Characterization of Aerosol Deposition in Children and Infants Using Idealized Extrathoracic Geometries

by

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Abstract

This thesis describes a number of experimental studies performed with the common goal of characterizing pharmaceutical aerosol deposition in children and infants using idealized extrathoracic geometries. First, an *in vitro* study of the recently proposed Alberta Idealized Child Throat showed that this idealized child oral extrathoracic airway model accurately replicates average deposition of pharmaceutical aerosol from pressurized metered dose inhalers and dry powder inhalers in school age children. This successful validation confirms that the Alberta Idealized Child Throat may indeed fulfill the existing requirement for a standardized platform in which benchtop testing of delivery devices and therapeutic formulations developed for children can be examined. Second, a joint *in vitro – in silico* methodology was employed to characterize deposition in an idealized infant nasal extrathoracic airway geometry. Using a novel flow system, total lung dose from two pressurized metered dose inhalers, delivered via valved holding chamber and facemask under a realistic breath profile, was approximated by the dose delivered distal to the idealized geometry. *In silico* simulations using this estimate of total lung dose provided insight on regional deposition in the lungs and on the concentration of drug in the airway surface liquid. From a clinical perspective, this *in vitro – in silico* methodology provides valuable guidance on the dosing required for efficacious use of aerosolized medications in infants. Finally, a comparison of *in vitro* deposition measured in two idealized geometries representative of the oral extrathoracic airways of children is described, illustrating the importance of considering the physics governing aerosol behavior in the human airways when developing idealized geometries meant to mimic *in vivo* deposition. It is hoped that the experiments undertaken as part of this thesis will aid in the development of new delivery devices and inhalation therapies for the treatment of disease in children and infants.

Preface

Chapter 2 of this thesis has been published as Ruzycki, C. A., L. Golshahi, R. Vehring, and W. H. Finlay. 2014. "Comparison of In Vitro Deposition of Pharmaceutical Aerosols in an Idealized Child Throat with in Vivo Deposition in the Upper Respiratory Tract of Children." *Pharmaceutical Research* 31 (6): 1525-1535. I was responsible for experimental design, data collection, analysis, and manuscript composition. L. Golshahi assisted with concept development, the initial experimental setup, provided advice regarding statistical analysis, and contributed to manuscript edits. R. Vehring provided access to critical components of the experimental setup and contributed numerous edits, concept development, and advice during manuscript formulation. W.H. Finlay was the supervisory author, involved with concept formulation, discussion of experimental results, and advice during manuscript composition.

Research described in Chapter 3, was performed as part of a collaboration with the Alberta Children's Hospital and the University of Calgary, led by Dr. David W Johnson, with Dr. Warren Finlay as the lead collaborator at the University of Alberta. The *in vitro* apparatus described in Chapter 3 was designed and built by myself. I also performed the mathematical filtering of particle size distributions as described in the initial portions of the *in silico* component of the study. Emadeddin Javaheri performed the one-dimensional numerical modeling of regional lung deposition and the calculations of drug concentrations in the airway surface liquid. The analysis of results of the joint *in vitro – in silico* methodology is my original work.

Portions of the results from Chapter 2 and Chapter 4 have been published as: Finlay, W. H., C. A. Ruzycki, L. Golshahi, and R. Vehring. 2014. "Validating and Scaling the Alberta Idealized Child Throat." *Respiratory Drug Delivery 2014* 1: 303-310. This paper composed the

results of Chapter 2 for pharmaceutical aerosol deposition in the Alberta Idealized Child Throat. Chapter 4 describes additional experiments to compare deposition in this throat model with that of one derived from a different design methodology. L. Golshahi was involved with the initial experimental setup used to examine pharmaceutical aerosol deposition in the Alberta Idealized Child Throat, and provided advice and edits during manuscript composition. R. Vehring provided access to critical components of the experimental setup, discussed methodology, and contributed edits to the manuscript. I was responsible for the experimental design, data collection, and analysis, including statistical analysis and calculations of hygroscopic growth. W.H. Finlay was the supervisory author and composed the manuscript. Chapter 4 expands on the data presented in this publication, including numerous additional measurements and a more in depth discussion of hygroscopic growth.

Acknowledgements

I must first express my sincere gratitude to my academic advisor, Dr. Warren Finlay. For an often-confused graduate student struggling through the obstacles of research, your advice, honesty, and generosity has been a constant source of motivation. Thank you for providing me the opportunity to work in a world-class research group, and for placing your trust in my abilities. You have afforded me many fantastic opportunities to grow as a researcher, and I have gained more than I could have dreamed over the course of the past two years. You are, without a doubt and confirmed by many, a supervisor and researcher of the highest quality, and I will be sad to leave the lab. You have been, and will continue to be, a wonderful role model (and I will understand if you want a rematch at billiards).

I must also thank the members of the Aerosol Research Lab, both past and present, for making the lab environment an enjoyable one. Mehdi, our conversations have been a constant source of entertainment, and I am sure we will cross paths again. Laleh, your encouragement and kind nature have been a major factor of my completion of this thesis, and I have yet to meet another researcher as pleasant as you. Andriy, your Russian language lessons have been invaluable, though I must admit I remember very little of it. Nick, I am glad to have had a friendly, similarly aged face in the lab, and wish you luck in your endeavors. Farzin, engineering deadly particles was made easier by our mutual hilarity. Azedah, you have provided me, on more than one occasion, the only means through which to continue my research. Helena, you are owed more gratification than you will admit. Your diligent chemical analysis, patent searches, and research have been of great assistance in my studies, as have been our conversations on popular culture and world news. Thank you for putting up with my tendencies to 'unpack' across the entire lab. Dr. Vehring, I must also thank you and your research group in NINT for allowing me

to expand my theoretical knowledge of particle engineering by actually making particles, a project that I will remain extremely proud of (few people can claim to have both engineered micro-particles, then imaged them with a scanning electron microscope!).

Assistance from the Department of Mechanical Engineering's machine shop and electronics shop has made this thesis possible. Special thanks are owed to Rick Conrad, Andrew Cambell, Tuula Hilvo, Roger Marchand, and Bernie Faulker. All of your experience has been instrumental in improving my often ambitious designs.

I would be nowhere without the family and friends that have been there to remind me of life outside of academia. You all hold a special place in my heart, and have helped me often through tough times both in research and in life. Kayley, as my sister you have showed me the value of loyalty, and have helped me keep track of the important things. Mom, your example has given me a strong desire to always improve. Dad, a proper acknowledgement would require more words than are contained in this thesis. You have provided me infinite support in all aspects of life, and I have you to thank for both my stubbornness and my drive to succeed. I'm looking forward to more endless discussion on topics too numerous to list.

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Chapter 1: Introduction

1.1 Background

Inhaled pharmaceutical aerosols have been used to great effect in the treatment of respiratory diseases including asthma, chronic obstructive pulmonary disease, and cystic fibrosis (Labiris and Dolovich 2003a). Recent work has hinted towards the utility of inhalation therapy as a non-invasive path for drug delivery via systemic circulation, with important implications in, for example, pain management (Macleod *et al.* 2012), antidote delivery (Corcoran *et al.* 2013), and treatment of diabetes with inhaled insulin (Patton, Bukar, and Eldon 2004). The lungs are a desirable target for drug delivery; rapid and predictable onset of action as well as the non-invasive and convenient nature of therapy has motivated the development of pharmaceutical aerosols. However, the use of aerosols for medication delivery is not without issue. A major consideration in the use of inhaled pharmaceutical aerosols is the filtering effect observed in the extrathoracic airways. Deposition in the extrathoracic region heavily influences the dose of drug delivered to the lungs (Borgström, Olsson, and Thorsson 2006; Finlay and Martin 2008), can reduce the efficacy of inhaled medications (Selroos, Pietinalho, and Riska 1996; Ruffin, Montgomery, and Newhouse 1978), and may lead to deleterious side effects (Zhang *et al.* 2013; Singh, Amin, and Loke 2009). Accurately characterizing extrathoracic deposition is therefore an important step in ensuring that patients receive a consistent, safe, and efficacious dose of medication when using a marketed inhalation device.

In vitro methods employing realistic extrathoracic airway replicas have been shown to successfully predict *in vivo* aerosol deposition in adults (Cheng *et al.* 2001; Ehtezazi *et al.* 2005; Finlay and Martin 2008). Given the complex fluid mechanics governing aerosol behavior in the

extrathoracic region, realistic replicas have proven useful in replicating and predicting extrathoracic deposition and total lung dose from pharmaceutical inhalers. For regulatory compliance and preclinical development, the use of a single standardized geometry is an attractive alternative to the various realistic replicas currently in use (Finlay *et al.* 2010; Byron *et al.* 2010). One such geometry, the United States Pharmacopeia Induction Port (USP IP), has seen much use as a common standard to compare various inhalers. However, the USP IP fails to replicate extrathoracic deposition observed in adults, likely as a result of its simple design that bears little resemblance to the human oral extrathoracic airway (Cheng *et al.* 2001; Zhang, Gilbertson, and Finlay 2007; Zhou, Sun, and Cheng 2011). The development of the Alberta Idealized Throat by our group (Stapleton *et al.* 2000) represented the first foray into the use of idealized geometries to characterize extrathoracic deposition. The Alberta Idealized Throat, incorporating simplified analogues of important anatomical features observed in adult oral extrathoracic airways, has been shown to successfully replicate extrathoracic aerosol deposition measured in adults (Grgic *et al.* 2004; Grgic, Finlay, and Heenan 2004; Zhang, Gilbertson, and Finlay 2007; Zhou, Sun, and Cheng 2011), and can be purchased commercially (Copley Scientific, UK).

Recent interest has turned towards the optimization of respiratory drug delivery in children and infants. Owing to differences in anatomy, physiology, and disease process, pediatric patients cannot simply be considered small adults (Everard 2003; Ahrens 2005). Clinical trials involving radiolabelled aerosols are difficult and expensive, and involve considerable ethical concerns, especially for trials involving pediatrics. This has effectively limited the number of *in vivo* studies examining aerosol deposition in children and infants, leading investigators to turn towards *in vitro* and *in silico* methods to characterize deposition in young patients (Carrigy *et al.*

2014b). While a number of *in vitro* studies using realistic replicas of child (Corcoran *et al.* 2003; Golshahi *et al.* 2011; Golshahi, Noga, and Finlay 2012; Golshahi *et al.* 2013) and infant (Cheng *et al.* 1995; Janssens *et al.* 2001; Storey-Bishoff, Noga, and Finlay 2008; Golshahi *et al.* 2010; Laube *et al.* 2010) extrathoracic airways have begun to characterize aerosol deposition in these age groups, there remains a need for standardized and validated geometries that can accurately predict extrathoracic deposition in pediatrics.

To this end, idealized child and infant geometries have recently been developed by our group. The Alberta Idealized Child Throat, described first by Golshahi and Finlay (2012), was developed by isotropic scaling of the Alberta Idealized Throat to replicate *in vitro* aerosol deposition observed in replicas of school-age child oral extrathoracic airways. Javaheri, Golshahi, and Finlay (2013) discussed the design of an idealized infant throat, representing the nasal extrathoracic airways of infants 3 to 18 months old. These are not the only idealized pediatric models; Bickmann *et al.* (2008) developed an idealized model by asymmetrically scaling the Alberta Idealized Throat based on MRI scans of 5 year old children. This thesis was undertaken to characterize deposition in these various idealized geometries using marketed pharmaceutical inhalers to determine the validity of using these geometries as standardized platforms.

1.2 Objectives

The main goal of this work is to characterize the deposition of pharmaceutical aerosols in idealized geometries representing the oral extrathoracic airways of children and the nasal extrathoracic airways of infants. It is hoped that the results of the present work validate the use of these idealized geometries as standardized platforms from which to characterize pharmaceutical aerosol deposition in young patients.

1.3 Thesis Structure

The presentation of this thesis follows a mixed format, including published and unpublished research. Chapter 1 is a brief introduction establishing the motivation of the research and the connections among remaining chapters. Chapter 2 describes the validation of the Alberta Idealized Child Throat, in which *in vitro* measurements of pharmaceutical aerosol deposition from two common delivery devices, including a pressurized metered dose inhaler and a dry powder inhaler, were compared to available *in vivo* scintigraphic data. The results of this chapter will be useful in determining if the Alberta Idealized Child Throat replicates pharmaceutical aerosol deposition observed in school-age children, an important step in the adoption of this model as a standardized platform for the optimization of inhaled aerosols and delivery devices. Chapter 3 illustrates the capabilities of a joint *in vitro – in silico* methodology to characterize deposition using the idealized infant geometry of Javaheri, Golshahi, and Finlay (2013). *In vitro* measurements of aerosol deposition provide the input for *in silico* simulations of regional deposition in the lung along with airway surface liquid drug concentrations. From a clinical perspective, this novel methodology provides guidance on the dosing required to ensure efficacious and safe drug delivery to infants using pressurized metered dose inhalers and valved holding chambers plus facemasks. Chapter 4 examines how the methodology governing the development of idealized geometries affects deposition by comparison measurements taken with the Alberta Idealized Child Throat and the Bickmann *et al.* (2008) idealized throat. This comparison of deposition will serve to illustrate the importance of careful consideration of the mechanisms governing aerosol deposition in the design of idealized geometries to ensure accurate replication of deposition expected in human patients. A summary of the thesis and directions for future work are presented in Chapter 5.

Chapter 2: Comparison of *In Vitro* Deposition of Pharmaceutical Aerosols in an Idealized Child Throat with *In Vivo* Deposition in the Upper Respiratory Tract of Children

A similar version of this chapter has been published as: Ruzycki, C. A., L. Golshahi, R. Vehring, and W. H. Finlay. 2014. "Comparison of In Vitro Deposition of Pharmaceutical Aerosols in an Idealized Child Throat with in Vivo Deposition in the Upper Respiratory Tract of Children." *Pharmaceutical Research* 31 (6): 1525-1535.[1]

2.1 Introduction

Inhaled pharmaceutical aerosols have been used to great effect in the treatment of respiratory diseases in adult and pediatric patients. A major consideration in the use of inhaled pharmaceutical aerosols is extrathoracic deposition, which plays an important role in determining the total lung dose from pharmaceutical inhalers (Stahlhofen, Rudolf, and James 1989; Borgström, Olsson, and Thorsson 2006; Finlay and Martin 2008). Drug lost to deposition in the extrathoracic region can reduce the efficacy of inhaled medications (Selroos, Pietinalho, and Riska 1996; Ruffin, Montgomery, and Newhouse 1978) and lead to deleterious side effects (Zhang *et al.* 2013; Singh, Amin, and Loke 2009). Furthermore, for many inhaled pharmaceutical aerosols, the total lung dose can often be approximated by the dose delivered distal to the extrathoracic region (Finlay and Martin 2008). With these considerations in mind, accurately characterizing extrathoracic deposition is an important step in ensuring that patients receive a consistent and appropriate dose when using marketed inhalation devices.

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The fluid mechanic interactions that occur in the extrathoracic region and in flow exiting an inhaler are inherently complex (DeHaan and Finlay 2004; Longest *et al.* 2012), making geometric models of the mouth-throat region useful in predicting extrathoracic deposition and total lung dose. *In vitro* methods using realistic oral airway replicas have been shown to successfully predict *in vivo* deposition in adults (Finlay and Martin 2008; Cheng *et al.* 2001; Ehtezazi *et al.* 2005), though issues stemming from intersubject variability and complex manufacturing of anatomical geometries are often encountered. From the point of view of regulatory compliance and preclinical development, the use of a single standardized geometry is an attractive alternative to realistic replicas (Finlay *et al.* 2010; Byron *et al.* 2010). Historically, the United States Pharmacopeia Induction Port (USP IP) has been used as a common standard to compare various inhalers, though its simple design, lacking resemblance to a human oral airway, fails to replicate mouth-throat deposition (Cheng *et al.* 2001; Zhang, Gilbertson, and Finlay 2007; Zhou, Sun, and Cheng 2011). To address the poor replication of *in vivo* deposition observed with the USP IP, work at the University of Alberta led to the development of the Alberta Idealized Throat (Stapleton *et al.* 2000). This idealized model, incorporating simplified analogues of important geometric features observed in adult extrathoracic airways, has been shown to accurately replicate average deposition in adults (Zhang, Gilbertson, and Finlay 2007; Zhou, Sun, and Cheng 2011; Grgic, Finlay, and Heenan 2004), and is commercially available (Copley Scientific, UK). Using an alternative methodology, Delvadia *et al.* recently developed a characteristic mouth-throat and upper airway model based on simplified anatomical data (Delvadia, Longest, and Byron 2012). This model captured mean *in vivo* deposition for five commercial dry powder inhalers (Delvadia *et al.* 2013), and together with complementarily

scaled versions, replicated the mean and variability of *in vivo* deposition from Budelin Novolizers (Delvadia, Longest, and Byron 2012).

Recent interest has turned towards optimizing respiratory drug delivery in pediatric patients. Despite differences in anatomy, physiology, disease processes, pathophysiology, and pharmacokinetics, children are commonly prescribed inhalers and formulations originally designed for adults (Ahrens 2005). Young patients may be treated off-label, necessitated by a lack of clinical trial data. Along with the traditional role of managing respiratory disease, recent developments (Macleod *et al.* 2012; Corcoran *et al.* 2013; Patton, Bukar, and Eldon 2004) have hinted towards the utility of aerosol therapy as a non-invasive path for drug delivery via systemic circulation. Therapies for systemic treatments are often subject to narrow margins between efficacious use and harmful systemic effects, and are thus subject to stringent dose quantification (Devadason 2006). As such, there is a vested interest in developing improved methods for testing pharmaceutical inhalers and formulations in pediatric patients for regulatory compliance and preclinical development.

While a limited number of *in vivo* studies have examined radiolabelled aerosol deposition from pharmaceutical inhalers in pediatric patients (Devadason *et al.* 1997; Wildhaber *et al.* 1998; Devadason *et al.* 2003; Roller *et al.* 2007), the ethical concerns associated with these types of investigations make *in vitro* methods a favorable option. *In vitro* methods allow for greater control over the variables that affect deposition of inhaled pharmaceutical aerosols, including environmental conditions such as temperature and humidity. Unfortunately, *in vivo* deposition studies rarely report the environmental conditions under which clinical data is obtained. This absence of such data complicates the validation of *in vitro* work via comparison to *in vivo* deposition, as environmental conditions, humidity in particular, are known to affect the

deposition of some pharmaceutical aerosols (Martin and Finlay 2005; Kwok and Chan 2008; Shemirani *et al.* 2013). To the authors' knowledge, only one *in vitro* study has examined the effects of humidity on inhaled pharmaceutical aerosol deposition in idealized mouth-throat models: Shemirani *et al.* (2013) recently demonstrated that extrathoracic deposition from solution and suspension pMDIs may increase significantly with increasing relative humidity (RH) through experiments with the Alberta Idealized Throat.

In vitro deposition has been examined in child (Corcoran *et al.* 2003; Golshahi *et al.* 2011; Golshahi, Noga, and Finlay 2012; Golshahi *et al.* 2013) and infant (Janssens *et al.* 2001; Storey-Bishoff, Noga, and Finlay 2008; Minocchieri *et al.* 2008; Golshahi *et al.* 2010; Laube *et al.* 2010) physical airway replicas, but the need for a standard idealized model for predicting average pediatric deposition remains. Bickmann *et al.* (2008) modified the Alberta Idealized Throat based on magnetic resonance imaging scans of 5-year-old children, altering the dimensions of the oral cavity, pharynx, larynx, and trachea to match that observed in younger patients. This idealized throat, representative of preschool children, was used to examine deposition from a Respimat® Soft Mist™ Inhaler and a pressurized metered dose inhaler (pMDI) plus spacer. More recent work with this geometry has focused on deposition measurements with Respimat Soft Mist Inhalers (Wachtel *et al.* 2010), and SalbuHexal® Easyhaler® and Salbu Novolizer® dry power inhalers (Below, Bickmann, and Breitkreutz 2013). Whether this 5-year-old child idealized throat replicates *in vivo* deposition in preschool children has not, to the authors' knowledge, been examined. With inhaler use being more common among children and adolescents over the age of 5, an idealized throat representative of children 6 to 14 years old has recently been developed by uniformly scaling the Alberta Idealized Throat to match the average characteristic diameter, defined as the airway volume divided by

surface area, measured in nine child oral airway replicas (Golshahi and Finlay 2012). This Alberta Idealized Child Throat has been shown to match average *in vitro* deposition under constant flow rates (Golshahi and Finlay 2012) and tidal breathing (Golshahi *et al.* 2013), but has yet to be compared to *in vivo* deposition from pharmaceutical aerosols.

It is thus the aim of the present chapter to validate the Alberta Idealized Child Throat with *in vivo* deposition data for inhalers commonly used in children. Specifically, a pMDI delivering beclomethasone dipropionate for asthma prophylaxis and maintenance treatment (QVAR®) and a multidose dry powder inhaler (DPI) delivering budesonide for the same indication (Pulmicort® Turbuhaler®) were tested with the Alberta Idealized Child Throat using simulated breathing profiles for comparison with published scintigraphic *in vivo* deposition studies (Devadason *et al.* 1997; Devadason *et al.* 2003). To account for potential discrepancies arising from differences in humidity between *in vitro* measurements in the present study and previously reported *in vivo* data, experiments were performed in an environmental chamber at various RH, thus allowing for an analysis of the effects of ambient humidity on deposition in the Alberta Idealized Child Throat.

2.2 Materials and Methods

2.2.1 Alberta Idealized Child Throat

The Alberta Idealized Child Throat was developed by uniformly scaling down the Alberta Idealized Throat by a factor of 0.62 to match the average characteristic diameter, defined as the airway volume divided by its surface area, of nine oral airway replicas of children age 6 to 14 years old (Golshahi and Finlay 2012). This mouth-throat geometry contains simplified analogues of anatomical features that heavily influence the transport and deposition of aerosols

in the extrathoracic airways (Finlay *et al.* 2010), and has been shown to replicate the *in vitro* deposition of micrometer-sized particles under constant flow rates (Golshahi and Finlay 2012) and tidal breathing (Golshahi *et al.* 2013). A rapid prototyped model of the Alberta Idealized Child Throat was made using stainless steel (Linear Mold & Engineering, Livonia, MI, USA), the use of which reduces artificial electrostatic surface charging effects and avoids solvent contamination issues during chemical assay.

Figure 1: One half of the Alberta Idealized Throat (left) and the Alberta Idealized Child Throat (right).

2.2.2 Experimental Setup

Two commercially available inhalers were selected for use in the present study, including a pMDI delivering beclomethasone dipropionate for asthma prophylaxis and maintenance treatment (label claim of 100 μg beclomethasone dipropionate, QVAR® pMDI, manufactured by

Medicis Pharmacetuical Corporation, Scottsdale, AZ, USA, distributed by Medicis Canada, Ltd., Toronto, Ontario, Canada) and a multidose DPI delivering budesonide for the same indication (label claim of 200µg budesonide, Pulmicort® Turbuhaler®, manufactured AstraZeneca Canada Inc., Mississauga, Ontario, Canada), owing to the availability of *in vivo* scintigraphic deposition data for comparison purposes (Devadason *et al.* 1997; Devadason *et al.* 2003). Devadason *et al.* (1997) examined deposition of radiolabeled budesonide delivered via Pulmicort Turbuhaler in children 4 to 16 years old with cystic fibrosis. A later study by the same group examined the deposition of radiolabeled QVAR administered via Autohaler™, a breath-actuated inhaler, in asthmatic children 5 to 14 years old (Devadason *et al.* 2003). QVAR pMDIs have been shown to achieve the same deposition as QVAR Autohalers for adult patients demonstrating proper inhalation techniques (Leach *et al.* 2005), and equivalent clinical efficacy for these inhalers has been demonstrated in children (Arshad *et al.* 1993). Gabrio Stein, and Velasquez (1999) found similar plume impact forces (31.9 mN and 39.1 mN), minimum plume temperatures (3.0°C and 2.2°C), and plume spray durations (282.3 ms and 292.5 ms) for a QVAR 100 µg pMDI and QVAR 100 µg Autohaler, respectively, further suggesting that similar deposition in the respiratory tract can be expected from these two devices. While further information on particle size would be useful in comparing these devices, the aforementioned references suggest that the use of a pMDI rather than an Autohaler in the present study may be considered a negligible source of error. To replicate patient use, inhalers were handled and operated according to product insert instructions. Prior to testing, the QVAR pMDI was primed by firing to waste four times at 1 minute intervals.

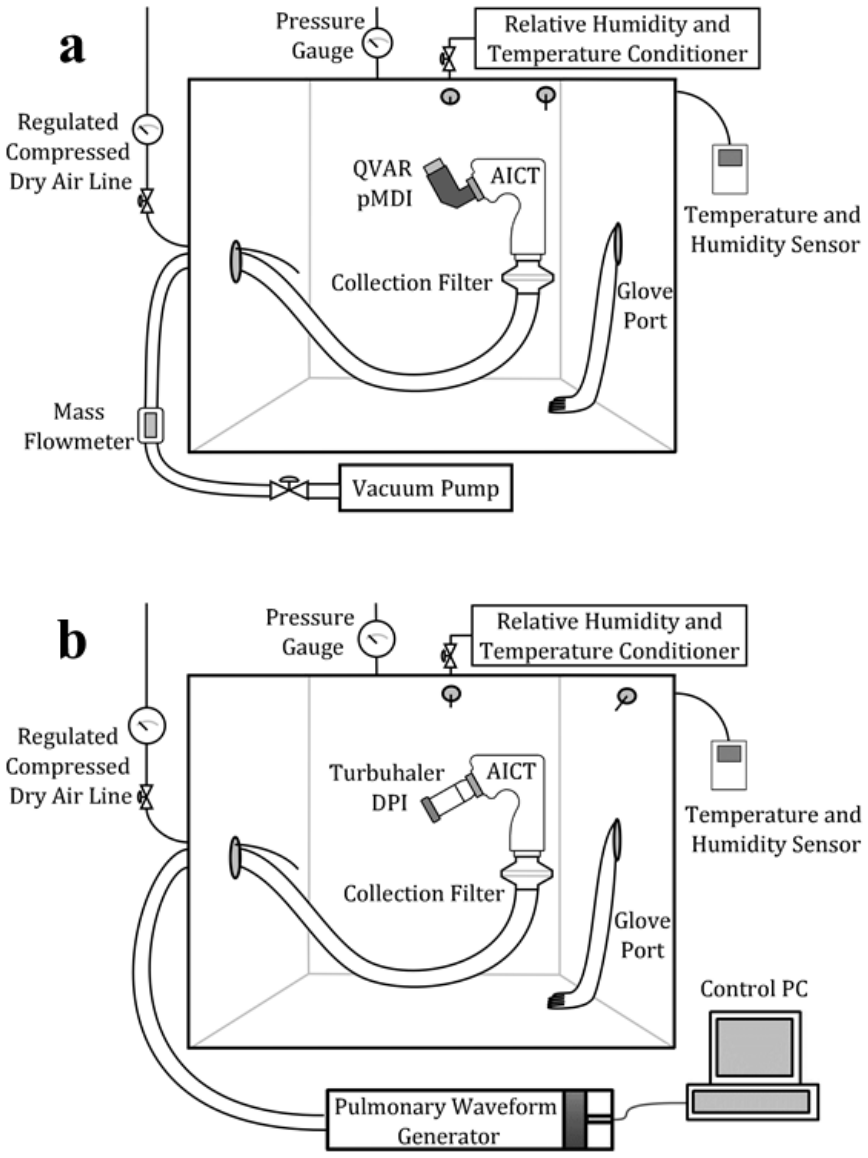

Figure 2: Experimental setup for a) QVAR pMDI with a constant flow rate set by a vacuum pump and b) Turbuhaler DPI with a time-variant flow profile supplied by a pulmonary waveform generator. AICT = Alberta Idealized Child Throat.

Schematic diagrams of the experimental setup for the QVAR pMDI and Turbuhaler DPI are shown in Figure 2. The Alberta Idealized Child Throat was coupled to a collection filter with

a pore size of 0.3 μm (Respirgard II™ bacterial/viral filters; Vital Signs Inc., Englewood, CO, USA) and placed within a modified environmental chamber with glove ports (CEO-910W-4; Lunair Environmental, Williamsport, PA, USA) and an integrated compressed dry air line (<1% RH). Conditions within the chamber were monitored using a humidity and temperature meter (Vaisala HUMICAP® HM70; Helsinki, Finland) accurate to ±1% RH of reading for 0-90% RH and ±0.2°C at 20°C. Inhalers were attached to the Alberta Idealized Child Throat prior to being placed in the environmental chamber using custom-built adapters. Separate flow systems were used to draw air through the setup owing to differences in device operation for press-and-breath QVAR pMDIs and breath-actuated Turbuhaler DPIs. The QVAR pMDI was examined under a constant flow rate generated by a vacuum pump (Model 0523; Gast Manufacturing Inc., Benton Harbor, MI, USA) and measured using a digital mass flow meter (Model 4043; TSI Incorporated, Shoreview, MN, USA) accurate to 2% of reading. In contrast, the Turbuhaler DPI was tested using a time-variant inhalation flow profile, generated by a pulmonary waveform generator (MH Custom Design & Mfg. L.C., Midvale, UT, USA).

Prior to each test run, the two halves of the Alberta Idealized Child Throat were coated with silicone oil (Molykote 316; Dow Corning Corporation, Midland, MI, USA) to minimize particle bounce. After allowing 15 minutes for solvent evaporation, the idealized throat was assembled, connected to the inhaler and downstream filter, and placed within the environmental chamber. The chamber was closed, and conditions were set to the desired temperature and relative humidity; deposition from each inhaler was examined under several RH values (10, 30, 50, 70, 90% RH) at a temperature of 23.5°C. After allowing for a sufficient period of time for conditions to stabilize within the chamber, approximately 5 minutes, inhalers were actuated into the Alberta Idealized Child Throat under simulated breathing. To achieve realistic *in vitro*

assessment of deposition in the Alberta Idealized Child Throat, breathing parameters were chosen to closely mirror those observed *in vivo* for each inhaler. A summary of simulated breathing parameters is presented in Table 1.

Table 1: Summary of simulated breathing parameters used to examine deposition in the Alberta Idealized Child Throat.

Inhaler	Inhaled Volume (L)	Inspiratory Flow Rate[2] (L/min)	Flow Increase Rate (L/s^2)
QVAR pMDI	1.6	45	-
Turbuhaler DPI	1.5	53	2

Deposition from the QVAR pMDI was examined using a constant inhalation flow rate, set to equal the average inhalation flow rate generated by patients examined in the Devadason *et al.* (2003) study on radiolabeled QVAR deposition. Reported heights of subjects from this study (weighted average of 136.8 cm, all male patients) were used to estimate the average inspiratory capacity of enrolled patients, 1.6 L, using the reference equations of Cook and Haman (1961) – see

These equations, recommended by Stocks and Quanjer (1995), are for patients 5 to 38 years old and were obtained helium gas dilution measurements of lung volumes. The average *in vivo* inhalation flow rate was estimated using the 1.6 L estimate of average inspiratory capacity and reported data concerning inspiratory time (Devadason *et al.* 2003); mean inspiratory time, 2.12 seconds, was calculated by subtracting the time to actuation of the Autohaler, 0.31 seconds,

[2] Value represents peak inspiratory flow rate for Turbuhaler DPI generated by the pulmonary waveform generator

from the total inspiratory time, 2.43 seconds. From these values of inspiratory capacity and inspiratory time, the average *in vivo* flow rate was calculated to be approximately 45 L/min. Thus, for QVAR, the vacuum pump was set to draw air at a constant rate of 45 L/min through the Alberta Idealized Child Throat. With the idealized throat, QVAR pMDI, and collection filter connected to the flow system inside the environmental chamber, the vacuum pump was turned on, and the flow rate was allowed to stabilize at 45 L/min. The pMDI was then actuated into the Alberta Idealized Child Throat, and a stopwatch (accurate to ± 0.1 seconds) was used to manually measure the time required for 1.6 L of air to be drawn through the idealized throat, equal to 2.1 seconds for the 45 L/min inhalation flow rate. The vacuum pump was then turned off, and the idealized throat, inhaler, and collection filter were removed from the chamber for deposition analysis. It should be noted that the use of a stopwatch is a somewhat inaccurate timing method susceptible to human error. However, errors in timing with this method will likely not significantly alter deposition, given that the spray duration from the QVAR pMDI is less than 300 ms (Gabrio, Stein, and Velasquez 1999), and the internal volume of the Alberta Idealized Child Throat is 18 mL. The aerosol bolus emitted by the pMDI can be expected to travel through the throat at roughly the inhalation flow rate, 45 L/min (equivalent to 750 mL/s), meaning a particle entering the inlet of the Alberta Idealized Child Throat will exit the outlet in approximately 24 ms. With the short spray duration of the QVAR pMDI, deposition of the aerosol bolus can be expected to occur well before 2.1 seconds, meaning errors in timing are likely to have no effect on deposition beyond this point in time.

Unlike most pMDIs, the Turbuhaler DPI is a breath-actuated device that relies on the patient to supply the energy required to adequately aerosolize the medicated powder for delivery

to the lungs[3]. Studies have demonstrated the performance of the Turbuhaler as being heavily dependent on flow parameters including peak inspiratory flow rate and flow increase rate (De Boer, Gjaltema, and Hagedoorn 1996; De Boer *et al.* 1997; Everard, Devadason, and Le Souëf 1997; Kamin *et al.* 2002; Martin, Marriott, and Zeng 2007), necessitating the use of appropriate values of these parameters when aiming for accurate *in vitro* characterization of *in vivo* deposition. Initial experiments using the same setup as the QVAR pMDI, with a vacuum pump used to draw air through the inhaler, were complicated by the breath-actuated nature of the Turbuhaler. The vacuum pump, when turned on, behaved similarly to a step-excited first-order system with a time constant on the order of 0.35 seconds, dependent on the peak flow rate. De Boer *et al.* (1997) posited that the majority of powder is dispersed and released from the Turbuhaler after the flow passes a threshold of 20 L/min, and suggest that the flow increase rate is of greatest importance between 20 L/min and 30 L/min. Using this metric, the vacuum pump delivered an average flow increase rate (calculated for 20 L/min to 30 L/min) of 1.04 L/s^2 when the peak flow rate was 45 L/min, and 1.80 L/s^2 when the peak flow rate was 60 L/min. Tiddens *et al.* (2006) showed that a majority of children between the ages of 6 and 18 years old with cystic fibrosis generated a flow increase rate of at least 2 L/s^2 in inhalers with device resistances similar to that of the Turbuhaler DPI, suggesting that the vacuum pump could not produce a high enough flow increase rate to accurately replicate *in vivo* conditions. Thus, to facilitate a more

[3] As noted earlier, QVAR was delivered via Autohaler in the *in vivo* study referenced here (Devadason et al. 2003). While the Autohaler is a breath-actuated device, the mechanism providing energy for aerosolization relates to the rapid expansion of propellant in the propellant/drug solution of QVAR. As such, this device is considerably less dependent on inhalation flow parameters as compared to the Turbuhaler DPI.

representative inhalation profile than could be provided with the vacuum pump, a pulmonary waveform generator was used to deliver a time-variant trapezoidal inhalation profile, consisting of a constant flow increase rate from zero to peak inspiratory flow rate, followed by a period of constant inhalation, then a linear decrease back to zero flow, for *in vitro* experiments with the Turbuhaler DPI.

To approximate *in vivo* breathing parameters, the pulmonary waveform generator was configured to deliver appropriate values of flow increase rate and peak inspiratory flow rate for the patients under consideration in the Devadason *et al.* (1997) study focused on children with cystic fibrosis. From reported data of patient-specific peak inspiratory flow rate, the average peak inspiratory flow rate of children in the study found to be 53 L/min. An estimate of average flow increase rate generated by children with cystic fibrosis through the Turbuhaler, 2 L/s^2, was selected based on the study of Tiddens *et al.* (2006). Patient demographics and age-appropriate estimates of body height (average age of 10 years, average height of 136 cm, both male and female patients) allowed for an estimation of average inspiratory capacity using appropriate reference equations (Cook and Hamann 1961), yielding 1.5 L. These values for peak inspiratory flow rate, flow increase rate, and inspiratory capacity were used to fully define the time-variant inhalation profile supplied by the pulmonary waveform generator. After connecting the Turbuhaler DPI, Alberta Idealized Child Throat, and filter to the experimental setup, sufficient time was allowed for the environmental conditions to stabilize, after which the Turbuhaler DPI was primed. As exposure to high RH can negatively influence powder aerosolization over a relatively brief period of time (Jashnani, Byron, and Dalby 1995), the pulmonary waveform generator was triggered immediately after priming, drawing the simulated breathing profile

through the Turbuhaler DPI. The idealized throat, Turbuhaler DPI, and filter were then removed from the environmental chamber for deposition analysis.

Table 2: Reference equations for calculating total lung capacity and functional residual capacity based on patient height H (in cm) in patients age 5 to 38 years, measured using the helium dilution technique (Cook and Hamann 1961). Inspiratory capacity used for *in vitro* testing was calculated by subtracting the functional residual capacity from the total lung capacity.

Gender	Total Lung Capacity (mL)	Functional Residual Capacity (mL)
Boys	$0.95 \cdot 10^{-3} \cdot H^{3.039}$	$0.125 \cdot 10^{-3} \cdot H^{3.298}$
Girls	$1.698 \cdot 10^{-3} \cdot H^{2.909}$	$0.286 \cdot 10^{-3} \cdot H^{3.136}$

Following inhaler actuation into the Alberta Idealized Child Throat and removal from the environmental chamber, the idealized throat and filter were rinsed, respectively, with 10 mL and 5 mL of methanol. The solution collected from each deposition site was transferred to volumetric flasks, and adjusted to volume using methanol. Samples were subjected to chemical assay by UV spectroscopy (Model 8452A; Hewlett Packard, Greely, Ontario, Canada) at wavelengths of 238nm for beclomethasone dipropionate and 244nm for budesonide to determine the mass of drug depositing in the Alberta Idealized Child Throat and collection filter.

The delivered dose was calculated as the sum of active pharmaceutical ingredient recovered from the Alberta Idealized Child Throat and collection filter. The mass of drug depositing in the Alberta Idealized Child Throat, defined as mouth-throat deposition, was considered an *in vitro* measure of *in vivo* extrathoracic deposition. The mass collected on the

filter, defined as the lung dose, was considered analogous to *in vivo* lung deposition plus exhaled dose, given that only inspiratory flow was considered with the present setup. For the initial *in vitro* analysis of the effects of humidity, the delivered dose, mouth-throat deposition, and lung dose were reported as a percentage of the label claim for each inhaler as reported in Canada, equivalent to the ex-valve dose for pMDIs. However, mouth-throat deposition was also reported as a percentage of delivered dose for further *in vitro* analysis to be comparable with *in vivo* data sets from Devadason *et al.* (1997, 2003), which were reported as the percentage of the total recovered dose within the body. Experimental conditions were not explicitly reported in the *in vivo* studies by Devadason *et al.* (1997, 2003). However, assuming these studies were performed in a heated, ventilated, and air-conditioned location, typically designed to maintain humidity ranging from 40% to 60%, a reasonable estimate of 50% RH can be assumed. Therefore, for comparisons to *in vivo* data were performed with *in vitro* deposition measurements obtained at 50% RH.

Five measurements were performed at each RH, for a total of 25 runs with each inhaler. Deposition results were subjected to one-way ANOVA with post-hoc Tukey's Honestly Significant Difference test for a comparison of deposition at different RH, and unpaired Student's t-tests with Welch's correction for comparisons between *in vitro* and *in vivo* data (Prism 6.02; GraphPad Software, Inc., La Jolla, CA, USA), where a p value < 0.05 was considered significant.

2.3 Results

2.3.1 Effect of Humidity

The delivered dose, deposition in the idealized throat, and lung dose for the QVAR pMDI and Turbuhaler DPI under varying RH are shown in Figure 3, expressed as percentage of label claim. For the QVAR pMDI, no significant difference was observed in the delivered dose ($p=0.722$) for increasing RH, while significant differences were noted in mouth-throat deposition ($p=0.015$) and lung dose ($p<0.0001$). Average delivered dose was 77.4 ± 2.4 μg beclomethasone dipropionate (n=25), equal to 77.4±2.4% of label claim. For Turbuhaler, no significant differences were noted in the delivered dose ($p=0.727$), mouth-throat deposition ($p=0.567$), or lung dose ($p=0.774$) for increasing RH. Average delivered dose was 116.7 ± 27.5 μg budesonide (n=25), equivalent to 58.4±13.7% of label claim. In terms of dose variability, the coefficient of variation of the average delivered dose was 0.032 for the QVAR pMDI, and 0.235 for the Turbuhaler.

Mouth-throat deposition in the idealized model, expressed as a percentage of the delivered dose, is shown in Figure 4. For the QVAR pMDI, significant differences in mouth-throat deposition were noted for varying RH ($p<0.0001$ via ANOVA), with a slight trend of increasing mouth-throat deposition for increasing RH. For QVAR, the lowest mouth-throat deposition (36.2±1.2%) was measured at 10% RH, while the highest (42.6±2.0%) was measured at 90% RH. Post-hoc analysis showed that for moderate ranges in humidity (30% to 70%), the effect of humidity on mouth-throat deposition was not significant, except for a slight difference between 30% and 70% RH ($p=0.014$). This indicates that the deposition measured at 50% RH provides a good estimate of the typical deposition values expected in air conditioned spaces. At

50% RH, mouth-throat deposition of beclomethasone dipropionate via QVAR pMDI was 39.2±2.3% of delivered dose (n=5).

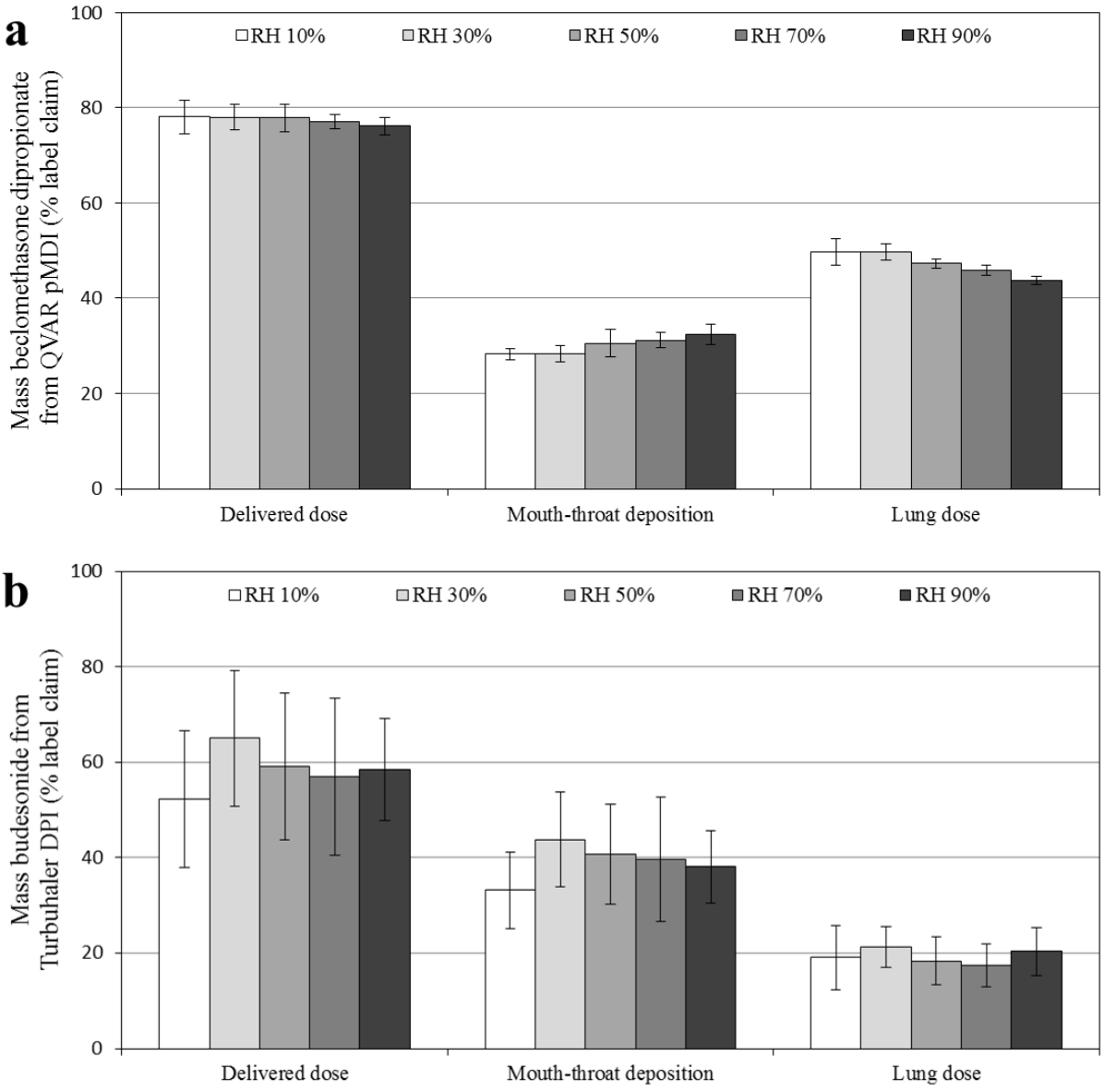

Figure 3: Mean deposition of a) the QVAR pMDI and b) the Turbuhaler DPI measured in the Alberta Idealized Child Throat under varying RH. Delivered dose, mouth-throat deposition, and lung dose are expressed as a percentage of the label claim for each device. Error bars denote standard deviation (n=5).

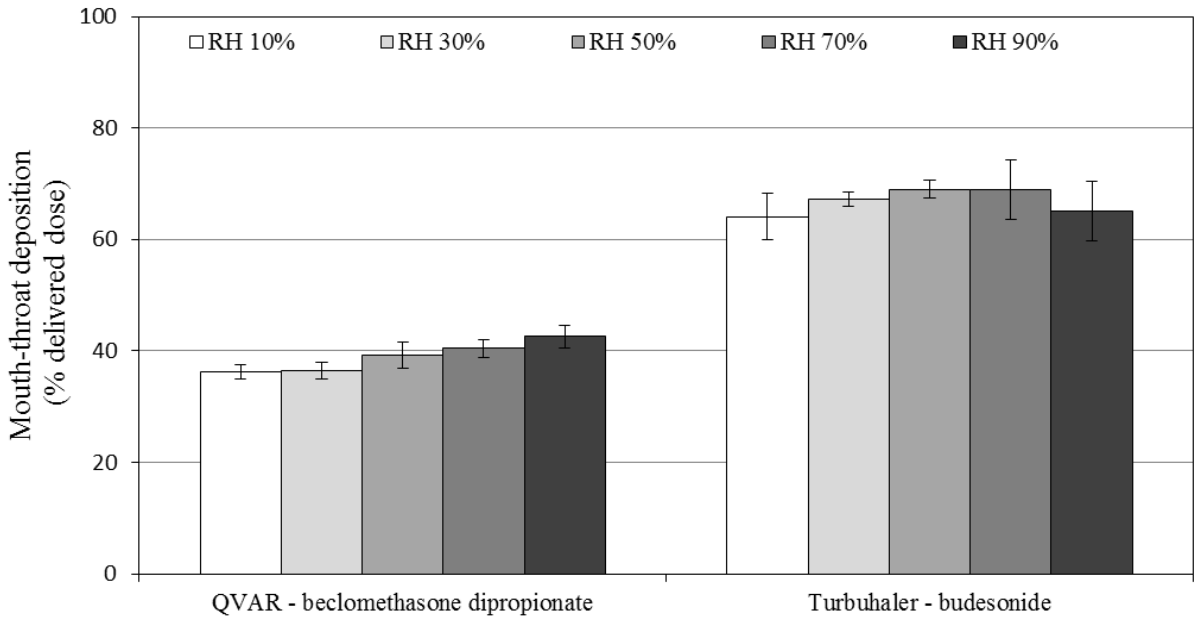

Figure 4: Mouth-throat deposition for the QVAR pMDI and Turbuhaler DPI in the Alberta Idealized Child Throat setup at varying RH, expressed as a percentage of delivered dose. Error bars denote standard deviations (n=5).

For Turbuhaler, no significant differences in mouth-throat deposition were observed for increasing RH (p=0.210 via ANOVA). The lowest deposition measured in the mouth-throat (64.1±4.2% of delivered dose) was measured at 10% RH, while the highest was observed at 70% RH (69.0±5.3%). Mouth-throat deposition of budesonide via Turbuhaler at 50% RH was 69.0±1.5% (n=5).

2.3.2 *In Vitro – In Vivo* Comparison

Devadason *et al.* (2003) reported deposition of radiolabeled QVAR in children age 5 to 14 in terms of the total dose depositing in the body or exhaled, equivalent to the delivered dose defined in the present work. Extrathoracic deposition was measured to be 59.7±8.2% (n=5), 48.9±12.3% (n=7), and 40.3±11.8% (n=4) of delivered dose, respectively, for children age 5 to 7,

8 to 10, and 11 to 14. No significant difference was observed between *in vitro* mouth-throat deposition at 50% RH and *in vivo* extrathoracic deposition for children age 11 to 14 (p=0.865) and 8 to 10 (p=0.084), while a significant difference was observed for deposition in children age 5 to 7 (p=0.004). Pooling the two oldest age groups, which are similar to the range of subjects upon which the Alberta Idealized Child Throat was based (Golshahi and Finlay 2012), mouth-throat deposition of QVAR in the Alberta Idealized Child Throat at 50% RH agreed well with the *in vivo* average for children age 8 to 14 of 45.8±12.3% (p=0.113).

In the *in vivo* study examining deposition from the Turbuhaler DPI, Devadason *et al.* (1997) reported extrathoracic deposition separately in terms of the oropharynx and the stomach. From their reported data, equivalent extrathoracic deposition was recalculated by adding deposition in the oropharynx and stomach; this gave estimates of *in vivo* extrathoracic deposition equal to 70.4±20.5%, 75.6±24.5%, and 65.1±21.1% of delivered dose in children age 6 to 8, 9 to 12, and 13 to 16, respectively. No significant difference was observed between *in vitro* and *in vivo* deposition for these age groups of 6 to 8 (p=0.874), 9 to 12 (p=0.539) and 13 to 16 (p=0.670); mouth-throat deposition in the Alberta Idealized Child Throat thus compares well with *in vivo* deposition in children age 6 to 16 with cystic fibrosis of 70.4±21.2% (p=0.424).

A summary of these comparisons is shown in Figure 5, where *in vitro* mouth-throat deposition is compared to *in vivo* extrathoracic deposition in children age 8 to 14 using QVAR and children age 6 to 16 using Turbuhaler.

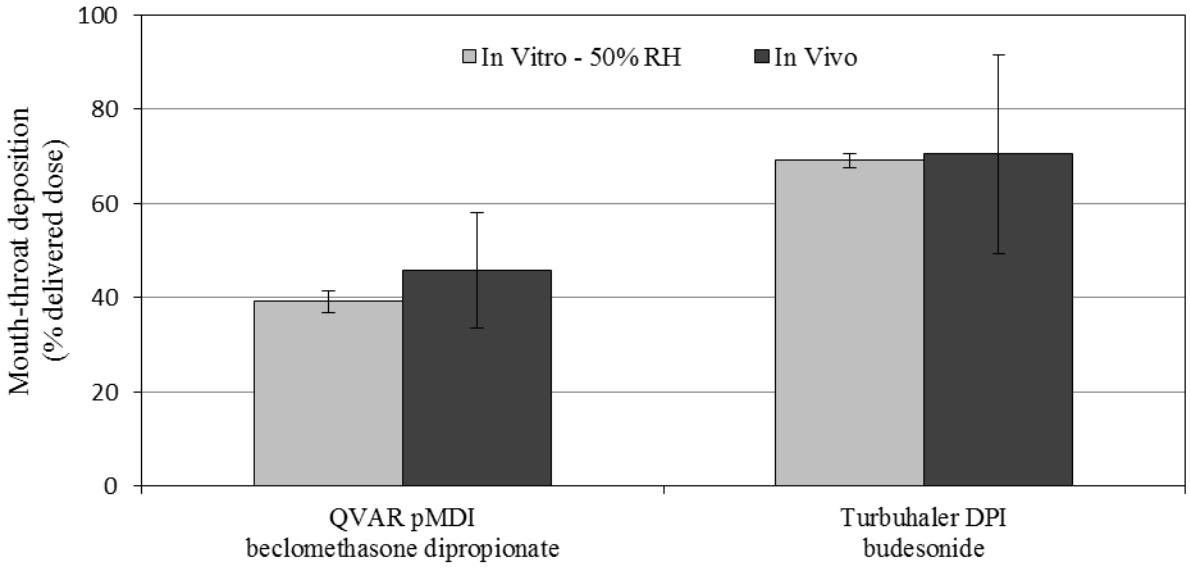

Figure 5: Mouth-throat deposition in the Alberta Idealized Child Throat at 50% RH compared to in vivo extrathoracic deposition in children age 8 to 14 for the QVAR pMDI and 6 to 16 for the Turbuhaler DPI. Error bars denote standard deviation (n=5).

2.4 Discussion

2.4.1 Humidity Effects

While the delivered dose from the QVAR pMDI remained consistent at varying RH, significant differences in regional deposition were observed. The relatively weak trend of increasing mouth-throat deposition with increasing RH, illustrated in Figure 4, mirrors the results of a recent study in which the deposition of a beclomethasone dipropionate pMDI (100 µg beclomethasone dipropionate per dose, 13% w/w ethanol, 1.3% w/w glycerol, in HFA134a - a similar formulation to QVAR) was examined in the Alberta Idealized Throat (Shemirani *et al.* 2013). In that study, Shemirani *et al.* (2013) found no difference in deposition for the HFA-134a beclomethasone dipropionate pMDI between 0% and 35% RH, but a significant difference between 35% and 80% RH, at a temperature of 20°C and flow rate of 60 L/min. Between 35%

and 80% RH, mouth-throat deposition increased from 43.5% to 50.8%, while the lung dose decreased from 56.5% to 48.0%, reported as a percentage of recovered dose (including retained dose within the pMDI actuator). This effect of humidity on deposition from pMDIs is believed to relate to the condensation of water onto propellant-cooled residual dry particles (Martin and Finlay 2005). As noted by Shemirani *et al.* (2013), higher RH would likely cause an increase in particle diameter, leading to increased throat deposition and a correspondingly lower lung dose. In the Alberta Idealized Child Throat, this effect was observed in the relatively minor 6% increase in mouth-throat deposition for RH increasing from 10% to 90%.

Unlike the QVAR pMDI, regional deposition with the Turbuhaler DPI showed no significant dependence on humidity, with mouth-throat deposition and lung dose remaining consistent between 10% and 90% RH. As evident in Figure 3, the Turbuhaler DPI yielded a high variability in delivered dose compared to the QVAR pMDI. This reflects the considerable variability in the performance of the Pulmicort Turbuhaler DPI that has been well-documented in the literature (Hindle and Byron 1995; Steckel and Müller 1997; Kamin *et al.* 2002; Kwok and Chan 2008). Despite a high variability in delivered dose, percentage deposition in the mouth-throat and the lung dose remained consistent across all examined RH for the Turbuhaler DPI. For the QVAR pMDI, no significant difference in delivered dose was measured at varying RH, while mouth-throat deposition increased slightly from 36.2% to 42.6% for RH increasing from 10% to 90%. Considering deposition in the mouth-throat and the lung dose, with no significant differences for the Turbuhaler DPI and minor differences for the QVAR pMDI, environmental conditions under which *in vivo* studies on the QVAR pMDI and Turbuhaler DPI were performed likely played a minor role on regional deposition measurements. This may not always be the case however, as demonstrated by the 30% decrease in lung dose from the Flixotide Evohaler

measured by Shemirani *et al.* (2013) for RH increasing from 0% to 80% at a temperature of 20°C. Thus, it is recommended that authors of *in vivo* studies report the environmental conditions under which experiments are performed to aid in proper drug delivery comparisons.

2.4.2 *In Vivo – In Vitro* Comparison

Deposition in the Alberta Idealized Child Throat compared well with the *in vivo* measurements by Devadason *et al.* (2003) for children age 8 to 14 using the QVAR pMDI. Extrathoracic deposition in children age 11 to 14, at 40.3±11.8% of delivered dose, matched mouth-throat deposition measured in the idealized throat at 50% RH, 39.2±2.3%. Good agreement was also found for *in vivo* extrathoracic deposition in children age 8 to 10. However, children age 5 to 7 demonstrated considerably higher mouth-throat deposition compared to older patients, with average extrathoracic deposition in this young age group equaling 59.7±8.2%, resulting in a poor comparison to deposition in the Alberta Idealized Child Throat. This likely stems from age-related differences in the size of the extrathoracic region. The average age of children used to develop the Alberta Idealized Child Throat was 11 years (Golshahi and Finlay 2012), and as such the size of the Alberta Idealized Child Throat is more in line with the dimensions of the extrathoracic regions of older patients in the Devadason *et al.* (2003) study. Increased impaction of the spray emitted from the QVAR pMDI would be expected in younger patients due to the decreased distance between the back of the throat and the mouthpiece of the inhaler, resulting in the increased extrathoracic deposition observed *in vivo*. Measurements in the larger Alberta Idealized Throat support this theory; in an examination of deposition from a QVAR pMDI in the Alberta Idealized Throat, Zhang, Gilbertson, and Finlay (2007) measured a mouth-throat deposition of 25.8±4.2% of delivered dose, considerably lower than that observed here in the Alberta Idealized Child Throat. *In vivo* deposition measurements of 100 μg QVAR in

older patients also support this trend, with Leach, Davidson, and Boudreau (1998) reporting an extrathoracic deposition of 29.0±18.0% of delivered dose in adult males age 18 to 55.

For the Turbuhaler DPI, deposition in the Alberta Idealized Child Throat compared well with that observed in children age 6 to 16 with cystic fibrosis (Devadason *et al.* 1997). Devadason *et al.* (1997) also measured deposition in two patients 3 to 5 years old, measuring a rather large average extrathoracic deposition of 86.8% of delivered dose (recalculated from reported deposition in the oropharynx and stomach). However, these two patients were much younger than the age represented by the Alberta Idealized Child Throat, and a proper statistical comparison to *in vitro* data could not be performed with only two subjects. For the time-variable flow profile used in the present work, flow increase rates and peak inspiratory flow rates representative of appropriate *in vivo* values for children with cystic fibrosis capture average *in vivo* deposition effectively. However, the parameters of flow increase rate and peak inspiratory flow rate are patient dependent, and given the dependence of Turbuhaler performance on these parameters (De Boer, Gjaltema, and Hagedoorn 1996; De Boer *et al.* 1997; Kamin *et al.* 2002; Martin, Marriott, and Zeng 2007; Everard, Devadason, and Le Souëf 1997), it is important to use values representative of the patient group under consideration in rigorous *in vitro* analyses. This importance is illustrated by a comparison of deposition in the Alberta Idealized Child Throat to that measured by Wildhaber *et al.* (1998) for radiolabel budesonide via Turbuhaler in asthmatic children age 6 to 16. The asthmatic patients in that *in vivo* study generated a peak inspiratory flow rate of 65 L/min, notably higher than that obtained by the cystic fibrosis patients of Devadason *et al.* (1997), though no data was reported concerning flow increase rate. The extrathoracic dose was recalculated as a percentage of the delivered dose from reported data (delivered dose equaling oropharyngeal deposition plus lung deposition), yielding an average of

55.4% of delivered dose, considerably less than the mouth-throat deposition measured in the Alberta Idealized Child Throat at 50% RH (69.0±1.5%) and the average extrathoracic deposition measured *in vivo* for children with cystic fibrosis at 70.4±21.2% (Devadason *et al.* 1997). Increased peak inspiratory flow rates achieved by the asthmatic patients in the study of Wildhaber and colleagues (1998) suggest that these subjects were likely able to generate more energy through the breath-actuated Turbuhaler DPI, resulting in improved aerosolization of the budesonide powder and improved delivery to the lungs. From this difference in deposition among two patient groups of similar ages, it is clear that realistic *in vitro* breath parameters for the patient group under consideration must be employed to achieve a good comparison to *in vivo* data.

The simulated breathing patterns in the present study were relatively simple, with a constant flow rate for the pMDI and a trapezoidal time-variant flow profile for the DPI. It has been suggested that the use of more realistic profiles may provide better comparisons between *in vivo* and *in vitro* deposition. For example, Delvadia *et al.* (2013) recently demonstrated a good comparison of deposition in an adult mouth-throat and upper airway model with *in vivo* data for five commercial dry powder inhalers using a breathing simulator and flow profiles more typical of patient use. While the methods employed in the present study were successful in replicating *in vivo* deposition in school age children, there remains room to study the effect of realistic breathing profiles on deposition in idealized pediatric geometries, as has been examined previously in the adult Alberta idealized Throat (Finlay and Gehmlich 2000). As a final note, it should be observed that *in vitro* analysis of the type performed here assay for drug content in determining deposition, while *in vivo* studies of a scintigraphic nature assess deposition using radiolabeled markers (typically ^{99M}TC); proper comparison of these data require that the

aerodynamic characteristics of drug and radiolabel are similar, and the radiolabeling process of drug formulations is not without issue (Scheuch *et al.* 2010). Radiotracers of DPIs may only coat the outside of solid particles, resulting in particle size-dependent proportions of drug to radiolabel, while for pMDIs, the distribution of drug and radiolabel among different sized liquid droplets may be notably uneven (Devadason *et al.* 2012). With this in mind, acceptance criteria regarding the aerodynamic characteristics of radiotracer and drug have recently been published (Devadason *et al.* 2012) that should address this issue and aid in future *in vivo – in vitro* comparisons.

2.5 Conclusions

The recently developed Alberta Idealized Child Throat has been compared with *in vivo* scintigraphic deposition data in school age children. For QVAR pMDIs, mouth-throat deposition in the Alberta Idealized Child Throat at 50% RH (39.2±2.3% of delivered dose) compared well with *in vivo* deposition in asthmatic children age 8 to 14 (45.8±12.3%). For Turbuhaler DPIs, *in vitro* mouth-throat deposition at 50% RH (69.0±1.5%) matched *in vivo* deposition in 6 to 16 year old children with cystic fibrosis (70.4±21.2%). Humidity ranging from 10% to 90% RH was found to have a small effect on the deposition from the QVAR pMDI and an insignificant effect on deposition from the Turbuhaler DPI at a temperature of 23.5°C. It is recommended that *in vivo* studies report the environmental conditions under which data is collected to aid in future comparisons between *in vivo* and *in vitro* data.

The current focus on pediatric respiratory drug delivery has outlined the need for improved *in vitro* methods for predicting aerosol deposition in young patients. The Alberta Idealized Child Throat, here shown to mimic *in vivo* deposition data for two common types of pharmaceutical inhalers – pressurized metered dose inhalers and dry powder inhalers – may

provide a standard platform for optimizing the treatment of school age children with inhaled pharmaceutical aerosols.

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Chapter 3: *In Vitro* and *In Silico* Characterization of Pharmaceutical Aerosol Deposition in an Idealized Infant Extrathoracic Geometry

3.1 Introduction

For infants and children, the treatment of respiratory disease using inhaled pharmaceutical aerosols, while providing an important modality of medication delivery, requires certain considerations. From a clinical perspective, pediatric patients cannot be considered small adults. Differences in anatomy, physiology, and disease process between pediatric and adult patients mean that response to a given treatment can vary considerably (Everard 2003; Ahrens 2005; Fink 2012). Clinical trials on infants and small children are difficult, expensive, and carry considerable ethical concerns while offering little in the way of incentive for pharmaceutical companies (McIntyre *et al.* 2000), resulting in a general lack of information regarding the safety and efficacy of inhaled pharmaceutical therapies for young patients. As such, pediatric clinicians are often left to balance the risks and benefits of an off-label or unproven administration of therapeutic agent (Tauer 2002; Smyth *et al.* 2010).

The human extrathoracic airways act a filter that limits the dose of inhaled pharmaceutical aerosol delivered to the lungs, thereby playing an important role in the determination of the total lung dose of pharmaceutical aerosols (Stahlhofen, Rudolf, and James 1989; Borgström, Olsson, and Thorsson 2006; Finlay and Martin 2008). Accurate characterization of extrathoracic deposition is an important step in ensuring that patients receive consistent, efficacious, and safe doses of medication with inhalation therapies. Extrathoracic deposition is heavily dependent on the mode of breathing (oral versus nasal, tidal versus single inhalation), the age of the subject (affecting the dimensions of the airways), and the inhalation

flow rate. Smaller airways in younger patients would suggest increased deposition in the extrathoracic airways due to impaction. However, young patients generally inhale at lower flow rates, yielding the opposite effect. The overall effects of age on deposition are complicated, and depend on the patient, the drug, and the method of delivery (Everard 2004; Fink 2012; Carrigy *et al.* 2014b).

Infants, as obligate nose breathers, are typically administered medications using either nebulizers or pressurized metered dose inhalers with valved holding chambers and facemasks. A number of studies have quantified *in vivo* nasal deposition in adults (Hounam, Black, and Walsh 1969; Heyder *et al.* 1986; Rasmussen, Andersen, and Pedersen 2000; Cheng 2003) and in both adults and children (Becquemin *et al.* 1991; Bennett, Zeman, and Jarabek 2008). Though a limited number of *in vivo* studies have been performed on infant subjects (Alderson *et al.* 1974; Chua *et al.* 1994; Mallol *et al.* 1996; Erzinger *et al.* 2007; Schueepp *et al.* 2009), the costs, difficulties, and ethical concerns discussed previously prevent the widespread use of these types of studies in examining aerosol deposition in very young patients. To address these difficulties, an increasing number of investigators have made use of realistic replicas of pediatric nasal airways to examine deposition *in vitro* (Swift 1991; Cheng *et al.* 1995; Janssens *et al.* 2001; Janssens *et al.* 2004; Mitchell 2008; Storey-Bishoff, Noga, and Finlay 2008; Laube *et al.* 2010; Golshahi *et al.* 2010). Additional *in silico* studies, employing three dimensional computational fluid dynamics simulations in realistic nasal airway geometries, have provided further insight into the mechanisms governing deposition in the nasal airways of infants in comparison to subjects of older ages (Xi *et al.* 2012; Xi, Kim, and Si 2012) and in nebulizer hoods (Kim *et al.* 2014).

An alternative to the use of realistic airways in benchtop testing of aerosol deposition lies in idealized geometries. The first of these geometries, the Alberta Idealized Throat (Stapleton *et al.* 2000), incorporated simplified analogues of important anatomical features of adult extrathoracic oral airways, and has been shown to replicate average deposition in adults (Grgic *et al.* 2004; Zhang, Gilbertson, and Finlay 2007; Zhou, Sun, and Cheng 2011). An isotropically-scaled child version of this idealized adult throat (Golshahi and Finlay 2012) has, as described earlier in Chapter 2, been shown to replicate the average deposition of pharmaceutical aerosols in pediatric subjects. An additional idealized geometry representing children 4 to 5 years old was developed through asymmetric scaling of the Alberta Idealized Throat (Bickmann *et al.* 2008), though the ability of this model to replicate *in vivo* deposition has yet to be demonstrated. Recently, an idealized infant geometry (Javaheri, Golshahi, and Finlay 2013) was designed based on deposition measurements of micrometer sized particles using previously-characterized realistic nasal airway replicas of 10 infants 3 to 18 months old (Storey-Bishoff, Noga, and Finlay 2008). This idealized infant geometry has been shown to replicate *in vitro* deposition of micrometer sized jojoba oil particles in realistic airways (Javaheri, Golshahi, and Finlay 2013), but has yet to be used to characterize deposition of pharmaceutical aerosols.

A number of recent reviews have hinted towards the utility of combining *in vitro* experiments and *in silico* simulations, leading to improved understanding of particle deposition (Longest and Holbrook 2012; Ruzycki, Javaheri, and Finlay 2013; Carrigy *et al.* 2014b). Of interest in examining regional deposition in lungs are one-dimensional models, which make use of known correlations to predict deposition by various mechanisms including impaction, sedimentation, and diffusion in simplified models of the respiratory tract. In these models, the lungs are commonly represented as a series of bifurcating tubes, originating from the trachea and

branching into deeper generations. Various correlations and lung models have been proposed and are reviewed elsewhere (Finlay 2001; Hofmann 2011; Longest and Holbrook 2012; Carrigy *et al.* 2014b). Javaheri *et al.* (2013) recently described one such model incorporating the symmetric lung model of Finlay *et al.* (2000) to estimate the effects of hygroscopic size changes on deposition. This model, which has yet to be applied towards deposition modeling in infants, provides a robust platform from which to model aerosol deposition throughout the respiratory tract. Following particle deposition, further information can be gained by modeling the effects of mucus production and tracheal clearance velocity on the concentration of drug in the airway surface liquid. As drug concentration plays an important role in the absorption and distribution of therapeutic agent (Chillistone and Hardman 2008), knowledge of the airway surface liquid concentration can provide guidance on proper dosing. One such model was proposed by Lange *et al.* (2001), who developed a numerical scheme to estimate the airway surface liquid concentration in a study on aerosolized antibiotics, using the results to specify required dosing from a jet nebulizer to reach minimum inhibitory levels of a liposomal peptide in pediatric and adult models.

The goal of the present study was to characterize pharmaceutical aerosol deposition in infants using a join *in vitro – in silico* methodology to ultimately provide guidance with regards to safe and efficacious dosing of marketed pharmaceutical inhalers in infants. Estimates for the total lung dose from two pressurized metered dose inhalers (Clenil® Modulite®, label claim 250 µg beclomethasone dipropionate, and Clenil® Compositum, label claim 250 µg beclomethasone dipropionate plus 100 µg salbutamol, Chiesi Farmaceutici S.p.A., Italy) delivered via valved holding chamber and facemask with a realistic breathing profile were obtained *in vitro* using the idealized infant geometry of Javaheri, Golshahi, and Finlay (2013). The one-dimensional lung

deposition model of Javaheri *et al.* (2013), with a scaled lung morphology representative of infants, was then used to estimate regional respiratory tract deposition of the active therapeutic agents from each inhaler. Further simulation of the airway surface concentration throughout the lung was performed using the model of Lange *et al.* (2001).

3.2 Materials and Methods

3.2.1 Idealized Infant Geometry

The idealized infant geometry recently described by Javaheri, Golshahi, and Finlay (2013) was designed to replicate average nasal airway deposition measured in 10 realistic airway replicas of infants 3 to 18 months old, previously characterized by Storey-Bishoff *et al.* (Storey-Bishoff, Noga, and Finlay 2008) for micrometer-sized particles inhaled during tidal breathing. Using cross-sections of the realistic replicas, Javaheri and colleagues designed the idealized infant geometry to incorporate simplified analogues of important anatomical features with significant influences on inertial impaction - including the septum, turbinates, and the nasopharynx - while ensuring that the hydraulic diameter[4] of the idealized geometry matched the average of the realistic replicas at 4.8 mm. Experimental measurements of deposition confirmed that the idealized infant geometry successfully captured average *in vitro* deposition of micrometer-sized particles measured in the realistic airway replicas (Javaheri, Golshahi, and Finlay 2013). To avoid artificial electrostatic surface charging effects, a stainless steel model of the idealized infant geometry was rapid-prototyped (Linear Mold & Engineering, Livonia, MI, USA) for *in vitro* deposition measurements in the present study.

[4] The hydraulic diameter D_h is calculated as the 4 times the airway volume V divided by the airway surface area A_S.

3.2.2 *In Vitro* Experimental Setup

Two pressurized metered dose inhalers (pMDIs) were selected for use in the present study, including a solution pMDI delivering beclomethasone dipropionate (label claim 250 µg beclomethasone dipropionate, Clenil® Modulite® 250 mcg; Chiesi Farmaceutici S.p.A., Italy) and a suspension pMDI delivering both beclomethasone dipropionate and salbutamol (label claim 250 µg beclomethasone dipropionate plus 100 µg salbutamol, Clenil® Compositum 250 mcg + 100 mcg; Chiesi Farmaceutici S.p.A., Italy). Each pMDI was administered using a valved holding chamber and face mask (AeroChamber Plus Flow-Vu Hospital Medium Mask; Trudell Medical International, London, Ontario, Canada), allowing for tidal inhalation of pharmaceutical aerosol through the idealized infant geometry. Valved holding chambers were used directly out-of-package according to product inserts, and to prevent contamination of samples separate valved holding chambers were used for each type of pMDI. Inhalers were handled and operated according to product insert instructions; Clenil Modulite was primed by firing once to waste, while Clenil Compositum was shaken vigorously prior to each actuation.

A diagram of the setup used to affix the valved holding chamber to the idealized infant geometry is shown in Figure 6. The facemask was sealed with sufficient force to the face of the idealized geometry to prevent leakage, a factor known to significantly reduce drug delivery efficiency from pMDIs coupled with valved holding chambers (Esposito-Festen *et al.* 2004; Carrigy *et al.* 2014a), though care was taken to not deform the facemask significantly. During breathing simulation, the quality of the seal was confirmed by observing the motion of the Flow-Vu seal indicator valve located on the AeroChamber facemask. The dose of aerosol delivered distal to the idealized infant geometry *in vitro* under simulated breathing is analogous to *in vivo* lung deposition (Finlay and Martin 2008). The *in vitro* lung dose was captured using a bias flow

system consisting of a compressed building air supply line and an in-house breathing machine upstream of the infant geometry, and a collection filter (Respirgard II™ 303, Vital Signs Incorporated; Englewood CO, USA) and vacuum pump (Model 0523; Gast Manufacturing Inc., Benton Harbor, MI, USA) downstream, shown schematically in Figure 7. The idealized infant geometry was coupled to the bias flow system using a custom tee designed to minimize loss of aerosol. Volumetric flow rates in the compressed air line and the vacuum pump line were measured using thermal mass flowmeters (TSI Model 4043; Shoreview, MN USA) and set to 45 L/min. 45 L/min was selected based on efficient aerosol capture at this flow rate in earlier experiments with the Alberta Idealized Child Throat (see Chapter 2). Use of this bias flow setup allowed for the delivery of time variant breathing patterns though the idealized infant geometry using the breathing machine while maintaining a sufficiently large, steady flow rate through the collection filter to ensure efficient capture of aerosol. The bias flow system also eliminated the effects of the approximate 20 mL dead space of the filter housing, which would otherwise lead to an underestimation of the lung dose, as all airflow passing through the geometry reached the filter.

The breathing machine was configured to deliver a sinusoidal breathing pattern representative of that expected in an average 1 year old infant. Reference equations[5] (Nguyen *et al.* 2013) were used to calculate appropriate values for tidal volume (94 mL) and number of breaths per minute (28 breaths per minute) for a 1 year old infant of average weight and height at 10 kg and 75 cm respectively (Stocks and Hislop 2002). The duty cycle, defined as the ratio of inspiratory time

[5] From Nguyen et al. 2013, equations for calculating the respiratory rate RR and the tidal volume V_T are presented as $RR = 2.588 + 1876.034/L + 38.906/A$ and $V_T = -38.347 + 1.128L + 0.204A + 3.688W$, based on patient height L (in cm), age A (in weeks), and weight W (in kg)

over the total respiratory maneuver time, was taken as 0.42 to match that expected in healthy infants (Stocks and Hislop 2002). The breathing pattern was cycled continuously, with no pause between inhalation and exhalation. Six complete breaths were drawn during each test in accordance with AeroChamber valved holding chamber directions for use.

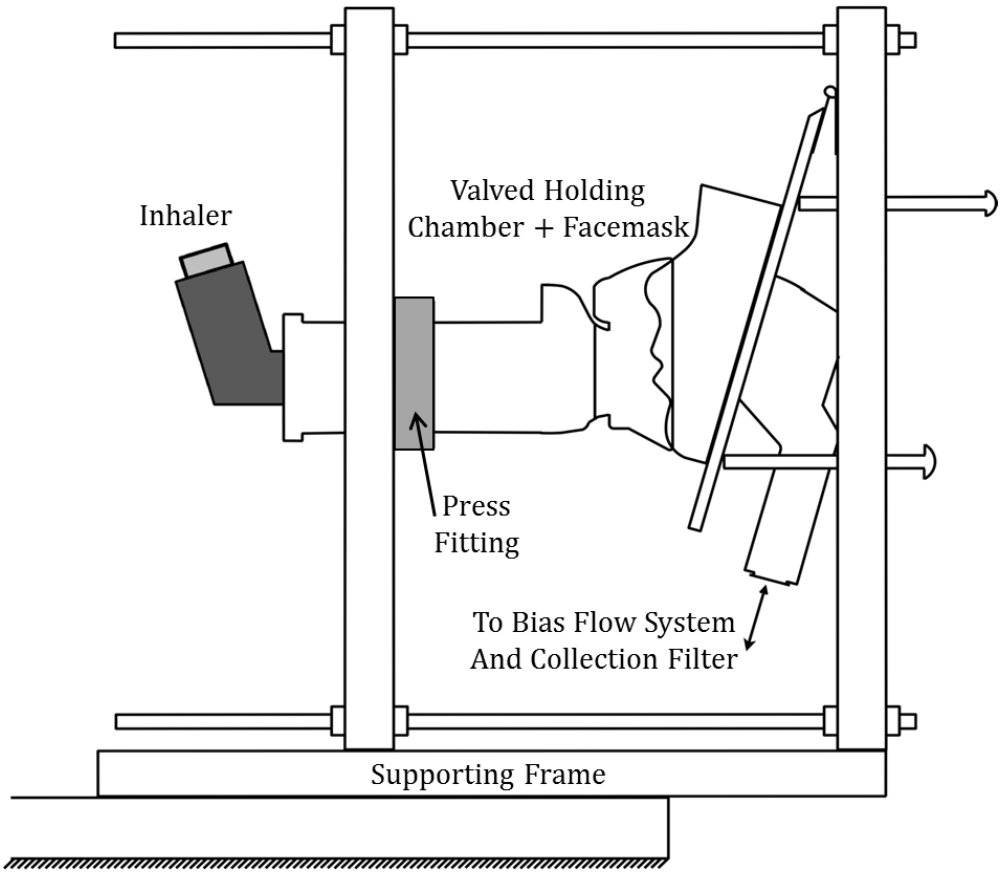

Figure 6: Experimental setup used to attach the valved holding chamber plus inhaler to the idealized infant geometry, with the outlet of the geometry leading to the bias flow system and collection filter.

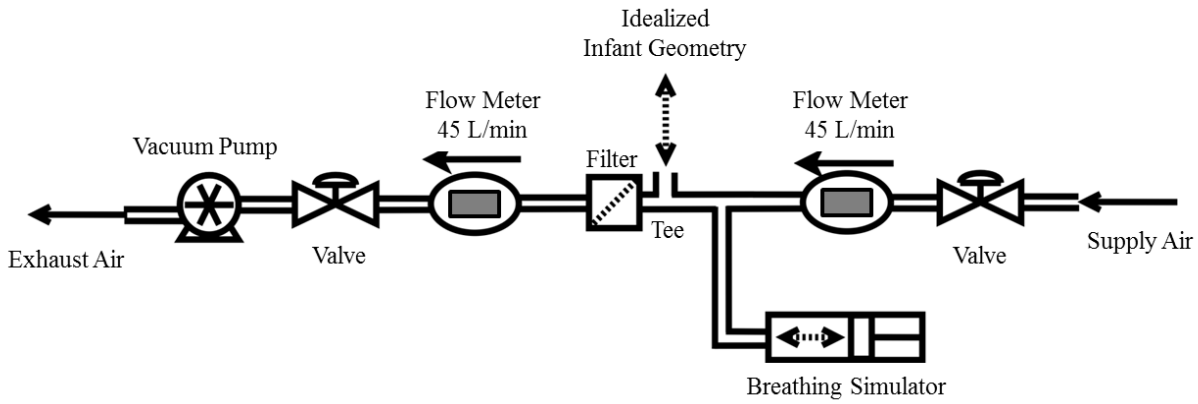

Figure 7: Schematic of the bias flow system used to provide simulated breathing patterns to the idealized infant geometry and ensure efficient aerosol collection on the downstream filter.

The use of non-symmetric inhalation and exhalation segments required the construction of a suitable breath profile. With a respiratory rate of 28 breaths per minute, 1 breath occurred every 2.14 seconds. For a duty cycle of 0.42, the inspiratory time was thus 0.9 seconds, while the expiratory time was 1.24 seconds, assuming no pause between inhalation and exhalation. In this case, the inhalation and exhalation curves were defined by separate sine waves with periods of 1.8 s and 2.48 s, respectively, with the same tidal volume of 94 mL. From the general equation for a sinusoidal flow signal Q:

$$Q = A\sin(2\pi f t) \tag{1}$$

where A is the amplitude, f is the frequency, and t is the time, integration over half of the period t' allows for calculation of the tidal volume:

$$V_T = \int_0^{\frac{t'}{2}} Q dt \tag{2}$$

Inserting Equation (1) into (2) and noting $f = 1/t'$, it follows that $A = \pi V_T/t'$. Expanding on Equation (1), the flow rate for a sinusoidal breath profile can be calculated as:

$$Q = \frac{\pi V_T}{t'} \sin\left(\frac{2\pi t}{t'}\right) \tag{3}$$

Inhalation and exhalation portions of the curve were defined separately based on the respiratory rate, duty cycle, and tidal volume described previously, with t' = 1.8 s for inhalation, and t' = 2.48 s for exhalation, then programmed into the in-house breathing machine. To confirm that the breath profile delivered through the idealized infant geometry matched that programmed into the breathing machine, a third thermal mass flowmeter (TSI Model 4043; Shoreview, MN USA) affixed to the face of the idealized infant geometry in an airtight manner was used to measure the flow rate generated during operation of the bias flow system shown in Figure 7. As the TSI Model 4043 thermal mass flowmeters are calibrated for flow in one direction, the inhalation and exhalation portions were measured separately, with the meter oriented to measure flow in the appropriate direction. These tests revealed that correction factors to both the time and tidal volume were required to obtain the desired breathing profile through the idealized infant geometry; data defining the inhalation and exhalation curves were multiplied by the factors of 1.07 for time and 1.14 for tidal volume during inhalation, and 1.10 for time and 1.16 for tidal volume during exhalation. With these correction factors, the breath profile delivered through the infant geometry closely matched that of the nominal case defined above, with differences of less than 1% and 3% in the average inhalation and exhalation flow rates, respectively, between the nominal and measured cases. A comparison of the nominal and measured breath profiles is shown in Figure 8.

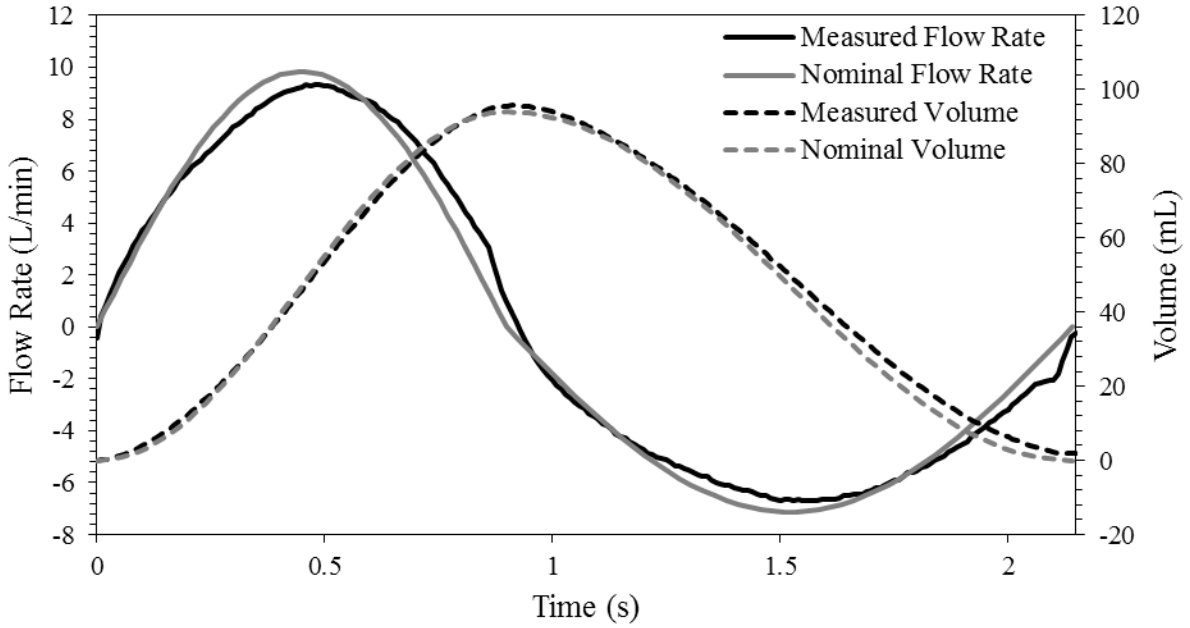

Figure 8: Comparison of measured and nominal breathing profiles delivered through the idealized infant geometry using the bias flow experimental setup. Positive flow rate indicates inhalation, negative flow rate indicates exhalation.

Prior to testing, interior surfaces of the idealized infant geometry were coated with silicone oil (Molykote 316; Dow Corning Corporation, Midland, MI, USA) to reduce the potential of particle bounce. After allowing 15 minutes for solvent evaporation, the idealized infant geometry was assembled, the valved holding chamber was affixed to the face, and the pMDI was inserted into the holding chamber. The bias flow system was then turned on and allowed to stabilize to 45 L/min, and a single inhalation/exhalation profile was delivered through the idealized geometry without pMDI actuation to confirm a good seal of the face mask via observation of the AeroChamber Flow-Vu seal indicator. The pMDI was then actuated into the valved holding chamber, with the inhalation cycle of the breathing machine triggered immediately after actuation. Following six simulated breaths, of both inhalation and exhalation,

the collection filter was disconnected from the flow system and subjected to chemical assay by UV spectroscopy. Beclomethasone dipropionate (from Clenil Modulite and Clenil Compositum) UV absorbance was measured in methanol at 238 nm in a regular Quartz cell, while salbutamol sulfate (from Clenil Compositum) was measured in water at 276 nm in a Malvern PC 1115 cell to determine the mass of pharmaceutical compound depositing in the collection filter for each inhaler. Note that samples from Clenil Compositum were analyzed for salbutamol sulfate, for which the expected mass is 120 μg per actuation, equivalent to 100 μg of salbutamol. Five repeated measurements were performed with each inhaler. To avoid sample contamination, the idealized infant geometry was disassembled and washed with acetone after each batch of measurements with a given inhaler. The filter deposition measured experimentally was then used as input for *in silico* modeling as discussed below.

3.2.3 *In Silico* Deposition Modeling[6]

In vitro filter deposition, while providing valuable information analogous to the mass of drug delivered to the lungs (Finlay and Martin 2008), does not yield information on the particle size distribution passing through the extrathoracic region. For the purpose of *in silico* deposition modeling, particle size must be known in order to apply the equations governing particle deposition in the respiratory tract. To this end, particle size distributions[7] for each inhaler were

[6] I am indebted to Emadeddin Javaheri for performing the one-dimensional numerical modeling of lung deposition and the calculations of airway surface liquid concentration described in this section. Special thanks are owed for his diligent work.

[7] Particle size distributions for Clenil Modulite and Clenil Compositum, measured downstream of a United States Pharmacopeia Induction Port, were obtained through personal communication with Tanya Church, Deputy Head of Laboratory, Chiesi Limited, 2013).

mathematically filtered to account for extrathoracic deposition in the idealized infant geometry using the empirical deposition equation of Javaheri, Golshahi and Finlay (2013):

$$\eta = 1 - \left[\frac{8.35 \times 10^7}{8.35 \times 10^7 + \mathrm{Re}^{2.812}\mathrm{Stk}^{1.094}}\right]^{0.4} \tag{4}$$

Here, η denotes the fraction of particles that deposit in the geometry, Re is the flow Reynolds number, and Stk is the Stokes number. The Reynolds number defines the ratio of inertial forces to viscous forces in a fluid and accounts for flow field effects, while the Stokes number, the ratio of a particle's stopping distance to a chosen characteristic dimension, governs inertial impaction. Particles with larger Stokes numbers deviate further from flow streamlines, leading to increased deposition as flow curves through the extrathoracic region. The Reynolds number and Stokes number are defined as:

$$\mathrm{Re} = \frac{4\rho_{air}\bar{Q}}{\pi\mu D_h} \tag{5}$$

$$\mathrm{Stk} = \frac{2\rho d^2 Cc\bar{Q}}{9\pi\mu {D_h}^3} \tag{6}$$

Variables influencing these dimensionless parameters include the density of air and the particle (ρ_{air} and ρ, respectively), the dynamic viscosity of air μ, the average flow rate during inhalation Q (equal to 6.15 L/min), the hydraulic diameter D_h (equal to 4.8 mm in the idealized infant geometry), the particle diameter d, and the Cunningham Correction Factor Cc, itself calculated as (Hinds 1999):

$$Cc = 1 + \frac{1}{Pd}[15.60 + 7.00\exp(-0.059Pd)] \quad (7)$$

Equation (7) allows for calculation of the Cunningham Correction Factor at a given absolute pressure (P), equal to 94 kPa during experiments performed in Edmonton, Alberta.

To implement Equation (4), the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of Clenil Modulite and Clenil Compositum were used to define log-normal mass distributions for each inhaler via Equation (8) (Finlay 2001):

$$m_{\text{norm}}(d) = \frac{1}{d\sqrt{2\pi}\ln GSD}\exp\left[\frac{-(\ln d - \ln MMAD)^2}{2(\ln GSD)^2}\right] \quad (8)$$

Equation (8) defines the fraction of aerosol mass contained in particles with diameters ranging from d to $d + d(d)$, where $d(d)$ denotes a small increment of particle diameter. Integrating the distribution across a range of particle diameters yields a cumulative mass distribution.

Application of the Javaheri, Golshahi, and Finlay (2013) correlation proceeded as follows. First, Equation (8) was used to plot the mass distribution for particle diameters ranging from 0 to 30 μm, in incremental steps of 0.1 μm, based on the unfiltered particle MMAD and GSD. The cumulative mass distribution was then calculated for bins of equal width, with a particle diameter d equal to the halfway point between bin edges ($d_{bin,i} = [d_{i+1} + d_i]/2$), with the mass in each bin (b_i) approximated with the Trapezoidal rule of integration: $b_i = [m_{\text{norm}}(d_{i+1}) + m_{\text{norm}}(d_i)]/2 \cdot [d_{i+1} - d_i]$. The filtered bin mass was then calculated by applying Equation (8) to each individual bin, i.e. $b_{i,filtered} = b_i \cdot [1 - \eta_i]$, with the particle aerodynamic diameter approximated by $d_{bin,i}$. The filtered distribution was then re-normalized

by dividing the filtered bin mass for each $d_{bin,i}$ by the total filtered normalized mass, allowing for the estimation of filtered MMAD and GSD using simple relations. Specifically, the MMAD was obtained by noting the particle diameter for which the cumulative mass distribution equaled 0.5, while the GSD was obtained from the square root of the ratio of d_{84} and d_{16}, the diameters at which the cumulative mass distribution was 0.16 and 0.84, respectively, or $GSD = \left(\frac{d_{84}}{d_{16}}\right)^{0.5}$ (Hinds 1999). Using this procedure, the MMADs and GSDs of aerosol escaping deposition in the idealized infant geometry and depositing on the filter were estimated for beclomethasone dipropionate from Clenil Modulite and beclomethasone dipropionate and salbutamol sulfate from Clenil Compositum.

A one-dimensional numerical dynamic lung deposition model (Javaheri *et al.* 2013) was then used to simulate tracheobronchial deposition of particles emitted from the Clenil Modulite and Clenil Compositum pMDIs, with the *in vitro* filter deposition providing a measure of the total lung dose. To examine the effects of age on respiratory tract deposition, two lung morphologies representing either a 7 month or a 22 month old infant were specified based on the lung model of Hofmann, Martonen, and Graham (1989). Deposition in the thoracic airways was calculated using the equations of Chan and Lippmann (1980) for inertial impaction, Heyder (1975) and Heyder and Gebhart (1977) for sedimentation, and Ingham (1975) for diffusion, under the assumption of non-hygroscopic particles. Following calculation of deposition in each trachea-bronchial generation, the concentration of drug in the airway surface liquid was estimated using the model of Lange *et al.* (2001). Three scenarios, consisting of varying tracheal clearance velocity and daily mucus production, were considered as summarized in Table 3. The largest daily mucus production rate for the pediatric lungs considered here, 18 mL/day, was obtained by scaling the upper bound of daily mucus production for the adult lung (40 mL/day)

considered by Lange *et al.* (2001) by the total surface area of the tracheal-bronchial airways in the 22 month old infant model. Slower tracheal clearance velocity and larger daily mucus production result in a thicker airway surface liquid layer that decreased drug concentration for a given deposited mass. The following six cases were investigated numerically using the aforementioned schemes:

i. Beclomethasone dipropionate via Clenil Modulite in a 7 month infant lung model
ii. Beclomethasone dipropionate via Clenil Modulite in a 22 month infant lung model
iii. Beclomethasone dipropionate via Clenil Compositum in a 7 month infant lung model
iv. Beclomethasone dipropionate via Clenil Compositum in a 22 month infant lung model
v. Salbutamol sulfate via Clenil Compositum in a 7 month infant lung model
vi. Salbutamol sulfate via Clenil Compositum in a 22 month infant lung model

Table 3: Tracheal clearance and mucus production conditions considered in simulations of particle deposition and airways surface liquid concentration.

Scenario	Airway Surface Liquid Drug Concentration	Tracheal Clearance Velocity (mm/min)	Daily Mucus Production (mL/day)
1	Low	5	18
2	Medium	10	10
3	High	15	5

3.3 Results

Filter deposition of beclomethasone dipropionate from Clenil Modulite was 44.6 ± 4.5 μg (average ± standard deviation), equivalent to 17.8±1.8% of labelled claim, with a corresponding coefficient of variation of 0.101. For Clenil Compositum, filter deposition was 60.2 ± 3.6 μg of beclomethasone dipropionate and 22.5 ± 1.1 μg salbutamol sulfate, equivalent to 24.1±1.4% and 18.8±0.9%, respectively, of labelled claim, with corresponding coefficients of variation of 0.060

and 0.049. Filter dose of beclomethasone dipropionate from Clenil Modulite was significantly less than that obtained with Clenil Compositum ($p < 0.05$). Results of the mathematical filtering of particle size distributions for Clenil Modulite and Clenil Compositum are summarized in Table 4. When accounting for extrathoracic deposition in the nasal airways, particle size distributions shifted to smaller MMADs with lower GSDs.

Table 4: *In vitro* filter deposition (average ± standard deviation, n = 5) and the effect of extrathoracic deposition on the MMAD and GSD of pharmaceutical aerosol exiting the idealized infant geometry. BDP = beclomethasone dipropionate, SS = salbutamol sulfate.

	Clenil Modulite	Clenil Compositum	
Compound	BDP	BDP	SS
Filter deposition (μg)	44.6 ± 4.5	60.2 ± 3.6	22.5 ± 1.1
Initial MMAD (μm)	3.45	2.25	3.55
Initial GSD	2.45	3.1	1.6
Filtered MMAD (μm)	2.79	1.79	3.3
Filtered GSD	2.28	2.85	1.58

From the *in* silico simulations, Table 5 summarizes the tracheobronchial, alveolar, and exhaled dose for each inhaler. Tracheobronchial deposition was higher for each inhaler and compound in the 7 month old infant model compared to the 22 month old infant; tracheobronchial deposition in the 7 month old infant was 12.5% greater for beclomethasone dipropionate from Clenil Modulite and 12.5% and 15% greater, respectively, for beclomethasone dipropionate and salbutamol sulfate from Clenil Compositum as compared to deposition in the 22 month old infant. Alveolar deposition was remarkably similar for both ages. Given the similarity of alveolar dose, increased tracheobronchial deposition in the younger infant model caused a corresponding decrease in the exhaled dose. The exhaled dose composed a significant fraction of the total lung dose in all simulations, ranging from 32% for salbutamol sulfate from

Clenil Compositum in the 7 month old infant model to 53% for beclomethasone dipropionate from Clenil Compositum in the 22 month old infant model. In terms of the label claim, tracheobronchial deposition across all cases ranged from 7.7% to 10.2%. Similarly, alveolar deposition ranged from 2.1% to 3.4% of label claim. The deposited dose in the lungs, calculated as tracheobronchial deposition plus alveolar deposition, ranged from 9.8% to 12.8% of labelled claim. Figure 9 to Figure 14 show the delivered mass and drug concentration in the airway surface liquid layer in the tracheobronchial region on a per-generational basis. Note that the tracheobronchial region is composed of generations 0 to 15 for the lung model of Hofmann, Martonen, and Graham (1989).

Table 5: Tracheobronchial region dose, alveolar region dose, and exhaled dose for the Clenil 250 mcg and Clenil Compositum pMDI pharmaceutical compounds in 7 and 22 month old infant models. BDP = beclomethasone dipropionate, SS = salbutamol sulfate.

Inhaler, Compound	Age (months)	Lung Dose (µg)	Tracheobronchial Deposition (µg)	Alveolar Deposition (µg)	Exhaled Dose (µg)
Clenil Modulite, BDP	7	44.6	21.6	5.5	17.5
Clenil Modulite, BDP	22	44.6	19.2	5.3	20.1
Clenil Compositum, BDP	7	60.2	23.4	8.4	28.4
Clenil Compositum, BDP	22	60.2	20.8	7.8	31.6
Clenil Compositum, SS	7	22.5	12.2	3.1	7.2
Clenil Compositum, SS	22	22.5	10.6	3.1	8.8

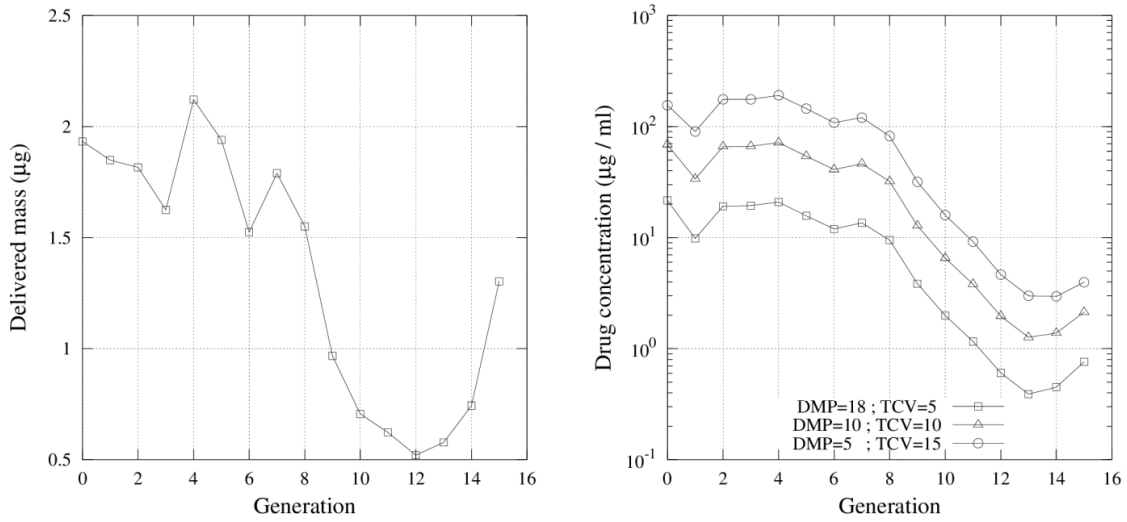

Figure 9: Delivered mass (left) and drug concentration in the airway surface liquid layer (right) of beclomethasone dipropionate from Clenil Modulite in the 7 month old infant model. DMP = daily mucus production (mL/day), TCV = tracheal clearance velocity (mm/min).

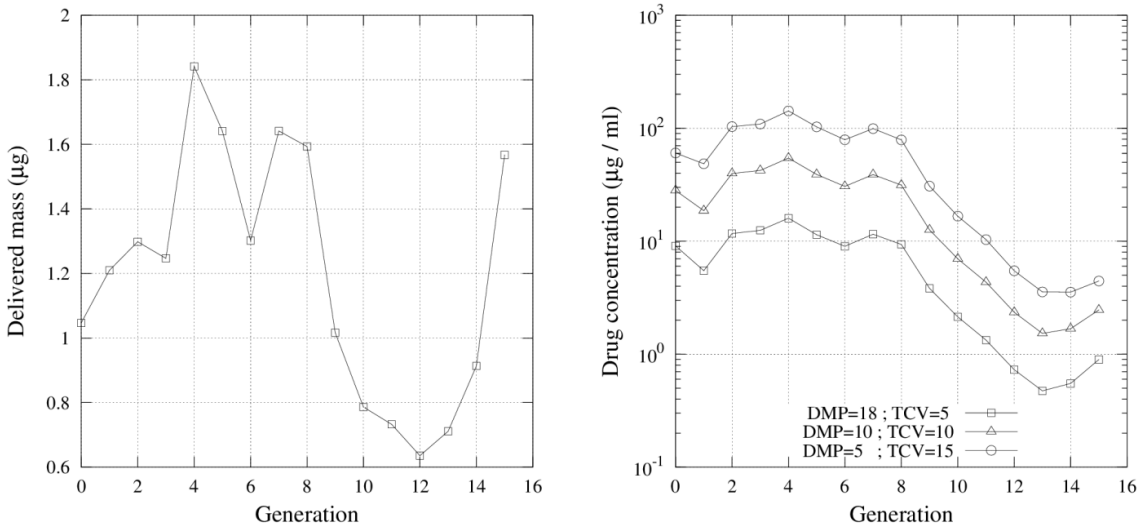

Figure 10: Delivered mass (left) and drug concentration in the airway surface liquid layer (right) of beclomethasone dipropionate from Clenil Modulite in the 22 month old infant model. DMP = daily mucus production (mL/day), TCV = tracheal clearance velocity (mm/min).

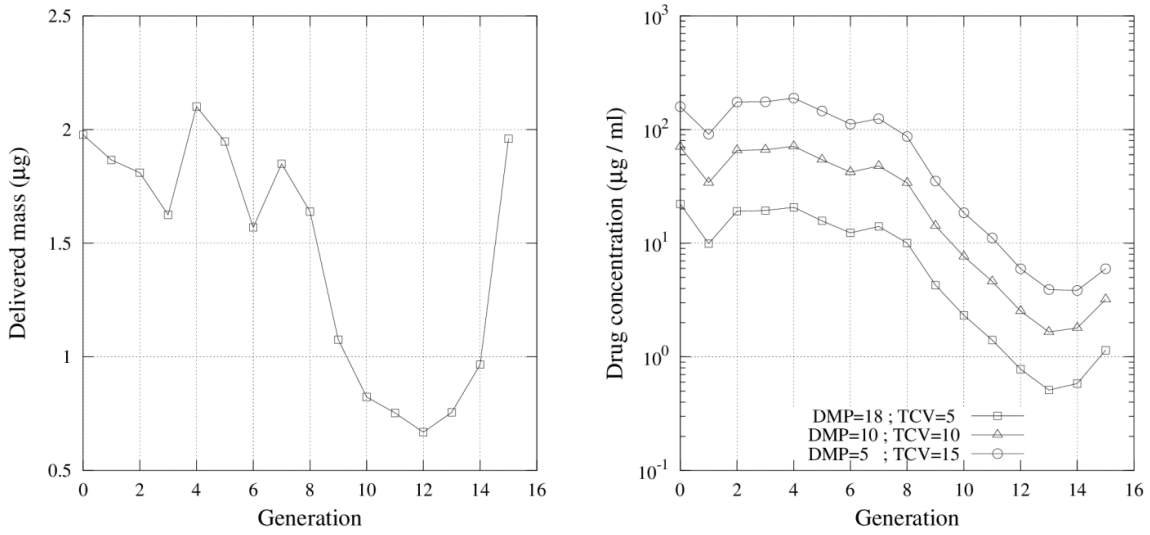

Figure 11: Delivered mass (left) and drug concentration in the airway surface liquid layer (right) of beclomethasone dipropionate from Clenil Compositum in the 7 month old infant model. DMP = daily mucus production (mL/day), TCV = tracheal clearance velocity (mm/min).

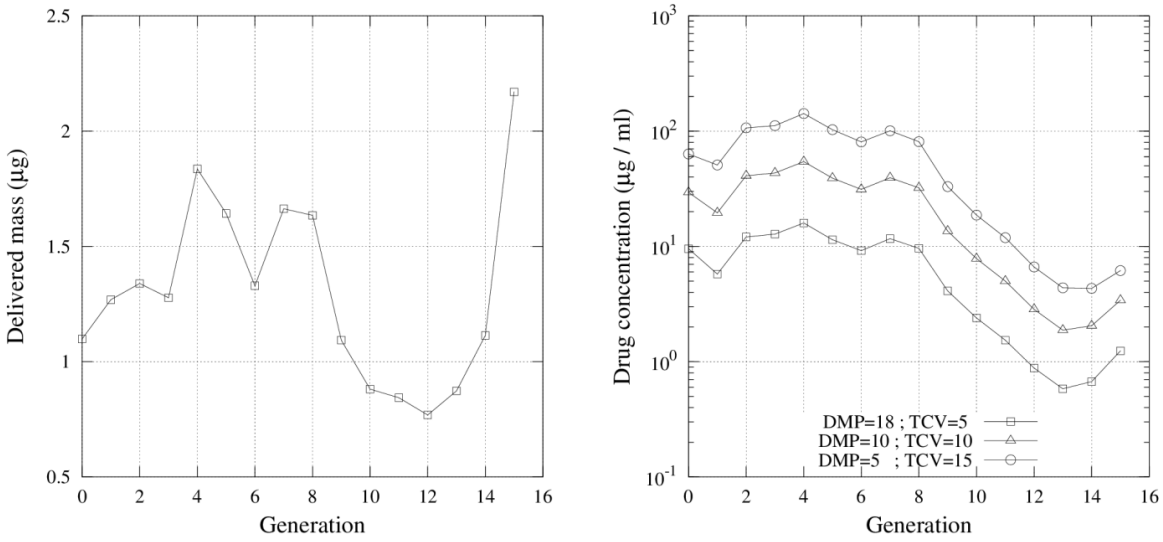

Figure 12: Delivered mass (left) and drug concentration in the airway surface liquid layer (right) of beclomethasone dipropionate from Clenil Compositum in the 22 month old infant model. DMP = daily mucus production (mL/day), TCV = tracheal clearance velocity (mm/min).

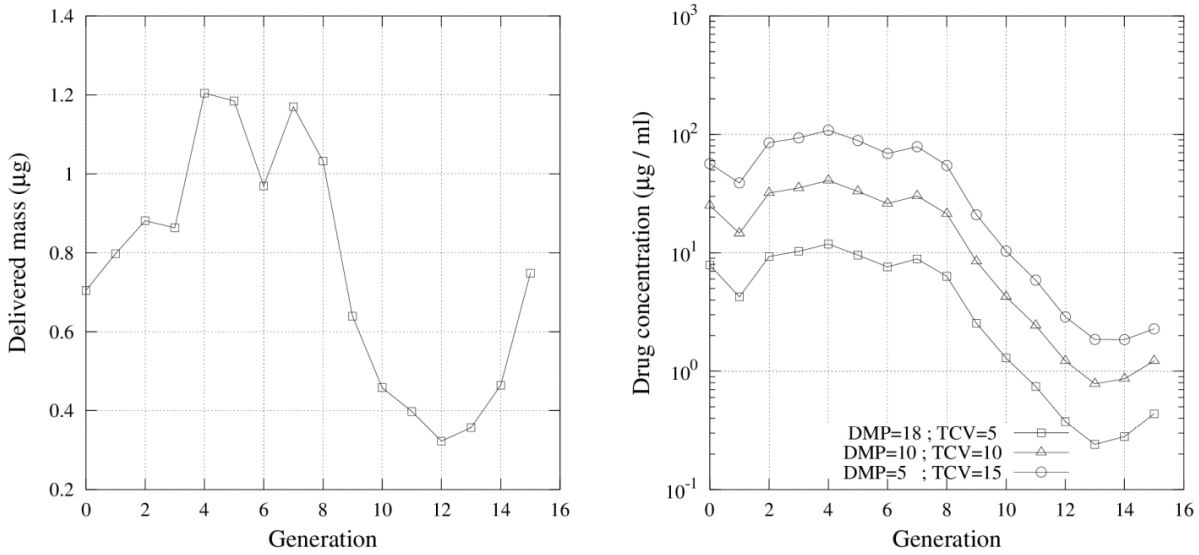

Figure 13: Delivered mass (left) and drug concentration in the airway surface liquid layer (right) of salbutamol sulfate from Clenil Compositum in the 7 month old infant model. DMP = daily mucus production (mL/day), TCV = tracheal clearance velocity (mm/min).

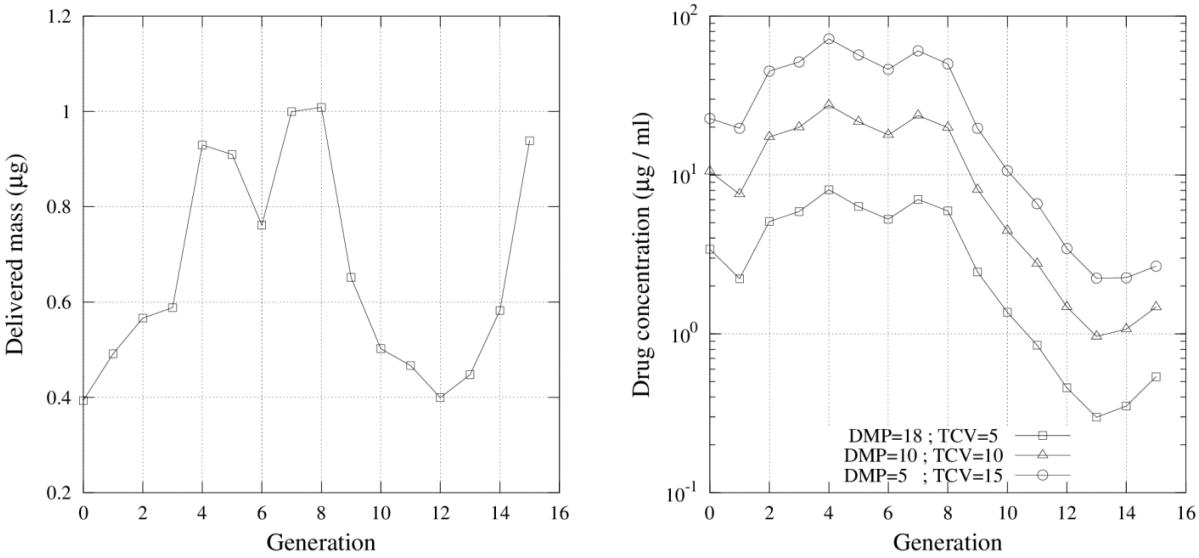

Figure 14: Delivered mass (left) and drug concentration in the airway surface liquid layer (right) of salbutamol sulfate from Clenil Compositum in the 22 month old infant model. DMP = daily mucus production (mL/day), TCV = tracheal clearance velocity (mm/min).

Deposition of beclomethasone dipropionate from Clenil Modulite showed similar trends for generations 2 to 15 in the 7 month and 22 month old infant models, though an increase in deposition was observed in generations 0 and 1 for the younger age, as evident in Figure 9 and Figure 10. A similar effect was observed in the deposition of beclomethasone dipropionate from Clenil Compositum (Figure 11, Figure 12). In contrast, salbutamol sulfate showed similar trends across all generations for both ages (Figure 13, Figure 14). In terms of the airway surface liquid, simulations involving low daily mucus production (5 mL/day) and high tracheal clearance velocity (15 mm/min) resulted in the highest drug concentrations in all tracheobronchial airway generations. Increased airways surface liquid concentration was also observed in the 7 month infant model compared to the 22 month model for each inhaler under consideration. In general, airways surface liquid concentration was highest in generations 2 to 8, with rapidly decreasing concentrations for generations 9 and greater. The highest drug concentrations (resulting from low daily mucus production and high tracheal clearance velocity) were roughly an order of magnitude greater than the lowest drug concentrations (resulting from high daily mucus production and low tracheal clearance velocity).

3.4 Discussion

In vitro filter deposition measurements suggest consistent delivery from both the Clenil Modulite and Clenil Compositum inhalers in the present setup, given that the maximum coefficient of variation for either inhaler or compound was 0.101 for beclomethasone dipropionate from Clenil Modulite. This consistent delivery was achieved likely in part by the good quality of seal between the facemask and the idealized infant geometry, confirmed using the AeroChamber Flow-Vu valve, given that the detrimental effects of a poor-sealing facemask have been well documented (Janssens *et al.* 2004; Carrigy *et al.* 2014a; Esposito-Festen *et al.*

2004). The significant difference in filter dose of beclomethasone dipropionate obtained with Clenil Modulite (44.6 ± 4.5 μg) compared to Clenil Compositum (60.2 ± 3.6 μg) likely arises due to differences in the particle size distributions for each inhaler; beclomethasone dipropionate from Clenil Modulite, with an MMAD of 3.45 μm prior to inhalation, is considerably larger than the pre-inhalation MMAD of Clenil Compositum at 2.25 μm. As deposition in the extrathoracic region is heavily dependent on inertial impaction, itself heavily dependent on particle size, the larger particles of Clenil Modulite can be expected to experience a higher degree of deposition in the nasal airways, leading to a lower lung dose.

Average filter deposition, expressed as a percentage of the labelled claim, ranged from 17.8% (for beclomethasone dipropionate from Clenil Modulite) to 24.1% (for beclomethasone dipropionate from Clenil Compositum). Similar experiments have measured filter deposition downstream of the SAINT model, a realistic nasal airway replica of a 9 month old infant, to estimate the total lung dose. Janssens *et al.* (2001) reported somewhat lower lung dose downstream of the SAINT model (8.8±0.3% of label claim) from Pulmicort budesonide pMDIs delivered through a spacer and attached facemask for a breathing pattern almost identical to that used in the present study (respiratory rate of 30 breaths per minute, tidal volume of 100 mL, sinusoidal pattern with a duty cycle of 0.43). In subsequent experiments using pMDIs delivered via valved holding chambers and facemasks, the same group has reported lung doses varying from 26.5% of label claim for a QVAR beclomethasone dipropionate pMDI to 4.8% of label claim for a Becotide beclomethasone dipropionate pMDI (Janssens *et al.* 2003), with additional filter measurements of 5 to 10% of label claim for a Pulmicort budesonide pMDI, and 15 to 20% of label claim for a Flixotide fluticasone pMDI (Janssens *et al.* 2004), all measured downstream of the SAINT model for a tidal volume of 100 mL and otherwise similar breathing patterns to

that used in the present study. Deposition in the idealized infant geometry tends towards the larger of these *in vitro* measurements in the SAINT model, though there are a few factors to consider that may cause differences in deposition. First, the SAINT model is a realistic replica representative of the infant from which CT scans were taken to model the geometry, while the idealized infant geometry of Javaheri, Golshahi, and Finlay (2013) is a simplified analog of the average nasal extrathoracic airways in infants 3 to 18 months old. Interestingly, the average age represented by the realistic replicas used to construct the idealized infant geometry of Javaheri, Golshahi, and Finlay (2013), at 9 months, matches that of the infant used to develop the SAINT model. Despite a similarity of age, however, the idealized infant geometry may, by virtue of its construction, possess inherent differences to the realistic geometry of the SAINT model that cause some variation in deposition. Second, particle size distributions of Clenil Modulite and Clenil Compositum, reported in Table 4, differ somewhat from those reported in the *in vitro* studies involving the SAINT model, inevitably causing differences in impaction during transit through the extrathoracic region. Additional experiments using identical pMDIs, valved holding chambers, and facemasks are required to assess differences in pharmaceutical aerosol deposition between the idealized infant geometry and the SAINT model in a more controlled manner.

In silico simulations defining regional deposition in the lungs indicated a considerable exhaled dose, ranging from 32% to 53% of the total lung dose. Accordingly, the simulated dose of drug deposited in the lungs was less than the total lung dose reported from *in vitro* experiments. The deposited dose in the lungs, equal to tracheobronchial deposition plus alveolar deposition in Table 5, provides a benchmark from which to compare to *in vivo* lung deposition data. Wildhaber *et al.* (2000) reported a lung deposition of 16.4±5.5% of metered dose from a radiolabeled salbutamol pMDI delivered via spacer and facemask in children under four years of

age, slightly higher than the deposited dose in the lungs calculated numerically (ranging from 9.8% to 12.7% of label claim). Erzinger *et al.* (2007) measured lung deposition ranging from 0.2% to 7.4% of labelled claim from a salbutamol pMDI plus spacer and facemask in infants 18 to 36 months old, attributing facemask seal and crying patients as the causes for the very small lung depositions (< 1% of labelled claim) measured in some subjects. The deposited lung dose simulated in the present study falls among the measured *in vivo* data, though caution is required in comparing deposition from different devices and formulations. Based on these *in vivo* studies, however, it appears that the *in vitro – in silico* methodology employed in the present study provides a reasonable estimate of the lung deposition to be expected *in vivo*.

Results from the one dimensional dynamic lung deposition model suggest that older infants receive slightly decreased doses to the tracheobronchial region despite similar deposition in the alveolar region for equivalent total lung doses, with corresponding increases in exhaled dose. This effect was observed for each inhaler and with both beclomethasone dipropionate and salbutamol sulfate for Clenil Compositum, and is likely related to differences in the size of the airways; older patients with larger airways are likely to experience less inertial deposition for equivalent flow rates. Interestingly, Hofmann, Martonen, and Graham (1989) calculated that total lung deposition would be smaller in a 7 month old infant than a 22 month old infant, though these simulations used age-variable breathing patterns that may account for the discrepancy. The considerable exhaled dose and surprisingly small alveolar dose for both ages is likely caused in part by the use of a continuous breathing pattern cycling with no breath hold. This limits the effective time for sedimentation and diffusion of particles penetrating into the alveolar region of the lungs, leading to a larger exhaled dose than would occur with the inclusion of a pause between inhalation and exhalation. For tidal breathing, however, the continuous cycle between

inhalation and exhalation used in the present study closely models the expected behavior *in vivo* (Stocks and Hislop 2002), and as such similarly large expired doses can be expected *in vivo*. Expiratory filter deposition data from Wildhaber *et al.* (2000), at 8.7±3.9% of labelled claim for a salbutamol pMDI, seems to support this conclusion.

Calculation of drug deposition and airway surface liquid concentration on a generational basis in the tracheobronchial region provides useful insight into the delivery of pharmaceutical aerosol to infants. As evident in Figure 9 to Figure 14, airway surface liquid drug concentration was greatest in generations 0 to 8, decreasing rapidly beyond the generation 9, a trend observed for both beclomethasone dipropionate and salbutamol sulfate. Therefore, both drugs appear to reach a maximum concentration in the more central components of the tracheobronchial region. However, with clinical drug efficacy depending on many factors including the ability of pharmaceutical aerosol to reach target cells and receptors and the dose-response relationship of a given drug (Zanen and Laube 2002; Labiris and Dolovich 2003b), it is difficult to speculate on the exact implications of the various cases studied here. Indeed, in the absence of more pertinent *in vivo* data for validation of the current methodology, the results of the present work should not be considered quantitative, but rather indicative of general trends. Nevertheless, the joint *in vitro – in silico* methodology employed here can provide a basic guideline for dosing through prediction of the deposition and concentration of drug in specific regions of the lungs.

3.5 Conclusions

The idealized infant geometry of Javaheri, Golshahi, and Finlay (2013) was used to characterize pharmaceutical aerosol deposition from two pMDIs delivered with valved holding chambers and facemasks under a realistic breathing profile. *In vitro* filter deposition measured downstream of the idealized infant geometry for beclomethasone dipropionate from Clenil

Modulite and both beclomethasone dipropionate and salbutamol sulfate from Clenil Compositum provided an estimate of the *in vivo* total lung dose. *In silico* simulations of regional lung deposition revealed that aerosol from both Clenil Modulite and Clenil Compositum deposited primarily in the tracheobronchial region (7.7% to 10.2% of labelled claim), with a relatively small amount of aerosol depositing in the alveolar region (2.1% to 3.4% of labelled claim). A significant exhaled dose was predicted in all simulations (32% to 53% of the total lung dose). Further simulations showed that the concentration of drug in the airway surface liquid was greatest in lung generations 0 to 8, with rapidly decreasing concentrations occurring beyond generation 9. The highest drug concentrations (resulting from low daily mucus production and high tracheal clearance velocity) were roughly an order of magnitude greater than the lowest drug concentrations in all generations of the tracheobronchial region. The combination of *in vitro* experimentation and *in silico* modeling used here may prove a guideline for dosing in a clinical perspective through the prediction of both regional deposition and the concentration of drug in the airway surface liquid, though validation of the method is required before results can be treated quantitatively.

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Chapter 4: Comparisons of Deposition in Two Idealized Child Throat Models

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4.1 Introduction

A major consideration in the use of inhaled pharmaceutical aerosols is extrathoracic deposition, which plays an important role in determining the total lung dose of medication from therapeutic inhalers (Finlay and Martin 2008). While a variety of mouth-throat replicas are available for *in vitro* estimation of extrathoracic deposition in adults (Byron *et al.* 2013), less work has been performed regarding oral inhalation in children (Carrigy *et al.* 2014b). The Alberta Idealized Throat (Stapleton *et al.* 2000), a simplified extrathoracic throat model shown to replicate average pharmaceutical aerosol deposition observed in adults (Grgic, Finlay, and Heenan 2004; Zhang, Gilbertson, and Finlay 2007; Zhou, Sun, and Cheng 2011), provides a standardized platform for inhalation product development and testing *in vitro*.

Recently, Golshahi and Finlay (2012) developed the Alberta Idealized Child Throat by isotropically scaling the Alberta Idealized Throat to capture the average deposition of aerosol in school age children. The isotropic scale factor was selected based on the average characteristic diameter $d = V/A_s$, where V is the airway volume and A_s is the airway surface area, measured in nine realistic oral extrathoracic airway replicas of children age 6 to 14 (Golshahi, Noga, and Finlay 2012; Golshahi *et al.* 2013). This characteristic diameter, accounting for intersubject

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variations in anatomical dimensions, provided a good reduction in scatter of deposition measured in the realistic child replicas, allowing for the accurate prediction of deposition with a relatively simple correlation (Golshahi, Noga, and Finlay 2012). Scaling the Alberta Idealized Throat by a factor of 0.62 yielded a characteristic diameter of 2.7 mm, equal to the average of the realistic child airway replicas (Golshahi and Finlay 2012). As shown in Chapter 2, the Alberta Idealized Child Throat successfully mimics average deposition of pharmaceutical aerosols in school age children.

Using an alternative methodology, Bickmann *et al.* (2008) proposed the modification of certain dimensions in the Alberta Idealized Throat based on measurements of anatomical features observed in two magnetic resonance image scans of five year old children, creating a non-uniformly scaled metal idealized child throat model meant to mimic deposition in a 5 year old child. This 5-year-old idealized throat has since been used in *in vitro* assessments of various inhalers (Wachtel *et al.* 2010; Below, Bickmann, and Breitkreutz 2013), but its ability to replicate *in vivo* data has not been established.

Given the separate methodologies used to create these idealized throat geometries from the same origin, the Alberta Idealized Throat, the question of how deposition in these idealized throat geometries compares naturally arises. The goal of the present work is to compare deposition in the Alberta Idealized Child Throat and the Bickmann *et al.* idealized throat to explore how differences in construction methodology affect deposition.

4.2 Materials and Methods

4.2.1 Pediatric Idealized Throat Models

A stainless steel model of the Alberta Idealized Child Throat was prototyped using additive manufacturing methods (Linear Mold & Engineering, Livonia, MI, USA). The same model was used to characterize pharmaceutical aerosol deposition in Chapter 2, and provided an accurate prediction of *in vivo* deposition in school age children. The Alberta Idealized Child Throat was developed based on deposition measurements in nine realistic child oral airway casts 6 to 14 years old, with a median age of 11 years. A plastic model of the Bickmann *et al.* idealized throat was prototyped in-house using VeroGrey polymer on a 3D printer (Objet Eden350V, Stratasys Ltd). The Bickmann *et al.* idealized throat was developed from anatomical measurements in children 5 years of age.

4.2.2 Experimental Setup

Deposition in both the Alberta Idealized Child Throat and Bickmann *et al.* idealized throat was examined using a Respimat® Soft Mist™ Inhaler (SMI, Boehringer Ingelheim GmbH) and a ciprofloxacin hydrochloride tracer aerosol. Unlike a conventional pressurized metered dose inhaler, the Respimat SMI uses mechanical spring to force a metered dose of solution through a nozzle, delivering a small volume of solution, 11.0 μL per actuation (Zierenberg 1999), over a long duration of 1.5 seconds (Hochrainer *et al.* 2005). A schematic diagram of the experimental setup is shown in Figure 15.

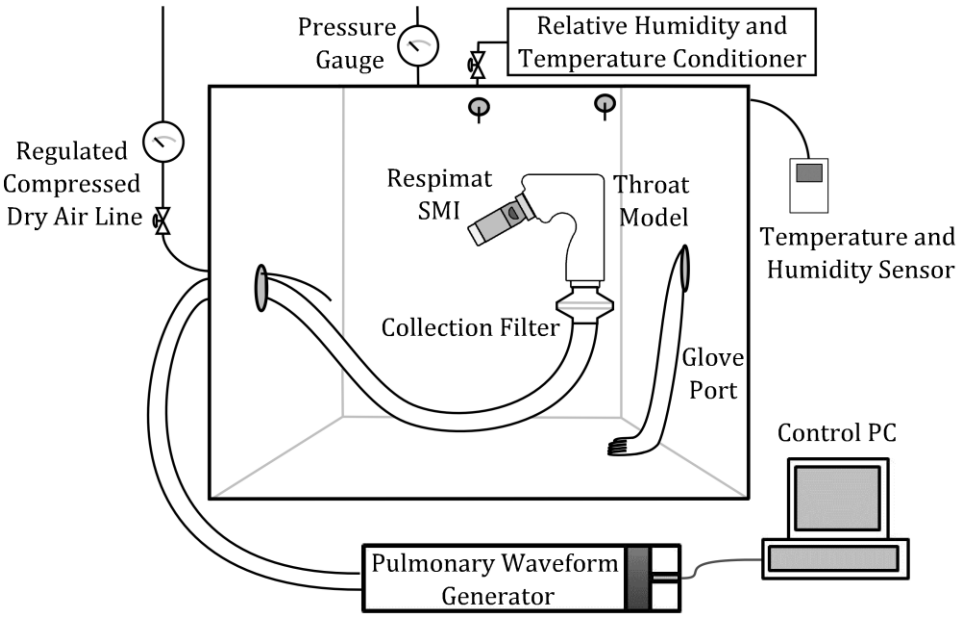

Figure 15: Experimental setup used to measure deposition from the Respimat SMI in the Alberta Idealized Child Throat and Bickmann *et al.* idealized throat under controlled environmental conditions.

A pulmonary waveform generator was used to draw air through either the Alberta Idealized Child Throat or the Bickmann *et al.* idealized throat using both fast (54 L/min) and slow (30 L/min) peak inhalation flow rates. For both flow rates, a total inhaled volume of 0.9 L and a flow acceleration of 1.8 L/s^2 were used, providing similar inhalation patterns to that used by Wachtel *et al.* (2010). A collection filter (Respirgard II bacterial/viral filters; Vital Signs Inc., Englewood, CO, USA) placed immediately downstream of the throat captured aerosol emitted distal to the throat geometry, indicative of the total lung dose *in vivo* (Finlay and Martin 2008). Ambient conditions were controlled to 50 ± 5% relative humidity and 24 ± 1 °C, equal to that reported by Wachtel *et al.* (2010), using an environmental chamber with glove ports (CEO-910W-4; Lunair Environmental, Williamsport, PA, USA).

Deposition in the Alberta Idealized Child Throat and Bickmann *et al.* idealized throat was analyzed using ciprofloxacin hydrochloride hydrate solutions with concentrations of 10.1 mg/mL and 10.0 mg/mL, respectively. Chemical assay using deionized water with UV spectroscopy were used to quantify the mass of drug depositing in either the Alberta Idealized Child Throat or the Bickmann *et al.* idealized throat (assayed twice with 10 mL), the collection filter (assayed first with 10 mL, then again with 5 mL), and the nozzle actuator (once with 5 mL). Trial runs showed that mass recovery of ciprofloxacin hydrochloride hydrate from the plastic Bickmann *et al.* idealized throat was enhanced significantly when the throat was coated with a layer of clear nail polish lacquer, a technique that was adopted for deposition testing in the present study. Further trial runs revealed very poor dose recovery of ciprofloxacin hydrochloride hydrate from the plastic throat when coated with a layer of silicone oil (Molykote 316; Dow Corning Corporation, Midland, MI, USA), meant to reduce particle bounce. For the sake of consistency, no silicone coating was applied to either the Alberta Idealized Child Throat or the Bickmann *et al.* idealized throat during the main experimental runs. Early measurements in the Alberta Idealized Child Throat suggested a small difference in mouth-throat deposition, less than 5% of delivered dose, for a silicone-coated versus uncoated throat geometry, indicating that particle bounce had a small effect on measured mouth-throat deposition.

The use of ciprofloxacin hydrochloride hydrate as a tracer allowed for the use of water as an assaying agent, assuaging any concerns related to solvent contamination issues with the plastic Bickmann *et al.* idealized throat. The ex-actuator dose was calculated as the sum of deposition on the nozzle actuator, mouth-throat, and filter. Mouth-throat deposition was expressed as a percentage of the delivered dose, calculated as the sum of deposition measured on the filter and in the idealized geometry. Five measurements were performed at each flow rate for

each throat model. Results were subjected to one-way ANOVA with post-hoc Tukey's Honestly Significant Difference test for comparison of the ex-actuator dose and the delivered dose measured in both idealized geometries at 30 L/min and 54 L/min, and unpaired Student's t-tests with Welch's correction for comparisons between mouth-throat deposition in the Alberta Idealized Child Throat and the Bickmann *et al.* idealized throat at each flow rate, with a p value < 0.05 considered significant (Prism 6.05; GraphPad Software, Inc., La Jolla, CA, USA).

4.2.3 Hygroscopic Growth

Initially, the intention of this work was to measure deposition in only the Alberta Idealized Child Throat, given the availability of *in vitro* deposition data of tiotropium delivered via Respimat SMI in the Bickmann *et al.* idealized throat (Wachtel *et al.* 2010). Calculations of hygroscopic growth were performed to investigate whether similar hygroscopic behavior of tiotropium and ciprofloxacin hydrochloride hydrate could be expected. The general theory describing hygroscopic aerosols is described by Finlay (2001). For a water droplet in air, the rate of change with respect to time t of a droplet with diameter d can, under a number of assumptions (Finlay 2001), be calculated using Equation (9):

$$\frac{dd}{dt} = -\frac{4D(c_s - c_\infty)}{\rho_{drop} d} \tag{9}$$

Here, ρ_{drop} is the droplet density, D is the diffusion coefficient of water vapour in air, and c_s and c_∞ are the water vapour concentrations at the droplet surface and in the ambient gas phase, respectively. Dissolved solutes such as tiotropium and ciprofloxacin hydrochloride hydrate alter intermolecular attractive forces in water, causing a change in the water vapour concentration at the droplet surface c_s that can be quantified with Equation (10):

$$c_s = S c_{s,\text{ pure } H_2O} \tag{10}$$

$c_{s,\text{ pure } H_2O}$ is the water vapour concentration at an air - pure water interface, calculated for a given ambient temperature T using the empirical relation of Equation (11):

$$c_{s,\text{ pure } H_2O} = (3.638 \times 10^5) e^{\frac{-4943}{T}} \tag{11}$$

S, the water activity coefficient, can be approximated using Raoult's law for dilute solutions containing multiple solutes:

$$S \approx 1 - \frac{\sum_j i_j x_{sj}}{x_w} \tag{12}$$

x_s is the molar concentration of the solute j, x_w is the molar concentration of water, and i is the van't Hoff factor, which describes the effect of a solute on colligative properties. The ideal van't Hoff factor is equal to the number of ions a molecule dissociates into in solution, and is typically a fair approximation of the actual factor in dilute solutions. The ambient water vapour concentration c_∞, dependent on relative humidity (RH), was calculated from $c_{s,\text{ pure } H_2O}$ as:

$$c_\infty = RH \cdot c_{s,\text{ pure } H_2O} \tag{13}$$

For the dilute ciprofloxacin hydrochloride hydrate solution (10.1 mg/mL) used in the Respimat SMI for deposition testing with the Alberta Idealized Child Throat, $x_{s,\text{CHH}} = 0.026$ mol/L, $x_{w,\text{CHH}} = 54.94$ mol/L, with an ideal van't Hoff factor of 2. From Equation (12), the water activity coefficient for the ciprofloxacin solution, S_{CHH}, was found to equal 0.9991, resulting in a water vapour concentration of $c_{s,\text{CHH}} = 0.02150$ kg/m^3 at the droplet surface.

The tiotropium Respimat SMI used by Wachtel *et al.* (2010) delivered 2.5 μg of tiotropium per puff (package leaflet, Spiriva Respimat 2.5 microgram solution for inhalation, Boehringer Ingelheim, 2010) corresponding to a molar concentration of tiotropium bromide monohydrate of $0.00058\ \mathrm{mol/L}$. Major additives included benzalkonium chloride and disodium edetate, with estimated molar concentrations of $0.00028\ \mathrm{mol/L}$ and $0.00067\ \mathrm{mol/L}$, respectively, based on available patent information (Drechsel *et al.* 2005). Assuming negligible amounts of other additives, and with ideal van't Hoff factors for tiotropium bromide monohydrate ($i = 2$), benzalkonium chloride ($i = 2$) and disodium edetate ($i = 3$), Equation (12) yielded a water activity coefficient for the tiotropium solution, $S_{\mathrm{TBM+additives}}$, of 0.9999, and a water vapour concentration of $c_{s,\mathrm{TBM+additives}} = 0.02152\ \mathrm{kg/m^3}$ at the droplet surface. It should be noted that the theoretical osmolarity for this solution, based on the above analysis, was 3.7 mOsm, which compared well with an experimental measurement of the actual osmolarity of the 2.5 μg tiotropium solution, at 3 mOsm. From Equation (9), assuming that droplet density, the diffusion coefficient of water vapour in air, the ambient water vapour concentration, and the initial droplet diameter are equivalent, the ratio of the rate of change of droplet diameter of the ciprofloxacin solution to the tiotropium solution is 0.998 at a relative humidity of 50%, indicating almost identical rates of hygroscopic size changes and therefore similar hygroscopic behavior. However, given the small volume of solution aerosolized by the Respimat SMI, further consideration was given towards the magnitude of hygroscopic size changes.

Finlay (1998) illustrated the importance of hygroscopic behavior based on the values of two dimensionless parameters. The first, ζ, represents the strength of hygroscopic driving forces, and is calculated as:

$$\zeta = \frac{12D\overline{\Delta c}\Delta t}{\rho_{drop} d^2} \tag{14}$$

For the present *in vitro* experiment, $\overline{\Delta c}$, the average value of $c_s - c_\infty$ over the time interval Δt, was approximated as initial difference $c_s - c_\infty$. Using the droplet lifetime $t_L = \rho_{drop} d_0^2 / [8D(c_s - c_\infty)]$ as an estimate for the time interval Δt (Finlay 2001), Equation (14) gives $\zeta = 1.5$. This value of ζ indicates a significant driving force for hygroscopic size change may exist, but requires the calculation of the second parameter γ to comment on the nature of the coupling between vapour evaporating from the droplet and the vapour present in ambient air. γ can be calculated as:

$$\gamma = \frac{\text{mass of droplets per unit volume}}{\Delta c^*} \tag{15}$$

Δc^*, the required amount of water vapour that would need to be exchanged between droplets and ambient air to reach equilibrium, was estimated as the initial difference $c_s - c_\infty$. The mass of droplets per unit volume was estimated using the mass delivered per dose from the Respimat SMI (11 µg for water), the duration of the spray (1.5 s), and a reference inhalation flow rate of 30 L/min, yielding a value of $0.0147\ \text{kg/m}^3$. This extremely small mass of droplets per unit volume imply that γ will be much less than 1 unless Δc^* is equally small in magnitude, which is not the case at an RH of 50%. Thus, aerosol emitted from the Respimat can be expected to experience significant hygroscopic effects, evaporating quickly to form dry particles.

The assumption of comparable deposition between tiotropium and ciprofloxacin would be valid if hygroscopic effects were negligible and if the Respimat SMI produced a similarly sized aerosol with each tracer. However, with the above analysis indicating that droplets emitted

from the Respimat SMI can be expected to undergo considerable evaporative size changes, and barring more information on the dry particle size for each compound delivered via Respimat SMI, a more thorough analysis requires the use of the same tracer in both the Alberta Idealized Child Throat and the Bickmann *et al.* idealized throat, as has been performed in the present study.

4.3 Results

Deposition measurements as a percentage of the ex-actuator dose and the delivered dose are summarized in Table 6 and Table 7, respectively. Analysis of variance revealed no significant difference in either the ex-actuator dose or delivered dose in the Alberta Idealized Child Throat or Bickmann *et al.* throat at peak inhalation flow rates of 30 L/min and 54 L/min. While measurements in the Bickmann *et al.* idealized throat at 30 L/min showed a somewhat larger variation than in the other experiments, Bartlett's test showed this difference in variance was not significant. Considerable deposition of ciprofloxacin was noted on the actuator nozzle for all experiments, with average values ranging from 24.0% to 30.7% of the ex-actuator dose.

Table 6: Deposition measurements in the filter, throat, and nozzle actuator expressed as a percentage of the ex-actuator dose. Reported as average value, with standard deviation in parenthesis (n = 5). AICT = Alberta Idealized Child Throat, PIFR = peak inhalation flow rate.

Throat	PIFR (L/min)	Ex-actuator dose (μg)	Filter Deposition (%)	Throat Deposition (%)	Actuator Deposition (%)
AICT	30	87.0 (12.8)	28.8 (1.3)	40.5 (7.1)	30.7 (7.7)
	54	90.5 (11.6)	20.1 (2.4)	50.5 (5.2)	29.3 (5.5)
Bickmann *et al.* throat	30	85.3 (22.8)	49.7 (12.9)	26.3 (5.1)	24.0 (8.1)
	54	90.1 (12.0)	31.1 (2.5)	41.5 (3.8)	27.5 (3.3)

Table 7: Deposition measurements in the filter and throat, expressed as a percentage of the delivered dose. Reported as average value, with standard deviation in parenthesis (n = 5). AICT = Alberta Idealized Child Throat, PIFR = peak inhalation flow rate.

Throat	PIFR (L/min)	Delivered dose (µg)	Filter Deposition (%)	Throat Deposition (%)
AICT	30	59.6 (4.3)	41.9 (3.9)	58.1 (3.9)
	54	63.5 (3.9)	28.5 (3.4)	71.5 (3.4)
Bickmann *et al.* throat	30	63.5 (12.0)	64.6 (9.8)	35.4 (9.8)
	54	65.3 (8.3)	42.9 (3.6)	57.1 (3.6)

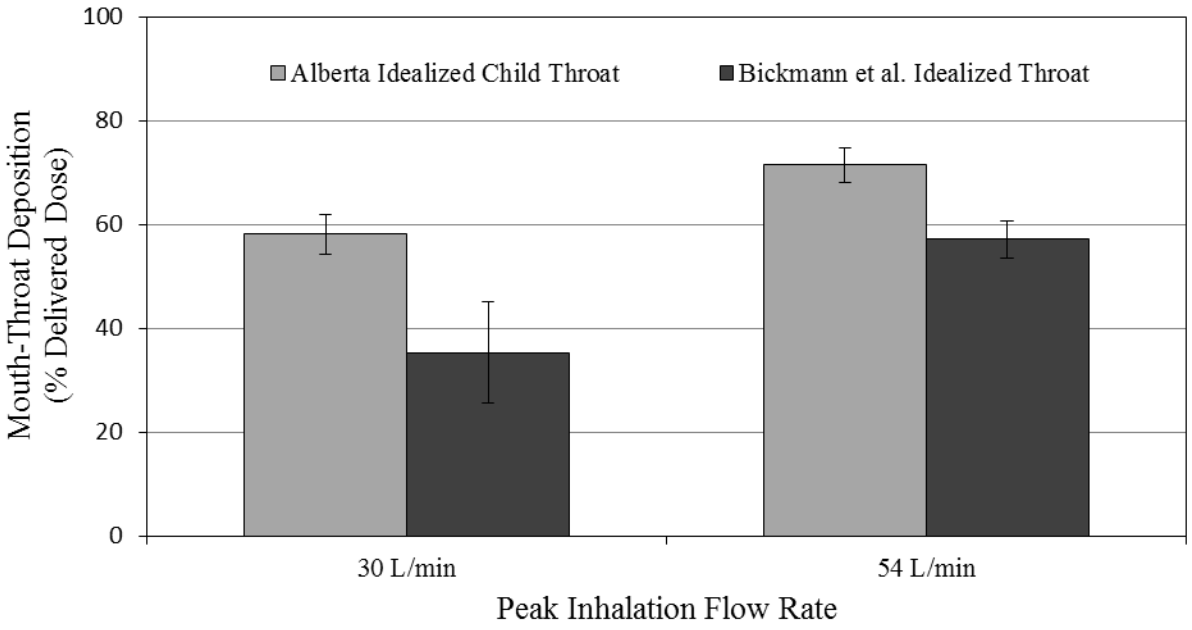

Figure 16: Mouth throat deposition in the Alberta Idealized Child Throat and the Bickmann *et al.* idealized throat expressed as a percentage of delivered dose. Error bars denote standard deviation (n = 5).

Mouth-throat deposition in the Alberta Idealized Child Throat and Bickmann *et al.* idealized throat, expressed as a percentage of delivered dose, is shown in Figure 16. At both 30

L/min and 54 L/min, mouth-throat deposition measured with the Alberta Idealized Child Throat was significantly higher than that measured in the Bickmann *et al.* idealized throat.

4.4 Discussion

As evident in Figure 16, mouth-throat deposition measured in the Alberta Idealized Child Throat was significantly higher than that measured in the Bickmann *et al.* idealized throat. At 30 L/min, the average mouth-throat deposition in the Alberta Idealized Child Throat exceeded that measured in the Bickmann *et al.* idealized throat by more than 20% of the delivered dose. Similarly, at 54 L/min, average deposition in the Alberta Idealized Child Throat was 14.4% of the delivered dose higher than that measured in the Bickmann *et al.* idealized throat. These differences in deposition are likely due to differences in geometry. As noted earlier, the characteristic diameter that best collapsed deposition in data in realistic child oral airway casts was the ratio of airway volume divided by airway surface area. The characteristic diameter[9] of the Alberta Idealized Child Throat, 2.7 mm, matches the average measured in nine realistic child oral airway replicas (Golshahi, Noga, and Finlay 2012; Golshahi *et al.* 2013). The characteristic diameter of the Bickmann *et al.* idealized throat, at 3.9 mm, is considerably larger. For reference, the ratio of airway volume to airway surface area of the Alberta Idealized Throat is 4.4 mm. It is also worth noting that in an *in vivo* deposition study in adults, Newman *et al.* (1998) measured an oropharyngeal deposition[10] of 47.4% of delivered dose of an aqueous fenoterol solution at an average inhalation flow rate of 24.8 L/min. Based on the smaller extrathoracic dimensions in children, along with the higher inhalation flow rate of 30 L/min, a higher mouth-throat

[9] Characteristic diameters were calculated based on volume and surface area measurements in SolidWorks 2013 (SolidWorks2013, Dassault Systems, Waltham Massachusetts, USA)

[10] Recalculated from regional deposition in the lungs, the oropharynx, and an expiratory filter.

deposition would be expected as compared to adults, as is seen in the Alberta Idealized Child Throat (58.1% of delivered dose). However, in the Bickmann *et al.* idealized throat, a lower mouth-throat deposition is observed (35.4% of delivered dose). *In vivo* measurements of deposition from the Respimat SMI in children would be useful to further examine the differences between the Alberta Idealized Child Throat and the Bickmann *et al.* idealized throat.

Hygroscopic calculations suggested the importance of using the same tracer delivered via Respimat SMI to measure deposition in the Alberta Idealized Child Throat and Bickmann *et al.* idealized throat geometries, owing to the lack of information on the dry particle sizes obtained with ciprofloxacin hydrochloride hydrate used in the present study and tiotropium used by Wachtel *et al.* (2010). Despite this lack of information, Wachtel *et al.* (2010) measured a mouth-throat deposition of 40.7% of the delivered dose using a realistic breathing profile in which the average inhalation flow rate was 30 L/min, matching the low flow rate used in the present study. Their measured mouth-throat deposition of tiotropium in the Bickmann *et al.* idealized throat, at 40.7% of delivered dose, compares well with the 35.4% mouth-throat deposition of ciprofloxacin observed here. This indicates that ciprofloxacin and tiotropium may, in fact, have similar aerodynamic size distributions, though additional *in vitro* particle size measurements are required to quantify this claim. An additional factor that may affect deposition measurements in the plastic Bickmann *et al.* idealized throat is the potential of artificial surface charging effects. While the stainless steel Alberta Idealized Child Throat allows for the dispersal of charge from deposited particles, the plastic material of the prototyped Bickmann *et al.* idealized throat is non-conductive; impacted particles on the plastic throat maintain charge and can affect the deposition of trailing particles through electrostatic forces. Unfortunately, there is appears to be no published data regarding the charge distribution of aqueous aerosol emitted from the Respimat

SMI, complicating the prediction of electrostatic effects. The Respimat SMI generates an aerosol via the convergence of two fine liquid jets at the outlet of the Respimat nozzle (Dalby, Spallek, and Voshaar 2004), a process that may generate charged droplets through spray electrification (Hinds 1999; Finlay 2001). While the magnitude of this effect is not known, it should be noted that the low number concentration of aerosol emitted by the Respimat SMI, on the order of 10^9 particles/m^3, acts to reduce space charge effects (Finlay 2001) and limits the number of particles within a few particle diameters of the interior surfaces of the mouth-throat, where electrostatic effects from deposited particles are strongest. Given the agreement in data between the measurements of ciprofloxacin in the plastic Bickmann *et al.* throat obtained here and of tiotropium in a metal version of the Bickmann *et al.* geometry by Wachtel *et al.* (2010), artificial electrostatic effects appear to play a relatively minor role. However, quantification of charge on aerosols emitted by the Respimat SMI is required if further consideration of this issue is to be made. To reduce electrostatic effects, metal throat geometries should be used, despite an increased cost of manufacture compared to plastic models.

4.5 Conclusion

Measurements of ciprofloxacin tracer aerosol in the Alberta Idealized Child Throat and idealized throat of Bickmann *et al.* (2008) were compared to examine the effects of geometry on deposition. Mouth-throat deposition in the Alberta Idealized Child Throat was consistently higher than in the Bickmann *et al.* idealized throat, likely due to the relatively large size of the Bickmann *et al.* idealized throat with regards to the ratio of airway volume divided by airway surface area. As the Alberta Idealized Child Throat has been shown to replicate deposition of pharmaceutical aerosols in school age children (see Chapter 2), it is likely that the Bickmann *et*

al. idealized throat overestimates deposition in young patients. *In vivo* data on Respimat deposition in children is required for further comparison.

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Chapter 5: Conclusion

5.1 Summary and Conclusions

In this thesis, aerosol deposition was characterized in new idealized extrathoracic geometries representative of children and infants. The Alberta Idealized Child Throat of Golshahi and Finlay (2012) was found to replicate *in vivo* pharmaceutical aerosol deposition from the QVAR pressurized metered dose inhaler in asthmatic children 6 to 14 years old (Devadason *et al.* 2003), and from the Pulmicort Turbuhaler dry powder inhaler in children with cystic fibrosis 6 to 16 years old (Devadason *et al.* 1997). This successful validation indicates that the Alberta Idealized Child Throat may indeed fulfill the existing requirement of a standardized platform for benchtop testing of aerosol delivery devices and therapeutic formulations oriented towards children.

The joint *in vitro – in silico* methodology employed in Chapter 3 revealed new insights into aerosol deposition in infants. Total lung dose was approximated *in vitro* using a realistic breathing profile and a novel bias flow system for two pressurized metered dose inhalers delivered via valved holding chamber and facemask using the idealized infant geometry of Javaheri, Golshahi, and Finlay (2013). This lung dose was then used as input for *in silico* modeling of regional lung deposition and airway surface liquid drug concentration. Simulations showed that a majority of the lung dose deposited in the tracheobronchial region, while a considerable amount of drug escaped deposition in the lungs and was exhaled. Less deposition was calculated to occur in the alveolar region. From a clinical perspective, this information provides valuable guidance with regards to dosing for infants, where limited *in vivo* data is available.

Finally, a comparison of deposition in the Alberta Idealized Child Throat and the idealized throat described by Bickmann *et al.* (2008) in Chapter 4 illustrated the importance of careful consideration of the physics governing aerosol behavior when designing idealized geometries meant to mimic *in vivo* deposition. The ratio of airway volume to airway surface area, a parameter shown to provide the best reduction in scatter of *in vitro* deposition data in realistic child throat replicas (Golshahi, Noga, and Finlay 2012; Golshahi *et al.* 2013), of the Bickmann *et al.* (2008) idealized throat (3.9 mm) was closer to that of the Alberta Idealized Throat (4.4 mm) than that of the Alberta Idealized Child Throat (2.7 mm). Thus, the observed disagreement in deposition, with the Alberta Idealized Child Throat measuring considerably higher extrathoracic deposition than the Bickmann *et al.* (2008) idealized throat, can be attributed to considerable geometrical differences between these geometries.

The research described in this thesis has the potential to both simplify and accelerate the development of new inhalable drugs and delivery devices targeting the pediatric population. It is hoped that this will aid in the treatment of respiratory disease and in the development of novel therapies that make use of the pulmonary route of delivery, for the benefit of both pediatric and adult patients alike.

5.2 Future Work

There remains room to study the effects of more realistic breathing profiles on measured deposition in the Alberta Idealized Child Throat. Experiments in the Alberta Idealized Throat suggest that judiciously chosen constant flow rates yield equivalent particle size distributions to those obtained with realistic profiles for two dry powder inhalers examined by Finlay and Gehmlich (2000), though it remains to be determined if the same can be said for dry powder inhalers and pressurized metered dose inhalers with the Alberta Idealized Child Throat.

Previous work has examined flow fields and aerosol deposition in the Alberta Idealized Throat using computational and experimental methods (Stapleton *et al.* 2000; Heenan *et al.* 2003; Matida *et al.* 2004; Grgic *et al.* 2004). Future study of the flow field generated in the Alberta Idealized Child Throat as compared to the Alberta Idealized Throat may provide further insight on how deposition differs between children and adults and improve the characterization of aerosol behavior in idealized geometries representative of different ages.

A limitation of the joint *in vitro – in silico* methodology in Chapter 3 lies in the absence of sufficient *in vivo* data for validation of the idealized infant geometry of Javaheri, Golshahi, and Finlay (2013). Available data for deposition in infants from pressurized metered dose inhalers delivered via valved holding chamber and facemask (Erzinger *et al.* 2007; Amirav *et al.* 2014) are hampered by small sample sizes and high variability in measurements. In addition, the *in silico* simulations of one-dimensional deposition and airway surface liquid concentration require validation before the results can be treated quantitatively. Unfortunately, advances in imaging techniques and expanded data sets will be required for validation of model predictions at generation-specific levels of the respiratory tract (Finlay and Martin 2008; Byron *et al.* 2010), leaving this issue to be addressed once suitable data becomes available.

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