13.3	24.5		
4	15.41	0.7	-0.1	5.8	11.2	slope	113.5
5	6.71	0.4	-0.4	3.1	5.4	intercept	11.2
6	3.64	0.2	-0.6	2.3	2.3	log D50	0.3
7	2.62					MMAD (D50)	2.2

2.2.9 Radiolabelling of drugs

The deposition studies in this thesis were performed using drug formulations radiolabelled with $^{99\text{m}}\text{Tc}$. Sodium pertechnetate ($\text{Na}^{99\text{m}}\text{TcO}_4$) was eluted from a $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator (Australian Radioisotopes, Lucas Heights, NSW, Australia) into a sterile evacuated vial in a volume of 10 mL. An activity of 500-1500 MBq was withdrawn from the vial in a shielded 2 mL syringe and diluted with physiological saline in a volume of 1.0-1.5 mL.

The $^{99\text{m}}\text{Tc}$ was dispensed into a Wheaton glass scintillation vial (Thomas Scientific, Swedesboro, NJ, USA). Organic solvent in a volume of 5-10 mL was dispensed into the glass vial. The radioactive mixture in the glass vial was placed in a lead pot and vigorously mixed for 2 min. The $^{99\text{m}}\text{Tc}$ was extracted into the organic layer. The organic phase was separated from the aqueous phase and filtered into a pre-weighed empty pMDI canister using a phase separating filter paper (Whatman 1PS, Crown

Scientific, Perth, WA, Australia) which was placed in a glass funnel clamped above an empty, preweighed pMDI canister.

The organic phase, containing ^{99m}Tc , was evaporated to dryness with a gentle stream of nitrogen (6-10 L/min) for approximately 25 minutes, resulting in a pMDI canister lined with ^{99m}Tc . The ^{99m}Tc lined pMDI canister weighed within 0.01 g of the pre-weighed canister, indicating negligible residual organic solvent.

The preweighed commercial reference canister containing drug was supercooled in dry ice or liquid nitrogen for 45-60 seconds, secured with a rubber mouth-piece adaptor (Copley Scientific, Nottingham, UK) and rapidly opened with a pipecutter (TC 1000 Imperial Eastman, USA) and the contents poured into the cooled ^{99m}Tc -lined canister and immediately crimped (Crimper Type 555G, Pamasol; Willi Mäder AG; Pfäffikon, Switzerland, Figure 2-5).



Figure 2-5: Pamasol Crimper used to clamp metering valve on radiolabelled pMDI canister.

The radiolabelled pMDI canister and empty commercial pMDI canister were reweighed to determine the percent transfer of contents. The recrimped pMDI canister was placed in a lead pot and vigorously shaken for 2 minutes. The radiolabelled pMDI canister and

empty commercial canister were weighed and the percent transfer of drug was evaluated. The radiolabelled pMDI canister was mixed in an ultrasonic water bath for 20 seconds, then returned to the lead pot and mixed for 30-60 minutes on a shaker (John Morris Scientific Pty. Ltd., Perth, Australia).

2.2.10 Validation of radiolabelling method

An essential step after the radiolabelling process is the rigorous validation experiments used to verify an aerodynamic match between drug and radiolabel.[314]

2.2.10.1 Before radiolabelling

In vitro mass output and aerodynamic particle size distributions of drug from the reference unlabelled commercial pMDI canisters were measured using Andersen Cascade Impaction. Several single actuations (5-20, depending on drug) were drawn into the impactor, as detailed in section 2.2.8.1. Mass of drug was measured according to section 2.2.4. The mass output was used to calculate MMAD and GSD, as shown in Table 2-6. The particle size distribution of BDP is shown in Figure 2-6.

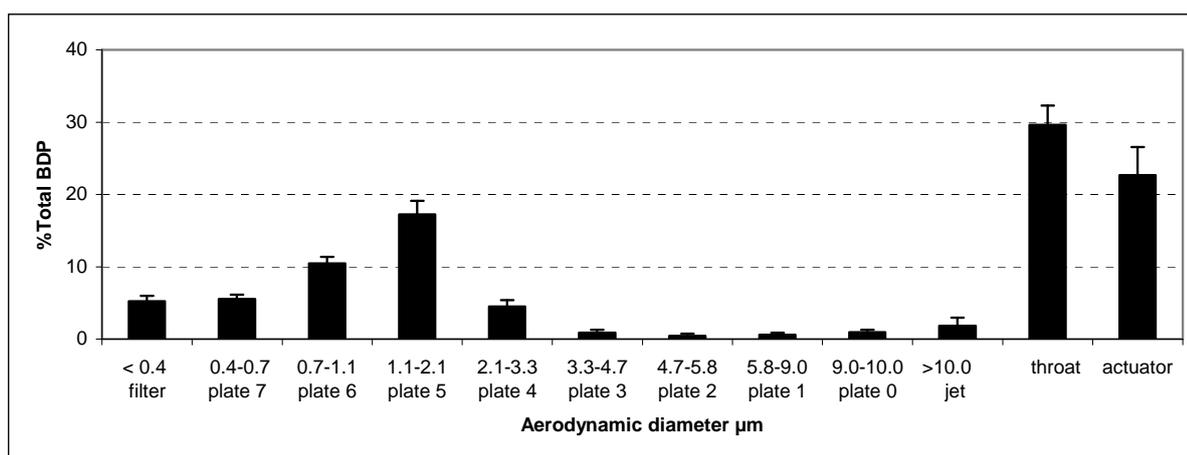


Figure 2-6: The y-axis shows the % total drug (BDP) output measured with Andersen Cascade Impaction (ACI) before radiolabelling, with the corresponding aerodynamic particle size fractions (µm) on the x-axis.

2.2.10.2 After radiolabelling

The particle size distributions of the radiolabelled pMDI canisters (QVAR™ and Flixotide®) were performed with ACI before and after radiolabelling to confirm that the contents of the commercial pMDI canister were not significantly changed by the radiolabelling procedure. The ^{99m}Tc activity and drug were washed from the actuator, ‘throat’, jet stage, impaction plates and absolute filter with 25 mL ethanol (BDP) or methanol (FP) and aliquoted into 25 mL volumetric flasks.

The corresponding ^{99m}Tc activity in each 25 mL volumetric flask was measured by lowering the volumetric flasks into a cylindrical ionisation chamber (Atomlab 200 dose calibrator; Gammasonics, Sydney, NSW, Australia) and the activity recorded. Drug levels in each volumetric flask were calculated as a percent of the total drug output from ACI, using UV-VIS spectrophotometry, as described in section 2.2.4. The ^{99m}Tc levels in each flask were calculated as a percentage of the total activity measured.

The fine particle fraction (FPF) of the delivered dose of radiolabel and drug was calculated as the proportion of the ex-valve dose in particles < 4.7 µm in diameter (sum of Andersen plate 3-filter). The fine particle mass of drug (FPM, measured in µg) and the FPF of both radiolabel and drug was calculated as the proportion of the ex-valve dose in particles < 4.7 µm in diameter (sum of Andersen plate 3-filter).

The particle size distribution of commercial drug delivered from the pMDI canister was measured both before and after radiolabelling in order to validate that the drug dose delivered from the commercial canister was not significantly altered by the radiolabelling procedure as shown in Figure 2-7.

2.2.10.3 Quality assurance of validation method

The delivered drug dose was defined as the drug exiting the pMDI or the drug exiting the pMDI-spacer, which would be delivered to the patient from the delivery device.

[432] The ^{99m}Tc label was confirmed to be a suitable marker for drug, when the ratio of the % ^{99m}Tc activity in the FPF and the % FPF of the delivered drug dose was within the specified reference range of 0.8 and 1.2, based on currently accepted consensus opinion.[314] [433, 434]

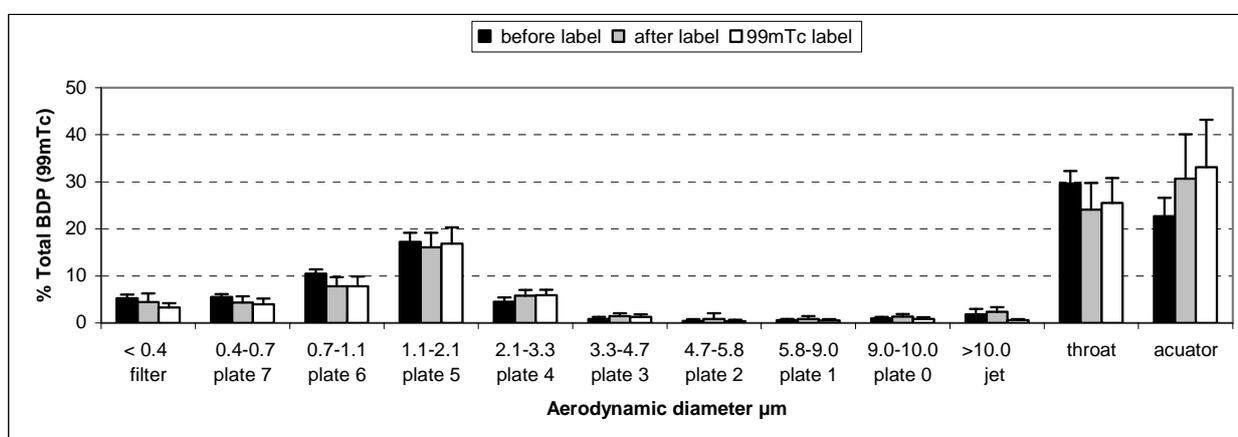


Figure 2-7: The y-axis shows the % total drug (BDP) delivered to the cascade impactor *via* pMDI ‘before label’ and ‘after label’ and % ^{99m}Tc -label (n=10). The x-axis shows the aerodynamic particle size fractions (µm).

2.2.10.4 Activity ^{99m}Tc per actuation

The ^{99m}Tc output from the radiolabelled pMDI canister was measured to ensure that the delivered dose of ^{99m}Tc inhaled by each subject did not exceed 2-4 MBq, depending on age, so that the radiation dose was within acceptable limits, as described in 2.2.7.2, Figure 2-3. [430]

Prior to inhalation, the total activity per actuation was measured by firing five actuations from the radiolabelled pMDI canister onto a glass wool filter, enclosed in a cylinder, attached to a suction pump at 28.3 L/min. The glass wool filter was measured in the

ionisation chamber to determine the activity per actuation. The activity of the radiolabelled canisters per actuation was in the range of 1-2 MBq, depending on the initial activity of ^{99m}Tc in each canister.

2.2.11 Gamma scintigraphy

The Toshiba GCA 7200DI (Toshiba Australia) and the E.CAM (Siemens Healthcare, USA) are dual-headed, large detector gamma cameras with an open gantry dedicated to planar and SPECT imaging of whole-body deposition (Toshiba GCA, Chapter 1, section 1.9.3.1, Figure 1-17 and E.CAM, Figure 2-8).[435] Low energy high resolution collimators were positioned on the dual detectors to simultaneously acquire planar anterior and posterior static images for 120 s with a 256×256 matrix. Recommended photopeak settings of 20% for the ^{99m}Tc energy peak (140 keV) were used and the sensitivity of the high resolution collimator was obtained from the manufacturer specifications for the camera/collimator system.



Figure 2-8: Dual detectors of the E.CAM gamma camera with patient bed positioned between collimators.

2.2.11.1 Transmission scan

An activity of 37 MBq $\text{NaTc}^{99\text{m}}\text{O}_4$ was drawn into a 2 mL shielded syringe and injected into a perspex flood source filled with water. The flood source was mixed vigorously for 10 minutes. The uniform $^{99\text{m}}\text{Tc}$ flood source was placed on the collimator of the gamma camera and an initial two minute flood source image was acquired. Each subject had a two minute anterior transmission scan in the supine position, with the uniform flood positioned on the collimator, underneath the subject, as described in Chapter 1, section 1.9.3.1, Figure 1-18.

2.2.11.2 Inhalation of radiolabelled drug

The radiolabelled pMDI canister was placed in an actuator and then into a lead shield. After the transmission scan each child inhaled 2-3 doses of the radiolabelled drug so that the dose was within 2-4 MBq, depending on age (Chapter 2, section 2.2.7.2). The radiolabelled canister was shaken between each administered dose. The child was repositioned between the two detectors of the gamma camera and simultaneous anterior and posterior planar scintigraphic images (120 seconds acquisition time) of the chest and abdomen and lateral images of upper airway were obtained (Figure 2-9).

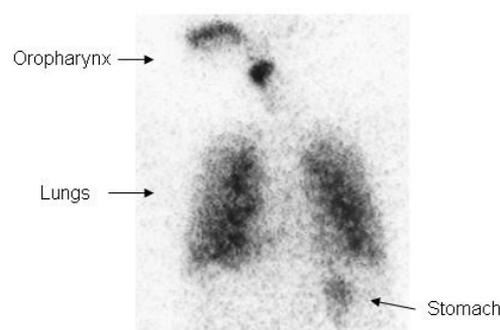


Figure 2-9: Anterior gamma scan showing $^{99\text{m}}\text{Tc}$ distribution in lungs, mouth, throat and stomach.

2.2.11.3 Exhaled dose

Immediately after inhaling the radiolabelled drug each child was instructed to exhale gently into a low resistance filter so that the exhaled fraction of radioactive drug could be assessed. The patient image was followed by a two minute image of the actuator, spacer and filter so that the total activity and the exhaled dose could be calculated.

Children commonly cough after inhaling from a pMDI and this was a technical limitation for an accurate measure of the exhaled dose.[436]

2.2.12 Gamma scan image analysis

2.2.12.1 Attenuation factors (AF)

The ratio of counts detected with the subject lying between the uniform flood source and the camera and counts detected without the subject between the flood source and the camera is related to the broad beam tissue attenuation (μ) and the tissue depth (x) by the following formula:[437]

$$N_t / N_o = e^{-\mu \cdot x}$$

An approximation for broad beam tissue attenuation and tissue depth is made using the geometric count rate. Anterior and posterior views allow calculation of a geometric mean (the geometric mean is the square root of the product of counts in the anterior and posterior regions of interest), which more consistently represents the amount of tracer in regions of interest independent of depth.[327]

Gamma photons emitted from the uniform flood source were attenuated by body tissues and attenuation factors were derived for each child due to absorption by their body tissues as described by Macey and Marshall.[328] Attenuation factors were generated using the square root of the counts per pixel in the transmission image of the ^{99m}Tc

flood source divided by the counts per pixel in regions of interest attenuated by each child.[328]

2.2.12.2 Regions of interest (ROI)

Gamma scintigraphic scans were analysed by defining ROI from the patient's transmission scan (pre-inhalation) and superimposing the ROI onto the anterior and posterior scans from the same patient (post-inhalation). Separate count rates were obtained for the right and left lungs, stomach, oesophagus, mouth, oropharynx, actuator, spacer and filter. Each total count was corrected for background counts and decay-corrected to the time of the patient scan.

The sensitivity of the gamma camera was estimated as the counts per second (cps) detected by the camera/collimator system per MBq. The count rate (cps/cm²) from a uniform flood source (No) and attenuation factors for each child were derived from the body attenuation of the uniform flood source image (Nt) and geometric means of corresponding anterior and posterior counts (G) were calculated to correct for tissue depth.[328] The counts per second (cps) detected by the camera were related to the ^{99m}Tc activity (A in MBq) and the camera sensitivity factor (E, cps/MBq) in the following equation (Table 2-8):

$$A = [G / E] \times [Nt / No]^{1/2}$$

Table 2-8: Example of count rates (cps) required to derive activity (MBq) in each ROI.

ROI (cps/cm ²)	G (cps/cm ²)	Nt/No (cps/cm ²)	E (cps/MBq)	MBq
Right lung	[Anterior*posterior] ^{1/2}	Transmission image / ^{99m} Tc flood source	Sensitivity	A

The dose deposited in the lungs was expressed as a percentage of the dose emitted from the inhaler, which is the sum of the total dose deposited in the body. The dose

deposited in the lungs was expressed as a percentage of the total dose delivered from the actuator (i.e. % ex-actuator).

The ^{99m}Tc may preferentially adhere to the plastic actuator if there is an electrostatic charge on the plastic. Static charge was reduced on the actuator and spacer by washing with detergent and drip drying, as this has been reported as reducing static charge.[168] Leach and Devadason have reported the % ex-actuator dose in previous deposition studies for both adults and children.[150] [112] Therefore the % ex-actuator dose was chosen as a more representative match for drug and label, however several reports show % ex-valve dose so these values were also given in selected tables for comparison.

Count rates (cps/cm^2) were also obtained for central and peripheral regions from each lung. The pulmonary regional distribution was determined by calculating the ratio of peripherally (P) and centrally (C) deposited activity. Deposition in the central region was measured as half the width of the lung and one-third the height and the remaining region was measured as the peripheral region Figure 2-10. [349]

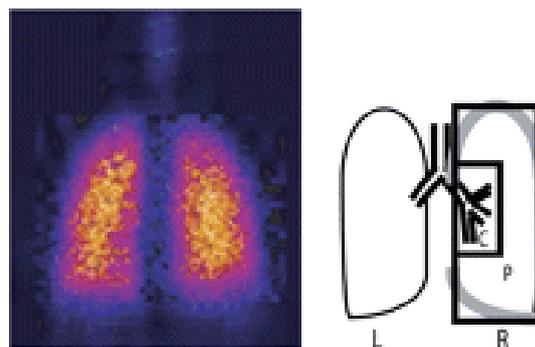


Figure 2-10: Gamma scan of lungs* (left) with ROI showing the central zone (C) and the peripheral zone (P, right).

*Reproduced with permission of the American Thoracic Society. Copyright © American Thoracic Society. Donaldson *et al*, [438] Proc Am Thorac Society 2007; 4(4): 399-405. Official Journal of the American Thoracic Society.

2.2.13 Breathing recordings

Thirty-four children, 5-17 years of age, were instructed to inhale *via* pMDI with an attached small volume spacer, either the Aerochamber Plus™ (AC+) or the Funhaler (FH), using either 5 tidal breaths or a slow single maximal inhalation followed by a ‘breath hold’ for 5-10 seconds. A subset of children (n=10) had simultaneous breathing recordings while inhaling radiolabelled drug (FP) *via* pMDI-spacer (AC+). The children’s breathing patterns were recorded using a linear pneumotachometer (RSS 100 Hans Rudolph, Kansas, USA) connected to a custom-built airtight perspex flow chamber containing the spacer device, without influencing airflow resistance.

A detergent-coated spacer device (AC+ or FH), with an attached placebo pMDI, was secured within the custom-built flow chamber as shown in Figure 2-11.[439] The placebo pMDI was actuated at the start of the inhalation technique and the breathing pattern was recorded by the pneumotachometer (Model RSS100-HR) attached to the flow chamber. The inlets to the flow chamber were sealed with dental putty in order to keep the flow chamber airtight during the recording procedure. Changes in flow and air pressure were measured through the membrane of the pneumotachometer.



Figure 2-11: Breathing recording equipment with pneumotachometer attached to the top of an airtight flow chamber with bias flow inlet tubing and spacer device (AC+) secured*.

*Reproduced with permission; Dr Schultz, Aerosol Research Group, School of Paediatrics and Child Health, UWA.

Each breathing pattern was recorded *via* computer software (Research Pneumotach System, KORR Medical Technologies; Windows Software Version 3.07b) and stored in an RSS file format. A bias flow of air was simultaneously delivered to the flow chamber at 4 L/min in order to prevent the build-up of carbon dioxide inside the flow chamber due to exhalation.[439]

2.2.13.1 Inspiratory parameters

The breathing recording program (Research Pneumotach System, KORR Medical Technologies; Windows Software Version 3.07b) was used to generate a signal file. This signal file was edited using Microsoft© Excel program to select the data for the corresponding first breath and saved as a text file. A custom made software, the breathing simulation calculation (BSC) program (Programmer Dr Brad Zhang, University of Western Australia), was used to calculate the various inspiratory parameters of the breath from the text file such as volume of inhalation, peak inspiratory flow and peak inspiratory time.

2.2.14 Flow-Volume Simulation (FVS)

The *in vivo* recorded breathing patterns were replicated *in vitro* with the Flow-Volume Simulator (FVS). The Series 1120 Flow-Volume Simulator is a device used for testing spirometers and other flow and volume measuring devices. The simulator consists of a rigid cylinder and a piston that is positioned in the cylinder by a linear actuator. A motion control computer controls the speed and displacement of the piston. The simulator is capable of generating all of the waveforms specified by the American Thoracic Society for testing spirometers. The simulator can be operated in a continuous mode to simulate breathing. The Waveform editor software is used to create the files that define the flow waveforms that are reproduced on the simulator.

2.2.14.1 Waveform conversion

Each child's recorded breathing waveform (acquired in RSS file format) was converted into FVW file format using a waveform conversion program (RSS Convert Software Version 1.04, Integrated Control System Incorporation) so that the waveform was compatible use with the Flow-Volume Simulator (FVS). The bias airflow was subtracted from the recording. The generated waveforms were edited so that each recorded breathing pattern commenced with the first inhalation. The breathing waveforms were replayed on the Flow-Volume Simulator (FVS) in order to simulate each subject's actual breathing pattern (Figure 2-12 and Figure 2-13).

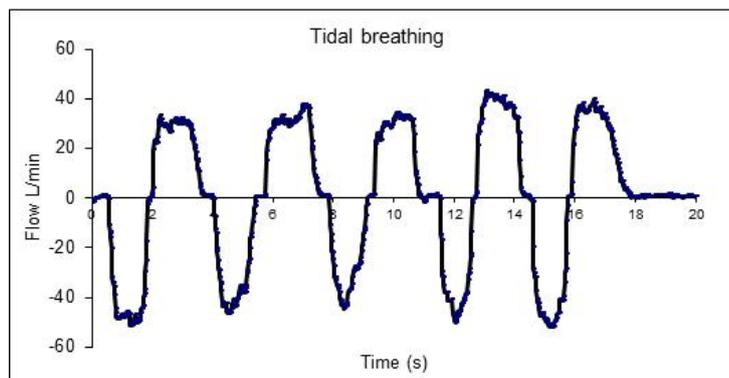


Figure 2-12: Representative waveform generated for 5 tidal breaths where negative flow indicates inhalation and positive flow indicates exhalation.

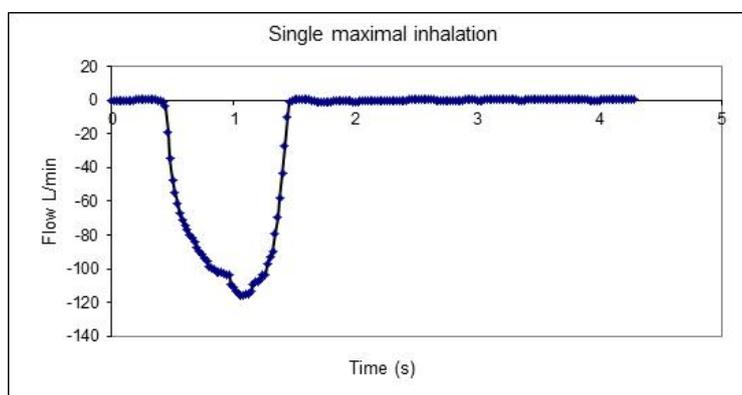


Figure 2-13: Representative waveform generated for single maximal inhalation.

2.2.14.2 Flow Calibration

Prior to each recording the pneumotachometer was calibrated using a 3 L syringe (Koko®, Pulmonary Data Service Instrumentation). The RSS100-HR calculates the error between the syringe volume and the measured inspiratory and expiratory volumes. The temperature and humidity were recorded. A correction factor was calculated for both inspiratory and expiratory flow-rates.

2.2.14.3 Validation of flow chamber for pMDI-spacer

In this study the children's breathing patterns were recorded in duplicate with the pMDI-spacer secured in a flow chamber, in order to create waveforms specific for each child. To ensure that the breathing patterns recorded in the flow chamber *in vivo* matched the experimental *in vitro* breathing patterns without the flow chamber, the *ex vivo* waveforms were replayed on the FVS (HR1120) and recorded a second time so that they could be replicated *in vitro*, as shown in Figure 2-14 (a).

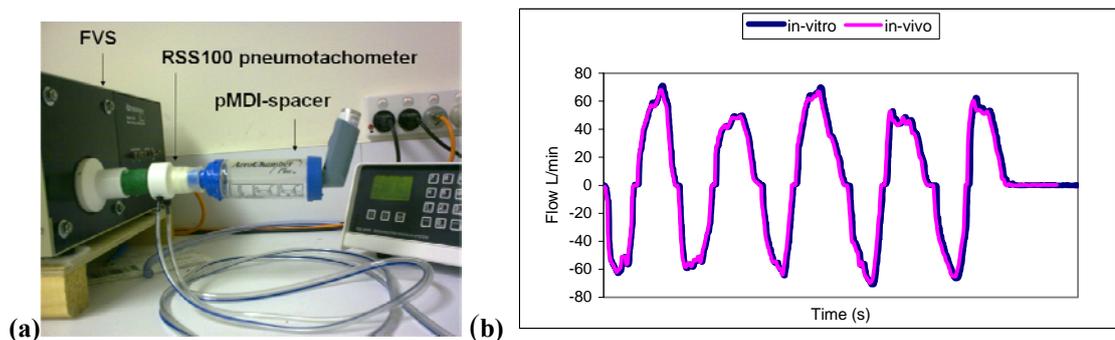


Figure 2-14: (a) FVS with attached pneumotachometer and pMDI-spacer (AC+)*. (b) The y-axis is flow in L/min and the time on the x-axis is recorded in time (s). Example of *in vivo* (pink) breathing pattern recorded with laminar flow chamber and *in vitro* replicated trace without the laminar flow chamber (blue).

*Reproduced with permission; Dr Looi, Aerosol Research Group, School of Paediatrics and Child Health, UWA.

Schultz *et al* have previously validated that the flow chamber did not affect the waveforms.[439] For this thesis, a set of waveforms were created in a subgroup of subjects (n=10) to validate that the *in vivo* waveforms matched the *in vitro* waveforms. The replicated waveforms (blue) created without the laminar flow chamber were shown to overlay the original *in vivo* waveforms (pink) as shown in Figure 2-14 (b).

2.2.14.4 FVS-filter 2 study

The outlet of the FVS was connected to an inspiratory filter (F2) and attached to the pMDI-spacer (Figure 2-15). Three puffs of HFA-FP were actuated with a synchronised replay of the child's waveform file on the FVS. The inspiratory filter, actuator and spacer were washed in 50 mL methanol so that the FP level could be assessed with UV-VIS spectrophotometry, section 2.2.4. The inspiratory filter (F2) was secured between the FVS and the spacer (F2-FVS) and a measure of drug output was later compared with the total body gamma deposition (TBD) *in vivo*.

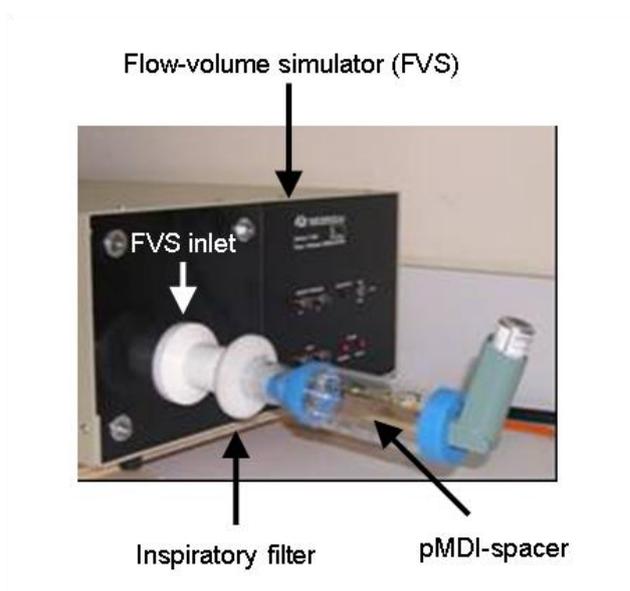


Figure 2-15: Flow-Volume Simulator with inspiratory filter attached to FVS inlet and pMDI-spacer*.

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2.2.14.5 FVS-NGI

The Flow-Volume Simulator (FVS) was connected to the Next Generation Impactor (NGI) *via* a Y-piece of plastic tubing connected to a T-piece as shown in Figure 2-16 and Figure 2-17. The right branch of the Y-piece of the circuit was attached to a pressurized air source with a constant flow of 100 L/min. The NGI was operated with a continuous flow regulated vacuum pump at 100 L/min. The left branch of the Y-piece was attached to the FVS. A linear pneumotachometer was attached to the T-piece and the air source was adjusted so that before the breathing simulator was turned on, the net airflow was zero at the T-piece in the circuit (Figure 2-16). In a similar experimental set-up Foss and Keppel have previously validated that airflow through the pMDI is regulated by the breathing simulator.[416]

The pneumotachometer was removed and the T-piece was attached to pMDI-spacer (Figure 2-17). The child's waveform was replayed with the FVS, which 'inhaled' drug into the impactor. The total drug (FP) output and particle size distribution were measured at the calibrated constant flow of 100 L/min, however the inhalation flow replayed with the FVS could not exceed that of the calibrated flow of the NGI.[84, 416]

2.2.14.6 Drug output from FVS-NGI

The aerosolised drug (FP) passes the T-piece and was drawn into the cascade impactor by the vacuum pump, as described by Foss and Keppel.[416] The particles in the inhaled aerosol cloud are collected and sorted by aerodynamic size as they pass through the cascade impactor at a flow of 100 L/min. Drug (FP) was washed from the actuator, 'throat', spacer and impaction cups and MOC filter and assayed with UV-VIS spectrophotometry, as described in section 2.2.4, Figure 2-2 and section 2.2.8.1. The mass of drug measured in particles smaller than 3.4 μm aerodynamic diameter (cups 3-7 + MOC filter) were defined as the extrafine particle or (EFPF < 3.4 μm). Newman and

Chan have suggested that the EFPF was a more realistic measure of lung deposition, section 2.2.8.1.[109] Drug particles less than $2.2\ \mu\text{m}$ (impaction cups 4-7 + MOC filter) were referred to as the ultrafine particle fraction (UFPF $< 2.2\ \mu\text{m}$). Newhouse *et al* suggested that this fraction of particles reflects lung deposition.[110]

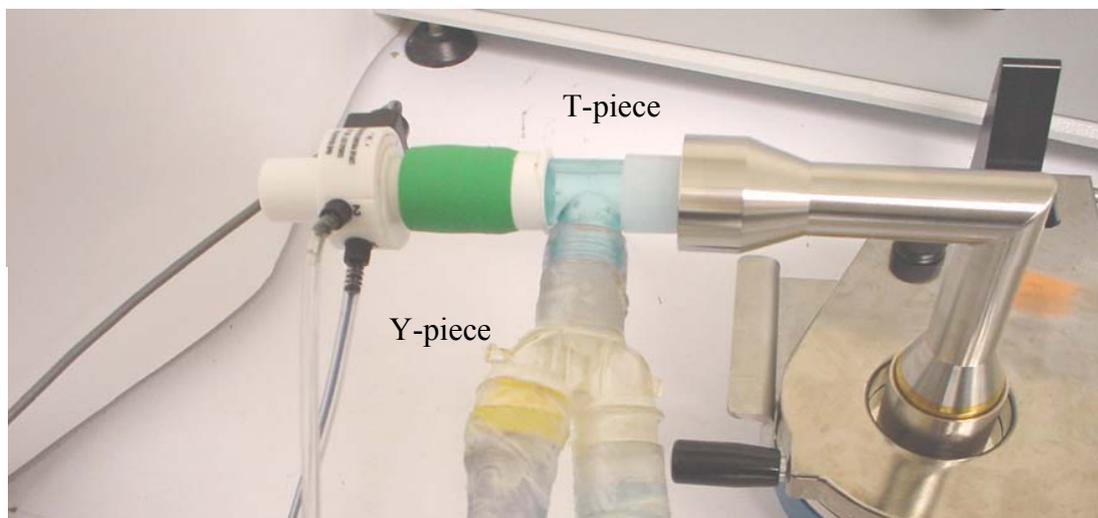


Figure 2-16: Pneumotachometer attached to green adaptor on the left branch of T-piece and right branch of T-piece attached to the USP throat model attached to NGI*. The left branch of the Y-piece is attached to the FVS and the right branch of the Y-piece has the positive airflow of 100 L/min.

*Reproduced with permission; Dr Looi, Aerosol Research Group, School of Paediatrics and Child Health, UWA.

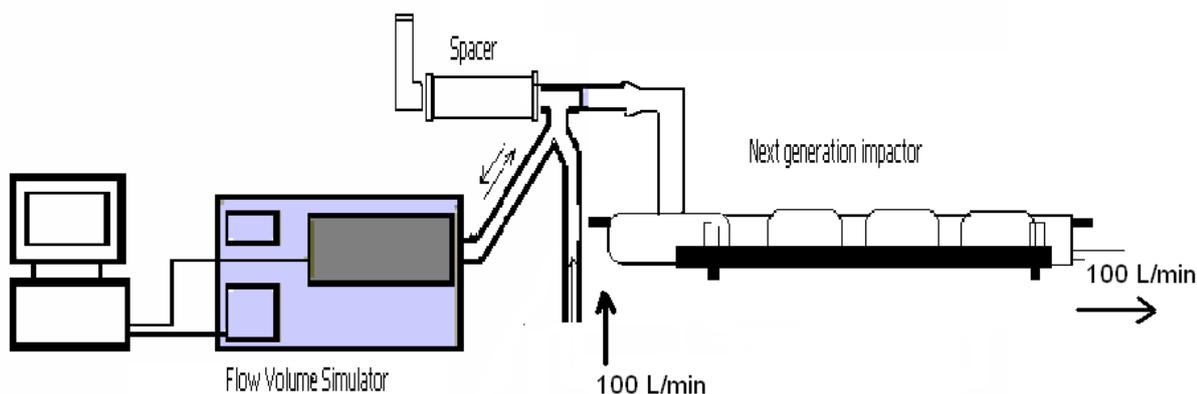


Figure 2-17: Schematic representation of Flow-Volume Simulator connected to left arm of the y-piece and pMDI-spacer attached to the left arm of the T-piece*. The right arm of the Y-piece has a positive flow of 100L/min. The right arm of T-piece is attached to the Next Generation Impactor with a suction of 100L/min.

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2.2.15 Statistical Analysis

2.2.15.1 Validation

Statistical analysis was carried out using Microsoft© Excel Analysis ToolPak (Microsoft Corporation, Redwood, WA, USA) using the paired t-test for sample means to compare the total drug output from the radiolabelled drug (FP) with the ^{99m}Tc label. The paired t-test for means was used when the same canister was compared. The unpaired t-test was used to compare the reference unlabelled commercial drug with radiolabelled drug, when the reference canisters were not the same canisters as the radiolabelled canisters. Differences between groups were deemed statistically significant when $p < .05$.

2.2.15.2 Gamma and FVS

Power calculations were performed, based on previous studies with radiolabelled QVAR™ and salbutamol by Devadason *et al* and Wildhaber *et al* respectively.[150] [171] To ensure adequate statistical power > 80%, I have recruited twenty-four children for the QVAR™ deposition study and thirty-six children for the Flixotide® deposition study. Lung doses, oropharyngeal and gastrointestinal doses and spacer retention of ^{99m}Tc-HFA-FP were presented with means and the corresponding standard deviations (SD). The variability was estimated as the coefficient of variation (CV%) which was calculated as the SD as a percentage of the mean. The spacer groups (AC+ and FH) were investigated with SPSS 16.0 to determine if the distributions approximated normal distributions.

The difference in the regional deposition was compared between ‘tidal’ and ‘breath hold’ groups using unpaired t-tests and between the three age-groups using analysis of variance (ANOVA) and analysis of covariance (ANCOVA). With Flixotide®, ANOVA was used to compare the difference between the three delivery methods: ‘tidal’ AC+, ‘breath hold’ AC+ and FH. Post-hoc tests were carried out with Fisher’s Least Significant Difference method. Independent sample T tests were used to compare the means of inspiratory parameters, drug output and gamma scintigraphy. The Pearson correlation coefficient (r) was estimated, using the bivariate correlations procedure. With the graphical representation of data produced with Microsoft© Excel, the squared correlation coefficient was given by r^2 .

In order to minimize the influence of lung function parameters, adjusted means and 95% confidence intervals of lung deposition were presented. The adjusted means were estimated using general linear models, assuming that children in ‘breath hold’ and ‘tidal’ groups had the same value of FVC. The adjustment aimed to minimize the influence of the difference in FVC on the lung deposition between the two groups. The interaction on lung and oropharyngeal and gastrointestinal deposition was also explored between age and groups (‘breath hold’ and ‘tidal’) using general linear models.

The bivariate correlations procedure and scatter plots were used to look for linearity in relationships between the independent variables and the main outcome variables, lung deposition and oropharyngeal and gastrointestinal deposition. Linear regression was used to determine the factors predictive for lung deposition and oropharyngeal and gastrointestinal deposition. The multiple correlation coefficient was given by R and adjusted R^2 gave an estimate of the predictability of the model in the general population. These statistical analyses were conducted using the SPSS 16.0 package.[440]

3 DEPOSITION OF HFA-BDP

The following peer reviewed article has been published from work carried out for this chapter of the thesis:

Spacer inhalation technique and deposition of extrafine aerosol in asthmatic children. Roller CM*, Zhang G*, Troedson RG¹, Leach CL², Le Souëf PN* and Devadason SG*.

European Respiratory Journal 2007; 29: 299-306.

ABSTRACT: The aim of the present study was to measure airway, oropharyngeal and gastrointestinal deposition of ^{99m}Tc labelled hydrofluoroalkane-beclomethasone dipropionate after inhalation *via* a pressurised metered-dose inhaler spacer (Aerochamber Plus™) in asthmatic children.

A group of 24 children (aged 5-17 yrs) with mild asthma inhaled the labelled drug. A total of 12 children took five tidal breaths after each actuation ('tidal' group). The other 12 children used a slow maximal inhalation followed by a 5-10 s breath hold ('breath hold' group). Simultaneous anterior and posterior planar scintigraphic scans (120 s acquisition) were recorded.

For the 'tidal' group, mean±SD lung deposition (% ex-actuator, attenuation corrected) was 35.4±18.3, 47.5±13.0 and 54.9±11.2 in patients aged 5-7 (n=4), 8-10 (n=4) and 11-17 yrs (n=4), respectively. Oropharyngeal and gastrointestinal deposition was 24.0±10.5, 10.3±4.4 and 10.1±6.2. With the 'breath hold' technique, lung deposition was 58.1±6.7, 56.6±5.2 and 58.4±9.2. Oropharyngeal and gastrointestinal deposition was 12.9±3.2, 20.1±9.5 and 20.8±8.8. Inhalation of the extrafine formulation with the 'breath hold' technique showed significantly improved lung deposition compared with tidal breathing across all ages. Oropharyngeal and gastrointestinal deposition was markedly decreased, regardless of which inhalation technique was applied, compared with a previous paediatric study using the same formulation delivered *via* a breath-actuated metered-dose inhaler.

KEYWORDS: Children, deposition study, inhalation technique, spacers

Co-authors contribution: *UWA Research Group; ¹Department of Nuclear Medicine, Princess Margaret Hospital; ²Lovelace Respiratory Research Institute, USA.

Roller, CM*	Experimental work, data analysis and manuscript writing	75%
Devadason, SG*	Aerosol research expert advice	
Le Souef, PN*	Respiratory research expert advice	
Zhang, G*	Statistical analysis advice and graphical presentation	
Troedson, RG ¹	Nuclear Medicine specialist	
Leach, CL ²	Respiratory research expert advice	

3.1 INTRODUCTION

Asthma is recognised as a chronic inflammatory disease affecting the large and small airways of both adults and children.[3-5] Inhaled corticosteroids (ICS) are recommended as prophylactic treatment of asthma in children with persistent asthma symptoms.[228, 229] Topical airway targeting largely determines the efficacy of ICS and aerosol particle size is one of the key determinants for airway targeting.[243, 441] Factors affecting efficacy include the child's age, the particle size of the aerosol, the delivery device, the inhalation profile and the geometry of the airways.[122]

Beclomethasone dipropionate (BDP) is one of the most commonly used corticosteroids for the treatment of asthma. With the environmental concerns about the ozone layer, chlorofluorocarbons (CFC) have largely been phased out as propellants in pressurised metered-dose inhalers (pMDI) and replaced by hydrofluoroalkanes (HFA).[442] By changing the type of propellant there has been a change in deposition characteristics, associated with the smaller particle size.[244, 443]

Beclomethasone dipropionate (BDP) reformulated with HFA-134a, with ethanol as a cosolvent (HFA-BDP or QVAR™, 3M Health Care Ltd, UK) produces an extrafine aerosol as the propellant evaporates, and the aerosol has a mass median aerodynamic diameter (MMAD) of approximately 1.1 µm.[318] The formulation has a lower spray force, a warmer temperature and is in solution, rather than suspension.[270] These changes in the properties of the aerosol are associated with improved lung deposition and improved penetration of the aerosol into the peripheral airways, and this may be associated with improved asthma control and health-related quality of life.[444, 445]

HFA-BDP has been recommended by the National Asthma Council of Australia for the treatment of childhood asthma because of its small particle size and its improved clinical effect at a lower dose.[201] Clinical studies have shown efficacy of HFA-BDP

at half the dose of CFC-BDP.[73, 112, 446] A 12 month study of 300 children with asthma aged 5-11 years demonstrated that extrafine HFA-BDP provided long-term maintenance of asthma control at approximately half the dose compared with CFC-BDP.[447] There were no clinically meaningful differences between HFA-BDP extrafine aerosol and conventional CFC-BDP with regard to growth or other systemic effects.[447]

High lung deposition of HFA-BDP delivered after inhalation *via* a breath-actuated MDI (Autohaler™), has been described by Devadason *et al* in a previous paediatric deposition study.[150] However, gastrointestinal drug deposition was as high as 60% in children from 5-14 years of age, after inhalation *via* Autohaler™. Drug reaching the gastrointestinal tract is unnecessary and may contribute to both local and systemic side-effects.[270, 448] Approximately 18% HFA-BDP dose in the gastrointestinal tract becomes systemically available.[257]

Spacer devices attached to pMDIs are recommended for children using inhaled corticosteroids, in order to reduce the impaction of the larger drug particles in the oropharynx and minimise drug reaching the gastrointestinal tract.[171, 449] Younger children are able to use tidal breathing with spacer devices, thereby minimising problems coordinating their inhalation with actuation. The GINA guidelines suggest that for children who can use the Aerochamber Plus™ spacer device, the optimal inhalation technique is a slow, deep breath in, followed by a 'breath hold' of about 10 seconds.[23] It is recommended that children who are unable to perform this inhalation technique use tidal breathing.[23]

Janssens *et al* described high lung deposition of extrafine QVAR™ delivered *via* pMDI-spacer (AC+) to an infant model using simulated tidal breathing.[417] However, another study reported that there was substantial dose variability in drug delivery in

children using a pMDI-spacer at home.[165] Spacer devices have been shown to reduce the oropharyngeal and gastrointestinal deposition of inhaled corticosteroids, thereby improving lung deposition.[183, 450] However different modes of inhalation are known to affect drug delivery from pMDIs.[74, 115, 174, 451] Therefore it would be important to evaluate the effect of two commonly used inhalation techniques on the delivery of BDP *via* pMDI-spacer to children with asthma.

Lung deposition of HFA-BDP delivered *via* pMDI-spacer with tidal breathing, compared with the slow single maximal inhalation technique followed by a 5-10 second 'breath hold', were assessed in this chapter in order to show the effect of the inhalation technique on the delivery of this extrafine formulation *via* pMDI-spacer. Both the tidal breathing technique and the 'breath hold' technique are commonly recommended by clinicians when training children to use pMDI-spacers. All children included in the study were able to maintain the 'breath hold' for a minimum of 5 seconds.

Gamma scintigraphy has been used to assess the deposition of radiolabelled HFA-BDP in both adults and children, however the delivery has not been previously assessed in children when HFA-BDP was delivered *via* pMDI-spacer (Aerochamber Plus™). A 'breath hold' technique has been shown to improve lung deposition of inhaled drugs but this technique has not been evaluated in children with gamma scintigraphy.[69, 425] Therefore children will be trained to inhale the radiolabelled drug *via* pMDI-spacer using either tidal breathing or a slow single maximal inhalation followed by a 'breath hold' for 5-10 seconds.

There are conflicting views about the exact measure of fine particle fraction in the literature. In this chapter the FPF is defined as the particles < 4.7 µm in aerodynamic diameter, as described by Mitchell *et al.*[84] Farr *et al* have suggested that a predicted lung deposition can be derived from the FPF measured by cascade impaction.[106]

Finlay *et al* have reported that the FPF consists of particles between 1.1-4.7 μm . [105] Newman and Chan have suggested that the extrafine particle fraction is predictive of lung deposition. [109] Newhouse *et al* suggest that the ultrafine particle fraction is the most clinically relevant. [110] In this chapter these *in vitro* measure of lung deposition will be compared with gamma scintigraphic measure of lung deposition. [106]

3.2 OBJECTIVES

- Assess lung deposition and total body distribution of QVAR™ (HFA-BDP) delivered *via* pMDI-spacer (Aerochamber Plus™) to children with mild asthma.
- Compare lung deposition and oropharyngeal and gastrointestinal deposition, using two different breathing techniques.
- Compare the total body deposition and lung deposition obtained with gamma scintigraphy with *in vitro* methods of drug output and fine particle fraction.
- Compare the predicted lung deposition methods based on the fine particle fraction exiting pMDI-spacer with Andersen Cascade Impaction.
- Compare the total body deposition with the inspiratory filter dose.

3.3 HYPOTHESES

- Extrafine QVAR™, in combination with pMDI-spacer, Aerochamber Plus™ (AC+), will show equivalent lung deposition compared to the breath-actuated pMDI device (Autohaler™), in children with mild asthma.
- The pMDI-spacer (AC+) will reduce oropharyngeal deposition of QVAR™ compared to Autohaler™.
- A ‘breath hold’ technique will improve lung deposition of the extrafine QVAR™ delivered *via* pMDI-spacer (AC+) in children, compared to tidal breathing.
- *In vitro* measures of drug output and fine particle fraction with Andersen Cascade Impaction (ACI), will overestimate lung deposition with gamma scintigraphy and underestimate variability.
- The total drug measured on an inspiratory filter will overestimate total body deposition (TBD).

3.4 METHODS

3.4.1 Study design

A series of *in vitro* and *in vivo* methods were carried out in order to assess the deposition of ^{99m}Tc-HFA-BDP delivered *via* pMDI-spacer (AC+) in asthmatic children 5-17 years of age (Table 3-1). On each study day written, informed consent was provided by parents or a legally acceptable representative (Chapter 2, section 2.2.7).

Table 3-1: Experimental steps required for the deposition study with QVAR™.

<i>In vitro</i> characterisation of drug output and fine particle fraction of HFA-BDP with ACI.
Height, weight, lung function and inhalation training.
Inspiratory filter drug deposition of HFA-BDP with two different inhalation techniques.
Validation of radiolabelling method for ^{99m} Tc-HFA-BDP delivered <i>via</i> pMDI.
Transmission scan with dual-headed gamma camera for each subject.
Gamma scan after inhalation of ^{99m} Tc-HFA-BDP delivered <i>via</i> pMDI-spacer (AC+) with two different inhalation techniques.

3.4.2 Study population

Twenty-five children (all male) aged 5-17 years, with mild, stable asthma were recruited from outpatient clinics at Princess Margaret Hospital for Children. Eligibility and exclusion criteria were presented in Chapter 2, section 2.2.1 and 2.2.2. Only those patients with FEV1 > 80% predicted values were enrolled in the study, as detailed in Chapter 2 section 2.2.3.[429]

One child did not receive the dose of Ventolin® prior to gamma scintigraphy, however his preliminary FEV1 was 98% predicted. One child was excluded from the study because he could not attain an FEV1 > 80% predicted. On the study day, each child had weight, height and lung function measured (Table 3-2).

Table 3-2: Means of height (cm), weight (kg), lung function (FEV1) (L) and FVC (L) in the children of ‘tidal’ and ‘breath hold’ groups*.

Age group (yrs)	N	Height (cm)	Weight (kg)	FEV1 (L)	FVC (L)
Tidal					
5-7	4	124.1±4.8	25.1±4.1	1.5±0.2	1.7±0.2
8-10	4	136.7±8.0	29.3±4.4	1.9±0.3	2.3±0.3
11-17	4	159.4±17.2	53.9±19.5	3.3±1.0	3.9±1.0
Breath hold					
5-7	4	119.1±5.0	23.6±0.6	1.8±0.3	1.8±0.1
8-10	4	130.7±14.0	30.4±8.6	1.7±0.5	2.0±0.6
11-17	4	155.3±8.5	42.9±5.3	2.4±0.3	3.0±0.3

*: Data are presented as mean±SD

3.4.3 Inhalation technique

Each child was trained to perform either tidal breathing (n=12) or a single maximal inhalation followed by a 5-10 s ‘breath hold’ (n=12) as described in Chapter 2, section 2.2.5. The training procedure for the inhalation technique was repeated 3-5 times, so that the child understood the correct technique before inhaling the radiolabelled BDP. The two inhalation groups were referred to as ‘tidal’ and ‘breath hold’ respectively.

The children were divided into three subgroups according to age: 5-7 years (n=4), 8-10 years (n=4) and 11-17 years (n=4), in order to compare results with the previous paediatric deposition study by Devadason *et al*, in which the Autohaler™ device was used to deliver the same radiolabelled formulation.

3.4.4 Inspiratory filter study

Prior to gamma scintigraphy each child performed a preliminary inspiratory filter study. Children were trained in the correct inhalation technique before inhaling the radiolabelled drug, as detailed in Chapter 2, section 2.2.5 and 2.2.6. The amount of

drug deposited on the filter was measured by UV-VIS spectrophotometry, Chapter 2 section 2.2.4 and compared with the total body deposition of radiolabelled drug.

3.4.5 *In vitro* particle sizing of HFA-BDP

The preliminary investigations were performed with cascade impaction in order to characterise commercial QVAR™ and assess the aerodynamic particle size distribution before radiolabelling (Chapter 2, section 2.2.8). Ten commercial pMDI canisters were primed and then inserted into the ‘throat’ of the cascade impactor. The particle size distribution of QVAR™ with pMDI alone was compared with pMDI with attached spacer in order to assess the effect of the spacer device on the drug delivery profile (Figure 3-1).

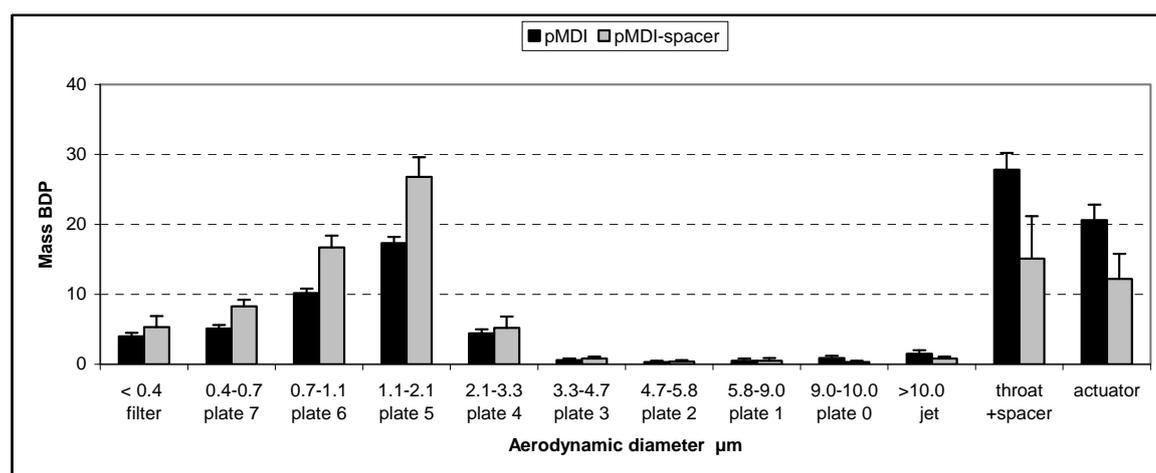


Figure 3-1: Preliminary particle size distribution with Andersen Cascade Impaction (ACI) and mean mass BDP µg delivered *via* pMDI and pMDI-spacer (n=10) with corresponding particle sizes (µm).

Twenty doses of HFA-BDP (100 µg/actuation) were drawn into the cascade impactor at a continuous flow of 28.3 L/min, in order to optimise analytical sensitivity and to minimise bounce effects.[452]

3.4.6 Validation of radiolabelling technique for QVAR™

The radiolabelling method for commercial QVAR™ (100 µg/actuation HFA-BDP) was performed using a method previously described by Newman *et al*, Leach *et al* and Devadason *et al*. Sodium pertechnetate (Na^{99m}TcO₄) was mixed with 10 mL chloroform, 6 µL tetraphenylarsonium chloride hydrochloride hydrate and 30 µL strong ammonium solution (28-30%) in a glass scintillation vial.

The ^{99m}Tc was extracted into 10 mL chloroform as tetraphenylarsonium pertechnetate (AsPh₄^{99m}TcO₄) and radiolabelled as detailed in Chapter 2, section 2.2.9. After the preliminary validation series, liquid nitrogen was used in place of dry ice to supercool the contents of the commercial canister. The pre-weighed commercial canister was lowered into a dewar flask containing liquid nitrogen for 50-60 s, before decrimping.

3.4.6.1 Validation

The particle size distribution (PSD) of the commercial QVAR™ was carried out with Andersen Cascade Impaction (ACI) before radiolabelling and the PSD of the radiolabelled pMDI canister was carried out after radiolabelling, to confirm that the contents of the commercial canister were not significantly changed by the radiolabelling procedure, as detailed in Chapter 2 section 2.2.10, Figure 2-7. The fine particle mass (FPM, measured in µg) and fine particle fraction (FPF, measured as a percentage of the total) were calculated as the proportion of the ex-valve dose in particles < 4.7 µm in diameter (sum of Andersen plate 3-filter).

More consistency was found between the drug (BDP) and radiolabel (^{99m}Tc) in the pre-patient validation series, when liquid nitrogen replaced dry ice as the coolant for the commercial pMDI canister. This technical modification to the supercooling method for radiolabelled BDP decreased the variability between drug and label, as shown by the error bars in Figure 3-2 compared to Figure 2-7 (Chapter 2, section 2.2.10).[346]

Leach *et al* have shown that BDP levels, measured by high-performance liquid chromatography (HPLC) match the radioactive counts of ^{99m}Tc , measured by ionisation chamber, when only three doses of ^{99m}Tc -HFA-BDP (100 μg /actuation) were sampled and the same radiolabelling procedure was used.[112] The ratio of % ^{99m}Tc measured in the fine particle fraction to % drug (BDP) in the fine particle fraction was used to confirm that ^{99m}Tc acts as a suitable marker for BDP (Chapter 2, section 2.2.10.3).

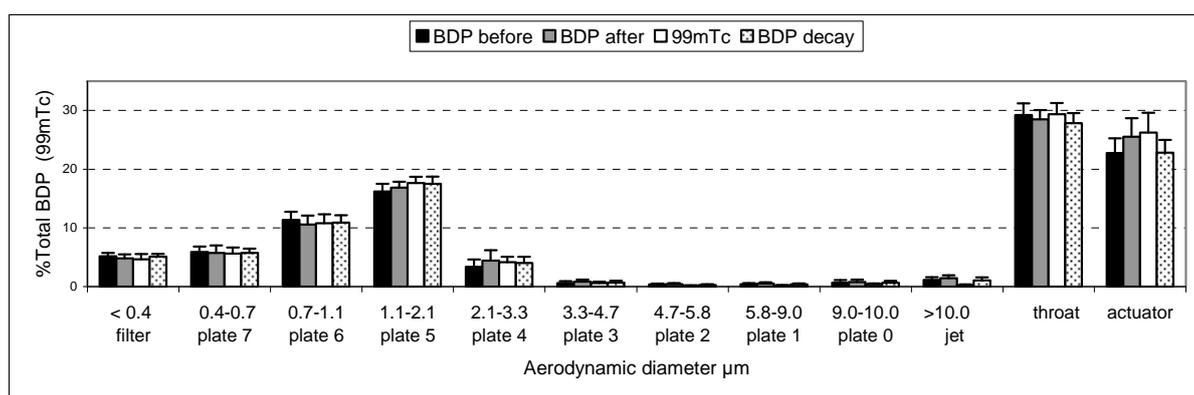


Figure 3-2: Pre-patient validation series showing the particle size distribution of commercial HFA-BDP ‘before label’, ‘after label’, ‘decay label’ and ^{99m}Tc radiolabel (n=15). The y-axis shows the % total BDP or % ^{99m}Tc *. The x-axis shows the aerodynamic diameter (μm). *Data are presented as mean (+SD) percentages of total dose.

3.4.6.2 Pre-patient radiolabel validation

The total drug output and particle size distribution of BDP from each QVAR™ canister was measured both before and after radiolabelling, as detailed in Chapter 2, sections 2.2.10.1 and 2.2.10.2. The ^{99m}Tc activity/actuation was measured to ensure that the delivered dose of ^{99m}Tc inhaled by each subject did not exceed 2-4 MBq, depending on age. After the decay of ^{99m}Tc , a third ‘decay label’ particle size distribution of BDP from the labelled canister was performed to verify the integrity of the transfer of contents from the commercial canister, as shown in section 3.4.6.1, Figure 3-2.

3.4.7 Gamma Scintigraphy

3.4.7.1 Transmission scan

Each subject had an initial two minute anterior transmission scan in the supine position, as detailed in Chapter 2, section 2.2.11.1.

3.4.7.2 Inhalation of ^{99m}Tc HFA-BDP

The activity per actuation was assessed before administering the radiolabelled BDP to the children (Chapter 2, section 2.2.10.4). After the transmission scan each child inhaled 2-3 doses of ^{99m}Tc -HFA-BDP (200-300 μg), as described in Chapter 2, section 2.2.11.2.

3.4.7.3 Image acquisition

Immediately after inhaling the ^{99m}Tc -HFA-BDP each child was instructed to exhale gently into a low resistance filter. The child was repositioned between the two detectors of the gamma camera and simultaneous anterior and posterior planar scintigraphic images were acquired. This was followed by an image of the actuator, spacer and filter as described in Chapter 2, section 2.2.11.3.

3.4.7.4 Image analysis

Attenuation factors were derived for each child due to absorption by body tissues as described by Macey and Marshall (Chapter 2, section 2.2.12.1).[328] Regions of interest (ROI) were defined for each of the images and total counts were derived, as described in Chapter 2, section 2.2.12.2.

The dose deposited in the lungs was expressed as a percentage of the total dose delivered from the actuator (i.e. % ex-actuator). The pulmonary regional distribution was determined by calculating the ratio of peripherally (P) and centrally (C) deposited activity, as shown in Chapter 2, section 2.2.12.2. [349]

3.4.8 Ethical considerations and effective dose

Approval for the study was granted by the Princess Margaret Hospital Ethics Committee. Informed consent was obtained from parents and children, refer to Chapter 2, section 2.2.7.

3.4.9 Statistical analysis

Lung, oropharyngeal and gastrointestinal deposition, together with spacer and filter deposition of ^{99m}Tc-HFA-BDP were presented as % ex-actuator dose, with means and the corresponding standard deviations (SD). With the sample size of 12 children in each of experiment groups: ‘tidal’ and ‘breath hold’, the study had more than 80% power to detect a 40% difference in terms of lung doses according to a pre-study calculation as detailed in Chapter 2, section 2.2.15. Adjusted means were presented with 95% confidence limits. All the statistical analyses were conducted using the SPSS package.[440]

3.5 RESULTS

3.5.1 *In vitro* particle size distribution

The mass distributions and corresponding particle size distributions of commercial QVAR™ (HFA-BDP) delivered *via* pMDI and pMDI-spacer were compared in order to determine the mass median aerodynamic diameter (MMAD), geometric standard deviation (GSD), and fine particle fraction particles < 4.7 μm (Table 3-3).

Table 3-3: Mass drug (BDP) (μg) delivered *via* pMDI (n=10) and pMDI-spacer (n=10) with Andersen Cascade Impaction (ACI).

Mass BDP (μg)	pMDI	pMDI-spacer
Total recovery	93.3 \pm 4.5	92.4 \pm 9.6
Delivered dose	72.6 \pm 4.1	66.1 \pm 6.4
Fine particle mass	41.6 \pm 2.5	63.0 \pm 6.4
MMAD	1.33 \pm 0.04	1.26 \pm 0.07
GSD	1.7 \pm 0.14	1.6 \pm 0.04

There was not a statistically significant difference between the delivered dose from pMDI alone compared to pMDI-spacer ($p=0.060$). However there was a significant increase in the fine particle mass (FPM) and a significant decrease in MMAD and GSD when QVAR™ was delivered *via* pMDI-spacer compared to pMDI alone ($p<0.000$ and $p=0.030$, $p=0.035$ respectively). The FPM represents 57% of the delivered dose with pMDI alone, compared to 95% with pMDI-spacer.

3.5.2 Inspiratory filter study

A comparison of the % mean (SD) *in vivo* inspiratory filter drug (BDP) dose as a proportion of the total ex-valve dose, % ex-actuator dose and the % mean (SD) BDP particles exiting the spacer *in vitro* are presented in Table 3-4 and Table 3-5. The ‘total body deposition’ (TBD) is defined as the total drug dose measured on the filter or exiting the spacer attached to the Andersen Cascade Impactor (ACI). It was noted that the drug dose on the actuator used with ACI is almost half the drug dose on the actuator used with the inspiratory filter study.

Table 3-4: Comparison of mean (\pm SD) total mass BDP (μ g) exiting pMDI-spacer (AC+) with ACI and inspiratory filter. Total body deposition (TBD) defined as drug exiting spacer and drug measured on inspiratory filter.

Mass BDP μg	Actuator	Spacer	TBD
ACI (n=10)	12.2 \pm 3.6	14.1 \pm 5.5	66.1 \pm 6.3
Inspiratory filter (n=24)	20.7 \pm 5.9	14.2 \pm 5.6	57.9 \pm 8.8
‘Tidal’ filter (n=12)	20.8 \pm 5.3	15.0 \pm 5.7	55.2 \pm 8.2
‘BH’ filter (n=12)	20.5 \pm 6.6	13.5 \pm 5.6	60.5 \pm 8.9
% BDP	Actuator	Spacer	TBD
ACI	13.1 \pm 2.8	15.1 \pm 5.0	71.9 \pm 6.9
Inspiratory filter	22.3 \pm 6.3	15.4 \pm 6.2	62.1 \pm 7.4
‘Tidal’ filter (n=12)	23.0 \pm 6.1	16.4 \pm 6.1	60.4 \pm 6.9
‘BH’ filter (n=12)	21.6 \pm 6.7	14.4 \pm 6.3	63.9 \pm 7.7
‘Tidal’ 5-7 yrs (n=4)	24.4 \pm 5.5	15.0 \pm 1.8	60.4 \pm 5.5
‘BH’ 5-7 yrs (n=4)	20.5 \pm 6.0	12.4 \pm 3.5	67.2 \pm 7.5

ANOVA was used to compare the mass of drug (BDP) measured on the inspiratory filters, after the two inhalation techniques. There was a statistically significant difference between the mass drug (BDP) on the actuator attached to the pMDI-spacer with the inspiratory filter for both ‘tidal’ and ‘breath hold’ compared with ACI ($p=0.000$, $p=0.002$, respectively). There was not a significant difference between the mass drug (BDP) retained on the spacer attached to the inspiratory filter and the spacer attached to ACI for ‘tidal’ or ‘breath hold’ ($p=0.724$, $p=0.795$).

There was a significant difference between the mass of drug (BDP) on the inspiratory filter for ‘tidal’ compared with drug exiting the ACI-spacer ($p=0.002$) whereas with

‘breath hold’ there was not a significant difference in the mass of drug on the inspiratory filter compared with drug exiting ACI-spacer ($p=0.110$). There was not a statistically significant difference between the mass drug (BDP) retained on the inspiratory filter, actuator or spacer with the two different inhalation techniques ($p=0.899$, $p=0.528$, $p=0.134$ respectively).

Table 3-5: Summary of % mean (SD) ex-valve and ex-actuator drug (BDP) measured on inspiratory filter and % drug (BDP) with ACI (n=10) delivered via pMDI-spacer (AC+).

Device	Ex-valve	Ex-actuator
Inspiratory filter (n=24)	62.1± 7.4	80.2±7.3
‘Tidal’ AC+ (n=12)	60.4 ± 6.9	78.8 ± 7.0
‘Breath hold’ AC+ (n=12)	63.9 ± 7.7	81.7 ± 7.5
% BDP exiting spacer (ACI)	71.9 ± 6.9	82.6 ± 6.1
% BDP particles < 4.7 µm	68.6 ± 7.2	78.8 ± 6.7
% BDP particles < 3.3 µm	67.7 ± 6.9	77.8 ± 6.5
% BDP particles < 2.2 µm	62.0 ± 5.3	71.3 ± 4.9
% BDP particles 1.1- 4.7µm	35.8 ± 6.0	41.1 ± 6.1

The mean % (SD) inspiratory filter dose was 60.4 (6.9) for ‘tidal’ and 63.9 (7.7) for ‘breath hold’. However the mean % (SD) inspiratory filter dose for the children in the 5-7 year age-group was 67.2 (7.5) for ‘breath hold’ and 60.4 (5.5) for ‘tidal’ and there was a statistically significant improvement in total drug deposition for ‘breath hold’ compared to ‘tidal’ ($t=0.045$) in this age-group.

The predicted *in vitro* % lung deposition estimated from the particle size distribution of BDP with ACI based on the definitions of the fine particle fraction (FPF) by Mitchell, Newman, Newhouse, Finlay and modified Farr *et al* is presented in Table 3-6. With extrafine QVAR™, the FPF (particles < 4.7 µm) measured with cascade impaction was

not significantly different to the extrafine particle fraction (EFPF, particles < 3.3 µm) for the ex-valve or the ex-actuator deposition (p=0.783, p=0.736 respectively).

Table 3-6: The % delivered drug (BDP) with ACI and the fine particle fractions and % predicted lung deposition BDP as defined by modified Farr *et al* as delivered dose * FPF/100.

Device	Ex valve	Ex actuator
% Delivered dose BDP with ACI	71.9 ± 6.9	82.6 ± 6.1
% BDP particles < 4.7 µm (Mitchell 2003)	68.6 ± 7.2	78.8 ± 6.7
% BDP particles < 3.3 µm (Newman 2008)	67.7 ± 6.9	77.8 ± 6.5
% BDP particles < 2.2 µm (Newhouse 1998)	62.0 ± 5.3	71.3 ± 4.9
Predicted lung deposition* Farr 2006 [106]	49.7 ± 9.5*	57.0 ± 9.6*
% BDP particles 1.1-4.7 µm (Finlay 2003)	35.8 ± 6.0	41.1 ± 6.1

3.5.3 Validation of radiolabelling method

The mean (SD) total amount of commercial drug (BDP, ex-valve dose) recovered from the actuator and cascade impactor *via* pMDI alone, for the commercial inhalers used for the patient studies (n=15), was 96.6 (4.2) µg before radiolabelling ('before label'), 102.5 (3.0) µg immediately after radiolabelling ('after label') and 95.1 (3.8) µg and after radioactive decay ('decay label').

Mean % ex-actuator (SD) fine particle fraction was 56.9 (2.5), 58.1 (2.5), 58.8 (2.7) and 59.4 (2.1) for 'before label', 'after label', radiolabel (^{99m}Tc) and 'decay label' respectively. The particle size distribution is shown in section 3.4.6.1, Figure 3-2. There was a statistically significant difference between total drug (BDP) output measured with Andersen Cascade Impaction 'before' and 'after' the radiolabelling process (p=0.035). However there was not a significant difference between the

delivered dose or the fine particle mass 'before' and 'after' radiolabelling ($p=0.553$ and $p=0.104$ respectively). The mean (SD) MMAD μm for commercial QVAR™ was 1.2 (0.1) before radiolabelling and 1.3 (0.1) after radiolabelling. Although this represented a statistically significant difference ($p=0.016$), a difference of 0.1 μm with MMAD would not be expected to cause a significant change in the body deposition of HFA-BDP.

The quality control ratio of the fine particle fraction of the $^{99\text{m}}\text{Tc}$ radiolabel and commercial drug (BDP) 'before label' was 0.9 (± 0.1). Therefore the radiolabelling method passed accepted quality control guidelines and the activity distribution of the radiolabel could reliably reflect the drug (BDP) distribution.

3.5.4 *In vivo* gamma scintigraphy

The children were divided into ‘tidal’ and ‘breath hold’ breathing groups. The children were further subdivided into age-groups, 5-7 years, 8-10 years and 11-17 years. The total body deposition is demonstrated in the anterior gamma scintigraphic images in Figure 3-3.

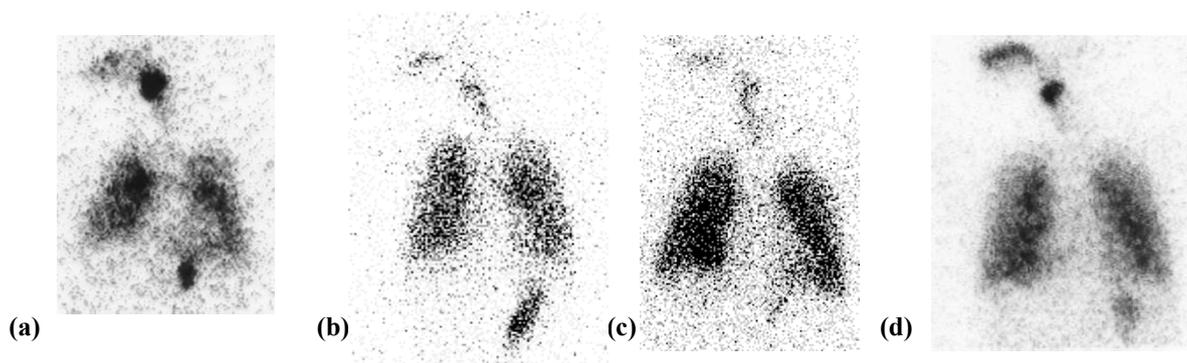


Figure 3-3: Anterior gamma scintigraphic scans showing regional distribution of radiolabelled QVAR™ in asthmatic children (a) age 5-7 ‘tidal’ and (b) age 5-7 ‘breath hold’; (c) age 11-17 ‘tidal’ and (d) age 11-17 ‘breath hold’.

Attenuation factors (AFs) were derived as detailed in Chapter 2, section 2.2.12.1. The AFs for lung, mouth, throat, oesophagus and stomach ROI ranged from 1.7-2.7. The regional distribution of ^{99m}Tc -HFA-BDP in lungs, oropharynx and gastrointestinal tract, spacer and expiratory filter in the ‘tidal’ group is shown in Table 3-7. The average proportion of lung deposition for the ‘tidal’ group was 45.9% (range from 14.4-67.9%). The coefficient of variation (CV) was 34.0%. The mean (SD) value for the peripheral to central (P:C) ratio for the ‘tidal’ group was 2.3 (0.5). The proportion of deposition in the lungs increased with age and lung function, although it was not statistically significant.

Lung deposition tended to increase with age and lung function and was positively correlated with FVC ($r^2=0.306$, $p=0.062$), FEV1 ($r^2=0.244$, $p=0.102$), height ($r^2=0.304$, $p=0.063$) and weight ($r^2=0.272$, $p=0.082$). Since only four children were studied in each of the age-groups the statistical power was 0.29 to detect the differences in lung depositions (shown in Table 3-7 between the age-groups).

Table 3-7: Regional distribution (% ex-actuator dose*) of extrafine aerosol ^{99m}Tc -HFA-BDP (QVAR™) in the tidal breathing groups (Tidal): 5-7 yrs, 8-10 yrs and 11-17 yrs.

Age yrs	Lungs %	OG* %	Spacer %	Expiratory filter %	P:C*
5-7	35.2 ± 18.7	25.0 ± 11.9	39.5 ± 8.2	0.39 ± 0.25	2.0 ± 0.5
8-10	47.5 ± 13.0	10.3 ± 4.4	41.5 ± 15.1	0.66 ± 0.78	2.2 ± 0.6
11-17	54.9 ± 11.2	10.1 ± 6.2	30.7 ± 11.5	4.3 ± 4.4	2.6 ± 0.5
5-17	45.9 ± 15.8	15.1 ± 10.4	37.2 ± 11.9	1.8 ± 3.0	2.3 ± 0.5

* Percentage of ex-actuator dose corrected for tissue attenuation and presented as means ± SD. OG-oropharyngeal and gastrointestinal and P:C-peripheral:central lung deposition ratio.

In the ‘breath hold’ group (Table 3-8), the average lung deposition of ^{99m}Tc -HFA-BDP was greater than 50% (range from 45.9% to 68.1%). The coefficient of variation was 11.4%. No significant difference in lung deposition was found between the three age groups. No correlation was found between lung dose, weight, height and lung function parameters FVC and FEV1. The mean (SD) P:C ratio for the ‘breath hold’ group was 2.3 (0.4).

Table 3-8: Regional distribution (% ex-actuator dose*) of HFA-BDP in groups using the single maximal inhalation with breath hold (Breath hold): 5-7 yrs, 8-10 yrs and 11-17 yrs.

Age yrs	Lungs %	OG %	Spacer %	Expiratory filter %	P:C
5-7	58.1 ± 6.7	12.9 ± 3.2	24.1 ± 7.0	4.9 ± 4.7	2.5 ± 0.2
8-10	56.3 ± 5.4	20.6 ± 9.8.	18.1 ± 1.6	5.0 ± 4.6	2.2 ± 0.4
11-17	58.4 ± 9.2	20.8 ± 8.8.	20.3 ± 4.5	0.55 ± 0.64	2.3 ± 0.4
5-17	57.6 ± 6.7	18.10 ± 8.1	20.8 ± 5.1	3.5 ± 4.1	2.3 ± 0.4

* Percentage of ex-actuator dose corrected for tissue attenuation and presented as means±SD. OG-oropharyngeal and gastrointestinal and P:C-peripheral:central lung deposition ratio.

Differences in lung deposition between ‘tidal’ and ‘breath hold’ were compared by adjusting for FVC, so that the difference between the two groups was independent of lung function and directly related to inhalation technique. Figure 3-4 compares the difference in lung deposition between ‘tidal’ and ‘breath hold’. The total difference in the adjusted means of lung deposition across the three age-groups was statistically significant (n=12, p=0.006).

With children aged 5-7 years, the adjusted mean of lung deposition in ‘breath hold’ was 1.6 times higher than that in ‘tidal’, although the difference was not statistically significant in the small sample size. In this youngest age-group the mean (SD) P:C was 2.5 (0.2) for ‘breath hold’ compared with 2.0 (0.5) for ‘tidal’. A comparison of lung deposition *via* pMDI-spacer with tidal breathing and the Autohaler™ device is shown in Figure 3-5.[150] The % oropharyngeal and gastrointestinal deposition (OG) is shown in Figure 3-6.

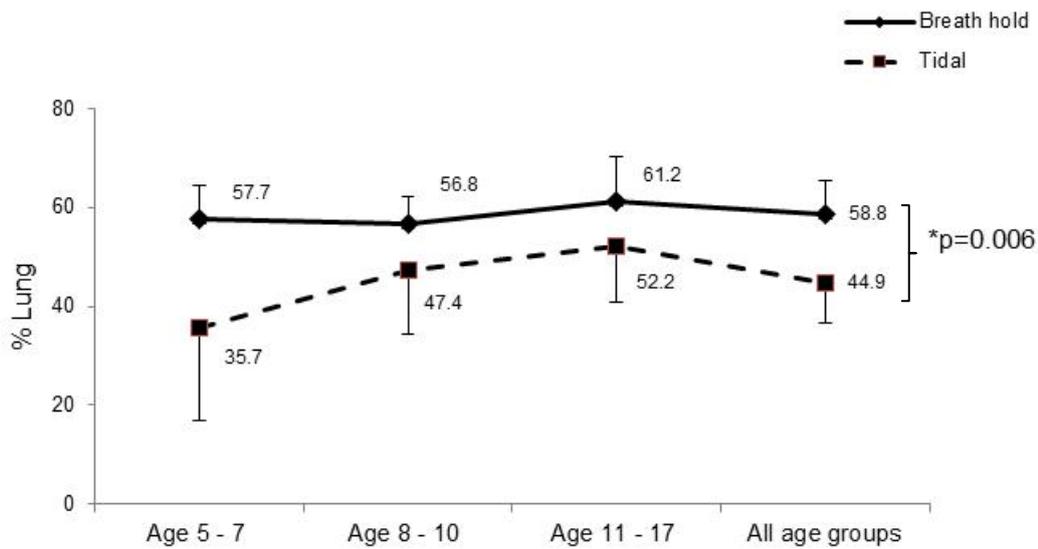


Figure 3-4: The y-axis shows the adjusted means (\pm SD) of lung deposition (% ex-actuator, adjusting for FVC) in the two study group: ‘breath hold’ and ‘tidal’.

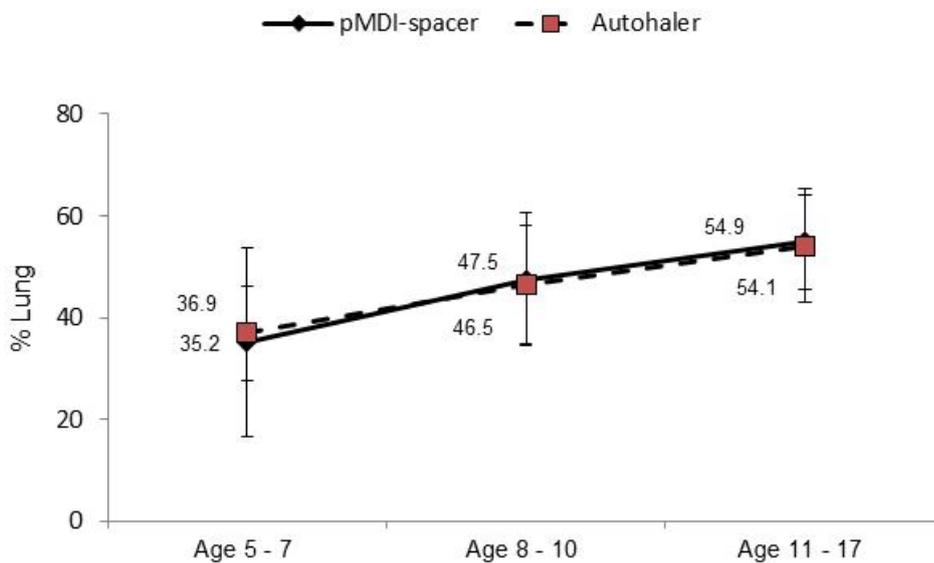


Figure 3-5: Comparison of mean (\pm SD) lung deposition HFA-BDP (% ex-actuator) with Autohaler™ and pMDI-spacer (Aerochamber Plus™) with tidal breathing.

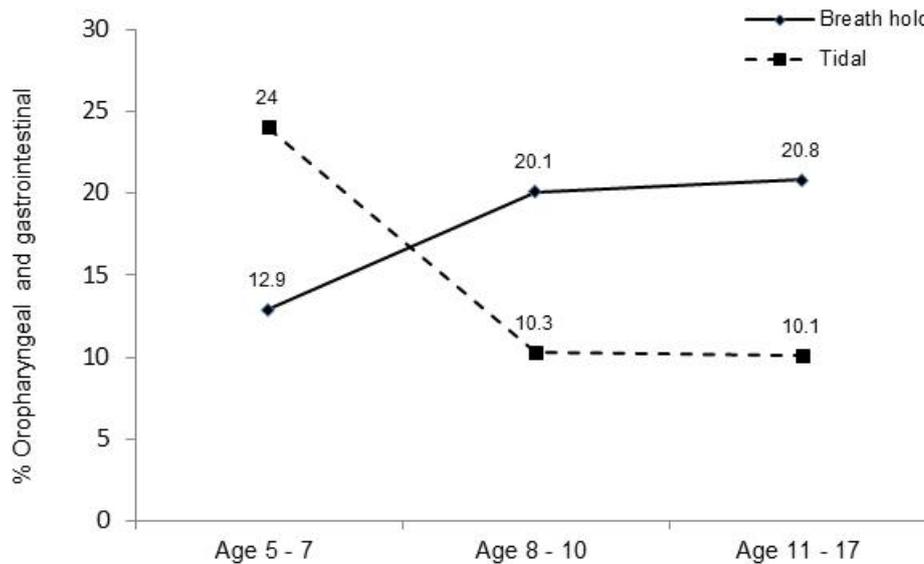


Figure 3-6: The y axis shows the adjusted mean oropharynx and gastrointestinal (OG) deposition HFA-BDP (% ex-actuator) for ‘breath hold’ and ‘tidal’.

With the ‘tidal’ group, children aged 5-7 years had a higher oropharyngeal and gastrointestinal (OG) deposition than ‘breath hold’. Conversely, children aged 8-17 years had higher OG deposition with ‘breath hold’ relative to ‘tidal’. The crossover interaction between age and ‘tidal’ and ‘breath hold’ groups for OG deposition was statistically significant ($p=0.016$). The OG deposition was reduced two to four-fold compared to the previously published dose of 40-60% obtained after inhalation of the same formulation *via* Autohaler™ (Figure 3-7).[150]

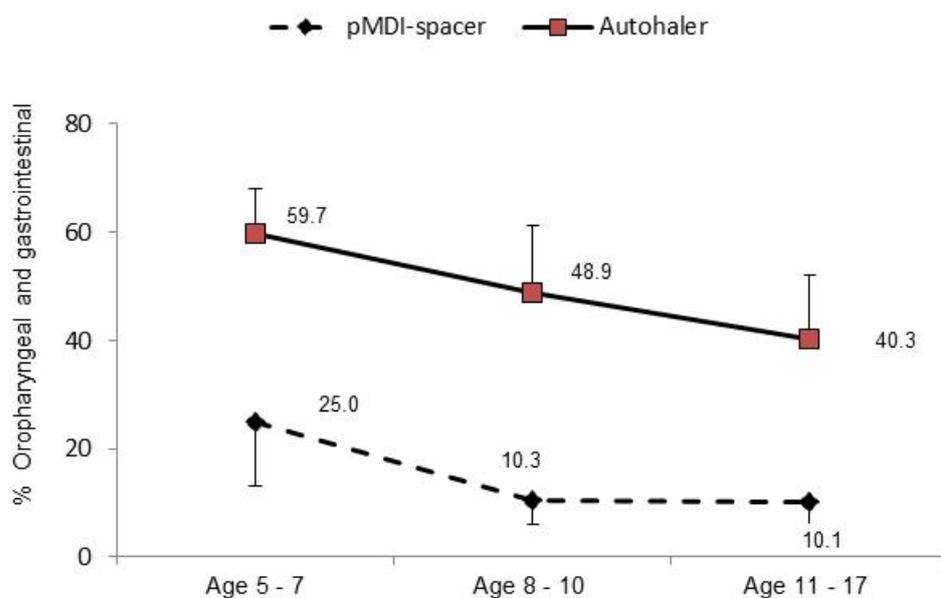


Figure 3-7: Comparison of mean (+SD) oropharyngeal and gastrointestinal (OG) deposition HFA-BDP (% ex-actuator) with Autohaler™ and pMDI-spacer (Aerochamber Plus™).

There was significantly more drug retained in the spacer with ‘tidal’ compared with ‘breath hold’ across all ages ($p < 0.001$, $n = 12$). The exhaled filter dose was difficult to obtain because some children would cough before breathing onto the filter.[28] A mean exhaled filter dose less than 5% across all ages was measured, which is comparable to the mean exhaled filter dose found with the same formulation and the Autohaler™ device.[150]

3.5.5 Gamma scintigraphy compared to ACI and filter study

With gamma scintigraphy, there was not a significant increase in the mass (μg) BDP on the actuator, after inhalation of the $^{99\text{m}}\text{Tc}$ -HFA-BDP, compared with the mass (μg) BDP on the actuator used for the inspiratory filter study with ‘tidal’ or ‘breath hold’ ($p = 0.126$, $p = 0.181$ respectively). Similarly there was not a significant difference between the mass BDP retained in the spacer used for the inspiratory filter study compared with the mass (μg) BDP retained in the spacer used for gamma scintigraphy

with 'tidal' or 'breath hold' ($p=0.068$, $p=0.599$ respectively). However, there was a significant difference between % BDP on the actuator used for the inspiratory filter study compared to the % BDP on the actuator used for gamma scintigraphy with both 'tidal' and 'breath hold' ($p=0.001$, $p=0.001$ respectively).

There was a significant increase in the % total BDP on the inspiratory filter compared with % total body deposition (TBD) measured with gamma scintigraphy for both 'tidal' and 'breath hold' ($p<0.001$, $p=0.001$). Similarly there was a significant increase in the % BDP exiting the spacer attached to the ACI compared with % TBD with gamma scintigraphy for both 'tidal' and 'breath hold' ($p<0.001$, $p<0.001$ respectively).

With 'breath hold', the spacer retention (% ex-actuator) with gamma scintigraphy was not significantly different to the spacer retention (% ex-actuator) with the inspiratory filter study ($p=0.402$). Similarly there was not a significant difference between % total BDP on the inspiratory filter dose (% ex-actuator) compared with TBD (% ex-actuator) measured with gamma scintigraphy with 'breath hold' ($p=0.078$).

With 'tidal' there was a significant difference between spacer retention (% ex-actuator) with the inspiratory filter study and spacer retention (% ex-actuator) measured with gamma scintigraphy ($p=0.001$). Similarly with 'tidal' there was a significant difference between the inspiratory filter dose (% ex-actuator) compared with TBD (% ex-actuator $p=0.001$) (Table 3-9, Table 3-10, Table 3-11).

Table 3-9: Comparison of mean (\pm SD) % total output BDP (ex-valve and ex-actuator TBD*) from pMDI-spacer with ACI, filter study and gamma deposition.

BDP %	N	Actuator	Spacer	TBD	ex-aTBD	FPF/lung	ex-a Lung/FPF
ACI	10	13.1 \pm 2.8	15.1 \pm 5.0	71.9 \pm 6.9	82.6 \pm 6.1	68.6 \pm 7.1	78.7 \pm 6.7
Filter study	24	21.9 \pm 6.2	15.5 \pm 6.2	62.1 \pm 7.4	80.2 \pm 7.3	-	-
Gamma	24	34.8 \pm 8.7	19.1 \pm 8.9	44.0 \pm 10.9	69.5 \pm 13.9	34.3 \pm 10.3	51.7 \pm 13.0

Table 3-10: Comparison of mean (\pm SD) % total output BDP (ex-valve TBD and ex-actuator TBD) from pMDI-spacer (AC+) with ACI, inspiratory filter study, gamma deposition and two different breathing techniques.

Tidal	N	Actuator	Spacer	TBD	ex-aTBD	ex-a Lung/ FPF)
ACI	10	13.1 \pm 2.8	15.1 \pm 5.0	71.9 \pm 6.9	82.6 \pm 6.1	78.7 \pm 6.7
Filter study	12	23.0 \pm 6.1	16.6 \pm 6.1	60.4 \pm 6.9	78.8 \pm 7.0	-
Gamma	12	35.2 \pm 8.4	24.0 \pm 9.5	39.6 \pm 9.5	61.0 \pm 10.5	45.9 \pm 15.7
Breath hold		Actuator	Spacer	TBD	ex-aTBD	ex-a Lung/FPF
Filter study	12	21.7 \pm 6.7	14.5 \pm 6.3	63.9 \pm 7.7	81.7 \pm 7.5	-
Gamma	12	33.6 \pm 8.8	14.0 \pm 4.2	50.0 \pm 7.0	75.7 \pm 7.1	57.6 \pm 6.7

* Total body deposition (TBD) defined as drug exiting spacer, drug on filter and total gamma deposition.

Table 3-11: Comparison of mean (\pm SD) lung deposition and total body deposition (TBD, % ex-valve) with gamma scintigraphy, fine particle fraction (FPF) and delivered dose (DD) with Andersen Cascade Impaction (ACI) and total dose on inspiratory filter with ‘tidal’ and with ‘breath hold’.

Tidal	Lung	FPF	TBD	ACI-DD	Filter dose
n=12	28.7 \pm 11.0	68.6 \pm 7.1	39.6 \pm 9.5	71.9 \pm 6.9	60.4 \pm 6.9
Breath hold	Lung	FPF	TBD	ACI-DD	Filter dose
n=12	39.1 \pm 6.7	68.6 \pm 7.1	50.0 \pm 7.0	71.9 \pm 6.9	63.9 \pm 7.7

With % ex-actuator BDP, the % fine particle fraction (% drug particles < 4.7 μ m) as reported by Mitchell *et al*, was significantly increased compared with the % lung deposition measured with gamma scintigraphy ($p < 0.001$). The predicted % lung deposition BDP *in vitro*, based on the modified Farr *et al* formula (i.e. the proportion of particles < 4.7 μ m and the delivered dose BDP emitted from the ACI), was not significantly different to % lung deposition measured with gamma scintigraphy *in vivo* with ‘breath hold’ ($p=0.874$) or ‘tidal’ ($p=0.056$), Chapter 2, section 2.2.8.1.

With the Finlay *et al* definition of % fine particle fraction (particles 1.1-4.7 μ m) the predicted % lung deposition BDP with ACI was significantly less than the % lung deposition measured with gamma scintigraphy with ‘breath hold’ ($p < 0.001$), indicating that ultrafine particles would be retained with ‘breath hold’. However there was not a significant difference with the Finlay *et al* predicted % lung deposition with ACI and % lung deposition measured with gamma scintigraphy with ‘tidal’ ($p=0.351$).

With extrafine QVAR™, the % extrafine particle fraction (EFPF, particles < 3.3 μ m), described by Newman and Chan as more representative of lung deposition, was significantly increased compared to the % lung deposition measured with gamma scintigraphy ($p < 0.001$). Similarly the % ultrafine particles in the FPF (particles

< 2.2 μm), described by Newhouse *et al* as the ‘lung targetable fraction’ were significantly increased compared with % lung deposition measured with gamma scintigraphy ($p < 0.001$).

3.6 DISCUSSION

Scintigraphic imaging has been widely used to assess pulmonary deposition of inhaled broncodilators and corticosteroids. Lung deposition of extrafine aerosol, delivered to children *via* pMDI-spacer, using different inhalation techniques, has not been previously reported in the literature. Two-dimensional (2D) planar scintigraphic images were recorded in order to minimise radiation exposure to the children. The HFA-BDP deposition study supported our hypothesis that inhalation of extrafine particles *via* pMDI-spacer would result in a high lung dose and show a marked decrease in oropharyngeal and gastrointestinal deposition compared with delivery of the same formulation *via* Autohaler.TM [150] The study was limited by the small number of subjects within each age-group; however, across all ages ($n=12$), the study had an acceptable power of 80%.

The *in vitro* drug output and fine particle fraction may be used to characterise a drug/device combination and estimate the expected total body deposition and lung deposition, however inertial sizing of aerosols has been shown to overestimate lung deposition and underestimate variability in adults.[109] Therefore, as expected the *in vitro* drug output measured with Andersen Cascade Impaction (ACI) significantly overestimated the expected total body deposition and did not show the degree of variability compared with *in vivo* total body deposition with gamma scintigraphy, confirming our hypothesis. Similarly the inspiratory filter study overestimated total body deposition and showed reduced variability compared with gamma scintigraphy.

The coefficients of variation with ACI, filter study and gamma scintigraphy were 9.5%, 12.0% and 21.7% respectively.

The *in vitro* ACI measure of the mean % ex-actuator drug exiting the spacer in the fine particle fraction was approximately 80% and the *in vivo* measure for the mean % ex-actuator lung deposition with gamma scintigraphy for young children aged 5-7 years using tidal breathing was 35%. This demonstrates that if the *in vitro* fine particle fraction was used to predict the *in vivo* outcome for this drug/device combination then the dosage regimen for a child in the 5-7 year age-group could be reduced by as much as 50% and this could have clinical relevance due to underdosing.

The mean predicted lung dose HFA-BDP derived from ACI *in vitro* with the modified Farr *et al* formula was 63% ex-actuator and this overestimated the lung deposition almost twofold for children aged 5-7 years.[337] However the Farr *et al* formula showed that the predicted lung deposition was in agreement with lung deposition with the ‘breath hold’ technique across all ages. Conversely, with the Finlay *et al* definition for FPF (1.1-4.7 μm), the predicted *in vitro* lung deposition was not significantly different to lung deposition with gamma scintigraphy and tidal breathing. This indicates that different *in vitro* definitions of the predicted lung dose, based on the the fine particle fraction of drug particles, may need to be adjusted for the particle size of the formulation and the inhalation technique in order to be predictive of lung deposition.[114]

There was not a significant increase in the actuator dose (μg) after inhalation of the radiolabelled drug compared with the actuator dose after the patient filter study for ‘tidal’ or ‘breath hold’. Similarly there was not a significant difference between the spacer dose (μg) after the patient filter study compared with the spacer dose (μg) after inhalation of the radiolabelled drug. However, the significant increase in the

% radiolabelled actuator dose may be related to a mis-match between drug and ^{99m}Tc with the larger drug particles emitted from the actuator, before evaporation of the propellant. Leach and Devadason have reported the % ex-actuator dose in previous deposition studies for both adults and children.[150],[112] Therefore the % ex-actuator dose was chosen as a more representative match for drug and label.

Filter studies have been widely used to estimate *in vivo* the total body deposition of inhaled drugs.[353] It was noted that although the filter study overestimated the total body deposition for children, the filter study was able to demonstrate that the ‘breath hold’ technique would provide significantly enhanced total drug deposition compared with tidal breathing for the youngest age-group. Furthermore both the filter study and gamma scintigraphy demonstrated that spacer retention of drug was increased with tidal breathing.

These findings show that some valuable predictive information about the drug/device combination may be obtained from the inspiratory filter study. However the filter drug deposition does not give any information about regional drug deposition and the proportion of drug distribution for lung deposition compared with oropharyngeal and gastrointestinal deposition. This is an important limitation of the filter study compared with gamma scintigraphy.

Tidal breathing, combined with pMDI and spacer, is a simple inhalation technique for children, as there are no coordination problems associated with inhalation and actuation. This study has shown that most of the children could obtain lung deposition of QVAR™ (HFA-BDP) greater than 30% ex-actuator dose, using tidal breathing with pMDI-spacer, confirming our hypothesis that the lung deposition would be equivalent to the previous Autohaler™ study. The amount of drug deposited in the lungs of children using tidal breathing tended to increase with both age and lung function, and was

remarkably consistent to that shown in children using the same extrafine formulation inhaled *via* the Autohaler™ device (37-54%).[150] However, the tidal breathing group showed a greater degree of intersubject variation with a coefficient of variation of 32%. The combined oropharyngeal and gastrointestinal dose deposited in children using pMDI-spacer, was markedly reduced (10-25%) in the tidal breathing technique, compared with Devadason's reported values for the Autohaler™ device (40-60%), confirming our hypothesis.

With the 'breath hold' technique, which requires a short training component for children, the QVAR™ study has shown high lung dose delivery (over 55% ex-actuator dose on average) of the extrafine formulation QVAR™, independent of age, FEV1, FVC, height and weight and consistent to that shown in adults using the same formulation *via* pMDI.[112] [319] There was less variability in the dose to the lungs across all ages, shown by a low intersubject CV of 11%. Regarding lung deposition in children aged 5-7 years, the QVAR™ study has shown that the single maximal inhalation technique can improve lung deposition almost twofold (range 51.5-64.8% for the % ex-actuator) when QVAR™ is delivered *via* pMDI-spacer (Aerochamber Plus™) compared with the previously reported Autohaler™ device (range 27.7-46.1% for the % ex-actuator).

Children aged 5-7 years also showed a higher mean P:C ratio for the 'breath hold' technique (2.5) compared with tidal breathing (2.0). This would indicate that there is more peripheral deposition of the extrafine formulation with the 'breath hold' technique for this age-group. The mean (range) P:C ratio in a previous deposition study with children aged 6-16 years, inhaling radiolabelled budesonide from Turbuhaler was 1.7 (1.0 to 2.4).[311] Children aged 5-7 years have a lower tidal volume and a lower inspiratory flow than children aged 8-17 years, although we did not record these

parameters. Inhalations < 60 L/min have been shown to improve peripheral penetration.[451]

Children aged 8-17 years received similar levels of lung deposition of the extrafine QVAR™ formulation, whether using tidal breathing or a slow single maximal inhalation followed by a ‘breath hold’ for 5-10 seconds. These children also exhibited similar peripheral penetration of the extrafine drug into the airways with either breathing technique. However, there was less variability in dosing associated with the single maximal inhalation technique. The Aerochamber Plus™ has a small chamber volume of 149 mL and was chosen because of its optimal *in vitro* characteristics, portability and ease of use. However, the small volume may have been the limiting factor which led to an increase in oropharyngeal and gastrointestinal dose in the children aged 8-17 years, using the ‘breath hold’ technique.

This study has confirmed the hypothesis that the single maximal inhalation technique, followed by a 5-10 s ‘breath hold’, in combination with Aerochamber Plus™, improves the delivery of QVAR™ to the peripheral airways of children. The increase in oropharyngeal and gastrointestinal deposition associated with ‘breath hold’ in patients aged 8-17 years may not be clinically relevant, whereas the decreased variability of drug delivery *via* pMDI-spacer with ‘breath hold’ in all age-groups is an important clinical consideration for drug delivery in children.

In conclusion, gamma scintigraphy demonstrated that spacer inhalation technique can significantly improve lung deposition of the extrafine aerosol delivered *via* pMDI-spacer in asthmatic children aged 5-17 years. This study has shown that tidal breathing with pMDI-spacer provided adequate lung deposition of the extrafine formulation, however the slow single maximal inhalation followed by a 5-10 s ‘breath hold’ produced less variability in lung dose in all age-groups and improved lung deposition in

children aged 5-7 years almost twofold. The delivery of extrafine QVAR™ *via* pMDI-spacer has not been previously reported in this age-group.

As expected the inspiratory filter study overestimated the total drug deposition and gave no information about regional drug deposition or lung deposition. However the filter study was able to demonstrate significant improvement in total drug deposition for young children using the ‘breath hold’ technique. *In vitro* measures of the % total drug output (72%) and fine particle fraction (69%) with cascade impaction, overestimated the total body deposition and lung deposition of BDP measured *in vivo* by gamma scintigraphy (45% and 34% respectively). Similarly the % ex-actuator drug (BDP) output with cascade impaction (87%) and % ex-actuator total dose for the filter study (80%), overestimated % total body deposition (68%) measured with gamma scintigraphy. Furthermore cascade impaction does not describe the variability in drug deposition to children.

The degree of variability in dosing is an important consideration when optimising formulation, delivery device and inhalation technique for the specific needs of children. Children from 5 years of age should be encouraged to use this spacer inhalation technique as soon as practicable. Future recommendations for optimising inhaled drug delivery to infants and young children who are unable to perform a slow single maximal inhalation technique could include the combination of pMDI-spacer with an extrafine formulation. Children from 2 years of age could be encouraged to perform a more consistent, regular tidal breathing pattern, perhaps with the aid of an incentive spacer device.

The National Asthma Council of Australia recommends extrafine QVAR™ and Flixotide® for children with persistent asthma.[201] In the next chapter of this thesis I will describe a radiolabelling method for fluticasone propionate (FP) reformulated with

HFA propellant (Flixotide®). HFA-FP is recommended at the same daily dose as HFA-BDP, however HFA-FP has a coarser particle size compared to QVAR™.

A radiolabelling method for Flixotide® had not been published prior to the work carried out in this thesis. In contrast, the radiolabelling method used for QVAR™ in this chapter had been previously developed. Therefore Chapter 4 outlines the steps involved in the development of a radiolabelling method, specifically for HFA-FP (Flixotide®) formulation. Chapter 4 also summarises the rigorous validation methods used for HFA-FP delivered *via* pMDI and pMDI-spacer, prior to a deposition study with radiolabelled HFA-FP in Chapter 5. Furthermore the work in Chapter 5 will investigate the effects of the increased particle size, two different breathing patterns and two different spacers on total body deposition and lung deposition and compare the results with extrafine QVAR™.

4 FLIXOTIDE® RADIOLABELLING METHOD

The following peer reviewed article has been published from work carried out for this chapter of the thesis:

***In vitro* validation of ^{99m}Tc-HFA-FP delivered via pMDI-spacer.** Roller CM, Schaefer NC, Zhang G and Devadason SG.

Journal of Aerosol Medicine 2006; 19(3): 254-260

ABSTRACT: The purpose of the study was to label Flixotide® (fluticasone propionate [FP] with HFA propellant), with technetium-99m and validate that ^{99m}Tc acts as a suitable marker for FP when delivered *via* pMDI-spacer. Sodium pertechnetate was mixed with 5 mL of butanone. ^{99m}Tc was extracted into butanone and transferred into an empty canister. The ^{99m}Tc lined canister was heated, and the butanone evaporated to dryness. A supercooled commercial Flixotide® canister was decrimped, and the contents transferred to the ^{99m}Tc lined canister and recrimped. The particle size distribution of FP and ^{99m}Tc from 10 radiolabeled canisters was measured using an Andersen cascade impactor calibrated to 28.3 L/min, and compared to commercial FP. The drug (FP) content of each particle size fraction was measured using ultraviolet spectrophotometry and the ^{99m}Tc level in each fraction was measured using an ionization chamber.

The percentage of particles in the fine particle fraction (< 4.7 µm) and the percentage of ^{99m}Tc from commercial and radiolabeled canisters were compared. The mean (SD) % FP in the fine particle fraction, before and after label was 43.2 (1.8)% and 43.9 (2.6)%, respectively. The mean (SD)% ^{99m}Tc in the fine particle fraction was 42.1 (5.1)%. The mean % FP exiting spacer at (< 4.7 µm) before labeling was not significantly different from the mean % FP exiting spacer at (< 4.7 µm) after labeling (p < 0.05). The mean % ^{99m}Tc attached to particles at (< 4.7 µm) after radiolabeling was not significantly different from the mean % FP levels (p < 0.05). The validation in this study indicates that ^{99m}Tc can act as a suitable marker for HFA FP, delivered *via* pMDI-spacer.

KEY WORDS: fluticasone propionate, validation, radiolabel, technetium-99m, pressurized metered-dose inhaler, spacer, particle size distribution, fine particle fraction

Co-author contribution: UWA Aerosol Research Group.

Roller, CM	Experimental work, data analysis and manuscript writing	80%
Schaefer, NC	Technical and laboratory assistance with cascade impaction	
Zhang, G	Expert statistical advice	
Devadason, SG	Aerosol research expert advice	

4.1 INTRODUCTION

The aerodynamic diameter of the particles of an inhaled drug formulation is a key determinant of the mass of drug particles deposited in the respiratory tract. Therefore *in vitro* characterization of the aerodynamic particle size distribution of inhaled drugs is essential for the evaluation of drug-device performance. The current ‘gold standard’ in terms of *in vitro* testing of inhalation formulations is inertial impaction assessment using Andersen Cascade Impaction (ACI) or Next Generation Impaction (NGI).

Impactors are recommended by the United States Pharmacopoeia (USP) aerosol testing regulations as the reference instrument used to characterise aerosols delivered from pressurised metered dose inhalers (pMDIs). Impaction methods measure the total drug output and the aerodynamic particle size distribution of a specific formulation delivered by a specific device. Several studies have demonstrated that the fine particle fraction (FPF) measured by cascade impaction can give an indication of *in vivo* lung deposition.[109] However, deposition studies with gamma scintigraphy provide a more realistic assessment of lung deposition of inhaled drug formulations from different devices.[453] Gamma scintigraphy can only be performed after the rigorous *in vitro* validation of the radiolabelling method used for a specific formulation.

Fluticasone propionate (FP) reformulated with hydrofluoroalkane (HFA) propellant is commonly used for inhaled asthma treatment in Australia, marketed as Flixotide® (Allen+Hanburys). A radiolabelling method for Flixotide® (HFA-FP) had not been developed prior to the publication of the modified butanone extraction method developed in this thesis.[346] The delivery of fluticasone propionate (FP) has been shown to be variable depending on the type of delivery device or spacer device used.[260] The Aerochamber Plus™, a small volume spacer attached to pMDI, has been shown to optimise the delivery of FP *in vitro*. [454-456] Lung deposition of

fluticasone propionate (FP) after inhalation *via* pMDI-spacer (Aerochamber Plus™) has not been investigated in children with asthma.

4.2 OBJECTIVES

- Characterize the *in vitro* drug output and particle size distribution of commercial Flixotide® (HFA-FP) delivered *via* pMDI and pMDI-spacer.
- Develop an *in vitro* radiolabelling method for HFA-FP with technetium-99m.
- Validate that the drug output and particle size distribution of HFA-FP is not significantly changed by the radiolabelling method and verify that ^{99m}Tc-HFA-FP acts a marker for commercial Flixotide®.

4.3 HYPOTHESES

- The particle size distribution and drug output of radiolabelled ^{99m}Tc-HFA-FP is not significantly different to the particle size distribution and drug output of commercial unlabelled HFA-FP.
- The particle size distribution of unlabelled drug (FP) and labelled drug (FP) matches the activity distribution of technetium-99m.
- Technetium-99m acts as a suitable marker for HFA-FP.

4.4 METHOD

4.4.1 Experimental design

The following series of *in vitro* studies were performed in order to characterise the drug output and particle size distribution of commercial Flixotide® (HFA-FP) and ^{99m}Tc-HFA-FP delivered *via* pMDI and pMDI-spacer (AC+) (Table 4-1).

Table 4-1: Experimental steps in the development of a radiolabelling method for HFA-FP.

<i>In vitro</i> cascade impaction and particle size distribution HFA-FP <i>via</i> pMDI.
<i>In vitro</i> cascade impaction and particle size distribution HFA-FP <i>via</i> pMDI-spacer.
Development of a radiolabelling method for ^{99m} Tc-HFA-FP.
Validation of radiolabelling method for ^{99m} Tc-HFA-FP delivered <i>via</i> pMDI.
Validation of radiolabelling method for ^{99m} Tc-HFA-FP delivered <i>via</i> pMDI-spacer.

4.4.2 Particle size distribution before radiolabelling

4.4.2.1 pMDI

In vitro experiments with Andersen Cascade Impaction (ACI) were performed in order to characterise the total drug output and aerodynamic particle size distribution (PSD) of commercial Flixotide® (HFA-FP) delivered *via* the pressurised metered-dose inhaler (pMDI) device. Ten commercial pMDI canisters were primed and then inserted into the standard USP ‘throat’ of the cascade impactor. Five doses of HFA-FP (250µg/actuation) were drawn into the cascade impactor at a continuous flow of 28.3 L/min and particle size distributions (PSD) were assessed, as described in detail in Chapter 2, section 2.2.8.1 and 2.2.10.

A second series of five commercial canisters had particle size distribution (PSD) measures with ACI, after modifications to the time of immersion of the commercial pMDI canister in liquid nitrogen (45-50 s), so that consecutive PSD could be evaluated. These particle size distributions were averaged to become the reference PSD of commercial Flixotide® ‘before’ radiolabelling (Figure 4-1).

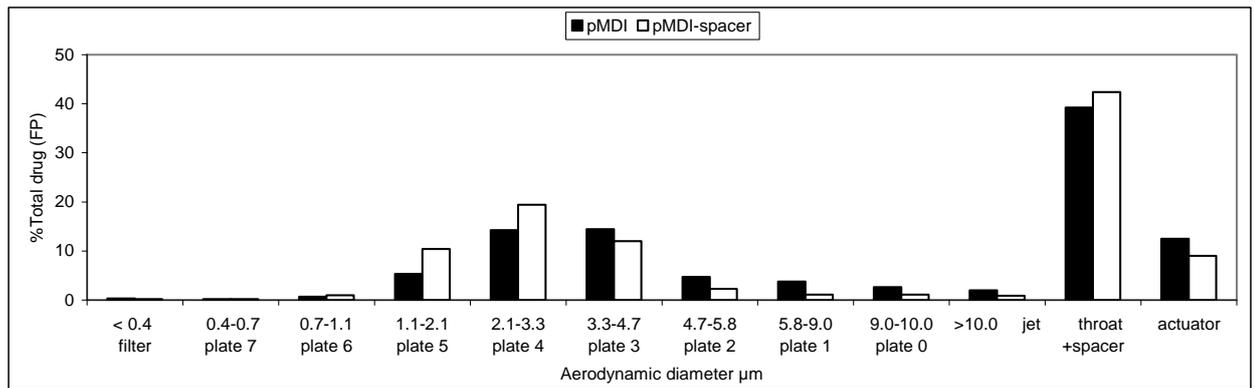


Figure 4-1: The y-axis shows the % total drug (FP) and the x-axis shows the aerodynamic diameter of drug particles (µm) measured with Andersen Cascade Impaction. The black bar shows HFA-FP delivered *via* pMDI (n=10) and the white bar shows HFA-FP delivered *via* pMDI spacer (AC (n=5)).

4.4.2.2 pMDI-spacer

After the assessment of the commercial Flixotide® pMDI canisters, a cascade impaction series was performed with HFA-FP delivered *via* pMDI-spacer (Aerochamber Plus™). Ten pMDI were primed and then inserted into the spacer attached to the ‘throat’ of the cascade impactor. Five doses of HFA-FP (250 µg/actuation) were drawn into the cascade impactor at a continuous flow of 28.3 L/min.

The particle size distribution of HFA-FP delivered to the cascade impactor with the pMDI device was compared with pMDI-spacer in order to assess the effect of the delivery device on drug delivery. The change in MMAD (3.5 µm → 2.9 µm) and the change in the aerodynamic diameter size range for the fine particle fraction (FPF) drug with pMDI and pMDI-spacer is shown in Figure 4-1 and Figure 4-2.

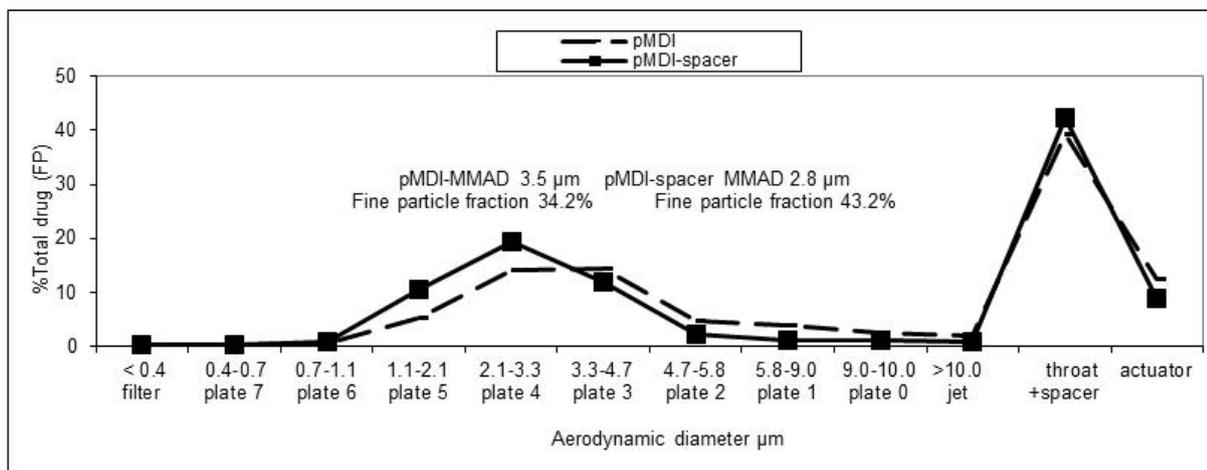


Figure 4-2: Line graph demonstrating the shift in the aerodynamic particle size distribution, MMAD and fine particle fraction of drug (FP) delivered via pMDI (n=10) and pMDI-spacer (n=5).

4.4.3 Development of radiolabelling technique

Commercial Flixotide®, reformulated with HFA propellant (HFA-FP) had not been previously radiolabelled with technetium-99m (^{99m}Tc) prior to the work carried out in this chapter. Two methods for radiolabelling Flixotide® with ^{99m}Tc were assessed. Leach *et al* had previously described a method to radiolabel QVAR™ (HFA-BDP) using a chloroform extraction method. [457] Newman *et al* used a chloroform extraction technique to radiolabel salbutamol.[149] Therefore the first radiolabelling method used to attempt to radiolabel Flixotide® was the chloroform extraction method that was used in Chapter 3 for QVAR™.

The second radiolabelling method used a modified butanone extraction technique, developed during the work carried out in this chapter.[346] Several butanone extraction methods have been described previously by Kohler *et al*, Summers *et al*, Wildhaber *et al*, Newman *et al*, Hardy *et al* and Bondesson *et al* to radiolabel fenoterol, nedocromil, salbutamol, flunisolide, nalcystelyn and lactose respectively.[171, 342, 345, 458, 459] After publishing the technique used in this chapter, fluticasone propionate

has been radiolabelled by Shrewsbury *et al* with a similar butanone extraction technique.[407]

4.4.3.1 Chloroform extraction method

HFA-FP was initially radiolabelled using the chloroform extraction method described by Leach *et al*. [457] The commercial formulation of Flixotide® delivered 250 µg FP per actuation. Sodium pertechnetate ($\text{Na}^{99\text{m}}\text{TcO}_4$) 500-1500 MBq, was eluted from a generator and made up to a volume of 1.0-1.5 mL with physiological saline and mixed with 6 µL tetraphenylarsonium chloride hydrochloride hydrate and 30 µL strong ammonium solution (28-30%), as described in detail in Chapter 2, section 2.2.9 and Chapter 3, section 3.4.6.

4.4.3.2 Assessment of particle size distribution after radiolabelling

The particle size distribution of the radiolabel $^{99\text{m}}\text{Tc}$ (after radiolabelling) was carried out with Andersen Cascade Impaction (ACI) (Figure 4-3).

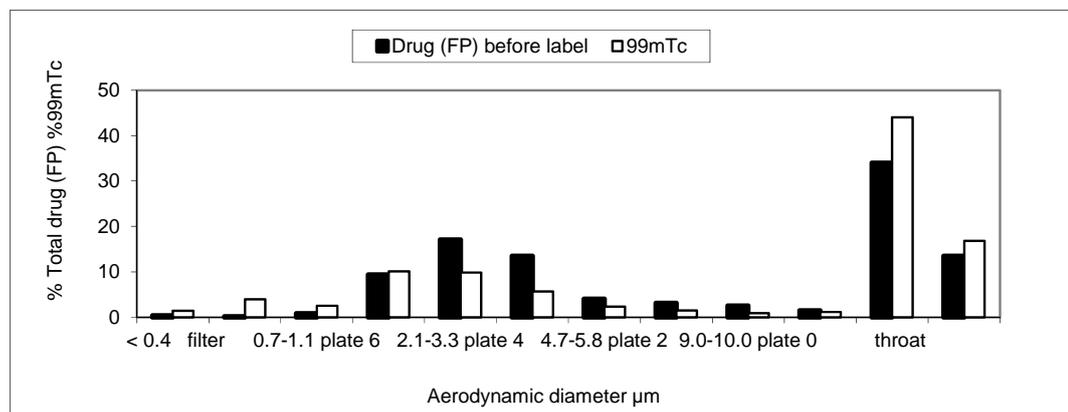


Figure 4-3: The y-axis shows the % total drug (FP) or % $^{99\text{m}}\text{Tc}$. The x-axis shows the aerodynamic diameter of drug (FP) and $^{99\text{m}}\text{Tc}$ -radiolabel after the chloroform extraction radiolabelling procedure (n=4).

4.4.3.3 Butanone extraction method

The second radiolabelling method that was investigated for radiolabelling commercial Flixotide® (HFA-FP), used an initial extraction of technetium-99m into butanone (BDH Chemicals Australia Pty., Ltd., Kilsyth, Victoria, Australia). The following experimental steps were used for the development of the radiolabelling procedure for Flixotide® (HFA-FP) in this thesis (Table 4-2).[346]

Table 4-2: Experimental steps required for radiolabelling HFA-FP.

Extract sodium pertechnetate ($\text{Na}^{99\text{m}}\text{TcO}_4$) into butanone.
Evaporate butanone under a gentle stream of nitrogen.
Heat $^{99\text{m}}\text{Tc}$ canister to 80°C for 30 minutes in heating block.
Supercool commercial canister in liquid nitrogen, then decrimp.
Pour contents into a $^{99\text{m}}\text{Tc}$ -lined canister and rerimp.
Mix $^{99\text{m}}\text{Tc}$ -canister in ultrasonic water bath 20 s.
Mix $^{99\text{m}}\text{Tc}$ -canister for 30-60 minutes.

HFA-FP was radiolabelled using a modification of the methods described by Hardy *et al* and Bondesson *et al*. [345, 459] For each canister containing 250 μg of HFA-FP per actuation, 500-1500 MBq sodium pertechnetate in a volume of 0.5-1.0 mL, was mixed with 5 mL ethyl methyl ketone (2-butanone, AnalaR grade) in a glass scintillation vial, according to the method described in Chapter 2, section 2.2.9.

The mixture in the glass vial was sonicated for 30 seconds in distilled water and poured into a phase separating filter into an empty, preweighed pMDI canister. The $^{99\text{m}}\text{Tc}$ lined canister was transferred to a lead pot and placed in a dry heating block (Barnstead

Thermolyne, Crown Scientific, Perth, WA, Australia) set at 80° C for 25 minutes, with a low flow of nitrogen (2-4 L/min) to evaporate any residual liquid phase.[345] The ^{99m}Tc-lined canister was supercooled in liquid nitrogen for 5 seconds. A preweighed commercial reference canister containing HFA-FP was supercooled in liquid nitrogen for 45-50 seconds and rapidly opened with a pipecutter and the contents poured into the cooled ^{99m}Tc-lined canister and immediately crimped as described in Chapter 2, section 2.2.9, Figure 2-5.

4.4.4 Preliminary validation pMDI

The particle size distribution (PSD) of 10 commercial pMDI canisters containing HFA-FP was performed with Andersen Cascade Impaction (ACI) before radiolabelling to obtain a ‘before label’ reference PSD. Five actuations of HFA-FP (250 µg/actuation) were measured with ACI, as detailed in Chapter 2, section 2.2.10. After the radiolabelling procedure another PSD was performed with each radiolabelled canister, in order to measure the ^{99m}Tc (‘label’) and drug level (FP, ‘after label’), Figure 4-4, as described in Chapter 2 section 2.2.8 and section 2.2.10.

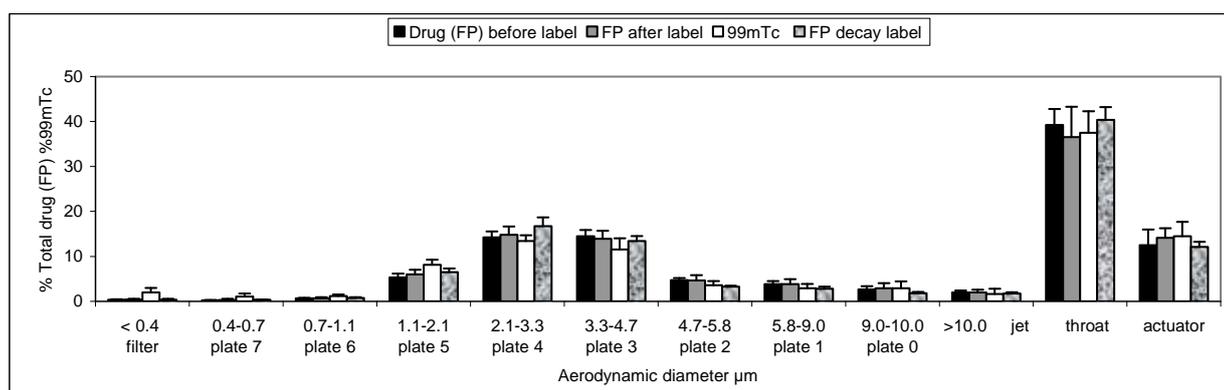


Figure 4-4: Particle size distribution drug (FP) ‘before label’, ‘after label’ and ‘decay label’ and ^{99m}Tc radiolabel delivered to Andersen Cascade Impactor *via* pMDI (n=10).

The ratio of the ^{99m}Tc -radiolabel to drug (FP) was used to confirm that ^{99m}Tc acts as a suitable marker for FP. After the decay of ^{99m}Tc , a ‘decay’ particle size distribution of drug (FP) from the labelled canister was performed to verify the integrity of the transfer of contents from the commercial canister (Figure 4-4).

4.4.5 Validation with consecutive pMDI

The particle size distribution (PSD) of the commercial Flixotide® pMDI canisters was carried out in order to obtain a ‘before label’ reference PSD. After modifications to the supercooling time from 45 seconds to 50 seconds, cascade impaction with 5 consecutive pMDI-canisters was carried out after radiolabelling, in order to confirm that the contents of the commercial canister were not significantly changed by the radiolabelling procedure (Figure 4-5, Figure 4-6).

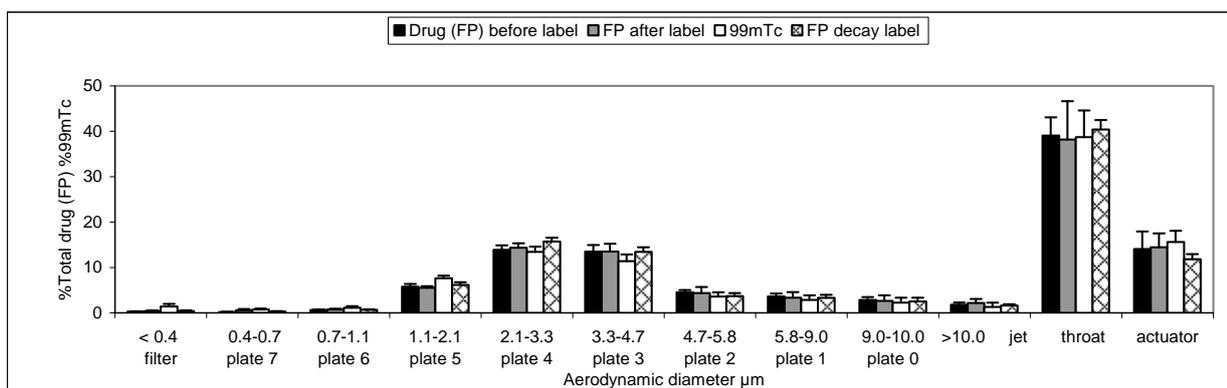


Figure 4-5: Particle size distribution FP delivered to Andersen Cascade Impactor *via* pMDI ‘before label’, ‘after label’ and ‘decay label’ and ^{99m}Tc radiolabel (n=5).

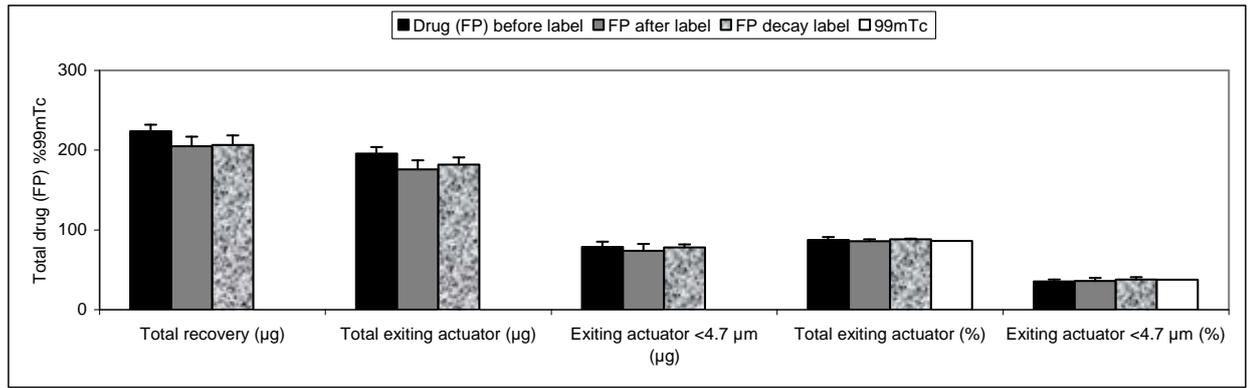


Figure 4-6: The y-axis shows the mean (+SD) mass (μg) drug (FP) or % $^{99\text{m}}\text{Tc}$ exiting actuator and in the fine particle fraction (particles $< 4.7 \mu\text{m}$) ‘before label’, ‘after label’ and after radioactive decay ‘decay label’. The x-axis shows drug output in μg and as a percentage of the total dose, with FP delivered *via* pMDI (n=5).

4.4.6 pMDI-spacer validation

Validation of the radiolabelling method *via* pMDI-spacer has not previously been reported in the literature. However in this thesis, the pMDI-spacer validation series was performed in order to reflect the clinical use of the spacer in the deposition study. On each study day a comparison was made between the reference particle size distribution (PSD) and output of drug (FP) ‘before label’ with the PSD of the corresponding $^{99\text{m}}\text{Tc}$ distribution. After radioactive decay, drug (FP) was measured in the corresponding fractions to determine the PSD of drug (FP) ‘after label’ (Figure 4-7).

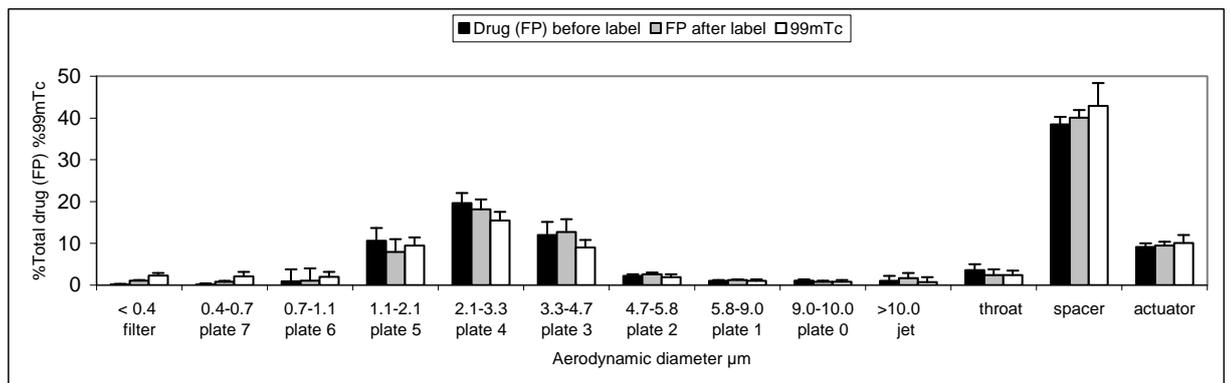


Figure 4-7: Validation with pMDI-spacer before patient use. The y-axis shows % total drug (FP) or $^{99\text{m}}\text{Tc}$. The x-axis shows the aerodynamic diameter of the drug (FP) particles (μm), ‘before label’, ‘after label’ and $^{99\text{m}}\text{Tc}$ (n=10).

4.4.7 pMDI-spacer validation after patient use

An additional fourth validation series was carried out directly after the children inhaled the radiolabelled drug (FP) in order to verify that the label was stable throughout the duration of the patient scintigraphic study (Figure 4-8, Figure 4-9).

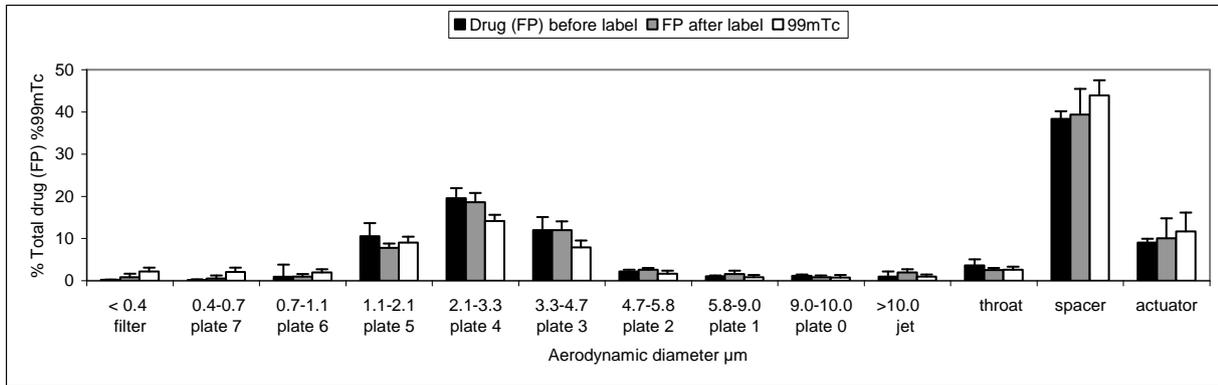


Figure 4-8: Validation with pMDI-spacer post-patient study. The y-axis shows % total drug (FP) or ^{99m}Tc. The x-axis shows the aerodynamic diameter of drug (FP) particles (µm) ‘before label’, ‘after label’ and ^{99m}Tc radiolabel.

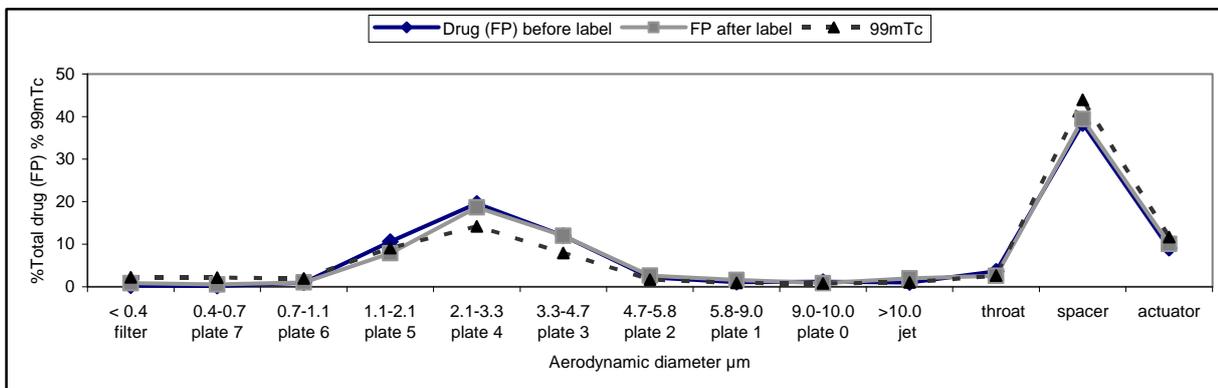


Figure 4-9: The y-axis shows % total drug (FP) or ^{99m}Tc. The x-axis shows the aerodynamic diameter of drug (FP) particles (µm) ‘before label’, ‘after label’ and ^{99m}Tc radiolabel. The line graph of the PSD shows the decreased output of ^{99m}Tc radiolabel on plates 3-5 after 1-2 hours (dotted line) compared with drug (FP).

4.4.8 Statistical analysis

Statistical methods were used to compare the particle size distributions of drug and radiolabel in validation experiments. Statistical analysis was carried out using Microsoft® Excel Analysis ToolPak (Microsoft Corporation, Redwood, WA, USA) using the paired t-test for sample means to compare the labelled drug with the ^{99m}Tc label and also to compare the radiolabelled drug with the drug after radioactive decay. The paired t-test for means was used if the same canister was compared. The unpaired t-test was used to compare the reference commercial unlabelled drug with radiolabelled drug, as the reference canisters were not the same canisters as the radiolabelled canisters. A p-value less than 0.05 was considered significant.

4.5 RESULTS

4.5.1 pMDI

4.5.1.1 Chloroform extraction method

The particle size distribution measured with cascade impaction, demonstrated a consistent mis-match between the ^{99m}Tc label and the drug (FP) particles using the chloroform extraction method, as shown in section 4.4.3.2, Figure 4-3.

4.5.1.2 Quality control

The match between the radiolabelled drug (FP) and ^{99m}Tc contained in the pMDI canister failed quality control. The ratio of ^{99m}Tc to FP should be in the range of between 0.8-1.2 for the fine particle fraction (FPF) of the delivered dose. This measure is recommended by the currently accepted consensus standards to verify the match between drug and label.[314] The ratio FPF ^{99m}Tc to drug (FP) was 1.4, which was outside the accepted limits. Therefore the distribution of the radiolabelled drug did not

match the distribution of the radiolabel, technetium-99m and the chloroform extraction technique was not suitable for the radiolabelling of fluticasone propionate (FP).

4.5.1.3 Butanone extraction method

Cascade impaction was used to measure the drug (FP) output from the actuator, ‘throat’, jet stage, 8 impaction plates and absolute filter from pMDI alone ‘before label’, ‘after label’ and ‘decay label’ (Table 4-3). The % ^{99m}Tc activity in each fraction was compared to % drug (FP) in each corresponding fraction. The mean percentage transfer of contents from the commercial Flixotide® canister (unlabelled FP ‘before label’) to the ^{99m}Tc-lined canister was approximately 95% (range 92.2% to 98.7%).

Table 4-3: Drug (FP) output with Andersen Cascade Impaction (ACI) with FP delivered via pMDI without spacer ‘before label’, ‘after label’ and ‘decay label’ (n=10).

FP-pMDI ACI	Before label	After label	Decay label
Total drug (FP) (µg)	231.4 ± 11.8	203.9 ± 20.3	214.0 ± 12.5
FP exiting actuator (µg)	203.0 ± 9.5	172.0 ± 17.7	188.0 ± 9.3
FP particles < 4.7 µm (µg)	79.0 ± 6.9	66.9 ± 11.8	74.8 ± 8.1
FP exiting actuator %	87.8 ± 1.2	84.4 ± 2.9	87.9 ± 1.5
FP particles < 4.7 µm %	34.2 ± 2.7	33.0 ± 5.9	35.1 ± 4.7
MMAD (µm)	3.5 ± 0.1	3.3 ± 0.2	3.2 ± 0.1
GSD	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1

The fine particle fraction (particles < 4.7 µm) delivered from the radiolabelled pMDI canisters after radiolabelling was compared to the FPF obtained from the commercial canisters before radiolabelling and there was not a significant difference in drug output

(p=0.361). The mean (SD) % FPF of drug (FP) ‘before’ label and ‘after’ label was 34.2 (2.7) and 33.0 (5.9) respectively.

Mean (SD) % ^{99m}Tc in the respirable fraction was 35.0 (4.4)%. The ratio ^{99m}Tc to drug (FP) in the FPF (particles < 4.7 μm) was 1.04 ± 0.03. The mean % drug (FP) in the FPF (particles < 4.7 μm) before radiolabelling and the mean % ^{99m}Tc, were not significantly (p=0.359). This confirmed that ^{99m}Tc acts as a suitable marker for HFA-FP for FP delivered *via* pMDI without spacer, when the butanone extraction method was used.

4.5.2 Consecutive pMDI

Table 4-4 shows the total mean (±SD) drug output for commercial drug, Flixotide® (HFA-FP), (‘before label’), radiolabelled FP (‘after label’), ‘decay’ radiolabelled drug (FP) after radioactive decay; the % mean (±SD) ^{99m}Tc and the MMAD and geometric standard deviation (GSD) of the particles.

Table 4-4: Drug output with Andersen Cascade Impaction for drug (FP) delivered *via* pMDI without spacer (n=5).

Drug (FP)	Commercial FP	Radioabelled FP	‘Decay’ FP	^{99m} Tc label
Total drug (FP) (μg)	217.3 ± 4.2	206.8 ± 11.3	209.8 ± 10.2	–
FP exiting actuator (μg)	186.7 ± 5.6	177.0 ± 9.5	184.5 ± 6.9	–
FP particles < 4.7 μm (μg)	74.2 ± 4.0	72.0 ± 8.9	77.6 ± 4.1	–
MMAD (μm)	3.5 ± 0.1	3.4 ± 0.2	3.3 ± 0.1	–
GSD	1.5 ± 0.01	1.5 ± 0.07	1.5 ± 0.07	–
% FP exiting actuator	86.0 ± 3.9	85.6 ± 3.1	88.0 ± 1.0	84.8 ± 2.7
% FP particles < 4.7 μm	34.1 ± 2.3	34.7 ± 2.8	37.0 ± 2.3	36.4 ± 2.0
% FP particles < 3.3 μm	20.6 ± 1.3	21.0 ± 1.5	23.6 ± 1.4	24.3 ± 1.3

There was not a significant difference between the % particles exiting the actuator for commercial FP ‘before label’ and ‘after label’ FP (p=0.88). Similarly there was not a significant difference between the % ^{99m}Tc exiting the actuator and % labelled FP (p=0.12); % particles <4.7 µm in radiolabelled FP and the % ^{99m}Tc (p=0.19). The MMAD for commercial FP was not significantly different to the MMAD for unlabelled FP (p=0.65) and radiolabelled FP and ‘decay’ FP (p=0.16). Figure 4-5, section 4.4.5, showed the mean (± SD) particle size distribution.

4.5.3 pMDI-spacer

Table 4-5 shows drug output HFA-FP when pMDI-spacer was used with ACI (n=10). The data is expressed as the mean (±SD). The statistical analysis found that there was not a significant difference in total drug (FP) output (p=0.19), drug exiting spacer (p=0.53), drug exiting spacer in the fine particle fraction (particles < 4.7 µm, (p=0.25) for commercial FP, ‘before label’ and radiolabelled FP, ‘after label’.

Table 4-5: Drug (FP) output with Andersen Cascade Impaction for unlabelled and labelled FP and ^{99m}Tc label delivered via pMDI-spacer (n=10).

Drug (FP)	Commercial FP	Radioabelled FP	^{99m} Tc label
Total FP (µg)	208.1 ± 8.8	200.9 ± 13.9	–
FP exiting spacer (µg)	108.6 ± 3.7	106.1 ± 12.0	–
FP particles < 4.7 µm (µg)	89.7 ± 2.3	86.9 ± 7.1	–
MMAD, µm	2.8 ± 0.1	2.9 ± 0.2	–
GSD	1.5 ± 0.02	1.5 ± 0.03	
% FP exiting spacer	52.3 ± 1.7	52.7 ± 4.7	49.1 ± 6.1
% FP particles < 4.7 µm	43.2 ± 1.8	43.9 ± 2.6	42.1 ± 5.1
% FP particles < 3.3 µm	31.1 ± 1.9	29.6 ± 2.2	31.9 ± 4.6

As a percentage of the total dose, there was not a significant difference between % drug (FP) exiting spacer in the % fine particle fraction (particles < 4.7 µm) for commercial FP and radiolabelled FP (p=0.48). Similarly there was not a significant difference between the % ^{99m}Tc radiolabel exiting the spacer in the fine particle fraction (FPF) and the % radiolabelled drug (FP) in the fine particle fraction (p=0.44).

The MMAD for the commercial drug (FP) ‘before label’ and radiolabelled drug (FP) ‘after label’ was statistically significantly different (p=0.01). Similarly there was a significant difference between the % radiolabelled FP ‘after label’ exiting the spacer in the extrafine particle fraction (EFPF, particles < 3.3 µm) and % ^{99m}Tc label (p=0.044). However, this was not matched with a significant difference between the reference commercial FP ‘before’ in the EFPF and % ^{99m}Tc label (p=0.62). The quality control showed that the mean ratio (±SD) of ^{99m}Tc/drug (FP) was 1.0 (± 0.03) in the FPF and 1.1 (± 0.1) in the EFPF, indicating that ^{99m}Tc levels could reflect FP in both the FPF and EFPF.[314] Table 4-6 shows the comparison of % commercial unlabelled FP exiting the spacer as a percentage of the total dose and delivered dose.

Table 4-6: Comparison of % total and delivered dose FP and ^{99m}Tc in the fine particle and extrafine particle fraction delivered with cascade impaction *via* pMDI-spacer.

Fine Particle Fraction	% Total dose	% Delivered dose
Commercial FP	43.2 ± 1.8	82.3 ± 2.6
^{99m} Tc	42.1 ± 5.1	85.5 ± 2.5
Ratio ^{99m} Tc/ commercial FP	-	1.04 ± 0.03
Extra-fine Particle	% Total dose	% Delivered dose
Commercial FP	31.1 ± 1.9	59.6 ± 3.0
^{99m} Tc	31.9 ± 4.6	65.0 ± 5.1
Ratio ^{99m} Tc/ commercial FP	-	1.09 ± 0.1

4.5.4 pMDI-spacer after patient use

An additional validation performed 1-2 hours after patient use was carried out for this thesis and this has not been previously reported. Table 4-7 shows the results of the final validation series that was carried out directly after a subgroup of children inhaled the radiolabelled canister (n=8). This was performed 1-2 hours after patient inhalation in order to validate that ^{99m}Tc acts as a marker for drug (FP) throughout the duration of the gamma scintigraphic study (Figure 4-8, section 4.4.7). The fine particle fraction (FPF) of the commercial drug, FP ‘before label’ and the ^{99m}Tc radiolabel was 41.9% and 37.5% respectively.

Table 4-7: Mean (SD)% drug output with ACI validation ‘post-patient’ inhalation of commercial ‘before’ FP, radiolabelled ‘after’ FP and ^{99m}Tc radiolabel (n=8).

Cascade Impactor	Commercial FP	Radiolabelled FP	^{99m}Tc label
% FP exiting spacer	51.1 ± 5.0	50.5 ± 4.3	44.4 ± 4.6
% FP particles < 4.7 µm	41.9 ± 3.5	40.9 ± 3.8	37.5 ± 3.3
% Particles < 3.3 µm	26.2 ± 10.1	28.9 ± 2.3	29.5 ± 3.4
MMAD µm	2.9 ± 0.5	2.9 ± 0.4	-
GSD	1.6 ± 0.1	1.6 ± 0.1	-

The ratio of the FPF of the delivered dose ^{99m}Tc label to commercial drug (FP) in the FPF was 0.83 which represented an acceptable match between drug (FP) and ^{99m}Tc . Although the total output of ^{99m}Tc was decreased in the delivered dose (44.4% compared to 49.1% with the previous ACI validation performed directly before the patient study), the match between drug and label was acceptable, based on current recommendations.[314, 434] Furthermore, in the ‘post-patient’ validation series there was not a significant difference between the % ^{99m}Tc in the delivered dose for particles

less than 4.7 μm and the % drug (FP) for particles less than 4.7 μm before radiolabelling ($p=0.190$). This 'post-patient' validation series verified that the match between $^{99\text{m}}\text{Tc}$ and drug had not degraded significantly during the duration of the gamma scintigraphic procedure.

4.6 DISCUSSION

Radiolabelling methods used for inhaled drug formulations require rigorous validation with cascade impaction to ensure that the aerodynamic particle size distribution of drug matches the aerodynamic particle size distribution of the technetium-99m ($^{99\text{m}}\text{Tc}$) radiolabel. A method to radiolabel Flixotide® (fluticasone propionate reformulated with hydrofluoroalkane, HFA-FP) with $^{99\text{m}}\text{Tc}$ was developed for this study.[346] HFA-FP had not been previously radiolabelled, prior to the study described in this chapter and published in the Journal of Aerosol Medicine.

Ideal validations show that there has been no significant change in the mass distribution and particle size distribution before and after the radiolabelling procedure.[314, 434, 460] In this study, a preliminary validation with five reference canisters of commercial Flixotide® (HFA-FP) showed that there was not a significant alteration of drug output of unlabelled FP or $^{99\text{m}}\text{Tc}$ -labelled FP, when HFA-FP was delivered *via* pMDI alone, providing indications that HFA-FP could be radiolabelled with $^{99\text{m}}\text{Tc}$ without causing significant changes to the characteristics of the drug.

As the ultimate aim was to deliver the drug (HFA-FP) *via* pMDI with attached spacer in a clinical deposition study, the preliminary validation with the commercial pMDI was followed with a validation of ten reference canisters of fluticasone propionate reformulated with hydrofluoroalkane (HFA-FP) delivered *via* pMDI-spacer (Aerochamber Plus™), in order to reflect clinical use.[115] This additional validation

with a pMDI with an attached spacer device has not been previously reported in the literature.

The pMDI-spacer validation showed that the drug output from ten pMDI with attached spacer (Aerochamber Plus™) was not significantly altered after radiolabelling. The mass of particles in the fine particle fraction (particles < 4.7 µm) before radiolabelling was not significantly different to the mass of particles in the fine particle fraction after radiolabelling. Furthermore, the % drug (FP) exiting the spacer and the % drug (FP) exiting the spacer in fine particle fraction (particles < 4.7 µm) before radiolabelling was not significantly different to the % ^{99m}Tc label in the fine particle fraction after radiolabelling.

In a clinical deposition study the % technetium-99m deposition should reflect the % drug deposition, so that estimations of drug deposition can be made based on the ^{99m}Tc radiolabel distribution measured *in vivo* by gamma scintigraphy. The information from gamma scintigraphic studies with radiolabelled drugs provides clinically useful information about total body deposition and regional deposition of drug/device combinations. Therefore consecutive radiolabels were used to demonstrate that the radiolabelling method was reliable and robust.

Some variability was observed in the aerodynamic properties of drug (FP) before and after radiolabelling when pMDI-spacer was used to deliver HFA-FP. There was a statistically significant difference in the MMAD for labelled FP and unlabelled FP. This may be associated with the different mass of HFA-FP in the reference canisters used for the 'before label' compared with the labelled canisters used 'after label'.

A small but consistent mismatch between drug level and ^{99m}Tc radiolabel was seen on plates 3 and 5 of the cascade impactor and this led to a small but statistically significant difference in the % labelled drug (FP) and % ^{99m}Tc level in the extrafine particle

fraction ($< 3.3 \mu\text{m}$). This may be due to an association between $^{99\text{m}}\text{Tc}$ and the propellant blend under some labelling conditions.[183, 337, 338] However, the % $^{99\text{m}}\text{Tc}$ exiting the spacer in the extrafine particle fraction (EFPF) was not significantly different to the % extrafine particles exiting the spacer in the reference unlabelled drug (FP). Furthermore the ratio of delivered $^{99\text{m}}\text{Tc}$ to delivered drug (FP) in the FPF and EFPF was within acceptable levels, according to current guidelines.[314]

This indicated that the $^{99\text{m}}\text{Tc}$ levels could reliably reflect drug (FP) levels. Before each clinical deposition study a pre-deposition validation procedure was carried out to ensure a consistent and acceptable match between drug and $^{99\text{m}}\text{Tc}$ label. Therefore we have confirmed the hypothesis that the particle size distribution of the radiolabelled drug had not been significantly altered by the radiolabelling procedure. Furthermore, with the radiolabelling method developed in this study, $^{99\text{m}}\text{Tc}$ can act as a suitable marker for HFA-FP.

A limitation of the validation procedure is that the $^{99\text{m}}\text{Tc}$ and drug match may degrade after patient use. This may mean that if several patients inhaled the radiolabelled drug from the same radiolabelled pMDI canister there could be a mis-match during the time course of the procedure. However, importantly, the final validation series performed in this chapter confirmed that 1-2 hours after the patient inhalation of radiolabelled drug, the match between $^{99\text{m}}\text{Tc}$ and FP remained acceptable, indicating that there was not a significant degradation of the radiolabelled drug throughout the duration of the patient study. This final validation procedure, performed 1-2 hours after patient use, has not been previously reported, however it provides valuable information about the duration of an acceptable match between drug and label in the labelled pMDI canister.

With propellant based suspension formulations such as fluticasone propionate, inconsistencies in method may affect the variability of the particle size distribution

(PSD). Several factors may influence the variability of the particle size distribution, such as the timing between shaking the pMDI canister, the rate of actuation and the drop in the temperature of the pMDI, the number of actuations, differences in delay times when firing multiple actuations and differences in the repetitive washing of drug from the cascade impactor plates. Other factors include differences in suspension resettling time, stage overload and particle bouncing. The particle size distribution may also be influenced by the limits of detection of the UV spectrophotometer, if small numbers of actuations are used.[365]

Validation of the radiolabelling method is essential before performing gamma scintigraphic studies in order to verify that technetium-99m acts as a valid marker for the commercial drug. Ideally the particle size distribution of the radiolabelled drug should match the particle size distribution of the commercial drug in order to verify that the radiolabelling process has not significantly altered the characteristics of the drug.

In vitro assessments of the fine particle fraction from the particle size distribution of a drug formulation have been shown to have some predictive power not only for drug deposition, but also for clinical effects.[109] The next chapter of this thesis will investigate whether whole-body deposition and lung deposition of ^{99m}Tc -HFA-FP measured with gamma scintigraphy correlates with total drug output and fine particle fraction measured by cascade impaction.

5 DEPOSITION OF HFA-FP

5.1 INTRODUCTION

Fluticasone propionate (FP) is a potent corticosteroid which is commonly used to treat childhood asthma. FP has been reformulated with hydrofluoroalkane (HFA) in pressurized metered-dose inhalers (pMDI), and has the tradename of Flixotide® (GlaxoSmithKline, UK). Flixotide® is the most widely used formulation in Australia. HFA-FP has retained the same particle size, deposition, and efficacy profile as CFC-FP.[442] [461, 462] Reformulation of FP with HFA propellant has produced a suspension formulation with the same impact force as CFC-FP.[86, 463] [464] The mass median aerodynamic diameter (MMAD) of HFA-FP has been reported to be 2.8 (± 0.1) μm when delivered *via* a pMDI-spacer (Aerochamber Plus™, Trudell Medical International, London, Canada).[346]

FP has greater anti-inflammatory effect compared to beclomethasone dipropionate and has been shown to be effective in improving lung function in infants and children.[250] [253] A review of randomised controlled trials in children have shown that fluticasone given at the same daily dose as extrafine beclomethasone or half the daily dose of budesonide can improve lung function (FEV1).[450, 465]

High drug deposition within the airways improves the efficacy of Flixotide®, but may lead to adverse systemic side-effects such as osteoporosis and adrenal suppression, if the dose of FP given to children is greater than 400 $\mu\text{g}/\text{day}$.[465] A randomized trial of 744 children from 6-11 years of age has shown that urine free cortisol levels decreased significantly with FP for doses approximately 200 $\mu\text{g}/\text{day}$ compared with ciclesonide.[466] The Australian Adverse Drug Reactions Bulletin has reported

worldwide cases of adrenal insufficiency developing in children using inhaled corticosteroids.[287]

In the 8 cases in Australia, the ages ranged from 3-10 years, and the doses of FP ranged from 250-1500 µg daily. Therefore delivery devices and inhalation techniques that markedly increase the dose delivered to the airways will increase plasma concentrations of FP and this can lead to adverse effects in children even at low doses.[286, 467]

Systemic side-effects of FP are directly related to lung deposition as FP has negligible systemic activity from gastrointestinal deposition of swallowed drug due to hepatic first pass inactivation.[461, 468] However local side-effects, such as hoarseness, dysphonia, pharyngitis and sore throat have been demonstrated after HFA-FP inhalation by children, therefore spacer devices that minimise oropharyngeal deposition are strongly recommended for children.[465]

Delivery devices and patient inhalation techniques are the primary determinants of the dose delivered to the lungs.[2, 115, 469] A pharmacokinetic (PK) study with children estimated that lung deposition of FP *via* the Diskus dry powder inhaler (DPI) was approximately 8% nominal dose in children.[257] However a PK study with adults has shown that lung deposition of FP *via* pMDI-spacer (OptiChamber) is approximately 4 times greater than lung deposition with a DPI device (Flovent Rotadisk Diskhaler).[468, 470] Higher therapeutic efficacy of FP has been shown in a group of school-age children (8-15 years) after inhalation *via* pMDI-spacer (AIR) compared with DPI (Diskus), indicating that the spacer device enhances lung deposition of FP.[259] Aerochamber Plus™, a small volume spacer, has been shown to optimise the delivery of fluticasone propionate (FP).[454-456] However *in vitro* investigations have shown that the delivery of FP can be variable depending on the type of spacer device used.[260] Short term knemometry studies in children inhaling FP *via* pMDI-spacer

(Aerochamber Plus™, AC+) or Diskhaler have shown significantly reduced lower leg growth.[471, 472] High lung deposition of FP would lead to increased systemic bioavailability and therefore increased side-effects in asthmatic children. High oropharyngeal deposition would be associated with increased local side-effects. Therefore it is important to ascertain both lung deposition and oropharyngeal deposition of FP from different spacer devices using different inhalation techniques in order to predict possible side-effects in children and as a guide to dosage regimens.

The National Asthma Council Australia has recommended HFA-FP and HFA-BDP at the same dosage for the treatment of paediatric asthma.[201] Extrafine HFA-BDP delivered *via* pMDI-Aerochamber Plus™ (AC+) using a slow, single maximal inhalation followed by a ‘breath hold’ has been shown to increase lung deposition in young asthmatic children almost twofold and reduce variability.[245] However device comparison studies have shown that spacer performance with one drug does not predict performance for other drugs.[456]

Previous studies have shown that the fine particle fraction (FPF), defined in terms of aerosol < 5.8 µm or < 6.8 µm diameter, systematically overestimated lung deposition for virtually all inhalers.[84] Newman and Chan reported that lung deposition was closer to the percentage of the aerosol dose smaller than 3 µm diameter.[109] Farr *et al* reported that the predicted lung deposition can be derived from the fine particle fraction and the emitted dose.[106]

Drug delivery from specific drug and device combinations provides important information about the likely clinical effect. HFA-BDP delivered *via* pMDI-AC+ has been shown to be equally effective as CFC-FP delivered *via* pMDI-Volumatic® at improving asthma control in children with mild-to-moderate asthma at the same daily dose.[473] The *in vitro* fine particle fraction of HFA-FP delivered

via pMDI-Volumatic® with a constant airflow has been shown to be equivalent to the fine particle fraction of HFA-FP delivered *via* pMDI-AC+.[454] However, in an adult study investigating cortisol suppression as a marker of lung dose, lung bioavailability of HFA-FP inhaled *via* pMDI-AC+ with a slow single maximal inhalation and ‘breath hold’ was shown to be higher than *via* pMDI-Volumatic®.[455]

The Funhaler, an incentive spacer device, has been shown to produce equivalent control of asthma symptoms compared with pMDI-spacer (AC+) in children less than 7 years of age, using tidal breathing.[189] However, variability in lung deposition remains high in children using different delivery devices, different formulations and different breathing patterns.[115] The residence time of the aerosol can be affected by the spacer volume and the spacer inhalation technique.[474] [475] Young children show irregular tidal breathing patterns and subsequent variability in the inhaled dose.[165]

Considerable variation in the fine particle fraction of salbutamol (MMAD 2.9 μm) has been shown with simulated pediatric tidal breathing with different spacer devices.[476]

The Funhaler spacer device may improve dose consistency with tidal breathing, by regulating the breathing pattern in response to the ‘toy and whistle’. Planar gamma scintigraphy has shown lung deposition values of approximately 50% for radiolabelled extrafine HFA-BDP (MMAD 1.1 μm) inhaled *via* pMDI-AC+ for children using a single maximal inhalation followed by a ‘breath hold’ for 5-10 s.[245] Children inhaling radiolabelled salbutamol with a slow single maximal inhalation and ‘breath hold’ *via* pMDI-Volumatic® have shown lung deposition values of approximately 40%.[171]

There is no published data describing the lung deposition of HFA-FP (MMAD 2.8 μm) delivered *via* pMDI with an attached small volume spacer in asthmatic children, using gamma scintigraphy. Scintigraphic studies have been shown to be a good indicator of

the degree of pulmonary targeting of inhaled drugs, proving a marked difference between different drug formulations, devices and inhalation techniques. Deposition studies with inhaled fluticasone propionate have not been measured in young children previously, therefore the expected variability between children with asthma is not known.

The experimental studies described in this chapter aimed to use gamma scintigraphy to assess the deposition of HFA-FP, delivered *via* pMDI with attached small volume spacer Aerochamber Plus™ in children 5-17 years of age and *via* Funhaler spacer in children 5-10 years of age. Spacer inhalation with tidal breathing or with a slow single maximal inhalation with a ‘breath hold’ for 10 s, are commonly recommended by clinicians when training children to use pMDI-spacers. All children included in the study were able to maintain the ‘breath hold’ for a minimum of 5 s.

5.2 OBJECTIVES

- Assess the *in vitro* drug output and fine particle fraction of HFA-FP using Andersen Cascade Impaction (ACI).
- Assess the total drug deposition of HFA-FP with two different breathing techniques and two different spacers with an inspiratory filter.
- Evaluate the lung deposition and total body distribution of HFA-FP delivered *via* pressured metered-dose inhaler (pMDI) with attached spacer (Aerochamber Plus™) and Funhaler to children with mild asthma, using two different breathing techniques and compare this with extrafine QVAR™.

5.3 HYPOTHESES

- *In vitro* estimations of body deposition with Andersen Cascade Impaction (ACI) and the inspiratory filter method will overestimate *in vivo* measures of drug deposition and underestimate the variability in dose.
- Lung deposition in asthmatic children will be reduced with the coarse particle size HFA-FP compared with extrafine QVAR™.
- A slow single maximal inhalation with ‘breath hold’ will enhance lung deposition of HFA-FP with pMDI-spacer (Aerochamber Plus™, AC+) and reduce variability compared with tidal breathing.
- The Funhaler (FH) incentive spacer device will show equivalent lung deposition compared with Aerochamber Plus (AC+).
- Oropharyngeal and gastrointestinal deposition will be increased with the coarse particle size HFA-FP compared with extrafine QVAR™.

5.4 METHODS

5.4.1 Study design

A series of *in vitro* and *in vivo* studies were performed in order to assess the deposition of ^{99m}Tc-HFA-FP delivered *via* pMDI-spacer (AC+ and FH) in asthmatic children from 5-17 years of age as shown in Table 5-1.

Table 5-1: Experimental steps involved in deposition of HFA-FP study.

<i>In vitro</i> characterisation of HFA-FP with cascade impaction.
Inspiratory filter drug output of HFA-FP with AC+ and FH
Validation of radiolabelling method for ^{99m} Tc-HFA-FP <i>via</i> pMDI and pMDI-spacer.
Deposition of ^{99m} Tc-HFA-FP delivered <i>via</i> pMDI-spacer (AC+) 5-17 years (n=26)
Deposition of ^{99m} Tc-HFA-FP delivered <i>via</i> pMDI-spacer (FH) 5-10 years (n=10)
Comparison <i>in vitro</i> cascade impaction, filter study and <i>in vivo</i> gamma scintigraphy.

5.4.2 Study population

Thirty-six children (28 male, 8 female) from 5-17 years of age, with mild, stable asthma were recruited from outpatient clinics at Princess Margaret Hospital for Children. On the study day, each child had weight, height and lung function measurements with Koko® spirometry Table 5-2. Only those patients with FEV1 > 80% predicted values were enrolled in the study, as detailed in Chapter 2, section 2.2.3.[429]

5.4.3 Inhalation technique

Each child was trained to perform either tidal breathing (n=22) or a single maximal inhalation followed by a 5-10 s ‘breath hold’ (n=14). Twelve children inhaled HFA-FP (250 µg FP/ actuation) with tidal breathing *via* pMDI-spacer (AC+) and ten children inhaled with Funhaler. Alternatively the child rehearsed with a slow single maximal inhalation, followed by a ‘breath hold’ for 5-10 s (n=14), as described in Chapter 2, section 2.2.5.

These groups are referred to as ‘tidal’ and ‘breath hold’ respectively. The two AC+ groups: ‘tidal’ (n=12) and ‘breath hold’ (n=14) were further sub-divided according to age-groups: 5-7 years and 8-17 years. The Funhaler age-groups were children 5-10 years (n=10), children 5-7 years (n=6) and 8-10 years (n=4).

Table 5-2: Height (cm), weight (kg), FEV1 (L) and FVC (L) in the children of ‘tidal’ and ‘breath hold’ groups with AC+ and Funhaler (FH)*.

Age group (yrs)	Spacer	N	Height (cm)	Weight (kg)	FEV1 (L)	FVC (L)
Tidal						
5-7	AC+	6	116.8 ± 5.2	23.1 ± 4.8	1.2 ± 0.1	1.5 ± 0.3
8-17	AC+	6	144.0 ± 14.5	42.9 ± 12.5	2.2 ± 0.6	2.5 ± 0.6
5-10	FH	10	124.4 ± 11.5	25.1 ± 5.8	1.5 ± 0.3	1.7 ± 0.4
5-7	FH	6	118.0 ± 9.4	21.8 ± 4.3	1.4 ± 0.3	1.5 ± 0.3
8-10	FH	4	134.1 ± 6.7	30.1 ± 3.7	1.7 ± 0.2	2.0 ± 0.2
Breath hold						
5-7	AC+	4	119.2 ± 7.7	23.5 ± 3.7	1.3 ± 0.2	1.4 ± 0.3
8-17	AC+	10	141.3 ± 15.4	37.6 ± 11.3	2.1 ± 0.9	2.1 ± 0.9

*Data are presented as mean ±SD

5.4.4 Inspiratory filter study

Prior to gamma scintigraphy the children inhaled *via* pMDI-spacer with attached inspiratory filter. A low resistance filter was placed between the pMDI-spacer and the child’s mouth-piece as described in Chapter 2, section 2.2.6. Each child inhaled 3 actuations of Flixotide® (250 µg/actuation), using the prescribed inhalation technique.

5.4.5 Particle size distribution of HFA-FP

The preliminary *in vitro* work involved determining the drug output and particle size distribution of commercial Flixotide® using cascade impaction, as detailed in as detailed in Chapter 2, section 2.2.8 and Chapter 4, section 4.4.2. The particle size distribution of HFA-FP with the pMDI alone was compared with QVAR™ in order to assess the effect of the different formulation on drug delivery (Figure 5-1).

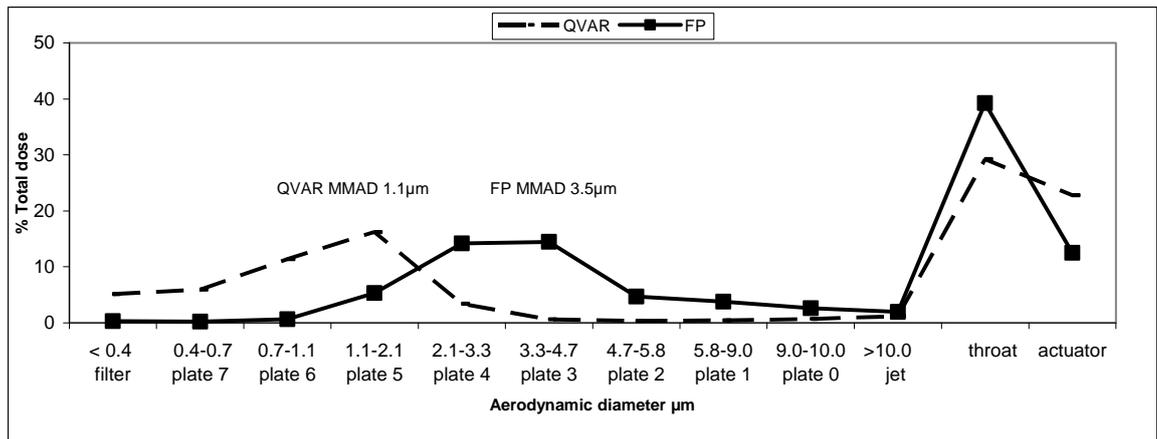


Figure 5-1: Preliminary line graph showing comparative particle size distribution from Andersen Cascade Impactor (ACI) for % total dose (FP and BDP) delivered *via* pMDI (n=10) and corresponding particle sizes (µm). **Note:** Increase in MMAD and aerodynamic diameter size range for fine particle fraction with FP.

5.4.6 Validation of radiolabelling method

In vitro particle size distributions of HFA-FP from ^{99m}Tc -labelled canisters was compared with the drug output and particle size distribution of drug (FP), delivered *via* pMDI-spacer (AC+), as described in detail in Chapter 4, section 4.4.6, Figure 4-7. The drug (FP) output, particle size distribution and fine particle fraction of labelled FP and ^{99m}Tc were compared to the commercial unlabelled reference Flixotide® canisters (n=10).

5.4.7 Gamma Scintigraphy

A double-headed gamma camera (Siemens E.CAM, GE Healthcare, USA) was used for the Flixotide® deposition study. After the transmission scan each child inhaled 2-3 doses of ^{99m}Tc -HFA-FP (500-750 µg) so that the dose was within 2-4 MBq, as detailed in Chapter 2, section 2.2.11. Two children in the AC+ ‘tidal’ group did not have P:C ratios calculated, as data could not be retrieved for technical reasons.

5.4.8 Ethical considerations

Approval for the study was granted by the Princess Margaret Hospital Ethics Committee. Informed consent was obtained from parents and children, as described in Chapter 2, section 2.2.7.

5.4.9 Statistical analysis

Statistical analysis of the radiolabelling validation method was carried out using Microsoft© Excel as detailed in Chapter 2, section 2.2.15.1. Statistical analyses for gamma deposition data were carried out using SPSS package 16.0, as detailed in Chapter 2, section 2.2.15.2. Lung doses, oropharyngeal and gastrointestinal doses and spacer retention of ^{99m}Tc -HFA-FP were presented with means and the corresponding standard deviations (SD). Analysis of variance (ANOVA) was used to compare lung deposition with the three delivery methods: AC+ ‘tidal’, AC+ ‘breath hold’ and FH. Post-hoc tests were performed with Fisher’s Least Significant Difference (LSD).

Based on a previous deposition study with radiolabelled salbutamol [171], there was a difference of 13.6% in lung deposition for children using tidal breathing compared with ‘breath hold’. In the present study we aimed to detect a difference of 10% in lung deposition between ‘tidal’ and ‘breath hold’. A statistical power >80% to detect the difference for a sample size of 5 in each of groups was determined. The power estimation was performed using the standard deviations reported in the previous study.

5.5 RESULTS

5.5.1 Particle size distribution

The mass distributions and corresponding particle size distributions of HFA-FP delivered *via* pMDI and pMDI-spacer were compared in order to determine the fine

particle mass (FPM), mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD), shown in Table 5-3.

Table 5-3: Drug (FP) output (µg) delivered *via* pMDI (n=10) and pMDI-spacer (n=10) to Andersen Cascade Impactor (ACI) with fine particle mass (FPM), MMAD and GSD.

Mass FP (µg)	pMDI	pMDI-spacer
Total recovery	231.4 ± 11.8	208.1 ± 8.8
Delivered dose	203.0 ± 9.5	108.6 ± 3.7
FPM (particles < 4.7 µm)	79.0 ± 6.9	89.7 ± 2.3
MMAD µm	3.5 ± 0.1	2.8 ± 0.1
GSD	1.6 ± 0.1	1.5 ± 0.02

There was a statistically significant difference between the delivered dose from pMDI alone compared to pMDI-spacer ($p < 0.001$). Similarly there was a significant increase in the fine particle mass (FPM, particles < 4.7 µm) and a significant decrease in MMAD when FP was delivered *via* pMDI-spacer compared to pMDI alone ($p < 0.000$, $p < 0.000$) respectively). Table 5-4 shows a comparison of drug output on the cascade impactor with Aerochamber Plus (AC+) and Funhaler.

Table 5-4: Comparison of total drug (FP) output (µg) on the actuator, spacer and ACI, representing available dose for total body deposition (TBD) for Aerochamber Plus™ (AC+) and Funhaler (FH).

Mass FP µg	N	Device	Actuator	Spacer	TBD
ACI	10	AC+	19.1 ± 2.3	80.5 ± 4.5	108.6 ± 3.7
ACI	10	FH	15.5 ± 3.2	89.8 ± 11.4	122.1 ± 10.4
% FP	N	Device	Actuator	Spacer	TBD
ACI	10	AC+	9.1 ± 0.9	38.4 ± 1.8	52.3 ± 2.0
ACI	10	FH	6.8 ± 1.3	39.5 ± 5.1	53.7 ± 4.1

5.5.2 Inspiratory filter study

A comparison of the % mean (SD) *in vivo* inspiratory filter drug delivery ($\mu\text{g}/250\mu\text{g}$ FP) as a proportion of ex-valve dose, ex-actuator dose and the % mean (SD) drug particles exiting the spacer *in vitro* are presented in Table 5-5 and Table 5-6. With pMDI-spacer (AC+), the mean % (SD) filter dose was 44.1 (6.1) for the ‘tidal’ group (n=12) and 48.0 (4.7) for the ‘breath hold’ group (n=14). There was an increase in the mass drug (FP) recovered on the inspiratory filter with ‘breath hold’ compared with ‘tidal’, however the difference was not significant (p=0.064).

Table 5-5: Comparison of mean (\pm SD) total mass FP (μg) exiting pMDI-spacer (AC+ and FH) with ACI and inspiratory filter.

Drug (FP) μg	N	Device	Actuator	Spacer	Total dose
ACI	10	AC+	19.1 \pm 2.3	80.5 \pm 4.5	110.2 \pm 5.3
ACI	10	FH	15.5 \pm 3.2	89.8 \pm 11.4	122.1 \pm 10.4
Filter	26	AC+	31.8 \pm 10.1	91.7 \pm 16.3	106.1 \pm 20.5
‘Tidal’ filter	12	AC+	29.7 \pm 9.2	95.8 \pm 19.0	98.9 \pm 18.5
‘BH’ filter	14	AC+	32.5 \pm 10.5	87.4 \pm 13.6	110.5 \pm 20.4
Filter	10	FH	28.8 \pm 9.7	119.2 \pm 16.4	103.5 \pm 7.4
% FP	N	Device	Actuator	Spacer	Total dose
ACI	10	AC+	9.1 \pm 0.9	38.4 \pm 1.8	52.3 \pm 2.0
ACI	10	FH	6.8 \pm 1.3	39.5 \pm 5.1	53.7 \pm 4.1
Filter	26	AC+	13.8 \pm 3.6	40.0 \pm 4.5	46.2 \pm 5.6
‘Tidal’ filter	12	AC+	13.2 \pm 4.2	42.5 \pm 4.2	44.1 \pm 6.1
‘BH’ filter	14	AC+	14.1 \pm 3.1	37.9 \pm 3.5	48.1 \pm 4.6
Filter	10	FH	10.8 \pm 2.4	45.6 \pm 3.9	39.9 \pm 4.3

Table 5-6: Summary of % mean (SD) ex-valve and ex-actuator *in vivo* drug (FP) delivery collected on inspiratory filter and *in vitro* drug output from ACI (n=10) with FP delivered *via* pMDI-spacer (AC+ and FH).

Device	ex-valve	ex-actuator
Inspiratory filter AC+ (n=26)	46.2 ± 5.6	53.5 ± 5.4
‘Tidal’ AC+ (n=12)	44.1 ± 6.1	50.8 ± 5.6
‘BH’ AC+ (n=14)	48.1 ± 4.6	55.7 ± 4.4
Funhaler (FH) (n=10)	39.9 ± 4.3	44.7 ± 4.2
% FP exiting spacer (ACI)	52.3 ± 1.7	57.8 ± 1.9
% FP particles < 4.7 µm (AC+)	43.2 ± 1.8	47.8 ± 1.6
% FP particles < 4.7 µm (FH)	49.4 ± 3.7	53.0 ± 4.6
% FP particles 1.1- 4.7 µm	43.1 ± 1.8	47.3 ± 1.7
% FP particles < 3.4 µm	31.1 ± 1.9	34.6 ± 1.9
% FP particles < 2.2 µm	11.9 ± 1.0	13.1 ± 1.0

With pMDI-spacer (Funhaler), the mean % (SD) filter dose was 39.9 (4.3). There was a significantly increased mass of drug retained on the actuator and spacer after patient inhalation *via* pMDI-spacer (Funhaler) during the inspiratory filter study compared with *in vitro* ACI data (p=0.002, p=0.000 respectively).

The % CV for the actuator drug dose increased from 12% with *in vitro* ACI, to 34% with the inspiratory filter. Similarly the % CV for spacer drug retention increased from 6% with ACI to 20% on the spacer attached to the inspiratory filter. The % CV for the total drug dose increased from 5% with ACI to 19% with the inspiratory filter. With the ‘tidal’ AC+ group, the ‘breath hold’ AC+ group and the FH group, there were statistically significant decreases in the percentage of drug (FP) recovered from the inspiratory filter compared with drug (FP) exiting the pMDI-spacer attached to the

cascade impactor (p=0.000, p=0.011, p=0.000). The fine particle fractions described by Mitchell, Finlay, Newman, Farr and Newhouse, as described in Chapter 2, section 2.2.8.1 are shown in Table 5-7.

Table 5-7: The % drug (FP) delivered to Andersen Cascade Impactor (ACI) with fine particle fractions. *Predicted lung deposition for AC+ as derived by modified Farr *et al* formula. [106]

% Drug (FP) output from ACI	ex-valve	ex-actuator
% Delivered drug (FP)	52.3 ± 1.7	57.8 ± 1.9
% FP particles < 4.7 µm (Mitchell)	43.2 ± 1.8	47.8 ± 1.6
% FP particles 1.1- 4.7µm (Finlay)	42.1 ± 1.7	46.3 ± 1.6
% FP particles < 3.3 µm (Newman)	31.1 ± 1.9	34.6 ± 1.9
*% Predicted lung deposition (Farr)	22.6 ± 1.6	25.1 ± 1.7
% FP particles < 2 µm (Newhouse)	11.9 ± 1.0	13.1 ± 1.0

The fine particle fraction (FPF) delivered from the cascade impactor, based on particles < 4.7 µm, was not significantly different to the FPF within particle sizes 1.1-4.7 µm, for ex-valve or ex-actuator deposition (p=0.109, p=0.064 respectively). However these values overestimated the predicted lung deposition by the modified Farr *et al* formula almost twofold.

5.5.3 Validation of radiolabelling method

The results of the validation were described in detail in Chapter 4, section 4.4.6. The mean ratio ^{99m}Tc/FP (±SD) was 1.0 (±0.03) in the fine particle fraction, indicating that ^{99m}Tc levels could reliably reflect FP. The mean (SD) amount of radioactivity per actuation was 1.5 (0.4) MBq. The total dose delivered to each patient was 2-4 MBq, as described in Chapter 2, section 2.2.11.

5.5.4 *In vivo* gamma scintigraphy

Attenuation factors (AFs) were derived as detailed in Chapter 2, section 2.2.12. AFs for the lung, mouth, throat, oesophagus and stomach ROI ranged from 1.5-4.0. The total body distribution of ^{99m}Tc -HFA-FP is demonstrated in the anterior planar gamma scintigraphic images in Figure 5-2.

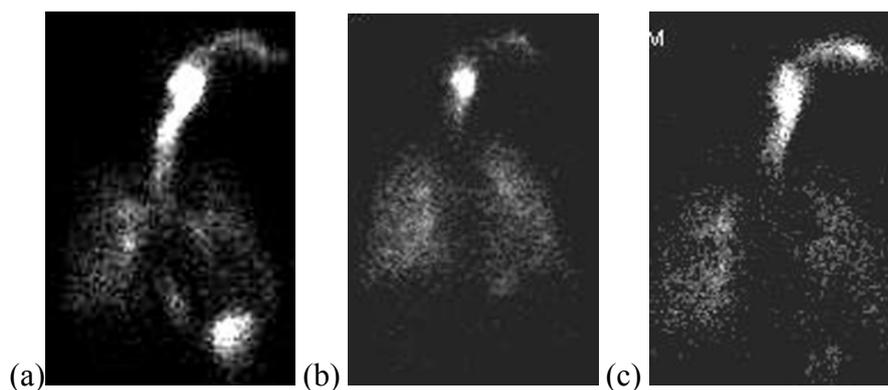


Figure 5-2: Anterior scintigraphic images of total body distribution of ^{99m}Tc -HFA-FP. (a) Child aged 6 years with 'tidal'. (b) Child aged 9 years with 'breath hold'. (c) Child aged 13 years with 'tidal'.

5.5.5 Aerochamber Plus™ spacer device

The regional distribution of ^{99m}Tc -HFA-FP in lungs, oropharynx and gastrointestinal tract, spacer and expiratory filter in the 'tidal' group is shown in Table 5-8. Across all ages the mean (SD) % ex-actuator lung, OG and spacer deposition for 'tidal' was 20.8 (10.7), 20.1 (7.7) and 57.5 (15.0) and mean (SD) % ex-actuator lung, OG and spacer for 'breath hold' was 24.1 (8.7); 23.1 (8.5) and 52.2 (14.1) and these were not significantly different ($p=0.392$, $p=0.368$, $p=0.0368$ respectively), Table 5-8, Table 5-9, Figure 5-3 and Figure 5-4.

Table 5-8: Regional distribution (% ex-actuator dose*) of ^{99m}Tc-HFA-FP in the tidal breathing groups (Tidal) with AC+: 5-7 years, 8-17 years and 5-17 years. ‘OG’ defined as oropharyngeal and gastrointestinal deposition.

Age (yrs)	N	Lungs %	OG %	Spacer %	Expiratory filter %	P:C
5-7	6	15.7 ± 7.8	17.2 ± 9.2	65.4 ± 14.9	1.4 ± 1.3	1.6 ± 0.3 (n=4)*
8-17	4	25.9 ± 11.3	22.7 ± 5.5	49.6 ± 12.1	1.8 ± 2.5	1.6 ± 0.6
Total 5-17	12	20.8 ± 10.7	20.1 ± 7.7	57.5 ± 15.3	1.6 ± 1.9	1.6 ± 0.5 (n=10)

* Percentage of ex-actuator dose corrected for tissue attenuation and presented as means ± SD. *Two subjects aged 5-7 yrs did not have P:C ratio measurements.

Table 5-9: Regional distribution (% ex-actuator dose*) of ^{99m}Tc-HFA-FP in groups using the single maximal inhalation with breath hold (Breath hold) with AC+: 5-7 years , 8-17 years and 5-17 years. ‘OG’ defined as oropharyngeal and gastrointestinal deposition.

Age (yrs)	N	Lungs %	OG %	Spacer %	Expiratory filter %	P:C
5-7	4	21.4 ± 5.9	27.2 ± 9.6	50.8 ± 15.2	0.5 ± 0.3	2.1 ± 0.6
8-17	10	25.2 ± 9.6	21.4 ± 8.0	52.8 ± 14.5	0.6 ± 0.6	2.0 ± 0.6
Total 5-17	14	24.1 ± 8.7	23.1 ± 8.5	52.2 ± 14.1	0.6 ± 0.5	2.0 ± 0.6

* Percentage of ex-actuator dose corrected for tissue attenuation and presented as means ± SD.

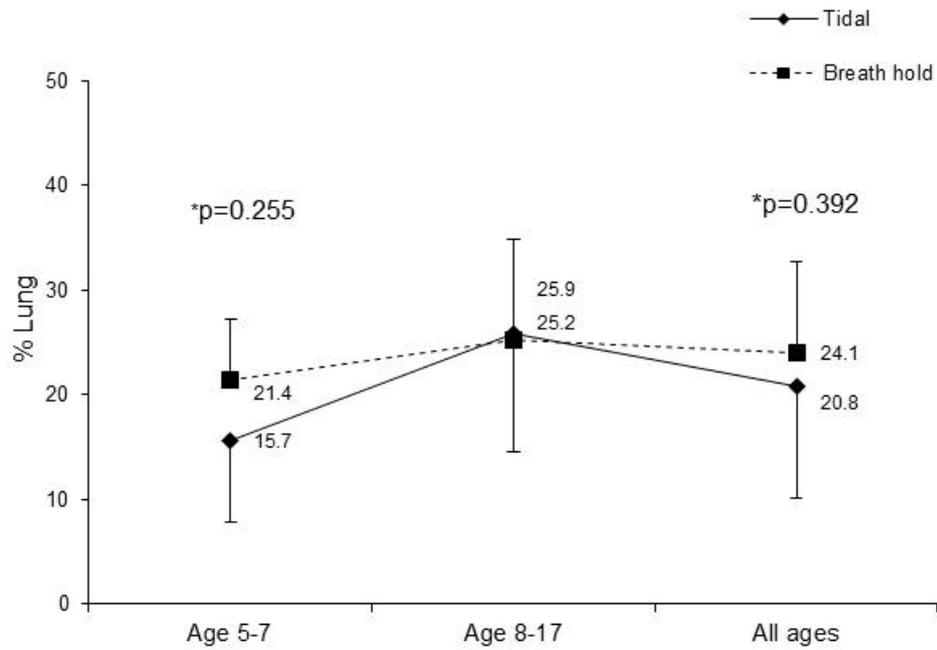


Figure 5-3: Mean (+SD) of lung deposition (% ex-actuator) with ‘breath hold’ and ‘tidal’ for children aged 5-7 years, 8-17 years and 5-17 years with Aerochamber Plus™ spacer device.

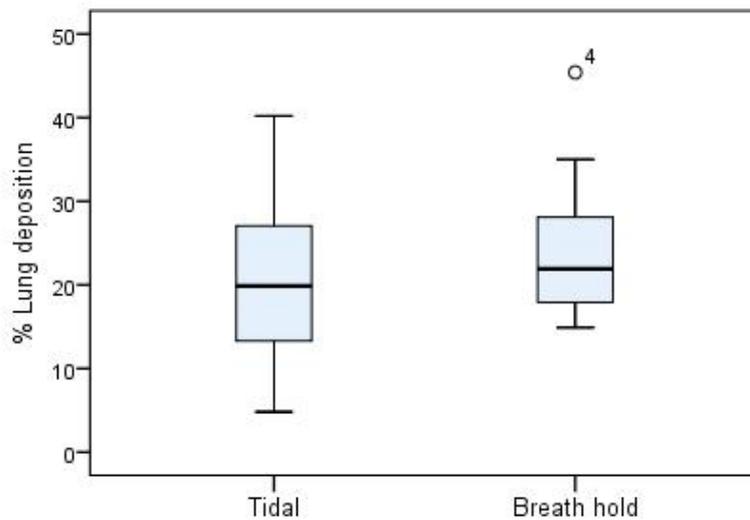


Figure 5-4: Box plot showing median and range of lung deposition (% ex-actuator) for ‘tidal’ and ‘breath hold’ groups with Aerochamber Plus™ spacer device.

Bivariate analysis showed that with tidal breathing, lung deposition significantly correlated with weight, height, and FVC ($r^2=0.596$, $p=0.041$; $r^2=0.605$, $p=0.037$; $r^2=0.611$, $p=0.035$ respectively), but not with age ($r^2=0.574$, $p=0.051$). Regression

analysis (stepwise) with factors age, weight, height and FVC found that with tidal breathing, lung function (FVC) was a significant predictor of lung deposition ($R=0.611$, $R^2=0.374$, $p=0.035$). However, with the ‘breath hold’ group there was not a statistically significant correlation between lung deposition and age, weight, height or FVC.

Adjusted means of lung deposition (adjusting for FVC) are shown in the three age-groups (Figure 5-5) that were investigated with QVAR™ in Chapter 3, section 3.5.4, Figure 3-4, although the tidal group aged 11-17 contained only two children.

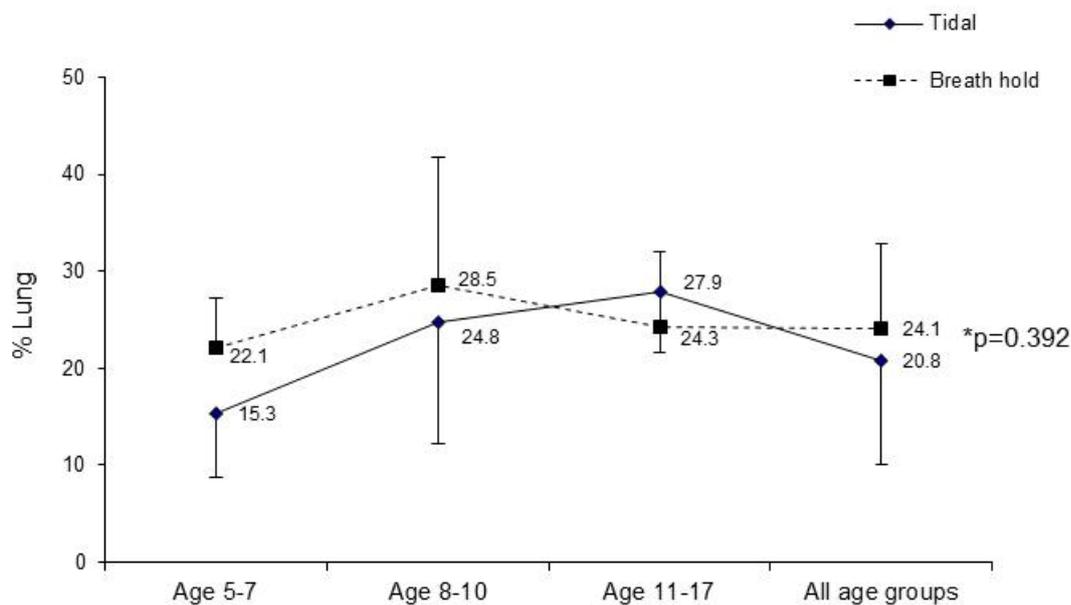


Figure 5-5: Adjusted means (\pm SD) of lung deposition (% ex-actuator, adjusting for FVC) in the two study group: ‘breath hold’ and ‘tidal’.

Differences in lung deposition between ‘tidal’ and ‘breath hold’ were compared by adjusting for FVC, so that the difference between the two groups was independent of lung function and directly related to inhalation technique. Regression analysis showed that breath type was not a significant predictor of lung deposition after adjusting for gender, age, weight, height, FEV1 and FVC ($p=0.336$). Similarly breath type was not a significant predictor of OG or spacer deposition ($p=0.072$, $p=0.121$ respectively). The difference in OG deposition between ‘tidal’ and ‘breath hold’ is shown in Figure 5-6.

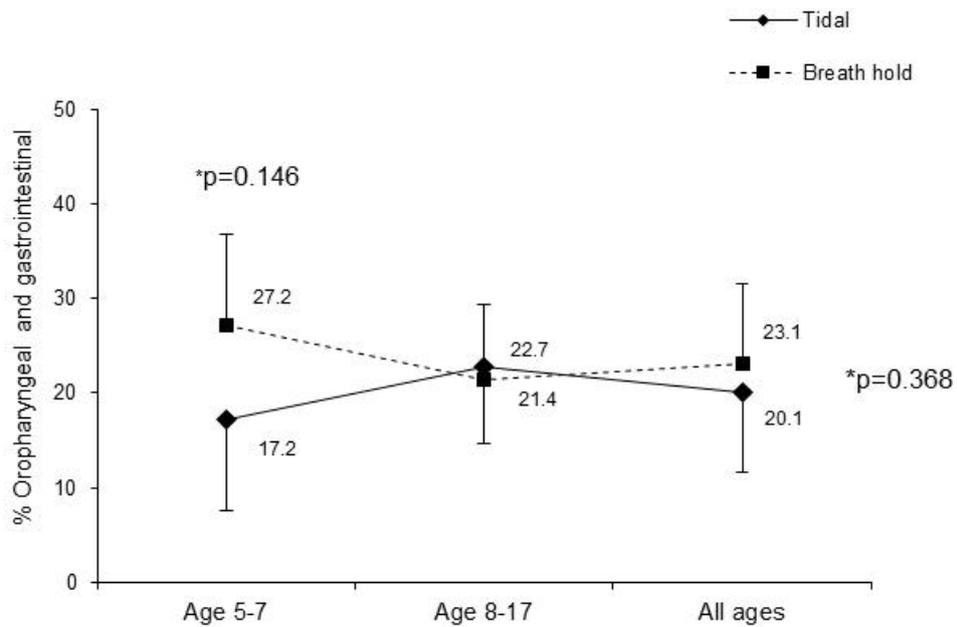


Figure 5-6: Mean (+SD) oropharyngeal and gastrointestinal (OG) deposition (% ex-actuator) with ‘breath hold’ and ‘tidal’ for children 5-7 years, 8-17 years and 5-17 years (all ages) with Aerochamber Plus™ spacer device.

As expected there was a highly significant negative correlation with % ex-actuator spacer retention and lung deposition and oropharyngeal and gastrointestinal (OG) deposition respectively ($r=-0.934$, $r^2=0.873$, $p=0.000$; $r=-0.899$, $r^2=0.809$, $p=0.000$).

The exhaled fraction increased with ‘tidal’ compared to ‘breath hold’, however the difference was not significant ($p=0.076$). The mean (SD) P:C ratio with the tidal breathing children was 1.6 (0.5, $n=10$). The mean (SD) P:C ratio with the ‘breath hold’ group was 2.0 (0.6, $n=14$). After regression analysis (stepwise) with age, spacer type, weight, height and FVC, breath type was a significant predictor of the P:C ratio ($R=0.450$, $R^2=0.203$, $p=0.008$).

For the youngest 5-7 year age-group ($n=10$) the mean (SD) % ex-actuator lung and OG deposition increased from 15.7 (7.8) and 17.2 (9.2) with ‘tidal’ ($n=6$) compared to 21.4 (5.9) and 27.2 (9.6) with ‘breath hold’ ($n=4$), however there was not a statistically significant difference between ‘tidal’ and ‘breath hold’ for lung deposition or OG

deposition ($p=0.255$, $p=0.146$). Mean (SD) % ex-actuator spacer retention HFA-FP decreased from 65.4 (14.9) with 'tidal' compared to 50.8 (15.2) with 'breath hold' in this youngest age-group, however the difference was not statistically significant ($p=0.169$). Similarly lung deposition, OG deposition or spacer retention for children aged ≤ 8 years using tidal breathing, were not significantly different to children aged > 8 years ($p=0.102$, $p=0.261$ and $p=0.071$ respectively).

Children aged ≤ 8 years using pMDI-spacer (AC+) with a slow single maximal inhalation and 'breath hold', did not show a significant difference in lung deposition, OG deposition or spacer retention compared to children aged > 8 years, $p=0.484$, $p=0.265$, $p=0.820$ respectively, Table 5-9. The exhaled filter dose ranged from 0-6.8% across all ages, however this was not considered representative of the actual exhaled dose because children would often cough after inhalation of the radiolabelled FP.[436]

As expected, lung deposition of HFA-FP was significantly less than lung deposition of extrafine QVAR™ for both 'tidal' and 'breath hold' ($p<0.001$, $p<0.001$ respectively), (Figure 5-7, Figure 5-8). There was not a statistically significant difference in OG deposition between QVAR™ and FP, with 'tidal' or 'breath hold' ($p=0.196$, $p=0.144$ respectively). A comparison table is also shown in the following section 5.5.5.1, Table 5-10.

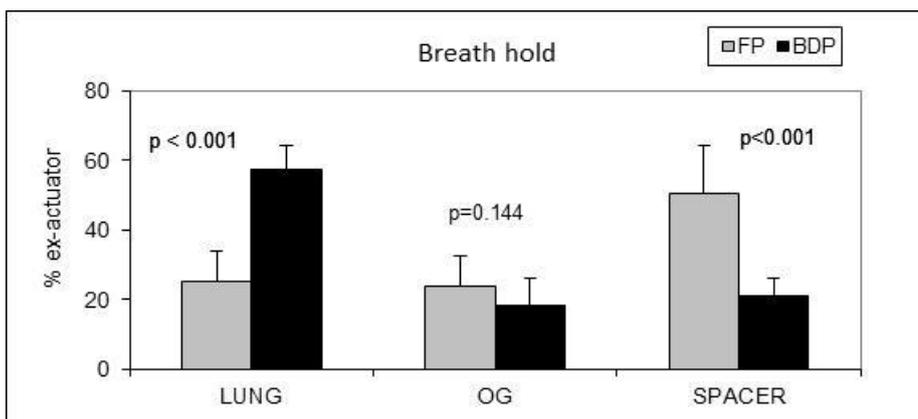


Figure 5-7: Comparison % ex-actuator lung deposition, oropharyngeal and gastrointestinal deposition (OG) and spacer retention of QVAR™ (HFA-BDP) and HFA-FP inhaled with ‘breath hold’.

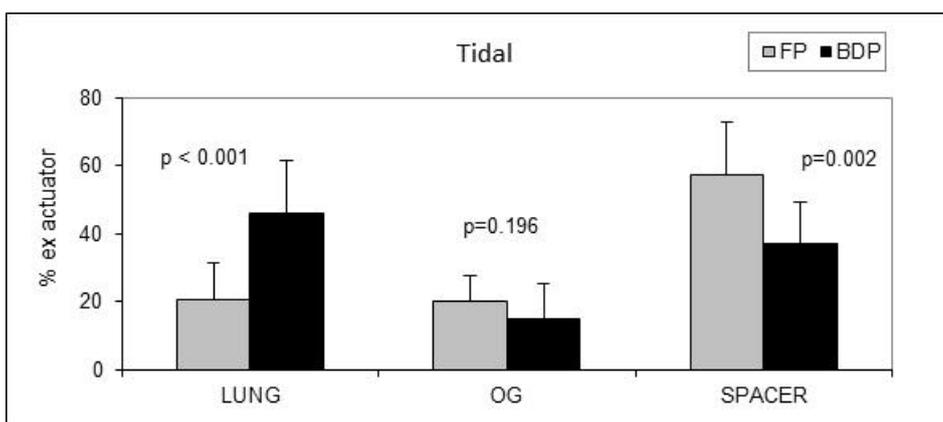


Figure 5-8: Comparison % ex-actuator lung deposition, oropharyngeal and gastrointestinal deposition (OG) and spacer retention of QVAR™ (HFA-BDP) and HFA-FP inhaled with ‘tidal’.

5.5.5.1 Comparison of Flixotide® with QVAR™

A comparison of *in vitro* and *in vivo* deposition of Flixotide® and QVAR™ is made in Table 5-10 and Table 5-11. It is noteworthy that although there were significant differences between lung deposition and spacer retention of extrafine QVAR™ and Flixotide®, there was not a significant difference in oropharyngeal and gastrointestinal

deposition of QVAR™ and Flixotide® with ‘tidal’ and ‘breath hold’ (p=0.196. p=0.144 respectively).

Table 5-10: Comparison % ex-valve deposition of QVAR™ and Flixotide® (HFA-FP) measured with ACI, inspiratory filter and gamma scintigraphy.

pMDI-AC+	QVAR™ tidal	FP tidal	QVAR™ BH	FP BH
% FP exiting spacer (ACI)	71.9 ± 6.9	52.3 ± 1.7	-	-
% particles < 4.7 µm (ACI)	68.6 ± 7.2	41.9 ± 4.3	-	-
% spacer retention (ACI)	14.1 ± 5.5	38.4 ± 5.0	-	-
Filter % total dose	55.2 ± 8.2	44.1 ± 6.1	60.7 ± 8.8	48.1 ± 4.6
Filter % spacer retention	15.0 ± 5.7	42.5 ± 4.2	13.5 ± 5.6	37.9 ± 3.5
Gamma % TBD	39.6 ± 9.5	35.8 ± 13.3	50.0 ± 7.0	37.8 ± 10.9
Gamma % lung	29.9 ± 11.4	17.6 ± 9.0	38.6 ± 7.1	19.1 ± 6.7
Gamma % OG	9.7 ± 6.8	16.9 ± 6.8	11.4 ± 5.9	18.2 ± 6.8
Gamma % spacer retention	24.0 ± 9.5	48.2 ± 12.6	14.0 ± 4.2	43.5 ± 13.1
% Lung / % OG	3.1	1.0	3.4	1.1

Table 5-11: Comparison Flixotide® and QVAR™ (% ex-actuator) with 95% confidence limits and corresponding p-values.

Tidal	Flixotide®	95% CI	QVAR™	95% CI	p-values
Lung	20.8 (10.7)	14.0-27.6	45.9 (15.7)	35.9-55.9	p=0.000
OG	20.1 (7.7)	15.2-25.0	15.1 (10.4)	8.5-21.7	p=0.196
Spacer	57.5 (15.3)	47.8-67.2	37.2 (11.9)	29.7-44.8	p=0.001
Breath hold	Flixotide®	95% CI	QVAR™	95% CI	p-values
Lung	24.1 (8.7)	19.1-29.1	57.6 (6.7)	53.4-61.8	p=0.000
OG	23.1 (8.5)	18.1-28.0	18.1 (8.0)	13.0-23.2	p=0.144
Spacer	52.2 (14.1)	44.1-60.4	20.8 (5.1)	17.6-24.1	p=0.000

5.5.5.2 Comparison of gamma scintigraphy with ACI and filter study

A comparison of the total dose with Andersen Cascade Impaction (ACI), the inspiratory filter and gamma scintigraphy is shown in Table 5-12. With the Aerochamber Plus™ (AC+) combined age-groups (n=26), the % mean ex-valve total body deposition of ^{99m}Tc-HFA-FP was significantly less than the % mean ex-valve dose exiting the spacer with cascade impaction (p=0.000). Similarly the % mean (SD, %CV) fine particle fraction (particles < 4.7 µm) with ACI was 43.2 (1.8, 4.1) with and this was significantly higher than the % mean (SD) ex-valve lung deposition value of 17.4 (4.6, 26), (p=0.000).

Table 5-12: Comparison of the mean (SD) % ex-valve drug (FP) delivered via pMDI-spacer (AC+) to ACI *in vitro* with the *in vivo* filter dose and total body deposition with gamma scintigraphy.

Total FP %	Actuator	Spacer	Total dose
ACI	9.1 ± 0.9	38.4 ± 1.8	52.2 ± 1.7
Inspiratory Filter	13.8 ± 3.6	40.0 ± 4.5	46.2 ± 5.6
Gamma	18.0 ± 7.3	44.5 ± 13.4	36.9 ± 11.8

With ‘tidal’ and ‘breath hold’ groups the % mean (SD, CV) ex-valve total body deposition was 35.8 (13.3, 37.2) and 37.8 (10.9, 28.8) respectively and these were not significantly different (p=0.682). In comparison, the % mean (SD) inspiratory filter dose with ‘tidal’ was 44.1 (6.1) and 48.0 (4.6) with ‘breath hold’ and these were significantly increased compared to the % total body deposition (TBD) obtained with gamma scintigraphy (p=0.047, p=0.013 respectively).

The predicted % lung deposition Flixotide® (FP) *in vitro*, based on the modified Farr *et al* formula (i.e. the proportion of particles < 4.7 µm and the delivered dose FP emitted from ACI, as described in Chapter 2, section 2.2.8.1), was not significantly different to

the % lung deposition obtained with gamma scintigraphy for ‘breath hold’ ($p=0.698$) or ‘tidal’ ($p=0.129$). As expected, there was low variability with cascade impaction (CV 7%), compared to ‘tidal’ (CV 51%) and ‘breath hold’ (CV 36%) with gamma scintigraphy.

The % fine particle fraction (FPF, particles $< 4.7 \mu\text{m}$) measured with cascade impaction was significantly higher than the % lung deposition obtained with gamma scintigraphy for ‘breath hold’ ($p<0.001$) and ‘tidal’ ($p<0.001$). The Newman and Chan predicted % lung deposition (based on particles $< 3.3 \mu\text{m}$ with ACI) showed that there was a highly statistically significant difference for both ‘breath hold’ and ‘tidal’ with cascade impaction compared to gamma scintigraphy ($p<0.001$, $p=0.001$ respectively).

The ‘lung targetable fraction’, particles $< 2 \mu\text{m}$, suggested by Newhouse *et al* was significantly less than lung deposition with gamma scintigraphy for both ‘tidal’ and ‘breath hold’ ($p<0.001$, $p=0.029$ respectively). Similarly the Finlay *et al* measure for lung deposition (drug particles $1.1-4.7 \mu\text{m}$) was significantly higher than lung deposition with gamma scintigraphy for both ‘tidal’ and ‘breath hold’ ($p<0.001$, $p<0.001$ respectively). However with the subgroup of children 5-7 years, the Newhouse ‘lung targetable fraction’ obtained from ACI was not significantly different to lung deposition obtained from gamma scintigraphy with both ‘tidal’ and ‘breath hold’ ($p=0.147$, $p=0.990$ respectively).

5.5.6 Funhaler spacer device

With the Funhaler group ($n=10$) the mean (SD) % total body deposition was 35.6 (8.4) and this was significantly less than the mean (SD) % inspiratory filter dose of 39.9 (4.3) ($p=0.046$). The mean (SD) % ex-actuator lung deposition, OG deposition and spacer retention were 17.0 (3.1), 18.4 (6.0) and 58.8 (9.1). In comparison, the children aged < 10 years ($n=10$) breathing tidally in the AC+ group, mean (SD) % ex-actuator lung

deposition, OG deposition and spacer retention was 19.4 (10.4), 20.1 (8.4) and 58.7 (15.5) as shown in Table 5-13.

Table 5-13: Comparison of % ex-actuator dose of ^{99m}Tc-HFA-FP (Flixotide®) between children < 10 years using the Funhaler (FH) spacer device and the Aerochamber Plus™ (AC+). * OG defined as oropharyngeal and gastrointestinal deposition.

Device	N	Lungs %	OG* %	Spacer %	Expiratory filter	P:C
AC+ <10	10	19.4 ± 10.4	20.1 ± 8.4	58.7 ± 15.5	1.7 ± 2.0	1.6 ± 0.6
FH <10 yrs	10	17.0 ± 3.1	18.4 ± 6.0	58.8 ± 9.1	5.9 ± 2.8	1.5 ± 0.3
FH 8-10 yrs	4	18.2 ± 1.5	16.8 ± 5.0	60.0 ± 5.9	5.0 ± 2.3	1.7 ± 0.4
FH 5-7 yrs	6	16.2 ± 3.7	19.5 ± 6.8	57.9 ± 11.2	6.5 ± 3.1	1.4 ± 0.2

There was not a significant difference in age, weight, height, FVC between the Funhaler and AC+ groups (p=0.941, p=0.279, p=0.882, p=0.656 respectively). Similarly there was not a significant difference between lung deposition, OG deposition or spacer retention for children < 10 years using either the AC+ or the Funhaler (p=0.472, p=0.678, p=0.993 respectively), however the CV% in lung deposition increased from 18% with FH to 54% with AC+ (Figure 5-9).

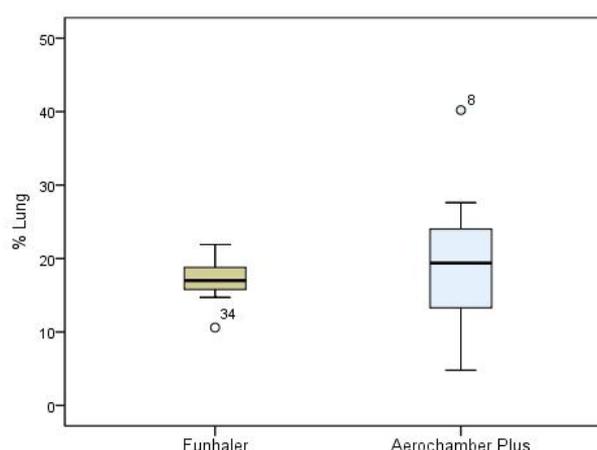


Figure 5-9: Box plot showing the median and range of lung deposition (% ex-actuator) for children aged < 10 years using Funhaler (FH) and Aerochamber Plus™ (AC+).

With the children 5-7 years, there was not a significant difference between lung deposition, OG deposition or spacer retention for children using either the AC+ or the Funhaler (p=0.909, p=0.676, p=0.347 respectively), however the CV% in lung deposition increased from 23% with Funhaler (FH) to 49% with AC+. Similarly there was not a significant difference in age, weight, height, FVC between the FH and AC+ groups 5-7 years (p=0.649, p=0.628, p=0.796, p=0.811 respectively).

With the children aged > 8 years, there was not a significant difference between lung deposition, OG deposition or spacer retention for children using either the AC+ or the FH (p=0.221, p=0.124, p=0.151 respectively), however the CV% in lung deposition increased from 8% with FH to 44% with AC+ (Figure 5-10).

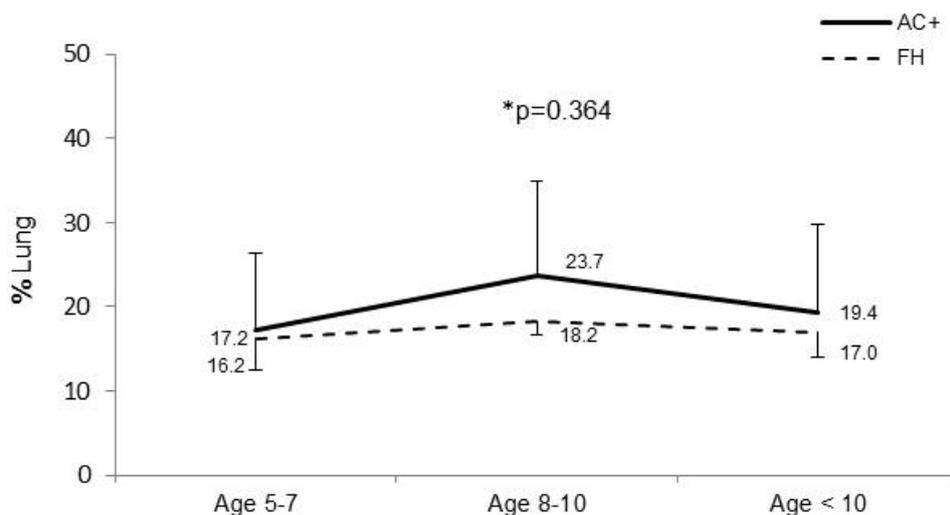


Figure 5-10: Mean (\pm SD) lung deposition (% ex-actuator) for Aerochamber Plus™ and Funhaler for children age 5-7 years, 8-10 years and all children less than 10 years.

The exhaled dose was trapped on the ‘toy’ of the Funhaler (FH) spacer device and the mean (SD) % ex actuator exhaled dose with FH was 5.9 (2.8) and this was significantly higher than the mean (SD) % ex-actuator exhaled dose of 1.8 (2.0) obtained with AC+ (p=0.002). With children aged < 10 years, the mean (SD) P:C ratio for FH was 1.5 (0.3) and 1.6 (0.5) for AC+ and this was not significantly different (p=0.565), although the

CV % increased from 20% with FH to 38 % with AC+. Similarly for children aged < 8 years the mean (SD) P:C ratio was 1.4 (0.2) for FH and 1.7 (0.4) for AC+, and these values were not significantly different (p=0.205). With children aged > 8 years, the mean (SD) P:C ratio for FH was 1.7 (0.4) and 1.6 (0.6) for AC+ and these were not significantly different (p=0.833). The mean (SD) P:C ratio with 'breath hold' was 2.0 (0.6, n=14) and this was significantly increased compared to the mean (SD) P:C ratio of 1.6 (0.5) for the children less than 10 years of age breathing tidally with AC+ and FH (n=20, p=0.008). ANOVA showed no statistically significant difference in lung deposition between the three delivery methods: AC+ 'tidal', AC+ 'breath hold' and FH. Post hoc tests with Fisher's Least Significant Difference showed no significant difference in lung deposition between AC+ 'tidal' and 'breath hold', AC+ 'tidal' and FH or AC+ 'breath hold' and FH (p=0.208, p=0.511, p=0.069).

5.6 DISCUSSION

Variability in drug delivery to the airways of children of different age-groups suggests that lung deposition data are needed so that clinical recommendations can be made for dosage regimens for children with asthma using different ICS, different devices and different inhalation techniques.[291] The choice of delivery device and inhalation technique can affect the fine particle fraction of fluticasone propionate (FP) in the delivered dose and this can alter lung deposition and therefore the clinical effect.[477-479] Fluticasone plasma concentrations are significantly greater after pMDI compared with DPI, and cortisol suppression is associated with higher fluticasone plasma concentrations.[468] [469, 480, 481] [482]

The use of pMDI with a large volume spacer (Volumatic®) has been shown to produce a twofold increase in the systemic bioavailability of FP as assessed by the relative

suppression of the overnight urinary cortisol:creatinine ratio.[483] The *in vitro* fine particle fraction of HFA-FP delivered *via* pMDI spacer (Volumatic®) has been shown to be equivalent to the fine particle fraction of HFA-FP delivered *via* pMDI-spacer (Aerochamber Plus™).[454] However an adult PK study has shown that Aerochamber Plus™ (AC+) can increase the bioavailable lung dose three-fold with a ‘breath hold’ technique, therefore lung deposition of FP delivered *via* small volume spacers would provide important information for dosage regimens in asthmatic children. [454]

In this chapter the fine particle fraction measured with cascade impaction overestimated the lung deposition and underestimated variability in delivery, confirming our hypothesis. Similarly the filter study overestimated total body deposition. The gamma scintigraphic study described in this chapter has shown both the total body deposition and regional deposition of fluticasone propionate (FP). The mean *in vivo* lung deposition of HFA-FP delivered *via* pMDI-spacer (Aerochamber Plus™) was comparable regardless of whether children inhaled with tidal breathing or with a single maximal inhalation with ‘breath hold’. This is consistent with data from a study by Schultz *et al*, who used simulated breathing patterns for children 2-7 years, and found that salbutamol delivery was not improved with a single maximal inhalation with ‘breath hold’.[426]

Lung deposition with tidal breathing increased with age, weight, height and lung function. Mean lung deposition increased from 16% for children aged 5-7 years in the ‘tidal’ group to 25% for children aged 8-17 years in the ‘breath hold’ group, however the 9% dose difference would not be expected to have a significant clinical effect and there was not a significant difference between these two groups when the dose was weight-corrected ($p=0.960$).[132] Therefore this study does not support the hypothesis that HFA-FP deposition will be significantly increased with a slow single maximal inhalation followed by a ‘breath hold’, when using pMDI with attached small volume

spacer, AC+. A limitation of this study was the small numbers of children in each age group, however across all ages (n=12, n=14) the study had 80% power to detect a significant difference of 10%.

With the 'breath hold' technique lung deposition was independent of age, weight, height and lung function. It is noteworthy that this study does support the hypothesis that the 'breath hold' technique reduced variability in lung dose. The interpatient variability in lung dose decreased significantly in the 'breath hold' group, with the coefficient of variation (CV) 36% compared to CV 51% in the 'tidal' group across all ages (p=0.049). Interestingly the peripheral to central (P:C) ratio was significantly increased from 1.6 (0.6) with 'tidal' to 2.0 (0.5) with 'breath hold' (p=0.008). In comparison the mean (SD) P:C ratio in a previous deposition study with extrafine QVAR™ (HFA-BDP) for the 'tidal' group, using the same spacer device, was 2.3 (0.5) and 2.3(0.4) for 'breath hold', indicating enhanced peripheral deposition of the extrafine aerosol, regardless of the inhalation technique.

The mean (SD) P:C ratio of 1.6 (0.4) obtained in the present study for tidal breathing is consistent with the mean (SD) P:C ratio of 1.6 (0.5) obtained with children aged 4-8 years obtained in a deposition study after inhalation of radiolabelled salbutamol (MMAD 2.9 µm) *via* pMDI-Volumatic® with tidal breathing.[171] However the mean (SD) lung deposition (% ex-valve) HFA-FP with tidal breathing *via* pMDI-AC+, 15.7% (7.8) was significantly lower than mean (SD) lung deposition (% ex-valve) radiolabelled salbutamol, 28.2% (6.7), obtained with pMDI-Volumatic® in the same age-group (p=0.020). Similarly the mean (SD) lung deposition (% ex-valve) radiolabelled salbutamol, for children aged > 8 years (n=5) inhaled with a 'breath hold' technique through a large volume spacer (Volumatic®), was 41.8% (3.8) and this was significantly higher than the mean(SD) lung deposition (% ex-valve) HFA-FP, 25.2% (9.6) (p=0.003) in this study in this age-group.[171]

In contrast the mean (SD) lung deposition of radiolabelled salbutamol delivered *via* pMDI-Volumatic® was 19% (8.9) in a group of adults using pMDI-Volumatic® with a ‘breath hold’ technique.[339] The improved lung deposition of radiolabelled salbutamol (MMAD 2.9 µm) for children aged > 8 years using pMDI-Volumatic® found by Wildhaber *et al* compared with the results of this study with radiolabelled Flixotide® (MMAD 2.8 µm) *via* pMDI-Aerochamber Plus™ [25.2% (9.6), n=10] indicates that there is less impaction of drug on the spacer wall with the large volume spacer, which may also retain the aerosol cloud longer than the small volume spacer, allowing increased droplet evaporation within the spacer.[86, 171] However, both studies are limited by small numbers.

The Aerochamber Plus™ (volume 149 mL) and Funhaler (volume 225 mL) were chosen because of their comparable *in vitro* characteristics. However the small volume may have been a limiting factor, as it is possible that children could have received an increased lung dose with a large volume spacer, as shown by Wildhaber *et al* with radiolabelled salbutamol and pMDI-Volumatic®, with lung deposition up to 40%.[167] Nevertheless, the small volume spacer demonstrated consistent mean lung deposition of 15-25% HFA-FP across all ages and this is an important clinical consideration for standardising dosage regimens in children.

HFA-FP deposition in this study was approximately half that of extrafine QVAR™ observed in a previous pediatric scintigraphic study.[245] This supports the hypothesis that the coarser particle size of HFA-FP will lead to reduced lung deposition compared with extrafine QVAR™. A halving of the dose of HFA-FP is consistent with the increased particle size of HFA-FP and the more potent anti-inflammatory effect of fluticasone propionate compared with QVAR™, resulting in equivalent clinical efficacy with the two formulations at the same dose levels.[473]

Lung deposition of the extrafine QVAR™ formulation increased almost twofold in children aged 5-7 years inhaling through Aerochamber Plus™ with a slow, single maximal inhalation followed by a ‘breath hold’ compared with tidal breathing. In this present study the mean lung deposition of HFA-FP in the same age-group increased marginally from 16% with ‘tidal’ to 21% with ‘breath hold’. However interpatient variability in lung dose decreased from CV 50% with ‘tidal’ to CV 28% in this youngest age-group with ‘breath hold’ and this is an important finding in relation to uniform dosing consistency of Flixotide® in young children. Similarly children less than 8 years using tidal breathing with the Funhaler incentive device demonstrated reduced variability in lung deposition, with CV 23% with Funhaler and CV 49% with AC+, although the difference in lung deposition was not statistically significant.

Mean OG deposition HFA-FP was not significantly different between ‘tidal’ (21%) and ‘breath hold’ (23%) across all ages, however variability decreased from CV 53% with ‘tidal’ to CV 35% with ‘breath hold’ for children aged 5-7 years. OG deposition increased in this youngest age-group with ‘breath hold’ with a corresponding decrease in spacer retention. The increased OG deposition in children aged 5-7 years may be related to narrower upper airways, decreased oropharyngeal length and shorter ‘breath hold’ times with younger children.[484] The increased spacer retention with tidal breathing in the youngest age-group may indicate a more pronounced mismatch between coordination of actuation and inhalation. More drug particles would be retained in the spacer when the initial inhalation with tidal breathing does not coincide with actuation. Spacer retention of extrafine QVAR™ (MMAD 1.1µm) was less than half that of HFA-FP (MMAD 2.8 µm) in the ‘breath hold’ group across all ages. This is consistent with the larger particles of FP being more likely to be retained in the spacer and therefore unavailable for lung deposition.

The deposition study in this chapter did not support the hypothesis that there would be a significant difference between the oropharyngeal and gastrointestinal (OG) deposition of HFA-FP compared with extrafine QVAR™. An adult deposition study by Leach *et al* showed that OG deposition was approximately 75% when CFC-FP was delivered *via* pMDI alone.[485] Therefore it follows that the use of the spacer device would significantly reduce local side-effects of fluticasone in children, such as hoarseness, pharyngitis and sore throat, as we have shown mean OG values of approximately 20% regardless of the breathing technique.[465]

The mean exhaled dose from the Funhaler device (6%) was significantly higher than AC+ (2%) and consistent with the exhaled dose of radiolabelled salbutamol (9%) reported in a previous deposition study by Wildhaber *et al* with pMDI-Babyhaler and an attached expiratory filter, which was able to collect the exhaled dose in children less than 4 years of age. The exhaled dose was measured more reliably with the Funhaler device because the expiratory ‘toy’ was able to capture the exhaled dose during tidal breathing before the child removed the spacer from their mouth.

The *in vitro* filter dose obtained when the children rehearsed the breathing technique overestimated the total body deposition of HFA-FP for both ‘tidal’ and ‘breath hold’ groups, confirming our hypothesis. However the filter study gave an indication that the ‘breath hold’ technique gave a higher dose than ‘tidal’. The filter study also gave an indication that there was less variability in dose with Funhaler compared with Aerochamber Plus™.

Similarly there was more variability in the actuator, spacer and filter dose compared with cascade impaction drug measures. There was also less variability in the dose with the filter study compared with gamma scintigraphy. This may be due to a combination

of the inspiratory filter capturing part of the exhaled dose and the effect of the different breathing patterns used during the filter study and the deposition study.

In summary this study indicates that the delivery of fluticasone propionate (HFA-FP) *via* the small volume spacer (Aerochamber Plus™) can produce comparable lung deposition with tidal breathing or with a slow, single maximal inhalation followed by a ‘breath hold’ for children aged 5-17 years. This study confirmed the hypothesis that there would be decreased variability in the lung dose of HFA-FP with the single maximal inhalation followed by a ‘breath hold’, especially for children aged 5-7 years.

Furthermore this study confirmed the hypothesis that the Funhaler incentive spacer device delivered equivalent lung deposition as the Aerochamber Plus™ spacer device. The Funhaler produced less variability in lung deposition and peripheral deposition to children less than 10 years age, using tidal breathing. These are important clinical findings in relation to drug/device/inhalation technique selection and dose reproducibility in young children. Across all ages there was a highly significant increase in peripheral deposition with the ‘breath hold’ technique compared with the tidal breathing children. The improved peripheral deposition associated with the ‘breath hold’ technique may be of clinical benefit, if concurrent dose titration is used to minimise adverse systemic effects.

The use of large volume spacers has been recommended for any inhaled asthma drug in young children, as a means of reducing systemic bioavailability of inhaled corticosteroids in children.[161] [162] *In vitro* tests have demonstrated that the fine particle dose of HFA-FP delivered *via* pMDI-Aerochamber Plus™ is comparable to pMDI-Volumatic®.[454] This chapter has shown that small volume spacers can achieve reasonably uniform and consistent lung deposition of the potent corticosteroid HFA-FP for asthmatic children aged 5-17 years, with half the lung deposition of

extrafine QVAR™. This is consistent with the National Asthma Council of Australia recommendations that HFA-FP is prescribed at the same dose as QVAR™ (100-250 µg/day).[201]

Previous studies have shown that there is significant variability in dosing associated with different breathing patterns and different devices. Variability in dosing can lead to suboptimal management and this may lead to inappropriate increases in the dosage regimen.[106, 486] The breathing pattern used by children with pMDI-spacer and attached inspiratory filter was different to the breathing pattern used by children before gamma scintigraphy and this may have accounted for the differences in the total body dose.

In vitro estimates of drug output and fine particle fraction have been used to evaluate different drug delivery devices, however the clinical effect can only be determined with *in vivo* studies. In this chapter the inspiratory filter study showed that there was a trend for the ‘breath hold’ technique to improve total body deposition, with decreased variability in dose *in vivo*, however it did not give any information about regional deposition. The potential for dose-related systemic effects for drug-inhaler combinations can be minimised by predicting the lung dose of fluticasone for each drug and inhaler device, as this information can be used to identify clinical effects.[271, 487] [155]

The modified Farr *et al* formula was shown to be predictive of lung deposition of HFA-FP. However variability in drug delivery for ‘tidal’ and ‘breath hold’ was not predicted with ACI measures. A correlation between *in vitro* and *in vivo* methods comparing drug delivery may be improved by measuring the aerodynamic particle size distribution (PSD) in ways that reflect clinical use.[109] There are few published data that relate PSD to the clinical response of inhaled drugs in a clear way.[109] However, patient-

specific variables such as breathing pattern, volume of inhalation and PIF were not investigated in this chapter.

Chapter 6 of this thesis addresses important questions about impact of the inhalation profile on lung deposition and compares lung deposition obtained *in vivo* with gamma scintigraphy to fine particle fraction obtained *in vitro* with breathing simulation in tandem with impaction. The effect of the inhalation profile and inspiratory parameters such as volume of inhalation, peak inspiratory flow and time of inhalation will be investigated in relation to total body deposition and regional deposition assessed by gamma scintigraphy. The effect of the child's inhalation technique on drug delivery will be assessed by recording their breathing pattern during inhalation. The child's recorded breathing pattern can then be replayed *in vitro* with a breathing simulator and the drug output and 'respirable' fraction of drug particles can be quantified with cascade impaction techniques.

In vitro cascade impaction in tandem with breathing simulation may reflect *in vivo* total body deposition and regional deposition with gamma scintigraphy. The aim will be to validate that the gamma scintigraphic body distribution obtained *in vivo* can be simulated *in vitro* using the recorded breathing pattern, the breathing simulator and cascade impaction. Clinicians may use this evidence-based model in making decisions regarding dosage regimens for asthmatic children of different age-groups who use different formulations and different breathing techniques.

6 BREATHING SIMULATION

6.1 INTRODUCTION

Breathing patterns can dramatically alter the drug delivery from different devices such as nebulisers, pMDIs and DPIs; and drug delivery can dramatically alter the therapeutic efficacy of inhaled corticosteroids (ICS). Therefore the inhalation technique, in combination with the delivery device, may alter the clinical response of ICS. In the previous chapter gamma scintigraphy was used to assess the total body deposition and lung deposition of Flixotide® (GlaxoSmithKline, UK), fluticasone propionate (FP) reformulated with hydrofluoroalkane (HFA-FP) in asthmatic children, using two different inhalation techniques and two different spacer devices. In this chapter the experimental studies will investigate whether the *in vitro* method of breathing simulation, used in combination with impaction methods, can be used to reflect the total body deposition *in vivo*, as shown with gamma scintigraphy.

Several studies have shown that there is considerable variability in aerosol delivery to young children using different spacer devices with different inhalation profiles.[75, 115, 451, 488] Magnetic resonance imaging models have shown that there are significant changes in the upper airway when different devices are used for inhalation.[489, 490] GINA guidelines recommend pMDI-spacer for children unable to use DPI and children less than 6 years of age.[44] An important limitation that occurs for children using the pMDI-spacer is the inconsistent dosing that occurs with incorrect use. There is a clinical need to establish inhalation techniques that can improve the reliability and consistency of dosing from pMDI-spacers.

Children can use pMDI-spacer as effectively as other inhaler devices if they are given regular feedback and instruction in the correct inhalation technique.[126] [115, 174,

177, 491] Tidal breathing with pMDI-spacer is the optimal inhalation technique recommended by clinicians for young asthmatic children, if they have difficulty coordinating actuation and inhalation.[154] The valved spacer device retains the majority of the drug plume for several seconds if the spacer volume is greater than 140 mL.[416] However, timing of inhalation with actuation can affect drug delivery from pMDIs and pMDI-spacer.[121, 492] Both the device and the inhalation pattern can alter the ‘respirable’ dose of FP [456, 493] The Aerochamber Plus™ (AC+) has been shown to significantly improve the ‘respirable’ dose of FP.[455] The Funhaler, an incentive spacer device, has been shown to provide equivalent control of asthma symptoms and equivalent deposition of radiolabelled HFA-FP in young children.[189]

A more effective use of the pMDI-spacer requires a low inspiratory flow, a deep inhalation and a ‘breath hold’.[146] A slow single maximal inhalation, followed by a ‘breath hold’ for 5-10 seconds, is recommended for older children in order to reduce inertial impaction in the oropharynx and upper airways and increase gravitational sedimentation of particles.[180, 488, 494] The ‘breath hold’ technique has been shown to enhance lung deposition of extrafine HFA-BDP and decrease variability in dosing of HFA-BDP and HFA-FP with young children.[245, 495] The flow-rate affects the fine particle dose with DPIs, however the inspiratory flow-rate has previously been shown to have less of an impact on drug delivery for pMDI devices.[368, 417, 496]

Schultz *et al* used simulated breathing patterns to show that for children 2-7 years, two tidal breaths were adequate to achieve approximately 40% salbutamol dose from pMDI-AC+.[426] Kamin *et al* used simulated breathing patterns to show that approximately 60-80% budesonide dose is delivered to young children from pMDI-AC+ with the first inhaled breath.[422] Few studies have evaluated the effect of the first breath and its inspiratory parameters, such as volume of inhalation, peak flow of inhalation and time

of inhalation, on inhaled *in vivo* drug delivery to children using small volume spacers.[422, 497]

Breathing simulation has been used to assess drug delivery from nebulisers, pMDI and DPI.[136, 404, 408, 409, 418, 498] The use of breathing simulators is based on the assumption that there is a correlation between the *in vitro* and *in vivo* inhaled mass of drug.[109] The breathing simulator has been described previously in the literature, in combination with particle sizing and cascade impaction [409, 416] and with the impactor inlets attached to upper airway models.[109, 142, 398]

Studies with breathing simulators and mathematical models indicate that there are significant variations in particle deposition patterns within the lungs for different inspiratory parameters including tidal volumes, inspiratory flows, respiratory rates and 'breath hold' times.[385, 388, 425] Several studies with breathing simulators have used computer generated sinusoidal or square waveforms based on the average tidal volume, inspiratory flow and breathing frequency to measure the influence of standardized breathing parameters on drug output.[421] However computer-generated breathing patterns show reduced variability compared to human breathing patterns and do not accurately reproduce the actual breathing pattern.[421]

Breathing simulation, operated with representative breathing patterns of children, has shown that inspiratory flows and tidal volume affect drug output from nebulisers and pMDI-spacer.[90, 136, 404] Deposition studies with adults and children have shown that different inspiratory flows, volume and inspiratory time can affect lung deposition and oropharyngeal and gastrointestinal deposition from pMDI.[485, 499, 500]

The correlation between *in vitro* and *in vivo* data may be improved by measuring the particle size distributions of inhaled drugs in ways that reflect clinical use.[109]

Gamma scintigraphic studies have been widely used to measure lung deposition of

radiolabelled inhaled drugs. Lung deposition data may be used as a surrogate for predicting the clinical response to inhaled asthma drugs.[163] However, scintigraphic studies are time-consuming and involve a radiation dose. There is a clinical need for prospectively estimating lung deposition of inhaled drug in the pediatric population so that effective recommendations for an inhaler type can be made based on evidence-based *in vitro* methods.

The proportion of the ‘respirable’ fraction of an inhaled drug measured *in vitro* that is available to the airways *in vivo* has not been resolved in the literature. Inertial sizing of aerosols, which operate at a constant airflow, may give a better approximation of the predicted body deposition, compared to gamma scintigraphy, when operated in tandem with breathing patterns. Andersen Cascade Impaction is operated at a constant flow of 28.3 L/min, whereas inhalation flow often exceeds 28.3 L/min. Therefore in this chapter, Next Generation Impaction (NGI), calibrated for particle sizing with a flow of 100 L/min, was chosen for the *in vitro* experimental work, as a more representative flow limit for children aged 5-17 years.

In a recent review article by Newman and Chan, the authors suggested that lung deposition was more closely linked to the percentage of the aerosol dose smaller than 3 micron diameter.[109] In Chapter 5 the EFPF measured with Andersen Cascade Impaction (ACI) at 28.3 L/min, was significantly different to lung deposition with gamma scintigraphy. The constant flow may have been a limiting factor, however Farr *et al* was able to predict lung deposition using the fine particle fraction (particles < 4.7 μm) with ACI. Therefore the primary aim of this study was to compare the delivered dose and the extrafine particle fraction (EFPF) exiting from pMDI-spacer attached to a breathing simulator in tandem with NGI, with the total body deposition and lung deposition obtained from gamma scintigraphy.[109]

The hypothesis of this study was that actual paediatric breathing patterns transformed into waveforms and transferred to the Flow-Volume Simulator (FVS) coupled to NGI, could be used to reflect total body deposition of HFA-FP with gamma scintigraphy. Furthermore, the EFPP measured with FVS-NGI could be used to reflect lung deposition. Lung deposition results from a combination of different factors, and breathing simulation may be used to identify and test the relative impact of different inspiratory variables, such as inspiratory flow, volume and time of inhalation, on drug delivery and deposition. Therefore the secondary aim of this study was to demonstrate that the inspiratory parameters of the first breath of the inhalation profile would have a significant effect on lung deposition outcomes for pMDI and attached small volume spacer for children aged 5-17 years. This chapter addresses the impact of the inhalation profile on lung deposition *in vivo* and *in vitro* with pMDI-spacer (Aerochamber Plus™). The Funhaler spacer device was investigated with an additional 10 children and the results are shown in the next chapter.

6.2 OBJECTIVES

- Compare the delivered dose and the extrafine particle fraction (EFPP) exiting from pMDI-spacer attached to a breathing simulator in tandem with Next Generation Impaction, with the total body deposition (TBD) and lung deposition obtained from gamma scintigraphy.
- Demonstrate that the inspiratory parameters, such as time of inhalation, volume of inhalation and PIF, associated with the first breath of the inhalation profile, will have a significant effect on lung deposition outcomes for pMDI and attached small volume spacer (Aerochamber Plus™) for children 5-17 years.

6.3 HYPOTHESES

- The delivered dose of HFA-FP measured with FVS-NGI can be used to reflect total body deposition measured with gamma scintigraphy.
- The extrafine particle fraction (EFPF) measured with FVS-NGI can be used to reflect lung deposition of HFA-FP.
- Lung deposition of HFA-FP will increase with time and volume of inhalation and decrease with increasing PIF.
- Oropharyngeal deposition of HFA-FP will increase with increasing PIF.

6.4 METHOD

6.4.1 Study population

Twenty-six children (20 male, 6 female) aged 5-17 years, with mild, stable asthma were recruited from outpatient clinics at Princess Margaret Hospital for Children. On the study day, each child had weight, height and lung function measured, as shown in Chapter 5, Table 5-2. Only those patients with FEV1 > 80% predicted values were enrolled in the study (Chapter 2, section 2.2.3).[429] The experimental steps are shown in Table 6-1.

Table 6-1: Experimental steps for comparison *in vitro* FVS-NGI with *in vivo* gamma scintigraphy.

Inspiratory filter (F1, n=26) and inhalation technique with breathing recordings (n=14)
Gamma scintigraphy with breathing recordings (n=10)
Flow-volume simulation (FVS) with attached inspiratory filter (F2, n=24)
FVS in tandem with Next Generation Impaction (NGI, n=22)

6.4.2 Inhalation technique - inspiratory filter 1

Twelve children were trained to perform tidal breathing with pMDI-Aerochamber Plus™ (AC+). Fourteen children were trained to perform a slow, single maximal inhalation with pMDI-AC+ followed by a 5-10 s ‘breath hold’ (n=14). A low resistance filter (F1) was attached to the mouth-piece of an Aerochamber Plus™ spacer as described in Chapter 2, section 2.2.6 and Chapter 5, 5.4.4.

6.4.3 Gamma scintigraphy

Ten children had their breathing patterns recorded with a pneumotachometer, whilst simultaneously inhaling radiolabelled Flixotide® (HFA-FP), as described in Chapter 2 section 2.2.13. This group of subjects will be referred to as the ‘simultaneous’ group. However, there were technical difficulties associated with adequate shaking of the shielded radiolabelled canister within the laminar flow chamber. In addition, there were concerns that the bulky set-up, (Chapter 2, section 2.2.13, Figure 2-11) required for the simultaneous measurements, combined with lead shielding, may have been intimidating or distracting for the children. These issues may have affected the way the children inhaled through the device and the subsequent lung deposition data would not have accurately reflected lung deposition from a standard pMDI-spacer.

Therefore after finding a correlation between the drug (FP) output on the inspiratory filter (F1) with gamma deposition, even though different breathing patterns were used, the remaining children had breathing patterns recorded prior to gamma scintigraphy. Two children in the ‘simultaneous’ group did not have their P:C ratio calculated from their lung deposition data due to technical difficulties retrieving the information on the ECAM gamma camera.

6.4.4 Recording of breathing patterns

Twenty-four children had their breathing patterns recorded (ten while simultaneously inhaling radiolabelled Flixotide® and fourteen prior to scintigraphy). Two subjects were unable to have their breathing patterns recorded on the study day. After the initial training in the inhalation technique and the inspiratory filter study (F1), the children had their breathing patterns recorded using a pneumotachometer with a placebo pMDI. Breathing patterns were recorded with the spacer device secured in a purpose-built airtight perspex flow chamber attached to the pneumotachometer, as described in Chapter 2, section 2.2.13.

6.4.5 Analysis of breathing patterns

The waveform conversion program was used to digitally select for the five breath waveform or the single full breath. The digitally selected breathing patterns were then converted to a FVW file format for compatible use with the FVS, as described in Chapter 2, section 2.2.14. Volume of inhalation, time of inhalation and peak inspiratory flow (PIF) were derived. The inspiratory parameters of the first inhaled breath with the ‘tidal’ group were compared with the ‘breath hold’ group.

6.4.6 Flow-Volume Simulator with inspiratory filter (F2)

A preliminary evaluation of total drug output from the FVS was performed by attaching a low resistance inspiratory filter (F2) to the outlet of the FVS and connecting the spacer (AC+). Three actuations of HFA-FP (250 µg FP/actuation) were fired into the spacer device as the waveform was replayed on the FVS. Drug (FP) collected on filter 2, was measured as described in Chapter 2, section 2.2.14.4, Figure 2-15.

6.4.7 Flow-Volume Simulation with Next Generation Impaction

The pMDI-spacer was attached to a Flow-Volume Simulator (FVS) connected in tandem with Next Generation Impactor (FVS-NGI) circuit in order to use the recorded breathing patterns with inertial impaction to determine drug output and fine particle fraction.[418] The vacuum pump had a suction of 100 L/min and the pressurized air source produced a positive airflow of 100 L/min.

The recorded PIF for two of the subjects was much greater than 100 L/min and consequently the waveforms for these two subjects were not suitable for FVS-NGI operated at 100 L/min.[416] The recorded PIF of one subject was 101.4 L/min and this was accepted for FVS-NGI as the mean suction on the NGI circuit was 100 ± 5 L/min. The FVS-NGI circuit is shown in Figure 6-1.

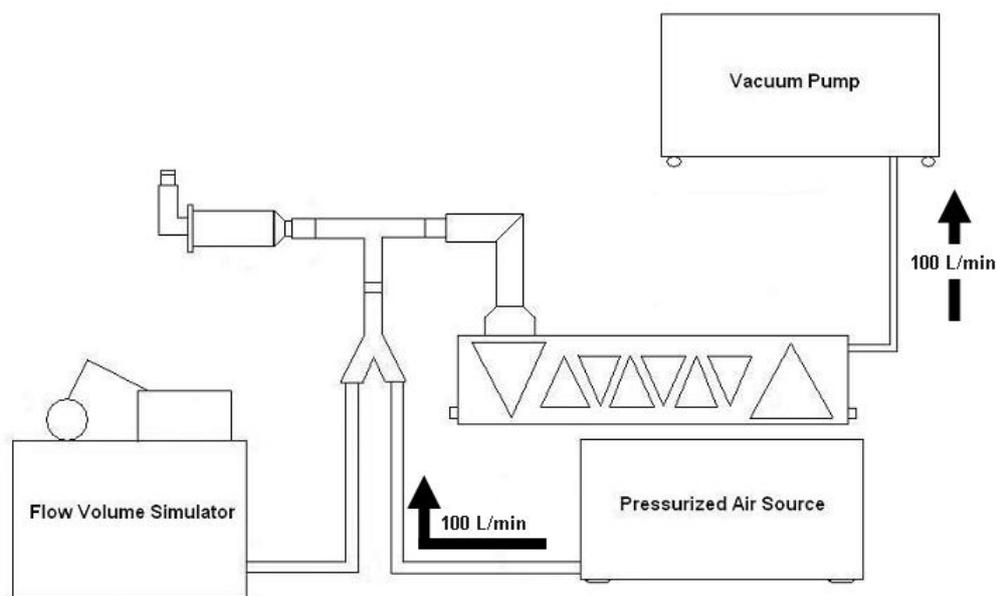


Figure 6-1: Schematic diagram of experimental set-up for the FVS-NGI*. Black arrows indicate the directional flow of air.

*Reproduced with permission; Dr Looi, Aerosol Research Group, School of Paediatrics and Child Health, UWA.

Before the impaction studies with FVS-NGI, the actuators and spacers were soaked in a dilute solution of detergent and air-dried, as described in Chapter 2, section 2.1.5.

There is a phase shift in the effective cut-off diameter (ECD) of particles with the NGI, with finer particles depositing on the upper impaction cups (Chapter 2, section 2.2.8.2).

Therefore the predicted lung deposition with the Farr *et al* formula in this chapter used the % particles < 6.1 μm , impaction cups 1-filter) as a proportion of the emitted dose for the NGI operated at 100 L/min. The Finlay *et al* definition was modified to include particles with ECD range 1.3-6.1 μm with NGI operated at 100 L/min (Chapter 2, sections 2.2.8.1 and 2.2.8.2). The two groups, 'tidal' (n=12) and 'breath hold' (n=14) were subdivided according to breath type and age.

6.4.7.1 UF-EF Algorithm

The extrafine fine particle fraction (EFPPF, particles < 3.4 μm) measured with cascade impaction has been proposed to reflect lung deposition by Newman and Chan, as described in Chapter 2, section 2.2.8.1 and 2.2.14.6.[109] The ultrafine particle fraction (UFPF, particles < 2.2 μm) has been suggested to reflect lung deposition by Newhouse *et al*.[110] A novel algorithm (UF-EF particle size range) was investigated in this chapter, whereby either the UFPF or the EFPPF was chosen to reflect lung deposition, depending on the volume of inhalation and the time of inhalation of each individual breathing pattern.

With the UF-EF algorithm, the % ultrafine particle fraction (UFPF, particles < 2.2 μm) was chosen to represent lung deposition, if the time of inhalation was less than 1 second or the volume of inhalation was less than 200 mL. The % extrafine particle fraction (EFPPF, particles < 3.4 μm) was chosen to represent lung deposition, if time of inhalation was greater than 1 second. This algorithm has not been previously suggested in the literature.

6.4.8 Statistical analysis

All data was stored in Excel (Microsoft© Office) and analysed with SPSS version 16.0 statistical software (Chapter 2, section 2.2.15). Based on a previous study by Farr *et al* there was a difference in lung deposition and the fine particle fraction of 13.2% with a standard deviation of 7.0.[106] With a sample size of 10, there was a power >80 % to detect a 10% difference, at the 0.05 significance level. Independent sample T tests were used to determine the statistical significance between breath type and inspiratory parameters. Paired samples t-tests were used to compare drug output from inspiratory filter 1 (F1), inspiratory filter 2 (F2)-FVS and FVS-NGI to total body deposition with gamma scintigraphy. Bivariate analysis was used to estimate the Pearson correlation coefficient (r) and general linear models were used to determine the effects of age, gender, breath type, weight, height, FVC, volume, PIF and time of inhalation on body deposition and spacer retention. Regression analysis was used to build a predictive model from *in vitro* data and inspiratory parameters for the main outcome variables, lung deposition and oropharyngeal and gastrointestinal deposition.[440]

6.5 RESULTS

6.5.1 Inspiratory filter - F1

The total drug (FP) dose measured on the inspiratory filter gave an indication of the total body deposition (TBD) measured with gamma scintigraphy (Table 6-2).

Table 6-2: Mean % (SD) drug (FP) measured on actuator, spacer and inspiratory filter (F1).

% FP	N	Actuator	Spacer	Total dose
Filter 1	26	13.8 ± 3.6	40.0 ± 4.5	46.2 ± 5.6
'Tidal' filter 1	12	13.2 ± 4.2	42.5 ± 4.2	44.1 ± 6.1
'BH' filter 1	14	14.1 ± 3.1	37.9 ± 3.5	48.1 ± 4.6

There was not a statistically significant difference between the total drug dose retained on the inspiratory filter (F1) between ‘tidal’ and ‘breath hold’, (p=0.075).

6.5.2 Gamma Scintigraphy

The results from the gamma scans are described in detail in Table 6-3 and Chapter 5 section 5.5.5. Across all ages there was not a significant difference between % TBD with gamma scintigraphy between ‘tidal’ and ‘breath hold’ (p=0.392).

Table 6-3: Mean (SD) % ex-valve lung and OG deposition, spacer retention, exhaled dose and total body deposition (TBD) measured with radiolabelled HFA-FP and gamma scintigraphy.

pMDI-AC+	N	Lung	OG	Spacer	Exhaled	TBD
Tidal	12	17.6 ± 9.1	16.9 ± 6.7	48.2 ± 12.6	1.3 ± 1.6	35.8 ± 13.3
Breath hold	14	19.1 ± 6.7	18.2 ± 6.8	43.6 ± 13.1	0.5 ± 0.4	37.9 ± 10.9

6.5.3 Inspiratory filter (F2) - FVS

An inspiratory filter (F2) was attached to the Flow-Volume Simulator and the waveforms were replayed *in vitro*, as described in Chapter 2, section 2.2.14.4. Results for the inspiratory filter dose (F1) and the inspiratory filter dose (F2) attached to the Flow-Volume Simulator (F2-FVS) are recorded in Table 6-4.

Table 6-4: Mean (SD) inspiratory filter 1 drug (FP) dose (% ex-valve) compared with inspiratory filter 2-FVS and % TBD with gamma scintigraphy.

Mean	N	Breath hold (n=14)	Tidal (n=12)
Filter 1	26	48.0 ± 4.6	44.1 ± 6.1
Filter 2- FVS	24*	37.9 ± 6.5	38.1 ± 3.6
Gamma (TBD)	26	37.8 ± 10.9	35.8 ± 13.3

* n=12 for F2-FVS with ‘breath hold’ as two subjects in the ‘breath hold’ group did not have their breathing patterns recorded.

There was not a statistically significant difference between the % ex-valve drug (FP) dose on F2-FVS compared with % total body deposition with gamma scintigraphy ($p=0.576$), however there was a significant difference between F1 and F2-FVS and F1 and gamma ($p=0.000$, $p=0.001$ respectively). Different breathing patterns were used for F1 and gamma.

6.5.4 Flow-Volume Simulation - Next Generation Impaction

6.5.4.1 ‘Simultaneous’ recordings

Ten children inhaled radiolabelled HFA-FP, while simultaneously recording their breathing pattern with a pneumotachometer, as described in Chapter 2, section 2.2.13. The ‘simultaneous’ group results are shown in Table 6-5.

Table 6-5: Subject age, respiratory parameters, gamma deposition, % drug (FP) exiting spacer attached to FVS-NGI, lung deposition (% ex-valve) and % ultrafine particle fraction (UFPF) for the simultaneously recorded subjects. *N/A ...PIF > 100 ±5 L/min and not used in FVS-NGI circuit.

Subject	age	FVC	Vol	PIF	Time	Gamma	FVS-	Lung	UFPF
1	10.14	2.6	1215	118.9	1.2	26.9	*N/A	12.9	*N/A
2	9.56	2.8	993	55.7	1.7	42.8	39.3	18.8	20.7
3	8.92	1.8	900	101.4	1.3	60.4	49.3	36.0	28.8
4	8.86	2.0	605	38.2	1.7	51.5	45.0	34.2	21.9
5	8.75	2.1	616	90.2	0.6	29.4	43.1	8.3	23.4
6	6.95	1.7	750	78.6	1.3	54.4	48.0	22.5	20.7
7	6.55	1.5	580	65.0	1.2	41.6	38.9	14.3	19.6
8	6.55	1.2	90	18.9	0.7	10.5	33.4	4.8	15.9
9	6.02	1.6	255	29.4	0.9	33.0	39.5	17.6	25.4
10	6.02	2.1	195	25.0	0.7	27.6	36.9	14.0	16.2

With the ‘simultaneous’ group of children, the mean (SD) % ex-valve total drug exiting the spacer from FVS-NGI was 41.5 (5.3), CV 13% and the mean (SD) % ex-valve total body deposition was 39.0 (15.6), CV 40% with gamma scintigraphy. It was noted that the % mean (SD) total drug (FP) output on the training inspiratory filter (F1) for this group of children, 42.0 (5.3), CV 12.6%, recorded prior to the simultaneous recordings and directly after inhalation technique training, was not significantly different to either the % total body deposition measured with gamma scintigraphy ($p=0.417$) or the % drug (FP) output from spacer-FVS-NGI ($p=0.988$). There was not a statistically significant difference between % drug (FP) exiting spacer attached to FVS-NGI and the % total body deposition with gamma scintigraphy ($p=0.522$). However, as expected, there was more variability in drug output with gamma deposition, as seen by the higher % CV 40%. There was a highly significant correlation between total body deposition (% ex-valve) with gamma scintigraphy and % drug (FP) exiting spacer-FVS-NGI ($r^2=0.774$, $p=0.002$) (Figure 6-2).

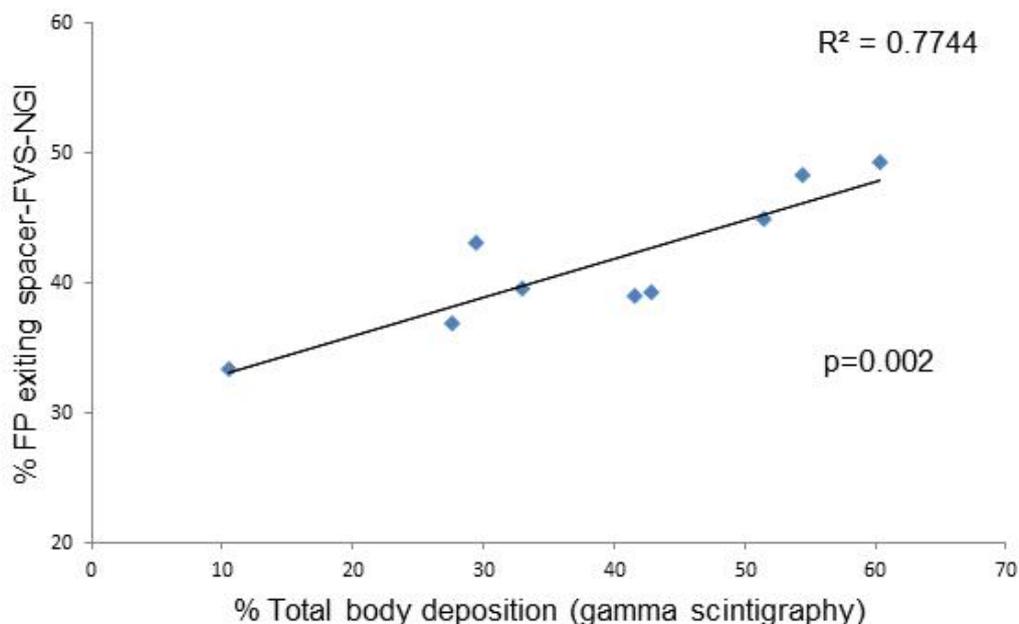


Figure 6-2: The y-axis shows the % drug (FP) exiting spacer attached to FVS-NGI and the x-axis shows the % total body deposition with gamma scintigraphy.

The mean (SD) radiolabelled Flixotide® canister weight (g), 13.4 (0.7), used for the gamma scintigraphic studies was significantly less than the mean (SD) commercial Flixotide® canister weight (g) used with FVS-NGI, 14.9 (1.0), ($p=0.010$). However there was not a significant correlation between canister weight and total body deposition or lung deposition ($p=0.974$, $p=0.676$). Similarly there was not a significant correlation between canister weight and drug (FP) or the extrafine particle fraction of drug particles exiting from the spacer attached to FVS-NGI, ($p=0.494$, $p=0.450$).

Mean (SD) drug output (% FP) on the inspiratory filter (F1) was 42.0 (5.3), CV 12.6%. F1 was carried out before recording the breathing pattern, and it was statistically significantly different to the mean (SD) drug (% FP) output measured on inspiratory filter 2 attached to the FVS (F2-FVS), 38.2 (4.1), CV 10.7%, ($p=0.013$), however there was a correlation between F1 and F2-FVS, ($r^2=0.469$, $p=0.029$), even though different breathing patterns were used for each filter study.

The mean (SD) % ex-valve lung deposition and the mean (SD) % ultrafine particle fraction (UFPF, particles $< 2.2 \mu\text{m}$) exiting the spacer attached to FVS-NGI was 18.2 (10.3), CV 57% and 20.5 (4.1), CV 20% respectively and these values were not significantly different ($p=0.587$). The mean (SD) % extrafine particle fraction (EFPF, particles $< 3.4 \mu\text{m}$) exiting the spacer-FVS-NGI was 29.1 (4.7) and this was significantly different to lung deposition ($p=0.005$). The mean (SD) % ex-actuator lung deposition was 22.7 (13.3), CV 59% and this was not significantly different to the % UFPF ($p=0.555$) or the mean (SD) % EFPF, 29.1 (4.7), ($p=0.098$).

The % EFPF exiting spacer-FVS-NGI and the % UFPF exiting the spacer-FVS-NGI significantly correlated with % ex-actuator lung deposition for the ‘simultaneous’ group of children ($r=0.742$, $p=0.022$; $r=0.685$, $p=0.042$ respectively). Regression analysis (stepwise) with breath type, age, gender, weight, height and FVC, showed that the %

EFPF and the % UFPF were predictive of lung deposition (% ex-actuator) ($R=0.742$, $R^2=0.550$, $p=0.022$ and $R=0.685$, $R^2=0.479$, $p=0.042$ respectively). The 95% confidence limits are shown in Figure 6-3.

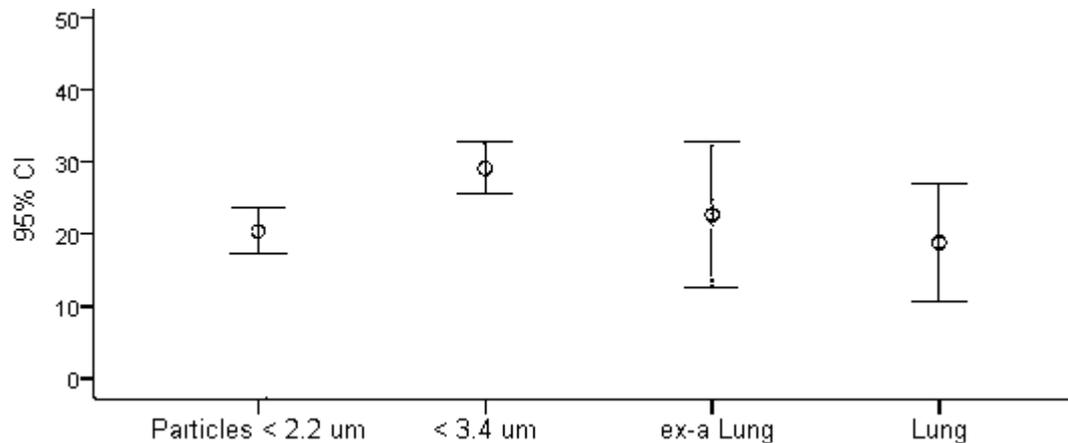


Figure 6-3: The y-axis shows 95% confidence limits. The x-axis shows % UFPF (particles < 2.2 μm) and % EFPF (particles < 3.4 μm) exiting the spacer attached to the Flow-Volume Simulator and Next Generation Impactor (FVS-NGI) and % ex-actuator lung (ex-a) and % ex-valve lung deposition.

Figure 6-3 shows that lung deposition (% ex-actuator) incorporates the range of values for the % UFPF (particles < 2.2 μm) and the % EFPF (particles < 3.4 μm) exiting the spacer-FVS-NGI. A Bland-Altman plot shows that the difference between lung deposition (% ex-actuator) with gamma scintigraphy and the % extrafine particle fraction (EFPF) exiting spacer-FVS-NGI falls within the mean \pm 2SD (Figure 6-4). Subject 1 did not have FVS-NGI, as the PIF was > 100 L/min.

There was not a significant difference between the predicted lung deposition (Farr *et al*) and lung deposition with gamma scintigraphy ($p=0.230$). There was a significant correlation between the predicted lung deposition and lung deposition (ex-valve and ex-actuator) from gamma scintigraphy ($r=0.758$, $r^2=0.574$, $p=0.018$ and $r=0.783$, $r^2=0.613$, $p=0.013$ respectively). Regression analysis (stepwise) with factors age, gender, weight,

height and FVC, showed that the *in vitro* predicted lung deposition (Farr *et al*) was a predictor of *in vivo* lung deposition (ex-valve and ex-actuator), ($R=0.758$, adjusted $R^2=0.513$ and $p=0.018$ and $R=0.783$, adjusted $R^2=0.557$, $p=0.013$ respectively).

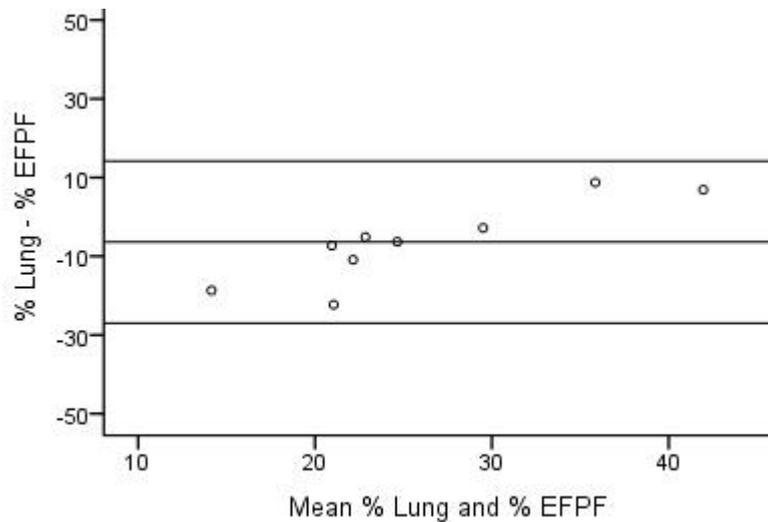


Figure 6-4: Bland-Altman plot showing the difference between lung deposition (% ex-actuator) and the % EFPF on the y-axis and the mean lung deposition (% ex-actuator) and % EFPF on the x-axis (n=9). Reference lines are drawn at the mean difference and $\pm 2SD$.

6.5.4.2 Inspiratory parameters ‘simultaneous’ group

The volume of inhalation, peak inspiratory flow (PIF) and time of inhalation ranged from 90-1215 L, 19-119 L/min and 0.6-1.7 s respectively. Regression analysis (stepwise) with breath type, gender, age, weight, height, FVC, volume of inhalation, PIF and time of inhalation showed that the time of inhalation was a significant predictor of % ex-valve and % ex-actuator lung deposition ($R=0.722$, $R^2=0.522$, $p=0.018$, $R=0.712$, $R^2=0.506$, $p=0.021$ respectively). The relationship between the % ex-actuator lung deposition and time of inhalation is shown in Figure 6-5. Linear regression with volume, PIF and time showed that PIF was a significant predictor of oropharyngeal and gastrointestinal (OG) deposition (% ex-actuator, $R=0.947$, adjusted $R^2=0.814$, $p=0.040$),

adjusted for age (Figure 6-6). Time of inhalation and breath type were significant predictors of spacer retention (% ex-actuator, $R=0.881$, $R^2=0.775$, $p=0.017$ and $p=0.027$ respectively).

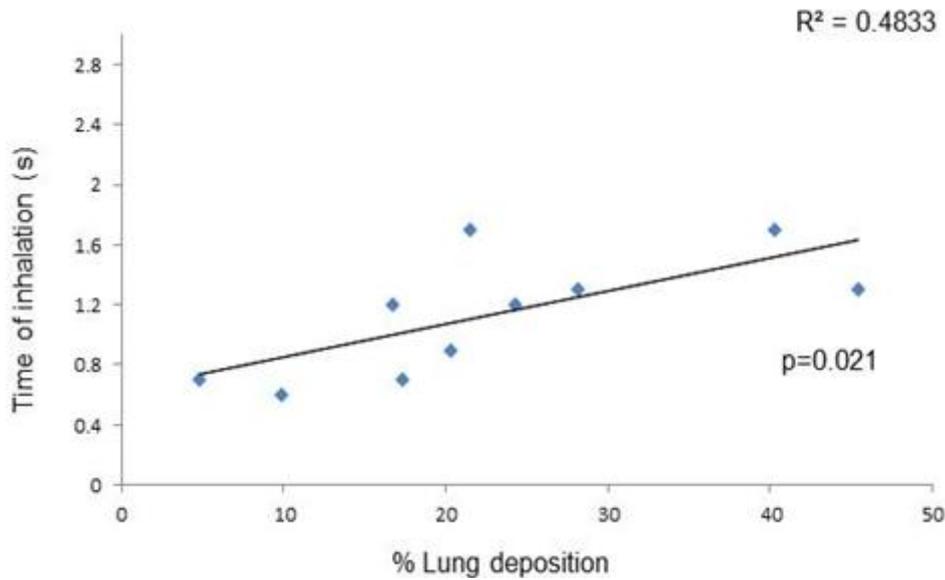


Figure 6-5: The y-axis shows the time of inhalation (s) of 1st inhaled breath and the x-axis shows the lung deposition (% ex-actuator), n=10.

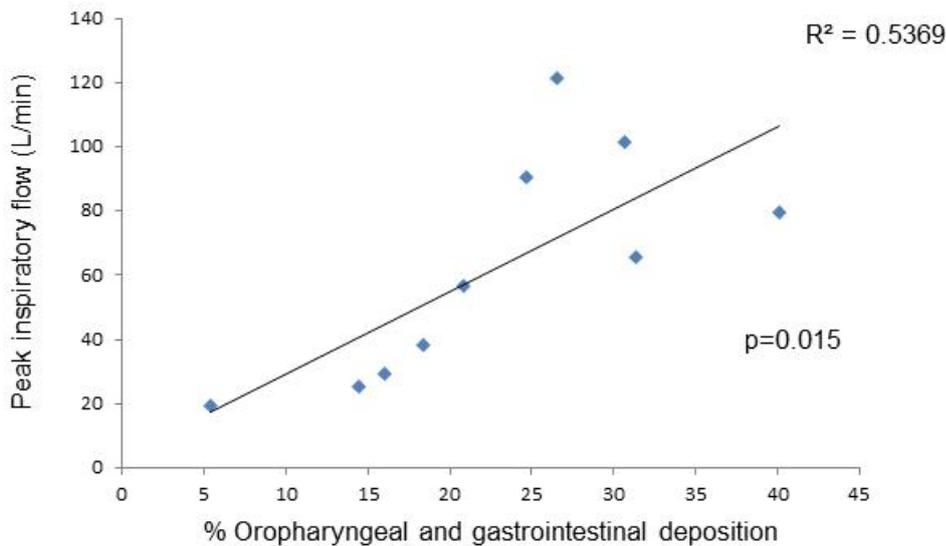


Figure 6-6: The y-axis shows the peak inspiratory flow (L/min) of the 1st inhaled breath and the x-axis shows the % oropharyngeal and gastrointestinal deposition (ex-actuator), n=10.

There was a significant correlation between volume of inhalation and PIF ($r=0.845$, $r^2=0.714$, $p=0.002$) as shown in Figure 6-7. Figure 6-8 shows the relationship between time of inhalation and volume.

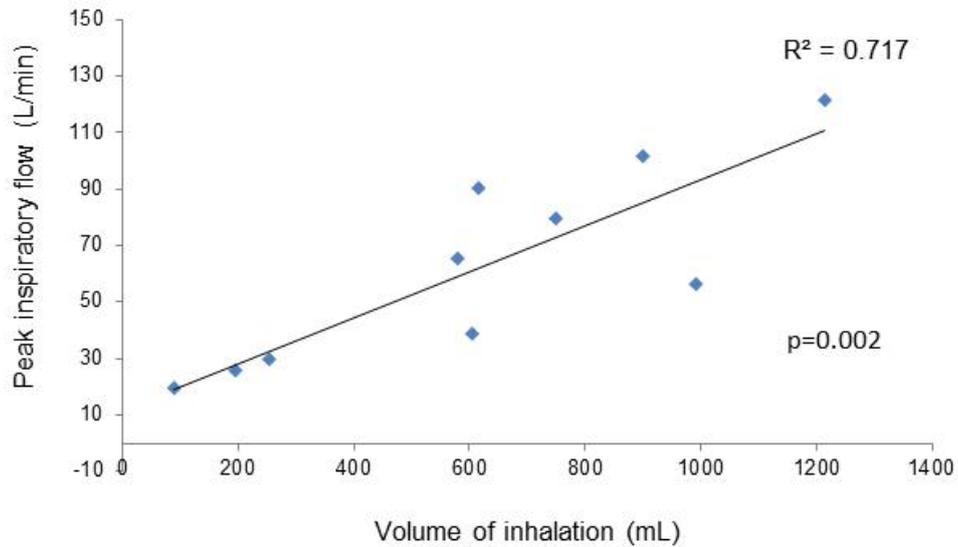


Figure 6-7: The y-axis shows the peak inspiratory flow 1st inhaled breath (L/min) and the x-axis shows the volume of inhalation 1st breath (mL), $n=10$.

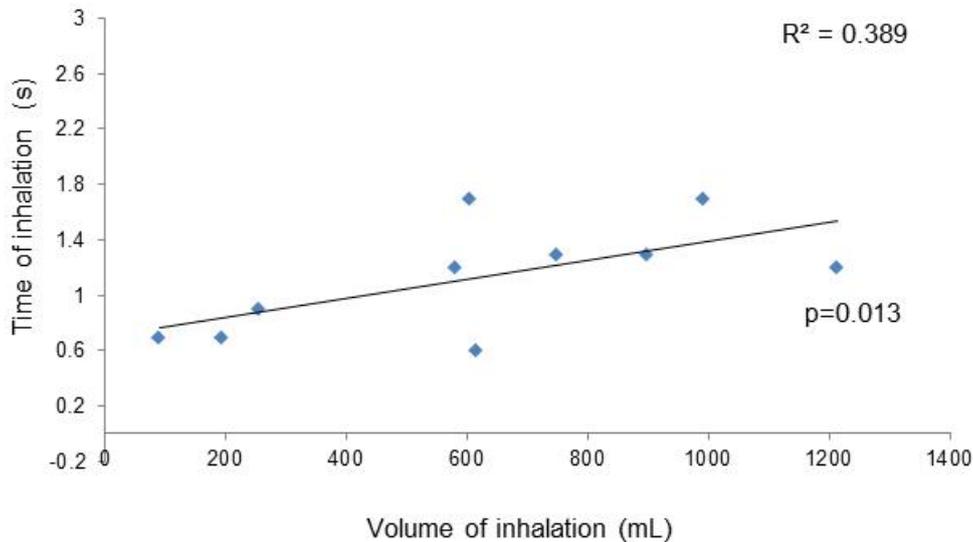


Figure 6-8: The y-axis shows the time of inhalation (s) 1st inhaled breath and the x-axis shows the volume of inhalation (mL), $n=10$.

Linear regression with age, weight, height, FVC showed that volume of inhalation was a significant predictor of PIF ($R=0.996$, adjusted $R^2=0.976$, $p=0.002$) and time of

inhalation ($R=0.988$, adjusted $R^2=0.927$, $p=0.003$). The volume of inhalation was a significant predictor of the extrafine particle mass ($R=0.678$, $R^2=0.460$, $p=0.045$). As expected there was significantly more drug retained in the spacer with ‘tidal’ compared to ‘breath hold’ ($p=0.036$). Regression analysis with factors age, gender, weight, height, volume, PIF and time showed that time of inhalation was a significant predictor of peripheral deposition (P:C ratio) ($R=0.936$, adjusted $R^2=0.827$, $p=0.002$), Figure 6-9.

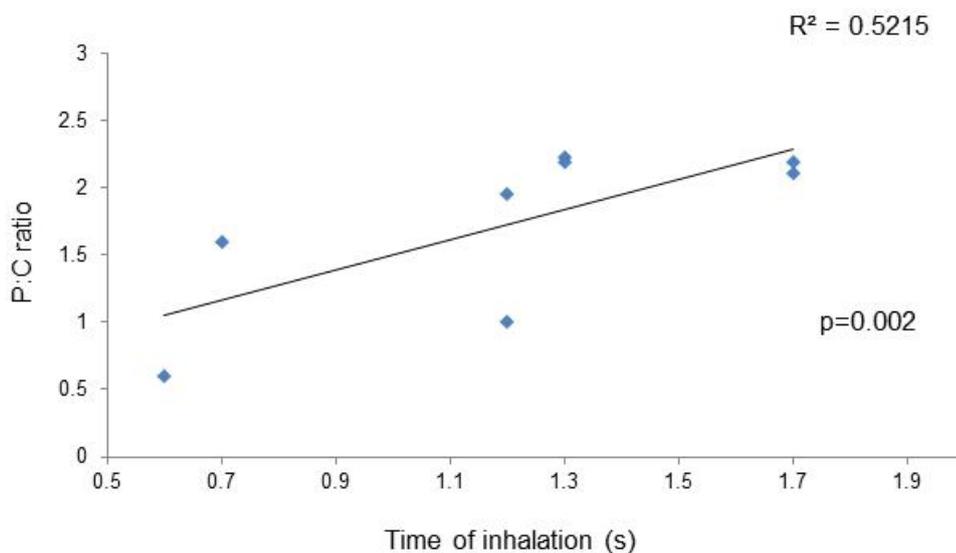


Figure 6-9: The y-axis shows the peripheral to central deposition (P:C ratio) and the x-axis shows the time of inhalation of the 1st inhaled breath (s), n=8.

6.5.4.3 Inhalation technique ‘simultaneous’ group

With ‘tidal’ breathing in the ‘simultaneous’ group (n=7) the mean (SD) volume of inhalation, PIF and time of inhalation was 569 (391), 55.2 (35) and 1.1 (0.4) respectively. The mean (SD) % ex-valve total body deposition and FVS-NGI was 32.2 (12.2), CV 37.9% and 39.4 (3.8), CV 9.6% respectively and these values were not significantly different ($p=0.142$). As a proportion of total dose the mean (SD) % ex-valve lung deposition and mean (SD) % UFPF (particles < 2.2 μm) exiting the spacer-

FVS-NGI was 15.9 (9.5), CV 59.7% and 19.2 (3.0), 15.6% respectively. These values were not significantly different ($p=0.365$), however the variability was much higher with *in vivo* lung deposition. With ‘breath hold’ there were only 3 children in the ‘simultaneous’ group. The mean (SD) volume of inhalation, PIF and time of inhalation was 955 (237), 99.6 (20.2) and 1.3 (0.1) respectively. There was a statistically significant increase in both volume and PIF with the ‘breath hold’ subjects ($p=0.046$, $p=0.013$), although the number of children in the group was very small. The mean (SD) % ex-valve total body deposition and FVS-NGI was 47.1 (17.8) and 48.7 (0.9) respectively and these values were not significantly different ($p=0.915$), however the variability was much higher with the *in vivo* deposition. The extrafine particle mass of drug (FP) increased from 68.8 (12.7) μg with ‘tidal’ to 87.4 (0.4) μg with ‘breath hold’, however the difference was not statistically significant ($p=0.088$).

For the ‘simultaneous’ group, stepwise regression with factors age, breath type, gender, FVC, weight, height, volume, PIF and time showed that the time of inhalation was significantly predictive of the difference between % gamma deposition and the % drug exiting the spacer-FVS-NGI ($R=0.838$, adjusted $R^2=0.661$, $p=0.005$). Similarly time of inhalation was significantly predictive of the difference between % lung deposition and % UFPF exiting the spacer-FVS-NGI ($R=0.893$, adjusted $R^2=0.730$, $p=0.004$). The mean (SD) % OG deposition increased significantly from 20.1 (10.2) with $\text{PIF} < 60$ L/min to 24.2 (9.2) with $\text{PIF} > 60$ L/min ($p=0.001$). The % spacer retention decreased significantly from 62.6 (13.5) with $\text{PIF} < 60$ L/min to 49.7 (13.4) with $\text{PIF} > 60$ L/min ($p=0.025$).

6.5.4.4 Lung deposition algorithm

With the UF-EF algorithm introduced in section 6.4.7.1, the % UFPF (particles $< 2.2 \mu\text{m}$) was chosen to represent lung deposition for subjects with a time of inhalation

< 1 s or volume of inhalation less than 200 mL (n=4), and the % EFPF (particles < 3.4 μm) was chosen to represent lung deposition for subjects with time of inhalation > 1 s (n=6). The % UF-EF particle size range had a strong correlation with % ex-valve and % ex-actuator lung deposition ($r=0.972$, $r^2=0.945$, $p=0.003$ and $r=0.862$, $r^2=0.743$, $p=0.003$), as shown in Figure 6-10.

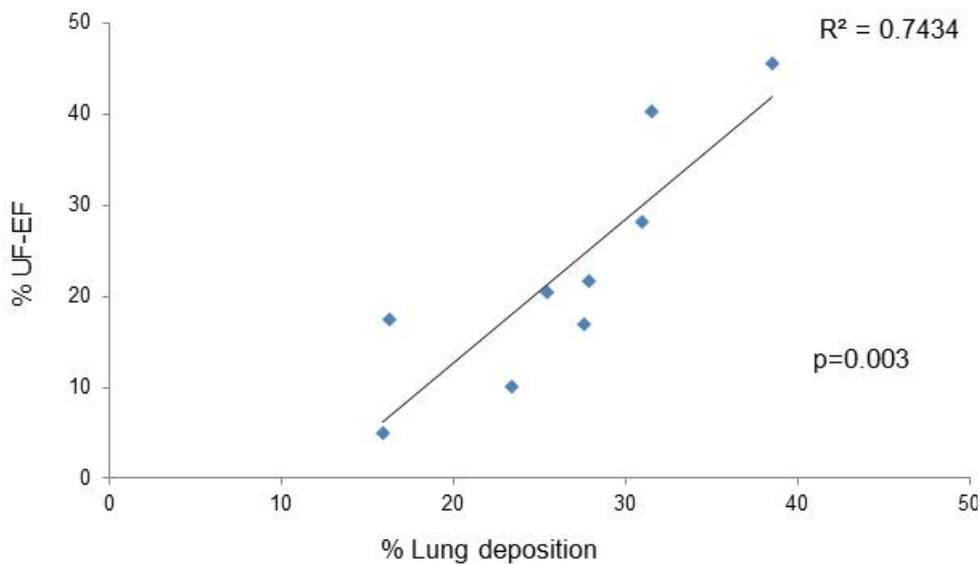


Figure 6-10: Relationship between % UF-EF particle size range on NGI (cups 3-filter) on the y-axis and % lung deposition (ex-actuator) on the x-axis.

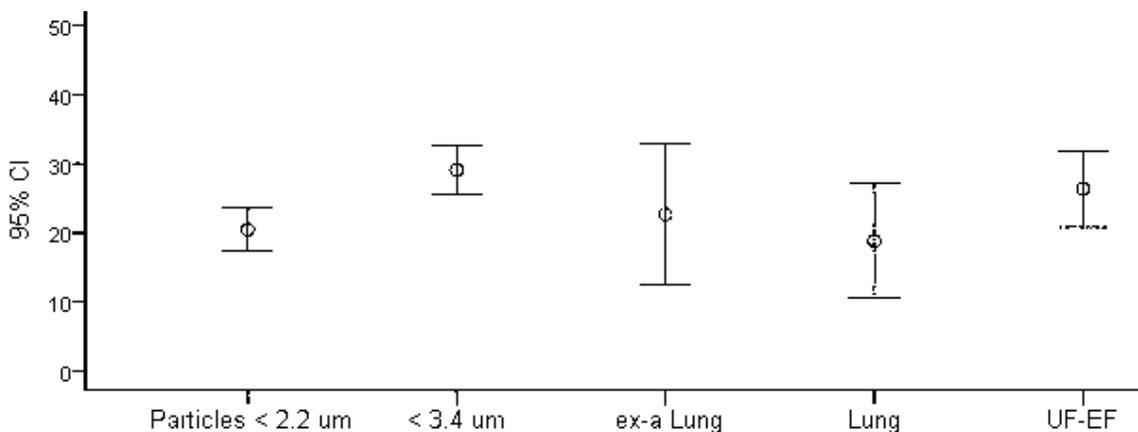


Figure 6-11: Error chart showing the 95% confidence limits for % lung, % ex-actuator lung (ex-a), % UFPF (particles < 2.2 μm) exiting FVS-NGI, % EFPF (particles < 3.4 μm) and % UF-EF for the ‘simultaneous’ group.

Regression analysis (stepwise) with factors age, gender, weight, height, FVC, volume, PIF and time of inhalation showed that % UF-EF particle size range was highly predictive of lung deposition (with $R=0.864$, adjusted $R^2=0.707$ and $p=0.003$ and $R=0.862$, adjusted $R^2=0.704$, $p=0.003$ respectively). The prediction model accounted for 70% of the variance in lung deposition. The particle size range incorporated in the % UF-EF was more consistent with the 95% confidence interval range of lung deposition (% ex-actuator) values obtained with gamma scintigraphy (Figure 6-11) as shown with the Bland-Altman plot (Figure 6-12).

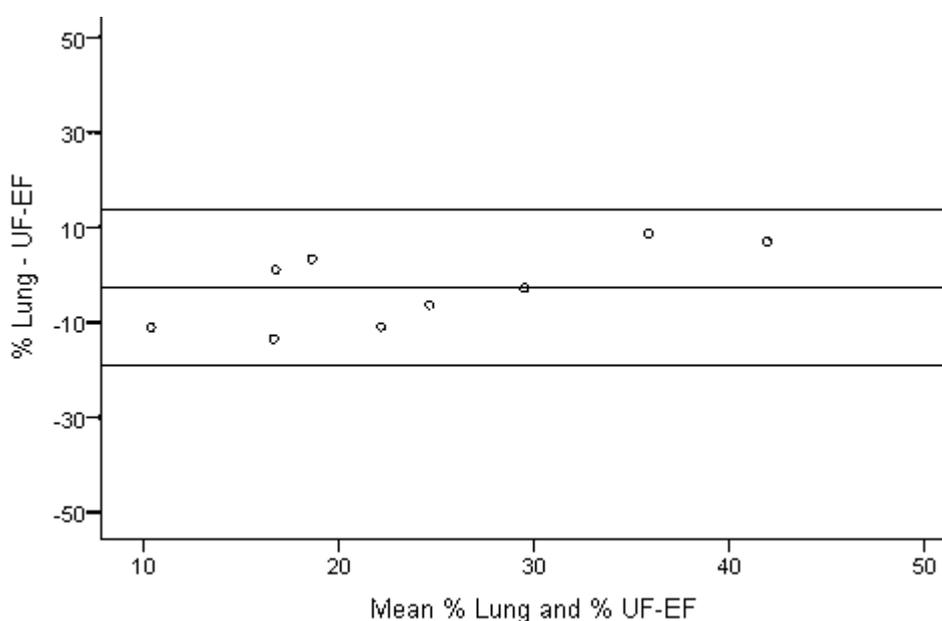


Figure 6-12: Bland-Altman plot showing the difference between lung deposition (% ex-actuator) and % UF-EF with FVS-NGI on the y-axis and the mean of % lung deposition and % UF-EF on the x-axis. Reference lines on the y-axis are at the mean difference and $\pm 2SD$.

6.5.4.5 ‘Non-simultaneous’ group

Fourteen children had their breathing pattern recorded prior to gamma scintigraphy, before inhaling the radiolabelled Flixotide®. This group is referred to as the ‘non-simultaneous’ group of children. The mean (SD) % ex-valve total drug (FP) measured

on inspiratory filter 2 (F2-FVS) and % ex-valve total drug exiting the spacer from FVS-NGI was 37.8 (5.9), CV 16 % and 44.0 (5.8), CV 13%. The mean (SD) % ex-valve total body deposition (TBD) was 36.3 (9.7), CV 27% with gamma scintigraphy. Gamma deposition with the ‘non-simultaneous’ group was not significantly different to the ‘simultaneous’ group ($p=0.760$). Similarly there was not a significant difference between the two groups for drug (FP) output measured on the inspiratory F2-FVS and drug output from spacer-FVS-NGI ($p=0.867$, $p=0.326$ respectively). With the ‘non-simultaneous’ group there was not a significant difference between % drug (FP) measured on filter 2 (F2-FVS) and % TBD with gamma scintigraphy ($p=0.460$), however there was a significant difference between the drug exiting spacer-FVS-NGI and % TBD with gamma scintigraphy ($p=0.015$). The mean (SD) % ex-valve lung deposition and mean (SD) % UFPF (particles $< 2.2 \mu\text{m}$ exiting the spacer) was 18.5 (6.0), CV 32 % and 20.4 (2.5), CV 12 % respectively and these values were not significantly different ($p=0.132$). The mean (SD) % ex-actuator lung deposition was 22.4 (7.7), CV 34 % and this was not significantly different to the % UFPF ($p=0.666$), however lung deposition was significantly different to the mean (SD) % EFPF (particles $< 3.4 \mu\text{m}$), 29.5 (3.8), ($p=0.005$). The extrafine particle mass was not significantly different between ‘tidal’ and ‘breath hold’ ($p=0.496$).

6.5.4.6 Inspiratory parameters ‘non-simultaneous’ group

The mean (SD) volume of inhalation (mL) was 930 (605) range 130-2415 mL; the mean (SD) PIF (L/min) was 67.5 (31.7) range 24-154 L/min and the mean (SD) time of inhalation (s) was 1.4 (0.7), range 0.6-2.9 s. Lung function (FVC) was a significant predictor of lung deposition ($R=0.689$, adjusted $R^2=0.431$, $p=0.022$), after stepwise regression analysis with factors age, gender, weight, height, FVC, volume, PIF and time. The ‘non-simultaneous’ group was not significantly different to the

‘simultaneous’ group for age, weight, height, FVC, Volume, PIF and time (p=0.134, p=0.297, p=0.235, p=0.471, p=0.164, p=0.712, p=0.249 respectively). Therefore the data were pooled in section 6.5.4.7 in order to improve the group size and statistics.

6.5.4.7 Inspiratory parameters pooled data

Twenty-four children using pMDI-Aerochamber Plus™ had their breathing pattern recorded with a pneumotachometer and the recorded patterns were transformed into waveforms using RSS software as described in Chapter 2, sections 2.2.13 and 2.2.14. One child in the tidal group had an outlier volume > 2400 mL and PIF > 150 L/min. A comparison was made between ‘tidal’ and ‘breath hold’ with and without the outlier value (Figure 4-5). There was a significant decrease in volume of inhalation for ‘tidal’ (p=0.035) compared with ‘breath hold’, when the ‘tidal’ outlier value was removed. However when the outlier value was included, there was not a significant increase in volume of inhalation between the two groups (p=0.362). Time of inhalation was significantly increased with ‘tidal’ compared with ‘breath hold’ (p=0.042). There was not a significant difference between the FVC or PIF with ‘tidal’ and ‘breath hold’ (p=0.668, p=0.387). The mean (±SD) inspiratory parameters for the ‘tidal’ and ‘breath hold’ groups are summarized in Table 6-6. Regression analysis showed that volume of inhalation and PIF were significant predictors of the extrafine particle mass exiting the spacer-FVS-NGI (R=0.626, adjusted R²=0.290, p=0.017 and p=0.004 respectively).

Table 6-6: Mean (SD) inspiratory parameters for total breath hold and tidal subjects. Data presented with and without* tidal outlier value.

Mean	Breath hold (n=12) 95% CI	Tidal (n=12) 95% CI	*Tidal (n=11) 95% CI
Volume (mL)	902 ± 411 (641-1163)	700 ± 182 (298-1101)	544 ± 344 (312-775)
PIF (L/min)	71.2 ± 25.9 (54.7-84.6)	59.5 ± 38.0 (35.3-83.6)	50.9 ± 24.8 (34.2-67.5)
Time (s)	1.5 ± 0.7 (1.1-2.0)	1.1 ± 0.4 (0.8-1.3)	1.0 ± 0.4 (0.8-1.3)

With time of inhalation < 1 s, there was a significant difference between the % total body deposition (TBD) with gamma scintigraphy and % drug exiting spacer-FVS-NGI (p=0.000). However with time of inhalation > 1 s, there was not a significant difference between % TBD with gamma scintigraphy and % drug exiting spacer-FVS-NGI (p=0.515, Figure 6-13).

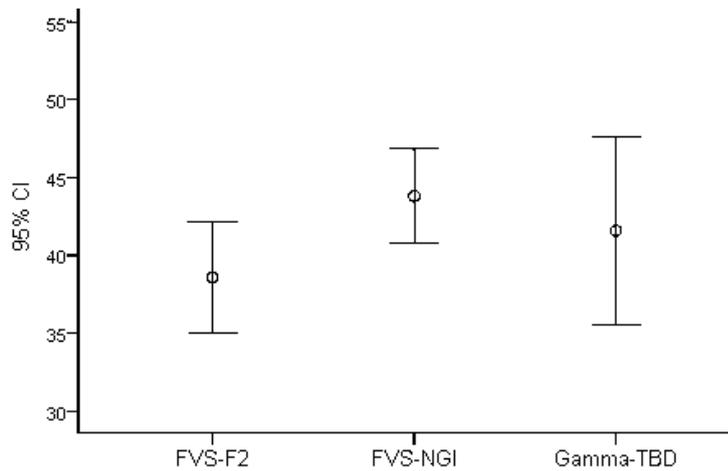


Figure 6-13: The y-axis shows the 95% confidence limits for % drug (FP) on filter 2 attached to FVS, % drug (FP) exiting spacer-FVS-NGI and % total body deposition (TBD) with gamma scintigraphy when time of inhalation of 1st inhaled breath is greater than 1 second (s).

The effect of a decreased time of inhalation and volume of inhalation was clearly demonstrated by comparing a subgroup of subjects with time of inhalation < 1 s (n=8) and volume of inhalation < 200 mL (n= 4), with the subjects with an increased time of inhalation > 1 s (n=16) and volume of inhalation > 200 mL (n= 20), as shown in Table 6-7 and Table 6-8.

Table 6-7: Comparison of mean (\pm SD) % ex-valve gamma deposition with drug (FP) on the spacer and exiting spacer-FVS-NGI and p-values between subjects with time of inhalation > 1 s (n=16) and subjects with time of inhalation < 1 s (n=8).

% Dose	Time > 1 s	Time < 1 s	p values
Lung (Gamma)	26.3 \pm 9.4	15.3 \pm 6.7	0.007
OG (Gamma)	23.9 \pm 7.3	16.4 \pm 7.3	0.026
Spacer (Gamma)	48.8 \pm 13.0	66.9 \pm 11.8	0.003
TBD (Gamma)	41.3 \pm 10.8	27.3 \pm 9.1	0.005
FP on spacer with FVS-NGI	44.3 \pm 3.5	45.4 \pm 5.2	0.556
FP exiting spacer-FVS-NGI	43.8 \pm 5.3	41.5 \pm 6.1	0.362
EFPP (particles < 3.4 μ m)	30.0 \pm 3.7	28.2 \pm 4.7	0.331
UFPF (particles < 2.2 μ m)	21.0 \pm 2.9	19.4 \pm 3.5	0.285
UF-EF	29.9 \pm 4.0	22.9 \pm 6.9	0.007

As expected, this subgroup of children was found to have significantly decreased % lung deposition, % OG deposition, % total body deposition (TBD) with gamma scintigraphy and significantly increased % spacer retention. However this was not matched with a significant difference in % drug exiting spacer-FVS-NGI or % EFPP exiting the spacer-FVS-NGI. As with lung deposition, there was a significant decrease in the % UF-EF exiting the spacer-FVS-NGI with a decreased time and volume of inhalation.

Table 6-8: Comparison of mean (\pm SD) % ex-valve gamma deposition with % drug (FP) on the spacer and exiting the spacer-FVS-NGI and p-values between subjects with volume of inhalation > 200 mL (n=20) and subjects with volume of inhalation < 200 mL (n=4).

% Dose	Volume > 200 mL	Volume < 200 mL	p values
Lung (gamma)	24.7 \pm 9.3	12.3 \pm 6.6	0.005
OG (gamma)	23.0 \pm 7.4	13.6 \pm 6.7	0.032
Spacer (gamma)	51.3 \pm 13.4	72.5 \pm 12.1	0.002
TBD (gamma)	39.4 \pm 10.7	22.8 \pm 9.8	0.004
FP on spacer with FVS-NGI	44.3 \pm 3.8	46.8 \pm 5.3	0.274
FP exiting spacer-FVS-NGI	43.6 \pm 5.1	37.8 \pm 2.7	0.755
EFPF (FP particles < 3.4 μ m)	29.8 \pm 3.9	27.4 \pm 4.9	0.294
UFPF (FP particles < 2.2 μ m)	20.8 \pm 3.0	18.6 \pm 3.8	0.212
UF-EF	29.3 \pm 4.7	18.6 \pm 3.8	0.000

There was a significant difference between % drug (FP) retained on the spacer with FVS-NGI and the % ^{99m}Tc retained on the spacer used with gamma scintigraphy with regards to the time < 1 s and volume of inhalation < 200 mL (p=0.000 and p=0.007 respectively). Similarly with time of inhalation > 1 s there was a significantly increased % ^{99m}Tc retained on the spacer compared to drug (FP) retained on the spacer-FVS-NGI (p=0.023).

For the subjects with volume of inhalation > 200 mL, there was not a significant difference between the % ^{99m}Tc retained on the spacer and % FP retained on the spacer with FVS-NGI (p=0.184). These differences in spacer retention may be explained by a mismatch between ^{99m}Tc and the drug particles of FP. Alternatively the spacer-FVS-NGI may not retain as much drug (FP) as *in vivo*, due to a mistiming of inhalation with actuation with gamma scintigraphy. Actuation was synchronized with ‘inhalation’ by the waveforms replayed on FVS-NGI, however during gamma

scintigraphy the subjects may not have inhaled the ^{99m}Tc -HFA-FP at the time of actuation.

The mean (SD) % OG deposition with PIF < 60 L/min (n=10), 15.3 (4.6) was significantly increased to 25.8 (7.0) with PIF > 60 L/min (n=14, p=0.000). The mean (SD) % spacer retention decreased from 62.6 (6.2) with PIF < 60 L/min to 49.3 (14.1) with PIF > 60 L/min (p=0.029). However there was not a significant difference between lung deposition for subjects with PIF < 60 L/min compared to PIF > 60 L/min (p=0.293).

6.5.4.8 FVS-NGI pooled data

Twenty-six children had gamma scintigraphic scans according to Chapter 5, section 5.4.7. The results are recorded in section 5.5.4. Twenty-four children had their breathing patterns recorded (Chapter 2, section 2.2.13 and 2.2.14). Waveforms were replayed on the FVS-NGI as described in Chapter 2, section 2.2.14. Of the 24 children who had their breathing patterns recorded, two children had PIF > 100 ± 5 L/min and could not be used with FVS-NGI, therefore only 22 children had their waveforms transferred to FVS-NGI, with 11 children in the ‘tidal’ group and 11 children in the ‘breath hold’ group.

The mean (SD) % total drug (FP) exiting the spacer was 42.9 (5.6, CV 13%) and mean (SD) % gamma deposition was 36.9 (11.8, CV 32%) and these values were significantly different (p=0.017). Although a mean difference of 6% may not be clinically relevant, the variability (CV%) of 13% with *in vitro* FVS-NGI compared to 32 % *in vivo* gamma scintigraphy may be clinically relevant.

The results comparing ‘tidal’ and ‘breath hold’ are shown in Table 6-9.

Table 6-9: Mean (SD) comparison of FP exiting FVS-NGI with total body deposition (% ex-valve) and comparison of the EFPF exiting FVS-NGI with lung deposition (% ex-actuator). Predicted lung deposition values are shown based on Newman, Newhouse, Finlay and Farr.

Method	N	Breath hold (n=14)	Tidal (n=12)
FP exiting spacer-FVS-NGI*	22*	46.3 ± 5.1 (n=11)	39.6 ± 3.9 (n=11)
Total body deposition (gamma)	26	37.8 ± 10.9	35.8 ± 13.3
Lung deposition (gamma)	26	24.1 ± 8.7	20.8 ± 10.7
EFPF (Newman)	22	31.4 ± 4.1	29.3 ± 4.1
UF-EF algorithm	22	31.2 ± 4.5	23.4 ± 5.0
Particles 1.3-6.1µm (Finlay)	22	24.5 ± 3.1	22.2 ± 2.2
UFPF (Newhouse)	22	21.8 ± 3.0	20.4 ± 3.1
Predicted lung (modified Farr)	22	18.0 ± 3.8	13.8 ± 2.8

*Twenty-six children had gamma scans. Two children did not have breathing patterns recorded. Two children had PIF > 100 ± 5 L/min and could not be used with FVS-NGI.

The extrafine particle mass (µg) of drug (FP) was a significant predictor of lung deposition, after stepwise regression analysis with factors breath type, age, gender, weight, height, FVC, volume, PIF and time (R=0.635, adjusted R²=0.340, p=0.009). Similarly, the % UFPF (particles < 2.2 µm) exiting spacer-FVS-NGI was a significant predictor of % ex-actuator lung deposition (R=0.598, adjusted R²=0.289, p=0.019), after stepwise regression analysis with factors, breath type, age, weight, height, gender, FVC and UFPF. A comparison of *in vitro* measures is shown with the *in vivo* lung deposition in Figure 6-14.

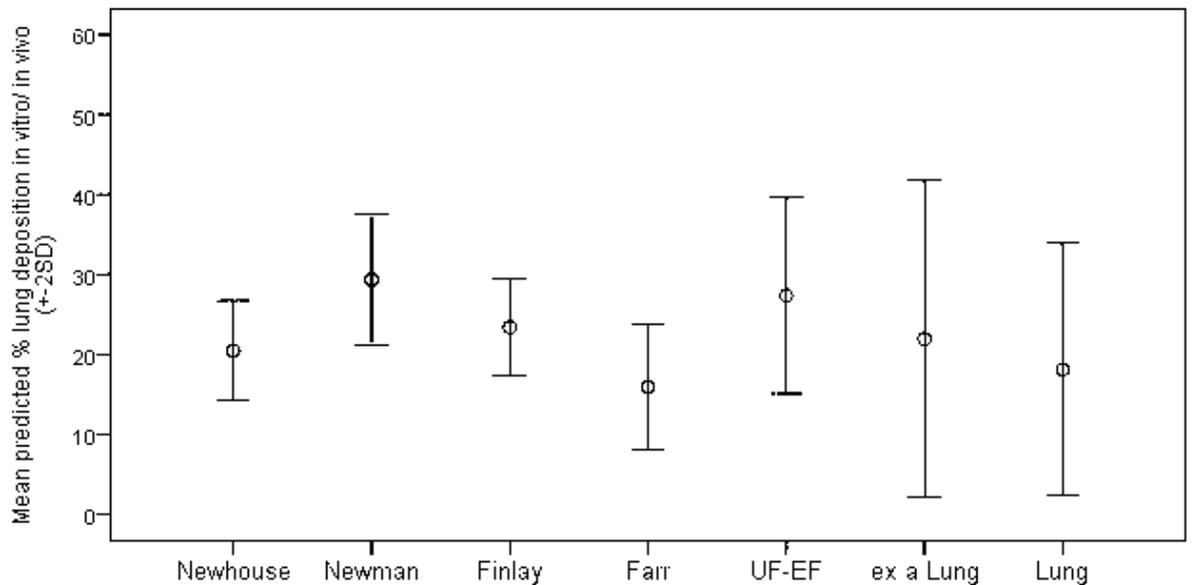


Figure 6-14: Error chart showing mean ($\pm 2SD$) predicted % lung deposition *in vitro* (FVS-NGI) based on Newhouse, Newman, Finlay, Farr and UF-EF algorithm compared to % ex-actuator lung and % ex-valve lung deposition with gamma scintigraphy.

Mean canister weight for the pooled data with FVS-NGI was 14.9 (1.0). The radiolabelled Flixotide® canister weight used for gamma scintigraphy was significantly less than the commercial Flixotide® canister weight used for FVS-NGI ($p=0.002$), however there was not a significant correlation between gamma deposition or lung deposition with canister weight ($p=0.916$, $p=0.906$). Similarly there was not a significant correlation between % total drug output and % EFPF (particles $< 3.4 \mu\text{m}$) or % UFPF (particles $< 2.2 \mu\text{m}$) exiting spacer-FVS-NGI with canister weight ($p=0.425$, $p=0.178$). When the UF-EF algorithm was applied, there was a highly significant correlation between % UF-EF and % lung deposition (ex-actuator) ($r^2=0.507$, $p=0.000$, Figure 6-15). Linear regression showed that % UF-EF was predictive of lung deposition ($R=0.771$, adjusted $R^2=0.527$, $p=0.000$) after adjusting for breath type and FVC. A Bland-Altman plot shows the difference between the two methods was within $\pm 2SD$, Figure 6-16.

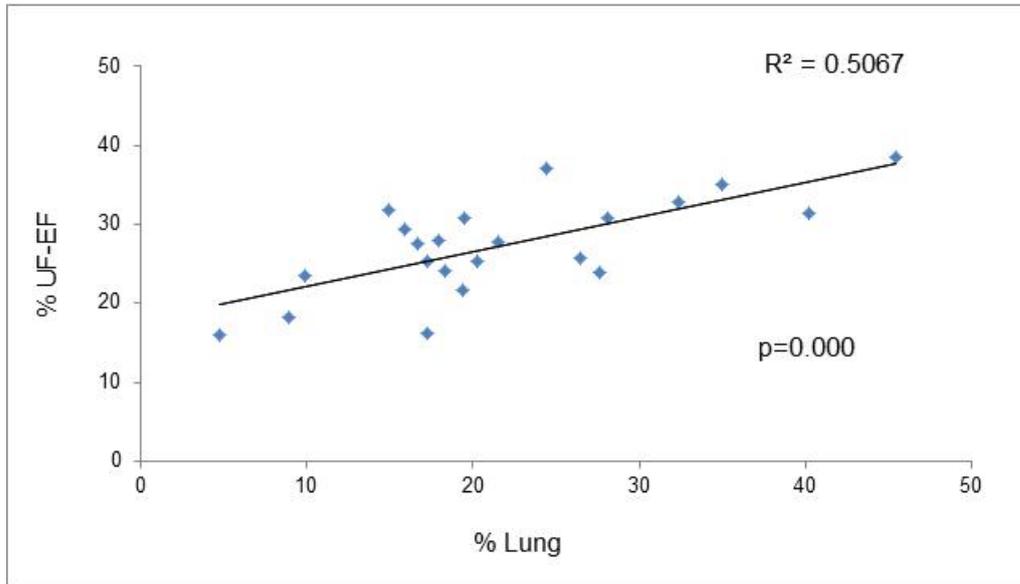


Figure 6-15: Relationship between % drug (FP) exiting spacer attached to FVS-NGI in the UF-EF particle fraction on the y-axis and % lung deposition (ex-actuator) on the x-axis.

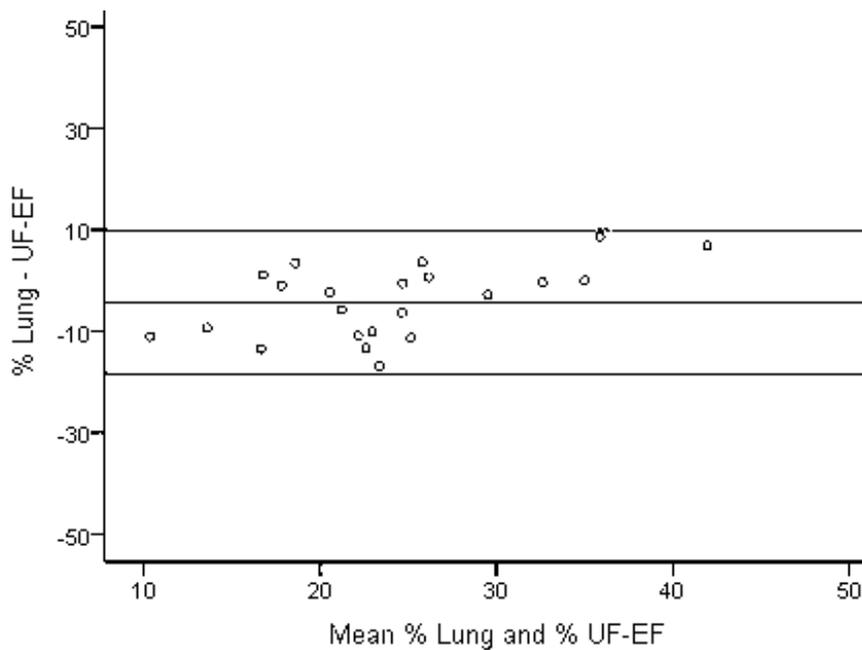


Figure 6-16: Bland-Altman plot showing the difference between % lung deposition (ex-actuator) and % UF-EF particles exiting spacer-FVS-NGI on the y-axis. The x-axis shows the mean % lung deposition and % UF-EF. Reference lines are at the mean \pm 2SD.

Regression analysis (stepwise) with factors, breath type, age, gender, weight, height, volume of inhalation, PIF and time of inhalation, showed that the volume of inhalation was predictive of lung deposition ($R=0.463$, adjusted $R^2=0.214$, $p=0.023$). As with the

‘simultaneous’ group, PIF was significantly predictive of OG deposition and spacer retention ($R=0.533$, adjusted $R^2=0.251$, $p=0.007$ and $R=0.543$, adjusted $R^2=0.370$, $p=0.006$ respectively). The peripheral lung deposition, reflected by the P:C ratio measured with gamma scintigraphy, correlated with time of inhalation ($r=0.454$, $r^2=0.206$, $p=0.034$). Regression analysis (stepwise) with factors breath type, age, gender, weight, height, FVC, volume of inhalation, PIF and time of inhalation showed that time of inhalation and volume of inhalation were significant predictors of the P:C ratio ($R=0.623$, adjusted $R^2=0.323$, $p=0.003$ and $p=0.028$ respectively). Similarly the volume of inhalation and PIF were predictive of the extrafine mass of drug particles exiting the spacer attached to the FVS-NGI ($R=0.770$, adjusted $R^2=0.286$, $p=0.016$ and $p=0.013$ respectively).

6.5.4.9 Pooled data – ‘tidal’

The mean (SD) volume of inhalation, PIF and time of inhalation was 544 (344), 59.5 (38.0) and 1.1 (0.4) respectively ($n=11$). With the ‘tidal’ group ($n=12$) the mean (SD) % total body deposition (TBD) was 34.4 (12.9), CV 37.5% and the mean (SD) % drug exiting the spacer-FVS-NGI ($n=11$) was 39.6 (3.9), CV 9.8% and these were not significantly different ($p=0.198$).

There was not a significant difference between % TBD measured with gamma scintigraphy and % drug (FP) measured on the inspiratory filter 2 attached to FVS (F2-FVS, $p=0.545$). Similarly there was not a significant difference between % drug (FP) measured on the inspiratory filter 2 (F2-FVS) and % drug exiting the spacer-FVS-NGI ($p=0.333$). The % drug (FP) measured on the inspiratory filter 1 (F1) was not significantly different to the % drug exiting the spacer-FVS-NGI ($p=0.077$), even though a different breathing pattern was used for F1. The % TBD was significantly different to the drug output on inspiratory filter 1 ($p=0.047$).

Mean (SD) % ex-actuator lung deposition was 20.8 (10.7), CV 51% and the mean (SD) % UFPF was 19.0 (2.6), CV 13.7% and these were not significantly different ($p=0.897$). However there was a significant difference between % EFPF (particles $< 3.4 \mu\text{m}$) exiting the spacer-FVS-NGI and % ex-valve and % ex-actuator lung deposition ($p=0.001$, $p=0.022$ respectively). Lung deposition correlated with weight, height, FVC, volume of inhalation, time of inhalation and spacer retention ($p=0.041$, $p=0.037$, $p=0.035$, $p=0.019$, $p=0.003$, $p=0.000$), but not with age ($p=0.051$).

When the time of inhalation < 1 s mean (SD) lung deposition was 13.4 (6.0) and this was significantly decreased compared to the mean (SD) lung deposition of 28.2 (8.9), with time of inhalation > 1 s ($p=0.008$). Stepwise linear regression with factors age, weight, height, gender, FVC, volume of inhalation, PIF and time of inhalation showed that the time of inhalation was significantly predictive of lung deposition, with tidal breathing ($R=0.772$, adjusted $R^2=0.555$, $p=0.003$).

As expected OG deposition showed a strong negative correlation with spacer retention ($r=-0.766$, $r^2=0.587$, $p=0.004$). Linear regression with factors age, FVC, volume, PIF and time showed that volume of inhalation, time of inhalation and PIF were significant predictors of OG deposition ($R=0.929$, adjusted $R^2=0.750$, $p=0.039$, $p=0.007$ and $p=0.004$). Time of inhalation and PIF were predictors of spacer retention of drug ($R=0.889$, $R^2=0.743$, $p=0.001$ and $p=0.037$ respectively). There was a significant correlation between the UF-EF algorithm and lung deposition with 'tidal' ($r=0.744$, $r^2=0.554$, $p=0.009$).

With the 'tidal' group the radiolabelled Flixotide® canister weight used with gamma scintigraphy was significantly less compared with the commercial Flixotide® canister weight used with FVS-NGI ($p=0.001$), however there was not a significant correlation between the radiolabelled canister weight and % gamma deposition or % lung deposition ($p=0.356$, $p=0.523$). There was not a significant correlation between the

canister weight and the total drug mass exiting the spacer-FVS-NGI ($p=0.368$).

Similarly there was not a significant correlation between the extrafine particle mass and canister weight ($p=0.158$).

6.5.4.10 Pooled data – ‘breath hold’

With ‘breath hold’ ($n=14$) the mean (SD) volume of inhalation, PIF and time of inhalation was 902 (411), 71 (26) and 1.5 (0.7) respectively. The mean (SD) % total body deposition (TBD) with gamma scintigraphy was 37.7 (10.8), CV 29% and the % drug (FP) exiting the spacer-FVS-NGI was 46.3 (5.1), CV 11%. There was a significant difference between between % TBD with gamma scintigraphy and % drug (FP) exiting FVS-NGI ($p=0.046$), however there was not a significant difference between % TBD and the % drug (FP) on the inspiratory filter 2 attached to FVS (F2-FVS) ($p=0.902$).

There was not a significant difference between the % drug measured on the inspiratory filter 1 and % drug exiting the spacer-FVS-NGI ($p=0.218$), even though two different breathing patterns were used. However, there was a significant difference between % drug (FP) on the inspiratory filter 2 attached to FVS (F2-FVS) and % drug (FP) exiting spacer-FVS-NGI ($p=0.003$), even though the same breathing patterns were used.

Mean (SD) % ex-actuator lung deposition was 24.1 (8.7), CV 36% and % UFPF particle fraction was 21.9 (3.1), CV 14%. There was not a significant difference between the % UFPF (particles $< 2.2 \mu\text{m}$) exiting spacer-FVS-NGI compared with % ex-valve or % ex-actuator lung deposition measured with gamma scintigraphy ($p=0.297$, $p=0.337$ respectively). However, there was a significant difference between the % EFPF (particles $< 3.4 \mu\text{m}$) exiting spacer-FVS-NGI and % ex-valve and % ex-actuator lung deposition ($p=0.000$, $p=0.025$ respectively).

Stepwise regression with factors age, weight, height, gender, FVC, volume, PIF and time showed that the UF-EF algorithm predicted lung deposition ($R=0.683$, adjusted

$R^2=0.408$, $p=0.018$). Lung deposition with 'breath hold' was not dependent on age, weight, height, FVC, volume of inhalation, PIF or time of inhalation, however the time of inhalation was significantly increased with 'breath hold' compared with 'tidal' ($p=0.042$).

As expected OG deposition showed a strong negative correlation with spacer retention ($r=-0.745$, $p=0.002$). The P:C ratio increased significantly with 'breath hold' ($p=0.015$). The % EFPF (particles $< 3.4 \mu\text{m}$) exiting the spacer-FVS-NGI with 'breath hold' was significantly higher than with 'tidal' ($p=0.027$). Spacer retention was highly negatively correlated to both lung deposition and OG deposition ($p=0.000$, $p=0.000$ respectively).

There was not a significant difference between the radiolabelled Flixotide® canister weight used with gamma scintigraphy compared with the commercial Flixotide® canister weight used with FVS-NGI ($p=0.170$). The commercial Flixotide® canister weight used with FVS-NGI did not correlate with total drug (FP) output or % UFPF ($p=0.627$, $p=0.579$ respectively).

When subgroups of children aged ≤ 8 years were compared with children aged > 8 years, time of inhalation, volume of inhalation and PIF decreased and spacer retention increased for children in the 'tidal' group ≤ 8 years of age compared with the children in the 'tidal' group > 8 years of age, however the difference was not significant ($p=0.138$, $p=0.075$, $p=0.060$, $p=0.071$ respectively).

With 'breath hold' the time of inhalation, volume of inhalation and PIF increased for children aged > 8 years, however the difference was not significant ($p=0.623$, $p=0.061$ and $p=0.303$). The % UFPF (particles $< 2.2 \mu\text{m}$) exiting spacer-FVS-NGI with the 'tidal' group ≤ 8 years of age was significantly less than % UFPF for the children in the 'tidal' group > 8 years of age ($p=0.029$), Table 6-10. Similarly there was significantly

more drug measured in the fine particle mass with children > 8 years of age compared to children ≤ 8 years of age (p=0.032).

Table 6-10: Comparison of mean (SD) inspiratory parameters, % lung deposition (ex-valve and ex-actuator) and % extrafine particle fraction exiting the spacer-FVS-NGI for children > 8 years age and children ≤ 8 years age, using ‘tidal’ and ‘breath hold’ (BH).

Mean ± SD	BH > 8 (n=10)	Tidal > 8 (n=6)	BH ≤ 8 (n=4)	Tidal ≤ 8 (n=6)
Volume (mL)	1057 ± 368	744 ± 215*	592 ± 335	377 ± 355
PIF (L/min)	77.0 ± 27.0	79.8 ± 40.5	59.8 ± 22.3	39.2 ± 23.4
Time (s)	1.6 ± 0.7	1.2 ± 0.5	1.4 ± 0.7	0.9 ± 0.2
% Lung (ex-a)*	25.2 ± 9.6	25.9 ± 11.3	21.4 ± 5.9	15.8 ± 7.8
% OG (ex-a)	21.4 ± 8.0	22.47 ± 5.2	27.2 ± 9.6	17.5 ± 9.2
% Spacer (ex-a)	52.8 ± 14.5	49.6 ± 12.1	50.8 ± 15.2	65.4 ± 14.9
% Lung	19.8 ± 7.5	22.0 ± 9.7	17.4 ± 4.3	13.1 ± 6.2
% OG	16.7 ± 6.4	19.2 ± 5.0	21.9 ± 7.0	14.6 ± 7.7
% Spacer	44.4 ± 13.3	41.9 ± 9.4	41.7 ± 14.3	54.6 ± 12.8
% EFPP < 3.4 µm	30.2 ± 4.4	29.2 ± 3.3	33.5 ± 2.8	25.7 ± 1.5
% UFPP < 2.2 µm	21.2 ± 3.5	20.8 ± 2.7	23.0 ± 1.9	17.5 ± 1.4
% UF-EF	31.4 ± 4.5	25.7 ± 3.9	31.0 ± 5.3	21.5 ± 5.3

*With the tidal group > 8 years, an outlier value for volume of inhalation > 2400 mL was excluded from the analysis (n=5). *Ex-actuator given by (ex-a). Two children in the BH group > 8 did not have FVS-NGI; one child had PIF > 100 L/min and was excluded from FVS-NGI (n=7).

With the subgroup of children < 10 years of age there was significantly higher % drug (FP) exiting spacer-FVS-NGI, % UFPP and % EFPP with ‘breath hold’ compared with ‘tidal’ (p=0.001, p=0.015, p=0.007 respectively). There was an increase in fine particle mass for children < 10 years of age using ‘breath hold’ (p=0.003). Similarly, for children ≤ 7 years of age, there was a significantly higher % drug exiting spacer with FVS-NGI, UFPP and EFPP with ‘breath hold’ compared with ‘tidal’ (p=0.000, p=0.002 and p=0.001 respectively) and shown in Table 6-11.

Table 6-11: Comparison of mean inspiratory parameters, % gamma deposition (ex-valve), EFPF, UFPF, UF-EF exiting the spacer-FVS-NGI and % lung deposition (ex-actuator) for children < 10 years age, using ‘tidal’ and ‘breath hold’ (BH) with AC+.

Mean ± SD	BH < 10 (n=9)	Tidal < 10 (n=10)	BH 5-7 (n=4)	Tidal 5-7 (n=6)
Volume (mL)	732 ± 394	543 ± 363	592 ± 335	377 ± 355
PIF (L/min)	67.8 ± 23.9	48.2 ± 24.4	59.8 ± 22.3	39.2 ± 23.4
Time (s)	1.3 ± 0.5	1.1 ± 0.4	1.4 ± 0.7	0.9 ± 0.2
% Lung	19.7 ± 7.6	16.4 ± 8.9	17.4 ± 4.3	13.1 ± 6.2
% OG	20.3 ± 7.3	17.0 ± 7.2	21.9 ± 7.0	14.6 ± 7.7
% Spacer	41.6 ± 13.4	49.4 ± 12.8	41.7 ± 14.3	54.6 ± 12.8
% Spacer FVS	46.6 ± 3.1	46.9 ± 3.1	40.4 ± 4.6	48.2 ± 2.6
% EFPF < 3.4 µm	33.5 ± 2.8	26.9 ± 3.0	33.5 ± 2.8	25.7 ± 1.5
% UFPF < 2.2 µm	22.5 ± 3.3	18.7 ± 2.6	23.0 ± 1.9	17.5 ± 1.4
% UF-EF	31.1 ± 5.2	23.6 ± 5.2	31.0 ± 5.3	21.5 ± 5.3
% ex-a Lung	24.2 ± 9.8	19.1 ± 10.4	21.4 ± 5.9	15.8 ± 7.8

Subjects < 10 years of age did not show a significant difference between volume of inhalation, PIF or time of inhalation with ‘breath hold’ and ‘tidal’ (p=0.308, p=0.108 and p=0.287 respectively). Similarly there was not a significant difference between lung deposition, OG and spacer retention with ‘breath hold’ and ‘tidal’ (p=0.319, p=0.244, p=0.247 respectively) in this age-group. Regression (stepwise) with factors age, gender, weight, height, FVC, time, volume and PIF showed that the time of inhalation was significantly predictive of lung deposition for the tidal breathing group aged < 10 years (R=0.761, adjusted R²=0.532, p=0.007) and the UF-EF algorithm was predictive of lung deposition (R=0.744, adjusted R²=0.504, p=0.009).

6.6 DISCUSSION

There is a clinical need for prospectively estimating lung deposition of inhaled drug in the pediatric population so that effective recommendations for an inhaler type can be made with evidence-based *in vitro* methods. There is significant variability in dosing associated with different breathing patterns. The reliability of inhalation therapy with pMDI-spacer and consequently the quality of long-term control of asthma might improve when choosing more effective spacer inhalation techniques.[501]

Experimental studies that can reflect *in vivo* results are needed in order to improve particle deposition simulations.

Children using tidal breathing with pMDI-spacer have different inhalation profiles compared to children using a slow, single maximal inhalation with ‘breath hold’. In Chapter 5, gamma scintigraphy was used to show that with tidal breathing *via* pMDI-spacer (Aerochamber Plus™), there was comparable lung deposition of HFA-FP compared with a slow, single maximal inhalation with ‘breath hold’. However the ‘breath hold’ technique was associated with decreased variability in lung deposition with CV 36% for ‘breath hold’ compared to 51% with ‘tidal’. The ‘breath hold’ technique was also associated with enhanced peripheral deposition of HFA-FP.

Similarly chapter 5 demonstrated that for children less than 10 years of age, the small volume incentive spacer, significantly reduced variability in lung deposition for young children using tidal breathing with CV 18% with Funhaler compared to CV 51% with Aerochamber Plus™. The *in vitro* analysis of the Funhaler spacer will be investigated in the next Chapter.

The studies in this chapter supported our hypothesis that patient-specific waveforms replayed on FVS-NGI could be used to estimate total body deposition and lung deposition *in vivo*. A subgroup of ten children had their breathing patterns recorded

while they simultaneously inhaled radiolabelled HFA-FP and there was a significant correlation between % gamma deposition and the % drug exiting the spacer attached to the FVS-NGI. There was a significant correlation between the extrafine particle fraction (EFPF) of drug (FP) and ultrafine particle fraction (UFPF) exiting the spacer-FVS-NGI and lung deposition. The time of inhalation was predictive of lung deposition and peripheral deposition. PIF was predictive of oropharyngeal and gastrointestinal (OG) deposition. The volume of inhalation correlated significantly with both time of inhalation and PIF and was a significant predictor of the extrafine particle mass. Furthermore the UF-EF algorithm was a predictor of lung deposition. These findings confirmed the hypotheses.

It can be argued that a direct comparison of the results from gamma scintigraphy cannot be made with FVS-NGI, as some of the breathing patterns were recorded prior to gamma scintigraphy. Interestingly, however, after some initial training in the correct inhalation technique, the % drug measured on the inspiratory filter (F1) also correlated significantly with both the % drug exiting the spacer attached to the FVS-NGI and the % gamma deposition, even though different breathing patterns were used for each procedure. Therefore for logistic reasons and because of difficulties shaking the radiolabelled canister contained in the laminar flow box, the remaining subject's breathing patterns were recorded prior to gamma scintigraphy.

There was not a significant difference between the simultaneously recorded group and the non-simultaneously recorded group for age, weight, height, FVC, volume, PIF and time of inhalation. Therefore the data were pooled to investigate correlations with lung deposition, OG deposition and spacer retention. With the pooled data, there was a significant difference between % drug exiting the spacer-FVS-NGI and % gamma deposition. However, the % drug measured on the filter attached to FVS (F2) was not

significantly different to % total body deposition with gamma scintigraphy and was also significantly correlated with FVS-NGI.

Interpatient variability of greater than 50% in drug deposition has been demonstrated in both adults and children. However, the *in vitro* variability in the fine particle fraction is relatively low.[91, 502] The novel UF-EF algorithm was proposed in this chapter in order to link the UFPF and EFPF to lung deposition, according to the individual subject's volume of inhalation and time of inhalation. The UF-EF algorithm proposed in this study was highly predictive of lung deposition and produced an increase in variability with CV 22%, compared to the CV 14% for % EFPF exiting the spacer-FVS-NGI.

Exact cut-offs for fine particle and extrafine particle fractions are fixed, however a range between UFPF and EFPF is more likely to be related to *in vivo* inspiratory parameters and inhalation technique. The UF-EF algorithm strongly indicated a significant relationship between lung deposition and particles in the UF-EF range exiting FVS-NGI. Therefore the algorithm improved the predictive power of the *in vitro* measures of lung deposition in this chapter. This has not been previously described in the literature.

There were several methodological limitations in this study which may have affected the match between *in vitro* and *in vivo* data. The breathing recordings were not all simultaneously recorded during inhalation of the radiolabelled drug and this may have altered both drug output and inspiratory parameters. The FVS-NGI was operated 'in-phase' with the start of the recorded first breath and this may not have coincided with the subject's actual time of inhalation of radiolabelled Flixotide® *via* pMDI-spacer. Dose inconsistencies may have been associated with the different canister weights for commercial Flixotide®.[146] Ideally subjects should use the same

number of actuations from the pMDI with gamma scintigraphy and FVS-NGI.[107]

The volume of the small spacer device may have been a limiting factor with regard to the finding of comparable lung deposition with ‘tidal’ and ‘breath hold’ inhalation techniques.[503]

Schultz *et al* demonstrated that the FVS is able to replicate *in vivo* breathing traces over a wide range of inspiratory flows, however there was underestimation of *in vivo* values at low flows, and overestimation at higher flows.[439] PIF values greater than 100 ± 5 mL/s were excluded from our FVS-NGI circuit, as the inhalation flow should not exceed the impactor flow.[416] [114] There may be inaccuracies due to the dead space in the Y-piece of the FVS-NGI circuit and air turbulence associated with mixing bi-directional variable airflow from the replayed waveforms with the one-directional positive airflow in the Y-piece. Flow-rate variability is an important source of measurement uncertainty with particle size distributions and inertial impaction.[365] The NGI is calibrated to operate with a constant flow with well-defined stage cut-offs, rather than in a circuit with the FVS and varying inspiratory flows from different breathing patterns.[114]

However, despite the limitations in experimental design, this study has demonstrated that there are specific factors associated with the inhalation waveform that improve drug delivery, consistent with Schultz *et al*. [426] This study has shown that with small volume spacers, PIF, volume of inhalation and time of inhalation of the first inhaled breath are important inspiratory parameters which interact and have an effect on lung deposition, OG deposition and spacer retention of HFA-FP *via* pMDI-spacer.

Schultz *et al* reported that two tidal breaths are adequate for drug delivery *via* pMDI-spacer for children 2-7 years.[426] Kamin *et al* carried out an *in vitro* study with budesonide and have reported that up to 80% of drug output from pMDI-spacers can be

inhaled with the first breath.[422] Therefore the first inhaled breath makes a significant contribution to the overall dose delivered from a small volume pMDI-spacer. In this chapter the interaction of a low volume of inhalation, PIF and time of inhalation significantly decreased *in vitro* and *in vivo* deposition via pMDI-spacer for children 5-17 years.

With the simultaneously recorded group, time of inhalation correlated significantly with lung deposition. The time of inhalation was also significantly increased with the ‘breath hold’ technique compared with ‘tidal’ breathing ($p=0.036$) and this increased time of inhalation, as well as the 5-10 s ‘breath hold’, may have contributed to the improved the P:C ratio and reduced variability with the ‘breath hold’ technique. Volume of inhalation and PIF were increased with ‘breath hold’, although the study was limited by small numbers.

With the pooled data, this study found that the *in vitro* extrafine particle mass of drug (FP) correlated with lung deposition, confirming the hypothesis. Furthermore the novel UF-EF algorithm was predictive of lung deposition. The volume of inhalation was a strong predictor of lung deposition and PIF was a strong predictor of both OG deposition and spacer retention. The time of inhalation was significantly predictive of lung deposition for the tidal breathing children aged <10 years. These findings added further support to the experimental hypotheses.

Previously, studies have shown that with adults, slow inspiratory flow-rates improve drug deposition with DPIs, although flow-rates are less likely to affect pMDI-spacer.[183, 488] [75] Inspiratory flows < 30-45 L/min have been recommended for pMDI.[416] In this paediatric study, peak inspiratory flows for the first inhaled breath ranged from 20 L/min to 154 L/min. Peak inspiratory flows less than 60 L/min were generally associated with ‘tidal’ breathing and decreased lung deposition, an increased

retention of drug in the spacer and a decreased volume of inhalation and time of inhalation. Synchronizing the coordination of actuation with the first inhaled breath is likely to be more important with small volume spacers and this study has shown that the time of inhalation and volume of inhalation *via* pMDI-spacer significantly impact on dose consistency and regional lung deposition for children using tidal breathing.[492] [504]

Regular clinical review of the inhalation technique used by young asthmatics is important. However, studies have shown that even with education and review, pMDIs are often used incorrectly. Further research is required for the development of more incentive inhalation devices. An incentive spacer, Funhaler, is investigated in the following chapter. The coordination of inhalation with actuation, as well as the time of inhalation may be improved with future spacer devices if they are designed to regulate the breathing pattern of young children by triggering a ‘toy’ or ‘whistle’ during inspiration, after a set time of inhalation and volume of inhalation has been achieved.

In summary, *in vitro* inertial sizing of aerosols can give a better approximation of predicted body deposition with gamma scintigraphy, when operated in tandem with children’s breathing patterns, rather than at a constant airflow. In a recent review article by Newman and Chan, the authors suggested that lung deposition was more closely linked to the percentage of the aerosol dose smaller than 3 μm diameter.[109] This chapter supports the view that the particles < 3.4 μm in the delivered dose are reflective of lung deposition *in vivo*. The Farr *et al* formula, using the product of the proportion of drug particles < 6.1 μm and the delivered dose, was predictive of lung deposition. However, more than 80% of the dose of drug particles < 6.1 μm were below 3.4 μm , with the NGI at 100 L/min. The studies in this chapter confirm the hypothesis that *in vitro* methods of FVS-NGI can be used to reflect *in vivo* gamma scintigraphy with suspension aerosols such as Flixotide® (MMAD 2.8 μm).

Inspiratory parameters of the first inhaled breath with pMDI-spacer interact to affect drug delivery. The use of a novel algorithm with *in vitro* methods of particle sizing and predictions of lung deposition could be further refined by using a variable cut-off particle size range, depending on the volume of inhalation and time of inhalation associated with individual breathing patterns. Further data are needed to explore and confirm the reproducibility of the algorithm introduced in this chapter.

7 INCENTIVE SPACER with FVS-NGI

7.1 INTRODUCTION

The inhaler delivery device plays an essential role in asthma management for the younger paediatric population. It is important for parents and/or carers to choose a delivery device that encourages both adherence to the dosage regimen and compliance with the correct use of the device. Poor patient and parental compliance with the correct use of an inhaler device can reduce drug delivery and this may lead to an exacerbation of the child's asthma symptoms and a subsequent increased dosage regimen.[505]

The device needs to be age-appropriate, easy to use and provide optimal drug delivery to each individual child.[122] The Funhaler is an incentive spacer device (valved holding chamber) that has been designed to improve adherence and compliance with inhaled drug delivery in young asthmatic children who use a pressurized metered-dose inhaler (pMDI). The incentive is a 'toy' attached to the spacer and isolated from the main inspiratory circuit by a valve.[506] As the child exhales, the 'toy' spins and a whistle sounds. The device encourages a rhythmic, regular inhalation/exhalation breathing pattern.

Monitoring devices attached to pMDI provide an important objective guide to patient adherence with asthma medications. Burgess *et al* used an electronic monitoring device (Smartinhaler) to demonstrate that the Funhaler spacer device improves patient adherence and compliance compared to Aerochamber Plus™ over a short period of two weeks in a group of children aged 18 months to 7 years.[189] However the use of the Funhaler device, with regular monthly review, was not associated with significant improvement in adherence or disease control over a three month period.[189] This may

indicate that more frequent review of young children's inhaler technique is necessary in order to maintain optimal adherence. Importantly however, the Funhaler device was preferred by both parents and children, even though seven of the Funhalers broke during the study. The Funhaler device did not compromise drug delivery, so it may provide a useful strategy for the initial inhalation technique training and management of young asthmatics.

In Chapter 5 of this thesis, I investigated the total body deposition of radiolabelled Flixotide® with gamma scintigraphy. Ten children aged 5-10 years used the Funhaler spacer device to inhale ^{99m}Tc -HFA-FP. In Chapter 6, I investigated the relationship between *in vitro* Flow-Volume Simulation (FVS) with recorded breathing patterns for children using pMDI with Aerochamber Plus™, in combination with Next Generation Impaction (NGI), with the results of gamma scintigraphy from Chapter 5.

The study summarized in this chapter was designed to compare the *in vivo* total body deposition and *in vitro* drug (FP) output with FVS-NGI, using pMDI with the incentive Funhaler (225 mL) spacer device and compare the results with the currently used 'gold-standard' small volume spacer device, Aerochamber Plus™ (149 mL). Schultz *et al* has reported that inhalation volumes for young children using Funhaler and Aerochamber Plus™ are larger than expected and only two tidal breaths are required for efficient drug delivery from pMDI-spacer in children aged 2-7 years.[426] Few studies have investigated the relationship between inspiratory parameters associated with the first breath of the recorded breathing patterns with lung deposition from pMDI with an attached small volume spacer. Therefore in this chapter the effect of the first inhaled breath with Funhaler spacer device will also be investigated with children aged 5-10 years.

7.2 OBJECTIVE

The aim of the study was to compare the total body deposition and lung deposition of ^{99m}Tc -HFA-FP delivered *via* pMDI-Funhaler (FH) spacer device in asthmatic children aged 5-10 years to % drug exiting spacer-FVS-NGI and % extrafine particle fraction (EFPF) exiting the spacer-FVS-NGI.

7.3 HYPOTHESES

- Total body deposition and lung deposition of inhaled ^{99m}Tc -HFA-FP inhaled *via* pMDI-spacer (Funhaler) can be predicted from *in vitro* recorded breathing patterns and FVS-NGI.
- Lung deposition of HFA-FP delivered *via* pMDI-Funhaler can be predicted from the UF-EF algorithm, proposed in Chapter 6.
- Increasing the volume of inhalation of the first inhaled breath will increase lung deposition of HFA-FP *via* pMDI-Funhaler.
- Increasing PIF of the first inhaled breath will increase oropharyngeal and gastrointestinal (OG) deposition of HFA-FP *via* pMDI-Funhaler.
- Inspiratory parameters of the first inhaled breath with pMDI-Funhaler spacer device will be equivalent to pMDI-AC+.

7.4 METHODS

7.4.1 Study population

Ten children (6 male, 4 female) 5-10 years of age, with mild, stable asthma were recruited from outpatient clinics at Princess Margaret Hospital for Children. Approval for the study was given by the Ethics Committee. On the study day, each child had

weight, height and lung function measured, documented in Chapter 5, Table 5-2. Only those patients with FEV1 > 80% predicted values were enrolled in the study (Chapter 2 section 2.2.3). The study design is shown in Table 7-1.[429]

Table 7-1: Experimental steps in Funhaler study for comparison *in vitro* FVS-NGI with *in vivo* gamma scintigraphy.

Inhalation technique with breathing recordings (n=10)
Calculation of the inspiratory parameters associated with the first inhaled breath
Validation of radiolabelling with pMDI-Funhaler (n=4)
Gamma scintigraphy (n=10, Chapter 5)
Flow-Volume Simulation (FVS) with attached inspiratory filter (F2) n=10)
FVS in tandem with Next Generation Impaction (NGI, n=10)

7.4.2 Experimental design

Variation in the amount of drug deposited in the airways using the same delivery device can occur from one inhalation to another. Younger children using the incentive spacer device would be more likely to have different breathing patterns on separate occasions because of their curiosity with the expiratory ‘toy’. Therefore the *in vitro* FVS-NGI data obtained with the Funhaler group was not used in Chapter 6 of this thesis.

7.4.3 Validation

Chapter 4 described a series of validation experiments to show that commercial Flixotide® (HFA-FP), delivered *via* pMDi-spacer (Aerochamber Plus™) could be radiolabelled with technetium-99m (^{99m}Tc). In this Chapter, pMDI-Funhaler was used to deliver radiolabelled Flixotide®. Several subjects using the Funhaler device were scheduled on the same day as children using the Aerochamber Plus™ device, therefore the AC+ was used for all pre-patient validations.

7.4.4 Recording of breathing patterns

Breathing patterns were recorded with ten children inhaling 3 actuations of placebo *via* pMDI with the attached Funhaler spacer device secured in a purpose-built, airtight perspex flow chamber attached to the RSS100 pneumotachometer, Chapter 2 section 2.2.13. The children were instructed to inhale with 5 tidal breaths.

7.4.5 Analysis of breathing patterns

The waveform conversion program was used to digitally select for the five breath waveform. The digitally selected breathing patterns were then converted to a FVW file format for compatible use with the FVS. Volume, time of inhalation and peak inspiratory flow were derived using the BSC calculation, as described in Chapter 2 section 2.2.13.1. The inspiratory parameters of the first breath were recorded and compared with children using Aerochamber Plus™ (AC+).

7.4.6 Flow-Volume Simulator with inspiratory filter (F2)

A preliminary evaluation of total drug (FP) output from the FVS was performed by attaching a low resistance inspiratory filter (F2) to the outlet of the FVS and connected to the Funhaler. Three actuations of HFA-FP (250 µg FP/actuation) were fired into the spacer device as the waveform was replayed on the FVS. Drug (FP) was collected on filter 2, which was placed between the spacer and FVS (Chapter 2, section 2.2.14.4).

7.4.7 Flow-Volume Simulator with Next Generation Impaction

The pMDI-spacer was attached to a Flow-Volume Simulator (FVS) connected in tandem with Next Generation Impactor (FVS-NGI) in order to use the recorded breathing patterns with inertial impaction to determine drug (FP) output and fine particle fraction.[415] The NGI was connected to a vacuum pump with a suction of

100 L/min. The FVS-NGI circuit was connected to a pressurized air source with a positive airflow of 100 L/min, as shown in Figure 6-1, Chapter 6, section 6.4.7.

7.4.8 Statistical analysis

All data was stored in Excel (Microsoft© Office) and analysed with SPSS version 16.0 statistical software (Chapter 2, section 2.2.15). Based on a previous study by Farr *et al*, there was a difference in lung deposition and the fine particle fraction of 13.2% with a standard deviation of 7.0.[106] With a sample size of 10, there was a power >80 % to detect a 10% difference, at the 0.05 significance level. Independent sample T tests were used to determine the statistical significance between spacer type and inspiratory parameters. Paired samples t-tests were used to compare total drug output measured on inspiratory filter 1 (F1), inspiratory filter 2 (F2)-FVS and FVS-NGI to total deposition with gamma scintigraphy. Pearson correlations (r) were estimated using the bivariate analysis procedure in SPSS 16.0. Linear regression analysis was used to determine if the UF-EF algorithm or inspiratory parameters were predictive of the main outcome variables, lung deposition and oropharyngeal and gastrointestinal deposition.[440]

7.5 RESULTS

7.5.1 Validation

The validation of a match between drug (FP) before labelling and ^{99m}Tc radiolabel was carried out with Andersen Cascade Impaction (ACI) at a constant flow of 28.3 L/min. Validation experiments with HFA-FP delivered *via* pMDI-Funhaler were carried out in duplicate and the results are shown in Figure 7-1. The mean (SD) ratio of ^{99m}Tc to drug (FP) before labelling was 0.92 (0.1) and this passed quality control. The Funhaler valve and spacer components detached from the adaptor during actuation with cascade

impaction with two validation experiments and these experiments did not pass quality control, as shown in Figure 7-2, with mean (SD) ratio of ^{99m}Tc to drug (FP) = 0.71 (0.1).

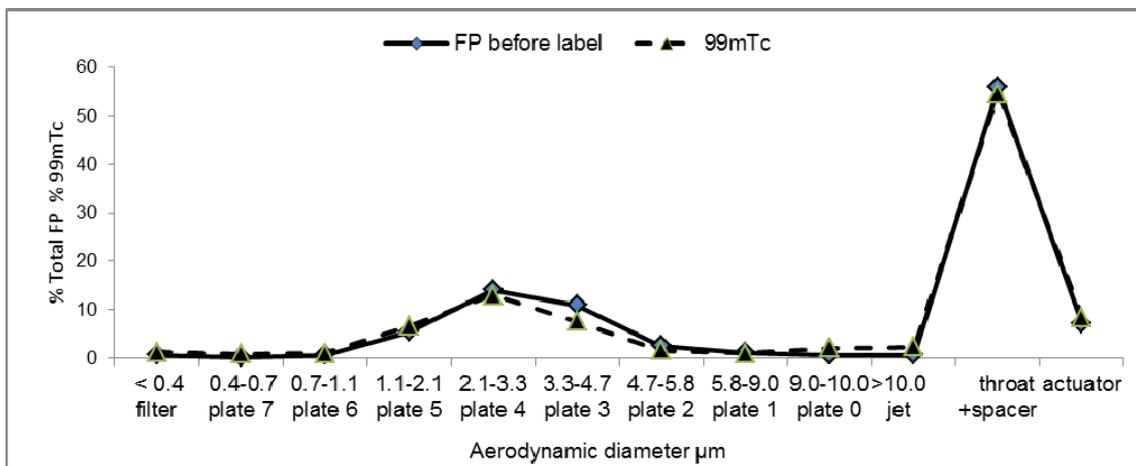


Figure 7-1: Validation between commercial drug (FP) before radiolabelling, FP ‘before label’ and ^{99m}Tc using ACI with pMDI-Funhaler (n=2). The y-axis shows the % total drug (FP) or % ^{99m}Tc and the x-axis shows the aerodynamic particle size (μm).

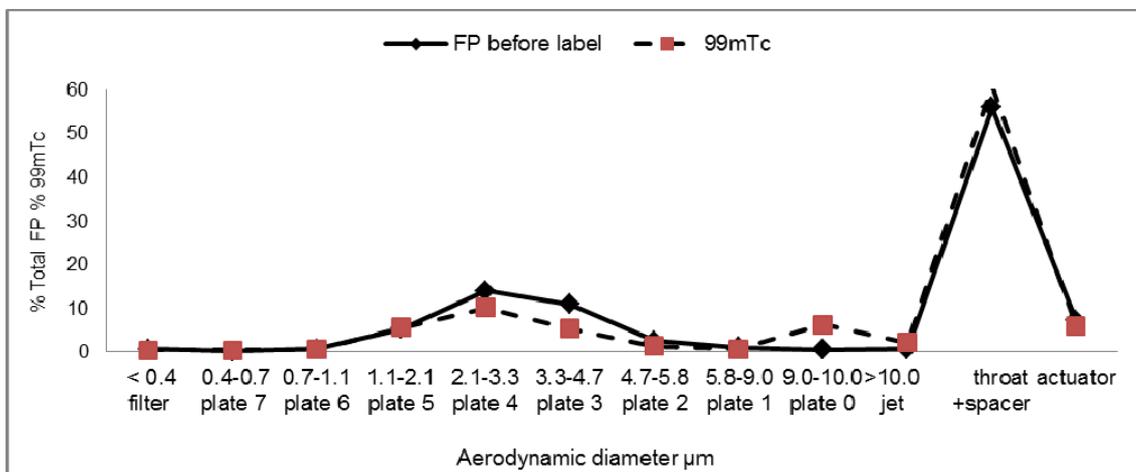


Figure 7-2: Failed validation of commercial drug (FP) before radiolabelling, FP ‘before label’ and ^{99m}Tc using ACI with pMDI-Funhaler (n=2). The y-axis shows the % total drug (FP) or % ^{99m}Tc and the x-axis shows the aerodynamic particle size (μm).

7.5.2 Funhaler data summary

The age, lung function (FEV1), inspiratory parameters of the first inhaled breath, total body deposition and lung deposition, % drug exiting spacer-FVS-NGI and % drug

particles in the extrafine particle fraction are summarised in Table 7-2. The children in the Funhaler group were compared to children aged < 10 years in the Aerochamber Plus™ group, previously investigated in Chapter 6, in section 7.5.4. Results from a subgroup of children aged < 8 years, were also reviewed in section 7.5.5.

Table 7-2: Age, respiratory parameters, total body deposition (TBD) with gamma scintigraphy, % drug (FP) exiting spacer (Funhaler) attached to FVS-NGI, lung deposition (% ex-actuator) and the % ultrafine particle fraction (UFPF).

Subject	Age yrs	FVC	Volume mL	PIF L/min	Time s	Gamma (TBD)	FVS - NGI	Lung	UFPF
1	6.2	1.66	685	81	0.7	43.4	51.2	16.2	21.3
2	8.0	2.10	540	84	1.0	38.4	58.3	19.7	18.5
3	5.6	1.07	170	36	0.6	39.4	37.2	17.8	15.4
4	6.5	1.39	320	32	1.3	43.5	50.7	14.7	24.8
5	9.0	1.70	745	55	1.1	37.0	47.5	18.8	21.0
6	6.9	1.63	565	56	0.9	43.4	56.8	21.9	25.0
7	8.9	2.10	130	15	0.7	27.6	49.2	16.2	25.5
8	6.6	1.46	480	43	1.4	18.0	49.3	10.6	23.3
9	9.0	2.14	800	51	1.5	36.7	50.1	18.0	22.7
10	7.1	2.07	345	25	1.5	28.2	51.2	15.8	22.1

The mean (SD) lung deposition was 17.0 (3.1) and mean (SD) % ultrafine particle fraction (UFPF, particles < 2.2 µm) exiting spacer was 22.0 (3.1) and these were significantly different (p=0.010), however the variability was similar with CV 18% with FH and CV 14% with the % UFPF exiting the spacer-FVS-NGI. There was not a significant correlation between lung deposition and % UFPF exiting the spacer (p=0.560) or % EFPF (particles < 3.4µm), (p=0.775). Similarly there was not a significant correlation between lung deposition and the UF-EF algorithm (p=0.507).

However there was a significant correlation between the UFPF (particles < 2.2 µm) and the *in vivo* peripheral lung deposition (P:C ratio) with $r=0.634$, $r^2=0.401$ and $p=0.049$.

7.5.3 Gamma deposition, F2-FVS and FVS-NGI

With Funhaler, mean (SD) % gamma deposition was 35.6 (8.4); mean (SD) % drug exiting spacer-FVS-NGI was 48.1 (6.0) and these were significantly different ($p=0.004$). However, mean % (SD) filter 2-FVS was 40.7 (7.6) and this was not significantly different to gamma ($p=0.248$). There was a significant negative correlation for spacer retention with lung and oropharyngeal and gastrointestinal (OG) deposition ($r=-0.713$, $r^2=0.508$, $p=0.021$; $r=-0.923$, $r^2=0.851$, $p=0.000$ respectively).

7.5.4 Comparison Funhaler with Aerochamber plus™

The *in vitro* commercial Flixotide® canister weight used with FVS-NGI was significantly less for FH-spacer compared to AC+ ($p=0.026$). There was a significant increase in the % UFPF and % EFPF measured with Funhaler and FVS-NGI compared to AC+ with FVS-NGI ($p=0.021$ and $p=0.021$ respectively). There was a significantly increased radiolabelled canister weight used for gamma scintigraphy with Funhaler (FH) compared to FH with FVS-NGI ($p=0.039$). There was not a significant difference between AC+ and FH with regards to lung deposition, OG deposition or spacer retention ($p=0.494$, $p=0.619$, $p=0.993$), previously described in Chapter 5, section 5.5.6 Table 5-13. However the variability in lung deposition, given by CV%, decreased markedly from 54% with AC+ to 18 % with FH, Chapter 5, section 5.5.6, Figure 5-9.

With children aged < 10 years, the mean (SD) % drug (FP) exiting spacer-FVS-NGI with Funhaler, 48.1 (6.0), CV12.5%, was significantly more than AC+, 39.6 (3.9) CV 9.8%, ($p=0.001$). There was not a significant difference between FH and AC+ with regards to the volume of inhalation, PIF or time of inhalation ($p=0.637$, $p=0.997$ and

p=0.972), as shown in Table 7-3. In contrast to pMDI-spacer (AC+), lung deposition *via* pMDI-Funhaler was not significantly increased when time of inhalation was > 1 s (p=0.436). With Funhaler, the mean OG (SD) deposition increased from 16.7 (5.5) with PIF < 60 L/min (n=8) to 25.1 (2.3) with PIF > 60 L/min (n=2), although the difference was not significant (p=0.076) and the results should be viewed with caution, as there were only two subjects in the latter group. Spacer retention had the most significant correlation with OG deposition, as shown in Figure 7-3 ($r=-0.923$, $r^2=0.852$, $p=0.000$).

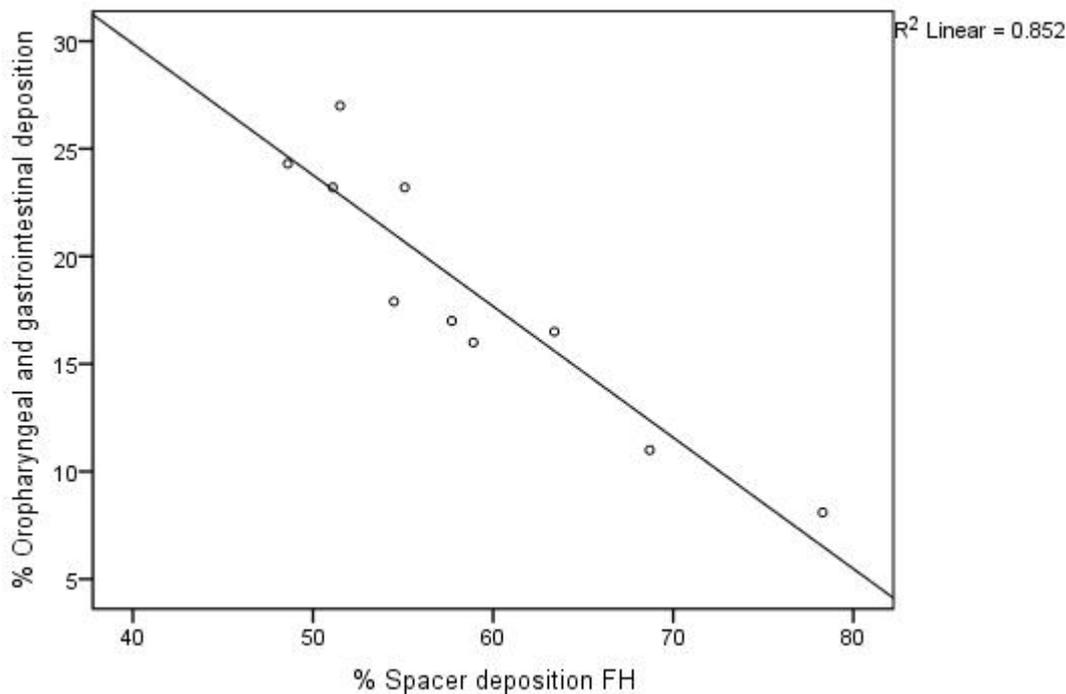


Figure 7-3: Relationship between the oropharyngeal and gastrointestinal deposition drug (FP) on the y-axis and spacer deposition with Funhaler on the x-axis.

PIF with Funhaler did not correlate significantly with OG deposition (p=0.062). PIF correlated significantly with volume of inhalation ($r=0.726$, $r^2=0.527$, $p=0.018$). Linear regression with factors PIF and volume showed that PIF was not predictive of OG deposition ($R=0.649$, $R^2=0.421$, $p=0.083$).

Table 7-3: Inspiratory parameters of the 1st inhaled breath for FH and AC+ with children < 10 years and children < 8 years and comparison with % lung deposition (ex-actuator), % OG deposition (ex-actuator) and the % UFPF and % EFPP.

Mean < 10 years	FH (n=10)	AC+ (n=10)	t-test
Volume mL	478 ± 233	544 ± 363	p= 0.637
PIF L/min	48.2 ± 22.7	48.2 ± 24.4	p=0.997
Time s	1.1 ± 0.3	1.1 ± 0.4	p=0.972
% Lung	17.0 ± 3.1	19.4 ± 10.4	p=0.472
% OG	18.4 ± 6.0	20.1 ± 8.4	p=0.678
% Spacer	58.8 ± 9.1	58.7 ± 15.5	p=0.993
UFPF-2.2 µm	22.0 ± 3.1	18.7 ± 2.6	p=0.021
EFPP-3.4 µm	31.2 ± 4.3	26.9 ± 3.0	p=0.021
UF-EF	24.7 ± 4.7	23.6 ± 5.2	p=0.639
Mean < 8 years	FH (n=6)	AC+ (n=6)	t-test
Volume mL	428 ± 186	377 ± 356	p= 0.763
PIF L/min	45.5 ± 20.6	39.2 ± 23.4	p=0.629
Time s	1.1 ± 0.4	0.9 ± 0.2	p=0.355
% Lung	16.2 ± 3.7	15.8 ± 7.8	p=0.909
% OG	19.5 ± 6.8	17.5 ± 9.2	p=0.676
% Spacer	57.9 ± 11.2	65.4 ± 14.9	p=0.347
UFPF-2.2 µm	22.0 ± 3.5	17.5 ± 1.4	p=0.016
EFPP-3.4 µm	31.3 ± 5.2	25.7 ± 1.5	p=0.029
UF-EF	22.0 ± 3.5	21.05 ± 5.3	p=0.866

With Funhaler, the mean (SD) volume of inhalation was 388 (279) with time of inhalation < 1 s (n=4). Volume of inhalation increased to mean (SD) of 538 (200) with time of inhalation > 1 s (n=6), however this increase was not significantly different (p=0.345). In contrast, the the mean (SD) volume of inhalation, 305 (221) with

Aerochamber Plus™ and time of inhalation < 1 s was significantly increased to 830 (217) with time of inhalation > 1s (p=0.004).

7.5.5 Children < 8 years

There were no significant differences in % ex-actuator lung deposition, OG deposition, or spacer retention for children aged 5-7 years, with Funhaler compared to AC+ (p=0.909, p=0.676, p=0.347 respectively), as previously shown in Chapter 5, section 5.5.6. Similarly there were no significant differences in time of inhalation, volume of inhalation or PIF between Funhaler and AC+ in this age-group (p=0.763, p=0.629 and p=0.355 respectively), although breathing patterns with pMDI-spacer (FH) tended to be more regular than pMDI-spacer (AC+), as demonstrated in Figure 7-4.

Note that the waveform for Funhaler in Figure 7-4 shows a match between the inhalation and exhalation phase whereas the Aerochamber Plus™ pattern shows a mismatch between the inhalation and exhalation phase.

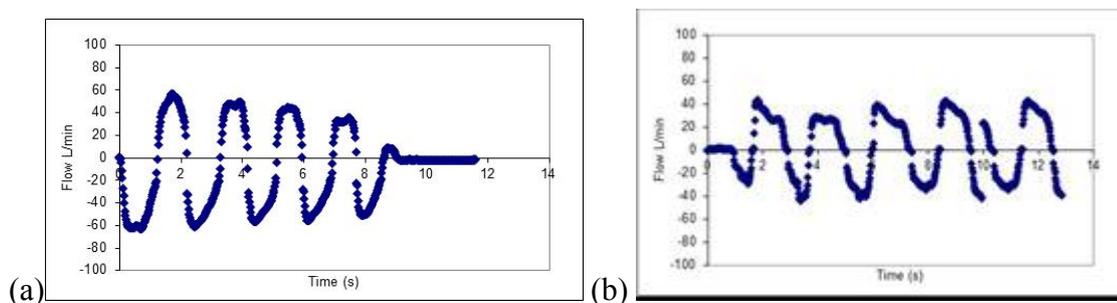


Figure 7-4: Representative waveforms (a) show regular breathing patterns with pMD-spacer (FH) and (b) irregular waveform with AC+. The y-axis shows flow in L/min; negative flow represents inhalation and positive flow represents exhalation. The x-axis shows time in seconds .

The volume of inhalation correlated significantly with PIF ($r=0.872$, $r^2=0.760$, $p=0.000$). The variability in the volume of inhalation was high with AC+, with CV% value of approximately 90 % with AC+ compared to 40 % with FH in this age-group.

Regression analysis (stepwise) with factors age, weight, height, time of inhalation, volume of inhalation and PIF showed that volume of inhalation was a significant predictor of lung deposition ($R=0.648$, $R^2=0.420$, $p=0.023$) in the tidal breathing children aged < 8 years.

7.5.6 Combined tidal breathing children (FH and AC+)

When the tidal breathing groups (pMDI-AC+ and pMDI-Funhaler) were pooled ($n=22$), mean (SD) lung deposition was 15.3 (5.5) with time of inhalation < 1 s ($n=12$) was significantly increased to 22.2 (8.9) with time of inhalation > 1 s ($n=10$), ($p=0.045$). Similarly there was significantly higher volume inhaled in the first breath with tidal breathing when the time of inhalation was > 1 s ($p=0.020$). The 95% confidence limits for the volume of inhalation in tidal breathing children are shown in Figure 7-5. The mean volume of inhalation was 338 mL (235) with time of inhalation < 1 s and 816 mL (557) with time of inhalation > 1 s in the combined group of children using tidal breathing.

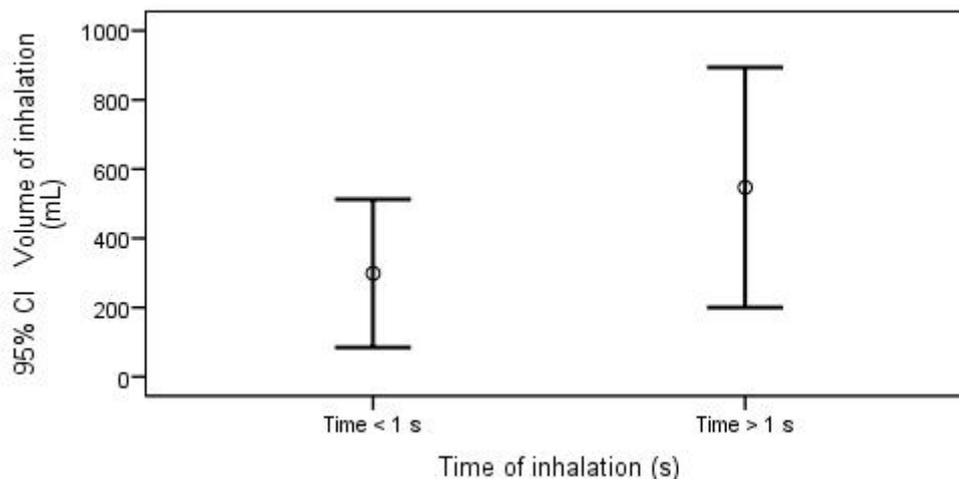


Figure 7-5: Error chart showing 95% confidence intervals for volume of inhalation of 1st inhaled breath with tidal breathing (AC+ and FH) in children aged < 10 years.

Spacer retention decreased significantly from 64.4 (11.6) with time < 1 s to 52.8 (11.2) with time of inhalation > 1 s ($p=0.028$). Mean (SD) OG deposition increased from 15.9 (5.3) with PIF < 60 L/min ($n=14$) to 25.4 (4.9) with PIF > 60 L/min ($n=8$) and this represented a highly significant increase ($p=0.000$). With the combined groups of tidal breathing children there was a significant correlation between lung deposition and the UF-EF algorithm, with % UF-EF exiting spacer-FVS-NGI ($r=0.543$, $r^2=0.295$ and $p=0.011$). Linear regression showed that the UF-EF algorithm was predictive of lung deposition with tidal breathing, after adjusting for spacer type ($R=0.594$, adjusted $R^2=0.281$ and $p=0.008$).

7.5.7 All children

When all data were pooled and the 'breath hold' group was included, regression analysis (stepwise) with factors age, weight, height, volume of inhalation, time of inhalation, PIF, spacer type and breath type showed that volume of inhalation was a significant predictor of lung deposition ($R=0.502$, $R^2=0.252$, $p=0.002$). Linear regression showed that PIF was a strong predictor of OG deposition, after adjusting for age and FVC ($R=0.675$, $R^2=0.456$ and $p=0.000$). Regression analysis with factors age, gender, weight, height, breath type, spacer type, time, volume and PIF showed that PIF was also a predictor of spacer retention ($R=0.539$, $R^2=0.291$, $p=0.001$). Spacer retention was significantly increased when PIF was < 60 L/min ($p=0.008$) and OG deposition was significantly reduced when PIF < 60 L/min. Lung deposition increased with PIF > 60 L/min, however the increase was not significant ($p=0.088$).

With the combined groups of children who had FVS-NGI measurements ($n=32$), there was a highly significant correlation between lung deposition and the UF-EF algorithm, with the % UF-EF exiting spacer-FVS-NGI ($r=0.665$, $r^2=0.443$, $p=0.000$), as shown in

Figure 7-6. Linear regression with age, FVC, volume, PIF and time of inhalation showed that % UF-EF was predictive of lung deposition ($R=0.717$, $R^2=0.513$, $p=0.001$).

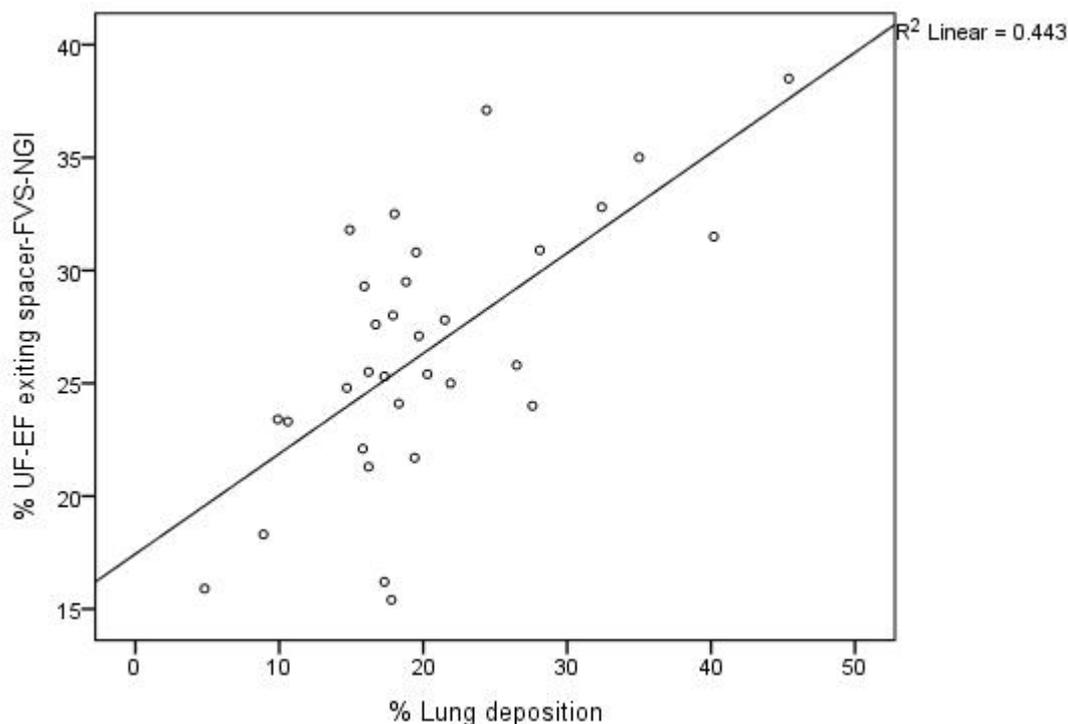


Figure 7-6: The y-axis shows the % UF-EF drug (FP) exiting the spacer attached to FVS-NGI (n=32) and the x-axis shows lung deposition (% ex-actuator).

7.6 DISCUSSION

Adherence to inhaled therapy remains a key issue in the treatment of asthma. Incentive spacer devices, such as Funhaler, have been designed as a strategy to improve adherence to asthma treatment in young children. Chapter 5 summarized the deposition study with gamma scintigraphy in children less than 10 years of age, inhaling ^{99m}Tc -HFA-FP with the Funhaler spacer. Prior to gamma scintigraphy, the Funhaler group of children had their breathing patterns recorded. After gamma scintigraphy the recorded breathing patterns were transformed into waveforms and replayed on the Flow-Volume Simulator (FVS).

The experimental work summarized in this chapter was carried out to investigate whether the *in vivo* results from gamma scintigraphy with the Funhaler spacer device could be matched by *in vitro* FVS-NGI, as in Chapter 6 with the Aerochamber Plus™. Children using the incentive spacer device tended to use a different breathing pattern during the recording of their breathing pattern, before gamma scintigraphy as they were learning to use the incentive spacer device. Therefore, the Funhaler data were not included in the analysis of FVS-NGI in Chapter 6.

The Funhaler group showed no correlation between *in vivo* lung deposition with gamma scintigraphy and the *in vitro* UFPF or EFPF exiting the spacer-FVS-NGI or the UF-EF algorithm. Therefore these results did not support the hypothesis that *in vitro* FVS-NGI could be used to reflect total body deposition with gamma scintigraphy, with Flixotide® delivered *via* pMDI-Funhaler. Inconsistencies with the canister weight may have contributed to differences between total body deposition measured with gamma scintigraphy and drug output measured with FVS-NGI. However there was a significant correlation between the drug (FP) measured on filter 2 attached to the FVS (F2-FVS) and total body deposition with gamma scintigraphy. Similarly there was a significant correlation between the ultrafine particle fraction (particles < 2.2 µm (UFPF) and the *in vivo* peripheral lung deposition (P:C ratio).

With Funhaler there was an increase in OG deposition with increasing PIF and volume of inhalation correlated with PIF, supporting the hypothesis. However there was not a significant increase in lung deposition with time of inhalation > 1 s. The volume of inhalation of the first inhaled breath was not significantly different with Funhaler, when time of inhalation was < 1 s compared with time of inhalation > 1 s. In contrast, the time of inhalation was significantly predictive of lung deposition with tidal breathing group aged < 10 years using Aerochamber Plus™ (p=0.007) and the UF-EF algorithm was predictive of lung deposition (p=0.009). This suggests that a sufficient volume of

drug (FP) particles was inhaled in the first breath with the Funhaler spacer device and this may be due to the ‘incentive’ for children to hear the whistle.

The variability in the volume of inhalation was much higher with Aerochamber Plus™, than with Funhaler. These findings suggest that the breathing pattern was more regular with the Funhaler device. This is consistent with the results of gamma scintigraphy in Chapter 5, which demonstrated markedly reduced variability in lung deposition with Funhaler (CV, 18%) compared to AC+ (CV, 54%). Janssens *et al* used simulated breathing patterns for sleeping infants to demonstrate that a more regular breathing pattern, with less variability in tidal volume and respiratory rate, showed increased deposition of budesonide in an *in vitro* model. [498]

Schultz *et al* reported that two tidal breaths were adequate for effective drug delivery *via* small volume spacer for children 2-7 years.[426] The study in this chapter looked at the relationship of drug output with the first inhaled breath and this may have been a limiting factor with regards to the finding that the volume of inhalation was equivalent for AC+ and FH. With the combined group of children using tidal breathing (AC+ and FH), lung deposition increased significantly with time of inhalation > 1 s. Similarly volume of inhalation was significantly increased with time of inhalation > 1 s. As expected OG deposition increased with PIF > 60 L/min, with a corresponding decrease in spacer retention.

The novel UF-EF algorithm was significantly predictive of lung deposition (p=0.008) for children using tidal breathing (AC+ and FH). Furthermore when all data were pooled the volume of the first inhaled breath and the UF-EF algorithm were strongly predictive of lung deposition (p=0.002, p=0.000), confirming the hypothesis. This indicates that the range of the ultrafine and extrafine particle fraction exiting the spacer

attached to FVS-NGI, based on individual inspiratory parameters of time of inhalation and volume of inhalation, was predictive of lung deposition.

Young children aged < 8 years may be encouraged to inhale their medication with an incentive spacer as an aid for learning the correct inhalation technique with pMDI-spacer. While the impact of the incentive spacer on adherence was short-lived in the study by Burgess *et al*, the incentive spacer may teach children to take a slow, deep inhalation.[189] An incentive spacer device that encourages regular breathing patterns would be advantageous in this age-group. Schultz *et al* reported that the Funhaler improved spacer technique in a randomised controlled trial with 132 children aged 2-6 years.[507] Al-Showair *et al* reported that good inhalation technique for pMDI can be achieved in adults by using a 'two-tone' trainer, which sounds one 'tone' when the flow is optimal and time of inhalation is 5 s and another 'tone' when the flow is too high.[508] In this chapter volume of inhalation of the first inhaled breath was significantly related to lung deposition in children aged 5-10 years. Children need to be trained to inhale slowly, whether using tidal breathing or a single maximal inhalation with 'breath hold'. More effective drug delivery may be possible with incentive spacer devices, specifically designed to improve the inhalation technique, with improved adherence and compliance in young children.

8 GENERAL DISCUSSION

8.1 INTRODUCTION

Current asthma management guidelines recommend the prevention of asthma symptoms as the key goal to maintain best lung function and prevent irreversible remodeling of the airway wall as a consequence of chronic inflammation. Randomised clinical trials have produced evidence to guide clinicians in how to manage children with asthma.[126]

However despite the increase in our knowledge of asthma, morbidity and mortality continue to be the end result of poor asthma management. Children with mild persistent asthma rarely see their doctor and mild asthma is generally undertreated.[509] With inadequate control of their symptoms, asthmatic children frequently require hospitalization.[510]

Strategies that optimize drug delivery to the airways remain important areas of research in childhood asthma management. Approximately 80% of asthma cases are diagnosed by age 6 and children represent the most difficult to treat age-group. Optimizing drug delivery to children can lead to more appropriate choice of drug delivery devices and therefore improved compliance and adherence as well as more effective use of asthma medications.

Inhaled corticosteroids (ICS) have become indispensable for the treatment of childhood asthma and play a key role in modifying the clinical expression of asthma. Research into the role of inflammatory mechanisms in the pathogenesis of asthma has led to the implementation of novel and specific drugs and delivery devices designed to improve adherence and optimise treatment. Significant progress has been made toward improving the therapeutic index of inhaled corticosteroids by balancing the safety and efficacy of new drug formulations with lung targeting strategies, aimed at reducing

dosage regimens and thereby minimizing unwanted side-effects. However there are several unresolved issues regarding the effective delivery of ICS to children. The key questions investigated in the studies in this thesis were:

- What is the optimal particle size for efficient delivery of ICS to the airways of children with asthma?
- What is the optimal inhalation technique for children using pMDI with an attached small volume spacer?
- Can *in vivo* methods of gamma scintigraphy, used for assessing the delivery of ICS, be reproduced *in vitro*?

8.1.1 *In vitro* estimations of lung deposition

Optimizing lung deposition of ICS is an important goal in the paediatric population in order to improve clinical effect and reduce side-effects. Comparisons of the effectiveness of different drug delivery devices is frequently made using *in vitro* measures, before using gamma scintigraphy. However, the relationship between different *in vitro* measures and *in vivo* gamma scintigraphy has not been clearly established.

Particle size distribution of delivered aerosols and the total mass of drug delivered from the inhaler are important determinants of pulmonary deposition and response to inhalation therapy. However, although *in vitro* drug output and fine particle fraction may be used to characterise a drug/device combination and estimate the expected total body deposition and lung deposition, there are conflicting definitions of the fine particle fraction in the literature and inertial sizing of aerosols has been shown to overestimate lung deposition and underestimate variability in drug delivery to children.

In Chapter 3, the *in vitro* cascade impaction measure of the mean % ex-actuator QVAR™ exiting the spacer (AC+) in the fine particle fraction was approximately 80%, whilst the *in vivo* measure of the mean % ex-actuator lung deposition with gamma scintigraphy was 35% for children aged 5-7 years using tidal breathing. If the *in vitro* fine particle fraction was used to predict the *in vivo* outcome for this drug/device combination then the dosage regimen for a child in the 5-7 year age-group could be reduced by more than 50% and this could have clinical relevance due to underdosing. Similarly, the predicted lung dose QVAR™ derived from cascade impaction, using the product of the emitted dose and the fine particle fraction, as reported by Farr *et al* was 63% ex-actuator and this overestimated lung deposition almost twofold for children aged 5-7 years.[295] Newman and Chan have suggested that the fraction of extrafine particles less than 3 µm is more predictive of lung deposition. The *in vitro* data in Chapter 3 showed that up to 68% QVAR™ was available as extrafine particles, whereas *in vitro* data in Chapter 5 showed that only 31% of drug particles were extrafine with HFA-FP.

With extrafine formulations the Newhouse measure for the ‘lung targetable fraction’, particles with an aerodynamic size < 2 µm, showed more consistency with lung deposition of QVAR™ for young children using tidal breathing, whereas with Flixotide®, the modified Finlay measure i.e. particles within the aerodynamic size range 1.1-4.7 µm, was more predictive of lung deposition, as there was minimal drug (FP) in the lower cut-off diameter particle size range, indicating that ultrafine particles do not play a significant role in drug (FP) delivery to the airways. There needs to be a standardised way to interpret *in vitro* impaction data with different formulations and different devices in order to make reliable predictions of lung deposition. The interpretation of the fine particle fraction needs to be tailored to the MMAD, the impaction device, the flow and the delivery device.

8.1.2 Inspiratory filter studies

Low resistance filters attached to delivery devices can be used to gain some preliminary clinically relevant information by estimating the expected total body deposition from inhaled aerosols. The inspiratory filter study is designed to estimate the total amount of drug that would be inhaled by children in a clinical setting. However, the filter dose does not show what proportion of the drug will deposit in the lungs compared to the mouth, throat and stomach or the exhaled dose.

In chapter 3, the inspiratory filter drug dose overestimated the total body deposition of QVAR™ compared with gamma scintigraphy, however the filter study was able to demonstrate that the single maximal inhalation with ‘breath hold’ (5-10 s) would provide significantly enhanced total drug deposition, compared with tidal breathing, for children 5-7 years. Furthermore both the filter study and gamma scintigraphy demonstrated that spacer retention of drug was increased with tidal breathing.

Similarly in Chapter 5, the inspiratory filter study overestimated total body deposition of Flixotide® compared with gamma scintigraphy. However, the filter study gave an indication that the ‘breath hold’ technique improved the delivery of Flixotide® *via* pMDI-spacer compared to tidal breathing. The filter study also gave an indication that there was less variability in dose with the Funhaler spacer device compared with Aerochamber Plus™ spacer.

Drug collected on inspiratory filters attached to breathing simulators can be performed *in vitro* with pre-recorded breathing patterns to compare different devices. Schultz *et al* reported that the *in vitro* simulated drug delivery approximates *in vivo* drug delivery onto an inspiratory filter.[439] Kamin *et al* used inspiratory filters attached to a breathing simulator to show that for young children, the time of inhalation, PIF and

volume of inhalation were important inspiratory factors for mass output of corticosteroids from different inhalation devices.[422, 527]

In Chapter 6 of this thesis, the FVS with an attached inspiratory filter showed that drug output was significantly correlated to total body deposition, as measured with gamma scintigraphy. These findings support the view that some valuable predictive information about the drug/device combination may be obtained from the inspiratory filter study. The filter studies described in Chapters 3, 5 and 6 have shown that inspiratory filter studies remain a valid focus of research investigations, providing comparisons of the efficiency of drug delivery from different devices.

8.1.3 *In vitro* FVS-NGI compared to gamma scintigraphy

Lung deposition studies provide key data documenting drug delivery *in vivo* for new and established products and lung deposition of ICS is closely linked to subsequent clinical effects. However, it is not possible to perform gamma scintigraphy for every new drug/device combination. Improvements to *in vitro* methods may be used to determine which optimal drug/device combinations may be candidates for clinical deposition studies.

Validation of a reliable *in vitro* method that is predictive of drug delivery *in vivo* may help to optimise dose consistency and minimise variability in the therapeutic dosing of ICS to children. Therefore many studies have focused on the development of *in vitro* methods, with ‘real-life’ breathing patterns, to predict drug deposition. Laboratory results may be matched to *in vivo* performance more realistically when breathing simulation is performed in tandem with standard *in vitro* impaction methods. This is a practical step that could lead to a flow-profiling for an individual child, providing closer *in vitro* and *in vivo* comparisons and the prediction of clinical outcomes for different drug/device combinations.[91]

In vitro data that can be measured objectively and evaluated as an indicator of *in vivo* performance, have to be reliable, measurable, specific, and predicative. In Chapter 6 of this thesis I have combined two different *in vitro* methods, FVS and NGI, in an attempt to show that with the suspension formulation, Flixotide®, patient-specific waveforms replayed on FVS-NGI could be used to estimate total body deposition and lung deposition *in vivo*.

With the subgroup of ten children who had their breathing patterns recorded while they simultaneously inhaled radiolabelled HFA-FP, there was a significant correlation between % gamma deposition and the % drug exiting the spacer attached to the FVS-NGI. Similarly there was a significant correlation between the fine particle fraction and the extrafine particle fraction of HFA-FP and lung deposition. The time of inhalation was predictive of gamma deposition and lung deposition. Both the extrafine particle mass and the extrafine particle fraction correlated significantly with lung deposition. When the results from 32 children were pooled, the volume of inhalation was shown to be predictive of lung deposition and PIF was predictive of OG deposition. These results were consistent with Schultz *et al* who reported that drug delivery (filter dose) through a pMDI-spacer attached to a Flow-Volume Simulator was related to inhalation volume and flow.[426] Similarly, Barry and O'Callaghan used sinusoidal breathing patterns to show that drug delivery from Aerochamber continued to increase at inhalation volumes > 200 mL.[404]

The results in Chapter 6 suggest that the application of a novel algorithm, using a variable cut-off particle size selection, depending on the volume of inhalation and time of inhalation, may be used to improve predictions of lung deposition with *in vitro* FVS-NGI. The novel UF-EF algorithm could lead to better characterisation of the fraction of fine particles available for lung deposition from different delivery devices.

8.1.4 Limitations of the study design

Certain aspects of the study design must be considered when interpreting the results from the studies carried out and described in this thesis. Small numbers are commonly used in gamma scintigraphic studies and gamma scans are not repeated on the same child using different devices and different formulations, which reduces the power of the study. There is still an incomplete understanding of the relationship between particle size and mass of drug delivered at the mouth and distal airway deposition and distribution, and these studies were not designed to specifically provide this information.[110]

Haughney *et al* reported that the fine particle mass of the emitted dose should be reported rather than fine particle fraction.[520] However, in order to compare the distribution of ^{99m}Tc , the % fine particle fraction was compared with the % fine particle fraction of delivered drug. Dolovich has reported that deposition studies should not be used in isolation and study protocols should incorporate clinical tests which provide parallel therapeutic data in response to inhalation of the drug by the different patient populations being studied.[75]

There were several methodological limitations in this thesis which may have affected the match between *in vitro* and *in vivo* data. For direct comparison *in vitro* doses used for particle size analysis should be the same as the number of doses used for gamma scintigraphy.[107] The breathing recordings were not all simultaneously recorded during inhalation of the radiolabelled drug and it can be argued that this may have altered both drug output and inspiratory parameters. Small sample sizes of 10 children with Funhaler and 12 children with Aerochamber PlusTM, were used to compare the two different spacer devices. The FVS-NGI was operated 'in-phase' with the start of the recorded first breath and this may not have coincided with the subject's actual time of

inhalation of ^{99m}Tc -HFA-FP *via* pMDI-spacer, prior to gamma scintigraphy. The volume of the small spacer devices may have been a limiting factor with regard to comparable doses of the suspension Flixotide® (HFA-FP) with ‘tidal’ and ‘breath hold’ inhalation techniques.[503]

There may be inaccuracies due to the dead space in the Y-piece of the FVS-NGI circuit and air turbulence associated with mixing bi-directional variable airflow from the replayed waveforms with the one-directional pressurized airflow in the Y-piece. A Copley ‘mixer’ has been designed specifically for this purpose, but this equipment was not available for the FVS-NGI measurements in Chapter 6 and 7. Flow-rate variability is an important source of measurement uncertainty with particle size distributions and inertial impaction.[365] The NGI is calibrated to operate with a constant flow with well-defined stage cut-offs, rather than in a circuit with the FVS and varying flows from different breathing patterns.[114] Dose inconsistencies may have been associated with the different pMDI canister weights of commercial Flixotide®.[146]

8.1.5 Effect of inhalation profile on delivery of ICS

Correct inhalation technique remains a major issue in achieving asthma control.[520] Strategies aimed at optimizing drug delivery to asthmatic children must involve not only the choice of an appropriate drug delivery device and formulation, but also an appropriate breathing technique. Optimization of the inhalation technique should improve lung deposition and therefore the clinical effect. Inspiratory parameters have been shown to affect lung deposition from nebulisers, dry-powder inhalers and pMDI. Therefore an important focus of research which leads to optimizing drug delivery to asthmatic children *via* pMDI-spacer is to consider the effect of the inhalation profile. Studies in this thesis have shown the effect of the inhalation technique on the delivery of QVAR™ and Flixotide® *in vivo* when children use pMDI with a small volume

spacer. Janssens *et al* used an upper airway model of an infant to show that drug delivery of extrafine QVAR™ was less dependent on the breathing pattern and this thesis supports the view that high lung deposition of QVAR™ occurs *via* pMDI-spacer (Aerochamber Plus™), regardless of inhalation technique.[417] Muller *et al* have also reported that with extrafine particles, the inhalation pattern has less of an effect on drug delivery.[464]

With potent ICS such as Flixotide® (HFA-FP) it is important to evaluate the effect of the inhalation technique on drug delivery before increasing inhaled corticosteroid dosage. With HFA-BDP and HFA-FP the ‘breath hold’ technique increased the P:C ratio and decreased variability and this may provide a clinically relevant advantage associated with both the improved peripheral deposition and dose consistency.

However, increased lung deposition of HFA-FP may lead to undesirable side-effects, without dose titration. Therefore in this thesis the inhalation profile was recorded for children inhaling the suspension HFA-formulation, Flixotide®.

Regular assessments of individual inspiratory parameters such as time of inhalation, volume of inhalation and peak inspiratory flow (PIF) may provide crucial evidence to support the choice of a particular drug delivery device.[424] Schultz *et al* used breathing simulation with an inspiratory filter study to show that most of the salbutamol inhaled *via* pMDI-spacer (Aerochamber Plus™) to children 2-7 years of age was delivered with the first two tidal breaths.[426] Kamin *et al* used tidal breathing patterns of toddlers and young children to show that approximately 60-80% of budesonide inhaled *via* pMDI-spacer (Aerochamber Plus™) can be delivered in the first inhaled breath.[422] Louca *et al* used breathing simulation with a standard waveform for a small child to investigate the delivery of HFA-BDP *via* pMDI-spacer. They suggested that most of the dose of HFA-BDP would be removed from pMDI with an attached small volume spacer after the first inspiration.[521] In Chapter 6 of this this thesis I

investigated the association of inspiratory parameters of the first inhaled breath with drug delivery of Flixotide® from pMDI-spacer to children 5-17 years.

The delivery of Flixotide® by pMDI with attached small volume spacer, Aerochamber Plus™, was shown to be dependent on the interaction of time of inhalation, volume of inhalation and the peak inspiratory flow, when children inhale *via* pMDI-spacer with tidal breathing. However I have shown that when children inhale *via* pMDI-spacer with the slow single maximal inhalation with ‘breath hold’, lung deposition of HFA-FP was independent of the inspiratory factors associated with the first inhaled breath. This is an important distinction, indicating that the time of inhalation and volume of inhalation were adequate with this inhalation technique.

There is significant variability in dosing associated with different breathing patterns. Poor inhaler technique with pMDI-spacer can significantly reduce drug delivery.[174] Failure to use delivery devices effectively has been associated with poor clinical response and limited patient adherence to therapy.[522] Several studies have reported that mis-timing of inhalation with actuation is the main error associated with pMDI.[148] [416] However despite these reports in the literature, the importance of timing of inhalation with actuation has not been emphasised with regards to small volume spacers.

Small volume spacers and holding chambers reduce the amount of available aerosol generated due to the impaction of the drug particles on their walls and the aerosol cloud will be retained for a shorter time in a small volume spacer, therefore mis-timing of actuation with inhalation may be more critical for effective drug delivery.[523] Poor coordination of actuation with inhalation, high PIF and low volume of inhalation interact to reduce the aerosol dose delivered to the lower airways, thereby reducing the effectiveness of therapy.[524] Al-Showair *et al* reported that the focus of inhalation

training should be to inhale at a slower rate and actuate with or just after the start of a slow inhalation.[508]

Inspiratory flows vary between patients and from dose to dose.[496] Lower tidal volumes and inspiratory flows are known to affect the efficacy of inhalation therapy, however little is known about the effect of inspiratory parameters on the regional deposition of inhaled drug. Al-Showair *et al* have reported that the slow inhalation technique can be improved with a training aid and the inspiratory flow should be less than 90 L/min with adults, however many patients inhale faster.[508]

In a study of adults, Laube *et al* reported that there was significantly less lung deposition of radiolabelled cromolyn sodium (CS) with fast inhalation (> 70 L/min) compared to slow inhalation (< 30 L/min). Similarly Newman *et al* reported less lung deposition and less peripheral penetration of radiolabelled CS with fast inhalation.[183] Tomlinson *et al* used a pharmacokinetic study to show that inhalation *via* pMDI at 20 L/min, over a 5 second time interval, improves urinary excretion of salbutamol, indicating higher lung deposition.[180] Kamin *et al* reported difficulty training children to inhale *via* pMDI with a constant flow of 40-90 L/min.[525] Thiel *et al* used an algebraic model for lung deposition and reported that MMAD, inhaled volume, inhalation rate and 'breath hold' have predictive value for lung deposition.[526]

Drug delivery with different inhalation techniques is device-dependent. Barry *et al* used breathing simulation to show that higher doses of budesonide were delivered *via* different pMDI-spacer with increasing tidal volumes.[404] Chapter 6 of this thesis has provided further evidence that with small volume spacers, time of inhalation, PIF and volume of inhalation of the first inhaled breath affect lung deposition because of their direct effect on oropharyngeal and gastrointestinal deposition and spacer retention of

drug particles. Furthermore, time and volume of inhalation were significant predictors of peripheral deposition.

In Chapter 6, a subset of ten children had their breathing pattern recorded while simultaneously inhaling ^{99m}Tc -HFA-FP *via* pMDI-spacer (Aerochamber PlusTM). Time of inhalation of the first inhaled breath correlated significantly with lung deposition. As expected the time of inhalation was significantly increased with the ‘breath hold’ technique compared with ‘tidal’ breathing ($p=0.036$) and this increased time of inhalation, as well as the 5-10 s ‘breath hold’, may have contributed to the improved peripheral deposition and reduced variability with the ‘breath hold’ technique.

An additional fourteen children using pMDI-Aerochamber PlusTM had their breathing pattern recorded prior to gamma scintigraphy. With the pooled data from twenty-four children, the volume of inhalation of the first inhaled breath was a strong predictor of lung deposition of HFA-FP and PIF was a strong predictor of both OG deposition and spacer retention. However the time of inhalation was significantly predictive of lung deposition for the tidal breathing group. These results concur with findings by Bennett *et al* who reported that the deposition fraction of 2 μm particles was most strongly predicted by tidal volume in children aged 6-13 years.[400] As expected the volume of inhalation increased with time of inhalation > 1 s. Inhalations of < 60 L/min have been shown to improve peripheral penetration.[451] For the children using pMDI-spacer with tidal breathing, the combined results for Aerochamber PlusTM and Funhaler, summarized in Chapter 7 of this thesis, showed that there was a significant increase with P:C ratio when PIF < 60 L/min ($p=0.045$). As expected the oropharyngeal and gastrointestinal deposition of HFA-FP increased with PIF > 60 L/min. This suggests that the slow, deep inhalation with tidal breathing will improve the laminar flow of particles into the peripheral airways and enhance gravitational sedimentation.

8.1.6 Validation of radiolabelled ICS

Gamma scintigraphy provides the best indication of lung deposition of ICS to the airways of asthmatic children, using different devices. However the acceptance of gamma scintigraphic results is based on validation experiments. It is essential to validate the ^{99m}Tc radiolabelling technique *in vitro*, before assessing delivery to the airways *in vivo*. Previous validation methods focused on drug delivery *via* pMDI, however the rigorous validation in Chapter 4 was carried out *via* pMDI and pMDI-spacer in order to reflect the clinical use of the inhaler. Furthermore, a post-patient validation series was performed to verify the integrity of the radiolabelling method throughout the duration of the clinical study. This additional validation step has not been previously reported in the literature.

Radiolabelling of Flixotide® (HFA-FP) had not been previously reported prior to the experimental work carried out in Chapter 4. I published a modified butanone extraction radiolabelling technique with HFA-FP delivered *via* pMDI and pMDI-spacer (Aerochamber Plus™). However, Chapter 4 highlighted the need for a standardized approach to the quality control of validation methods. There are still issues associated with producing radiolabelled ^{99m}Tc aerosols that are precise markers for the drug formulation being tested and in quantitating absolute doses deposited in the lung.[75]

In the discussion in Chapter 4, I noted that there was a consistent mis-match between drug and ^{99m}Tc radiolabel for plates 3-5, although the ratio between the fine particle fraction of ^{99m}Tc radiolabel and drug in the delivered dose was within acceptable current consensus limits used for the quality assurance of validation methods.[314] With FP 80% delivered dose settles on plates 3-5. However there was a higher proportion of ^{99m}Tc in the extrafine particle size range, plates 4-7, despite passing quality control,

which may have made a minor contribution to lung deposition values. These issues need to be addressed in standardising quality control measures between laboratories in future validation studies. Consistent ratios across different size bands may be better for quality control purposes.

8.1.7 Optimising lung deposition of ICS with different particle sizes

In this thesis I have used gamma scintigraphy to assess lung deposition and total body deposition of two ICS with different particle sizes, extrafine QVAR™ and Flixotide®, delivered *via* pMDI-spacer. Guidelines from the Global Initiative for Asthma (GINA) recommend a pMDI with a valved holding chamber (spacer) plus a mouth-piece for children 4-6 years of age; and a dry-powder inhaler (DPI), breath-actuated pMDI, or pMDI with a spacer for children aged 6 years or older. [44, 154]

The European Respiratory Society (ERS) and the International Society for Aerosols in Medicine (ISAM) task force have recently developed a consensus statement which highlights that correct inhaler use is essential for effective drug delivery.[155] An observational study of 364 children reported that 78% of children using a pMDI make at least one error when using their inhaler.[511, 512] Mis-use of inhalers is directly linked to decreased asthma stability.[148] The focus of this thesis has been optimizing the delivery of ICS *via* pMDI-spacer to children 5-17 years.

The clinical relevance of gamma scintigraphy is related to the fact that drug distribution patterns obtained from scintigraphic studies provide information on the effectiveness of aerosol delivery to the lungs and therefore provide an important guide to optimal delivery devices and dosage regimens.[513] Reports of gamma scintigraphic measures of total body deposition and regional deposition of QVAR™ (HFA-BDP) and Flixotide® (HFA-FP), inhaled by asthmatic children *via* pMDI-spacer, have not been previously published. Ciclesonide has a similar MMAD to QVAR™ and would be

expected to show a similar deposition pattern to QVAR™, as shown in Chapter 3.

Similarly new formulations of budesonide and mometasone have a similar MMAD to Flixotide® and lung deposition and regional deposition of these ICS would be expected to show similar deposition to Flixotide®, as shown in Chapter 5.[90, 442] Therefore the results of this thesis may be extrapolated to provide an indication of potential lung deposition and total body deposition for several ICS delivered to children, *via* pMDI with an attached small volume spacer.

Few studies have compared drug delivery of ICS from different spacers *in vivo* and the optimum spacer size for children remains unresolved. Drug delivery from different spacers can be highly variable.[514] Finlay *et al* reported that the mass of drug, inhaled in fine particles during pediatric tidal breathing from spacers, is dependent on the spacer type, the drug formulation used and the breathing pattern.[419] Drug delivery from high performing small volume spacers (with well-designed valves) has been reported to be comparable to large volume spacers *in vitro* and the small sized spacer devices may offer significant advantages to children with regards to compliance with therapy.[426, 454] Therefore, in this thesis I have used small volume spacers because of their ease of use for young children using a pMDI-spacer.

A Cochrane review by Lasserson *et al* observed that there is limited evidence of the deposition of extrafine formulation to children in the literature.[450] Aerosols with a small MMAD and a narrow GSD have been shown to improve lung deposition of ICS to infants and children.[116, 120, 150, 309, 347, 351, 355] The delivery of extrafine HFA-BDP delivered *via* pMDI with an attached small volume spacer (Aerochamber Plus™) to asthmatic children had not been previously reported in the literature using gamma scintigraphy prior to the work carried out in this thesis. I have published the deposition study in Chapter 3 and have reported the total body deposition and the

regional deposition of extrafine HFA-BDP delivered *via* pMDI-Aerochamber Plus™, with children using pMDI-spacer with two different inhalation techniques.

I have shown that the extrafine formulation shows an even, diffuse deposition throughout the airways of asthmatic children aged 5-17 years. The results of Chapter 3 confirmed the results of a previous pediatric deposition study by Devadason *et al*, that reported high lung deposition of extrafine HFA-BDP delivered with the breath-actuated pMDI device (Autohaler™).[150] However, oropharyngeal and gastrointestinal deposition *via* Autohaler™ ranged from 40-60% of the delivered dose. An important finding in Chapter 3 of this thesis was that the high lung deposition of HFA-BDP could be maintained in young asthmatic children using tidal breathing *via* pMDI-spacer, with oropharyngeal and gastrointestinal deposition from 10-25%, which is markedly reduced compared to Autohaler™, thereby reducing possible local and systemic side-effects during long-term use of QVAR™ and confirming the experimental hypothesis of Chapter 3.

Another important finding in Chapter 3 was that when children inhaled with a slow single maximal inhalation with ‘breath hold’ (5-10 s), high lung deposition (over 55% ex-actuator dose on average) of the extrafine formulation QVAR™ was independent of age, FEV1, FVC, height and weight and consistent to that shown in adults using the same formulation *via* pMDI.[112] [319] Furthermore there was less variability in lung deposition of QVAR™ across all ages, when children inhaled with a slow single maximal inhalation with ‘breath hold’, as shown by a low intersubject CV of 11%. The children using pMDI-spacer with tidal breathing showed greater variability in dosing with a coefficient of variation of 32%.

The QVAR™ study highlighted that the slow single maximal inhalation technique, can improve lung deposition almost twofold (range 51.5-64.8% ex-actuator) to children

5-7 years, when QVAR™ is delivered *via* pMDI-spacer (Aerochamber Plus™) compared with the previously reported Autohaler™ device (range 27.7-46.1% ex-actuator). There was also improved peripheral deposition of the extrafine formulation in this age-group, with this inhalation technique and this has important implications for optimising drug delivery of extrafine formulations. This study confirmed the hypothesis that the slow single maximal inhalation technique, followed by a 5-10 s ‘breath hold’, in combination with pMDI-Aerochamber Plus™, improves the delivery of QVAR™ to the peripheral airways of children. This inhalation technique has been recommended by the recent ERS and ISAM task force, for children 6 years or older. [155]

The children aged 5-7 years tended to have more oropharyngeal and gastrointestinal drug (HFA-BDP) deposition associated with tidal breathing. This observation may be due to reduced laminar flow of the drug particles and more deposition by inertial impaction in the oropharyngeal region. The improved laminar flow and enhanced gravitational sedimentation associated with a slow single maximal inhalation and ‘breath hold’, would be an advantage for younger children with smaller airways.

Children aged 8-17 years received similar levels of lung deposition of the extrafine QVAR™ formulation, whether using tidal breathing or a single maximal inhalation with a ‘breath hold’ for 5-10 s. These children also exhibited similar peripheral penetration of the extrafine drug into the airways with either breathing technique. However, there was less variability in dosing associated with the single maximal inhalation technique and this has important implications for consistency in dosing.

It has been suggested that high lung deposition, associated with increased absorption *via* alveolar deposition, may be associated with higher systemic effects and therefore an increased risk/benefit ratio.[444] Efficacy of QVAR™ (HFA-BDP) at half the dose of

CFC-BDP [446] means that with regular clinical review and titration of the dose, the improved therapeutic effect associated with targeting the airways could be maintained, while minimising the systemic dose from both lung and oropharyngeal and gastrointestinal deposition. Improved efficacy at a lower dose may result in equivalent control and fewer side-effects.[228] [271, 444, 515]

Gamma scintigraphy has demonstrated that the extrafine formulation results in an even diffuse distribution of QVAR™ throughout the lungs of adults and children [112] [73] and the increased peripheral deposition may be associated with improved asthma control.[5] Corticosteroid receptors are located throughout the airways [516] and inflammation extends to the alveoli.[5, 228, 229] Adcock *et al* used in-situ hybridization studies to show that the glucocorticosteroid receptor mRNA showed the highest concentration in the alveolar walls and vascular endothelium and smooth muscle.[516]

Computed tomography has been used to detect structural changes to the airways of infants and children.[517] Functional high resolution computed tomography (HRCT) imaging has shown that there is reduced air-trapping and improved efficacy when extrafine formulations reach the distal lung.[517] [248] Penetration of aerosol to the lung periphery may be reduced in asthmatic patients, because of the preferential deposition in central airways of the lungs.[339] This would indicate the need for more efficient delivery of ICS to the small airways in children with persistent asthma. New inhaled corticosteroid formulations with an extrafine particle size, such as ciclesonide, should offer both even, diffuse lung deposition, as can be obtained with QVAR™, as well as an improved safety profile.[518]

In Chapter 5, mean lung deposition of Flixotide® (HFA-FP) was approximately 20% i.e. approximately half that of extrafine QVAR™ observed in the scintigraphic study in

Chapter 3.[245] This finding supports the hypothesis that the coarser particle size of HFA-FP will lead to reduced lung deposition compared with extrafine QVAR™. A halving of the dose of HFA-FP is consistent with the increased particle size of HFA-FP and the more potent anti-inflammatory effect of fluticasone propionate compared with QVAR™, resulting in equivalent clinical efficacy with the two formulations recommended at the same dose levels.[473] The lower lung deposition of HFA-FP compared with QVAR™ is offset by the greater potency of FP.

The mean lung deposition (approximately 20% ex-actuator across all ages) of HFA-FP in asthmatic children using the small volume spacer (Aerochamber Plus™) in Chapter 5 was consistent with the mean systemic bioavailability (21%) that Thorsson *et al* found in an adult pharmacokinetic (PK) study with CFC-FP delivered *via* pMDI alone with an inhalation flow of 30 L/min.[519] In contrast, in an adult scintigraphic study with radiolabelled CFC-FP inhaled *via* pMDI alone, Leach *et al* found the mean % ex-actuator lung deposition was 13% with an inspiratory flow that was > 100 L/min.[485] However, with the HFA-FP formulation, an adult PK study by Nair *et al* found that the respirable dose of HFA-FP delivered *via* pMDI-spacer (Aerochamber Plus™) was significantly increased compared with pMDI alone.[455] Clearly the different methodologies, breathing patterns, formulations, propellants and delivery devices account for the variability in drug (FP) deposition in the literature.

The oropharyngeal and gastrointestinal (OG) deposition was not significantly different for Flixotide®, 17-27%, compared with QVAR™, 10-25%, regardless of the inhalation technique. This suggests that the small volume spacer had sufficient volume to retain the larger particles which would impact in the OG region, regardless of the particle size. These are important clinical findings in relation to drug/device selection and dose reproducibility in young children.

8.1.8 Future directions

The focus of managing asthma has undergone a paradigm shift from the concept of assessing severity to assessing control. There has been a shift in the efficacy of clinical practice with extrafine particles, and in this thesis, high lung deposition of the extrafine formulation was achieved regardless of the inhalation technique. In addition, variability decreased with the ‘breath hold’ technique. Furthermore, consistent lung deposition of Flixotide®, delivered *via* pMDI with an attached small volume spacer using the ‘breath hold’ technique, was demonstrated across all age-groups with reduced variability.

Just as monitoring non-invasive biomarkers of inflammation is an important adjunct in asthma therapy, monitoring inspiratory parameters with pMDI-spacer may be an invaluable adjunct in asthma management for children using small volume spacers. The coordination of dose release with inhalation, time of inhalation, volume of inhalation and the inspiration rate could be monitored regularly. Consistent dosing every time a child uses a pMDI-spacer device would ensure that any variability or change in clinical control is due to the child’s asthma and not erratic dosing.[528] Dose reproducibility contributes to optimized treatment of childhood asthma.[107] Furthermore, a measure of the inspiratory parameters combined with FVS-NGI may provide sensitive predictors of lung deposition. The novel algorithm introduced in Chapter 6 in this thesis is a possible new *in vitro* application which may provide more clinically meaningful predictions of the effectiveness of drug/device combinations. While promising, more thorough investigations with greater patient numbers would be required, in order to evaluate the applicability of this algorithm.

There remains uncertainty in the community regarding the effectiveness and suitability of different drugs and delivery devices for individual children. The potential benefits of

monitoring breathing patterns and patient inhalation technique with different devices may provide improved efficacy. Effectiveness of asthma treatment may be predicted with an evidence-based *in vitro* assessment tool such as FVS-NGI, if a database of inspiratory parameters is established as a guide to correct performance. This information may play a key role in determining which subgroups of children are at greatest risk for exacerbation due to poor performance with the device. Instruction given to the parents/caregivers can ensure correct performance.

By describing the relationship between *in vivo* gamma scintigraphy and *in vitro* FVS-NGI in Chapter 6, there may be improved *in vitro* interpretation of drug output with FVS-NGI, which combines two different techniques and provides the combination of breathing patterns and cascade impaction. Large multi-centre studies could be possible so that FVS-NGI could be used as a tool to predict lung deposition and the effect of different formulations and inhalers in different populations.

8.2 SUMMARY

The primary pharmacologic management of childhood asthma is with ICS. There are several modes of delivery and many different formulations. For many children, asthma exacerbations and unnecessary, adverse side-effects may be prevented by administering ICS *via* pMDI-spacer, monitoring the inhalation technique, using a small particle size formulation and keeping the total dose of ICS within safe limits.

On the basis of the findings of this thesis, I conclude that with small volume spacers, the ‘breath hold’ technique is more efficacious than tidal breathing for the delivery of both QVAR™ and Flixotide® to young asthmatic children. A slow deep inhalation followed by a ‘breath hold’ should be recommended for young children as soon as they are able to perform this inhalation technique. The extrafine formulation maximizes lung deposition and this may lead to minimal side-effects with dose titration. With the

suspension formulation, Flixotide®, consistent drug delivery was achievable with either breathing technique, however the ‘breath hold’ technique reduced variability. The incentive spacer device, Funhaler, offered an advantage to young children using pMDI-spacer with tidal breathing, as there was a marked reduction in the variability of Flixotide®. These are particularly important issue with regards to dosing consistency in young children and the Funhaler device may have a role as a training aid to improve spacer inhalation technique for children using tidal breathing.[507]

Furthermore the ‘take home message’ of this thesis is that the integration of *in vitro* impaction with ‘real-life’ *in vivo* breathing patterns may be a useful strategy that can improve clinical insight into how inhalation techniques and inspiratory parameters work together to improve drug delivery to asthmatic children. A greater understanding of the effect of individual breathing patterns in asthmatic children will increase our understanding of the drug/device interaction, and also provide guidelines for the development of therapeutic drug delivery strategies. Future clinical studies based on the FVS-NGI model in this thesis may allow clinicians to make more informed decisions regarding the relative efficiency of different devices for delivering ICS. Perhaps more importantly, these studies lay the groundwork for further investigations aimed at optimising inhaled drug delivery.

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