TITLE: *In vivo* gamma scintigraphy comparison of inhaled corticosteroid monotherapy delivered by pressurised metered dose inhaler with and without a spacer in adolescents with asthma

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This Thesis is presented in partial fulfilment of the requirements for the degree of Master of Child Health Research of the University of Western Australia.

2017
THESIS DECLARATION

I, Natalie Johnson, certify that:

This thesis has been substantially accomplished during enrolment in the degree.

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Written patient consent has been received and archived for the research involving patient data reported in this thesis.

The following approvals were obtained prior to commencing the relevant work described in this thesis: Ethics ID 2013105EP

The work described in this thesis was funded by [PMH (now PCH) Foundation].

Technical assistance was kindly provided by Joyce Wilson and Karen Hindley to perform the Nuclear Medicine scans described in 2.2.6.3.

This thesis does not contain work that I have published, nor work under review for publication.

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Spacer use is considered essential for children using inhaled corticosteroids (ICS) delivered via pressurised metered dose inhaler (pMDI) and recommended for all ages by the Asthma Foundation of Australia. Spacer recommendation is primarily to reduce oropharyngeal deposition and associated corticosteroid-induced side effects. Additionally, spacers minimise the effects of incorrect inhaler use, which commonly occurs and is associated with reduced medication compliance. However, anecdotal evidence indicates that spacers are seldom used as recommended, particularly by adolescents.

The recommendation for spacer use with a pMDI may not be necessary, with some asthma medications available in Australia generating fine and extrafine aerosols (fine= <2.5 μm mass median aerodynamic diameter (MMAD), extrafine= <2.0 μm). These aerosols result in less oropharyngeal deposition compared to coarse aerosols (>2.5–10 μm MMAD). Thus, the aims of this study were 1) to establish if spacers are required for use with a pMDI in adolescents and 2) investigate the effect of pMDI aerosol MMAD on lung distribution, both with and without a spacer.

To investigate these aims, this thesis evaluates in vivo lung deposition of two ICS pMDI produced aerosols, one coarse, one fine, each delivered either with or without a spacer, utilising a radiolabelling technique validated in vitro. Retrospective analysis to confirm the integrity of previously used radiolabelling methodology for the drugs (fluticasone propionate formulated as Flixotide® (coarse, 2.8 μm MMAD) and beclomethasone dipropionate (BDP) formulated as QVAR® (extrafine, 1.2 μm MMAD)), as used in this study, was also reported on.

We recruited fourteen adolescents aged 13–17 years (6 male, 8 female) with mild stable asthma for a randomised crossover study on the use of one of the two pMDIs mentioned above, with or without a spacer. On the study day, after validation of the radiolabelled drug was confirmed successful, radiolabelled drug was inhaled by the participant using a single maximal inhalation and breath-hold technique. Dose of drug deposited was quantified immediately with 2D gamma scintigraphy. Drug deposition was compared in adolescents inhaling the same
drug with and without a spacer, then further analysed with multivariate statistical modelling.

Univariate analysis showed no significant difference in total lung deposition with the coarse aerosol when a spacer was used, compared to pMDI use alone (p=0.31), or in the group inhaling the extrafine aerosol (p=0.52). There was a mean decrease of 32% in oropharyngeal deposition when using a coarse aerosol with a spacer, compared to a decrease of only 10% when using an extrafine aerosol. The mean ratio of peripheral to central lung deposition achieved with the extrafine aerosol was not significantly different with and without spacer use (p=0.82), and similar results were seen in users of the coarse aerosol (p=0.15), although a more peripheral deposition was seen visually with the extrafine aerosol. After multivariate analysis, deposition in the actuator (p=<0.0001), sex, age and BMI were significantly associated with lung deposition with the extrafine aerosol only.

Dependent on further investigation in a larger cohort we recommend that BDP as QVAR™ (MMAD 1.2 μm) may be used without a spacer in adolescents, assuming regular inhaler technique training (every 30 days) with an appropriately trained clinical professional. Spacer use should still be recommended for FP as Flixotide®.
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STATEMENT OF CANDIDATE CONTRIBUTION

The candidate performed all of the work described in this thesis, except where contributions of other individuals have been acknowledged below. Estimated percentages of contributions (where not 100%) are shown below.

Funding for the study was obtained from the PMH foundation by Associate Professor Devadason.

Recruitment of participants was completed, and consent obtained, by Ms Charlotte Allen

Validation of radiolabelled preparations, and preparation of the radiolabelled study drug each day was completed by the candidate, with assistance from Dr Ditcham (45%)

Participant technique training and clinical assessment was performed by Ms Charlotte Allen

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Participants were instructed how to inhale the study drug by Dr Ditcham (95%)

Gamma scintigraphy imaging was performed by Ms Joyce Wilson, Ms Karen Hindley, Ms Jill Summers, and Mr Simon Ferrero.

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Data analysis was carried out by the candidate with assistance from Ms Charley Budgeon at the Centre for Applied Statistics, University of Western Australia.

The candidate wrote all sections of the thesis, with critical feedback and editing provided by Associate Professor Devadason (35%) and Drs Trend (35%) and Ditcham (30%).

Critical feedback that contributed to the discussion and conclusion was given by examiners, Drs Fink, and Shah.
PUBLICATIONS ARISING FROM THIS THESIS

Publications in preparation

Does improved delivery of ICS monotherapy to adolescents with asthma still require spacer use?

Oral Presentations/Abstracts

Annual Rottnest Respiratory Symposium, New Investigator Award Session Nov 2016: Spacer use in asthmatic adolescents

Annual Scientific Meeting, Thoracic Society of Australia and New Zealand March 2017: The effect of particle size delivered by pressurised meter dose inhaler (pMDI) in asthmatic adolescents
DEFINITIONS

Adherence; adhering to the recommended regimen and use of the prescribed medication

Coarse aerosol; an aerosol containing a mass median aerodynamic particle size distribution of ≥2.5 μm–10 μm

Compliance; complying with the intended use of a device

Extrafine aerosol; an aerosol containing a mass median aerodynamic particle size distribution of ≤2 μm but not ≤ 0.1 μm

Fine aerosol; an aerosol containing a mass median aerodynamic particle size distribution of ≤2.5 μm but not ≤ 0.1 μm

Ultrafine aerosol; an aerosol containing a mass median aerodynamic particle size distribution of ≤0.1 μm
## ABBREVIATIONS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACI</td>
<td>Anderson cascade impactor</td>
</tr>
<tr>
<td>APSD</td>
<td>Aerodynamic particle size distribution</td>
</tr>
<tr>
<td>ARPANSA</td>
<td>Australian Radiation Protection and Nuclear Safety Agency</td>
</tr>
<tr>
<td>BDP</td>
<td>Beclometasone dipropionate</td>
</tr>
<tr>
<td>FP</td>
<td>Fluticasone propionate</td>
</tr>
<tr>
<td>LUT</td>
<td>Look up table</td>
</tr>
<tr>
<td>PBS</td>
<td>Pharmaceutical Benefits Scheme</td>
</tr>
<tr>
<td>pMDI</td>
<td>Pressurised metered dose inhaler</td>
</tr>
<tr>
<td>PMH</td>
<td>Princess Margaret Hospital</td>
</tr>
<tr>
<td>PSD</td>
<td>Particle size distribution</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>$^{99m}\text{Tc}$</td>
<td>Technetium$^{99m}$</td>
</tr>
<tr>
<td>TPA</td>
<td>Tetraphenylarsonium chloride</td>
</tr>
<tr>
<td>TGA</td>
<td>Therapeutic Goods Administration</td>
</tr>
<tr>
<td>WHO</td>
<td>The World Health Organisation</td>
</tr>
<tr>
<td>GINA</td>
<td>The Global Initiative for Asthma</td>
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### UNITS

<table>
<thead>
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<th>Unit</th>
<th>Description</th>
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<tbody>
<tr>
<td>LPM</td>
<td>Litres per minute</td>
</tr>
<tr>
<td>MBq</td>
<td>Megabecquerel</td>
</tr>
<tr>
<td>mSv</td>
<td>Millisievert</td>
</tr>
<tr>
<td>cps</td>
<td>Counts per second</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>s</td>
<td>seconds</td>
</tr>
<tr>
<td>w/v</td>
<td>weight for volume</td>
</tr>
<tr>
<td>FEV₁</td>
<td>one second forced expiratory volume</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
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1 CHAPTER I: INTRODUCTION

1.1 ASTHMA OVERVIEW

Asthma is defined as a single disease with variable phenotypes, characterised by chronic airway inflammation and respiratory symptoms which have multiple triggers and vary in severity and frequency (Program., 2017). The variable presentation of symptoms give rise to asthma being under or over diagnosed, although when appropriately diagnosed and treated, symptoms and severity can be reduced with medication in most individuals (Aaron et al., 2017; Boulet, FitzGerald, & Reddel, 2015; Rabe et al., 2004). According to prescription dispensing data, in Australia, asthma is over diagnosed, thus over treated in these individuals, and in those with correct diagnosis, undertreated due to lack of adherence to medication regime (AIHW: Correll PK, 2015). Asthma is estimated to contribute to one in every 250 deaths worldwide, but deaths resulting from exacerbation can be preventable in most individuals when treatment is adhered to (Masoli, Fabian, Holt, Beasley, & Global Initiative for Asthma, 2004). Higher levels of adherence to medication are associated with decreased risk of exacerbations, however medication adherence is generally low (Engelkes, Janssens, de Jongste, Sturkenboom, & Verhamme, 2015). Low adherence to medication leads to decreased asthma control levels, the latter which are already low and likely to be over-estimated (AIHW: Correll PK, 2015), so it is critical to find effective treatment that is easy to implement in practice.

The prevalence of asthma in Australia is high compared to international rates; one in ten adult Australians have asthma, and the direct health costs of asthma to the Australian economy were estimated to be AUD 1.2 billion in 2015 (Australian Centre for Asthma Monitoring, 2011; National Asthma Council Australia, 2016a). In 2015 there were 421 asthma-related deaths in Australia although the mortality rate has decreased over the last 10 years as has the prevalence of asthma in children and young adults (15–34), but remained stable in adults over 35 (Australian Centre for Asthma Monitoring, 2011). Childhood asthma is the leading non-communicable disease worldwide and prevalence in Australian children and adolescents (ages 5–17) still remains high compared to other age groups (Australian Centre for Asthma Monitoring, 2011; World Health
Organisation, 2013). In Australia, more males than females have asthma during childhood (ages 0–14), similar levels of both sexes experience asthma during adolescence, and from adulthood onwards more females than males have asthma (ages 25+) (Australian Centre for Asthma Monitoring, 2011).

1.2 DISEASE PROCESS AND PRESENTATION

The lack of consensus on a single, environmental or genetic, pathophysiological aetiology for asthma has led to reassessment of this condition as a syndrome (Lötvall et al., 2011; Program., 2017). Asthma disease phenotypes are important clinically, reduce complexity of diagnosis, and improve asthma management (Agache, Akdis, Jutel, & Virchow, 2012), however it is now understood that phenotypes are not directly related to disease process, and disease variants exist (Lötvall et al., 2011). Common clinically identifiable phenotypes include; allergic asthma, non-allergic asthma, late-onset asthma, asthma with fixed airflow limitation, and asthma with obesity (Program., 2017). It is estimated that approximately 50% of asthma cases involve the allergic asthma phenotype which is considered an early onset, or childhood, asthma, that can persist into adulthood (Haldar et al., 2008; Wenzel, 2012; Woodruff et al., 2009).

1.2.1 DISEASE PROCESS IN ALLERGIC ASTHMA

The T-helper type two (Th2) response (Figure 1-1) appropriately describes a proposed biological mechanism of the allergic asthma phenotype in adults and children (Anderson, 2008; Wenzel, 2012).

![Classical disease model of Th2 mediated asthma](figure1-1.png)

**Figure 1-1 Classical disease model of Th2 mediated asthma.** Aeroallergen incites Th2 cytokine response, causing inflammation, damage and airway remodelling, airways become hyperresponsive and broncho-constriction results. Picture retrieved from; (Anderson, 2008).
A typical immune response to aeroallergen results in Th2 specific cytokine production; IL-4, IL-5, and IL-13 (Grünig et al., 1998; Watanabe et al., 1997). These inflammatory cytokines induce various forms of inflammation; recruitment of eosinophils to the airways, B-cell class switching to produce IgE, and mucus production in airway epithelium via goblet cells. Damage, resulting from inflammation, leads to airway hyperresponsiveness and bronchoconstriction, and once established, airway hyperresponsiveness can be worsened by inflammation (Denham et al., 2008; Henderson et al., 2003; Winkler & Venegas, 2007). Long term inflammation can lead to airway remodelling, and thickening of the sub-epithelial basement membrane that contributes significantly to asthma pathophysiology (Brusselle, Kips, Joos, Bluethmann, & Pauwels, 1995; Dabbagh et al., 1999; Manetsch et al., 2012; Snapper, Finkelman, & Paul, 1988). Individuals with an allergic asthma phenotype, defined as having eosinophilic airway inflammation, usually with an early onset, respond well to inhaled corticosteroid (Lötvall et al., 2011; Program., 2017; Woodruff et al., 2009).

1.3 TREATMENT OF ASTHMA

Initial treatment recommendations for those not already using preventer treatment are determined by disease presentation. For children aged six and over, disease presentation is initially classed as: infrequent intermittent, frequent intermittent, or persistent asthma (mild, moderate or severe) (National Asthma Council Australia, 2016a; Van Asperen PP, Mellis CM, Sly PD, & C., 2010). These categories are defined by the pattern and intensity of the presenting symptoms. Persistent asthma is scaled by percent predicted FEV₁ score: \( \geq 80\% \) = mild, \( 60\% \) to \( < 80\% \) = moderate, and \( \leq 60\% \) = severe. It is estimated that 74% of young children have infrequent intermittent asthma, which does not require inhaled corticosteroid (ICS) use (Van Asperen PP et al., 2010). Initial treatment recommended for frequent intermittent and mild persistent asthma, is daily low dose ICS monotherapy, if no clinical response is seen after an initial four week trial of non-corticosteroid therapy. Moderate to severe asthma symptoms are initially recommended a low dose inhaled corticosteroid, to be reviewed after four weeks (Van Asperen PP et al., 2010).

Adolescents from 14–16 years onwards are recommended to be treated as adults for diagnosis and medical management purposes, with additional
consideration for confidentiality and psychosocial status (National Asthma Council Australia, 2016a). Acknowledged difficulties exist however, when investigating symptoms in adolescents due to their denial or overplay of symptoms (National Asthma Council Australia, 2016a). Additionally this population may be less likely to adhere to medication regimens (Dima et al., 2015) and special consideration in treatment and diagnosis must therefore be taken. A particular consideration for mental health issues, increased likelihood of risk-taking behaviours, cigarette smoking and/or exposure to second hand smoke is recommended in the diagnostic approach (National Asthma Council Australia, 2016a). Spirometry can be used to confirm a diagnosis even if asthma was experienced during childhood (National Asthma Council Australia, 2016a).

Adults presenting with asthma symptoms are prescribed medication according to symptom frequency (National Asthma Council Australia, 2016a). The initial treatment for those experiencing symptoms more than twice per month is to prescribe a short acting beta-2 agonist and regular, low dose, ICS monotherapy. Combination ICS/Long acting beta-2 agonists are optionally recommended for those additionally suffering persistent daytime symptoms. A regular high dose ICS monotherapy is recommended for very uncontrolled asthma (National Asthma Council Australia, 2016a).

1.4 MEDICATION FOR ASTHMA TREATMENT
The mainstay of treatment for asthma is drug therapy consisting of preventative medication to decrease the airway inflammation, and reliever medication to relieve airway constriction (National Asthma Council Australia, 2016a). Medication can be delivered by oral tablet, or as orally inhaled drug direct to the lungs, with dose and frequency adjusted according to individual symptoms and severity (National Asthma Council Australia, 2016a). Nasal inhalation therapy is not used for asthma although those also experiencing allergic rhinitis do receive some improvement of asthma specific outcomes (Lohia, Schlosser, & Soler, 2013).

1.4.1 RELIEVER MEDICATION
Reliever medication is given to reduce airway constriction, which causes wheezing and breathlessness during an asthma attack. Short acting beta-2
agonists are the main bronchodilators prescribed to treat this condition in Australia, on a taken as-needed basis (National Asthma Council Australia, 2016a).

1.4.1.1 Short acting beta 2 agonists
The most commonly recommended beta-2 agonists in Australia are salbutamol delivered by pMDI, suitable for all ages, and terbutaline delivered by breath actuated inhaler, suitable for all ages over six years. Terbutaline, due to the type of delivery device, requires a threshold acceleration for activation that is not possible for many small children to achieve (National Asthma Council Australia, 2016a).

1.4.1.2 Long acting beta 2 agonists
Long acting beta-2 agonists (LABA) are usually formulated in combination with inhaled corticosteroids and not recommended at all for treatment of asthma in children 0–5 years, where studies are absent (Program., 2017; Van Asperen PP et al., 2010). In adults and adolescents ICS/LABA combination is generally recommended as a step up treatment for those whose symptoms both persist despite daily low-dose ICSs, and are not due to lack of adherence or poor technique (Program., 2017). Certain combination LABAs have been shown effective in adults, but not in children, to reduce the risk of flare ups compared with inhaled corticosteroid treatment alone (Chauhan, Chartrand, Ni Chroinin, Milan, & Ducharme, 2015; Ducharme, Ni Chroinin, Greenstone, & Lasserson, 2010).

1.4.2 PREVENTER MEDICATION
There are two main types of preventer medications, non-corticosteroid therapy and corticosteroid therapy (CS), which can be oral, or inhaled (ICS). Preventer medication acts to target airway inflammation, and with regular, correct use, reduces the risk of exacerbations and hospitalisation.

1.4.2.1 Non-corticosteroid therapy
Non-steroid preventer therapy is more commonly used in children or can be used to prevent exercise-induced bronchoconstriction, although pre-dosing before exercise with a short acting beta-2 agonist is the preferred treatment (National Asthma Council Australia, 2016a). In children, non-steroidal therapy is initially prescribed for 2–4 weeks and if no clinical response is seen, it is
recommended to proceed to inhaled corticosteroid monotherapy (National Asthma Council Australia, 2016a; Van Asperen PP et al., 2010).

Anti-IgE monoclonal antibody therapy (Omalizumab, Xolair®) has only recently been made available by prescription in Australia for children aged 6–12 years (2016), although was previously available to adolescents and adults. It is recommended as an add-on therapy in those who have documented exacerbations despite daily high dose inhaled corticosteroid treatment, often those with moderate-severe persistent allergic asthma (AIHW: Correll PK, 2015).

1.4.2.2 Oral corticosteroids
Oral corticosteroids are currently used only in short courses (less than two weeks) for acute asthma management, as this duration is considered safe and effective, excluding children aged 0–5 years where efficacy has not been validated (National Asthma Council Australia, 2016a). Oral corticosteroid treatment with prednisolone is recommended in Australia for 5–10 day courses, for acute asthma flare-ups, usually after presentation at an emergency department (National Asthma Council Australia, 2016a). Due to the benefits of ICS, they largely replaced oral corticosteroids as a mainstream treatment in the 80’s (Petrisko, Skoner, & Skoner, 2008). Limitations of oral corticosteroids include greater systemic side effects, and higher doses required to be effective compared to ICS (Davies, Stampone, & O’Connor, 1998).

1.4.2.3 Inhaled corticosteroids
Inhaled corticosteroids (ICS) are the mainstay of asthma treatment in Australia and in 2013 they were dispensed to 6.9% of the population (AIHW: Correll PK, 2015) (National Asthma Council Australia, 2016a). If optimally administered, some orally inhaled therapies, usually those with a fine (<2.5 μm mass median aerodynamic diameter, MMAD) aerosol, can deliver a higher fraction of total drug dose directly to the lungs, and a correspondingly lower fraction to the oropharyngeal (C. L. Leach, Kuehl, Chand, & McDonald, 2015). ICSs therefore require a smaller therapeutic dose to be effective when compared to oral corticosteroids. Glucocorticoid receptors are present in almost every cell type in the body and so the targeted delivery of inhaled drugs to these receptors in lung epithelium reduces systemic side effects caused by non-specific exposure
(Barnes, 2004). Additionally when using a drug with a fine (<2.5 µm) MMAD, the theoretical assumption is a higher fraction of total dose is depositing in the peripheral airways (<2 µm diameter), the main site of airway inflammation in asthma (Hamid et al., 1997; Tsuda, Henry, & Butler, 2011).

Various corticosteroids drugs are available in Australia for inhalation and are prescribed either as monotherapy, or as combined therapy with LABA in more severe persistent cases. Equivalence has been shown therapeutically between drug variations, although sub-optimal delivery affects therapeutic efficacy, and side effects may differ between drugs (Chet Leach, Colice, & Luskin, 2009; Lipworth, 1999).

**LOCAL SIDE EFFECTS**

A recognised local side effect of ICS is oral thrush, which can be avoided through mouth rinsing immediately after administration. Hoarseness can be experienced in adults, dependent on device and formulation used, but rarely reported in children using a spacer and pMDI (National Asthma Council Australia, 2016a). Aerosol formulations with a pH under 5.5 pH, usually dry powders, can dissolve tooth enamel, particularly in children (National Asthma Council Australia, 2016a).

**SYSTEMIC SIDE EFFECTS**

Systemic side effects can occur due to gastrointestinal or lung absorption and are dose, treatment, and treatment duration dependent (Lipworth, 1999; Loke, Blanco, Thavarajah, & Wilson, 2015; National Asthma Council Australia, 2016a; Petrisko et al., 2008; Zhang, Prietsch, & Ducharme, 2014). If the duration of a low to medium daily dose ICS treatment continues for a year, growth can be reduced at a rate of 0.48 cm during that year, however growth suppression decreases in the subsequent years if treatment continues, and there is little effect on final adult height of children (Loke et al., 2015; Zhang et al., 2014). Upper dose limits in children for ICS in Australia are 500 μg for fluticasone propionate (FP), and 400 μg for beclomethasone di-propionate (BDP), much lower than the daily dose that causes marked adrenal suppression (750 μg FP, or 1500 μg BDP) (Lipworth, 1999; National Asthma Council Australia, 2016a; Program., 2017). Doses above these limits do not equate to increased
therapeutic effect, are not prescribed, and adhering to prescribed dose limits strongly encouraged, so adrenal suppression due to high daily dosing is unlikely to occur (National Asthma Council Australia, 2016a; Randell et al., 2003; Van Asperen PP et al., 2010).

To reduce potential side effects some investigations are focusing on increasing drug receptor site affinity and absorption time in the lung epithelium (Salter et al., 2007; Van Den Berge et al., 2010). An ideal delivery is where action is exerted in the peripheral airways <2 μm (target region) and excess drug does not move into the systemic circulation where side effects can potentiate (Hamid et al., 1997; Van Asperen PP et al., 2010).

**CORTICOSTEROID INSENSITIVITY**

There are small subsets of the asthmatic population, usually with severe asthma, who are corticosteroid insensitive (Adcock & Lane, 2003). Investigating the mechanism of action of corticosteroids has been a focus for this reason (Kobayashi, Mercado, Barnes, & Ito, 2011; Nair et al., 2017; Quante et al., 2008). The mechanism of action by which corticosteroids treat airway inflammation is not yet fully understood, although recent studies have elucidated some mechanisms. Corticosteroids act, in part, by decreasing transcription of inflammatory cytokines, including IL-4, IL-5 and IL-13 (Barnes, 1998). Furthermore, they suppress inflammation in airway smooth muscle, by up-regulation of an endogenous mitogen-activated protein kinase (MAPK) inhibitor, which contributes to the repression of IL-6 secretion (Manetsch et al., 2012; Quante et al., 2008). Glucocorticoid activation of Kruppel-like factor 15 represses airway smooth muscle hypertrophy (Sasse et al., 2017).

Severe asthma can be corticosteroid insensitive, which similarly manifests in Chronic Obstructive Pulmonary Disease and may be reflective of a different disease variant entirely (Barnes, 2013). Increasing evidence shows that true severe asthma is distinct in pathophysiological presentation to mild-moderate asthma, and most likely established in early life, remaining unchanged over time (Anderson, 2008; Phelan, Robertson, & Olinsky, 2002; Wenzel et al., 1999). Despite true severe asthma being pathologically different in presentation, mild to moderate asthma can deteriorate to present as severe when prescribed
treatment is not followed, and severe-untreated asthma also exists in some populations (Chung et al., 2014; Romagnoli et al., 2007).

1.5 ORALLY INHALED DRUG THERAPIES: ADVANTAGES AND DISADVANTAGES
Due to the lung site specificity and minimised side effects, delivery via oral inhalation is most common for both preventative and reliever medications. There are various delivery devices available, each with their benefits and limitations. Children under five years of age, who have trouble coordinating inhalation instructions, are recommended to use a facemask in addition to an inhaled therapy (National Asthma Council Australia, 2016a).

1.5.1 NEBULISERS
Nebulisers are devices that produce inhalable drug as a “wet” aerosol, administered via either the mouth or nose. The aerosol is commonly produced by a jet nebuliser, where drug solution is drawn through an orifice by a pressure drop, generated by a venturi and a compressor, to provide the driving airflow. Alternatively, pressure drop generation can be via a vibrating mesh nebuliser, where the aerosol is generated by the vibration of a thin flexible piece of metal perforated by many tiny holes, and driven by the vibration of a piezo-electric crystal. Most machines are either costly, in the case of vibrating mesh nebulisers, or noisy and time consuming to use, in the case of jet nebulisers. Additionally, those devices that deliver drug via the nose are often uncomfortable to administer, especially with young children.

Nebulisers are not recommended as an initial treatment for asthma in Australia, only to be considered if the child cannot be taught to use an inhaler, usually relevant to children under five years (National Asthma Council Australia, 2016a).

1.5.2 DRY POWDER INHALERS
Dry powder inhalers (DPIs) are devices that aerosolise particles, after actuation by a deep in-breath, and cannot be used by young children (under 5 years) or in those who cannot generate adequate inspiratory flow to de-aggregate the powder, activating the device (Federico Lavorini, 2013). They are recommended as a secondary option, after pMDI’s, for children over 6 years.
Similar to pMDIs, the lung deposition of a DPI depends on inhalation parameters and device design. DPIs exhibit similar issues to pMDIs, with oropharyngeal deposition although unlike a pMDI these cannot be avoided with addition of a spacer device and instead would require a slower inhalation flow for activation if to reduce the risk of oropharyngeal deposition (Federico Lavorini, 2013). Another disadvantage is that not all DPIs provide multiple dose capacity. Those that do are either designed as multiple dose reservoirs (i.e. Turbuhaler®) or as multiple dose units (i.e. Accuhaler™, Elliptor®). The former do not always provide consistency of dosage, and the latter do but are more expensive (Federico Lavorini, 2013).

1.5.3 BREATH ACTUATED INHALERS
Breath actuated inhalers require a threshold inspiratory flow to activate them, and therefore may not use be used optimally or consistently by young children, whom are instead prescribed pMDIs with spacers (National Asthma Council Australia, 2016a). They are recommended for patients who have difficulty using their hands to connect spacer devices to their pMDI (National Asthma Council Australia, 2016a). Breath actuated inhalers can be used with a spacer, however a much larger inspiratory flow must be used, to adequately draw airflow through the additional spacer device, and thus limits it general use and recommendation (National Asthma Council Australia, 2016a). Additionally, problems potentiate with non-autonomous operation; increased oropharyngeal deposition occurs if breathing ceases after device activation usually if the aerosol plume is distasteful or cold when hitting the back of the throat.

1.5.4 PRESSURISED METERED DOSE INHALERS
The most commonly used device to administer orally inhaled products is the pressurised metered dose inhaler (pMDI). It is cheap and portable, and reported to have equivalent or improved delivery to other available devices, factors that contribute to their higher prescription rate (AIHW: Correll PK, 2015; Brocklebank, Wright, & Cates, 2001; Labiris & Dolovich, 2003; F. Lavorini et al., 2011). PMDs aerosolise medication into carrier particles ranging in size between 1.1–10 μm in diameter. The contents of the pMDI consist of a drug in suspension or a solution, propellant, dissolved excipients, and a co-solvent (Stein, Sheth, Younis, Mogalian, & Myrdal, 2015). A pMDI's ability to target
therapy to the lung is dependent on formulation parameters, device design, clinical factors and particle size distribution (Stein et al., 2015).

1.5.4.1 PMDI device compliance

Coordinating timing of actuation with inhalation is required for correct administration of aerosolised drug from a pMDI. Inhaler misuse is demonstrated regardless of age, which compounds already low, adherence-related levels of asthma control, by sub-optimal use of prescribed devices (Brennan, Osman, Graham, Critchlow, & Everard, 2005; Desai & Oppenheimer, 2011; Levy, Hardwell, McKnight, & Holmes, 2013)(Sanchis, Gich, & Pedersen, 2016). Sub-optimal use may be avoided with repetitive training, focused at the stage of initial diagnosis, as compliance is highest after visiting a clinical professional and subsequently decreases (Haahtela et al., 2006; Kamps, Ewijk, Roorda, & Brand, 2000). For those who aren’t coordinated, particularly children under five, spacer use is recommended with a pMDI to minimise oropharyngeal deposition and associated side effects (National Asthma Council Australia, 2016a).

1.5.4.2 Spacer use

Due to the limitations of pMDIs, spacers are currently recommended in Australia as an accessory device for anyone prescribed ICS delivered by a pMDI, and for those who cannot coordinate, with any pMDI (National Asthma Council Australia, 2016a). The concept of using a pMDI with a spacer, was introduced in the 1950’s with the primary aim to minimise oropharyngeal deposition, a result achieved when larger particles impact in the spacer first rather than in the mouth or throat (Newman, Woodman, Clarke, & Sackner, 1986; Stein & Thiel, 2017; Toogood, Baskerville, Jennings, Lefcoe, & Johansson, 1984). Spacers are essential in groups who either cannot perform a breath hold considered to be adequate (>5 s), or cannot coordinate timing actuation with inhalation (National Asthma Council Australia, 2016a). These groups are usually young children who lack these skills due to developmental age, but can also be elderly or disabled patients (Brennan, Osman, Graham, Critchlow, & Everard, 2005). Spacers require cleaning between use with warm soapy water and drip-drying to avoid electrostatic build up (Wildhaber et al., 1996).
1.5.4.3 Spacer non-compliance
Spacer devices are bulky and time consuming to use, as opposed to a pMDI alone, and non-compliance with use is frequently reported, (Brennan et al., 2005) and observed at Princess Margaret hospital for Children. Even in studies where patients can demonstrate effective technique with a spacer, they can still use their device incorrectly (Brennan et al., 2005). If the subject perceives use as time-consuming or conspicuous, they may release multiple actuations into the spacer device before inhalation, to speed up the process (Barry & O'Callaghan, 1995). Others can delay inhalation in error. Both result in a decrease in respirable fraction available for inhalation (Barry & O'Callaghan, 1995; Nikander, Nicholls, Denyer, & Pritchard, 2014; Rubin & Fink, 2003). The inconvenience of the device, to use and transport, often results in disuse (Shim, 2000). The outcome when a spacer device is prescribed to those who do not comply with use, may be a decrease in asthma control, or reversion to pMDI use alone (Brennan et al., 2005). To address the reality and consequences of this common problem, pMDI drug formulations must be considered for use without a spacer.

1.5.4.4 Drug formulations available by pMDI
Chlorofluorocarbon (CFC) propellants, implicated in ozone depletion, were phased out with the ratification of the Montreal Protocol of 1987, and replaced with hydrofluoroalkane propellants (HFAs) (The Secretariat for The Vienna Convention for the Protection of the Ozone Layer & The Montreal Protocol on Substances that Deplete the Ozone Layer, 2003). The required reformulation of pMDIs with HFA propellant provided the opportunity for other desired changes to formulation and devices, such as formulations with a fine aerosol (<2.5μm MMAD) or extrafine aerosol (<2 μm MMAD), or devices producing a softer spray force (Gabrio, Stein, & Velasquez, 1999; Gentile & Skoner, 2010; Secretariat, 2003). A softer spray force results in less oropharyngeal deposition as the decreased velocity of particles released causes less impaction in the upper respiratory tract, and leaves a larger drug fraction available for lung deposition (Figure 1-2). Fine aerosols (≤2.5 μm MMAD) give increased lung deposition when compared to coarse aerosols (MMAD >2.5–10 μm) (Australian Government, 2004; Davies et al., 1998). Additionally, fine aerosols can achieve equivalent lung deposition to coarse aerosols, with smaller total doses (Davies
et al., 1998). Two pMDIs producing fine aerosol, HFA, formulations, were approved by the Therapeutic Goods Association (TGA) Australia in early 2000, however few studies have re-assessed associated clinical practices, such as spacer use (C. L. Leach & Colice, 2010).

Evidence shows that adults can achieve equivalent lung deposition using extrafine aerosols with or without a spacer (C. L. Leach & Colice, 2010). Additionally, when using a pMDI producing an extrafine aerosol, adolescents with a spacer can achieve lung deposition consistent to the adult amounts without a spacer (Devadason, Huang, Walker, Troedson, & Le Souéf, 2003; C. M. Roller, Zhang, Troedson, Leach, Le Souef, et al., 2007).

![Image of Andersen Cascade Impactor](image)

**Figure 1-2** Presentation of regional airway deposition relative to Anderson Cascade Impactor stage size range (28.3 LPM) adapted from (Dunbar & Mitchell, 2005). Showing approximate cut offs for Anderson Cascade Impactor stages Throat, Jet, and stages 0–Filter.
1.5.4.5 Inhaled corticosteroids delivered by pressurised metered dose inhaler (pMDI) in Australia

In Australia there are three inhaled corticosteroids delivered via pressurised metered dose inhaler (pMDI) that do not include other drugs in combination (monotherapies), a small subset of the orally inhaled asthma medication available in Australia (National Asthma Council Australia, 2016a). Drug choice by consumer or recommending clinician may be affected by whether that drug is listed on the Pharmaceutical Benefits Scheme (PBS), which subsidises medications to all Australian permanent residents. Fluticasone propionate (FP) formulated as Flixotide®, a coarse aerosol delivered via pMDI (2.8 µm MMAD), is the most widely used of the monotherapies according to 2013–2015 PBS data. Ciclesonide formulated as Alvesco® and beclomethasone dipropionate (BDP) formulated as QVAR®, are both extrafine aerosols (1.2 µm MMAD), and lesser used than Flixotide®.

1.6 ADHERENCE TO MEDICATION

Adherence to medication usage regimes was recognised as an issue and formally addressed in Australia and worldwide nearly 20 years ago, with the aim of improving asthma management and subsequent long term health benefits for affected individuals (Haahtela et al., 2006; National Asthma Council Australia, 1999). According to the Australian Institute for Health and Welfare, in 2013, only 17 out of 100 people received and used their prescribed inhaled asthma drugs correctly (Figure 1-3).
Figure 1-3 People dispensed preventer inhalers in 2013, adapted from:

1.6.1 NEAR FATAL ASTHMA AND NON-COMPLIANCE
Near-fatal asthma is defined as the most severe clinical presentation of asthma, usually requiring intensive care unit admission (Restrepo & Peters, 2008). It may be possible to characterise a more exacerbation-prone asthmatic type, however increased exacerbation episodes are not necessarily an indication of an individual having a severe asthmatic phenotype (Restrepo & Peters, 2008; Romagnoli et al., 2007). A near-fatal asthma phenotype is described as having mild-moderate asthma with reduced medication compliance, therefore regular use of prescribed ICS medications can protect against near-fatal asthma and fatal asthma exacerbations (Restrepo & Peters, 2008; Romagnoli et al., 2007).

1.6.2 MEDICATION COMPLIANCE IN ADOLESCENT PATIENTS
Adolescents (12–18 years) are considered a special population in health care and increased sensitivity in the approach with health care, and thus when treating asthma is advised (Michaud, 2007; Program., 2017). Management advice addresses the adolescents privacy and their transitioning to more autonomous functioning, with raised awareness on the increased risk taking behaviours exhibited by this age group, such as smoking, which is a known trigger for exacerbation (National Asthma Council Australia, 2016a; Program., 2017).
There are a number of psychosocial and socioenvironmental barriers to medication adherence in adolescents that can negatively influence symptom control, thus increase morbidity, and risk of hospitalisation (Chen, Bloomberg, Fisher Jr, & Strunk, 2003). Good family psychosocial functioning (support) is a particularly important to reduce these barriers, and a reduction can lead to improved asthma control, and increased quality of life scores (improvements in emotional functioning, symptom reduction and decreased limitations on daily activity) (Rhee, Belyea, & Brasch, 2010). Barriers that exist are both general and specific - those experienced by all chronic disease sufferers, and those specific to the adolescent population with asthma.

Particularly in the adolescent age group, it is reported that forgetting to take medication, or not wanting to when at school, are contributors to non-adherence (Koster, Philbert, de Vries, van Dijk, & Bouvy, 2015). During adolescence, the responsibility for taking their medication becomes theirs, including getting their own prescriptions (Orrell-Valente, Jarlsberg, Hill, & Cabana, 2008). This responsibility comes at a transitional time, and it has been identified that key factors, such as forgetfulness, social pressures, and conspicuousness of taking medication can influence both medication adherence, or incorrect prescribed use, and even shared non-prescription use of inhalers (Boyd, McCabe, & Teter, 2006; Desai & Oppenheimer, 2011; Rhee et al., 2010). These issues are anecdotally evident in focus group interviews (Koster et al., 2015), where individuals describe not taking their medication until they have symptoms and feeling embarrassed about taking their medication. A good relationship with the family medical practitioner (Haskard Zolnierek & DiMatteo, 2009) and parent or guardian is important to improving medication adherence during this time. It has been shown that increased adherence to medication results if there is continuing and repeated instruction for use of inhaler device (every 30 days), and high level supervision by a care giver (Park et al., 2015). Whilst it is clear there are a multitude of reasons effecting adherence to medication in adolescents, some are clearly more difficult to address than others, such as family psychosocial functioning. Furthermore, it is unclear whether the conspicuousness associated with spacer use is the primary reason, as this is difficult to study directly without risk of bias. Regardless, there are obvious inefficiencies in the current delivery methods of ICS medication, so reducing
these, by re-visiting the requirement for spacer use will ultimately benefit this population.

Non-adherence is associated with exacerbation, and hospitalization, and potentially life threatening (Milgrom et al., 1996). It is increasingly critical to find ways to encourage adherence, particularly in this population. If it is apparent that adolescents may be less inclined to adhere to their treatment regimen due to psychosocial, and cognitive changes at this time, then it is evident that making treatment more effective, less conspicuous, and easier to implement in practice will benefit this population group.

1.7 METHODS OF ASSESSMENT OF INHALER USE AND EFFICACY

1.7.1 THEORETICAL IN VITRO ASSESSMENT (MATHEMATICAL MODELLING)

Mathematical modelling of the lung deposition of inhaled particles can be used to support lung deposition measurements obtained from investigational studies by describing underlying physical mechanisms (Asgharian, Price, & Hofmann, 2006). Considering this is important, as the lung geometry of an adult is not proportionately smaller for a child (Yu & Xu, 1987) and lung deposition studies in children are few. A realistic representation of particle deposition in the lungs via a mathematical model is dependent on accurate lung geometry and ventilation, and aerosol-particle transport models. Lung geometry can be obtained with models or with computed tomography lung geometries. Lung ventilation can be uniform or non-uniform, depending on the lung geometries (Asgharian et al., 2006). Aerosol-particle transport models can be determined mathematically using the aerodynamic diameter and density of the spherical envelope of a particle. These parameters affect the deposition efficiency that occurs via impaction and sedimentation, interception and diffusion (Cai & Yu, 1988; Yu & Xu, 1987). However, mathematical models cannot predict accurately a particle’s trajectory, as most models use a typical–path, symmetric, airway geometry, and multiple-path, asymmetric, airway geometry makes determining lung ventilation more difficult, and most cannot consider the turbulence of the upper airway (Asgharian, Hofmann, & Bergmann, 2001; Chang, 1999; R Lambert, O'Shaughnessy, Tawhai, Hoffman, & Lin, 2011).
Computational fluid dynamic (CFD) models of lung deposition can however, with the use of 3D technologies, particularly those that use accurate whole airway ventilation models and are able to create moving lung geometries in three dimensions, more reflective of a realistic airway. Flow is typically described using Reynolds Averaged Navier–Stokes, an equation of movement for fluid flow, or Large Eddy Simulation, a mathematical model for turbulence. Flow under normal conditions in the lung is considered laminar and in the upper airway, turbulent (Cai & Yu, 1988; Mead-Hunter, King, Larcombe, & Mullins, 2013). CFD has largely been performed with static lung models, due to the complexity of the computational design in creating a moving lung, although (Mead-Hunter et al., 2013) have considered both static and moving lung geometries.

1.7.2 IN VITRO LABORATORY STUDIES

*In vitro* studies are used to estimate lung deposition of an aerosol based on its aerosol particle size distribution. This measurement can then theoretically estimate what should be received regionally in the mouth, throat, conducting airways and peripheral regions of the lung by an individual who uses that drug. Testing is carried out with a particle-sizing device, (or flow domain), a device designed to separate aerosol particle sizes within fractions, simulating the lungs of a breathing individual, with deposition in the device or domain mimicking that within the lungs. To administer drug into the particle-sizing device, either a single rate of flow, a simulated breath pattern, or a recorded breath pattern from a patient can be used, although the rate of flow through the particle-sizing device must remain constant. These devices are unable to simulate the expansion of lungs, thus do not mimic accurate lung ventilation, and can consequently overestimate lung deposition. They also do not take into account user variability, and are not generalisable of administration in practice. *In vitro* studies are good however, at accounting for total body dose, and measuring the MMAD of an aerosolised drug. For this reason they are a required result when assessing therapeutic efficacy of an inhaled drug (Australian Government Department of Health Therapeutic Goods Administration, 2013; European Medicines Agency Committee for Medicinal Products for Human Use, 2009).
1.7.3 **IN VIVO DRUG DELIVERY STUDIES**

Drug delivery to inspiratory filters in studies is designed to measure the amount of drug emitted by a device that will potentially enter the airways, or the estimate of total body dose. They are useful for studies in small children where ethical approval for studies involving administration of drugs is difficult to obtain. A limitation of filter studies is that no information is obtained about the dose that would be delivered to the lungs.

1.7.4 **IN VIVO LUNG DEPOSITION STUDIES**

*In vivo* testing is required to estimate airway deposition, as no current *in vitro* study is able to accurately predict patterns of airway deposition *in vivo*, although considerable progress has been made with CFD (Stephen P. Newman & Chan, 2008). Deposition studies allow us to understand the characteristics of an aerosolised test drug *in vivo*, and attempt to relate this to its *in vitro* particle size distribution (Stephen P. Newman & Chan, 2008). Lung deposition studies have an advantage over other studies because they account for an individuals variability. Variability in lung deposition is dependent on patient factors, such as peak inspiratory flow, whether the participant completes a full exhalation before inhalation, and airway diameter and characteristics, which vary according to the individual (Labiris & Dolovich, 2003). As such, lung deposition in studies is shown to decrease with inhalation errors such as decreased breath hold time and delaying actuation after inhalation (C. L. Leach, Davidson, Hasselquist, & Boudreau, 2005; Newman, Pavia, Garland, & Clarke, 1982). The mechanisms by which drug deposits, both in the lung and higher in the respiratory tract, varies according to aerosol particle size distribution. Theoretically, particles (>5 μm) impact by inertia and do not easily pass through the oropharyngeal tract to the lungs, where the drug is required, especially in children (Xu & Yu, 1986). Small particles (<2 μm) theoretically deposit by gravitational sedimentation and were thought to be exhaled after an inadequate breath hold, however studies have since disproved this although the mechanism is not fully understood (C. L. Leach et al., 2015; Rubin & Fink, 2003). Combining the theoretical deposition of a particle based on its size, with an aerosol particle size distribution measurement obtained with *in vitro* laboratory studies, and then evaluating the same aerosol via lung deposition studies provides a complete assessment of
most factors contributing to lung deposition, excluding transport or absorption at a cellular level.

1.7.4.1 Lung deposition studies using radiolabelled Technetium
Deposition studies using a gamma emitter, Technetium ($^{99m}$Tc) as a radiotracer, with 2D gamma scintigraphy imaging is the current best practice for assessing drug deposition patterns in vivo (Adams et al., 2010). As there is no way to chemically combine the drug and a radioisotope with suitable decay requirements, (i.e. long enough to complete the study requirements, and short enough to minimise radiation exposure to the individual), indirect labelling with $^{99m}$Tc (half-life of 6 hours) is used with aerosolised drugs (S. P. Newman, 1996). Indirect labelling creates a physiochemical-association between label and drug (Farr, 1996). After inhalation of the aerosolised radiolabelled drug by the study participant, the radioactivity deposited in the lungs and elsewhere can be measured to estimate aerosol deposition with a gamma scintigraphy scan. Due to this indirect assessment, validation that the labelling method does not change the characteristics of the aerosol or drug, when compared to the reference drug, is essential to ensure quality control (Corcoran, Devadason, & Kuehl, 2012)

1.7.4.2 Validation of drug radiolabelling techniques
The validation process for radiolabelled drugs requires that two separate testing processes be carried out. One is to characterise the reference drug, and the other is to characterise the radiolabelled drug. The reference drug is a canister of commercially available drug, unaltered. Characterisation is carried out using an in vitro particle size testing device, usually a multistage cascade impactor, and the resulting data is presented as three sets of information; percentages of total recovery for; labelled drug, radiation, and commercial drug, for each impactor stage.

These data are displayed mathematically as ratios of labelled to unlabelled drug, of radiotracer to labelled drug and of unlabelled drug to radioactivity for each stage individually, or as combined stages with a minimum of four groupings. With most particle-sizing devices, the information from stages three through to filter, <3 μm, (Figure 1-2) can be interpreted in combination as the fine particle fraction, and is considered respirable (not swallowed) (Ditcham et al., 2014).
The validation process involves *in vitro* testing of the radiolabelled drug and if carried out successfully it will ensure that:

1. The radiolabelled drug formulation is an accurate representation of the commercial drug formulation;

2. That the radiotracer Technetium ($^{99m}$Tc) has created a physiochemical association with the test drug and;

3. That the radiolabelling process has not altered the aerodynamic characteristics of the labelled aerosol from that of the reference drug aerosol.

### 1.7.4.3 International guidelines for interpreting successful radiolabelling of inhaled drugs

Point one of the international guidelines for radiolabelling of inhaled drugs, Devadason et al. (2012), suggests that when reported mathematically, the acceptability range for the mean ratio of drug dose for the labelled to unlabelled drug be between 0.85–1.18. If regional (inner and outer lung regions) lung deposition is being measured, this ratio is obtained per impactor stage or as grouped stages (min. four groups) (point four). Point two suggests that the ratio of labelled drug in the fine particle fraction, to that of the radiotracer itself also falls within 0.85–1.18 for measurement of total lung deposition, and that ratios are measured per impactor stage when regional deposition is of interest (point three) (Devadason et al., 2012). Point five requires that 90 percent confidence intervals are calculated for all of the above points, and point six requires that total emitted dose be measured prior to *in vivo* testing. Point seven recommends that data for the aerodynamic particle size distribution (drug on each impactor stage) be presented visually with a histogram or similar (Fig. 1.6.2) (Devadason et al., 2012).

### 1.7.4.4 Importance of international standards for inhaled radiolabelled preparations

Changes in the validation process for inhaled radiolabelled preparations have been reflective of field developments in methodology, device design, and reporting guidelines over time, with notable developments displayed in Table 1-1.
Table 1-1  A Reflection on Guidelines for Validation Practices. Table shows key developments of the validation guidelines since their inception.

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<tr>
<td>Data reporting</td>
<td>No standard for data reporting discussed</td>
<td>No standard for data reporting discussed</td>
<td>Minimum criteria for reporting data, histogram recommended for validation data display</td>
</tr>
<tr>
<td>Particle-sizing devices</td>
<td>Recommended that the entry for the sizing device be standardized. Particle-sizing methods not routinely used. Some devices with glass throats in use (Clarke et al., 1992)</td>
<td>Throat used, Variable number of sizing device stages</td>
<td>Throat used, Minimum of five stages</td>
</tr>
<tr>
<td>Impactor flow rate</td>
<td>Particle-sizing methods not routinely used. Inhalation rate limited to device design, if used.</td>
<td>Inhalation rate limited to device design</td>
<td>Inhalation rate must be an accurate representation of the study population</td>
</tr>
<tr>
<td>Number of replicates in validation phase</td>
<td>Not discussed</td>
<td>Testing to be carried out prior to dosing participant, on study day, a minimum of five unlabelled and five labelled inhalers be tested prior to study day.</td>
<td>Minimum of six inhalers to be tested (labelled and unlabelled comparisons) before study day, as well as study day testing of inhaler, If time elapses (&gt;3 months) between testing and study day, two acceptable tests must be conducted additionally prior to study day.</td>
</tr>
<tr>
<td>Validation acceptability range</td>
<td>No acceptability range mentioned</td>
<td>Three key points to determine acceptability. Ratio of labelled to unlabelled to fall within 0.8–1.2</td>
<td>Seven key points to determine acceptability. Ratio of labelled to unlabelled to fall within 0.85–1.18</td>
</tr>
</tbody>
</table>
The currently proposed international guidelines for radiolabelled drug preparations offer improvement on previous practices (Table 1-1). Minimum requirements for data reporting allow easy comparison of methodology, and provide ability for study replication or improvement. Reporting of the particlesizing device used, for example, is essential, as validation data characterised on a particle-sizing device without a throat would give readings of higher proportions and with a larger particle size than are representative of the in vivo deposited drug. Furthermore, a larger range of stage cut-offs in the particlesizing device allows particle size distribution to be determined with greater resolution, which could affect quality of results negatively if not reported, but if reported could be taken into consideration. A particular point to note is the requirement for number of replicates involved in validation, which allows future meta/collaborative studies to have consistent base numbers to work with. An extension of the acceptability range criteria to seven key points (1.7.5.3) allows ease of interpretation that has never previously been established, and should provide improved consistency of reporting and thus more easily comparable results. Retrospective analysis of previous validation data, using current international guidelines can provide confirmation that data obtained with earlier versions of the guidelines are still relevant, and can be used for future comparison. Additionally, assessment will support the integrity of the radiolabelling method used, for use in current and future studies.

1.7.4.5 Gamma scintigraphy to assess in vivo lung deposition
Gamma scintigraphy is a 2D imaging process where gamma radiation from a known source (i.e. inhaled radiolabelled (Tc\textsuperscript{99m}) drug), released by disintegration of the radioactive nucleus, is captured when the camera detects this release, as disintegrations (counts) per second, or Becquerels. To quantify the radiation emitted from the deposited radiolabelled drug, simultaneous anterior and posterior images are taken. Some activity is not detected as emitted rays not entering the collimators are not counted. Depending on the size of the individual the amount lost will vary, but is estimated to be minimal (C. L. Leach et al., 2015).
1.7.4.6 Single photon emission computed tomography 3D Imaging

Single photon emission computed tomography utilises a 360° rotating camera, taking longer to acquire than the 2D gamma scintigraphy planar image, and thus requiring a higher radiation dose. For this reason 3D imaging is rarely used in lung deposition studies. Recent studies have used this method to compare the 2D and 3D imaging, which confirmed that only small amounts of radiation are lost to the cameras with 2D, and that 3D imaging provides a more accurate estimate of regional lung deposition when estimating peripheral and central deposition ratio (C. L. Leach et al., 2006).

1.7.4.7 Positron Emission Tomography Imaging

Positron Emission Tomography is where a radiotracer can be directly incorporated into a drug molecule, usually with a positron emitter such as $^{11}$C or $^{18}$F. The method is expensive and the radioisotopes required do not have suitable decay requirements for the experimental process involved in lung deposition studies.

1.7.5 CLINICAL OBSERVATION OF INHALER TECHNIQUE

Inhaler competence according to the Asthma Council Australia is defined according to device used (National Asthma Council Australia, 2016a). It is understood that failure to utilise a device with correct technique leads to poor asthma control (Engelkes et al., 2015; Levy, Hardwell, McKnight, & Holmes, 2013). Furthermore, it is evident that clinically observing technique is not necessarily a reliable measure (Biswas, Patel, Mohsin, Hanania, & Sabharwal, 2016).

1.7.6 CLINICAL TRIALS

Clinical trials are used to assess drug therapeutic efficacy via improvement in symptom related outcomes, and can also be used to compare drug efficacy via these means (Lasserson, Cates, Lasserson, & White, 2010). They can also be used for investigating adherence to medication regimens (Rand et al., 1992). Trials provide evidence that asthma can be controlled in the majority of individuals when treatment is both adhered and delivered correctly (de Groot, Kreggemeijer, & Brand, 2015; Engelkes et al., 2015). While symptom presentation and asthma control are important clinically, clinical studies investigating these are limited in their ability to determine that the prescribed
dose reaches its destination in the lungs, and how efficiently. Furthermore, if a participant is using incorrect technique, this may not always be observable clinically and drug loss to incorrect technique, results in decreased asthma control and an unnecessary increase of treatment regimen (Biswas et al., 2016; Bousquet et al., 2010). Thus, clinical trials, in combination with in vitro particle size distribution studies, are used to provide therapeutic efficacy of an orally inhaled product by the Australian Therapeutic Goods Administration (Australian Government Department of Health Therapeutic Goods Administration, 2013).

1.8 SUMMARY AND THESIS AIMS

1.8.1 BACKGROUND SUMMARY
Asthma is a major cause of morbidity worldwide with the highest prevalence in children and adolescents, making it critical to find effective methods of treatment in these age groups. There is a distinction between those who suffer from exacerbations and severe asthmatics. Exacerbation and near-fatal asthma is often an indicator of non-adherence to medication, and individuals who suffer from exacerbations can be mild asthmatics. There is evidence that exacerbations and hospitalisations can be prevented in many individuals, when preventative treatment is adhered to. A spacer is an additional device used with pMDIs to maximise therapeutic benefit, although this device may be unnecessary in the adolescent age group with the invention of fine and extrafine aerosol formulations. Furthermore, adolescence is recognised as a challenging environment, a time of complex cognitive and social change, and so it is increasingly critical to find new treatments with improved efficacy that are easy to implement in practice, encouraging both adherence and compliance in this age group. If a spacer was not required with use of a pMDI producing an extrafine aerosol, it would highlight the need of regular user training (every 30 days), with an appropriately trained clinical professional, for correct administration of a pMDI (Park et al., 2015).

1.8.2 PRIMARY STUDY OBJECTIVES
The primary objective of this study is to quantify total drug deposition and distribution in the airways of a group of adolescents, following inhalation of radiolabelled aerosols with different particle size distributions. Both an extrafine aerosol; beclomethasone dipropionate (BDP) as QVAR™, MMAD=1.2 µm, and
a coarse aerosol; fluticasone propionate (FP) as Flixotide®, MMAD=2.8 µm will be used to represent a range of the ICS monotherapies available. Inhalation of aerosol via a single maximal inhalation and breath-hold technique will be used. Oropharyngeal and gastrointestinal deposition will be assessed to identify if decreased deposition in these regions occurs when using an extrafine aerosol with a spacer/pMDI combination with breath hold technique, as previously reported (Devadason et al., 2003). If use of the pMDI producing an extrafine aerosol without a spacer can achieve both similar levels of oropharyngeal deposition and lung deposition to that achieved by the same aerosol with a spacer, it may suggest a spacer is not necessary. Future studies would be required to confirm this finding in larger cohorts in order to impact on clinical recommendation to use a spacer alone.

1.8.3 PRIMARY AIMS AND HYPOTHESES

1.8.3.1 Aims

1. To assess if equivalent total lung deposition can be achieved with an adolescent participant using an extrafine aerosol with or without a spacer, whom has been trained to use the device correctly immediately prior to use, and witnessed doing so by a trained professional during administration in the study.

2. To assess if equivalent total lung deposition can be achieved with an adolescent participant, using a coarse aerosol with or without a spacer whom has been trained to use the device correctly immediately prior to use, and witnessed doing so by a trained professional during administration in the study.

3. To assess decrease in oropharyngeal deposition of an extrafine aerosol with and without a spacer

4. To assess decrease in oropharyngeal deposition of a coarse aerosol with and without a spacer

1.8.3.2 Hypotheses

1. Adolescents with good technique who use extrafine aerosolised drugs will achieve equivalent lung deposition of drug using a pMDI with or without a spacer
2. The mean difference in oropharyngeal drug deposition with and without a spacer will be lower with an extrafine aerosol than a coarse aerosol.

1.8.4 SECONDARY STUDY OBJECTIVE
To retrospectively analyse the validation data from two previous radiolabelling studies completed in our laboratory, using current validation guidelines for inhaled radiolabelled products. If data analysed falls within the current guidelines, this will provide confirmation of the integrity of previously obtained data, the radiolabelling methodology, and thus results obtained in this study.
2 CHAPTER II: MATERIALS AND METHODS

All experiments were initially conducted by the author under the direct supervision of a senior member of laboratory staff with an appropriate level of experience in the methods described here. After deemed competent by the supervisor, methods were completed by the author unsupervised. A radiation handling and safety course was attended by the author to enable the completion of methods described here.

2.1 MATERIALS

2.1.1 MATERIALS AND EQUIPMENT

Materials and equipment used for gamma scintigraphy are described in appendix 5-1. Materials used for radiolabelling and particle size distribution assessment are described in Appendix 5-1 and 5-2 respectively. Drugs (Appendix 5-3) were radiolabelled with the gamma radiation emitter, $^{99m}$Technetium ($^{99m}$Tc, Global Medical Solutions, Perth Radiopharmacy, Australia).

2.1.2 MATERIALS AND EQUIPMENT PREPARATION EACH STUDY DAY

A study day was any day where a recruited participant completed a visit that contributed to the result of the study.

2.1.2.1 Reagents preparation (Appendices 5.1)

A fresh 10 mg/mL solution of tetraphenylarsonium chloride (Sigma Aldrich) was prepared with room temperature ultrapure water (Milli-Q, Ultrapure Water System, Millipore Australia) each study day.

A stock solution of either fluticasone propionate (FP, Sigma Aldrich) or beclomethasone di-propionate (BDP, Sigma Aldrich) at a concentration of 100μg/mL in methanol (Thermofisher Scientific, Australia) was prepared and stored at 4 °C until required.

2.1.2.2 Preparation of spacers for study day use with radiolabelled pMDI

To reduce electrostatic effects (Wildhaber et al., 1996), the spacers (Compact Space Chamber Plus®, Allersearch, VIC, Australia) were left to soak for a minimum of one hour in a 1:5000 w/v detergent solution (Pyroneg, JohnsonDiversey Pty Ltd, Australia), then left to dry without rinsing in an
incubator at 37 °C. A control spacer was soaked in the same water as the study spacer.

2.1.2.3 Pneumotachometer calibration
A 160 litres per minute (LPM) pneumotachometer head was attached directly to a Research Pneumotach Systems 3 L syringe, and calibrated using Research Pneumotach Systems software (Hans Rudolph), to detect a flow (inspiratory and expiratory) of 3 L ± 30 mL, with a flow rate of 80 LPM ± 8 LPM.

2.1.2.4 Airflow calibration, Anderson Cascade Impactor (ACI)
The Anderson Cascade Impactor (ACI) was attached to the vacuum pump, and the pneumotachometer head was placed in the ACI throat using an actuator mouthpiece adaptor and airtight laboratory film. The airflow drawn through the Anderson Cascade Impactor (ACI) was checked with Research Pneumotach Systems software and adjusted on each study day to be 28.3 LPM.

2.2 METHODS

2.2.1 RADIOLABELLING METHOD
The radiolabelling technique used has been described previously (CL Leach, Davidson, & Boudreau, 1998) and validated in our laboratory prior to the project (Christina M. Roller, Schaefer, Zhang, & Devadason, 2006; C. M. Roller, Zhang, Troedson, Leach, Le Souëf, et al., 2007). All radiolabelling procedures carried out on a study day were undertaken in a class II laminar flow hood (Standardised Protection Pty Ltd). Each radiolabelled canister contained 500–600 MBq of $^{99m}$Tc as sodium pertechnetate ($\text{Na}^{99m}\text{TcO}_4$). $\text{Na}^{99m}\text{TcO}_4$ (2 mL) in 0.9% sodium chloride was mixed with 6 μL tetraphenylarsonium chloride (10 mg/mL in ultrapure water) (ICN Biomedicals, Ohio, USA) and 30 μL ammonium hydroxide solution (28–30% NH₃), (Sigma Aldrich, Australia). Butanone (6 mL) was added and $^{99m}$Tc was extracted as tetraphenylarsonium pertechnetate by shaking. The emulsion was transferred into a glass separating funnel, allowed to separate and the aqueous components run to waste. The butanone, containing the radiotracer, was transferred into a new aluminium pMDI canister and evaporated to dryness with a gentle stream of nitrogen. A commercial pMDI (Appendix 2-1) was supercooled in liquid nitrogen for 50 seconds, the valve removed with a pipe cutter, and the entire contents transferred into the $^{99m}$Tc–coated dried canister. For the BDP radiolabelled
canister, 80 μL of ethanol was then added. The vial was re-crimped with a new valve (manual crimper, Pamasol, Willi Mader, AG) and weighed to ensure that 95% of the contents of the original pMDI by mass had been transferred. The newly crimped canister was placed on a shaker at 200 rpm for 30 minutes then re-weighed to ensure the seal was maintained.

2.2.1.1 Total radiation dose given to participants
Ethical approval for the radiation dose delivered to adolescents (aged 11–17 years) was given by the Princess Margaret Hospital (PMH) Ethics Committee. Data obtained from the International Commission on Radiological Protection, (International Commission on Radiological Protection, 1988) for $^{99m}$Tc pertechnetate was used to calculate dose estimates by Clinical Physics, Department of Medical, Engineering and Physics at Royal Perth Hospital (appendix, Item 2). The maximum radiation dose estimates for $^{99m}$Tc pertechnetate (as a radiolabelled preparation) in the protocol for children between 11–17 years, (up to six MBq dispensed in total) range from approximately 0.04 to 0.4 mSv. Using Australian Radiation Protection and Nuclear Safety Agency guidelines, the risk associated with this level of activity is believed to be ‘very low’ (dose range, 0.02 mSv ≤ 2 mSv).

2.2.1.2 Radiation clearance
Dose estimates calculated by Clinical Physics, Department of Medical Engineering and Physics at Royal Perth Hospital (appendix, Item 3) were based on both quick-clearing and slow-clearing aerosols, with lung clearance rates (biological half-life times) of 1 hr for QVAR™ and 24 hrs for Flixotide®.

2.2.1.3 Determination of radioactivity dispensed per pMDI actuation
Radioactivity emitted per actuation was measured by using a glass wool filter connected to the vacuum pump drawing air through the filter at 28.3 L/min, and actuating the pMDI five times into the filter. The canister was weighed pre- and post- five actuations, and the radioactivity of the canister and the glass filter were measured before and after the actuations, and mean activity per actuation calculated.

2.2.1.4 Radiation dose accountability
The total radioactivity of the labelled canister was measured immediately prior to study drug inhalation (Atomlab 200 dose calibrator, Gammasonics, Sydney,
Australia). A single dose was emitted to waste then the activity of the radiolabelled pMDI immediately measured, to ensure radiation emitted did not exceed the approved dose. If the activity/actuation exceeded two MBq, but was not greater than three MBq, then a single actuation of the pMDI was administered to the participant. If the dose measured was less than two MBq, but not less than one MBq, then two actuations of the pMDI were administered to the patient.

2.2.2 PARTICLE SIZE DISTRIBUTION ASSESSMENT OF LABELLED AND UNLABELLED DRUG AND DISTRIBUTION OF RADIONUCLEIC TRACER

Particle size distribution (PSD) of study drugs were assessed before and after radiolabelling, using an eight stage non-viable Anderson Cascade Impactor (ACI) (Copley Scientific, Nottingham UK) at a continuous flow of 28.3 L/min. The measurement was taken by firing ten actuations into the entraining airflow at the entrance of the ACI throat, with ~5 seconds of shaking in between firing. Once complete, the flow was stopped and the ACI was dismantled. The actuator, throat, and each stage of the ACI was then held above a funnel resting on a 25 mL volumetric vial with clamp tongs, and rinsed back and front with ~22 mL methanol, with a further ~3 mL used to rinse the funnel of residual drug. The concentration of drug rinsed from each item or stage (each vial) was then measured using ultraviolet spectrophotometry. Associated radioactivity of each 25 mL vial (radiotracer) was measured separately, prior to spectrophotometry, in an ionization chamber (Atomlab 200 dose calibrator, Gammasonics). The particle size distribution was considered the staging of drug content from each vial, as percent of sum-total recovery of drug from all vials. The distribution of the radiotracer was assessed similarly.
2.2.2.1 Ultraviolet visible spectrophotometry to interpolate drug concentrations

Spectrophotometry measurements were taken either after 24–48 hrs decay time for a radiolabelled pMDI, or immediately after particle size distribution assessment for unlabelled drug. Prior to measurement on the spectrophotometer (Shimadzu, Model 1601) a zero reading was taken with methanol, for all the samples except the spacer, where the zero was a methanol rinse of a detergent coated spacer. Each 25 mL volumetric flask was inverted 2–3 times, then 2–3 mL were transferred, by pouring into a quartz cuvette, and the absorbance at λ=238 nm for BDP or λ=247 nm for FP was measured for each sample in duplicate. The quantity of drug in tested samples was determined by interpolation of these absorbance readings, using a standard curve generated on the same day (Figures 2-1–2-2).

2.2.2.2 Standard curve generation to interpolate drug concentrations

The lower limits of detection for the concentration of drug standards used previously in our laboratory (range 1–20 µg/mL) were insufficient for this study. Therefore, the method was optimised to decrease the lowest detectable concentrations of drug in samples. An assay with a range of standards (range 0.625–40 µg/mL) was prepared by diluting a stock solution of 100 µg/mL in 10 mL for both FP and BDP (Figures 2-1 and 2-2 respectively). A standard curve was created from the absorbance reading of each standard at wavelength λ=238 (BDP) or λ=247 (FP). The correlation, of absorbance to µg/mL, for BDP and FP was linear and repeatable, $r^2 0.997$ (n=5), $r^2 0.999$ (n=5), respectively.
Figure 2-1 Comparison of original and improved assay for a standard curve generated for fluticasone propionate drug interpolation on semi log (x) scale. Data show absorbance of light at $\lambda=247$ nm of fluticasone propionate standards prepared for the original assay, for each concentration (inverted triangles); 1 μg/mL, 2 μg/mL, 5 μg/mL, 10 μg/mL and 20 μg/mL, and with optimised detection limits (circles); 0.625 μg/mL, 1.25 μg/mL, 2.5 μg/mL, 5 μg/mL, 10 μg/mL, 20 μg/mL and 40 μg/mL.

Figure 2-2 Comparison of original and improved assay for a standard curve generated for beclomethasone dipropionate drug interpolation on a semi log (x) scale. Data show absorbance of light at $\lambda=238$ nm of beclomethasone dipropionate standards prepared for the original assay, for each concentration (inverted triangles); 1 μg/mL, 2 μg/mL, 5 μg/mL, 10 μg/mL, and 20 μg/mL, and with optimised detection limits (circles); 0.125 μg/mL, 0.25 μg/mL, 0.5 μg/mL, 1 μg/mL, 2 μg/mL, 5 μg/mL, 10 μg/mL, 20 μg/mL, 30 μg/mL and 40 μg/mL.
2.2.3 PRE-STUDY QUALITY CHECK OF RADIOLABELLING METHOD (VALIDATION)

Validation of a radiolabelled inhaled drug is essential to ensure quality control and standardised reporting within the field (1.7.3.2). The process was determined by assessing the PSD (2.2.2) before and after labelling, and the distribution of the radotracer (2.2.2). A total of six PSD assessments were completed and analysed using the international guidelines as quality criteria, prior to administering the study drug preparation to participants (Devadason et al., 2012).

2.2.3.1 In vitro validation analysis using international guidelines as quality criteria

Pre-study quality checks were done using international guidelines as quality criteria, obtained from (Devadason et al., 2012) (1.7.4.2–1.7.4.3):

1. The ratio of total drug recovery (µg) of the labelled drug and reference drug was within the range 0.85–1.18.
2. The ratio of drug recovery of the labelled drug’s respirable fraction as percent of total drug recovery, when compared to its reference drug counterpart, was within the range 0.85–1.18.
3. The ratio of radioactivity recovery for the labelled drug’s respirable fraction as percent of total radioactivity recovery, when compared to its reference drug counterpart was within the range 0.85–1.18.
4. The ratio of the amount of drug recovered from each stage obtained from the PSD assessment, as a percentage of total drug recovery, for labelled drug and radotracer, to the reference drug, was to be within the range of 0.85–1.18. If amount of drug in any one group was under 10% of the total recovered dose, an absolute difference of both the labelled and radotracer to the reference drug of ±2 percent was deemed allowable.
5. The respirable fraction as described above is obtained from the PSD assessment as percent of total of the combined ACI stages three to filter (2.2.2).
6. Minor deviations from the ratio were considered acceptable (≤±4).
To verify the integrity of radiolabelling (2.2.2) after validation (2.2.3), on each study day, the PSD was reassessed. If a radiolabelling failure was suspected, due to the ratios described above not falling within acceptable guidelines, (n=2, FP), PSD testing was repeated, and only accepted if the ratio was within limits on the repeat (n=1). Failure was confirmed if repeat testing was not acceptable (n=1), and another radiolabelled canister was prepared (n=1).

2.2.4 RETROSPECTIVE ANALYSIS OF PAST RADIOLABELLED VALIDATION FOR DRUGS AS USED IN THIS STUDY

Radiolabelling methodology used previously in our labs, as described in (C. Roller, 2012) for FP and in (C. M. Roller, Zhang, Troedson, Leach, Le Souef, et al., 2007) for BDP, had been validated using now superseded guidelines. To verify the integrity of the radiolabelling method, and the data obtained, we obtained previous abovementioned raw data and re-analysed this using the current guidelines for validation of radiolabelled preparations (1.7.4.2–1.7.4.3). We then compared this studies radiolabelling validation results for each drug with previous radiolabelling results for each drug to assess the robustness of the methods.

2.2.5 IN VIVO AEROSOL DEPOSITION STUDY METHODS

2.2.5.1 Study Design

In order to investigate the requirement for a spacer, and the effect of aerosol particle size distribution on this recommendation, our study assessed lung deposition of two ICS drugs, with and without a spacer. The study was designed as a randomised cross-over trial (Figure 2-3), where participants completed two visits, with a washout period of 3–7 days between each visit. The asthma ICS pMDIs used in this study, did not include other drugs in combination, and were chosen from those available in Australia, based on their particle size distribution comparison, and whether they had been previously used in similar studies within our laboratory. PMDs used were; fluticasone propionate (FP), formulated as Flixotide®, (MMAD 3.5 μm), and beclomethasone dipropionate (BDP) formulated as QVAR® (MMAD of 1.1 μm).
Figure 2-3  A randomised cross over design study, where each participant completed one of two possible arms.
2.2.5.2 Participants
Adolescent participants, between ages 11–17, with mild stable asthma were recruited from a general paediatrics outpatient clinic at PMH, Perth Western Australia (n=14). Participants and their parents gave informed consent (see appendices 5.4), and participants were screened for clinical wellness by the respiratory clinical nurse specialist at PMH. Screening for clinical wellness included a physical examination and obtaining vital signs (oxygen levels, respiratory rate, heart rate, blood pressure, temperature) (see appendices 5.5 and 5.6). All participants were within normal ranges of clinical wellness and included in the study. Participants were also interviewed at the screening visit to assess asthma medication use and compliance. No participant had any of the following exclusion criteria: 1) previous involvement in a radiolabelling deposition study for research purposes; 2) previous involvement in a clinical trial within the past four weeks; 3) a known previous adverse reaction to $^{99m}$Technetium (Tc) pertechnetate, beclomethasone dipropionate or fluticasone propionate or; 4) a past or present diagnosis of cardiovascular, renal or liver disease.

2.2.5.3 Participant randomisation and allocation
Prior to their first study day visit, participants were randomised to device according to their recruitment number and a sequence of 28 binary integers (1=pMDI or 2=spacer) generated by an internet application (random.org). Drug group was allocated on the day according to which drug had been radiolabelled. The complexity and lengthiness of the laboratory process in combination with the requirements of validation verification meant that it was not feasible to label two drugs on one day. Each participant in the randomised cross-over study received the same drug at both visit one and two (Figure 2-3). However, on visit two, each participant used the device they were not randomised to receive on their first visit. Clinical cases were prioritised over research access to the gamma camera, and so, to maximise time, multiple participants were scheduled on a single study day, hence receiving the same drug.
2.2.6 ASSESSMENTS

2.2.6.1 Screening questionnaire
Participants were required to complete a screening questionnaire, similar to that used clinically at PMH, concerning adherence to medication and related issues (see appendices item 5.7). The survey was administered with an open, non-judgemental approach, by the clinical nurse asthma specialist at PMH. Survey response data was then interpreted by a single user according to Table 2-1.
### Table 2-1 User interpretation of adherence to medication survey responses as described in 2.2.6.1.

<table>
<thead>
<tr>
<th>Question as per Case Report (CRF) Form</th>
<th>Interpretation in survey</th>
<th>Examples of CRF answers</th>
<th>Interpretation of CRF answers in survey results</th>
</tr>
</thead>
<tbody>
<tr>
<td>What medication are you prescribed for your asthma?</td>
<td>Dry powder use Yes/No</td>
<td>All were prescribed Ventolin as the reliever medication. Inhaled corticosteroids reported were various and most reported being on more than one medication; Flutiform (1) Flixotide (2), Symbicort Turbuhaler (3), Alvesco (2), Seretide (6), Seretide Accuhaler (1), Singulair (2), Spiriva (1).</td>
<td>Those who reported using either Seretide Accuhaler or Symibcort Turbuhaler were classified as dry powder users. Those who reported using Flutiform, Flixotide, Alvesco, Seretide, or Spiriva were classified as pMDI users. The individuals who reported using Singular used this in combination with their inhaled drug and so singular was not included in survey results. One participant was only prescribed Ventolin, indicated in survey as “not prescribed”.</td>
</tr>
<tr>
<td>Many people forget to take their medications occasionally. How often do you forget to take it?</td>
<td>Two possible outcomes from this question; Adherence to prescription Y/N, Daily adherence Y/N</td>
<td>Often (1), Rarely (2), Occasionally (1), Frequently (2), Never (3), Not really (1), 2-3times/week (1), 1-2times/week (3).</td>
<td>Those who answered “never” (3), were allocated YES in daily adherence. All other answers were NO. Those who answered either frequently or often were allocated NO to adherence to prescription. All others were YES.</td>
</tr>
<tr>
<td>Do you take your medication with or without a spacer?</td>
<td>Consistency of spacer use Y/N</td>
<td>With and without (2), no-DPI (4), with (5), without/occasionally with (2), without.</td>
<td>Those who answered ONLY “with” were allocated YES, all others were allocated NO. Answers were ranked most to least consistent; “with”, “with and without” and “without/occ. with”</td>
</tr>
</tbody>
</table>
2.2.6.2 Inhalation technique

On study day, immediately prior to inhalation of the study drug, all participants (n=14) were trained by the clinical nurse asthma specialist to use their pMDI or pMDI/spacer combination. PMDI training was in accordance with National Asthma Council guidelines for inhalation technique using a single maximal inhalation and breath-hold, either with or without a spacer (National Asthma Council Australia, 2016b). Training involved the nurse specialist first demonstrating the technique required, then asking the adolescent to demonstrate this, and training was repeated until correct demonstration was observed. All participants were assessed as competent and included in the study.

2.2.6.3 Gamma scintigraphy to assess drug deposition in vivo

Four gamma scintigraphy scans were successively taken using the 2D gamma camera (Ecaml, Siemens), at PMH Nuclear Medicine department, all with a 120 second acquisition time. Firstly, prior to inhalation of the study drug, a scan was taken of a planar flood source containing ~37 MBq Tc$^{99m}$. Secondly, a transmission scan was taken of the participant using the same planar flood source to allow for assessment of attenuation of body tissues by gamma radiation (Macey & Marshall, 1982). Thirdly, immediately after the participant had inhaled the study drug, simultaneous anterior and posterior planar scintigraphy scans were taken of the chest and abdomen with lateral positioning of the head and neck. Lastly, Device components (pMDI actuator, spacer, exhalation filters) were scanned immediately after the participant. Times between all abovementioned scans were recorded in order to calculate decay correction in analysis stages. There was not more than a 15 minute duration between the initial scan and the final scan.

2.2.6.4 Gamma scintigraphy image acquisition and analysis

Images were acquired using Syngo VE31F software. Each participant had regions of interest (ROI) subjectively delineated for their transmission, anterior and posterior scan, by a single trained user. ROI were each whole lung, central and peripheral lung regions, oropharyngeal, oesophagus, and stomach. The central lung region was defined as a rectangle one third of the length and half
the width of a rectangular ROI drawn to just contain the entire lung, centred on the proximal boundary of the rectangle. The lung ROI, (Figure 2-4) except for the central box, was defined as peripheral lung (Figure 2-5). Total counts and the area in pixels were obtained for each ROI, the flood source, and a background count for transmission, device component, anterior and posterior scans.

Figure 2-4 Subjective delineation of the lungs depicting method for quantifying central and peripheral regions as described in 2.2.6.4. The total region of the lung (orange/red) is drawn around the whole lung (green) in order to measure a third of the length and half the width (blue box).

Figure 2-5 Depiction of peripheral to central ratios. Central region of lung depicted inside blue box. Anything not inside this region, but within the lung region (red/orange delineation) was considered peripheral deposition.
2.2.6.5 Using gamma scintigraphy data to calculate an attenuation corrected interpretation of emitted radiation in each region of interest (ROI)

Calculations were completed using the three steps below. Attenuation correction was done in accordance with (Macey & Marshall, 1982). All means below are geometric for the purpose of log-linear decay.

1) **Obtaining and calculating the geometric means of each ROI**

Corrected counts (one count is one disintegration of one radioactive nucleus) were determined (Equation 2-1) for all ROI; the lungs, oropharyngeal, oesophagus, stomach, and central and peripheral lung regions.

**Equation 2-1** Where: \( A \) = the background count in the 2D scan from an area equal to the ROI, subtracted from the total counts in each ROI, and divided by the area of the ROI in pixels. \( A \) was then divided by the acquisition time (120 seconds) to get the corrected counts per second (cps);

\[
\frac{A}{120} = \text{corrected (cps)}
\]

**Equation 2-2** The mean of the corresponding anterior and posterior (cps) was calculated;

\[
\sqrt{\text{anterior (cps)} \times \text{Posterior (cps)}} = \text{mean (cps)}
\]

2) **Obtaining and calculating an attenuation correction factor specific to each ROI.**

**Equation 2-3** Counts per pixel were obtained for the flood source by dividing area in pixels by the counts;

\[
\frac{\text{flood source counts}}{\text{area in pixels}} = \text{flood source counts per pixel}
\]
**Equation 2-4** Using the decay correction formula the flood source counts per pixel were adjusted to the time of the patient scan. Where \( A \) = the negative time from the patient scan to the flood source scan divide by the half-life of technetium in minutes;

\[
\text{flood source counts per pixel} \times 2^{(A)} = \text{decay corrected flood source counts per pixel}
\]

**Equation 2-5** Transmission counts per pixel were also corrected to the time of the patient scan using the decay correction formula. Where \( A \) = the negative time from the patient scan to the transmission scan divide by the half-life of technetium;

\[
\text{transmission counts per pixel} \times 2^{(A)} = \text{decay corrected transmission counts per pixel}
\]

**Equation 2-6** Transmission counts per pixel were then calculated by;

\[
\frac{\text{decay corrected transmission counts}}{\text{area in pixels}} = \text{transmission counts per pixel}
\]

**Equation 2-7** The attenuation of activity of the flood source counts per pixel by body tissues are then calculated for each ROI;

\[
\sqrt{\frac{\text{decay corrected flood source counts}}{\text{transmission counts per pixel}}} = \text{attenuation factor}
\]

**Equation 2-8** The mean (cps) is then multiplied by the attenuation correction factor to obtain attenuation corrected (cps) in a region of interest;

\[
\text{mean (cps) in region of interest} \\
\times \text{attenuation correction factor} \\
= \text{attenuation corrected mean cps}
\]
3) Using the attenuation corrected means to interpret radiation per ROI.

Equation 2-9 Attenuation corrected (cps) of the means were converted to a reading of MBq in each ROI;

\[
\frac{\text{mean (cps)} \times \text{attenuation factor}}{\text{camera efficiency}} = \text{Mbq in ROI}
\]

This MBq reading in each ROI is defined as the percent of total detected dose.

2.2.6.6 Camera efficiency

Camera efficiency was measured as cps per MBq, and this measurement was taken on the study day by using a uniform flood source of known concentration with a lookup table (Radpharm®). The lookup table was based on known constant decay rates for a Cobalt-57 flood source (see appendices 5.8). The average of measurements taken (n=6) was used to interpret all participant data (105 cps/MBq). The efficiency was confirmed by camera servicing history to have little inter-day variation.

2.2.7 STATISTICAL ANALYSIS

Gamma scintigraphy deposition data was preliminarily assessed via t-test in paired analysis with and without a spacer for each drug group using GraphPad Prism. Group demographics for FP and BDP were assessed with a Chi-Square test (SAS) for categorical variables; sex, age, height, weight and BMI.

To complete multivariate analysis, drug and actuator deposition variables were transformed using the arcsine square root transformation for analysis with SAS ver. 9.4. The BMI variable was used to simplify variables of weight and height. Univariate assessment first looked at the relationship on total lung deposition with each variable separately, in each drug group; age, sex, body mass index (BMI), visit order, sequence, actuator deposition, and spacer. Multivariate assessment was then employed to look at total lung deposition in each drug group (proc mixed, SAS) in a full model including variables: age, sex, body mass index (BMI), visit order, sequence, actuator deposition, spacer and random effect (a compulsory variable for mixed models). Multivariate analysis with backward selection (significance level of 0.05) further assessed the relationship.
All statistical analyses using SAS version 9.4 for Windows (SAS Institute Inc., USA) were performed with assistance from the Centre for Applied Statistics, at the University of Western Australia. Analysis done with GraphPad Prism v. 7.02 (GraphPad Software, Inc.) were performed unassisted.

2.2.7.1 Power calculations
A pre-study calculation based on previous data within our group (C. M. Roller, Zhang, Troedson, Leach, Le Souef, et al., 2007) showed that with a sample size of (n=28), 14 in each of the experimental groups, the study had 80% power to detect a 10% difference in lung dose at alpha=0.5.
3 CHAPTER III: RESULTS

3.1 IN VITRO VALIDATION OF RADIOLABELLED DRUG PREPARATION TO DETERMINE IN VIVO REPORTING

To confirm successful validation, particle size distributions (with stages divided into four groups), are shown for fluticasone propionate (FP) and beclomethasone dipropionate (BDP) in Figure 3-1 and 3-2 respectively, with the corresponding ratio comparison displayed in Table 3-1 and 3-2 respectively. Additional confirmation that radiolabelling the commercial drug has not significantly altered the aerodynamic diameter of the labelled drug is shown via total recoveries of mass (Figure 3-1, A, C FP, and Figure 3-2, A, C, BDP) for pre-study and study day reference and labelled drugs.
Figure 3-1 (A, C) Recovery of FP 125 μg per actuation, comparison of reference to labelled drug. The corresponding ratios of reference to labelled drug (A, C) were 0.89 and 0.97 respectively. Particle size distribution was divided into four groups, to determine if the labelled drug and radiotracer counterpart fall within the 95% CI of the reference drug (B, D).

Table 3-1 Fluticasone propionate particle size distribution assessment comparisons. Ratios confirmed that validation was successful when within the range of 0.85–1.18 or ±2% for the groups that had under 10% of the total drug.

<table>
<thead>
<tr>
<th>Stage of ACI:</th>
<th>Actuator to throat (difference from reference)</th>
<th>Jet to 0</th>
<th>One to two (difference from reference)</th>
<th>Three to filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio obtained from</td>
<td>Pre-study</td>
<td>Study day</td>
<td>Pre-study</td>
<td>Study day</td>
</tr>
<tr>
<td>Reference /labelled canister</td>
<td>1.12</td>
<td>1.06</td>
<td>±2%</td>
<td>±2%</td>
</tr>
<tr>
<td>Reference/ radiotracer</td>
<td>0.95</td>
<td>1.07</td>
<td>±2%</td>
<td>±2%</td>
</tr>
</tbody>
</table>
Figure 3-2 (A, C) Recovery of BDP 100 μg per actuation, comparison of reference to labelled, The corresponding ratios of reference to labelled drug (A,C) were 1.03, and 0.97 respectively. Particle size distribution was divided into four groups, to determine if the labelled drug and radiotracer counterpart fall within the 95% CI of the reference drug (B, D).

Table 3-2 Beclomethasone di-propionate particle size distribution assessment comparison. Ratios confirmed that validation was successful when within the range of 0.85–1.18 or ±2% for the groups that had under 10% of the total drug.

<table>
<thead>
<tr>
<th>Stage of ACI</th>
<th>Actuator to throat</th>
<th>Jet to 0 (difference from reference)</th>
<th>One to two (difference from reference)</th>
<th>Three to filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio obtained from</td>
<td>Pre-study</td>
<td>Study day</td>
<td>Pre-study</td>
<td>Study day</td>
</tr>
<tr>
<td>Reference /labelled canister</td>
<td>1.08</td>
<td>1.11</td>
<td>±2%</td>
<td>±2%</td>
</tr>
<tr>
<td>Reference/radiotracer</td>
<td>1.07</td>
<td>1.04</td>
<td>±2%</td>
<td>±2%</td>
</tr>
</tbody>
</table>
3.2 RETROSPECTIVE ANALYSIS OF RADIOLABELLED FP AND BDP

Confirmation of successful validation of previous data, as described in C. Roller (2012) for FP and C. M. Roller, Zhang, Troedson, Leach, Le Souef, et al. (2007) for BDP, particle size distributions (with stages divided into four groups), is shown, reanalysed, for fluticasone propionate (FP) and beclomethasone dipropionate (BDP) in Figure 3-4 and 3-6 respectively. Corresponding ratio comparisons are displayed in table 3-3 and 3-4 respectively. Additional confirmation that radiolabelling the commercial drug has not significantly altered the aerodynamic diameter is shown via total recoveries of mass (Figure 3-4, A, C, FP, and Figure 3-6, A, C, BDP) for pre-study and study day reference and labelled drugs. Radiolabelling validation results for FP from this study (3.1, Figure 3-1) were compared to retrospectively analysed results (3.2, Figure 3-4) confirming that the radiolabelling method is robust over time. FP particle size distribution varied with each study due to the difference in mass delivered per actuation (125 and 250 μg), Figure 3-3, which was not the case for BDP (Figure 3-5) as both studies used 100 μg per actuation. The lower dose used for FP in this study resulted in a higher respirable fraction (Third to Filter), and lower Actuator to Throat fraction. Radiolabelling validation results for BDP from this study (3.1, Figure 3-2) were compared to retrospectively analysed results (3.2, Figure 3-6) confirming that the radiolabelling method is robust over time.
Figure 3-3 Grouped comparison of two different radionuclide validation results for FP, 125 μg (hatched) and 250 μg per actuation. Grouped comparison of mean radiotracer and labelled to mean reference (95% CI) for FP radiolabelling method shows little variation over time or between users. Labelled drug (Dark Grey) and Radiotracer (Black) were marginally outside the 95% CI of the Reference (Light Grey) drug, similar over time from 2006 (filled columns) to 2016 (hatched columns).
Figure 3-4 (A, C) Recovery of FP 250 μg per actuation, comparison of reference to labelled, (B, D) Corresponding ratio between the reference to labelled drug (A,C) was 1.13, and 1.09 respectively. Particle size distribution was divided into four groups, to determine if the labelled drug and radiotracer counterpart fall within the 95% CI of the reference drug (B, D).

Table 3-3 Fluticasone propionate particle size distribution assessment comparisons. Ratios confirmed that validation was successful when within the range of 0.85–1.18 or ±2% for the groups that had under 10% of the total drug.

<table>
<thead>
<tr>
<th>Stage of ACI</th>
<th>Actuator to throat (difference from reference)</th>
<th>Jet to 0 (difference from reference)</th>
<th>One to two (difference from reference)</th>
<th>Three to filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio obtained from</td>
<td>Pre-study</td>
<td>Study day</td>
<td>Pre-study</td>
<td>Study day</td>
</tr>
<tr>
<td>Reference /labelled canister</td>
<td>0.91</td>
<td>1.02</td>
<td>±2%</td>
<td>±2%</td>
</tr>
<tr>
<td>Reference/radiotracer</td>
<td>0.93</td>
<td>0.99</td>
<td>±2%</td>
<td>±2%</td>
</tr>
</tbody>
</table>
Figure 3-5 Grouped fraction comparison of two different radiolabelled validation results for BDP, both used 100 μg per actuation. Grouped comparison of mean radiotracer and labelled to mean reference (95% CI) for BDP radiolabelling method shows no variation over time or between users. Labelled drug (Dark Grey) and Radiotracer (Black) were comparable to the 95% CI of the Reference (Light Grey) drug, the integrity of the radiolabelling method unchanging over time from 2006 (filled columns) to 2016 (hatched columns).
Figure 3-6 (A, C) Recovery of BDP 100 μg per actuation, comparison of reference to labelled. Corresponding ratio between the reference to labelled drug (A,C) was 0.93, 0.94 respectively. Particle size distribution was divided into four groups, to determine if the labelled drug and radiotracer counterpart fall within the 95% CI of the reference drug (B, D).

Table 3-3 Beclomethasone di-propionate particle size distribution assessment comparison. Ratios confirmed that validation was successful when within the range of 0.85–1.18 or ±2% for the groups which had under 10% of the total drug.

<table>
<thead>
<tr>
<th>Stage of ACI</th>
<th>Actuator to throat</th>
<th>Jet to zero (difference from reference)</th>
<th>One to two (difference from reference)</th>
<th>Three to filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio obtained from</td>
<td>Pre-study</td>
<td>Study day</td>
<td>Pre-study</td>
<td>Study day</td>
</tr>
<tr>
<td>Reference /labelled canister</td>
<td>0.98</td>
<td>0.97</td>
<td>±2%</td>
<td>±2%</td>
</tr>
<tr>
<td>Reference/radiotracer</td>
<td>0.90</td>
<td>0.93</td>
<td>±2%</td>
<td>±2%</td>
</tr>
</tbody>
</table>
3.3  IN VIVO DRUG DEPOSITION STUDY RESULTS

3.3.1  STUDY POPULATION

The demographic information for the cohort is displayed in Table 3-5 for the full cohort. The mean demographics assessed were similar within drug groups (Table 3-6) and to the full cohort demographic means (Table 3-5). There was no significant difference between groups in any variable (Table 3-6).

Table 3-4 Full cohort (n=14) demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean, Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (n)</td>
<td>6 Male, 8 Female</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>15 y, 13–17 years</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169, 158–179</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67, 46–110</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.4, 16–40</td>
</tr>
</tbody>
</table>
Table 3-5 Group demographics separated for BDP and FP

<table>
<thead>
<tr>
<th>Variable</th>
<th>BDP</th>
<th></th>
<th></th>
<th>FP</th>
<th></th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
<td>P-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>1 Male, 6 Female</td>
<td>5 Male, 2 Female</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>15 years</td>
<td>14–16 years</td>
<td>15 years</td>
<td>13–17 years</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td></td>
<td>166 cm</td>
<td>158–175 cm</td>
<td>172 cm</td>
<td>157–179 cm</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td>64 kg</td>
<td>46–89 kg</td>
<td>71 kg</td>
<td>47–110 kg</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td>23</td>
<td>18–29</td>
<td>24</td>
<td>17–40</td>
<td>0.76</td>
<td></td>
</tr>
</tbody>
</table>
3.3.1.1 Screening questionnaire data

All participants (n=14) completed a screening questionnaire (Figure 3-7), involving adherence to medication and related issues. Approximately 50% of participants who required a spacer used it regularly. Over 75% of participants who were prescribed inhaled corticosteroids for their asthma reported regularly taking them, but only 25% of those participants said they take them every day (Figure 3-7).

![Survey of adherence to use of asthma drugs by study participants (n=14)](image)

Figure 3-7  Survey of adherence to pMDI delivered corticosteroid asthma drugs and related questions in mildly asthmatic adolescents. Nearly 70 % of participants did not adhere to their prescribed daily puffer medication, and of those who did approximately half used their spacers.
3.3.2 SHOT WEIGHT DELIVERED BY PMDI

The dose of drug delivered to each participant, measured via mass, is shown in Figure 3-8. Two significant outliers were observed with BDP, and two with FP, both confirmed to be due to inhaler variability per shot (Figure 3-8).

![Shot weight (mean SD, FP labelled)](image)

![Shot weight (mean SD, BDP labelled)](image)

Figure 3-8 Mass of drug delivered to each participant on study day reduced in variability with multiple shots per mean. Variability of the same labelled canister reduced when obtaining the mean of 5 and 10 actuations, showing natural variability, and not an effect of a flawed radiolabelled drug. Additionally, inter user variability was present at n=2. Timepoints were obtained successively for n=5, 10 and 2.

3.3.3 GAMMA SCINTIGRAPHY IN VIVO DEPOSITION AFTER INHALATION OF RADIOLABELLED DRUG

Gamma scintigraphy images were taken, obtained, and analysed, according to methods sections 2.2.6.3, 2.2.6.4, and 2.3.6.5, respectively. Results of gamma scintigraphy deposition data for FP and BDP are displayed regionally in Figure 3-9 and Table 3-7. Deposition data was defined as percentage of total ex-valve dose as detected via gamma camera (MBq). Data show a fivefold decrease in oropharyngeal deposition with a spacer with FP, (p= 0.001), although only twofold decrease was seen with BDP (p=0.03) (Figure 3-9, A). Lung deposition was higher with a spacer, although not significantly higher with FP (p=0.31), or BDP (p=0.52) (Figure 3-9, B). Mean peripheral to central ratio with was higher with a spacer (FP p=0.16, BDP p=0.81).
Figure 3-9 Univariate analysis after transformation of gamma scintigraphy deposition data, as percentage of ex-valve, for FP (n=7) and BDP (n=7): A) mean±SD paired oropharyngeal deposition, after inhalation with radiolabelled drug, B) mean±SD paired lung deposition after inhalation of radiolabelled drug C) mean±SD regional lung deposition after inhalation of radiolabelled drug as a peripheral to central ratio.
Table 3-6 Comparison of mean (SD) deposition data for purpose of cross study comparison. Deposition values are displayed for each region of interest as percent of ex-valve or ex-actuator

<table>
<thead>
<tr>
<th>Region</th>
<th>FP Spacing</th>
<th>pMDI only</th>
<th>BDP Spacing</th>
<th>pMDI only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ex-valve (%)</td>
<td>Ex-actuator (%)</td>
<td>Ex-valve (%)</td>
<td>Ex-actuator (%)</td>
</tr>
<tr>
<td><strong>Actuator</strong></td>
<td>20±9</td>
<td>-</td>
<td>18±8</td>
<td>-</td>
</tr>
<tr>
<td><strong>Lung</strong></td>
<td>37±6</td>
<td>46±5</td>
<td>33±9</td>
<td>40±13</td>
</tr>
<tr>
<td><strong>OP</strong></td>
<td>9±7</td>
<td>11±8</td>
<td>49±12</td>
<td>59±13</td>
</tr>
<tr>
<td><strong>Spacer</strong></td>
<td>33±9</td>
<td>41±11</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Exhaled</strong></td>
<td>1±2</td>
<td>2±2</td>
<td>&lt;0.5±1</td>
<td>&lt;1±1</td>
</tr>
</tbody>
</table>
Visual review of the gamma scintigraphy images, shown for FP in Figure 3-10, revealed higher oropharyngeal deposition (OP) and deposition around the major bifurcations, with hot spots in the lungs, particularly when drug was administered via a pMDI without a spacer. BDP deposition visually showed an even spread in the lungs with either device, and a slight decrease in OP deposition when a spacer was used (Figure 3-11). Images were not altered after obtaining from software.

Figure 3-10 Qualitative analysis of gamma scintigraphy lung deposition images (FP). Image data show paired data; (A) Deposition with pMDI alone, (B) Deposition with pMDI/spacer combination.

Figure 3-11 Qualitative analysis of gamma scintigraphy lung deposition images (BDP). Image data show paired data; (A) Deposition with pMDI alone, (B) Deposition with pMDI/spacer combination.
3.3.4 STATISTICAL ANALYSIS OF GAMMA SCINTIGRAPHY DEPOSITION DATA AFTER TRANSFORMATION

When each variable was analysed separately with drug with BDP, percent deposition in the actuator significantly reduced lung deposition (Table 3-8). After adjustment in a full model, age, sex and BMI additionally affected lung deposition with BDP only, and remained significant after backward elimination at significance level alpha 0.05 (Table 3-8).

Table 3-7 Analysis of regional deposition data after transformation. Lung deposition from each drug was analysed: with a single variable (Spacer etc.), in a full model to adjust for confounders, then variables were eliminated via backward selection at 0.05 level of significance, until the final model was left (exit point p-values displayed).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Full Model (adjusted)</th>
<th>Final Model (backward selection)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FP</td>
<td>BDP</td>
<td>FP</td>
</tr>
<tr>
<td>Drug</td>
<td>FP</td>
<td>BDP</td>
<td>FP</td>
</tr>
<tr>
<td>Spacer</td>
<td>0.27</td>
<td>0.45</td>
<td>0.51</td>
</tr>
<tr>
<td>Age</td>
<td>0.92</td>
<td>0.57</td>
<td>0.44</td>
</tr>
<tr>
<td>Sex</td>
<td>0.28</td>
<td>0.36</td>
<td>0.61</td>
</tr>
<tr>
<td>Randomisation order</td>
<td>0.90</td>
<td>0.70</td>
<td>0.25</td>
</tr>
<tr>
<td>Visit order</td>
<td>0.16</td>
<td>0.74</td>
<td>0.33</td>
</tr>
<tr>
<td>BMI</td>
<td>0.12</td>
<td>0.20</td>
<td>0.09</td>
</tr>
<tr>
<td>Actuator</td>
<td>0.54</td>
<td>&lt;0.0001</td>
<td>0.58</td>
</tr>
</tbody>
</table>
CHAPTER IV: DISCUSSION

Lung deposition with use of the pMDI producing the extrafine aerosol (BDP), or the coarse aerosol (FP) by adolescents in this study was not significantly different with or without use of a spacer, and peripheral airway deposition was equivalent regardless of spacer use. Whilst the MMAD for FP was assessed here to be 2.8 μm and is therefore marginally a coarse aerosol, it appears to have some of the characteristics demonstrated by a fine aerosol (≤2.5 μm MMAD) in this study, perhaps as it is very marginally coarse. Our findings for BDP, QVAR™, are consistent with a previous study of this drug formulation that reported equivalent lung deposition with or without a spacer in an adult cohort (C. L. Leach & Colice, 2010). Furthermore, levels of lung deposition found here with pMDI producing an extrafine aerosol are consistent in both adult populations without a spacer (CL Leach et al., 1998; C. L. Leach, Davidson, Hasselquist, & Boudreau, 2002; C. L. Leach et al., 2005), and child populations with a spacer, assuming correct and reproducible inhalation technique (C. M. Roller, Zhang, Troedson, Leach, Le Souef, et al., 2007). The recommendation for a spacer as an additional device would thus be unnecessary with an extrafine preventer aerosol (≤~2 μm MMAD), assuming reproducible results in a larger adolescent cohort or other supportive evidence such as that obtained by Guilbert et al. (2017), who saw no clinical improvement with use of a spacer compared to pMDI alone in a retrospective study of patient clinical data. A combination of epidemiological and/or survey methods combined with evidence based studies such as ours, would provide the best approach to supporting change in practice.

Implementing this change in practice would make progress toward addressing the long overdue issue of incorrect pMDI usage, shown to be unchanged over the 40 years prior to 2014 in a systematic review by Sanchis et al. (2016). The Global Initiative for Asthma identifies incorrect pMDI use as a contributor to decreased asthma control levels (Reddel et al., 2015) which are shown to be low - 24% of adolescents with asthma in the Asia Pacific region have uncontrolled asthma, a further 63% only partly controlled, and only 13% well controlled (Gold, Thompson, Salvi, Faruqi, & Sullivan, 2014). Sanchis et al. (2016) also found that, despite spacer use, pMDI technique remained poor.
Interestingly, the recommendation for use of a spacer currently attempts to address incorrect inhaler use. Furthermore, spacer use has proved difficult to implement in practice, particularly in populations that require special clinical management to encourage medication compliance, such as adolescents (Michaud, 2007), and so it seems that change to this practice is also long overdue and would particularly benefit the adolescent population.

Adolescents are a special population whose adherence to medication is directly influenced by healthy family psychosocial functioning, where family support can improve autonomy in the journey toward adulthood and help foster a positive attitude toward medication self-management (Rhee et al., 2010). Positive attitude toward self-management can include increased likelihood of visiting a health professional when required – and a good relationship with the health care provider is key to improving asthma control levels (Haskard Zolnierek & DiMatteo, 2009). Conversely, when this support is not available, issues with self-management become apparent and negatively impact asthma control, increasing morbidity and the risk of hospitalisation (Chen et al., 2003; Rhee et al., 2010). These issues can be further compounded by a multitude of generally accepted issues with asthma treatment: inadequacies with the medication delivery itself, inadequate health education contributing to false beliefs about the safety of asthma medication or the chronic nature of the disease, and false perceptions about the ease of effective drug delivery contributing to non-compliance with treatment recommendations (spacer use). Whilst improving family psychosocial functioning is an infinitely more challenging task, those compounding issues can more easily be addressed, such as, by improving drug delivery, and investigating the requirement (or not) for spacer use, (particularly as adolescents display an aversion to using them). Real-life outcomes of spacer use have been studied by Guilbert et al. (2017), however, to our knowledge ours is the first study to assess the clinical recommendation, via lung deposition study, for spacer use with ICS formulations produced by pMDI, both with and without a spacer, in a solely adolescent population. Our study widens already established findings that show increased delivery efficacy in adults, of an extrafine aerosol, by extending that finding to the adolescent age group.

Our study showed a high lung to oropharyngeal deposition ratio per dose with the extrafine aerosol only (i.e. 60:40 lung to oropharyngeal). The findings in our
study are supported extensively with previous studies for the extrafine aerosol, showing consistent lung deposition of ~55% (ex-actuator dose) and oropharyngeal deposition of ~40% (ex-actuator dose) with a pMDI alone (CL Leach et al., 1998; C. L. Leach et al., 2002, 2005). Limited lung deposition studies were available to compare our coarse aerosol data with, none with and without a spacer in any population. With the coarse aerosol pMDI alone, we recorded lung deposition of ~40% (ex-actuator), which contrasted a previous adult study reporting ~25% (ex-actuator). With a pMDI+spacer, however, we reported lung deposition of ~45% (ex-actuator), comparable to a previous study (C. Roller, 2012) reporting ~25% (SD 10), range 15–45%, (ex-actuator) in a child cohort. It is reasonable to conceive a higher lung deposition would be observed in an older cohort and lung deposition may have been additionally increased by the lower dose per actuation used in our study. As seen in our validation retrospective analysis, the higher dose per actuation (250 μg) used by C. Roller (2012) resulted in a decreased respirable fraction, compared to our lower dose (125 μg). At higher doses, particles may coagulate after aerosolisation, resulting in larger particles, and thus a decreased dose delivered to the lungs (Tolpekin, Duits, van den Ende, & Mellema, 2004; Tsuda et al., 2011). Use of a lower dose may reduce the potential of this occurring. Considering a 44 μg dose was used by C. L. Leach et al. (2015) this may also confirm that the dose used in our study was optimal for a pMDI producing a coarse aerosol. Alternatively, a lower dose estimate consistent with that obtained by C. L. Leach et al. (2015) may be more reasonable, which could highlight the importance of recognising even minor inconsistencies from radiolabelling validation guidelines, as seen with fluticasone propionate in our study. Radiolabelling validation of fluticasone propionate as previously reported by C. L. Leach et al. (2015) and C. Roller (2012) however, demonstrably similar inconsistencies to our study, such as the “tailing effect”, where <5% of total un-incorporated radiation is observed. Thus, it is unlikely to affect study comparability, although exact ratios and confidence intervals were not provided by C. L. Leach et al. (2015) and so a more accurate reflection cannot be made. To recommend the required change against spacer use in clinical practice, evidence of high lung to oropharyngeal deposition ratio per dose is necessary. The concern where this is absent is an increased systemic ICS bioavailability and resultant side effects. Our results confirm that when using a pMDI
producing extrafine aerosol, you receive a higher respirable fraction of the total dose, which corresponds to an increased therapeutic dose.

Our study in adolescents demonstrated equivalent lung deposition regardless of spacer use, or drug formulation, as has been found previously with an extrafine aerosol only (C. L. Leach & Colice, 2010). This indicates a spacer may not be needed to improve lung deposition with a coarse aerosol or with an extrafine aerosol, building on a previous epidemiological study where spacer use did not improve asthma outcomes for a fine or extrafine aerosol (Guilbert et al., 2017). The observed equivalence in lung deposition regardless of spacer use or drug in our study, may be due to the relatively small difference in MMAD of the chosen drugs (2.8 µm versus 1.2 µm), with the coarse aerosol consideration for a fine aerosol (fine aerosol= >0.1–2.5 µm) (Australian Government, 2004). Alternatively, it could be an effect of repeated inhaler technique training, (Kamps et al., 2000) although statistical analysis in this study did not find an association, i.e. there was no evidence of greater lung deposition on the second visit compared with the first. It is commonly accepted that inhaler technique remains poor in practice (Price et al., 2013; Sanchis et al., 2016) regardless of technique assessment by clinical observation (Biswas et al., 2016), yet this may be substantially due to insufficient or incomplete initial training programs (Haahtela et al., 2006; Papi et al., 2011). In this study adolescents were considered able to coordinate an adequate inhalation technique, during training with a clinical nurse asthma specialist, which suggests a spacer may not necessarily be required to decrease the effects caused by lack of coordination under these circumstances. Whilst our small sample size limits generalisable conclusions, our data emphasises the potential of an appropriately and repeatedly trained, clinically observed, adolescent to use a pMDI producing an inhaled corticosteroid aerosol <2.8 µm MMAD to achieve approximately equivalent lung deposition to that seen with use of the same drug with a spacer. A short acting beta 2 agonist, even if formulated with a <2.8 µm MMAD may not produce a similar result, as a symptomatic patient may give a different pattern of inhalation; usually an increase in rate of inhalation and a resulting more central and oropharyngeal deposition (Laube, Norman, & Adams, 1992). Additionally, coordination may not be optimal when patients are symptomatic and so the user may still benefit from use of a spacer.
With the pMDI producing an extrafine aerosol we reported an increased proportion of the total dose depositing in the peripheral airways regardless of spacer use. This finding is comparable to other studies and demonstrates no effect of spacer on regional deposition (C. L. Leach et al., 2006; C. L. Leach et al., 2015). Whilst the 2D imaging method used in this study may be limited in its ability to estimate regional lung deposition compared to 3D methods, C. L. Leach et al. (2006) confirmed this systematic under- and over-estimation was marginal. Additionally, a recently validated diagnostic method for 2D assessment of lung deposition showed increased sensitivity in detecting regional deposition, and could reduce this bias in future studies (Bennett, Xie, Zeman, Hurd, & Donaldson, 2014). Based on our study, it is reasonable to assume a spacer is not required for use, additional to a pMDI producing an extrafine aerosol, to increase peripheral deposition in this age group. This may mean that a future focus on pMDIs producing a fine aerosol, particularly those considered extrafine (≤~2 μm MMAD) as used in our study, would be a useful approach to target peripheral lung disease.

If clinical practice was to change, based on this and larger future studies, it would highlight the need for improved clinician and patient pMDI technique training programs, particularly from the point of initial treatment (Fink & Rubin, 2005; Haahtela et al., 2006). PMDs are cheap and portable devices for orally inhaled delivery of corticosteroids, and the most recommended device worldwide for the treatment of asthma, (Brocklebank et al., 2001) however, clearly, the problem of inhaler technique compliance still exists outside of a clinical setting, and is associated with an increased risk of exacerbation and hospitalisation (Levy et al., 2013; Sanchis et al., 2016). An improvement in education and training for the delivery of ICS, if combined with easy to implement therapies is advantageous to the wider population, and particularly valuable to special populations with reduced medication adherence, such as adolescents (Dima et al., 2015). The extrafine aerosol formulation produced by pMDI used in this study shows a repeatable increase in lung deposition when compared to coarse aerosols, and is already available in Australia and worldwide, emphasising the possibility for imminent changes to clinical recommendation (CL Leach et al., 1998; C. L. Leach et al., 2006; C. L. Leach et al., 2002, 2005; C. L. Leach et al., 2012; C. L. Leach et al., 2015).
Based on results of this study, significant effectors of lung deposition with an extrafine aerosol, such as deposition of drug in the actuator, BMI, sex, and age, may be useful inclusions of future study designs. The effect of repeated training (inclusion of a visit order variable) is a particularly recommended inclusion.

The introduction of the international guidelines for inhaled radiolabelled preparations in 2012 (Devadason et al., 2012), should lead to improved consistency of reporting and so it is increasingly possible that studies completed under the new guidelines, such as ours, are easily comparable. Evidence from this and previous studies suggests that the MMAD of an aerosol has a significant effect on lung deposition with ICS asthma drug formulations. PMDs producing a fine aerosol (MMAD<2.5 µm), specifically those considered extra-fine (MMAD ~2 µm), result in almost double the lung deposition and half the oropharyngeal deposition, achieved in previous studies using coarse (>2.5–10 µm MMAD) formulations. This emphasises the necessity of changes to clinical recommendations, such as spacer use, particularly for the adolescent population, where ease of implementation is especially important.

4.1 CONCLUSION

Results presented here investigate the MMAD of two inhaled corticosteroids, and pragmatically addresses the recommendation for spacer use in a population whom are less likely to manage their disease optimally, more likely to have poor asthma control, and express an aversion to use the spacer device. It builds on prior work done in adults showing that an extrafine aerosol can achieve equivalent lung deposition with or without a spacer, when inhaled with good technique, by expanding these same findings to an adolescent age group. It supports prior epidemiological work showing no improvements in asthma outcomes are evident with spacer use with a fine ICS aerosol. This thesis shows that, whether or not a spacer is used, an extrafine preventer aerosol achieves equivalent regional and total lung deposition and comparable oropharyngeal dose. Considering our findings in the extrafine aerosol are supported by previous work, discontinuing spacer recommendation for this formulation could potentially simplify asthma treatment in non-compliant patients.


disease (COPD) in adults and for use in the treatment of asthma in children and adolescents.


Leach, C. L., Davidson, P. J., Hasselquist, B. E., & Boudreau, R. J. (2002). Lung deposition of hydrofluoroalkane-134a beclometasone is greater than that of chlorofluorocarbon fluticasone and chlorofluorocarbon beclomethasone: A cross-over study in healthy volunteers. Chest, 122(2), 510. doi:info:doi/


Lipworth, B. J. (1999). Systemic adverse effects of inhaled corticosteroid therapy: A systematic review and meta-analysis. *Archives of Internal Medicine, 159*(9), 941-955. doi:10.1001/archinte.159.9.941


doi:10.1111/all.13212


National Asthma Council Australia, M. (2016b). Inhaler technique for people with asthma or COPD.


Quante, T., Ng, Y. C., Ramsay, E. E., Henness, S., Allen, J. C., Parmentier, J., . . . Ammit, A. J. (2008). Corticosteroids reduce IL-6 in ASM cells via up-


Van Den Berge, M., Luijk, B., Bareille, P., Dallow, N., Postma, D. S., & Lammers, J. W. J. (2010). Prolonged protection of the new inhaled corticosteroid fluticasone furoate against AMP hyperresponsiveness in...
patients with asthma. *Allergy, 65*(12), 1531-1535. doi:10.1111/j.1398-9995.2010.02414.x


5 APPENDICES

5.1 Materials and equipment for radiolabelled drug preparation, administration and gamma scintigraphy

<table>
<thead>
<tr>
<th>Chemical/Reagent</th>
<th>Source</th>
<th>Lot Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium Hydroxide</td>
<td>Sigma-Aldrich</td>
<td>11308JB</td>
</tr>
<tr>
<td>Beclomethasone dipropionate</td>
<td>Sigma-Aldrich</td>
<td>SLBC702 3V</td>
</tr>
<tr>
<td>Butanone</td>
<td>Sigma-Aldrich</td>
<td>K3148317 8 248</td>
</tr>
<tr>
<td>Fluticasone propionate</td>
<td>Sigma-Aldrich</td>
<td>034M472 2V</td>
</tr>
<tr>
<td>Liquid nitrogen</td>
<td>BOC, Perth Australia.</td>
<td></td>
</tr>
<tr>
<td>Methanol (High Performance Liquid Chromatography grade)</td>
<td>Thermofischer</td>
<td></td>
</tr>
<tr>
<td>Nitrogen gas</td>
<td>BOC, Perth Australia.</td>
<td>1956A</td>
</tr>
<tr>
<td>$^{99m}$Tc $^{99m}$Technetium sodium pertechnetate (Na$^{99m}$TcO$_4$)</td>
<td>Molybdenum/$^9$ $^{9m}$Tc generator, Australian Radioisotopes, Lucas Heights NSW OR $^{99m}$Tc pertechnetate Global Medical Solutions, Perth Radiopharmacy. Australia</td>
<td></td>
</tr>
<tr>
<td>Tetraphenylarsonium chloride</td>
<td>Sigma-Aldrich</td>
<td></td>
</tr>
<tr>
<td>Pyroneg Detergent</td>
<td>JohnsonDiversey PTY LTD, Australia</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Source</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma Camera ECAM</td>
<td>Siemens, GE Healthcare, USA.</td>
<td></td>
</tr>
<tr>
<td>Perspex collimator flood source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High resolution collimators</td>
<td>Siemens, GE Healthcare, USA.</td>
<td></td>
</tr>
</tbody>
</table>
## 5.2 Materials and equipment for assessment of drug particle size distribution

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson cascade impactor</td>
<td>Copley Scientific, Nottingham UK.</td>
</tr>
<tr>
<td>Air Pump</td>
<td>Copley Scientific, Nottingham UK.</td>
</tr>
<tr>
<td>Boss head clamp</td>
<td></td>
</tr>
<tr>
<td>Clamp stand</td>
<td></td>
</tr>
<tr>
<td>Clamp scissors</td>
<td></td>
</tr>
<tr>
<td>Gilson’s pipettes, 20 μl, 200 μl, 1000 μl (Pipetteman Neo)</td>
<td>John Morris Scientific, Willoughby, NSW, Australia.</td>
</tr>
<tr>
<td>Glass cuvette</td>
<td>Starna PTY LTD,</td>
</tr>
<tr>
<td>Funnel</td>
<td>Labfriend Australia</td>
</tr>
<tr>
<td>Heatbox with wells</td>
<td>Barnstead International</td>
</tr>
<tr>
<td>Manual Crimper</td>
<td>Willi mader AG, Pfaffikon Switzerland.</td>
</tr>
<tr>
<td>Lead Box</td>
<td>Custom made, PMH Physical resources department.</td>
</tr>
<tr>
<td>Lead containers and Lead-glass shielding</td>
<td>Global medical solutions, Osbourne park, WA</td>
</tr>
<tr>
<td>O ring clamp</td>
<td></td>
</tr>
<tr>
<td>Platform shaker</td>
<td>Ratek Instruments Pty LTD</td>
</tr>
<tr>
<td>Scintillation counter</td>
<td>Capintec Inc.</td>
</tr>
<tr>
<td>Scales</td>
<td>A &amp; D Company Ltd</td>
</tr>
<tr>
<td>Separation funnel</td>
<td></td>
</tr>
<tr>
<td>Tweezers</td>
<td></td>
</tr>
<tr>
<td>Tongs</td>
<td>Global Medical solutions, Osbourne park, Western Australia.</td>
</tr>
<tr>
<td>Ultra Violet-Visible Spectrophotometer, Model 1601</td>
<td>Shimadzu Scientific, Japan</td>
</tr>
<tr>
<td>Pipe cutter</td>
<td>Newman Tools Inc. Hartford, CT USA.</td>
</tr>
<tr>
<td>25 mL volumetrics glassware</td>
<td></td>
</tr>
<tr>
<td>Wheaton Glass scintillation vials</td>
<td>Thomas scientific, Swedesboro, NJ USA.</td>
</tr>
</tbody>
</table>
5.3 Consumables for radiolabelling and gamma scintigraphy on study day

<table>
<thead>
<tr>
<th>Consumables</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compact Space Chamber Plus® spacer</td>
<td>Allersearch, VIC, Australia. Owned by EBOS Group Pty Ltd.</td>
</tr>
<tr>
<td>Exhalation Filters</td>
<td></td>
</tr>
<tr>
<td>QVAR™, 100 μg beclomethasone di-propionate per actuation</td>
<td>iNova Pharmaceuticals (Aust) Pty Ltd</td>
</tr>
<tr>
<td>Flixotide®, 125 μg fluticasone propionate per actuation</td>
<td>Allen &amp; Hanburys respiratory care. Division of GlaxoSmithKline</td>
</tr>
</tbody>
</table>
5.4 Parent information and consent form

PARENT/GUARDIAN INFORMATION SHEET

Project title
The effect of particle size on the efficacy of pressurised inhalers (pMDIs) in adolescent asthma - is a spacer really required for pMDI use in this age group?

Study contact details
 Principal Investigator  Miss Charlotte Allen  9340 8713
 Study Coordinator  A/Prof Sunalene Devadason  9340 8985

General information
Your child is being asked to take part in a research study to investigate how a drug is deposited into the lungs of young people with asthma after inhalation training, with and without the use of a spacer device with a puffer. Your child can be in this study because he or she is between 11 and 17 years old, and has been diagnosed with asthma.

Please read this consent form carefully. This form explains what your child will be asked to do and explains possible side effects your child might have when participating. You may have questions about the study or what some words mean. Please ask the study investigator if you have any questions. You can ask as many questions as you need to so that you understand what your child will be asked to do. If you decide that you would like your child to take part in this study you will be asked to sign this consent form because you are his/her parent or legal guardian. The study will also be explained to your child in words that they can understand. It is important that he/she understand what he/she is being asked to do and that he/she agrees.

Why are we doing the study?
The main purpose of this study is to measure the amount of drug depositing in the lungs of children between 11 to 17 year of age when using their puffers both with and without a spacer, to determine the age at which we can recommend the use of the puffers alone. As the droplet size emitted from the puffers can also affect the amount of drug depositing in the lungs, we will also be comparing a puffer that delivers normal droplet sized aerosols with one that delivers smaller droplets. We will be assessing inhalers currently commonly used for asthma therapy in Australia.

Who is doing the study?
This study will be carried out by the Princess Margaret Hospital department of Paediatric Medicine, with the University of Western Australia Paediatric Aerosol Research Group.

What will the study tell us?
This study will tell us whether older children (pre-teens and teenagers), with appropriate training, are able to use their puffers alone or whether they still need to use spacers with their puffers. Being able to use their puffers alone may mean that some teenagers will take their medication more regularly because the puffers are smaller and more convenient to carry than
the spacers, but we don’t want to recommend that they use the puffers only, without first measuring the amount delivered to the lungs.

**Does my child have to take part?**

It is important that you understand that your child does not have to take part in this study. At any stage during the study you may change your mind and withdraw your child from the study. This will not affect any treatment your child may receive from the hospital in the future.

**Study design**

This is a randomized, open label, cross over study. See below for an explanation of these terms:

**Randomised:** This means that your child will have a 50:50 chance of using the spacer with the device first, or the device without the spacer first.

**Open label:** This means that the study doctors and you will know which drug is administered to your child. In this study it is called technetium ($^{99m}$Tc) labelled Flioxidone or QVAR.

**Cross-over:** This means that your child will use the puffer with and without a spacer at two separate visits to deliver drug.

**What will you be asked to do if you decide to take part in the study?**

If you choose to take part in the study we will ask you and your child to attend two or three visits to the hospital, and one follow-up phone call.

**What will my child need to do to take part in the study?**

The schedule for each of the visits and what will be required from you and your child are outlined below:

**Screening Visit**

This visit will take 10 to 20 minutes, and we will complete:

- **Medical history:** Questions will be asked all about your child’s health up to now. This includes allergies, illnesses, conditions, treatments, operations, general health and medicines.
- **Physical examination:** This will include an examination of your child’s respiratory system by a clinical nurse specialist.
- **Height and weight:** These measurements will be taken with your child’s shoes off.
- **Vital signs:** This will include pulse rate, oxygen saturation and respiration rate.

If your child is well, and it is convenient for you to stay for a longer visit, we can proceed straight to the next stage of the study (Visit One)

**Visit One**

This visit will take 40 to 60 minutes and we will complete:

- **Questionnaire:** Your child will be asked to complete a questionnaire indicating their thoughts and opinions on the use of a puffer with a spacer and without.
- **Respiratory physical examination and clinical review by a clinical nurse specialist** (unless on the same day as the screening visit).
- **Vital signs:** This will include pulse rate, oxygen saturation and respiration rate.
Training: Your child will be trained on how to coordinate breathing in with the actuation of the puffer without a spacer device.

Study drug administration: $^{99m}$Tc labelled study drug will be administered to your child without a spacer.

Scanning: In one scan we will take a picture of your child’s nose, mouth, airways, lungs and stomach.

Safety review including vital signs

Visit Two
This visit will take 40 to 60 minutes and we will complete:

Training: Your child will be trained on how to coordinate breathing in with the actuation of the puffer with a spacer device.

Respiratory physical examination and clinical review by a clinical nurse specialist (unless on the same day as the screening visit).

Vital signs: This will include pulse rate, oxygen saturation and respiration rate.

Study drug administration: $^{99m}$Tc labelled study drug will be administered to your child.

Scanning: In one scan we will take a picture of your child’s nose, mouth, airways, lungs and stomach.

Adverse events: You will be asked if your child had any problems since your last visit.

Safety review including vital signs

Follow-up Phone Call
We will call you seven days (plus or minus two days) following your final visit to hospital. We will ask you how you child is feeling and if they had any adverse reactions to the procedure.

### Is there likely to be any benefit to my child?

Your child’s inhaler technique will be reviewed by the asthma clinical nurse specialist and additional training and guidance will be provided for the use of their inhaler medication, which may be beneficial if their technique is improved.

### Is there likely to be a benefit to other people in the future?

The outcomes of this research may simplify the treatment regime in adolescents with asthma. Patients who either present at the Emergency Department or are admitted as inpatients at Princess Margaret Hospital with asthma exacerbations will be referred to the Asthma Liaison nurses for education and advice on how to manage their symptoms.

The information obtained from this study will also be communicated to the wider community of adolescent asthmatics through education sessions at the Asthma Foundation and other presentations of the findings.

### What are the possible risks and/or side effects?

This research study involves inhalation of a very small amount of a radioactive substance called technetium. As part of everyday living, everyone is exposed to naturally occurring background radiation and receives approximately 2 millisieverts (mSv) per year. The total radiation dose will vary between participants ranging from approximately 0.04 – 0.4 mSv. The type of radioactivity used in this study will clear quickly from the body; about half the activity will be removed from the lungs within 15-20 minutes after inhaling the radioactive dose, and there will be no detectable radioactivity in the body after 24 hours.
What are the possible discomforts and/or inconveniences

If you agree to participate in this study, you and your child will need to come in to PMH for at least two appointments that may last 40 to 60 minutes. There is a possibility that there might need to be a third visit scheduled. Also, you child will need to keep still while lying under a gamma camera for two minutes at a time, on two occasions at each study visit (Visits One and Two), which may be difficult for them.

Costs for participating in this study

There will be no cost to you and your child for participating in this study. Any equipment or materials required for this study will be provided free of cost to you.

Treatment related injury

If your child becomes physically sick or is injured as a direct result of a study procedure related to this study appropriate medical care for the immediate treatment of the illness or injury will be given to him/her.

If your child experiences an injury or side effects, you should contact the study doctor, Prof Mark Everard at 9340 8830 during the day from 9am to 5pm. After hours you can call the hospital switchboard on 9340 8222 and ask to be put through to Prof Mark Everard.

Where is your information kept?

Your information will only be accessible to authorised researchers on this study. Information will be recorded and stored in a secure manner in line with the WA Health Practice Code for the Use of Personal Health Information and the NHMRC National Statement on Ethical Conduct in Human Research (2007), and will be stored for 25 years after the research is completed.

What about my privacy?

Only authorized staff will have access to the study data. We will collect information about your child’s medical history and medications, as well clinical progress from the information that is collected as part of normal care. The laboratory researchers will give each participant a unique study code and all information will be identified by this code and not by you or your child’s name.

Information from this study will remain confidential or will only be released with your permission. As part of their responsibility to ensure that clinical studies are carried out in accordance with international standards, representatives of the Australian government regulatory agencies may require access to your child’s records. Information obtained from this study may be presented at meetings, or published in scientific or medical journals, but you and your child will not be identified in any way.

Who has approved this study?

This study has been approved by the Princess Margaret Hospital ethics committee.
Who to contact if you have any concerns about the organisation or running of the study?

If you have any concerns or complaints regarding this study, you can contact the Director of Medical Services at PMH (Telephone No: (08) 9340 8222). Your concerns will be drawn to the attention of the Ethics Committee who is monitoring the study.

What to do next if you would like your child to take part in this research

If you would like to take part in this research study, please read and sign the consent form provided.

THANK YOU FOR YOUR TIME
FORM OF CONSENT  
(For Parent/Guardian)

PLEASE NOTE THAT PARTICIPATION IN RESEARCH STUDIES IS VOLUNTARY AND SUBJECTS CAN WITHDRAW AT ANY TIME WITH NO IMPACT ON CURRENT OR FUTURE CARE.

I have read the information explaining the study entitled

I have read and understood the information given to me. Any questions I have asked have been answered to my satisfaction.

I agree to allow

(full name of participant and relationship of participant to signatory)

to participate in the study.

I understand my child may withdraw from the study at any stage and withdrawal will not interfere with routine care.

I agree that research data gathered from the results of this study may be published, provided that names are not used.

Dated .................................. day of ................................................ 20 ........

Child's Signature .................................................................  
(Where appropriate)

Parent or Guardian's Signature ...................................................

I, .......................................................... have explained the above to the

(Investigator's full name)

signatories who stated that he/she understood the same.

Signature

Version 3

Date: 12th March 2015

S. Trend
## 5.5 Physical examination

Case Report Form  
Confidential  

Protocol Title: The effect of particle size on the efficacy of pressurised inhalers (pMDIs) in adolescent asthma - is a spacer really required for pMDI use in this age group?  
Investigator: Charlotte Allen  

<table>
<thead>
<tr>
<th>Participant Initials:</th>
<th>Participant ID:</th>
</tr>
</thead>
</table>

### Physical Examination

Time: [ ]: [ ] (using 24 hour format)  
(e.g. hh:mm)  

**Physical Examination not performed**

Visit Number (check one):

- [ ] Visit #Screening  
- [ ] Visit #Deposition 1  
- [ ] Visit #Deposition 2

<table>
<thead>
<tr>
<th>Body System</th>
<th>Finding* (check one)</th>
<th>Comments (required if Abnormal)</th>
<th>Clinically Significant (Y/N)</th>
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<tbody>
<tr>
<td><strong>Respiratory system</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
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</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Other (specify in Comments)</strong></td>
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<td></td>
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Additional Notes:

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Is there any chance you are pregnant?  
[ ] Yes  
[ ] No

If Yes participant should be withdrawn from the study

Physical Examination performed by: ________________________________

Principal Investigator Signature: __________________ Date: ____________

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Version 0.3 updated by NJ 09Jun2016  
4 of 40  
Initials: ______
5.6 Vital sign measurements

Case Report Form
Confidential

Protocol Title: The effect of particle size on the efficacy of pressurised inhalers (pMDIs) in adolescent asthma - is a spacer really required for pMDI use in this age group?
Investigator: Charlotte Allen

Participant Initials: ___________  Participant ID: ___________

Vital Sign Measurements

Visit Number (check one):
☐ Visit #Screening  ☐ Visit #Deposition 1  ☐ Visit #Deposition 2

☐ Height Measurements not performed

Height: _______ cm  Weight: _______ kg

☐ Height not measured  ☐ Weight not measured

Time: _______ : _______ (using 24 hour format of hh:mm)

Temperature: _______ ◦ Celcius  ☐ Temperature not measured

Method: (check one) ☐ Oral  ☐ Axillary  ☐ Tympanic

Respiratory Rate: _______ breaths/min  ☐ Respiratory Rate not measured

Heart Rate: _______ beats/min  ☐ Heart Rate not measured

Blood Pressure _______ / _______ mmHg  ☐ Blood Pressure not measured

Method: (check one) ☐ Manual  ☐ Automated  Oxygen Saturation % _______

Location: (check one) ☐ Left Arm  ☐ Right Arm

Position: (check one) ☐ Sitting  ☐ Supine  ☐ Standing

Additional Notes: _____________________________________________________________

Vital Sign Measurements obtained by: ________________________________

Version 0.3 updated by NJ 09Jun2016  3 of 40  Initials: _______
5.7 Screening questionnaire

Case Report Form
Confidential

Protocol Title: The effect of particle size on the efficacy of pressurised inhalers (pMDIs) in adolescent asthma - is a spacer really required for pMDI use in this age group?
Investigator: Charlotte Allen

Participant Initials: ___________    Participant ID: ___________

Visit 1 study procedures

Date of Visit: __________/________/________

Questionnaire

1. What medication are you prescribed for your asthma?

2. Many people forget to take their medications occasionally. How often do you forget to take it?

3. Do you find it easier to remember to take your medication in the morning or the afternoon?

4. If you do forget to take your medication, why do you think you have difficulty remembering to take it?

5. Do you take your medication with or without a spacer?

6. Which spacer do you use- volumatic or SVS?

7. Do you wash your spacer in soapy water?

8. Does it bother you to take daily medication?

9. Have you considered using dry powder asthma devices?

Version 0.3 updated by NJ 09Jun2016  8 of 40  Initials: _______
5.8 Look up table

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* mCi and MBq values are reference values only, this is not a calibrated source