School of Population Health

Progress Towards the Integration of Experimental and Computational Techniques to Better Understand the Delivered Dose of Aerosolised Medication

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This thesis is presented for the Degree of

Doctor of Philosophy

of

Curtin University

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Declaration Page

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Date: ....19/01/2023.....

Natalie Victoria Elaine Anderson

Acknowledgement of Country

Much of the writing of this thesis was done at my home on Mooro Country and the lands of Doondalup and Yellagonga. This land belonged to the Oor-dal-kalla people and it is from the Oor-dal-kalla people that the Noongar word Doondalup originates, which means 'the lake that glistens'. These lands are part of Noongar Whadjuk Boodja and I would like to pay my respects to elders past present and emerging.

Acknowledgement by Curtin University

We acknowledge that Curtin University works across hundreds of traditional lands and custodial groups in Australia, and with First Nations people around the globe.

We wish to pay our deepest respects to their ancestors and members of their communities, past, present, and to their emerging leaders. Our passion and commitment to work with all Australians and peoples from across the world, including our First Nations peoples are at the core of the work we do, reflective of our institutions' values and commitment to our role as leaders in the Reconciliation space in Australia.

Abstract

The overarching goal of this thesis was to gain improved understanding of the delivery efficacy and safety of aerosolised medications used in an off-label manner via laboratory and computational investigation. The series of experiments described were intended to generate an empirical evidence base for solid particles and liquid droplet aerosols – the two forms of aerosols generated by devices that produce aerosolised medications. The computational investigation was intended to study the physics of aerosolised medicines that comprise of solid particles and to make considerable progress towards the development of improved computational models. A long-term goal of the work is to further develop the computational methods to have suitable validity to be integrated with (or eventually replace) laboratory investigations for applications involving both solid and liquid aerosolised medications.

The aerosolised medications investigated were primarily those given to children off-label in a hospital setting (Chapters 2, 3 & 5) but also the widely used and "unapproved" electronic cigarette (Chapter 6) – used "off-label" by all users. Specifically:

- Chapter 2, 3 & 5: inhaled salbutamol produced by a pressurised metered dose inhaler to children using an artificial airway (a solid particle aerosol);
- Chapter 4 inhaled tobramycin produced by a nebuliser to children using a tracheostomy airway (a liquid droplet aerosol);
- Chapter 6 inhaled "e-liquid" aerosol produced by the electronic cigarette (a liquid droplet aerosol). The "e-liquid" is the "base" excipient component to which nicotine can be added.

As the first two studies involved drugs which have already been approved in children (who breathe through their native airways) and therefore have known safety profiles, the aim of the first two studies was primarily to determine the efficacy of delivery of the

aerosolised medication. Efficacy was determined via obtaining data on the estimated delivered dose, which was previously unknown in the settings described above (i.e. to patients with artificial airways or using a tracheostomy airway). The first two studies were conducted via laboratory investigation. The first experimental study (involving a solid particle aerosol) was then supplemented with computer modeling and simulation.

Results from Chapter 3 indicated that aerosolised delivery was not comparable in efficacy across all methods used to deliver aerosolised salbutamol and, in some cases, methods were completely inefficacious and therefore unlikely to deliver expected doses. These results helped inform clinical practice at Perth Children's Hospital and those methods that were completely inefficacious (where delivered dose was negligible) were discontinued. Results from Chapter 4 indicated that the delivered dose of aerosolised medication was comparable to the dose that a child breathing through their native airway would receive, and similar to the dose that an adult would receive via a tracheostomy airway. However, results also indicated that delivery of the medication was unlikely to be via aerosolization as expected – it was more likely that aerosol delivery was via larger droplets formed when aerosol formed liquid film in the tracheostomy tube. This has important implications, as if large droplets are the primary method of medicine delivery, medication is unlikely to reach the lungs to provide therapeutic effect. Results from Chapter 4 will be used to help inform the development of standardized guidelines, which do not exist for children using tracheostomy airway. While efficacy of delivery was the primary reason for investigation in both Chapter 3 & 4, it was found that safety is also an important concern where the delivered dose is not being received, or unlikely to be received at all, particularly if the medication is potentially lifesaving, as with salbutamol.

In Chapter 6 the primary concern was to determine safety before efficacy given the completely “unapproved” (in any population) status of the electronic cigarette. To study safety, my research developed a novel method to produce an electronic cigarette aerosol in a way that enabled it to be easily quantified and that was improved on current more

difficult and time-consuming methods. This novel method was then used in further laboratory investigation, to determine the safety of the inhaled aerosol, via quantification of the chemical composition of the produced (thermochemically altered) aerosol, which appears in the appendix of this thesis. Results from study (3) (both the thesis and the appendix) indicated that the aerosol produced by the base e-liquid component contained chemicals that were unsafe to be inhaled, either at all, or at certain quantities, and some chemicals/ingredients should be entirely banned. The results in the appendix of this thesis were used to inform government consultation processes that eventuated in an update to the Therapeutic Goods Order 110 in 2021 with a list of banned ingredients. Later in 2023, a full ban on importation of electronic cigarettes was announced.

In summary, this work has:

- **Added to the empirical evidence base to guide clinical best practice of off-label use for two commonly prescribed aerosolised medicines in Australia.**
- **Added to the empirical evidence base which can be used to develop international standardized guidelines for inhaled medicine delivery to children with tracheostomy airways.**
- **Generated an empirical evidence base to support development of credible computational models to investigate solid and liquid particle aerosolised medication delivery to children.**
- **Made substantial progress toward a credible *in silico* method to study solid particle aerosolised medications, particularly in off-label settings, in addition to identifying key issues/limitations with current approaches.**
- **Developed an improved method to study the chemical composition and safety of the e-liquids that are used with electronic cigarettes.**
- **Advised government consultation for the safe use of electronic cigarettes.**

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Position paper: ***The Environmental Impact of Inhaled Medicines: A TSANZ Position Paper.*** Danielle Wurzel, Brett Montgomery, **Natalie Anderson**, Elena Schneider-Futschik, Johnson George, Sinthia Bosnic-Anticevich, Emily Stone, Bob Hancox, James Fingleton, Helen Tope, John Blakey **(submitted, Respirology)**

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- Title: *"In-silico medical aerosol delivery to spontaneously-breathing pediatric patients with artificial airways"*

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- Poster presentation
- Title: *"In-silico aerosol delivery to spontaneously-breathing pediatric patients with artificial airways"*

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- Speaker
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- Poster presentation
- Title, *"In-silico aerosol delivery to spontaneously-breathing paediatric patients via oral (south-facing) RAE endotracheal tubes"*

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Introduction (brief)

This thesis forms a significant contribution to our understanding of how inhaled medicines, used in "off-label" situations behave in a clinical setting, thereby providing important delivery efficacy/safety information. It employs both empirical (laboratory experimental) and computational approaches, which together form an integrated approach to assess medical aerosol delivery efficacy and/or safety.

Aerosolised drug delivery is particularly difficult to study *in situ* due to both the constant clearance mechanisms of the lung, and that the currently adopted study methods are either relatively simplistic, (i.e. laboratory experimentation), or ethically questionable (i.e. radiolabeled lung deposition studies). Aerosols of a complex nature – that is those containing a mixture of chemicals and/or comprised of both solid particles and liquid droplets – are even more difficult to assess in terms of safety and delivery efficacy. Current study methods (laboratory experimentation or radiolabeled deposition studies) give the regional location of aerosol deposition, but do not contribute important knowledge on the mechanisms (the physics) behind aerosol delivery which is critical in informing and improving clinical use or practice. Therefore, much of this thesis seeks to expand the knowledge of aerosolised drug delivery gained in empirical laboratory experiments by supplementing these studies with advanced computer modelling and simulation techniques. The use of computational study here aims to give some depth to the understanding of the problem gained by experimental studies while ensuring the outcomes remain explained in a way that is clinically meaningful for medical staff. As such it avoids both the use of overly complex engineering jargon and detailed explanation of engineering principles.

The focus of this thesis is primarily on inhaled drugs that act locally to deliver therapeutic effect to the lungs of children or adolescents, as many inhaled drugs are first developed for use in adults and adapted minimally (if at all) for use in children. Therefore, a significant gap exists in our knowledge of how to deliver inhaled medicines most effectively and safely to this population, resulting in off-label respiratory drug use in pediatrics being common. This is particularly true in settings which require the inhaled drug to also be used in a different manner mechanistically from that for which it was first developed, such as in children using a breathing tube (artificial airway) during surgery. Investigation in children using artificial airways is therefore a key focus of this thesis. Off-label delivery of a therapeutic is at the discretion and responsibility of the prescribing physician and therefore the components of work that addresses off-label inhaled medicine use in children (Chapters 2, 3 and 5) are aimed at informing clinical decisions for pediatricians.

Secondly, this thesis considers the “unapproved” systemically-acting (if containing nicotine) inhaled aerosol produced by the electronic cigarette, commonly used by adolescents. The electronic cigarette creates a complex aerosolised mixture of chemicals which is difficult to characterize, partly due to varying degrees of thermochemical modification during generation/delivery. This means that downstream health effects are also difficult to comprehensively ascertain. Determining potential health effects is important, however, considering

this is an unapproved therapeutic good that is widely used. Thankfully, potential harm to Australian consumers were reduced when the new vaping reforms were announced, set to commence on 1st January 2024, involving a ban on the importation of non-therapeutic vapes and a ban on personal importation. However, a doctor may still prescribe nicotine to be added to an "e-liquid" and used with an electronic cigarette device for the purpose of smoking cessation, via an authorized prescriber (or equivalent) scheme. As the electronic cigarette is used entirely "off label", it has not been thoroughly tested for safety (or efficacy) as an inhaled therapeutic in any population, and with the new reforms, importers and manufacturers are required to notify the Australian Therapeutic Goods Administration of their products compliance with relevant product standards.

Overall, this thesis contributes knowledge to inhaled medicinal aerosols in underrepresented demographics and off-label settings where the aerosol safety and efficacy are undetermined and therefore so too, the health outcomes. The intention was to provide data which would help clinicians to make informed decisions around off-label inhaled medicine use, and to develop an evidence base for assisting with the development of future guidelines.

Chapter 1 **(Introduction and background)** is broken down as follows:

- CH1.1 describes the important aspects of aerosol physics and mechanics relevant to inhaled drug delivery.
- CH 1.2 describes the important aspects of human biology/physics relevant to inhaled drug delivery.
- CH 1.3 describes the respiratory diseases that have approved inhalation treatments (that are locally acting, and where those same drugs are used off-label).
- CH 1.4 describes the diseases that have approved inhalation treatments (that are systemically acting) (and those unapproved).
- CH. 1.5 describes why there is so much off-label usage and the problems with drug development.
- CH 1.6 describes the new technologies that can help improve drug delivery and/or design of new orally inhaled therapeutics.

Chapter 2 **(publication: Aerosolized drug delivery in awake and anesthetized children to treat bronchospasm)** focusses on the off-label use of inhaled treatments perioperatively and compares this to delivery in emergency settings via a review of the current devices that are used to deliver orally inhaled medicines that exert local therapeutic effects in the lungs of children experiencing bronchospasm, predominantly with the beta-2-antagonist drug, salbutamol (and example of a solid particle aerosol).

Chapter 3 **(publication: Laboratory assessment of artificial airways)** is an original article that examines the delivery of a solid particle aerosol: the inhaled medicinal salbutamol delivered via various types of artificial airways to children when simulated in the laboratory setting.

Chapter 4 **(Laboratory simulation of nebulized tobramycin through tracheostomy to spontaneous breathing children)** examines the delivery of a liquid particle aerosol: the inhaled antibiotic tobramycin, delivered via various types of artificial airways to children when simulated in the laboratory setting.

Chapter 5 **(*In silico* assessment of solid particle aerosols to support laboratory simulation of aerosolised drug delivery to intubated children)** examines the delivery of inhaled medicinal salbutamol delivered via various types of artificial airways when simulated via the computer modeling technique "computational fluid-particle dynamics" replicating the investigation in *Chapter 3*.

Chapter 6 (**A rapid semi-quantitative screening method to assess chemicals present in heated e-liquids and e-cigarette aerosols**) examines a novel method that can be used to rapidly test for ingredients in "e-liquids" that are banned by the Therapeutic Goods Administration, but which could be inhaled in the aerosol produced by the unapproved therapeutic good, the electronic cigarette.

Appendix 1 includes a co-authored article which tested for various chemicals produced by electronic cigarette aerosols and their base liquids to identify dangers to the consumer of this unapproved therapeutic good.

Appendix 2 includes a co-authored article investigating the environmental impact of inhaled medicines produced by a certain device that aerosolizes medicine by using pressure generated by a greenhouse gas propellant.

Chapter 1: Introduction and background

1.1 Physical principles of aerosols affecting therapeutic aerosol delivery

The lung represents a very large surface area proximal to a blood interface. Therefore, aerosol delivery to the lung represents an important pathway for both targeted delivery of medication for respiratory pathologies as well as general (non-respiratory) medicine (Smyth & Hickey, 2011). The lung also represents an important toxicological exposure pathway to exogenous agents (Widdicombe & Wine, 2015). There are many factors that make aerosol delivery to the lung effective and therefore therapeutic, however there are also many drawbacks to current delivery methods which prevent optimal aerosolised delivery. It is therefore important to understand the basic physical principles behind current delivery methods of the two key types of aerosols – solid or liquids – to improve aerosolised drug delivery to the lungs.

1.1.1 Overview of aerosol properties and terminology

1.1.1.1 Definitions

Aerosols are most commonly defined as solid or liquid particles suspended in a gas (usually air) (Hinds, 1999). The term aerosol includes the more commonly referred to terms: bioaerosols, dust, particulate matter, haze, mist and fog, smog, fumes, clouds, smoke, droplets, and sprays (Hinds, 1999). Similarly, aerosols generated for therapeutic delivery (medicinal aerosols) can also be described broadly as either liquid or solid particles. The physical aspects of both solid and liquid aerosols, such as density, surface tension and particle size distribution, need to be considered in computational and laboratory simulation of aerosol delivery to the lung.

1.1.1.2 Shape, size, and mass

When referring to the overall size of an aerosol, the classification of either monodisperse (within geometric standard deviation <1.22 μm) or polydisperse is used, but most aerosols are polydisperse, meaning the total aerosol has a geometric standard deviation of greater than 1.22 μm (Usmani et al., 2003). For example, an asthma inhaler such as Ventolin will produce particles within the range of ~0.1–30 μm in a single "puff" or dose, rather than them being all 5 μm in size (Alatrash & Matida, 2016). It is important to know the mass of drug delivered to a patient, and therefore therapeutic aerosols are usually measured by the mass median diameter, or mass median aerodynamic diameter, both of which assume particles to be spherical and have the density of water (Hinds, 1999). Aerosol measurement can either be by mass or by number count (Hinds, 1999). Mass and number count are both important measures as for some types of aerosol, more than 90% of particles are ultrafine particles (<0.1 μm) and therefore only account for 10% of the total mass (Kwon et al., 2020). However, the active pharmaceutical ingredients for solid particle aerosols are commonly different shapes, when examined with microscopy (Parisini et al., 2015), and so an "effective" diameter is used in this case, which is the spherical equivalent of the actual shape. Solid particles produced by spray drying can be made spherical but spray dry production of medical aerosols is a relatively new technology and primarily done for dry powders inhalers (DPIs) not pressurised metered

dose inhalers (pMDIs) (Focaroli et al., 2020). Liquid aerosols, however, are usually spherical unless experiencing high acceleration or deceleration (or the drag force to surface tension ratio is high).

1.1.1.3 Respiratory aerosol deposition

Whether or not an aerosol particle will deposit in the lungs, and by what mechanism, is predominantly based on the size (aerosol diameter), the velocity of the travelling aerosol (Figure 1.1.1.4) and the "flow Reynolds number", which is a determinant of whether the air the aerosol passes through is turbulent or smooth (laminar). Larger aerosol particles (>5 μm) usually deposit by impaction (momentum force) in the upper conducting airways, which is due to these particles travelling faster than in the lower airway, and the direction of the flow changing more frequently in the upper airway than the lower (turbulent flow) (Koullapis et al., 2018a). In the upper airways smaller particles will deposit by turbulent dispersion, which causes particles to be pushed toward the airway walls, where deposition is then by diffusion due to the zero-gradient condition (no flow) at the airway walls (Koullapis et al., 2018a, Koullapis et al., 2018b). In the small airways, the flow in the lung changes from turbulent to laminar as the velocity slows, and the smaller particles that reach the small airways deposit by diffusion and gravitational settling (Koullapis et al., 2018b).

The movement through the lungs and resultant lung deposition of an aerosol particle can also be determined by its shape. For example, fibre particles, i.e. particles that have a high aspect ratio (where aspect ratio is the term for ratio of length to width), usually 3:1 or 5:1, exhibit different deposition behaviors to spherical particles (Institute of Medicine (US) Committee on Asbestos Selected Health Effects, 2006). For example, fibrous particles like asbestos behave either by diameter or by length depending on whether the flow is convergent i.e. inhaling or divergent, i.e. when exhaling flow is divergent and particles turn sideways (behave by length) and get stuck.

1.1.1.4 Aerosol characterization (particle sizing)

Aerodynamic particle size distribution of an emitted (inhaled) drug is a critical quality attribute of all inhaled therapeutics (Nichols et al., 2013). Aerosol particle-sizing is required by therapeutic goods administrations for all orally inhaled drug products prior to marketing and helps to determine the product's therapeutic effect and also the repeatability of dosing (European Medicines Agency Committee for Medicinal Products for Human Use, 2009, United States Food and Drug Administration, 2002). Typically, a validated multistage impactor/impinger, like an Anderson cascade impactor (ACI) or Next Generation Impactor (NGI) with a United States Pharmacopoeia induction port and constant flow rate (or square wave) is used to measure particle size indirectly via particle mass fraction (Nichols et al., 2013, European Medicines Agency Committee for Medicinal Products for Human Use, 2009, United States Food and Drug Administration, 2002). However, the NGI, plus mixing inlet, breathing simulation, and a realistic mouth-throat model, such as those generated by Virginia Commonwealth University are upgrades that should be considered to ensure more accurate representation of *in vivo* data (Figure 1.1.1.4) (Tavernini et al., 2021). The "stages" or plates of either a NGI or ACI have "cut-offs" based on particle size (Nichols et al., 2013). To characterize aerosol particle size, flow through the impactor is constant at 30,60 or 100

L/min for assessment of pMDI and DPI products, and 15 L/min for nebulised products (Nichols et al., 2013). These aerosol particle size data are recommended to be used in combination with clinical imaging data where possible (European Medicines Agency Committee for Medicinal Products for Human Use, 2009). Using the standard approach, the ACI or NGI device requires a constant flow to take measurement, however, to make particle sizing results more accurate/realistic, flow-volume simulator equipment can be used that is programmed with inhalation parameters based on ventilation guidelines for adults or children (Wei et al., 2018). Simulated breathing is prefered in pharmacopeia in accordance with good practice and relevant International Organization for Standardization (ISO) standards for the laboraotry testing of oral inhaled products (Mitchell et al., 2023, International Organization for Standardization, 2023).

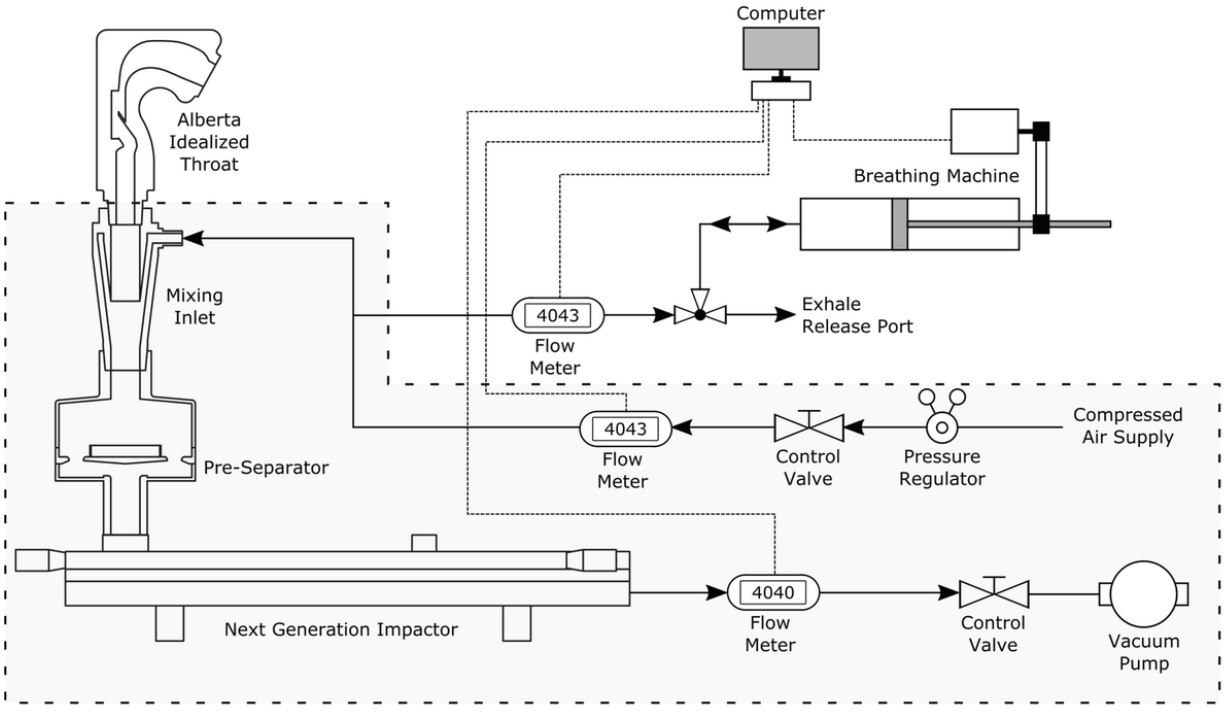


Figure -1.1.1.4 Recommended set-up for medical/therapeutic aerosol particle sizing of a solid particle aerosol.

The set up includes, in anticlockwise order: a realistic mouth-throat mode where aerosol is introduced (Alberta realistic throat), a mixing inlet, a Next Generation Impactor connected to flow meters and the breathing machine (breathing simulator). Modified and reprinted with permission from: Tavernini, et al., (2021). Using Filters to Estimate Regional Lung Deposition with Dry Powder Inhalers. *Pharmaceutical research*, *38*(9), 1601–1613. https://doi.org/10.1007/s11095-021-03082-0

There are limitations of particle sizing approaches such as the ACI or NGI as they are largely designed to conduct multiple tests rapidly are, therefore, somewhat of a compromised design (Mitchell & Nagel, 2003). All impactors suffer major issues with particle bounce for both solid and liquid particles. For liquid particle aerosols, such as those produced by a soft mist inhaler or nebuliser, more sophisticated measurement equipment must be used, which comes with its own set of limitations such as evaporation and redistribution of liquid by bounce (Mitchell & Nagel, 2003). This means that the particle size distribution could be skewed slightly from one measurement

component stage to the other. While most aerosol delivery devices produce solid particles (as these are easier to deliver than liquid particles), many drugs can only be formulated as liquid particles and so measurement of both solid and liquid particles is required in a medical context. Additionally, the medical context can change the particle size, and this is often increasingly difficult with liquid aerosols: such as when the aerosol moves through a humidified system or a confined ventilation or airway tube – making it difficult to measure aerosol particle size that is inhaled by the patient in real-world scenarios. To aid the study of these difficult scenarios, particle size data produced by the inhaler device could be obtained, and then computational 3D, physics-based models (like computational fluid dynamics) used to more realistically study delivery scenarios where accurate aerosol measurement would otherwise be difficult. Realistic conditions could include study of the effects of temperature and humidity in the nasal cavity, supersaturation, particle growth and water-latent heat in airway mucous (Xu et al., 2021).

The equipment used to measure a medical aerosol produced by an orally inhaled drug product will depend on the type and size range of aerosol being measured (Nichols et al., 2013). For example, an ACI, NGI or optical particle spectrometer (OPS) can be used to measure most aerosols, however OPS generally work best when the particle size is <100 nm (Vasilatou et al., 2021). Mass-based instruments such as ACI/NGI lose resolution at low sizes (<100 nm), meaning that electrical classifiers such as scanning mobility particle sizers are preferred for particles >100 nm (Moreno et al., 2020). Further, re-entrainment (re-capture) is an issue for all impactors, with both solid and liquid particles (Mitchell & Nagel, 2003). Liquid particle aerosols can only be measured accurately with liquid impingers, or OPSs if the aerosol is at low densities/concentrations. This is because with ACI or NGIs re-distribution of liquids can occur post collection, which skews the particle size distribution (Nichols et al., 2013) and with OPS measurement, there is a risk of coincidence error – or detecting two particles as one – at high concentrations (Sachweh et al., 1998). This is because OPSs are designed to measure ambient air quality in critical environments – such as manufacturing warehouses – where airborne particulate matter is expected to be low and therefore most can only detect up to 10^6 particles per cubic centimeter, however this is dependent on the instrument (Vasilatou et al., 2021).

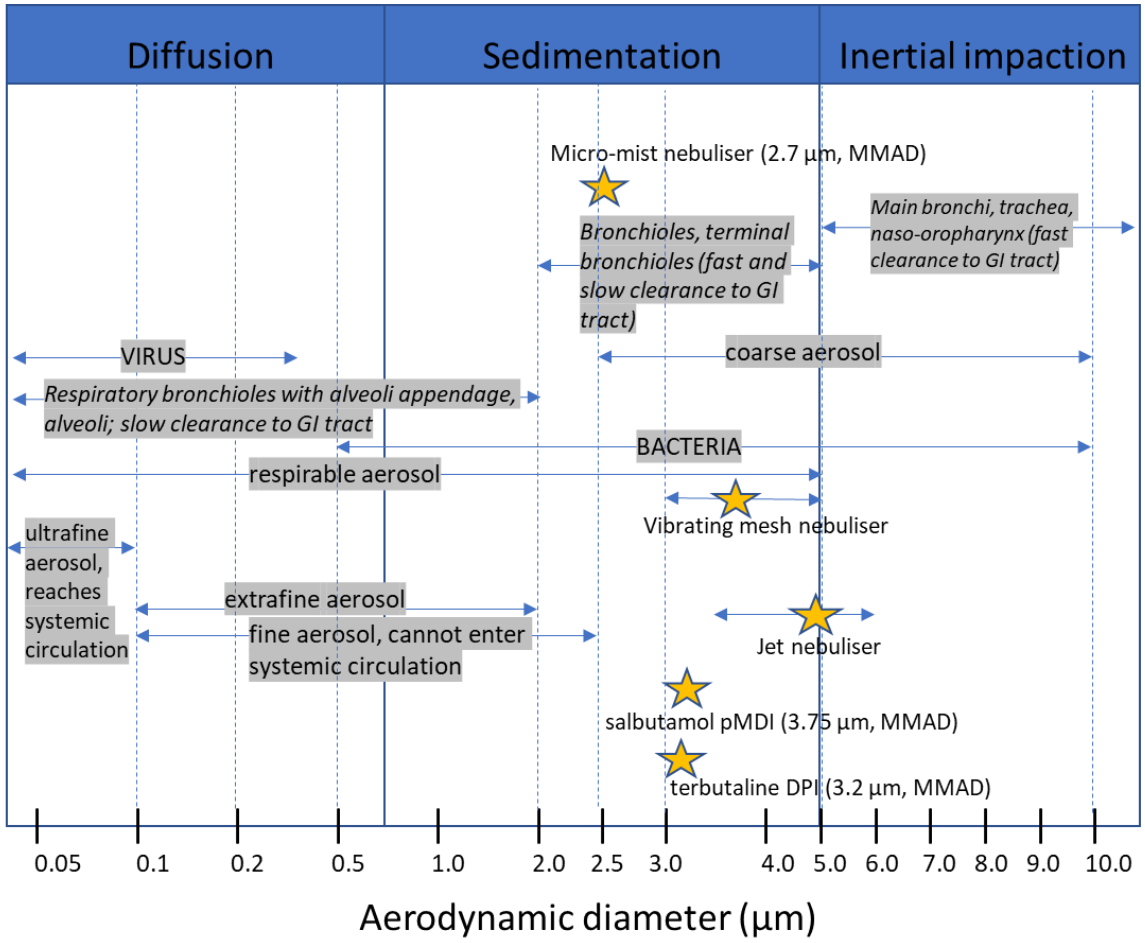


Figure 1.1.2 Mechanisms of aerosol deposition of different types and sizes in the lung.

Adapted with permissions from (Labiris & Dolovich, 2003). GI= gastrointestinal*, MMAD= mass median aerodynamic diameter DPI= dry powder inhaler. Clearance = mucocillary clearance. Aerosol larger than 5 µm will be cleared quickly to the mouth and swallowed, aerosol between 0.1 and 5 µm will deposit in the bronchioles and exhibit slow clearance (allowing longer for dissolution and absorption), unless the bronchioles are functional for respiratory mechanisms and thus have alveoli appendages, slowing clearance further. Ultra-fine particles (under 0.1 µm) reach the systemic circulation and so treat systemic conditions rather than conditions local to the lung. Aerosol particles larger than 0.1 µm cannot enter the systemic circulation but are cleared slowly allowing longer for dissolution and absorption. *Some particles are sequestered in the epithelium and will be cleared to the lymph nodes, not GI tract.

1.1.2 Aerosol physical phenomena

Aerosol phenomena relevant to medical aerosol lung deposition are the physical events which alter the shape, size or physical properties of an aerosol particle and therefore influence where (and if) it will deposit in the lungs (Figure 1.1.2) (Labiris & Dolovich, 2003). The basic aspects of adhesion, coagulation, agglomeration, evaporation, and condensation will be discussed briefly below, only to the degree necessary to understand for an inhaled medicine and understanding physical phenomena that may be involved in programming of computational

models. Although these physical phenomena affect all inhaled aerosols, they are rarely measurable (or measured) in the context of inhaled medicines and not always present in computational models, therefore a complex explanation is outside the scope of this thesis.

1.1.2.1 Adhesive forces, agglomeration, and coagulation

When aerosol particles contact each other, they form agglomerates and aerosol particles are assumed to stick to any surface they meet, if kinetic energy is sufficiently small such that particle rebound is negligible (Hinds, 1999). The terms "agglomeration" and "coagulation" can both be used to describe solid particles whereas coagulation will refer to liquid particles only. The models investigated in this Thesis accounted only for solid particle interaction with the stationary phase, with a modified O'Rourke collision model – no agglomeration (collision) of particles other than with the boundary walls (O'Rourke et al., 2009). Likewise, rebound or imperfect adhesion has also not been considered. More complex models were outside the scope of investigation, however, the mechanisms they are based on are discussed here to give examples of why they might be necessary to include in future models. Liquid particle models are discussed in Chapter 7, Future Directions.

1.1.2.1.1 Adhesive forces

Adhesive forces include the Van Der Waals force, electrostatic forces and the force arising from the surface tension of adsorbed liquid films, with van der Waals and surface tension forces being greater than electrostatic forces (Hinds, 1999). Van Der Waals forces and surface tension forces are more predominant in liquid solutions than with solid particles therefore liquid aerosols are often more complex to deliver. Van Der Waals forces are also applicable in dry powder applications to overcome inter-particle forces with deagglomeration – the aerosol generation principle for these devices (Young et al., 2007, Le, et al., 2012). Electrostatic force is due to the net charge on the particle inducing an equal and opposite charge in the opposing surface and is found in particles 0.1 μm diameter or larger (Hinds, 1999). Electrostatic forces do affect aerosol deposition within some aerosol delivery devices such as spacer devices (devices used to improve aerosol delivery) and artificial airways (used perioperatively) (Piérart, et al., 1999). Current technological advancements in materials means that many such devices now contain an anti-static coating to prevent electrostatic effects on aerosol deposition (Chambers, et al., 2009). Van Der Waals and electrostatic force can also lead to changes in the aerosol particle size over time when in certain types of formulations (such as suspension-based products) due to adhesion and cohesion, where adhesive forces are between a particle and a surface of a material, and cohesive forces are between particles of the same predominant material (chemical composition) (Feng & Hays, 2003).

1.1.2.1.2 Agglomeration and coagulation

Cohesive forces can result in agglomeration of particles, dependent on adhesion number, defined as the ratio of the adhesive force to the drag force, which can be employed to characterize the degree of agglomeration (Liu, 2020). The rate of particle (solid or liquid) collision resulting in agglomeration or coagulation is measured by a corresponding decrease in the particle number concentration and is neglected in laboratory studies when aerosol

particle concentration is less than 10^6 particles per cubic centimeter (Hinds, 1999). For monodisperse particles, particle size increases by the inverse cube root of the particle number concentration (Hinds, 1999). Calculating the coagulation rate/increase in particle size/decrease in particle number concentration is far more difficult for polydisperse aerosols (Hinds, 1999). Agglomeration has been demonstrated to affect inhaled medicine aerosol deposition in radiolabeled lung deposition studies, when dose is high, for example when a 200 µg dose is delivered in a single dose, instead of two doses of 100 µg (Roller, 2012). Further the drug can be initially designed to prevent agglomeration, for example with the use of highly porous spherical particles (Stass et al., 2013).

1.1.2.1.3 Evaporation

Evaporation is the process by which molecules leave the surface of a droplet and it is usually described by a rate of evaporation during a droplet lifetime (or until complete evaporation) (Hinds, 1999). Many droplets contain a solid particle (low solubility) nucleus, such as with suspension formulated pressurised metered dose inhalers, and therefore will dry until the nucleus is left (Hinds, 1999). Factors that affect evaporation include relative humidity, and the material properties of the liquid (Hinds, 1999). Most inhaled medicines will be subject to minimal evaporation if they are inhaled through a mouthpiece, as they will be humidified immediately upon entering the lung environment (Haddrell et al., 2017, Morrow, 1986, Zhang et al., 2006).

1.1.2.1.4 Condensation

Whenever the amount of gas contained in a sample is equal to its water vapour capacity, the relative humidity is 100% and the gas is completely saturated (Al Ashry & Modrykamien, 2014). Condensation involves mass-transfer from gas to liquid phase (e.g., water vapour and water), and usually occurs when under conditions of supersaturation, (where the saturation ratio (or relative humidity) is greater than 1 (relative humidity over 100%) (Hinds 1999). At normal conditions, the lungs (beyond the isothermic saturation boundary, usually 5 cm below the carina) are at 37 ºC and 100% relative humidity which means that the lung contains the maximum amount of water vapour that it can contain given the volume of air (Al Ashry & Modrykamien, 2014). The relative humidity and air temperature reach 99.5% and 37 ºC by the fourth dichotomous branch of the airway and therefore aerosols that pass this point is almost saturated (Morrow, 1986, Zhang et al., 2006). The capacity of an aerosol particle to absorb water can be measured accurately up to 100% relative humidity for pharmaceutical aerosols using a refined electrodynamic balance technique (Hadrell et al., 2014, Rovelli et al., 2016). Some inhaled therapeutics are produced by evaporative condensation processes however, such as aerosols produced by electronic cigarettes (Spahn, et al., 2021). Particles formed of hygroscopic (or soluble) components such as saline or water, have the ability to grow by an order of magnitude in mass or diameter as the relative humidity passes 99.5% (Hadrell et al., 2017). This means that aerosol particles made of hygroscopic (or soluble) materials will increase in size more rapidly than particles that are not made of hygroscopic materials (Hadrell et al., 2017).

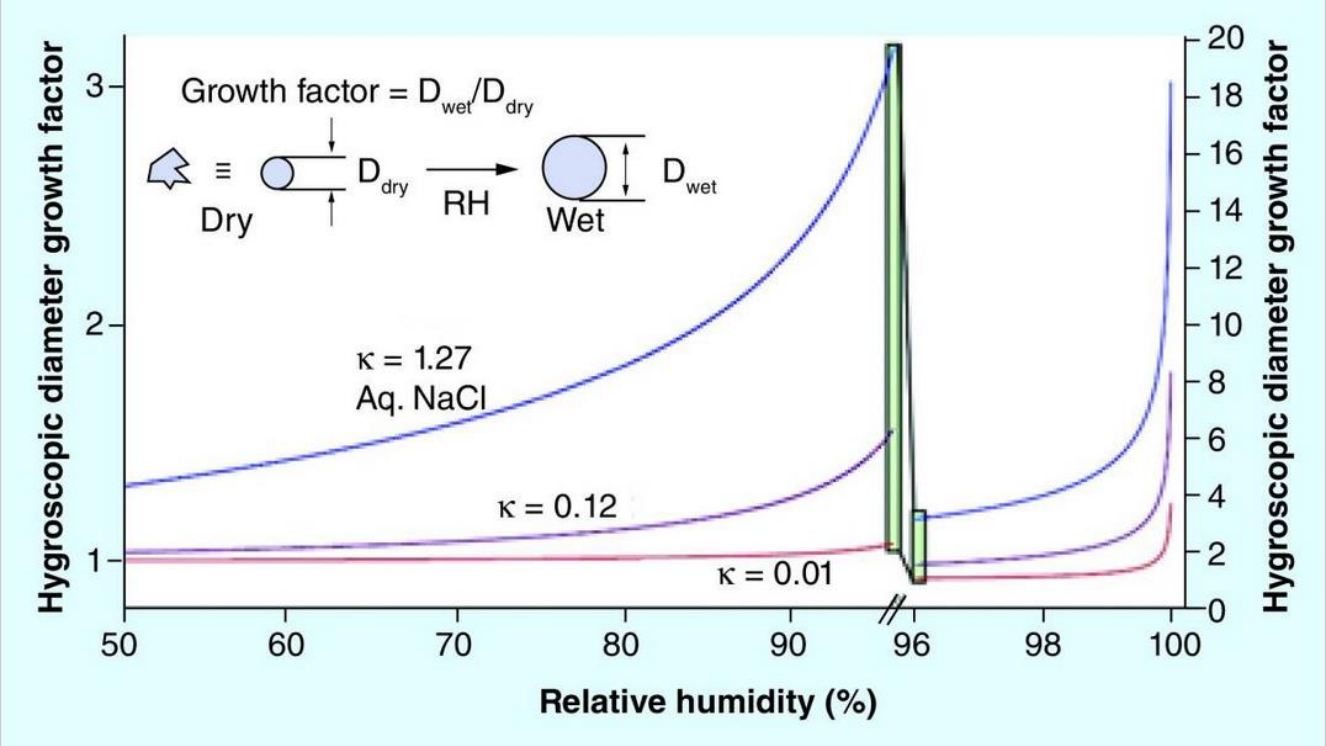


Figure 1.1.2.1.4. Hygroscopic growth of particles during relative humidity. Reprinted from Hadrell et al., 2017, with permissions under creative commons license (https://creativecommons.org/licenses/by-nc-nd/4.0/). Particles respond to changes in the surrounding relative humidity of the gas phase by absorbing water and growing in size. Here, the growth in size is represented as a diameter growth factor which reports the change in particle size relative to the dry particle as the relative humidity increases. Growth curves for particles of increasing hygroscopicity parameter (= 0.01, 0.12 and 1.27) are shown as examples of insoluble, low solubility and high solubility, respectively.

1.1.3 Aerosol generation

There are five key aerosol generation techniques commonly used to generate/deliver inhaled medicines which determine if the aerosol produced is a solid or liquid particle; 1) Electrical field generated (e.g. ultrasonic nebulisers, liquid particle), 2) Electrostatic (e.g. electrospray drying, solid particles), 3) Evaporation-condensation (e.g. e-cigarettes, liquid particles), 4) Mechanical (e.g. vibrating mesh, jet nebulisers, soft mist inhalers, liquid particles, or dry powder inhalers, solid particles), and 5) State change (e.g. pressurised meter dose inhalers, solid particles (suspension based) or liquid particles (solution based)). Each generation technique produces polydisperse aerosol particles, and as technology has improved most types of medical aerosol generation devices nowadays can produce particles of a size that can reach the small airways of the lungs (respirable particles, <5 μm). General concepts and principles of each aerosol generation are introduced below.

1.1.3.1 Electrical field (the ultrasonic nebuliser)

The ultrasonic nebuliser works by producing ultrasonic compression waves generated by a piezoelectric crystal. These are focused near the surface of the liquid to nebulize, which then create intense agitation in turn creating a conical fountain of aerosol.

1.1.3.2 Electrospray or electrostatic atomizers

Electrospray devices generate aerosol by feeding liquid slowly through an orifice (such as a hollow needle or perforated plate) which is facing downward and a few centimeters opposite a coaxial ring (Hinds, 1999). This creates a strong electrostatic field near the rip of the needle and causes the liquid exiting the needle to form a conical aerosol spray (Hinds, 1999). There are no medical aerosol devices to treat respiratory conditions that currently use this method to deliver aerosol, but electrospray or "spray-drying" (or spray-freeze drying) methods are used to create the aerosol particles themselves, such as is used to create various types of dry powder particles (Stass et al., 2013, Park et al., 2013, Mohan et al., 2022)

1.1.3.3 Evaporation-condensation aerosol generation

An evaporation-condensation device works by heating a coil wrapped around a wetted-wick, which produces liquid condensate droplets that quickly evaporate when an airflow is drawn through the device to form aerosol nuclei. An example of device that uses this method of aerosol generation is the electronic cigarette (Hon, 2003). The electronic cigarette is used to deliver inhaled nicotine which, in Australia, is a prescription medicine (Australian Government, Department of Health and Aged Care, Therapeutic Goods Administration, 2023). Evaporation-condensation aerosol generators produce a monodisperse aerosol, however, as they are produced in such high concentrations, agglomeration of particles creates a polydisperse aerosol.

1.1.3.4 Mechanical

1.1.3.4.1 Dry powder inhaler

Dry powder inhalers work on the principle of deagglomeration, which is triggered by a patient inhaling and bursting (or breaking) a dry powder capsule contained in the device. The ability for the powder to deagglomerate is largely determined by the physical properties of the drug formulation, however certain device designs can encourage deagglomeration better than others, such as the Turbuhaler™ device which increases/accelerates the inhalation speed generated by the patient by pulling air through a series of interweaving tunnels (Kuna & Kuprys, 2002).

1.1.3.4.2 Jet nebuliser

A jet nebuliser produces aerosol by high velocity liquid stream, which results in formation of droplets at certain distance from the nozzle (Hinds, 1999). The high velocity is usually driven by a gas source: compressed air or oxygen, for example a hospital wall air or compressed oxygen source, however jet nebulisers can have their own compressors for home use. Limitations of jet nebulisers are that they are often noisy and have large residual volumes (retention in the nebuliser of the dose intended for delivery) (Park et al., 2021).

1.1.3.4.3 Vibrating mesh nebuliser

Vibrating mesh nebulisers operate by using a syringe to drive a small volume of liquid through a small oscillating orifice which produces a thin liquid filament with each oscillation (Hinds, 1999). The vibrating mesh nebuliser does not require a compressed air source and the power required to generate the oscillation is by piezoelectric crystal (powered by electrical/battery source). Vibrating mesh nebulisers were designed to overcome the issues with jet nebulisers and are therefore quiet and have low to nil residual volume (Park et al., 2021).

1.1.3.4.4 Soft mist inhalers

The soft mist inhaler uses mechanical energy from a spring to force liquid through a specialised nozzle called a "uniblock" which produces two fine jet streams of liquid and converges them at a defined angle to create the soft mist (Dalby et al., 2004). An example is the RESPIMAT® softmist inhaler (Dalby et al., 2004).

1.1.3.5 State change (the pressurised metered dose inhaler)

The pressurised metered dose inhaler (pMDI) works in a similar way to an aerosol spray can; a small volume of pressurised liquid (propellant), mixed with drug in suspension, is delivered through a valve (Newman, 2005). The pressurised liquid provides the energy required to generate the aerosol, for example a hydrofluoroalkane 134a propellant (Sellers, 2017).

1.2 Anatomy and physiology of the human respiratory system

The lungs are the only internal organ to directly interface the external environment and they do so via a gas exchange surface roughly the size of a tennis court – 30 times that of the surface of the skin (Combs & Dickson, 2020). The average human adult inhales over 6000 liters of air every day, has 30,000 terminal bronchioles, and 300 million alveoli and thus is constantly exposed to exogenous agents such as particulate matter and environmental irritants which can cause lung damage and disease (Wang 2002, Mazzone & Undem, 2016). It is therefore essential to understand airway structure and function to better understand lung conditions during health and disease and identify the optimal drugs and conditions for drug delivery.

Respiratory disease conditions are characterized by changes to homeostasis in the lung environment. Changes can include; excess or altered mucous consistency, narrowing of normal airway tone (e.g. bronchoconstriction), altered immune response (e.g. inflammation), loss of healthy tissue (e.g. lung damage), impaired normal airway clearance mechanisms (e.g. altered mucocillary clearance) or changes in the normal microbiome and bacterial population (e.g. increased pathogen concentrations). These disruptions to homeostasis will impede aerosol delivery but it is essential to deliver drugs to the lung site where they can exert therapeutic effect (Tiddens, 2016). It is already known that some factors can improve aerosol delivery to patients with lung disease, such as a use of a fine particle aerosol or using a slow inhalation maneuver instead of a fast one (Usmani et al., 2003) however as this area is still in its infancy, further discussion on this topic is outside the scope of this thesis (Oakes et al., 2019).

This section will discuss anatomy and physiology of the lung within the context of the challenges it presents and benefits it brings with respect to aerosol delivery to the lung.

It is well established that there are multiple advantages to the lung as a site for delivery of medication:

1) the lungs have a large surface area (70–100 m^2 for the alveoli and 2.5 m^2 for the airways) for drug absorption and the endothelium of the alveolar capillaries is the most dense vascular bed in the human body (Wang 2002, Fröhlich et al., 2016, Wiebe & Laursen, 1995 Mercer et al., 1994);

2) when medicine is delivered to the alveoli, there is direct and quick access to the systemic blood stream, meaning a short therapeutic onset-of-action;

3) less drug is required compared with oral medication as drug will not be wasted by first-pass metabolism and therefore aerosolised medicine contributes minimally to systemic toxicity;

4) the lung is a non-invasive target for delivery of medicines compared with injection (intravenous, intramuscular, subcutaneous).

1.2.1 Human lung structure overview and its relevance to inhaled medicine delivery

The gross anatomy of the human lung includes three lobes on the right and two on the left (Rubenstein et al., 1949). The right lung is slightly wider (along the frontal/coronal plane) and shorter (along the sagittal plane) than the left lung, to accommodate the liver and heart (Rubenstein et al., 1949). For best aerosol drug delivery to both lobes a healthy patient should be standing or seated upright, however this is likely to vary if there is a disease condition and lung ventilation is uneven (Bachmann et al., 2018). For example, lung lobes with substantial structural damage will not receive as much aerosolised drug as aerosol particles and air will flow preferentially down the path of least resistance into the less damaged lobes (Holsbeke et al., 2013). Further the upper airway (trachea) has great variability between individuals and therefore lung deposition is highly dependent on this factor (Darquenne et al., 2016).

The respiratory system is functionally divided into the upper (conducting) airway which acts to conduct and filter air and the lower (functional) airways which act to exchange gas (Figure 1.2.1.1). Accordingly, an inhaled drug can be classified as acting locally or systemically dependent on whether it deposits in the upper or lower (respiratory) airways respectively, assuming good ventilation and blood circulation (Powers, 2023). The conducting airways include the nose, mouth, throat and the first 0–16 branches of airways, while the functional airways (also called the parenchyma) are from branches 17–23, which complete respiration (ICRP, 1994, ICRP 2015). The airway can alternatively be further divided into the tracheobronchial region (0–8 generations), the bronchiolar region (9–16 generations) and the respiratory (gas exchange) region (generations 17–23) comprised of the respiratory bronchioles and alveolar ducts and sacs (alveoli). These further divisions are often helpful to explain where certain types of drugs should deposit for optimal therapeutic effect. For example, drugs that are

required to exert a local therapeutic effect in either the tracheobronchial or bronchiolar regions of the airways include the class of drug called bronchodilators. Drugs that are required to exert local therapeutic effects in only the bronchiolar region of the airways include inhaled corticosteroids. Medications depositing in the tracheobronchial region of the upper airway will be subject to mucociliary clearance and therefore locally acting drugs are best designed to reach the bronchial region where they will have a longer duration of action before being metabolised and excreted (Hastedt et al., 2022).

1.2.1.1 Conducting (upper) airway structural overview

From a physiological perspective and through their influence on (air) flow development, cell types, airways sizes and branching angles all influence where a particle will deposit and how long it will reside before clearance (Espinosa-Moreno et al., 2023). Additionally, the optimal aerosol particle size influences lung deposition, which is debated however usually considered to be under 5 μm in size and not small enough to be exhaled (Darquenne et al., 2016, Bake et al., 2019). Both therefore influence therapeutic effectiveness of an inhaled drug.

Airway diameter and airway branching angle influence the deposition mechanisms for aerosol particles: airway diameter (and length) will determine the volume-flow rate of air and therefore largely, the deposition of each size of aerosol particle. Particles are generally considered "respirable" when they are less than 5 μm in size, with particles between 2 and 5 μm being less likely to deposit in the upper airway (Tiddens et al., 2014). Particles < 5 μm are also small enough to reach the small airways which are considered to be less than 2 mm in diameter, but need to not be too small that they will be exhaled (i.e. not less than ~1 μm) (Bake et al., 2019). The terminal bronchioles are the last of the air-conducting bronchioles – the respiratory bronchioles are so called as they have alveoli to some degree (ICRP 1994) and have an internal diameter of ~500 μm (Betts et al., 2013): an alveolus (or alveolar sac, comprising of the smallest airway and capillaries) ranges from 150 to 500 μm in size (Wang, 2002, Betts et al., 2013). The alveolar sacs, with the capillaries, form the blood-air barrier (Ball, 2023, Betts et al., 2013).

Branching angles for the bronchial and bronchiolar region range between 28 and 53 degrees, and 45 and 52 respectively for each region (ICRP 1994). Sharper degrees of branching mean a greater degree of change in airflow direction and more likelihood for turbulence and particle deposition (Koullapis et al., 2018a, P. Koullapis et al., 2018b).

Cell types do not directly influence the deposition mechanisms of impaction or sedimentation but will influence the retention time in the lung (Hastedt et al., 2022). For example, cells that have cilia and produce mucous will move an aerosol particle up the mucocillary escalator to be swallowed and therefore such particles will not be retained for as long as particles which deposit on cells without cilia.

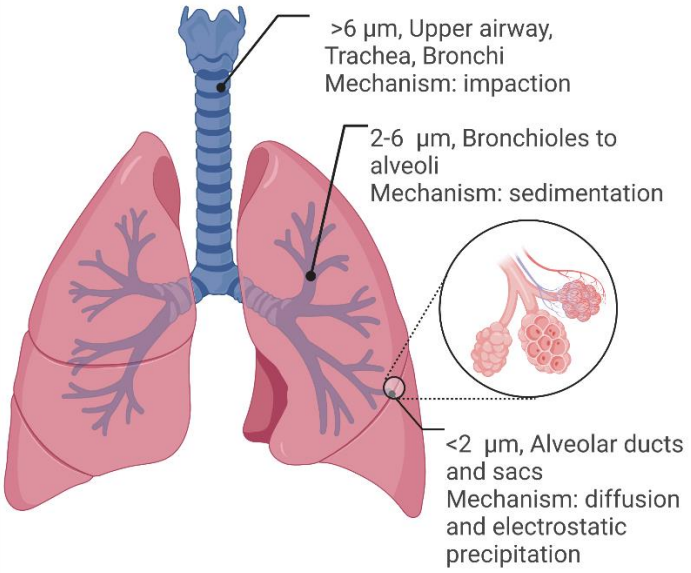


Figure 1.2.1.1 Main deposition mechanisms for each region of airway. Reprinted from Anderson et al, 2022. Upper airway, trachea, and bronchi: mechanism is impaction and commonly particles over 6 µm in size will deposit here. Bronchioles to alveoli: mechanism is sedimentation, and commonly particles between 2 and 6 µm in size will deposit here. Alveolar ducts and sacs: mechanisms are diffusion and electrostatic precipitation and commonly particles less than 2 µm in size will deposit here. Impaction occurs primarily at airway bifurcations/branches and does also occur in the upper bronchioles.

1.2.1.2 Lung surface area

The surface area of the lung can be separated into the upper airway, which includes the tracheobronchial region and bronchiolar region (surface area ~2–2.5 m^2) and the small (respiratory) airways (surface area ~70–100 m^2) which includes the alveoli and alveolar ducts (Wang 2002, Fröhlich et al., 2016, Hastedt et al., 2022, Wiebe & Laursen, 1995 Mercer et al., 1994). The lung epithelium is present everywhere in the lung except the larynx and pharynx (Kia'i, 2023) and the epithelium surface area values most used are either those published by the International Commission for Radiological Protection at 78 m^2 (ICRP, 1994) or 130 m^2 (Weibul, 2009), which roughly equate to 1 m^2/kg body weight (Fröhlich et al., 2016), however they vary dependent on lung inflation (for example, 35m^2 on exhalation, and 100 m^2 inhalation) (Von Hayek, 1960). The lung lining (epithelial) layer is thinner and more permeable in the respiratory airways, compared to the thicker epithelium of the conducting airways, and is thus more suitable for inhaled drug absorption (Hastedt et al., 2022, Boger & Wigström, 2018).

1.2.1.3 Lung lining fluid volume and composition (adults)

Drug that is delivered to the lung must be dissolved in the lung lining fluid if it is to evade the mucocillary clearance mechanisms in the upper respiratory tract or by phagocytosis from macrophages in the lower respiratory tract and take therapeutic effect (Radivojev et al., 2021, Whitsett & Weaver, 2002). Although the lung lining fluid volume and composition varies according to disease state and lung region, the volume of fluid is expected to be between 10–70 mL in adults and the layer thickness of fluid is ~0.5 µm in the alveoli and 3 µm in the peripheral

conducting airways (Figure 1.2.1.3, Hastedt et al., 2022). A mucous layer of 8 µm thickness is present in the upper airways (Figure 1.2.1.3, Hastedt et al., 2022). Historically, the composition of lung lining fluid has been simulated for testing of inhaled drugs. Simulated fluids include base solutions such as Gambles solution, Hanks balanced solution, or pure phosphate buffered saline, to which pulmonary phospholipids are added to increase solubility of poorly soluble drugs (Radivojev et al., 2021). More recently, the respiratory tract lining fluid has been characterised and contains; lipids – dipalmitoylphosphatidylcholine (DPPC), dipalmitoylphosphatidylglycerol (DPPG) and cholesterol; proteins - albumin, immunoglobulins, and transferrin; and antioxidants - ascorbate, glutathione and urate (Bicer, 2015). The microstructure of a particle (e.g., shape, porosity, pore size, agglomerative potential) contributes to its ability to be dissolved in the lung lining fluid, known as its dissolution rate (Hochhaus et al., 2021, Mohan et al., 2022).

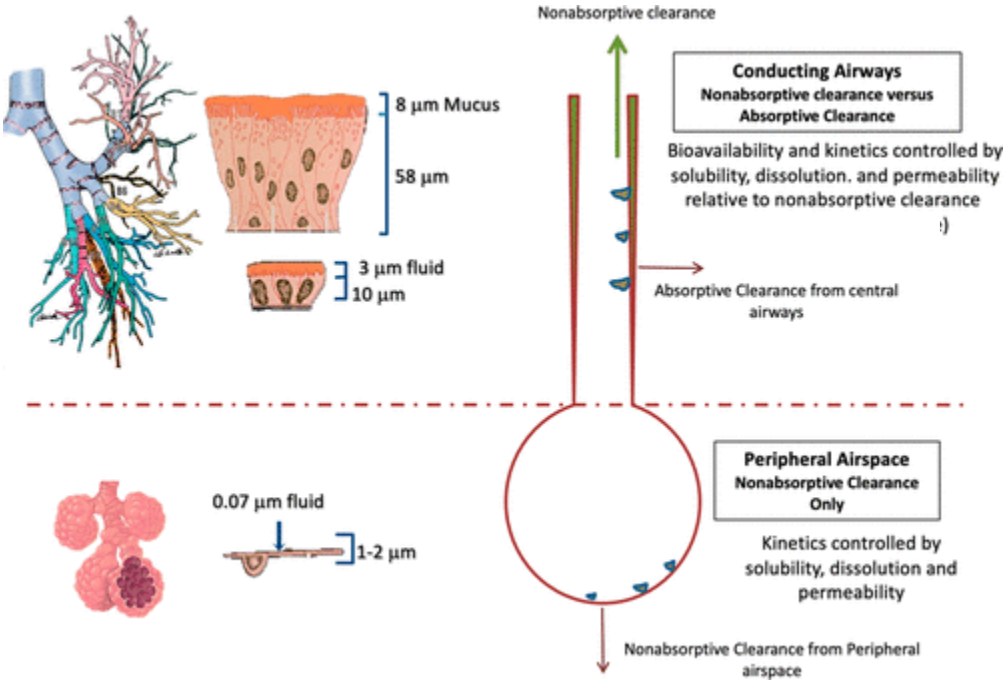


Figure 1.2.1.3. Lung lining fluid thickness in the conducting and peripheral/respiratory airways (top and bottom respectively). Reprinted From Hastedt et al., 2022, under creative commons licence.

1.2.1.4 Vascularization

To facilitate gas exchange, the lung is highly vascularised, and the alveoli represents a very large surface area proximal to a blood interface, ~ 70–100 m^2. The lungs are supplied with two separate blood supplies/vascular systems – the bronchial and the pulmonary arteries (Walker et al., 2015). The bronchial arteries arise directly from the aorta and supply blood to the lung tissue of the upper airway (tracheobronchial region): trachea, bronchi, and bronchial branches (Walker et al., 2015). The pulmonary artery provides blood supply for oxygenation (Betts et al., 2013). The bronchial circulation is also part of the systemic circulation (Betts et al., 2013).

The proximity of the submucosal bronchial venous system to the airway lumen allows ready access to the systemic circulation, and bypasses first-pass metabolism (McCullagh et al., 2010). Theoretically, once in the systemic circulation there may be redistribution to the lungs.

1.2.2 Function overview

Broadly speaking, the lungs' function is to contribute towards maintaining body homeostasis (e.g. blood gas composition and pH) by warming, humidifying, and filtering, (through mucocillary clearance and other cellular mechanisms) the air we breathe for microorganisms/potential pathogens/toxins (Al Ashry & Modrykamien, 2014). However, the primary function of the airways is extracting oxygen from the air to oxygenate blood and to excrete carbon dioxide. Secondary roles the lung performs that will not be discussed here include a range of immune and endocrine functions. This section will only give a brief overview of healthy lung function pertinent to its primary function. A detailed explanation of the structural and functional changes of each disease condition is outside the scope of this thesis. Although one day a personalized approach to drug delivery may be possible based on lung ventilation and perfusion in disease conditions, this option is still under investigation.

1.2.2.1 Motion of breathing

Normal breathing or gas exchange is initiated by the change of pulmonary pressure and is explained by Boyles Law (Betts et al., 2013). Boyles law is $P_1V_1 = P_2V_2$ where P represents pressure and V represents volume, and states that at a constant temperature the pressure of a system is inversely related to the volume of the system i.e., when volume increases pressure decreases (Betts et al., 2013). For example, when the ribcage, diaphragm and parietal pleura attached to the diaphragm move, the volume inside the lung increases (~20%), and the intrapleural pressure decreases, as does alveolar pressure, decreasing the intrapulmonary pressure by 1–2 mmHg and forcing the lungs to expand and move air inward (Betts et al., 2013 and Brinkman, 2023). Expiration is passive, owing to relaxation of the diaphragm and the lungs' elastic properties, and ideally these motions must be simulated in laboratory and computational studies, however it is not currently advised as best-practice (Brinkman, 2023, Mead-Hunter 2013, P. Koullapis et al., 2018a).

1.2.3 Nervous system (innervation) of the upper airway (airway smooth muscle) overview

The respiratory nervous system serves as the gateway between the structure and function of the lung. Innervation of the lung is via the vagus nerve, which is the 10^{th} cranial nerve and provides innervation to the neck, chest and abdomen and controls respiration (ICRP, 1994). Although evidence supports a primary role for airway nervous system (autonomic) dysfunction in airway disease, particularly asthma (Canning et al., 2012), and chronic cough or vagal afferent (neuronal) hypersensitivity (Chung et al., 2022), it is outside the scope of this thesis to discuss.

1.3 Disease conditions treated with orally inhaled medicines that exert local effects in the lung

According to the Global Burden of Diseases study, chronic respiratory diseases (including asthma and chronic obstructive pulmonary disease (COPD)) were the third leading cause of death worldwide in 2015, responsible for ~4 million deaths (Wang et al., 2016). Lower respiratory tract infections are the leading cause of death in developing nations and children are particularly affected (Ding et al., 2021). Common respiratory conditions treated with regulatory approved orally inhaled medicines include asthma, cystic fibrosis, and chronic obstructive pulmonary disease (COPD). Inhaled medicines used in these conditions are designed to treat or prevent the respiratory symptoms of the disease only rather than cure the underlying disease. Common inhaled medicines delivered to the lung to exert local (respiratory system) effects include; inhaled short or long acting beta 2 agonists (bronchodilators), inhaled short or long acting muscarinic antagonists (a class of bronchodilator), inhaled corticosteroids (ICS) (Figure 1.3), inhaled antibiotics and inhaled mucolytics.

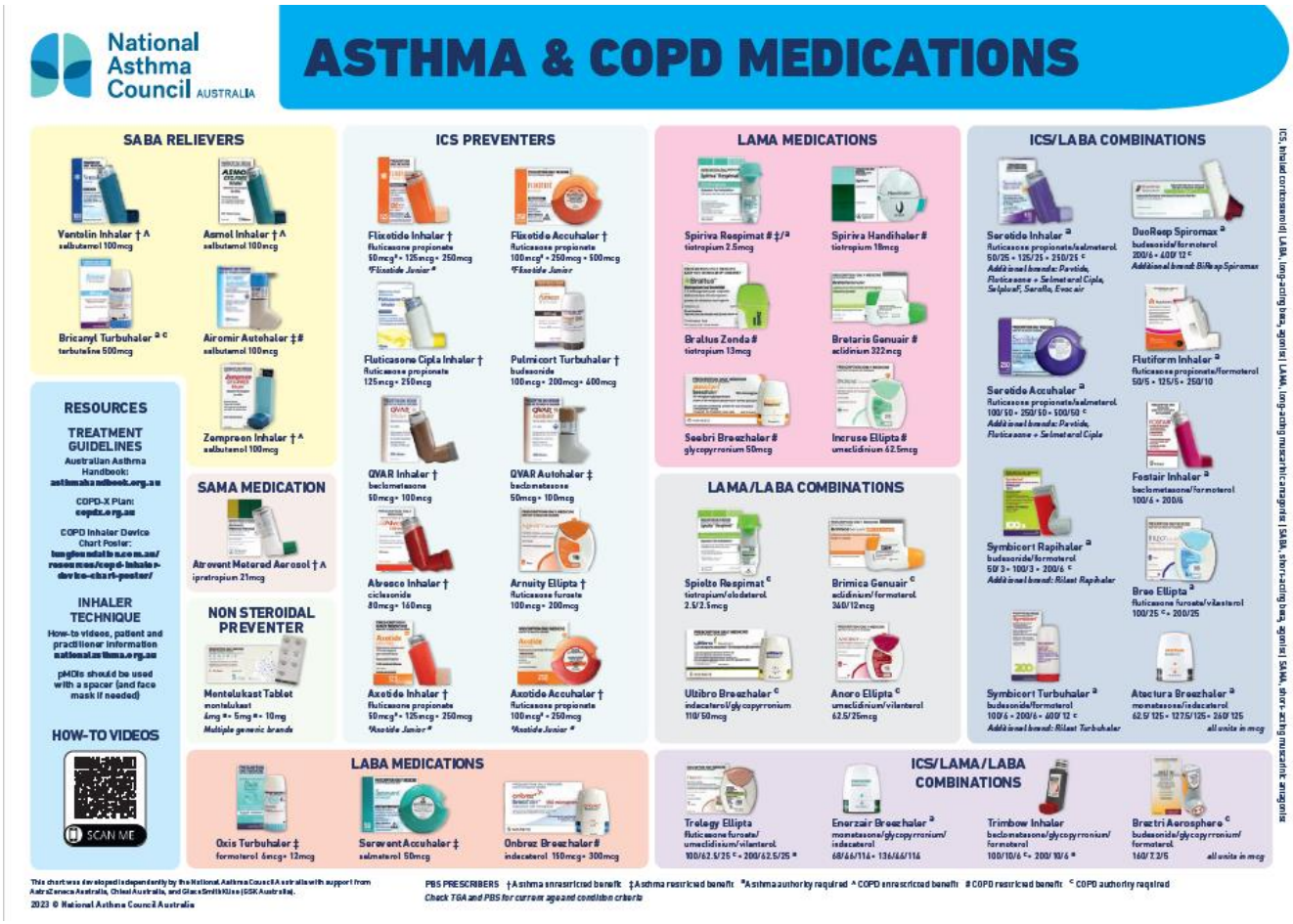


Figure 1.3 National Asthma Council of Australia medication chart for Asthma and Chronic Obstructive Pulmonary Disease. Retrieved (September 2023) from https://www.nationalasthma.org.au/living-with-asthma/resources/health-professionals/charts/asthma-copd-medications-chart

Inhaled medicines with local (respiratory) effects are those intended to be absorbed by the lung lining fluid of the bronchiolar region and stay in the fluid to exert therapeutic effect locally, as opposed to being delivered to the lower respiratory airways and absorbed straight into the systemic circulation. Formulation of locally acting medications is extremely important to extend the duration of action at the lung (Guo et al., 2021). Benefits of localized treatment of lung disease include; 1) minimized risk of side effects, as a fraction of the systemic dose can be delivered; 2) rapid clinical response, and; 3) bypass of first pass metabolism (Labiris & Dolovich, 2003).

Many respiratory diseases including asthma, COPD and cystic fibrosis are treated with inhaled medicines that have been approved as therapeutic goods. However, in some cases, respiratory conditions are treated with medicines that have not necessarily been approved for inhalation, otherwise known as "off-label treatment" (Schmiedl et al., 2014, Ding et al., 2021). For example, these off-label treatments can involve nebulizing an antibiotic solution intended for intravenous administration to treat airway bacterial infection, or the use of ICS and other inhaled medicines for COPD patients, where ICSs use was first approved for patients with asthma (not COPD) (Agustí, 2015). This section will introduce the most common respiratory conditions that are treated with inhaled medicines that act locally in the lung and include discussion of both approved and unapproved use of inhaled medicines for these conditions. Although there is off-label use of inhaled drugs (particularly antibiotics) in adults for conditions other than those mentioned here, (e.g. ventilator associated pneumonia or idiopathic pulmonary fibrosis) this thesis has focused on the common conditions that effect children.

1.3.1 Asthma (Inhaled bronchodilators and corticosteroids)

Worldwide, 334 million people have asthma, and it affects 14% of children globally (Sheffield, 2017). In Australia 10.7% of the population have asthma, with it being more common in females (Australian Bureau of Statistics, 2022). In 2019 ~40,000 people were hospitalized and there were ~440 deaths directly attributed to asthma in Australia (AIHW, 2020).

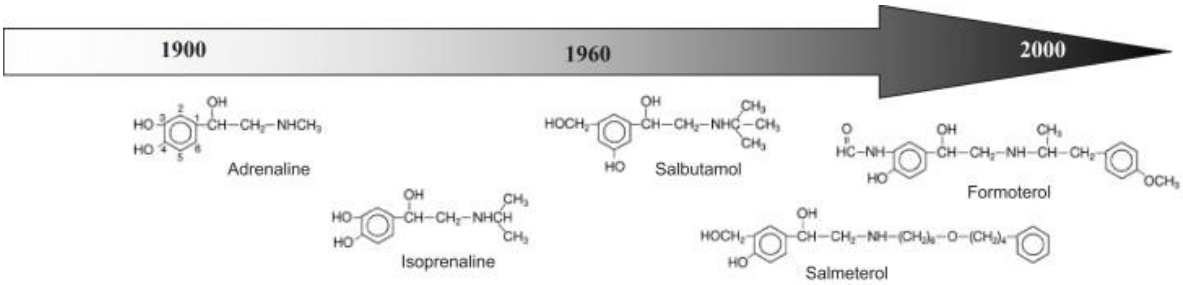
Except for patients with uncontrolled severe allergic (or severe eosinophilic) asthma, which can often be treated with an injectable monoclonal antibody, treatment for asthma typically involves targeting the conditions (or symptoms) of bronchoconstriction (bronchospasm) with inhaled bronchodilator drugs and airway inflammation with inhaled corticosteroid drugs (National Asthma Council, 2022, accessed 29th March 2023). Importantly, bronchospasm can also be experienced perioperatively in non-asthmatic patients and it occurs in roughly 1% of every 100 operations (Habre et al., 2017).

1.3.1.4 Bronchodilators

Bronchodilators are a class of drug that act on the airway (bronchial) smooth muscle to relieve (relax) bronchospasm or bronchoconstriction (Lemanske, 2023). Bronchodilators include beta-2-agonists and antimuscarinics, and can be short or long acting, with a fast or slow onset. For example, short-acting beta-agonists (SABAs) include salbutamol (albuterol) and terbutaline and long-acting beta-2 agonists include formoterol,

salmeterol and indacaterol (Figure 1.3.1.4). Short acting muscarinic antagonists (SAMAs) include ipratropium bromide, and oxitropium bromide and long acting antimuscarinics (LAMAs) include tiotropium bromide, aclidinium bromide, glycopyrronium bromide and umeclidinium bromide. The SABAs available at point-of-care only include adrenaline (epinephrine), which is an alpha- and beta-adrenergic receptor agonist.

Over time bronchodilators have changed from non-selective (i.e. target both alpha and beta receptors, (adrenaline)) to selective (target beta-2-agonist only), and longer acting formulations have been created (Figure 1.3.1.4, Diamant et al., 2007). Despite these advancements, the drug salbutamol, first identified in the 1960s, remains the most widely used bronchodilator and hence was the drug predominantly investigated in this thesis (Chapters 2 and 3) (Diamant et al.,2007). Bronchodilators are commonly delivered in the outpatient setting by either pressurised metered dose inhaler or dry powder inhaler, whereas in a hospital emergency department setting, delivery is either via pMDI or nebuliser depending on the severity of the emergency and managed in accordance with hospital protocols.



Name	Adrenaline	Isoprenaline	Salbutamol	Salmeterol	Formeterol
Specificity	α and β agonist	β_1 and β_2 agonist	Selective β_2 agonist	Selective β_2 agonist	Selective β_2 agonist
Mechanism of action	Fast onset and short duration	Fast onset and short duration	Fast onset and short duration	Slow onset and long duration	Fast onset and long duration
Preferred route of administration	Injected or nebulized	Sublingual or nebulized	MDI or nebulized	MDI or DPI	MDI or DPI
Side-effects	Cardiotoxicity Hypertension Tachycardia Tremor	Tachycardia Palpitations Tremor	Tremor	Tremor	Tremor

Figure 1.3.1.4. The progression of bronchodilator drug formulation over time. Reprinted from Diamant, Diderik Boot, & Christian Virchow, 2007, with permissions.

1.3.1.5 Inhaled corticosteroids

Inhaled corticosteroids act to relieve airway inflammation and must be used regularly to exert therapeutic effect (GINA, 2023). Inhaled corticosteroid drugs on the market in Australia approved for use with asthma include; fluticasone propionate, beclomethasone diproprionate, budesonide, ciclesonide and fluticasone furoate. They are not classified as short- or long-acting but do have different half-lives ranging from 0.1 hours (for beclomethasone

dipropionate) to 14.4 hours (fluticasone propionate) (Derendorf et al., 2006). The increased half-life is a result of pharmacological progress over time attempting to develop longer acting medications (Diamant et al., 2007).

1.3.2 Chronic Obstructive Pulmonary Disease (COPD) (Inhaled bronchodilators (antimuscarinics))

Worldwide, 200 million people have COPD, although, due to underdiagnosis this is expected to be much higher, and 3 million people die from COPD every year (Forum of International Respiratory Societies, 2021). COPD has major subtypes including pulmonary emphysema, chronic bronchiolitis, and chronic obstructive asthma (King Han, 2021) and each subtype may require a different approach with inhaled medicines. Increased (dysregulated) vagal cholinergic tone is the main reversible (treatable) element of airway obstruction in COPD (Heredia, 2009).

Inhaled medications for COPD are used to treat the conditions of bronchoconstriction (bronchospasm) with inhaled bronchodilators, increased airway (vagal cholinergic) tone with inhaled antimuscarinics, and sometimes bacterial infection with off-label inhaled antibiotics (Stass et al., 2013). The use of inhaled corticosteroids in COPD is of considerable debate and is only advisable as an add-on combination therapy treatment (i.e. ICS combined with long acting beta agonist) in patients (GOLD, 2022) who; 1) do not smoke – approximately a quarter of all patients with COPD, 2) respond to bronchodilators, and 3) have an eosinophilic subtype of COPD (Lamprecht et al., 2011). If not observing these considerations, there is an increased risk of airway conditions such as pneumonia due to the dampening of the immune system seen with high but not low-dose ICS (Lee et al., 2020), and of disease progression to cancer (Tantucci & Pini, 2020, GOLD, 2022). Inhaled hypertonic saline or mannitol is also under debate as a treatment for COPD (Mandru et al., 2021, Aaron, 2017), and may be recommended if symptoms are moderate to severe, however both treatments should be preceded by inhaled bronchodilators (Lemanske, 2023) as bronchospasm is a side effect of both therapies (Ralston et al., 2010).

1.3.3 Cystic Fibrosis (Inhaled antibiotics, inhaled mucolytics and airway surface restorers)

Approximately 3500 people in Australia have Cystic Fibrosis (CF), and it is the most common genetic condition worldwide in Caucasian populations, with progressive respiratory impairment the leading cause of morbidity and mortality (Ahern & Bell, 2020, Elborn, 2016, Scotet et al., 2020). The incidence of CF is estimated at between 1/3000 and 1/6000 live births in populations of European descent (Scotet et al., 2020). CF is characterized in the lungs by increased mucous retention (defective mucocillary clearance) and chronic infection (through failure to clear pathogens), both result in the dominant pathology of a pro-inflammatory airway which leads to airway damage, bronchiectasis, and small airways obstruction (Elborn, 2016). As it is a multi-system disease, CF is treated with a variety of inhaled and non-inhalation medications.

There are multiple types of inhaled treatment for patients with cystic fibrosis. These include inhaled bronchodilators (to open airways), inhaled corticosteroids (to reduce airway inflammation), inhaled hypertonic saline (to mobilize mucous), inhaled Dornase Alpha (Pulmozyme™) to thin mucous, inhaled antibiotics (to treat

bacterial infection), and inhaled mannitol for assisting sputum clearance (Anderson et al., 2022, Fodor et al., 2012).

1.3.3.4 Inhaled mucolytics and airway surface liquid restorers

Most inhaled mucolytics act to increase airway surface liquid which aids clearance of mucous from the airway and have only demonstrated efficacy (been approved for use) in patients with cystic fibrosis (Aaron, 2017). Three commonly used mucolytics include DNase I, hypertonic saline and mannitol, although DNase I is considered a mucous-changing drug as it does not act to increase airway surface liquid like the other mucolytics do (CFWA, 2017). DNase I and hypertonic saline are typically delivered with a nebuliser, however mannitol is formulated for delivery with a dry powder inhaler. Bronchodilators are required prior to use of inhaled mucolytics to ensure the nebulised solution reaches the parts of the lung which would ordinarily be hard to reach due to the presence of mucous (CFWA, 2017). There is some evidence that inhaled mucolytics are effective for patients with COPD (GOLD, 2022).

1.3.3.5 Inhaled antibiotics

Inhaled antibiotic medication is approved by all four key regulatory authorities, and primarily is used to treat chronic bacterial infection in patients with CF including infection with *Pseudomonas aeruginosa*, *Burkholderia cepacia*, or methicillin-resistant *Staphylococcus aureus* (Simon., 2023, Ding et al.,,2021). Patients with COPD, non-CF bronchiectasis and other nosocomial lung infections, such as ventilator-associated pneumonia, or TRacheostomy-Associated respiratory INfections (TRAINs) – including pneumonia and bacterial tracheitis – can also be treated with inhaled antibiotics, although use is off-label for all patients without CF (Ding et al., 2021, Simon., 2023).

Common inhaled antibiotics include: Aminoglycosides (tobramycin and amikacin), Fluoroquinolones (ciprofloxacin), Aztreonam, Colistin, and Vancomycin (Ding et al., 2021). Less common inhaled antibiotics or those still in development include; nitric oxide, antimicrobial peptides, Gallium-based agents and bacteriophage (Ding et al., 2021). Only antibiotic agents that are available in Australia and used currently to treat P. Aeruginosa airway infections will be discussed here; tobramycin, amikacin and colistin (Simon, 2023).

Antibiotics can treat planktonic (free-floating and non-communicative) bacteria or bacteria in dense biofilm "colony" formations which are communicative and adhered together with an extracellular polymeric substance, providing a protective physiology for the bacteria (Ding et al.,2021). However, the efficacy of antibiotics against bacteria in biofilm formation is relatively low, compared to free floating bacteria (Ding et al.,2021). Some ongoing challenges of inhaled therapy include designing medications with the ability to penetrate biofilms and penetrate areas of the lung which are poorly ventilated. Although under investigation, inhaled moxifloxacin and ciprofloxacin are not widely used (Chorepsima et al2018, Sethi et al., 2010) and not indicated or approved for use in Australia and therefore not discussed here.

Tobramycin and amikacin are the most common aminoglycosides used to treat aerobic, gram-negative bacteria-caused, respiratory infections (Krause et al., 2016). Tobramycin was the first inhaled antibiotic to be approved for the treatment of *P. aeruginosa* and thus has the greatest safety profile of all inhaled antibiotics (Simon., 2023). It has since been approved as a treatment for *B. cepacia* (Simon., 2023). Tobramycin is the preferred drug of choice to treat *P. aeruginosa,* although "cyclic" treatment, or cycling between tobramycin and another antibiotic is preferred to prevent antibiotic resistance (Simon., 2023). When the antibiotic binds to the ribosomal decoding (A) site in the 30 S ribosomal subunit of the prokaryotic (bacterial) ribosome (or ribosomal subunit) it inhibits protein synthesis, resulting in the production of non-functional proteins in bacteria (Krause et al., 2016).

Tobramycin and amikacin derive from the Kanamycin chemical family and are produced by the bacterial species *Streptomyces* spp. (Krause et al., 2016). Amikacin is indicated to treat *P. aeruginosa* in patients with CF but in Australia is only used if the patient cannot tolerate tobramycin or colistin. The antibiotic colistin (a polymyxin) is also used to treat P. Aeruginosa infection as an alternative to tobramycin.

1.4 Disease conditions treated with orally inhaled drug-products that exert systemic effects

Although the primary focus of inhaled medication is to target respiratory disease, the lung is also an attractive site for delivery of general (non-respiratory) drugs as it offers a non-invasive way to deliver medication, has a large surface area, and delivery to the lung bypasses the gastrointestinal tract (Cipolla, 2016). Since the application of recombinant DNA technologies to produce pharmaceuticals, and the introduction of human recombinant insulin, inhaled formulations have (since the 1990's) been increasingly considered to treat systemic conditions (Cipolla, 2016).

Orally inhaled drugs with systemic effects are those intended to be absorbed by the respiratory bronchioles and alveoli to exert a therapeutic effect on organs other than the lung after entering the systemic circulation. Drugs that usually have low bioavailability or can be excreted easily with first pass metabolism when administered orally benefit from this mode of delivery, assuming they can be formulated for aerosolised delivery (Fröhlich et al., 2016).

There are only three inhaled medicines with systemic effects that have been approved at all by any medicines regulatory agency worldwide (Ye, Ma, & Zhu, 2022) and these include; loxapine (as Adasuve®) to treat agitation associated with schizophrenia, insulin to treat diabetes (as Alfrezza®), and levadopa to treat Parkinson's disease motor deficits (as Inbrija®). The Voke or the e-Voke, a device that delivers nicotine aerosol (not vapour), was approved by the medical regulator in the United Kingdom in 2015 (Medical and healthcare products Regulatory Agency, 2015), but is no longer being marketed. Despite the theoretical superiority of inhaled medicine as a delivery route, in clinical trials, all inhaled drugs intended for systemic delivery have demonstrated side-effects and thus are contraindicated for patients with lung conditions (Ye et al., 2022). For example, all inhaled medicines used to treat conditions outside the lung (to exert systemic effects) approved in the United States have

demonstrated increased risk of cough and bronchospasm in clinical trials and thus are either contraindicated, or to be used after risk assessment, in patients with lung conditions (Ye et al., 2022). Only two of the three inhaled systemic drug-products (Inbrija® and Adasuve®) have been approved in the European Union, Alfrezza® was excluded as more significant side-effects were demonstrated (Oleck et al., 2016, Ye et al., 2022).

In Australia there are no systemically-acting orally-inhaled products currently listed on the Australia Register of Therapeutic Goods. However, the orally-inhaled nicotine delivery device – the electronic cigarette (or e-cigarette) – can be used and bought in Australia either without a nicotine containing e-liquid, or with a nicotine containing e-liquid if prescribed by a general practitioner under a special access scheme. Additionally, a type of orally inhaled drug that delivers nicotine called the Nicorette® Inhalator is approved in Australia, but it is absorbed primarily through the buccal (oral) cavity, not the lung..

Overall, there is a lack of development in this area and focus has been shifted to other biological pharmaceuticals, such as sustained release medications or transdermal patches, perhaps due to the huge initial failure of inhaled insulin (Oleck et al., 2016), and the difficulties with development of orally inhaled medicines that will be discussed further in Introduction 1.5.

Each non-respiratory condition is briefly discussed below to give an overview of the device type and treating indications. The electronic cigarette is discussed in more detail since it is an off-label use and the focus of Chapter 6 of this Thesis.

1.4.1 Diabetes (Inhaled insulin)

Inhaled insulin is a non-invasive option to the much more commonly used injectable insulin, however it is only available currently in the United States for adults under the brand name Alfrezza®. There are some concerns for the use of Alfrezza® use related to cancer development during clinical trials, possibly associated with insulin being a growth hormone (Oleck, Kassam, & Goldman, 2016). It is delivered with a dry powder inhaler device (Ye 2022) and is not recommended for use in patients with a history of lung disease, including asthma, COPD and lung cancer (Oleck et al., 2016). A previously marketed and failed therapeutic that delivered inhaled insulin is known by the brand name Exubera (Ye et al., 2022)

1.4.2 Schizophrenia/Psychosis (Inhaled loxapine)

Inhaled loxapine (also known as Staccato® Loxapine, the brand name Adasuve®) is an effective alternative to anti-psychotics delivered via intramuscular, intravenous, or oral dosing (de Berardis et al., 2017). Inhaled loxapine is delivered using the Staccato® inhalation system, which is a condensation-evaporation aerosol device; as it detects inhalation, it briefly heats a film of pure drug (no excipients) to 400ºC which is then inhaled (Dinh et al., 2011). It is contraindicated for patients with a history of lung disease due to the increased risk of bronchospasm (de Berardis et al., 2017).

1.4.3 Parkinson's disease (inhaled levodopa)

Levodopa is the gold-standard medication to treat Parkinson's disease motor deficits, however when taken orally, (by tablet) levodopa has a low bioavailability, and hence why the inhaled route was pursued (Ye et al., 2022). Licensed for use in the United States and European Union, the levodopa inhaler (also known by brand name Inbrija®) is a dry powder inhaler device (Ye et al., 2022). Inhaled levodopa is designed to be taken to ease "off period" or sudden symptom onset (Ye et al., 2022).

1.4.4 Nicotine dependence/cigarette addiction (Inhaled nicotine)

Oral inhalation of nicotine for delivery to the lung, and directly into the systemic blood circulation, is preferred over other forms of nicotine replacement currently on the market for patients who wish to quit smoking, as this mimics the nicotine "hit" that regular cigarettes offer (Cipolla & Gonda, 2015). However there is no product marketed as a therapeutic that delivers nicotine directly to the lungs yet – some products have been patented, trialed, and later failed to market, or develop, such as the AERx and the E-Voke (Voke®) (tobacco Tactics, AERx original patent number: US5823178A, Pecota, 2012). There are many non-inhalation types of nicotine replacement therapy that target delivery of nicotine to the oral cavity. For example, there are 91 different types of nicotine gums, mouth or nasal sprays, lozenges and patches registered on the Australian Register of Therapeutic Goods (Australian Government, 2023, keyword search nicotine, access date September 2023). Nasal delivery of nicotine does provide the immediate nicotine hit, but also irritates the nasal epithelium (Cipolla, 2016). Because most therapeutic nicotine replacement therapy currently on the market is not via inhaled delivery directly to the lung, meaning the nicotine "hit" takes longer to come about (Cipolla, 2016), patients are likely to seek alternate devices that do offer the nicotine hit, such as the e-cigarette, which also still provides the desirable hand-to-mouth action. The e-cigarette is an unapproved therapeutic product, for which nicotine may be prescribed by a medical practitioner in some jurisdictions (including Australia), and it does deliver nicotine directly to the lungs.

1.4.4.1 The electronic cigarette (e-cigarette)

The e-cigarette is a condensation-evaporation aerosol generation device, that heats a coil wrapped around a wetted wick (wetted with "e-liquid") to produce aerosol nuclei which then form a condensate (liquid aerosol). The liquids commonly used with e-cigarettes (called e-liquids) contain primarily excipients glycerin and propylene glycol, to which flavours, nicotine and other chemicals can be added, the most common flavours being tobacco, fruit, and menthol (Chaffee et al., 2022, Jongenelis, 2023). There are four "generations" of e-cigarette devices (Banks et al., 2023), which vary in many ways, although their main function is always to heat and aerosolize an e-liquid for inhalation. First-generation e-cigarette devices as we know them today, were first patented in 2003, however were in development as early as 1963 (Dutra et al., 2017). As described by the 2003 patent, e-cigarettes are intended to be used as a smoking cessation aid (Hon, 2010). There is moderate-certainty evidence to support their use as a smoking cessation aid compared to regular forms of nicotine replacement, but nicotine addiction remains an issue even after smoking (cigarette) cessation (Hartmann-Boyce et al., 2021). In Australia, nicotine is a prescription

medicine (listed as a schedule 4 drug) and can be prescribed by a general practitioner for use in an e-cigarette device, for the purpose of smoking cessation (Australian Government, 2021). However, it is recommended only when the patient has tried and failed other approved modalities for cessation or as a "second line" treatment (RACGP, 2021 Australian Government, 2021).

Extensive banning of ingredients in e-liquids can be found in guidelines and laws in the UK, Germany and France however they are difficult to enforce (European parliament, 2014). A lack of enforced regulation means that e-liquids can contain contaminants (Larcombe, 2021), and in many jurisdictions manufacturers are not required to list ingredients in a manner that is satisfactory to consumer standards. For example, the European Association for the Co-ordination of Consumers Representation in Standardisation (ANEC, 2021) released a position paper proposing that limits on chemicals in e-cigarette aerosols be derived from occupational exposure limits or indoor air quality, because adequate regulation was lacking to protect consumers (ANEC, 2021).

Some component ingredients in e-liquids were banned in Australia in October 2021, however there will be complete bans on importation of non-prescription electronic cigarettes brought into effect when Australia introduces the proposed regulatory reforms (Media Release, The Hon Mark Butler MP, 2023). The ban in Australia arose predominantly because young adults and teenagers are taking-up e-cigarettes at alarming rates, causing significant issues in schools with e-cigarette use a known gateway to smoking cigarettes (Banks et al., 2023). Importantly, fatalities have been reported due to e-cigarette or vaping use-associated lung injury (EVALI) when using tetrahydrocannabinol or vitamin E acetate with e-cigarette devices, and due to nicotine poisoning (Banks et al., 2023), demonstrating there will continue to be a risk to health of use. E-cigarettes remain a concern to health agencies worldwide as the main components of the inhaled aerosol, and the added flavours, are known to degrade into chemicals that can be carcinogens, mutagens, or teratogens (National Academies of Sciences, 2018). Particularly, compounds found in e-liquids that are potentially genotoxic, include (but are far from limited to) cinnamaldehyde compounds, diacetyl, acetone and benzaldehyde however, not all these compounds are on banned ingredient lists (Australian Government, Health Products Regulation Group, 2021).

1.5 Development of orally inhaled drugs and drug-products

Despite the known benefits of delivering certain medications directly to the lungs, inhaler device design has changed little over the last 60 years, and in particular devices that deliver inhaled medicines for asthma have evolved minimally since their first introduction. Changes in evolution of orally-inhaled medicines have been spurred mostly by necessity due to the Montreal Protocol in 1987 banning chlorofluorocarbons (the propellant in pMDIs at the time), and the resulting pharmaceutical competition (Stein & Thiel, 2017). Orally-inhaled medicines have many unique challenges which make their development more complex than other (non-inhaled) drugs, such as batch-batch variability, drug-device combination, patient device use variability and non-standard analytical methods (Newman, 2023). For these reasons they require larger clinical trial cohorts than other drug types and

can therefore take longer, cost more and be less likely to succeed/progress to market, compared with other medications. For these reasons model informed drug development (use of supportive *in silico* or *in vitro* studies) is particularly beneficial to reduce the clinical trial costs and increase their likelihood of success.

1.5.1 Standard testing for orally inhaled drug products

Traditionally, for a new-chemical entity (drug product) to proceed to first-in-human trials, it must first be tested for sub-chronic (18-26 weeks) or chronic (36 weeks) exposure safety testing in two non-human species, to account for species differences, and this is commonly a rodent and a non-rodent (Prior et al., 2020). However, an international review panel has re-assessed this requirement for two species, with the intention to reduce it to a single species, considering technological advances in both *in vitro* and *in silico* methods (Prior et al., 2020). These technological improvements include quantitative structure activity relationship (QSAR) analysis and pharmaco-kinetic and dynamic *in silico* approaches such as physiologically based pharmaco-kinetics (PBPK), both modalities having gained acceptance since animal-testing for cosmetics was banned (Gellatley, 2019). These improvements in technology have led to the ability to improve assessment of an orally-inhaled medicine, which is traditionally more complex than systemically-acting drugs, since the delivery mechanism can also determine the dose.

1.5.2 The Inhaled Bioequivalence Classification Scheme

For drugs that are absorbed by the gastrointestinal system, bio-simulation approaches such as PBPK are much more developed than drugs absorbed in the lungs, due to the bio-pharmaceutics classification scheme for the gastrointestinal system (giBCS) being first described in 1995 (Amidon et al., 1995). The giBSC is based on a thorough understanding of a drug-molecules' parameters (such as solubility and permeability) that contribute to absorption, distribution, metabolism and excretion by the body (ADME) and can thus be used to estimate *in vitro-in vivo* (or *in silico*) correlations (or *in vitro-in vivo* equivalence, (IVIVE)) and therefore support the claim for bio-equivalence of a generic molecule to its brand-name counterpart, during regulatory application (Hastedt et al., 2022). An inhalation biopharmaceutical classification scheme (iBSC), similar to the giBSC has been in development for many years culminating in an international workshop in 2015, aimed at helping to provide support to de-risk the development of inhaled drugs and drug products (Bäckman et al., 2022). Publication of Part 1 of the Principles and Framework for the iBCS occurred in May 2022 (Hastedt et al., 2022) and part 2 in July 2022 (Bäckman et al., 2022). Fundamentally, the iBCS must correlate key *in vitro* attributes of a drug or drug product to a development risk or a clinical risk (Bäckman et al., 2022).

1.5.3 Limitations of orally inhaled product development and future directions

Globally, there is a 70% failure rate for orally inhaled drug products which include both device only and drug-device combination products (Barnes et al., 2015). Importantly, both are largely tested in healthy adults in accordance with pharmacopeia (Barnes et al., 2015, Movia & Prina-Mello, 2020). Part of the failure has been the

ability to assess the dose received to the lung after delivery of a set-dose by the device, as the device can be used variably by the patient, resulting in dose variability. For example, there are many patient factors that can reduce the dose to the therapeutic site of the lung, including (but not limited to) variable inhalation rate and lung volume, presence of lung disease, angle of the inhaler, and timing of the actuation and inhalation (Darquenne et al., 2016). This in turn affects the absorption, distribution, metabolism, and elimination of the drug from the body, and the corresponding safety and efficacy profile of the drug. Therefore, it is essential to understand lung dosimetry of an orally inhaled product to improve therapeutic efficacy, particularly for products that are used off-label as a result of the slow drug approval process. The series of studies presented in this thesis help to improve our understanding of lung dosimetry of important orally inhaled medications.

1.5.4 Limits of drug development and orally inhaled drug product development for children

On top of the nine years it takes on average to produce a drug for adults, an additional seven to eight years are required to have that same product approved for use in children (Bi et al., 2019, Mehrotra et al., 2016). For inhaled medicines it is clearly stated in guidance that adult studies cannot be extrapolated to children (European Medicines Agency Committee for Medicinal Products for Human Use, 2009). Considering the difficulty in improving delivery systems for adults, it follows that pediatric improvements to aerosolised drug delivery, are even less common (Yackey et al., 2019, Roberts et al., 2003). Clinical trials and drug development specifically for children are increasingly important as chronic disease is becoming more prevalent in children but in the past clinical trials in children were complex and not profitable (Zimmerman et al., 2019, and Roberts et al., 2003). This means that devices are designed and tested in adults and minimally (if at all) adapted or safety-profiled for use in children, and that pediatricians must use many medications on a trial-and-error basis or that children miss out on potentially lifesaving medication if there is reluctance to use off-label prescribing (Roberts et al., 2003). Additionally, the gap between drug approval in adults and the time it takes to get the same drug approved in children leaves much time to develop off-label prescribing habits (Benning et al., 2021). Regulatory hurdles have been addressed in the US with the Best Pharmaceuticals for Children Act (BPCA) (2002) and the Pediatric Research Equity Act (PREA) (2003), similarly in the EU regulatory guidelines have been revised to ensure that pre-clinical animal studies are fit-for-purpose (ICH, EMA, 2020). As a result of the described difficulties, adaptations to adult medicines are often made at a clinical level to address unmet pediatric needs and then tested for evidence base in hindsight to allow the translation into clinical practice (Anderson et al., 2020).

1.5.5 Future Directions

Development guidelines by the European Medicines Agency (EMA) outline that particle size testing and radiolabeled lung deposition studies should be conducted when suitable to determine aerosol dosimetry, however these guidelines were last updated in 2009 and have been under review since 2015, when the medical devices regulation changed, and orally inhaled drug products (inhalation devices) were implicated (Santos et al., 2018).

Considering the EMA guidelines for PBPK reporting were updated last in 2018, it is likely that review will include consideration of advances in technologies to assess inhaled bio-equivalence.

The issue of unknown dosing in the development of orally inhaled drug products has been partially overcome with technological advances over the past decade – although *in vitro* and *in silico* "replacements" to traditional animal testing requirements are scientifically valid, they can be slow-to-uptake and subject to status-quo bias (Wilkonson, 2019). In vitro method advancement includes development of an apparatus that allows direct estimation of regional lung deposition by metal grid filters (Finlay et al., 2022). Advances include combining computational fluid dynamics models with physiologically based pharmacokinetic models or functional imaging (Usmani et al., 2021, Corley et al., 2021), improved imaging methodologies such as functional imaging technologies (Ohno et al., 2021), and advanced human-based *in vitro* systems, including micro-physiological systems such as "Lung-on-Chip" technologies (Huh, 2015). However, there are few successful regulatory applications using *in vitro* or *in silico* data in lieu of animal data in the USA and European Union (Corley et al., 2021, Ramanarayanan et al., 2022). Ongoing set-backs to orally inhaled drug and device development can broadly be attributed to the lack of an inhaled Biopharmaceutical Classification scheme, released in concept (and part) during 2022 (Hastedt et al., 2022, Bäckman et al., 2022) combined with the increased scrutiny of non-animal methods (Gallegos-Catalán et al., 2021).

1.6 Computer modelling and simulation and its use and relevance in orally inhaled medicine development

The inherent ethical and logistical difficulties in estimating lung dose with the current gold standard clinical study (radiolabeled lung deposition) has resulted in increased interest in the use of computational methods to study aerosol lung deposition as part of the drug development pipeline (Longest et al., 2019). Additionally, species differences in airway geometry means limited relevance of animal studies for this purpose (Movia & Prina-Mello, 2020), which has accelerated the interest in building a virtual human twin, based on theoretical (mathematical and statistical) understanding of the function of the human body (or lung in this case). In fact, various types of computer modeling and simulation (CM&S) are now used in orally inhaled medicine development, usually to assess either efficacy or safety at various stages of product development;

1) Preclinical: the potential (pre-screening) efficacy and safety of the drug molecule/new chemical entity, e.g. Quantitative Structure Activity Relationship (QSAR) (European Union Science Hub, 2023),

2) Preclinical: the safety and efficacy of the aerosol delivery device: its mechanistic ability to deliver specified dose of medication consistently and without failure for the complete content packaged, e.g. CFD/CFPD (Longest & Holbrook, 2012),

3) Clinical or Preclinical: the therapeutic efficacy, and safety, of the drug once the dose is administered to the body (the pharmacokinetics, primarily: adsorption, distribution, metabolism and excretion), e.g. PBPK and (European Union Science Hub 2023),

4) Clinical: the efficacy of delivery of the drug to the lung by the device when administered by a user according to manufacturer instructions (aerosol lung deposition), e.g. CFD/CFPD.

The types of computational models for each purpose above are shown in Table 1.6. however, the focus of this thesis is on models to predict aerosol lung deposition (point 4 above), rather than early phase (pre-pre-clinical) development of the device product or drug on its own or effects downstream of lung deposition such as pharmacokinetic models (PBPK).

When CM&S tools, such as CFD (Table 1.6), are used for the development of pharmaceutical products, the approach is called Model Informed Drug Development (MIDD) (Musuamba et al., 2021, Jean 2021) or Integrated Approaches to Testing and Assessment (European Union Science Hub, 2023b). The overall aim of MIDD is to gain a better mechanistic understanding of a drugs pharmacology, likewise IATA takes a flexible approach for chemical safety assessment based on the integration and translation of the data derived from multiple methods and sources (Jean 2021, European Union Science Hub, 2023b). The MIDD approach is particularly useful for certain applications, for example; 1) to attribute *in vitro* attributes of a drug or drug product to a development risk or a clinical risk, 2) to establish starting doses for human clinical trials or 3) to support use of medicines for children that have been approved in adults (Bi et al., 2019). The use of alternative (*in silico* and *in vitro*) approaches are increasingly being used and accepted by the US FDA to demonstrate bioequivalence of orally inhaled medicines. These approaches include using computational fluid dynamics to determine both aerosol lung deposition (regional and total), and spray velocity and evaporation rates, when they are combined with other *in vitro* tests, such as dissolution studies, *in vitro* aerosol particle sizing studies using realistic mouth-throat models, physiologically based pharmacokinetics and aerosol morphology studies (Table 1.6).

Table 1.6. Purposes for use of computational models: 1) the potential (pre-screening) efficacy and safety of a drug molecule/new chemical entity (perhaps through demonstrating bioequivalence to an already marketed product, based on physical or chemical attributes), 2) the safety and efficacy of the device: its mechanistic ability to deliver specified dose of medication consistently and without failure for the complete content packaged, 3) the therapeutic efficacy, and safety, of the drug once the dose is administered to the body (the pharmacokinetics, primarily: adsorption, distribution, metabolism and excretion), and 4) the efficacy of delivery of the drug to the lung by the device when administered by a user according to manufacturer instructions (aerosol lung dosimetry). Pichelstorfer et al., 2016.

Method/model name	**Purpose used for**	**Basis for Computational modelling tool**	**Example of Open Source software available**	**Outcome measure**	**Guidelines for best practice available from**
Physiologically based Pharmacokinetics (PBPK)	3	Mathematical equations	Applied Research Associates – MPPD (Price et al., 2002)	Absorption distribution, metabolism, and excretion of drug from the body.	European Medicines Agency US Food and Drug Administration International Committee for Radiological Protection.
Inhaled Biopharmaceutical classification system	2	Mathematical equations, based on known parameters such as surface area of the lung and volume of lung lining fluid		Residence time in the lung, absorption distribution, metabolism, and excretion	Hastedt et al., 2022
Computational Fluid particle dynamics (CFPD)	2,3,4	Maths and physics: equations of motion for fluid and particle flow, averaged over a discretized (finite) volume	OpenFOAM	Aerosol mouth throat and lung dosimetry, total and regional, plume characteristics: spray velocity, evaporation rate.	European Research Community on Flow Turbulence and Combustion, Koullapsis, 2018 et al. (a, b)
Multiple-particle path dosimetry	3	Was stochastic but is now increasingly quantitative	Applied Research	Aerosol Lung Dosimetry, total deposition, (less accurately – regional	Asgharian et al., 2022, Kuprat et al., 2023, Corley et al., 2021.

			Associates – MPPD (Price et al., 2002)	deposition unless incorporated improvements to alveolar spaces)	
Combined PBPK and CFPD	3 & 4	Maths and physics	OpenFOAM	Aerosol Lung Dosimetry, Adsorption distribution, metabolism and excretion of drug from the body	none
Quantitative Structure activity relationship (QSAR)	1	Database, artificial intelligence, statistical, chemical structure	StopTOX	Human toxicity endpoints relevant to the lung include: inhalation toxicity, respiratory sentisation, neurotoxicity,	Borba et al., 2022

Computational models for estimating aerosol lung deposition vary in complexity, however all are based on the premise of building the model to the simplest level of complexity required to validate the model within a context of use. The most widely used computational models to provide aerosol dosimetry estimates that are open source (freely available) include, computational fluid dynamics models using OpenFOAM, and multiple-path particle dosimetry (MPPD), Applied Research Associates (Price et al., 2002). The MPPD model is considered to be an upgrade of the IRCP single-path particle dosimetry model (1994) discussed earlier. While not open source, recently, the PBPK/dosimetric combined model software Mimetikos Preludium has been validated with *in vivo* imaging data, in accordance with the principles and framework of the inhalation biopharmaceutical classification system by Olsson et al, 2021. All computational models require a highly accurate model of the human lung as the processing domain which was discussed previously in section 1.2.4. As computational power has increased and imaging resolution has improved, more complex models of aerosol deposition have evolved and some are available to create and download (United States Environmental Protection Agency, 2023).

1.6.1 Introduction and history of human respiratory tract models

The complex computational models of the respiratory system as we know them today have their origins in the first "Human Respiratory Tract Model (HRTM) for Radiological Protection", (aerosol dosimetric mathematical model) which began its development during the period of 1949 to 1953 by the International Commission for Radiological Protection (ICRP 1994). The model simply included the mathematical calculation of the deposition, clearance, and retention of radioactive inhaled aerosols (including gases) (ICRP 1994). The need for a model was primarily to protect radiation workers but was also of benefit to estimate the inhaled doses for the public, for example due to high indoor radon levels (common with indoor heating/good window seals). As more information became available, the first HRTM, published in 1960 (ICRP publication 2), was revised and updated in 1966, 1979 (ICRP publication 30), and then again in 1994 (ICRP publication 66). Most recently, an updated human respiratory tract model with new information regarding major clearance pathways, particle transport and absorption to blood (Bailey et al., 2007) was included in ICRP publication 130, "Occupational Intakes for Radionuclides" (ICRP, 2015). A similar, but more extensive model (more generations of airway) was developed by the National Council for Radiological Protection in 1997 (Fröhlich et al., 2016).

The ICRP dosimetric model of the human respiratory tract includes six key elements; 1) morphometry (e.g., structure) of the lung, 2) respiratory physiology (e.g., rates and volumes of air intake), 3) radiation biology, 4) deposition of aerosols, 5) clearance of aerosols, and 6) dosimetry to all organs, of the radioactive energy absorbed. The dosimetric model uses mathematical equations to calculate aerosol dosimetry based on known lung morphometry, physiology, and radiation biology. Today computation is used to calculate dosimetry estimates and the HRTM has therefore become a "computational model".

1.6.1.1 Models of the human respiratory tract/human lung: Empirical, deterministic, and stochastic

The accuracy of aerosol lung deposition predictions relies on the accuracy of the lung model available, and these are limited as full reconstructions of the lung down to the alveolar (based on morphometric measurement, usually cadavers) are not possible with computed tomography (CT) or High-Resolution CT (HRCT) imaging (Asgharian et al., 2001, Montesantos et al., 2016). Therefore, a combined approach is generally taken where the upper airways and several generations that are imageable are used (if imaging is available) and a model is employed for the lower half/lower generations (Montesantos et al., 2016). If imaging was not conducted, then previously measured morphometries would be used (e.g. measurements taken from cadavers with histological methods). One of the most extensively used and cited morphological descriptions of the lung which many models are based on is that of Weibel (Weibel, 1963). Models of the lung used to study aerosol dosimetry over time have included both deterministic (non-random) and stochastic (random) approaches. If there is a high level of variability within species for a part of the lung, a stochastic approach is more suitable (Asgharian et al., 2001). An example of a deterministic (experimentally obtained) approach for modelling the lung is the conducting airways being selected from morphometric measurements in rats, and an eight-generation dichotomous branching symmetric acinar region (model) being attached to the terminal airways (Asgharian et al., 2001). This approach is useful for/employed when wanting to determine total aerosol dose, as opposed to regional dose (Asgharian et al., 2001). An example of a stochastic model is that the first few upper generations were selected based on previous measurements (Raabe et al., 1976) and the remaining airways in both conducting and pulmonary regions were reconstructed from frequency distributions of airways lengths, diameters, and branching angles based on morphometric measurements (Montesantos et al., 2016).

1.6.1.2 Single and Multiple path symmetrical (typical) and asymmetrical (stochastic) models of the lung

It is believed that the first mathematical model for deposition of inhaled particles was reported in 1935, which was considered a “single path [symmetrical or typical] model” (Wang, 2011). Single typical path models mean that the airway is represented by a single series of compartments where each generation of airway is assumed to have the same value for both diameter, length, and bifurcation angle (Wang, 2011). A five-lobe symmetrical model was introduced in 1980 (Yeh & Schum, 1980) and a five-lobe symmetrical model with additional tracheobronchial tree and an alveolar region in 2001 (Wang, 2011, Ashgarian et al., 2001). These models are also known as multiple-path [symmetrical] models or multiple path particle dosimetry (MPPD) models and both single path and multiple path models are still considered “typical-paths” as they do not involve randomness and are symmetrical. An asymmetric model that expanded on previous typical path models was later developed in 1988 (Wang, 2011, Koblinger & Hofmann, 1990). The multiple-path [asymmetrical] model of the lung, was different to the typical [symmetric or single path] path models, as it employed statistically distributed airway dimensions, i.e. where the diameter, length, and branching angle of airways vary within each airway generation (Wang, 2011). Single-path models are rarely used nowadays since the more accurate multiple-path models became available and since there are continuing improvements in technologies, even multiple-path models are now considered “1D

models", even those that extend to the alveolar region and use coordinates for each particle location (Longest & Holbrook, 2012).

1.6.1.2 MPPD Stochastic ("1D") versus CFPD 3D models

In silico models have been recently summarised and include two main categories: stochastic (or semi-empirical like previous MPPD models) or computational fluid dynamics models (CFD/CFPD) (Sperry et al., 2023). CFPD models use the laws of physics and mathematics to calculate aerosol deposition, rather than estimations based on stochastic/semi-empirical "1D", or Euler-Euler models with stochastic models (Sperry et al., 2023) being less accurate but also less computationally intensive. Stochastic models include the Aerosol Dynamics in confinement model, with the Inhalation, Deposition and Exhalation of Aerosols in/from the Lung-(IDEAL) model, and MPPD models depending on any quantitative upgrades (Asgharian et al., 2022, Kuprat et al., 2023, Koblinger & Hofmann, 1990). CFD/CFPD models can be either Euler-Euler models or Euler-Lagrangian models, with Euler-Euler models being less computationally intense and also less accurate. CFPD models are able to more accurately predict the transport of particles and any phase changes (gas to liquid etc) e.g. mass transfer between the liquid and vapour, which is important as phase change plays an important role in aerosol physics (and influences particle size) in the humidified environment of the lung. CFPD models are more likely to accurately reflect *in vivo* imaging data or studies as they are able to consider the complicated physics in the mouth throat region and the aerosol plume geometry. However, 1D models have been validated with imaging 2D data, and then extrapolated to 3D, such as with Mimetikos Preludium (Olsson, 2021). Since CFD and CFPD models provide the most physiologically accurate representation, they will be discussed in detail further.

1.6.2 Introduction to models of aerosol flow and aerosol lung deposition

The concept of investigating lung deposition of inspired particles in the respiratory tract dates back as early as 1870 when the first scientific observation of pulmonary deposition was reported by John Tyndall (Wang, 2011, Heyder et al.,1986). The deposition fraction is dependent on which type of morphometric model is used for the lungs. Morphometric models of the lungs began as a "single typical path" symmetrical model. They then developed in future years in attempts to more realistically model the human lung with upgrades: 1) to a five lobar model, and then 2) to an expanded five lobar model – a five lobar model with a tracheobronchial and alveolar region. Additional improvements included calculating the mean of several different variations of the expanded five lobar model or having different variations of sizes of airway diameters and lengths within each airway generation for the same model. Introducing variation into airway diameters, length and bifurcations more accurately represents human lung geometry. Each is discussed in more detail in 1.6.1.1.1.

Empirical lung deposition data have been obtained since the 1940s, but detailed studies began in the early 1970s and were used to support development of mathematical models with models being called semi-empirical (Wang, 2011). Between 1973–1985 early empirical lung deposition studies in humans involved inhaling either: 1) di-2-ethylhexyl sebacate aerosols produced by heterogeneous condensation (particle diameter larger than 0.08 μm), 2)

iron oxide aerosols produced by dispersion of suspensions (particle diameter larger than 0.5 µm), or 3) silver aerosols produced by homogeneous condensation and subsequently 'monodispersed" with an electrical mobility device (particle diameter smaller than 0.1 µm) (Heyder et al., 1986). To measure deposition with imaging, either radiotracer techniques (radiolabeling) or magneto-pneumography was used, where it was recognised that radiolabeling methods were more reliable (Heyder et al., 1986).

1.6.2.1 Computational software or tools to calculate deposition fractions

A software program based on a refined version of the multiple-path deposition model has been developed (Price et al., 2002). The related online Respiratory Deposition Calculator of the Aerosol Research Laboratory of Alberta provides a convenient method to calculate the whole-lung deposition of pharmaceutical aerosols. The multiple path particle deposition model (MPPD) (Price et al., 2002) is a whole-lung modelling tool that can provide estimates of delivery to individual airway branches (Longest & Holbrook, 2012). The later developed Monte Carlo program and computer code IDEAL-2 (Inhalation, Deposition, and Exhalation of Aerosols in/from the Lung, 2nd version) was able to estimate (statistical) deposition probabilities in the lung region (Pichelstorfer et al., 2016). Aerosol dynamics in Containments (ADiC) is also a stochastic (statistically based) model. Upgrades to the MPPD model mean that it now allows many estimates that are quantitative: quantitative total and site-specific deposition in human lung can be calculated, instead of statistical probabilities (Pichelstorfer et al., 2016). The MPPD model can simulate impaction, sedimentation, diffusion and particle evaporation and coagulation and accurately predict total lung deposition but less accurately regional deposition (Corley et al., 2021). Previously MPPD models underrepresented regional aerosol deposition however improvements in 2022 now allow aerosol mixing in the alveolar space (Asgharian et al., 2022), which greatly improves deposition predictions (Kuprat et al., 2023).

1.6.2.2 Computational Fluid Dynamics (CFD)

Computational fluid dynamics is a branch of fluid mechanics that uses numerical analysis to solve the governing equations of fluid-flow (Navier-Stokes equations), which are mathematical equations that obey the conservation laws of physics: the conservation of matter, newtons second law and the first law of thermodynamics (Versteeg, 2007). Therefore, with a finite element approach, the motion of fluid in three dimensions is described by a system of five partial differential equations: mass conservation, momentum equations (1, 2 or 3D) and energy equation (Versteeg, 2007). CFD refers to the flow of fluid without aerosol particles, and CFPD refers to aerosol particle flow through fluid.

1.6.2.3 Computational Fluid-Particle Dynamics (CFPD)

CFPD models are widely accepted to accurately predict aerosol deposition of inhaled particles. The CFPD model in OpenFOAM version 8 software (available from: https://openfoam.org/) mathematically couples a discrete (aerosol) phase with a continuous (air) phase, where the particle movement is considered with a Lagrangian view (not Eularian) and accounts for the coupled transport of a single kinematic particle cloud including the effect of the volume fraction of particles on the continuous phase (O'Rourke et al., 2009). Therefore, aggregation of

particles is not considered however the O'Rourke collision model is used for wall-particle interactions (O'Rourke et al., 2009). Particle-particle interaction can be resolved with a discrete element method of fluid dynamics, however it is more computationally intense.

1.6.2.3.1 CFPD coupled with 1D models (Multiscale models)

As there are limitations to the computational power required to model complex lung models (past the 6th airway generation), models are often coupled with lower dimensional models to resolve complete inhalation and exhalation when considering the entire lung. Indeed, there are very few cases where CFPD has been used consecutively from upper airway to lower bronchioles (Khoa et al., 2023). For example, coupled 3D and 1D modelling is achieved by allowing Lagrangian particles that reach the interface between the 3D and 1D domain to be summed up every 0.01s to give a mass-flux per second which was then converted to a concentration field and passed on to the 1D domain (Darequenne et al, 2016, Kuprat et al., 2023).

1.6.3 Credibility of Computer Modelling & Simulation (CM&S) pertaining to CFD

When a computer model or simulation is both verified and validated, it has credibility, which in computational terms is, "*the quality to elicit belief or trust in predictions of the computer modelling and simulation within a context of use*" (ASME 2018). To obtain credibility, the model should be built to a level of complexity that validates an experimental/empirical result and the calculations used verified to be correct within a margin of error and safety (ASME 2018). Verification and validation were first defined in 2003 (Oberkampf et al., 2003), pertaining to computational fluid dynamics simulation. Leading bodies that produced the original guidelines for the main rules for the conduct of CFD modeling studies are the European Research Community on Flow Turbulence and Combustion (ERCOFTAC) and the American Institute for Aeronautics and Astronautics. Later, guidelines for credibility of CM&S were established by the American Society for Mechanical Engineers that could be used in regulatory science, with application to medical devices (ASME, 2018).

1.6.3.1 Validation

Validation has been simply described, as "solving the right equations" and this process quantifies the uncertainty in the ability of the model to accurately reflect the real-life situation, or the "physical modeling uncertainty" (Oberkampf et al., 2003, Roache, 1998). To "solve the right equations" means that the model needs to properly understand the physical (and sometimes chemical or mechanical) conditions surrounding the context of the model (Roache, 1998). Often, validation of computational fluid dynamics models is obtained by matching experimental data with computational data.

1.6.3.2 Verification

Verification has been simply described as "solving the equations right" and this process quantifies the calculation errors (Roache, 1998). Usually verification includes grid-dependence assessment (discretisation error assessment), which is a test of the model's ability to solve the equations right, consistently, within a margin of

error (and safety factor). The use of a "Grid Convergence Indicator" (GCI) is recommended to quantify the numerical error, which includes the error multiplied by a safety factor (Roache, 1998). The success of the grid dependence study relies on the quality of the volume mesh which can be generated from a stereolithic file.

1.6.4 Methods for validation of computational fluid-particle dynamics (CFPD) models for the lung (experimental vs clinical)

Validation of a CFPD model to estimate aerosol dosimetry in the lung ideally uses the direct approach of combining *in vivo* human (clinical imaging) data with particle sizing data (European Medicines Agency Committee for Medicinal Products for Human Use, 2009). However, validation of a computational model assessing lung deposition must be indirect (experimental), where *in vivo* human data are lacking (Olsson & Kassinos, 2021). To validate a CFPD model of aerosol deposition to the lungs and provide aerosol dosimetry estimates, the ERCOFTAC guidelines (2017) for best practice should be used for an indirect approach. For a direct approach, radiolabeled lung deposition studies using gamma (Planar/2D) scintigraphy imaging to give peripheral and central regional lung deposition estimates, is best practice (Olsson & Kassinos, 2021). Use of radiolabeled lung deposition studies is recommended in clinical development guidelines for orally inhaled drug products, but not always possible/ethical (EMA, 2009).

1.6.4.1 Indirect (experimental/laboratory based) validation

ERCOFTAC advise that a 3D printed lung model (Lizal et al., 2012, and Koullapis 2018a), which is an estimation of the lung geometry, should be used as the flow domain to deliver an aerosol which is radiolabeled with Fluorine^{18}, and imaged using positron emission tomography (Lizal et al., 2012, and Koullapis 2018a). The 3D printed lung has five generations of airway and was taken using high resolution computer tomography on a cadaver lung from a healthy human adult male and combining this with an upper airway (Lizal et al., 2012). The 3D lung model (stl file) is publicly available allowing researchers to download and create the volume mesh files required for computation (Lizal et al., 2012). The new airway model is comparable to older models (Koullapsis, 2018b). An important limitation of experimental validation using airway models is that no natural movement of the airway occurs, i.e. the 3D model is rigid. The 3D model also conducts charge, and therefore particles must be passed through a charge equilibrator such as a Krypton 85 source. An additional limitation of the best practice advice case is that it does not recommend using an inhalation pattern, but rather a constant flow (less computationally intense). Other laboratory-based methods are in development such as apparatus that allows direct estimation of regional lung deposition by metal grid filters (Finlay et al., 2022).

1.6.4.2 Direct (clinical imaging) validation

Clinical imaging studies have been used to validate non-CFD aerosol dosimetry models, however generally the goal of directly imaging particles in the human lung *in vivo* is unobtainable with current methodologies (Martin, 2021, Olsson & Kassinos, 2021). New methods with low radiation dose (similar to high resolution computed tomogram) have been developed with a resolution down to 67 μm, but this is still not sufficient to resolve aerosol

particles usually with a MMAD less than 5 µm and the method is only available in a research capacity currently (Albers et al., 2023). Clinically, most *in vivo* data to support computational modelling comes from 2D gamma scintigraphy by radiolabeling aerosols to inhale, which is considered the industry standard and a supported/recommended approach during clinical development in regulatory science by the European Medicines Agency (EMA, 2009, Scheuch, 2010, Smaldone, 2001). Two-dimensional gamma scintigraphy allows total dose and regional dose to be assessed. Computational models can be validated using 2D gamma scintigraphy imaging *in vivo* data, when applying a correction factor to convert the 2D into 3D (Olsson & Kassinos, 2021). Importantly a radiolabeled aerosol must first be validated to not differ from its un-labeled counterpart (Devadason et al., 2012). For example, if a radiolabeled aerosol falls within 0.85 to 1.18 (ratio) of its unlabeled counterpart during aerosol characterization process (particle size assessment), it is deemed within an acceptable range and that the radiolabeling process has not significantly altered the aerodynamic properties of the drug (Devadason et al., 2012).

1.6.4.2.1 Imaging methodology used in humans to validate computational models

Imaging is used to view the locations where aerosols deposit in the lungs by combining an aerosol with a radioisotope that can then be imaged. Imaging methodologies usually harness the special qualities of radioisotopes to emit different types of radiation, primarily gamma (2D=Gamma Scintigraphy, 3D=Single-photon emission CT, PET), or Dark x-ray (x-ray (bremsstrahlung rays)). These imaging methods typically require doses of radiation that are the equivalent to airplane flights or x-ray/CTs and therefore radioisotopes are chosen that have short half-lives i.e. Technetium 99m (6 hours), Fluorine (1.5 hrs) etc. The most common imaging modality to assess aerosol lung deposition is Gamma Scintigraphy, due to its short acquisition times. Gamma scintigraphy is a 2D imaging method that measures the radiation emitted from a gamma producing radioisotope, commonly technetium 99m. By a process of radiolabeling, the radioisotope forms a physiochemical bond with an inhaled drug molecule, which must be validated so that the drug and the radiation accurately match (Devadason et al., 2012). Once validated the aerosol deposited in the lungs can be quantified for total and regional deposited dose, factoring in tissue attenuation. Peripheral to central estimates are considered *regional* dose as the scan is limited to 2D.

1.6.4.2.2 Limitations of human imaging methods to assess validity

The major limitation of current imaging methods used to assess aerosol dosimetry in the lungs is the difference in acquisition times and that only poor spatial resolution (2D) is possible with shorter acquisition times (Conway, 2012). For example, the radiation dose required for a 3D image (taking at least 20–60 mins to acquire) is too great for ethical approval in large cohorts of adults or altogether in children, whereas a 2D image, taking 2–5 minutes for acquisition, and having the radiation dose similar to an x-ray, is more likely to be approved (Conway, 2012). Even with high resolution CT scans the small airways are unable to be resolved, limiting imaging methods (Usmani et al., 2021). Other limitations include movement artifacts during long exposures which can result in difficulties when quantifying the radiolabeled drug.

1.6.4.3 Animal models for validation of computational methods

Animals can be used in research only when no valid alternative exists (NHMRC, 2013). As imaging methods can potentially be translated for use in humans, developing these methods first in animals (*in vivo* or *ex vivo*) or cadavers is popular. *Ex vivo* animal imaging models utilising magnetic resonance imaging and phase-contrast enhancement agents such as gadolinium or iodine have been successfully developed (Montigaud et al., 2020). Further, compact synchrotron light source can be used, combined with propagation based (grating), phase-contrast x-ray methods, to get excellent resolution of lung soft tissues, and particles of 50 μm, in a mouse model (Gradl et al., 2017, Gradl et al., 2018). Animal models experience the same issues as imaging in humans with movement artifacts, (i.e. the time taken between dosing and imaging will be subject to aerosol clearance mechanisms, and dose will be reduced/moved from the location deposited).

1.6.5 Credibility of Computational fluid particle dynamics for assessment of inhaled particles in the lungs of adults

The reliability of both upper and lower airway CFD models has been discussed in detail for airflow and for particle deposition with CFPD modelling (Khoa et al., 2023). Multiple studies have demonstrated the credibility of CFPD in the upper airway region (Khoa et al., 2020, Khoa et al., 2022, Kleinstreuer & Zhang, 2010, Phuong et al., 2020, Inthavong et al., 2021, Shang et al., 2022, Zhang & Kleinstreuer, 2004).

Introduction summary

This first four parts of this introduction highlight the physics of aerosol deposition, biological factors that influence lung deposition, patient conditions that are treated with inhaled medications for both conditions of the lung and generalised conditions. The last two parts highlight the problems inherent with development of aerosolised medicines in both general and paediatric populations, why off-label use of inhaled medicines is common, particularly in paediatric populations, and what new methods to study aerosol lung deposition are being developed to mitigate this problem within drug development process and what their level of regulatory acceptance and scientific validity is.

Research Case

Respiratory conditions are one of the leading causes of morbidity, death, and economic cost in the world. In many cases, these conditions are currently treated with aerosolised medicines. The properties of a medical aerosol particle that allows it to be inhaled and deposited in the lungs to exert therapeutic effect are varied (Chapter 1.1) and the mechanics of the lung in health and disease will influence this (Chapter 1.2). Respiratory conditions treated with inhaled medicines include asthma and chronic obstructive pulmonary disease (Chapter 1.3), however aerosolised medicines can also be used to treat conditions that are systemic or do not pertain to the respiratory system, such as nicotine to aid with smoking cessation, or inhaled levodopa to treat Parkinson's disease (Chapter 1.4). Over the last 60 years there have been few orally inhaled drug products developed to deliver aerosolised medications to treat respiratory conditions and relatively few improvements to the available devices during this time (Chapter 1.5). Children and patients with artificial airways are most affected by this lack of product development meaning that products first designed for use in other settings (or populations) require clinical adaptations to allow use in alternate population or settings – considered "off-label" use. Where off-label use is present, clinical efficacy is largely unknown and relies on good clinical practice to ensure a timely therapeutic outcome for the patient, with the responsibility for treatment outcome lying with the prescribing clinician. Therefore, Chapter 2 presents an education review aimed at a clinical readership, with a focus on aerosol delivery in a perioperative setting.

The inefficiency of available orally inhaled medicines has been particularly noticed in pediatrics. While technology evolves, the unmet need for innovation of inhaled drug products to children has primarily become the problem of clinical practice to address, resulting in "off-label" but clinically indicated use of inhaled therapies. The lack of approved treatment options means that patients may require prolonged aerosol therapy with limited clinical improvement, and supplemental intravenous treatment strategies to prevent serious adverse outcomes related to limited oxygen supply. It is therefore critical to reassess and substantially improve current aerosol delivery methods, particularly in populations at high risk of adverse events such as during perioperative or emergency settings (Chapter 2). Aerosol delivery perioperatively is particularly difficult to study *in situ* and yet respiratory adverse events such as bronchospasm (or "asthma attack") can be life threatening – especially for children who have higher demand for oxygen than adults. As reported in Chapter 3, I assessed experimentally in the laboratory a range of commonly used devices to deliver aerosolised medication to children experiencing bronchospasm whilst under anesthesia. In Chapter 4, I considered nebulised inhaled therapy used to treat children who use artificial airways which is also considered "off label" use, as the inhaled drug (tobramycin) was first approved to treat patients with cystic fibrosis.

As technology improves, there are increasingly more detailed computational methods available to assess aerosol delivery, device design and the safety and efficacy of an inhaled agent. These computer models can be used to

help understand the complex physical phenomenon underlying aerosol delivery and subsequently, to improve it. As discussed in Chapter 1.7, computer modeling and simulation (CM&S) has been present for decades as a method to estimate aerosol dosimetry and passage of aerosol through the body. Computational fluid-particle dynamics (CFPD) models are most physically correct as they account for aerosol particles and fluid (air) flow in three dimensions and in time. CFPD models must be informed first with preliminary experimental aerosol characterization including determination of particle size and concentration, and active and non-active ingredients of the inhaled substance, such as the data that were obtained in Chapter 6 for e-cigarettes. Once informed, the model then requires validation experimentally – either directly, with clinical validation and imaging or indirectly in the laboratory with simulation. Therefore, in Chapter 5, I replicated the laboratory experimental study from Chapter 3, *in silico*, with the aim to indirectly (by means of laboratory data) validate a CFPD model that could be used to assess and improve aerosol delivery.

The final off-label use of an inhaled drug investigated in this thesis was the aerosol produced by the electronic cigarette. As highlighted in Chapter 1.5, the electronic cigarette has not gone through the rigorous testing required to be an approved therapeutic good but is used to deliver a prescription medicine (nicotine) which is combined with other non-approved/prescription excipients for the purpose of smoking cessation. The electronic cigarette device produces a liquid aerosol by a condensation-evaporative process and since the start of work for this thesis became a prescription-only (unapproved) medication in Australia, meaning it is no longer allowed to be sold as a consumer product accessed by the public (May 2023). The electronic cigarette does not deliver a set dose of either prescription drug or excipient, meaning the inhaled ingredients and dose can vary greatly with each user and potentially be unsafe. Additionally, the e-liquid that is used to produce the aerosol by the electronic cigarette device via the condensation-evaporation process is known to contain and produce a complex mixture of chemicals which vary when heated. Therefore, it was determined that the most important component of experimental work to establish the safety of the inhaled good with the electronic cigarette was chemical testing of the heated e-liquid and the aerosol produced. In Chapter 6 I assessed experimentally the chemical composition of several different e-liquids and compared them in heated and unheated form and with two different methods of heating, which could improve testing times. The method tested in Chapter 6 was used to test other brands and flavors of e-liquids which was subsequently published (Appendix A).

Chapter 2 Literature review (published): Aerosolized drug delivery in awake and anesthetized children to treat bronchospasm.

Plain language introduction to Chapter 2:

This chapter was designed to investigate and educate clinicians on best practice for aerosol delivery when delivering bronchodilators to children in emergency settings and covers use of both nebuliser therapy and pressurised metered dose inhaler delivered drug therapy. The aim was to compare best practice in children who were awake and children who were anesthetised to see where there were consistencies or inconsistencies in delivery techniques or inhaler devices. Aerosol delivery to anesthetised children is considered an off-label use with the responsibility for health outcomes lying with the clinician, and therefore comparison to best practice in awake children is beneficial for good clinical practice.

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EDUCATIONAL REVIEW

Pediatric Anesthesia WILEY

Aerosolized drug delivery in awake and anesthetized children to treat bronchospasm

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Summary

Bronchospasm is a common respiratory adverse event in pediatric anesthesia. First-line treatment commonly includes inhaled salbutamol. This review focuses on the current best practice to deliver aerosolized medications to awake as well as anesthetized pediatric patients and discusses the advantages and disadvantages of various administration techniques. Additionally, we detail the differences between various airway devices used in anesthesia. We highlight the unmet need for innovation of orally inhaled drug products to deliver aerosolized medications during pediatric respiratory critical events such as bronchospasm. It is therefore important that clinicians remain up to date with the best clinical practice for aerosolized drug delivery in order to prevent and efficiently treat pediatric patients experiencing life-threatening respiratory emergencies.

KEYWORDS
aerosol, anesthesia, nebulizer, pediatric, perioperative adverse events, salbutamol

1 | INTRODUCTION

Bronchospasm is a common respiratory adverse event in pediatric anesthesia. First-line treatment for bronchospasm is usually inhaled salbutamol (albuterol), a short-acting beta-agonist (SABA), or adrenaline, a non-selective adrenergic agonist—to reduce airway constriction and/or edema. The aim of this review is to examine current best practice for the delivery of orally inhaled drug products to treat bronchospasm in awake and anesthetized children, and to identify clinical practice aimed at prevention of perioperative bronchospasm. Closing the gap between aerosol delivery science and clinical practice is essential to minimize morbidity and mortality by facilitating a safe recovery and by identifying potential areas for technological improvements.

Orally inhaled drug products, such as those delivering salbutamol or adrenaline, can be divided into two categories during drug administration 1) drug-device combination products (medicinal products—primary mode of action is attributable to the drug component), for example, pressurized metered dose inhalers (pMDI) and 2) device-only products, for example, spacer actuators (in-line spacers), universal adaptors (mini-spacers), spacers, or nebulizers.[1,2] Dry powder inhalers are not typically used to administer emergency medicines (since they commonly require a significant inspiratory flow) and are therefore outside the scope of this review.

Drug-device combination products are designed to be tested for safety together with a specific drug prior to going onto the market. In contrast, device-only products are intended to either replace the actuator/aerosol generator of an already existing drug-device product (eg, in-line spacers, mini-spacers) or to be used with an already existing drug product (eg, nebulizers, spacers). Clinicians have to keep in mind that device-only products are not required to conform with drug-device combination product legislation and that there are

no drug-device combination products approved for use in a ventilation line to date.[2-4] Additionally, aerosol delivery devices are designed for adults not children and thus "off-label" use, in this sense, is common.[5-7] Therefore, any clinical adaptations for pediatrics deemed necessary are tested for evidence base in hindsight—if at all—to allow the proper translation into clinical practice.[7,8]

In awake patients, salbutamol administration via a pMDI—a drug-device combination product—with an accessory spacer device, is the gold standard because they are both cheap, portable, and considered easy to use.[9] A pMDI, while seemingly easy to use, is in fact difficult to use correctly, as demonstrated by both patients and clinicians, and has not improved over time.[10,11] Furthermore, spacer devices are a post-market add-on and need to be carefully considered, since different spacers used with different drugs will deliver aerosol differently and should not be considered interchangeable.[12] Correct usage of a pMDI with a spacer involves a series of steps, as per the manufacturer instructions, which includes 1) "priming" the pMDI device by actuating two puffs into the air before use, in order to ensure no vapor or air bubbles are present in the metering chamber (that can form during storage and temperature cycling), 2) shaking the device well (for ~5 s) before and between doses, 3) holding the device upright and inhaling at the same time as actuating the device, and 4) waiting for ~30 s between dosing.[13] Shaking between use may not be required with newer formulations that are in a hydrofluoroalkane propellant, although this is yet to be confirmed.[11,14] Even when a salbutamol pMDI is used with a spacer and facemask correctly—according to the manufacturer instructions—it will deliver between approximately 2–7.5% of the labeled claim dose to awake young children (<5 years) in a non-acute setting, which is similar to that delivered by a nebulizer, but in a shorter time.[15,16] The dose administered will be further reduced if the device is not used as per the manufacturer instructions, or if there is a facemask leak.[15]

In anesthetized and/or ventilated children, the aerosolized drug is often administered in the inspiratory limb of the ventilator circuit with a device-only product as there are currently no drug-device combination products on the market designed for this use.[17] Similarly, there is currently only one drug-device product used to treat respiratory emergencies—the pMDI; however, even the pMDI is used with an untested-prior-to-market add-on device—the spacer. In fact, only two conceptually new drug-device products have been introduced since the pMDI in the 1950s—the dry powder inhaler and the soft-mist inhaler (or liquid metered dose inhaler), but neither are used in emergency medicine and nebulizers are a device-only product.[1-3,18] There have been many upgrades to the existing pMDI since its introduction, including 1) the switch to "F gases" as the propellant instead of chlorofluorocarbons to address the ozone hole as a result of the Montreal Protocol in 1987, and resultant changes to formulations,[14,19] 2) introduction of breath-actuated inhalers in the 1990s,[20] and 3) the addition of dose counters and combination-drugs to address the longstanding issue of severe asthma exacerbations associated with poor adherence to treatment by patients with mild asthma.[21] The reason for the lack of new products may well be due to the 70% failure rate for orally inhaled drug product development which means few new products reach the market.[22] Furthermore, orally inhaled drug products are considered a "complex therapeutic," and are thus more difficult to produce.[22] Considering this unmet need for innovation in orally inhaled drug products, it is therefore important that clinicians remain reliant on the most up to date, best clinical practice for aerosolized drug delivery in order to both prevent and efficiently treat pediatric patients experiencing life-threatening respiratory emergencies.

2 | SEARCH METHOD

Search period: 2011–2021.

Data bases: Pubmed, UpToDate.

Search Terms: bronchospasm, anesthesia, intraoperative bronchospasm, pediatric croup, post-extubation stridor, intraoperative bronchospasm pediatric, perioperative bronchospasm pediatric, nebulizer therapy, croup, medical aerosol in acute and critical care. Reference lists from identified articles were also included.

3 | EPIDEMIOLOGY

Acute asthma is the fourth most common reason for pediatric presentations to the emergency department.[23] Most asthma hospitalizations are in children and the hospitalization rate for children (aged 0–14) is 363 per 100 000 population with a death rate of 1.3 in 100 000.[24]

In the perioperative period, in general, 1 in 7 children experience a respiratory adverse event (eg, laryngospasm, bronchospasm, desaturation), with this rate further increasing in children with risk factors (eg, recent upper respiratory tract infection, nocturnal dry cough).[25,26] Approximately one in a hundred children undergoing surgery will experience bronchospasm.[25,26]

4 | TREATMENT

Treatment for bronchospasm often involves the delivery of aerosolized medication by a medicinal product (a drug-device combination product) or a medical device (a device-only product) that administers medicines for inhalation. The combination of a drug with a device has an essential impact on the safety and efficacy of the medicine, especially when it is intended to treat a life-threatening condition such as bronchospasm.[27] Only the manufacturer intended combination of drug-device product are properly tested for safety and efficacy with the medication they deliver before they go to market and thus will produce aerosolized medication: (1) without failure of the delivery mechanism, and (2) of an adequate, respirable size; both are essential to ensure drug will reach the lungs and provide relief of symptoms.[27] For example, the European Medicines Agency specifies that, before the product goes to market, the manufacturer must confirm that

the drug-device combination product can deliver the expected dose within +/-15% for the number of doses claimed to be provided.[28] Therefore, a delivery device should not involve "off label" use of a drug-device product, that is the use of the drug component of a drug-device combination product with a different device to that it was packaged with or vice versa. For example, the placement of a pMDI canister into a 50 ml syringe, which can risk patient safety, if the syringe was to fracture and fragments be inhaled.[29] Furthermore, use of a 50 ml syringe to deliver aerosol has been shown to be ineffective, as the valve cannot function as designed to produce the labeled claim dose.[8] To reduce this risk, increasingly, medications intended for use with nebulizers must specify which nebulizer they were tested with and many device-only products are recently being reclassified.[3,30]

A recent review of aerosol delivery to awake children has summarized all radiolabeled lung deposition aerosol studies for either nebulizer or pMDI generated treatments in children and shows that there is little difference between nebulized and pMDI delivery with conventional methods.[31] Regardless of pMDI or nebulizer delivery, approximately 2% of the labeled claim dose was delivered to awake infants and approximately 7% to children under 15 years.[31] A larger delivered dose was observed when newer technology was used: delivered aerosol dose increased up to 56% of labeled claim dose when using a vibrating mesh nebulizer or up to 54% of labeled claim dose when using a pMDI and (antistatic) spacer with an extra-fine-particle (1.1 μm mass median aerodynamic diameter [MMAD]) aerosol.[32,33] However, it has to be kept in mind that crying reduces the dose significantly.[32,33] Aerosol delivery to a group of sleeping (sedated) infants was reported as being between 0.3 and 2.1% when delivered by nebulizer and facemask, which is similar to that in awake children.[34] Cooperation during aerosol delivery remains the single most important factor when assessing efficiency—the delivery interface must be tolerated by the child; during mechanical ventilation, many other factors can reduce aerosol delivery (eg, airway device, humidity).[4,35]

The key considerations of any treatment option are therefore; 1) consider the type of product, that is, is it a drug-device combination product, for example, pMDI, or a device-only product, for example, nebulizer?, 2) Does the delivery setup allow the product/device to be used in accordance with delivery as per the manufacturer instruction leaflet?, 3) If the product is a device-only product, has the device been tested with the drug independently or before market (if it has it should/will be specified on the medicine leaflet), 4) Importantly, ensure tolerance and comfort of the interface with the child.

5 | AEROSOL PARTICLE SIZE

The aim of any aerosol generation device is to produce particles that are of a respirable size (<5 μm) and ensure that they reach the lung so the active drug can take effect and relieve symptoms. It is particularly important to have an appropriate respirable particle size for children whose lungs are smaller than those of adults, otherwise aerosol deposition in the mouth and throat will increase; typical aerosol deposition in adult airways can be seen in Figure 1.[31] Aerosol-generating devices produce different median particle sizes and the aerosol is classified based on its size when referring to a solid particle aerosol, typically; ultrafine (<0.1 μm), fine (<2.5 μm), and coarse (>10 μm).[36] However, when referring to medical aerosols, coarse is considered to be (>2.5 μm), and a third non-typical category—extra-fine - (0.1–2 μm) is also referred to.[36,37]

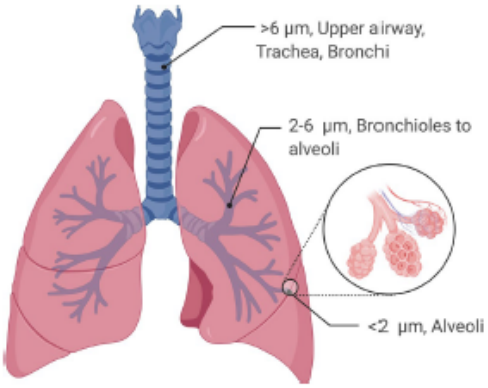

FIGURE 1 Aerosol particle size deposition mechanisms in the lungs. Particles over 6 μm will deposit by impaction in the mouth and throat, particles 2–6 μm will deposit by impaction, interception or sedimentation in the bronchi and terminal bronchioles, and particles <2 μm will reach the alveoli if they are not exhaled, and deposit by diffusion or electrostatic precipitation.[43] Image created with biorender, https://biorender.com/

There are many factors that affect particle size of both solid and liquid aerosols; condensation and evaporation processes, coagulation, electrical properties, and surface tension. Increases or decreases in air temperature will increase or decrease the density of the air, and thus, the movement of the aerosol particle through the air as it becomes more viscous. High concentration of aerosol can lead to coagulation or aggregation, and thus increase the aerosol particle size. All of these factors come into play when assessing the delivery efficacy of a medical aerosol.

Reported average particle size, based on mass, for salbutamol pMDI (as Ventolin®, GSK) is 3.75 μm.[38] Particle size generated by nebulizers is generally in a similar range, with slightly less deviation from median compared to pMDI and should be reported by the manufacturer based on nebulization of a base-liquid such as saline.[30,39] For conventional jet nebulizers, this is in the range of 4–6 μm median diameter; however, vibrating mesh nebulizers are reported to produce particles in the range of 3–5 μm and new small-volume "micro-mist" jet nebulizer technologies, or adaptations to conventional jet nebulizers for pediatrics can produce

even smaller particles.[39,40] For example, the Hudson Micro Mist® small-volume nebulizer produces an average particle size of 2.7 µm mass median aerodynamic diameter at the recommended flow rate of 8 L/min.[41] However, smaller particle size does not necessarily equate to better clinical outcomes for all conditions treated with inhaled medicines.[42] Factors such as half-life of the active ingredient drug, and whether the dose of an extra-fine formulation is available at an equivalent dose to a non-extra-fine formulation should be considered.

6 | PRESSURIZED METERED DOSE INHALER

The pressurized metered dose inhaler (pMDI) is a drug-device combination product which consists of; a drug formulation, a "container closure system"—involving the pMDI canister, actuator and the metering valve, and any additional features, such as an integrated spacer or dose counter.[1] The drug formulation component includes a drug substance either suspended or dissolved in a liquified, pressurized propellant—usually a fluorinated or "F gas" often HFC-134a or HFC-227—which provides the energy to generate the aerosol.[1] The propellant evaporates when at room temperature, leaving only the drug to be deposited in the patient's lungs. All pMDIs produce a polydisperse aerosol and the formulation can be considered a coarse (>2.5–10 µm), fine (0.1–2.5 µm), or an extra-fine aerosol (0.1–2 µm), if the mass median aerodynamic diameter is within said ranges.

The pressurized metered dose inhaler has changed little conceptually over the last 70 years since its introduction to treat asthma but has received many upgrades. The most recent adaptation/upgrade to the salbutamol pMDI to come to market (in 2021 in Australia), is the addition of a dose counter—first recommended by the Food and Drug Administration in 2003—that allows monitoring of drug usage and avoids use of near-empty canisters which deliver less than the labeled claim dose.[21] Formulations for pressurized metered dose inhaler are likely to change with new requirements for the phase out of the "F gases" currently used as propellants in pMDIs, as we saw previously with the phase out of the old CFC's (chlorofluorocarbons) in response to the Montreal Protocol of 1987.[44]

6.1 | Environmental impact of the "F gases" in pressurized metered dose inhalers

In 2016, the Kigali amendment to the 1987 Montreal protocol came into force, whereby the "F gases" such as those found in pressurized metered dose inhalers are to be phased out by 2037 for developed nations and 2047 for developing nations due to their contribution to greenhouse effect and global warming.[45,46] Since dry powder inhalers have a larger carbon footprint due to manufacturing, and pMDIs are often the best choice of device for a patient, propellants currently in pMDIs are being switched to those with a lower global warming potential.[46-48] Those with a lower global warming potential that are currently being tested for safety in pMDIs include, HFC-152a and HFO-1234ze(E) with safety data for HFC-152a expected to be lodged with regulatory bodies in early 2022.[46-48]

7 | DEVICES THAT INTERFACE WITH THE PATIENT

The device interface (eg, mouthpiece, face mask) should be chosen primarily based on patient age, the distress level of the child, and the child's tolerance for a particular interface.[49] Most commonly used device interfaces include; the spacer, mouthpiece, facemask, and nasal cannula. Less commonly used interfaces include valved holding chambers (spacers with one-way valve to prevent exhaling into), pacifier masks, blow-by (wafting aerosol from a nebulizer near the face by hand), nasal masks, and hoods (head boxes) which have been reviewed but will not be discussed here as they are outside the scope of this review.[49] Additionally, the American Association of Respiratory Care has recommended that blow-by be abandoned due to its unreliable dosing.[30]

7.1 | MOUTHPIECE, FACEMASK, OR NASAL CANNULA

A mouthpiece—preferably the mouthpiece of a spacer device if using a pMDI—is preferred over a facemask where possible (usually in children >4 years of age), as aerosol can be lost into the facemask and through leaks around the mask, decreasing the dose delivered to as low as 1% of the label claim.[49,50] However, valved facemasks, when used with the more efficient (compared to jet nebulizer) vibrating mesh nebulizer and low flow oxygen (<2 L/min), have been shown as effective as a mouthpiece at delivering nebulized medication in a recent study of pediatric patients with asthma.[51] Facemasks may not be well tolerated by some children and therefore a nasal cannula or other device that allows the child to still use a pacifier may be a better option.[49] Furthermore, novelty facemasks (eg, "Dragon" character, Carefusion) are available that may reduce the fear for young children of using a facemask and may improve compliance and cooperation.[49] Older children should use a mouthpiece if able to do so, as they can deliver up to twice the dose, compared to a facemask, if using a conventional jet nebulizer.[9,49]

Children under 18 months are either obligate (<6 months) or preferential nose breathers, and children 18 months to 4 years may be unable to use a mouthpiece properly, thus a facemask with pMDI, or facemask or nasal cannula with nebulizer, is preferred for these age groups.[49] Aerosol delivery through high-flow nasal cannula and nebulizer is being increasingly investigated as an alternative interface and was shown to be tolerated better than a face mask in several studies; however, flow rates should be less

than the normal respiration (volume flow rate) of the child in order for this therapy to effectively deliver aerosolized medication and oxygen.[52,53]

8 | AWAKE PATIENTS

8.1 | Spacer devices (holding chambers)

A spacer device is the gold standard interface for awake and asleep delivery of inhaled medication produced by pMDIs. The spacer is designed to remove particles larger than 5 μm, which would otherwise deposit in the mouth and throat, thereby making the aerosol exiting the device "respirable" (<5 μm) and able to reach the lungs of the child.[30] A spacer is not intended to replace any working component of the drug-device pMDI product but to be used in combination with it to further enhance the delivery efficacy. A spacer device can come with or without a one-way valve, and so is also called either a holding chamber, or a valved holding chamber. A one-way valve is incorporated into a spacer primarily to prevent exhalation into the chamber.[12] Compared to an un-valved device, it will stop particles from exiting the chamber until inhalation begins; however, the valve is also a point for impaction and would reduce aerosol delivery if faulty.[12,30] Regardless of valve presence, a spacer should ideally be made of an antistatic material to decrease any electrostatic deposition of particles within the spacer,[30] and of a small volume (but not less than 100 ml), as this requires less breaths for a child to clear from a single pMDI actuation.[9,30] Optimal use of a spacer device requires a slow single maximal inhalation timed with a single actuation of the pMDI; however, this is unlikely to occur in an acute setting.[9] Multiple actuations into the device, and tidal breathing can reduce aerosol delivery efficiency.[12,30] Spacer devices are generally not recommended for use with a nebulizer. It is important to know that drug type and inhalation technique influences drug delivery via a spacer, and that a spacer is regulated as an accessory device for a pMDI actuator (mouthpiece), not as a drug-device combination product, thus may not have been tested for efficacy with a particular pMDI drug prior to market.[12]

9 | ANESTHETIZED PATIENTS

9.1 | Artificial airway device

In vitro, drug delivery through artificial airways (laryngeal mask/supraglottic airway [LMA]), endotracheal tube or tracheostomy tube) shows that drug exiting the airway and thus delivered to the child, generally increased with increased internal tube/lumen diameter and decreased with any obstructions to the lumen such as presence of aperture bars.[8] However, when using standard weights per size of airway device, the mg/kg dose remains similar regardless of internal lumen diameter, as smaller tubes are used for smaller, lower weight children.[8]

Laryngeal mask airways have been shown to reduce perioperative respiratory adverse events even in young infants as compared with endotracheal tubes.[26,50] For a size 2 or 4 LMA, a larger lumen (8.7, 12.7 mm ID, respectively, for size 2 and 4 LMA) generally delivers more salbutamol than an equally sized (ie, patient size 4) smaller lumen (7 or 10 mm ID) LMA.[8]

If an endotracheal tube is used, a cuffed endotracheal tube is beneficial in maintaining tidal volume over time and allows for more efficient ventilation, particularly in children with increased airway resistance as compared to uncuffed endotracheal tubes.[54,55] Furthermore, cuffed endotracheal tubes are associated with lower rates of stridor compared with uncuffed endotracheal tubes.[54,55] Tracheostomy tubes deliver the same amount of drug with a pMDI used with or without a spacer due to their small length.[8] Pre-formed tracheal tubes (eg, RAE tubes) deliver less drug than standard tracheal tubes due to the standard (short) 90-degree angle of the elbow, which is commonly acknowledged in fluid dynamics engineering to have an increased resistance co-efficient (due to the flow having to change direction suddenly) compared to a longer radius 90-degree bends or a lesser angle.[56]

9.2 | "MINI-SPACERS" OR "UNIVERSAL/GENERAL ADAPTORS"—ACTUATORS

Spacer actuators (Figure 2A,C) are more effective than universal or general use actuators (mini-spacers) (Figure 2E,F) at delivering aerosol.[8] If a spacer actuator is not available, a universal or general actuator with a larger internal diameter is preferred (ie, 22 mm compared to 15 mm), as less of the spray exiting the valve will hit the wall of the adapter due to the increased lumen size. Accessory elbow pieces should be used with caution as they will likely reduce the dose if they are put between the aerosol generation device and the patient.[8,57] New accessory devices, such as streamlined T-pieces, Y pieces, and nasal cannula, are being tested for placement in ventilation lines that allow more efficient airflow and therefore aerosolized particle flow.[57]

In vitro testing showed no benefit of using a dual-spray adapter or "mini-spacer" when compared to a unidirectional spray adapter, and both devices were less efficient when compared to use of small-volume in-line spacers.[8] Additionally, creating in-line adapters via off-label use, such as placement of a pMDI canister inside a 50 ml syringe (Figure 2G) should be abandoned, as it can risk patient safety.[29]

9.3 | "INLINE" SPACERS—SPACER-ACTUATOR DEVICES

In ventilated children or children breathing spontaneously via a ventilator circuit, in-line spacers (Figure 2A,C) can be used to administer the inhaled medication without interrupting the ventilation. In-line spacers are commonly inserted into the inspiratory arm of the ventilation setup and are more efficient at delivering aerosol

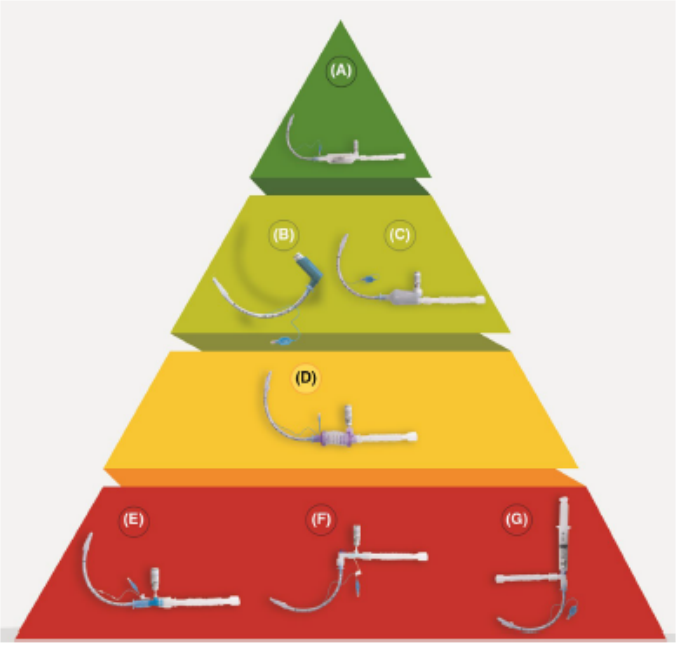

FIGURE 2 Devices that interface the patient for anesthetized patients. A = small-volume (145 ml) non-valved spacer actuator, B =direct pMDI administration, C = small-volume (110 ml) valved spacer actuator, D = collapsible, non-valved small-volume (~100 ml) spacer-actuator "in-line spacer," E = "off label" pMDI use—placement of pMDI inside 50 ml syringe, F = universal/general unidirectional actuator "adapter," G = dual-spray "mini-spacer." Images adapted and reprinted from[8] with permissions

than general actuators ("universal adapters").[8] A spacer-actuator device is intended to replace the actuator component of an approved pMDI drug-device product—for example, salbutamol as Ventolin®. Spacer actuators currently on the market have not been required to go through testing as a drug-device product, and therefore, the delivery efficacy of the spacer actuator with any pMDI canister is unknown and variable between different spacer actuators and pMDIs.[3] If not independently tested, this can lead to potentially unreliable drug administration.[2,3]

Small-volume spacers are better for younger children, and for the best efficiency should ideally be of a volume approximately equal to their tidal volume, so a single puff of aerosol can be cleared with a single breath. Additionally, larger volumes increase dead-space, particularly if a spacer is kept within the breathing circuit and cannot be collapsed (Figure 2D).[8,9]

10 | NEBULIZERS

A nebulizer is a medical device that produces aerosolized medication and is generally used for patients who are unable to use the gold standard delivery method (a pMDI and spacer) or where the medication needed is not available for delivery with pMDI.[39] Drug formulation as a liquid is easier than as a solid and there are many drugs formulated for use with nebulizers that cannot be formulated as a solid particle.[58] For example, muco-active drugs (eg, dornase alfa), tobramycin, and adrenaline are not available in pMDI form.[40]

A nebulizer produces a liquid particle aerosol, compared to a pMDI which ultimately produces a solid particle aerosol after propellant evaporation. Limitations of nebulizers compared with pMDIs include requirement for power, longer treatment times, and cleaning and maintenance; however, nebulizers have the advantage of being able to deliver medication, or multiple medications (eg, salbutamol, ipratropium bromide, and oxygen) continuously.[39,40] Furthermore, nebulizer technology is continually improving,[40,57] and thus nebulizers, particularly the vibrating mesh type, are becoming a viable alternative to the pMDI with regard to efficacy of treatment.[59,60] Particularly considering that many vibrating mesh nebulizers and "micro mist" small-volume nebulizers can produce an equivalently small particle size compared with a pMDI, but in contrast to a pMDI, consistently and continuously, without requiring removal of the larger particles with a spacer device. Furthermore, nebulized particle size usually is less variable than that produced by a pMDI.[30] Despite this, a pMDI with a spacer remains the gold standard for aerosol delivery for children of all ages. PMDIs are as effective or better at relieving symptoms than nebulizers, portable and easy to use, and have less risk of side effects due to the longer treatment time required by nebulizers.[30,40] Furthermore, pMDIs have a lower risk of transmission of respiratory infections compared with nebulizers, as they come with the medication pre-packaged in the pMDI canister (do not require a technician to prepare the solution), and do not require the use of a driving flow that could further generate/spread bio-aerosols.[9,61]

The most commonly used nebulizers are the jet nebulizer, the vibrating mesh nebulizer, and the ultrasonic nebulizer. The jet

nebulizer is reported as most commonly used for delivering aerosolized medication in the acute setting in the emergency department.[39,40,51,65] There is rarely a standardized requirement for a particular type or brand of nebulizer, this will vary between centers based on the types of drugs most commonly delivered, and many drugs (eg, salbutamol) are approved for "general nebulizer use."[30,40] Additionally, there are many drugs (eg, Tobramycin (as TOBI®) is approved for use with the PARI LC® Side stream Plus) that are only approved for use with certain types of nebulizer. Therefore, it is important that if a particular type of nebulizer is stated to be used with the drug on the packaging, that this instruction is observed, as not doing so will negatively impact on the efficacy of drug delivery to the patient.[30,39,40] There is a great interest in validating vibrating mesh nebulizers for standardized use in clinics due to their demonstrated improved performance compared to jet nebulizers during *in vitro* testing, and increasingly in clinical studies.[51,62–65]

10.1 | Jet nebulizers

The jet nebulizer was the "first generation" nebulizer; however, it is often known today as a "small volume jet nebulizer." "Micro mist" nebulizers are increasingly common as they deliver a smaller particle size compared with conventional jet nebulizers.[30,39,40] Jet nebulizers cost less than vibrating mesh nebulizers and ultrasonic nebulizers and have historically had the advantage that they can be used with oxygen which is indicated for severe life-threatening asthma. However new adapters (ie, Aerogen® Ultra) are available for vibrating mesh nebulizers that allow intermittent oxygen therapy and so this advantage is of lesser importance in the newer models.[30,39,40,51] To use a jet nebulizer successfully in clinical practice, the instructions given by the manufacturer must be followed—the nebulizer flow rate defined on the device label, usually, 6 L/min should match that of the flow meter for the driving source (usually oxygen).[30,39,40] If a compressor is used instead of a driving gas, it should match the pressure required by the jet nebulizer—usually 50 psi.[39] Changes to either of these parameters can result in less efficient drug delivery.[30,39,40]

10.2 | Smart nebulizers

Smart nebulizers were developed to overcome some of the limitations of continuous nebulizers, such as high residual volumes, and drug wastage due to fugitive aerosol caused by aerosol generation during exhalation.[40] These "second generation" jet nebulizers include breath-actuated and breath-enhanced nebulizers and are referred to as "adaptive" aerosol delivery, because they analyze the patients breathing and synchronize aerosol delivery in time with inhalation.[30,40] Breath-activated nebulizers require an inhalation rate of 15 L/min to activate the device and are therefore not suitable for many patients, particularly not for young infants.[30] Conventional jet nebulizers have been shown to deliver aerosol more efficiently than breath-activated nebulizers during ED presentation of asthma.[66]

10.3 | Driving gas for jet nebulizers

Oxygen is most often used to "drive" nebulizers as it is readily available in most hospitals, and the flow rate to be used with the nebulizer will be specified by the manufacturer. Commonly the flow rate is approximately 2 ml/kg. However, some newer studies indicate that if using nasal cannula to deliver aerosol to young children flow rate should be reduced to less than that of the natural tidal minute volume of the child.[52] For neonates, reducing the flow rate from 5 L/min to 0.65–1.5 L/min increases the aerosol delivery from 1% to 10% of dose given.[52] Similarly, decreasing the flow rate from 7.5 L/min to ~2 L/min improves aerosol delivery from 2% to 11.5% for toddlers (15 kg).[52] These results indicate perhaps low flow (<2 L/min) nasal cannula is more effective, although more studies are needed as lower flow rates may not be indicated for use with particular jet nebulizers.

The use of heliox (80%–20% or 70–30% helium to oxygen) as a driver, improves gas exchange and reduces the work of breathing as well as the need for mechanical ventilation for treatment of acute respiratory distress.[67] While it is not commonly used in routine care, it can be used as an adjunct therapy to improve outcomes only if the patient does not require >30% oxygen.[67]

10.4 | Mesh nebulizers

A vibrating mesh nebulizer uses electricity to generate aerosol through vibration of a mesh (a plate with small apertures); it is small and portable, and requires no driving flow, therefore has less risk of bioaerosol transmission.[39,40] Previous studies have shown repeatedly *in vitro* that vibrating mesh nebulizers increase the efficacy of drug delivery when compared with jet nebulizers and thus are generally considered the nebulizer of choice for new drug development.[39,40,64] There is growing evidence to suggest that vibrating mesh nebulizers are superior to jet nebulizers in the delivery of emergency medications, particularly if using a valved facemask.[51,63] A recent study showed that the use of a vibrating mesh nebulizer for asthma exacerbations in the emergency department reduced hospital admission rate by 39% compared to a jet nebulizer, when using a facemask.[51] The vibrating mesh nebulizer has been shown to improve clinical outcomes and increase the dose delivered to the patient, when compared to a jet nebulizer.[39,51,63,64] Particularly, the vibrating mesh nebulizer has been shown to deliver more drug than a conventional jet nebulizer.[68] Other key advantages of vibrating mesh nebulizers compared to jet nebulizers include smaller size aerosol generation with particles of 3–5 μm in size compared with conventional jet nebulizer generated particles of 4–6 μm. Additionally, mesh nebulizers use the entire drug volume while jet nebulizers can leave 25–50% of the nominal dose.[40]

10.5 | Ultrasonic nebulizers

Ultrasonic nebulizers use a piezoelectric crystal to generate an aerosol, creating oscillations, and then aerosol droplets, they were

originally developed to nebulize large volumes of saline for sputum induction.[39,40] They are not as commonly used due to being bulky and more costly. They also have the potential to degrade heat sensitive materials, such as proteins, and should not be used with suspensions such as budesonide, due to heat generation during aerosol generation process.[30,39,40]

10.6 | Nebulized delivery in presence of bio-aerosols, that is, during COVID-19 pandemic

Bio-aerosols generated by infectious patients, particularly during talking and coughing, are a major potential source of transmission for healthcare workers.[61,69] Nebulized treatment, high-flow nasal oxygen, and single and dual circuit non-invasive respiratory therapy have been associated with an increased risk of transmission of viral sources.[61,69] To mitigate risk, aerosol-generating procedures should be used with caution particularly in patients with airborne infections and great care should be taken to wear appropriate personal protective equipment (PPE).[61,70] If required to use a nebulizer during an emergency, initial risk mitigation should focus on containment—the device should be operated, if possible, in a negative pressure room, or if not possible, in a single room where clinical staff are in full PPE including N95 mask and the interface to the patient should be a single-use.[61,70] Secondary to this, the preference should be to avoid fugitive emissions; therefore, a mesh nebulizer or breath-enhanced or breath-activated jet nebulizer is preferred over a conventional jet nebulizer, in order to reduce fugitive emissions resulting from high driving flow.[61] To mitigate risk of using a jet nebulizer at any flow it is important to put filters on all expiration ports and use aseptic loading techniques for jet nebulizer cups.[61]

11 | CURRENT BEST PRACTICE TO *TREAT* BRONCHOSPASM

Aerosol delivery for anesthetized patients is largely adapted from awake practices. This is mainly due to innovation being absent, or poor in the case of the newly revised device-only category.[3,5]

12 | BRONCHOSPASM

Bronchospasm is commonly treated with inhaled salbutamol.[9,71] The short-acting anticholinergic, ipratropium bromide is also indicated in severe life-threatening asthma.[9,71] Optimal diagnosis and treatment can improve patient outcomes and reduce risk of hospitalization.[67]

12.1 | Awake

First-line awake treatment of a moderate-severe asthma exacerbation is delivery of an inhaled SABA intermittently with ipratropium bromide, with a pMDI and spacer (with facemask <5 years), if the patient is able to cooperate with treatment. Otherwise, nebulized salbutamol and ipratropium bromide (with facemask interface) were delivered either continuously for 1 h or every 20–30 min for three doses, with oxygen supplemented to SpO_2 >92%, and systemic glucocorticoids given.[9,71] If the patient is unable to cooperate with nebulized treatment, or if anaphylaxis is suspected as the cause of the exacerbation, an alternative treatment is intramuscular or subcutaneous adrenaline or terbutaline. For life-threatening (critical) asthma, where respiratory failure is imminent (the child is unable to use a pMDI with spacer), nebulized SABA, combined with intermittent nebulized ipratropium, driven by oxygen should be delivered by a tolerable interface—usually a facemask.[9,71]

12.2 | Anesthetized

First-line delivery for anesthetized spontaneously breathing children is with SABA via pMDI and spacer, which can be attached directly to the connecting port of the artificial airway if they have one. If a spacer is not available, the pMDI with proprietary actuator should be attached to the artificial airway directly instead (Figure 2B).[8]

13 | SEVERE LIFE-THREATENING VIRAL AND POST-EXTUBATION CROUP/STRIDOR

Upper airway obstruction and stridor caused by viral croup or post-extubation croup are treated with inhaled adrenaline, which is reported to reduce symptoms 30 min post-treatment.[72,73]

First-line awake delivery of inhaled adrenaline is by nebulizer with a facemask where a good seal should be formed with the facemask to ensure effective delivery of drug and avoid fugitive emissions reaching the eyes.[73] The nebulizer should be used in accordance with the manufacturer's instructions, that is, at the correct flow rate, to ensure drug is delivered efficiently.[30,39]

13.1 | SARS-CoV-2 infection with croup

There have been multiple reported cases of children who tested positive to SARS-CoV-2 and had stridor at rest which did not respond to adrenaline treatment.[74-76] All reported cases received multiple doses of dexamethasone treatment as glucocorticoid treatment. Dexamethasone is known to reduce croup symptoms, shorten hospital stays and reduce the rate of return visits. However, it is noted that further investigations are required to determine the optimal management of "COVID-19 croup."[74,77]

14 | CURRENT BEST PRACTICE TO *PREVENT* BRONCHOSPASM

It is important to optimize asthma therapy during the pre-operative period to reduce bronchial hyperreactivity.[78] On the day of surgery,

premedication with salbutamol is recommended to prevent perioperative respiratory adverse events, including bronchospasm in children with active respiratory symptoms (eg, upper respiratory tract infection, dry nocturnal cough, recurrent wheeze) and those undergoing ENT surgery.[79] If there is a longer time to prepare the child prior to surgery, then a general optimization of asthma therapy, for example, with an inhaled corticosteroid should be considered.[9,78]

15 | CONCLUSION

We have summarized that the current best practice for relieving bronchospasm caused by acute asthma exacerbation is with SABA delivered by a pMDI and spacer, assuming patient compliance with treatment, and for stridor caused by viral or post-extubation croup is by nebulized adrenaline. While nebulized aerosol delivery involves longer treatment time compared to pMDI and spacer, it offers the benefit of a continuous delivery of multiple medications and can thus be advantageous in a busy emergency department to ensure complete treatment adhering to guidelines. Vibrating mesh nebulizers offer improved delivery compared to jet nebulizers, shorter treatment times with greater efficiency/less drug waste and now come with adaptors to allow concomitant delivery with oxygen. While currently vibrating mesh nebulizers are more expensive than jet nebulizers, evidence is increasing for their use in emergency treatment of bronchospasm for these reasons. A complete system for emergency aerosol delivery, for example, a drug-device combination product, tested with a patient interface, for awake or anesthetized delivery, is yet to come to market and is required to improve drug delivery of aerosolized medications to pediatric patients.

16 | REFLECTIVE QUESTIONS

1. What is the optimal delivery interface for anesthetized patients?
2. What type of artificial airway is best to deliver aerosolized medications?
3. Why does the vibrating mesh nebulizer look likely to replace the jet nebulizer in the future?
4. What is the difference between a drug-device combination product and a device-only product?

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CONFLICT OF INTEREST

BvUS is a section editor for Pediatric Anesthesia.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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Chapter 3: Research article (published): Laboratory assessment of artificial airways (Pediatric Anesthesia, 2020):

Plain language introduction to Chapter 3:

Chapter three is an original article of experimental laboratory studies that were conducted to simulate aerosol delivery to children who use an artificial airway to breathe during surgery. It includes investigation of various types of artificial airways used commonly to intubate children for surgical procedures and the various delivery methods used. It outlines which methods are best at delivering bronchodilator aerosol and which ones should be discontinued. The results from this work were used to improve clinical practice at Perth Children's Hospital. The results from this chapter were necessary pre-requisites to inform forthcoming Chapter 5 – they provided laboratory measurements that were used to support the validation of the aerosol delivery computational model.

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RESEARCH REPORT

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Assessment of different techniques for the administration of inhaled salbutamol in children breathing spontaneously via tracheal tubes, supraglottic airway devices, and tracheostomies

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Abstract

Background: Perioperative respiratory adverse events account for a third of all perioperative cardiac arrests, with bronchospasm and laryngospasm being most common. Standard treatment for bronchospasm is administration of inhaled salbutamol, via pressurized metered dose inhaler. There is little evidence on the best method of attaching the pressurized metered dose inhaler to the artificial airway during general anesthesia.

Aim: The aim of this study is to investigate the best method to deliver aerosolized salbutamol via pressurized metered dose inhaler to the lungs of an anesthetized child.

Methods: We measured salbutamol delivered by pressurized metered dose inhaler through different sized tracheal tubes, supraglottic airway devices, and tracheostomies *in vitro* for methods commonly employed for connecting the pressurized metered dose inhaler to the artificial airway. Breathing was simulated for patients weighing 3, 16, 50, and 75 kg. Pressurized metered dose inhaler actuation coincided with inspiration.

Results: A pressurized metered dose inhaler combined with an in-line non-valved or valved spacer, or the direct method, when delivered via tracheal tube, was linked with improved delivered dose of salbutamol, compared to all other methods for 3 or 50 kg simulated patients weights. The delivered dose when using a non-valved spacer was greater than all methods for 16 and 75 kg patient weights. A spacer improved delivery for the flexible supraglottic airway device type, and there was no difference with or without a spacer for remaining types.

Conclusion: Via tracheal tube and non-valved spacer, the following doses should be delivered after single actuation of a 100 μg labeled-claim salbutamol dose: ~2 μg kg^{-1} per actuation to a 3 kg neonate, ~1 μg kg^{-1} per actuation to a 16 kg child, and ~ 0.5 μg kg^{-1} per actuation for a 50–75 kg child. The least effective methods were the syringe, and the uni- and bidirectional adaptor methods, which require replacement by the direct method if a spacer is unavailable.

KEYWORDS
administration: inhalation, artificial respiration, bronchospasm, child, general anesthetics, metered dose inhaler, Salbutamol

1 | INTRODUCTION

Severe critical events in pediatric anesthesia occur in roughly 5% of patients, with approximately 60% of these being perioperative respiratory adverse events.[1] Perioperative bronchospasm accounts for one third of these cases, that is, 1% of severe critical events, and is potentially life threatening without appropriate treatment.[1-3] Bronchospasm is more common among patients with current respiratory problems, resulting from the combined effect of underlying bronchial hyperresponsiveness, and direct airway stimulation by the artificial airway during airway management.[2,4] This is particularly true if there is a history of recent viral respiratory tract infection.[5] Appropriate and effective treatment is essential to facilitate recovery.

In awake patients without artificial airways, the orally inhaled B_2-agonist—salbutamol—delivered directly to the active (lung) site is the most effective treatment for acute bronchospasm. Patients generally self-administer the aerosol using a pressurized metered dose inhaler (pMDI) with or without a spacer/valved holding chamber or use a dry powder inhaler. The spacer/valved holding chamber is a device that reduces the need to coordinate inhalation with pMDI actuation—larger particles impact in the spacer, which makes the inhaled particles smaller and more likely to reach the lungs. A similar approach to that of awake patients without artificial airways is reported[6] as being the commonly used treatment when a patient develops acute bronchospasm during anesthesia, with orally inhaled salbutamol being delivered via the artificial airway device. Systematic studies[6] report best practice for orally inhaled salbutamol delivery in both ambulant children without artificial airways and mechanically ventilated intubated patients, to be via pMDI and spacer, but no such evidence is available to warrant the same recommendation for spontaneously breathing children with artificial airways.

A variety of approaches to delivering B_2-agonists from pMDIs to the lungs of spontaneously breathing anesthetized children with artificial airways have been proposed and used, but to date there has not been a systematic approach comparing the potential efficacy of these delivery systems. Delivery systems commonly used include the following: 1) direct administration method (interrupted circuit delivery)—direct insertion of manufacturers actuator/mouthpiece containing pMDI, into artificial airway 15 mm port, 2) by pMDI placed inside a syringe (50 mL) connected to luer-port of a right-angle piece, and 3) by pMDI inserted into an in-line unidirectional universal adaptor connected to right-angle piece.[7] Use of any of these systems requires deviation from manufacturer instructions, and thus, dose to the lower airways may be reduced.[8]

What is already known about the topic

More than three quarters of critical incidents in pediatric anesthesia are caused by perioperative respiratory adverse events. Perioperative bronchospasm can have severe consequences for a child and is commonly treated with aerosolized salbutamol.

What new information this study adds

The best method to deliver aerosolized salbutamol by pMDI, to spontaneously breathing child patients, was shown to be by non-valved spacer, and this is consistent with current evidence-based recommendations for ambulant children, and mechanically ventilated adult patients. Some methods such as the syringe and uni- or bidirectional adapter are not effective in administering the labeled-claim dose per actuation, and thus, dose needs increasing if to provide therapeutic benefit, or preferably, the direct method or a spacer should be used.

2 | AIMS AND HYPOTHESES

Our aim was to investigate with *in vitro* laboratory studies the best method to deliver aerosolized salbutamol via pressurized metered dose inhaler to the lungs of a simulated, anesthetized child patient, via tracheal tubes, supraglottic airway devices (SADs), and tracheostomy tubes (TTs), thus provide evidence-based recommendations. Our study had two hypotheses: 1. a spacer method would considerably improve delivery of aerosolized salbutamol to pediatric patients breathing spontaneously through artificial airways, when compared to other commonly used methods; 2. certain artificial airway types would be better for delivering aerosolized salbutamol with a spacer than others; that is, SADs may be better than tracheal tubes.

3 | METHODS

To investigate the amount of salbutamol delivered to spontaneously breathing children through artificial airways we performed two *in vitro* studies. First, total delivery of salbutamol (Ventolin®, GlaxoSmithKline, Boronia, Australia) to a filter was measured for different methods of connecting the pressurized metered dose inhaler (pMDI) to the artificial airway device (Figure 1). Five repeated measurements were taken for each of various combinations of airway type and size within a delivery method (Table 1). A second *in vitro* model was devised to explain what fraction of the delivered dose, obtained in filter studies, would be of a respirable particle size and therefore reach the lower airway. Particle sizing was performed on 5, 7, and 9 mm tracheal tubes with the direct administration method, valved and non-valved spacer methods only. Different experimental set-ups were used for filter and particle sizing experiments respectively (Appendix 1, Figures D and E). Experiments were completed in a temperature-controlled environment.

(A) The pMDI placed inside a 50 mL Syringe+90°angle piece with luer port and plug (Parker Healthcare, Mitcham, VIC, Australia)

(B) Universal Adaptor; 15M-15F, (Intersurgical, Berkshire, United Kingdom) attached to 90° angle piece with luer port and plug (Parker Healthcare, Mitcham, VIC, Australia)

(C) Dual spray in-line adapter - MiniSpacer® Dual Spray MDI adapter (Thayer medical, AZ, USA), 15M-15F

(D) Direct administration/interrupted circuit method: direct insertion of pMDI mouthpiece (Ventolin®, GlaxoSmithKline, Boronia, VIC, Australia) onto artificial airway 15 mm port

(E) In-line collapsible spacer, ~100 mL (Spirale DDS, Koala Medical Pty Ltd, Warriewood, NSW, Australia)

(F) In-line valved spacer, 110 mL (AeroChamber mini™, Trudell Medical, London, Ontario, Canada)

(G) In-line non-valved spacer, 145 mL (AeroChamber® MV, Trudell Medical, London, Ontario, Canada)

(H) Valved spacer (AeroTrach® Plus), 145 mL, attached to pMDI (Ventolin® GlaxoSmithKline, Boronia, VIC, Australia)

FIGURE 1 Methods used to connect pressurized metered dose inhaler (pMDI) to artificial airways. For all devices which are designed to be used in-line (A, B, C, E, F, G) tubing from anesthesia circuit (Meditech, Australia) was connected distal to the artificial airway and drug delivery method. Tracheal tube airways tested set-ups (A–G), supraglottic airway devices tested set-ups (D), and (G). tracheostomy airway tested set-ups (D) and (H) [Colour figure can be viewed at wileyonlinelibrary.com]

3.1 | *In vitro* filter studies

Aerosolized salbutamol was drawn through the artificial airway by simulated breathing patterns generated by a flow-volume simulator (FVS, Research Pneumotach systems, Kansas, USA) and captured on a filter (Uni-Filter, GE Healthcare, CareFusion, Australia). Simulated breathing patterns were created to be appropriate to the average size patient who would use the artificial airway tested (Table F,

TABLE 1 Description of artificial airway types and sizes tested

Airway	Type description	Size (internal diameter, mm)
Tracheal tube	3 mm; KimVent, MicroCuff for pediatrics; Kimberley Clark, Australia, 5,7,9 mm; Portex tracheal tube, soft-set, murphy eye, cuffed; Smiths, Brisbane, Australia 7 mm Reinforced (flexible); Portex tracheal tube, soft-set, murphy eye, cuffed; Smiths, Brisbane, Australia	3,5,7,9 (mm)
SAD	All types tested in both size 2 and 4: a) Second generation SAD #1 (The Dr Brain Supreme™, The Laryngeal Mask Company Ltd., Le Rocher; Victoria, Mahe, Seychelles) b) First generation SAD (PRO-BREATHE Laryngeal Mask Device, Well Lead Medical Co., Guangzhou, Peoples Republic of China) c) Flexible SAD (PRO-BREATHE ArmourFlex Laryngeal Mask Well Lead Medical Co) and; d) Second generation SAD #2 Ambu® Aura I (Ambu®, Glen Burnie, Maryland, USA)	Size (internal diameter, mm) by type and SAD size Type – SAD size (2, 4) a – 7, 12.7 b – 7, 10 c – 7, 10 d – 8.7, 12.7
TT	Pediatric tracheostomy tube cuffed, Shiley™, Covidien™, llc, Mansfield, MA, USA	5, 7, 9 (mm);

Tracheal tubes, of standard type, were tested in sizes 3, 5, 7, and 9 mm internal diameter, and the reinforced (flexible) airway type tested only in size 7 mm. Supraglottic airway devices (SADs) were tested in sizes 2 and 4 with various types (a-d). Tracheostomy tubes (TT) were tested in sizes 5, 7, and 9 mm internal diameter.

Appendix 1). Inspiratory filters, and components of the set-up, were separately rinsed with methanol (~25 mL) and methanol assayed for salbutamol using high-performance liquid chromatography, to obtain representative delivered dose (filters) and total-emitted dose (component set-up).

3.2 | High-performance liquid chromatography assay for salbutamol detection

The method has been previously described.[9] Briefly, the mobile phase was 35:65, methanol to phosphate buffer (20 mM, pH 8.0). The column was a reverse phase C18, 5 µm, 250 × 4.6 mm (Pinacle DB, Restek International, Bellafonte, PA, USA). The solvent delivery system (Varian ProStar Model 210) was used to generate a flow rate of 1 mL min^{-1}. Detection was at 227 nm for ultraviolet detection (Varian ProStar Model 310).

3.3 | *In vitro* assessment of particle size distribution

Subsequent to filter testing, particle size distribution assessment was performed using a Next Generation Impactor (NGI, Copley Scientific, Nottingham, UK). An airflow system was created by introducing air at the same flow rate as the vacuum pump was removing it (Appendix 1, Figure E) and air pumps were confirmed stable. Simulated inhalation occurred from the port-end of the tracheal tube—the end distal to the patient—and into the NGI—representing the patient—using an appropriate driving airflow for each patient size. Each NGI plate was rinsed with methanol and assayed for salbutamol.

3.4 | Drug administration—filter and particle size distribution assessment

After the first five actuations of the canister were wasted[10] and the canister shaken for five seconds, drug was administered in time with each of ten inhalations.

3.5 | Statistical analysis

Salbutamol captured on filters (delivered dose) for each delivery method was compared initially for each artificial airway type, by size (Table 1), assuming non-parametric distribution, using non-parametric ANOVA—the Kruskal-Wallis test, with the Dunn's multiple comparison test. Subsequently, a post hoc analysis was completed comparing the median delivered dose of salbutamol from each method, with the median of all other methods, separately, using a non-parametric t-test, the Mann-Whitney test. GraphPad Prism (v 8.0.1 for Windows, GraphPad Software, San Diego, California, USA) was used for all analysis and a 95% confidence level chosen. For clinical purposes, secondary analysis was completed; the delivered salbutamol dose was divided by the kilogram value for each respective age group in Table F, Appendix 1, to obtain the µg kg^{-1} per actuation dose, and analysis completed in an identical fashion to that described above for the delivered dose to confirm the differences

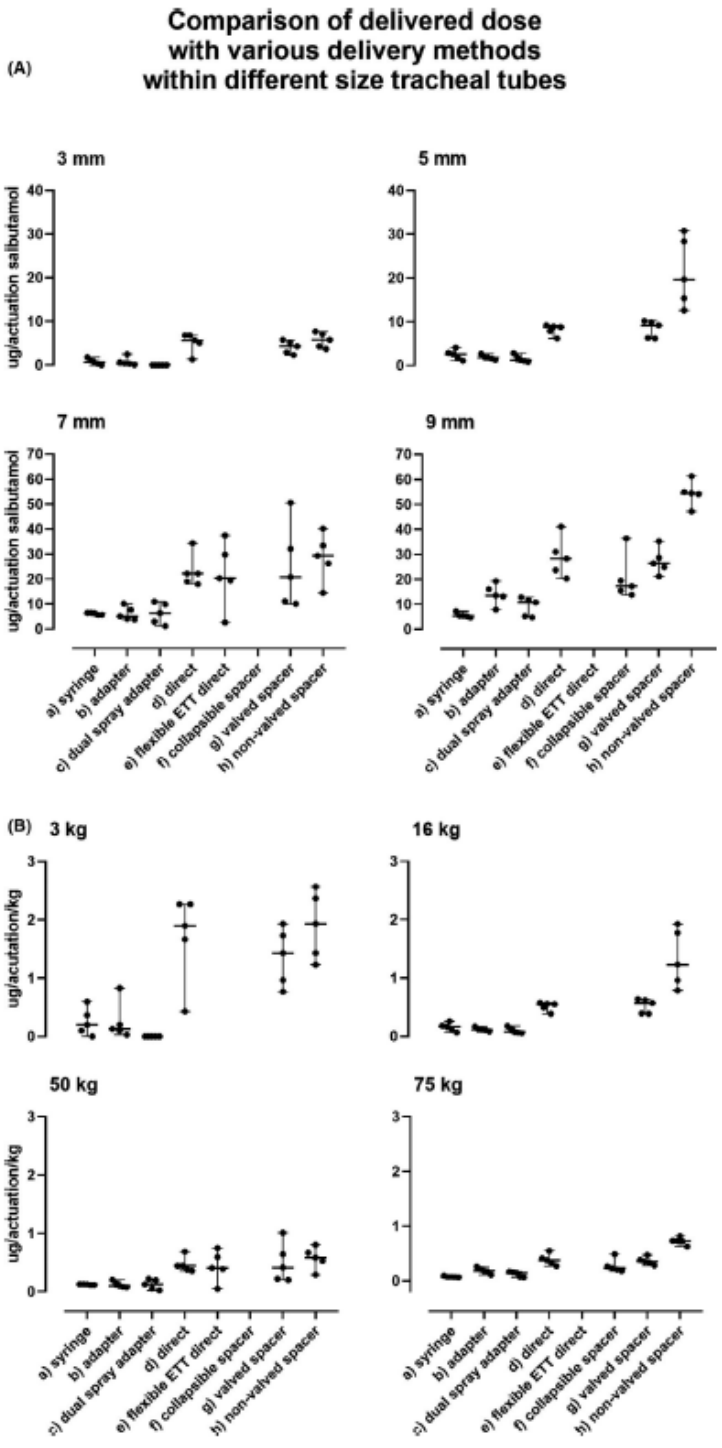

FIGURE 2 Median (95% CI) salbutamol µg/actuation (A) or µg kg^{-1} per actuation (B) exiting tracheal tube, when delivered through different sizes by pressurized metered dose inhaler with various delivery methods. Delivery methods include direct methods (A, B, C, D, E), and spacer methods (F, G, H): (A) 50 mL syringe inserted into 90° angle piece with luer port, (B) In-line unidirectional adapter, (C) Dual spray in-line adapter, (D) Direct administration/interrupted circuit method: direct insertion of pMDI actuator onto artificial airway 15 mm port, (E as per D), but with reinforced tracheal tube (flexible), (F) Collapsible in-line spacer – 100 mL, (G) In-line valved spacer, 110 mL, and (H) In-line non-valved spacer 145 mL. For all sizes, there was a difference between methods after non-parametric ANOVA. After t-test individual comparisons, the non-valved spacer was different to all other methods with a 5 and 9 mm size, but both spacers and the direct method were different to all other methods with the 3 and 7 mm size (there was no difference between spacers and direct method). All statistical differences and no differences remained with µg.kg^{-1} transformation. A representative 3 kg patient would receive the highest salbutamol dose, – 2 µg kg^{-1} of any patient size, when using spacer methods (F, G) or the direct method, (D) (B). Collapsible spacer only assessed for 9 mm tracheal tube. Direct method (flexible), (E), only assessed for 7 mm tracheal tube

remained. Although not directly statistically comparable, for clinical purpose different artificial airway types were compared graphically. For quality control, total-emitted dose was reported (Table B, D, E Appendix 1) and delivered dose data analyzed as percent of total-emitted (Appendix 1, Table A, C).

4 | RESULTS

4.1 | Tracheal tubes

For a 3 mm tracheal tube size, the delivered dose of salbutamol when using a non-valved spacer (median 5.8 μg) was more than that of the syringe, adapter, and dual spray adapter (median 0.6, 0.4, 0.0 μg, CIs = 2.6 to 7.1, 3.1 to 7.3, and 3.7 to 7.7 respectively, Figure 2A). The non-valved spacer and direct method, and non-valved spacer and valved spacer method, did not show evidence of a difference in delivered dose (CIs = −2.5 to 4.5, and −1.5 to 4.8, Figure 2A). For a 5 mm tracheal tube size, the delivered dose of salbutamol when using a non-valved spacer (median 20 μg) was greater than all other methods—the syringe, adapter, dual adapter, direct method, and valved spacer (median 2.6, 1.8, 1.2, 8.8, 9.2 μg, CIs = 10 to 28.2, 10.8 to 29, 11.4 to 29.6, 3.8 to 22.2, and 3.4 to 22.2 respectively, Figure 2A). For a 7 mm tracheal tube size the non-valved spacer delivered more drug (median 29 μg) than the syringe, adapter and dual adapter (median 6.3, 5.1, 6.4 μg, CIs = 8.2 to 34, 9.4 to 35.1, and 8.1 to 33.8, respectively). The non-valved spacer and direct method, and non-valved spacer and valved spacer, did not show evidence of a difference in delivered dose (CIs = −7.70 to 18, and −21.3 to 23.3, Figure 2A). For a 9 mm tracheal tube size, the non-valved spacer delivered more drug (median 55 μg) than all other methods—the syringe, adapter, dual adapter, direct method, collapsible and valved spacer methods (median 5.4, 14, 11, 28, 26 μg, CIs = 41.8 to 55.9, 33.7 to 47.8, 36.4 to 50.5, 13.4 to 34.6, 18.08 to 44.02, and 18.8 to 34.9 respectively, Figure 2A). Only the three most effective methods, the non-valved and valved spacer, and the direct method, generated at least the labeled-claim dose of 100 μg ± 10%, or higher (Appendix 1, Table B).

When drug delivered for each tracheal tube size was divided by patient weight to achieve a μg kg^{-1} per actuation dose, and assessed within each delivery method all differences and no differences from statistical analysis of μg delivered dose remained. The representative 3 kg patient received more drug per kilogram bodyweight than any other patient weight, when using any of the three most effective methods—the direct method or, the valved or non-valved spacer, 1.9, 1.4, 1.9 μg kg^{-1} per actuation, respectively (Figure 2B). This was followed by the 16 kg patient at 0.6, 0.6, and 1 μg kg^{-1} per actuation, respectively, for the top three methods, the 50 kg patient, 0.4, 0.4, 0.6 μg kg^{-1} per actuation, and the 75 kg patient, 0.4, 0.4, and 0.7 μg kg^{-1} per actuation, respectively (Figure 2B). All representative patient weights received negligible drug via adapters or syringe (Figure 2B).

4.2 | SADs

For all SAD types tested within size 2, the delivered dose of salbutamol was assessed when using the non-valved spacer method and compared to the direct method. The non-valved spacer only improved delivered dose for a flexible SAD type (median 7.8 μg with spacer, 5 μg direct, CI = 1.15 to 5.58), there was no evidence for a difference for all other SAD types (First generation with spacer 8.3 μg, direct 4.9 μg, CI = −2.9 to 5.6, second generation #1 with spacer 20.5 μg, direct 4.7 μg, CI = −0.7 to 21.5, second generation #2 with spacer 7.4 μg, direct 10.8 μg, CI = −12 to 7.4, Figure 3A). When size 2 SAD types were compared to each other, using the direct method, the delivered dose of salbutamol, with a second generation #2 SAD type, was greater (median 10.8 μg) than all other SAD types (median 5, 4.9, 4.7 μg, flexible, first generation, and second generation #1 SADs respectively, CIs = 1.2 to 14.5, 0.9 to 14.4, and 1.5 to 14.6, Figure 3A). There was no evidence of a difference in delivered dose between the flexible and first generation, flexible and second generation #1, or second generation#1 and first generation type SADs, (CIs = −3.2 to 2.3, −2.5 to 2.4, and −2.8 to 2.1). When size 4 SAD types were compared to each other, using the direct method, the delivered dose of salbutamol, with a second generation #2 SAD type, was greater (median 23.3 μg) than all other SAD types (median 12.4, 13.3, 17.2, μg, flexible, second generation #1, and first generation SADs respectively), and evidence for this increase can be seen for the first two comparisons, the third may only be seen as a trend (respective CIs = 6.2 to 43.5, 0.23 to 39.7 and −0.31 to 35.2, Figure 3A). When drug delivered by the direct method for each SAD size 2 type and size was divided by patient weight to achieve a μg kg^{-1} per actuation dose, all differences and no differences from statistical analysis of μg delivered dose remained. For a SAD size 2, the representative 16 kg child would receive the most drug, median 0.7 μg kg^{-1} per actuation, with the second generation #2 SAD type, and median 0.3 μg kg^{-1} with all other types (with direct method, Figure 3B). For a SAD size 4, the representative 50 kg child would receive the most drug, median 0.5 μg kg^{-1} with the second generation #2 SAD type, and 0.3 μg kg^{-1} for all other types.

4.3 | Tracheostomies

Tracheostomy tubes were compared for three sizes—5, 7 and 9 mm—when using the direct or spacer method to administer salbutamol. There was no evidence for an improvement of delivered dose with the addition of a spacer—the direct method was more effective for a 5 and 7 mm tracheal tube (median 9.3, 18 μg, CIs = 0.98 to 10.3, 0.37 to 4.2, for 5 and 7 mm respectively) and there was no evidence of a difference between methods with a 9 mm tracheal tube (22 and 24 μg for direct and spacer respectively, CI = −6.1 to 13.7, Figure 4A). When drug delivered for each tracheostomy tube size was divided by patient weight to achieve a μg kg^{-1} per actuation dose, statistical differences and no differences remained. The representative 16 kg child received the most drug with the direct method, median 0.6 μg kg^{-1} per actuation, and the 50 kg child,

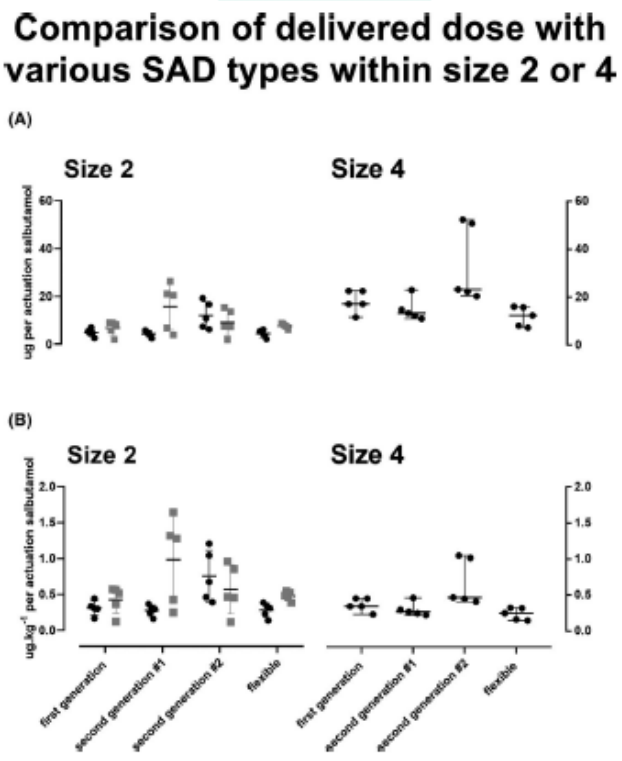

FIGURE 3 Median (95% CI) salbutamol µg per actuation exiting SAD (A), or µg kg^{-1} per actuation (B), compared within SAD size when using the either the direct administration method (circles) or by non-valved spacer delivery (squares) with various SAD types. SAD = Supraglottic airway device. SAD size two was divided by a 16 kg weight and size four by a 50 kg weight. With direct administration (circles), the second generation #2 SAD type resulted in the most drug exiting the SAD, in both size two and four. When size two SADs were compared between delivery with a spacer (squares) and the direct method (circles), a difference was seen only for the flexible type. SAD types used were as follows: (A) first generation SAD, (B) second generation #1, (C) second generation #2, and, d) flexible SAD

median 0.4 µg kg^{-1} (Figure 4B). The 75 kg patient, with either spacer or direct method, median −0.3 µg kg^{-1} per actuation (Figure 4B).

For our non-statistical comparison of different artificial airways used for a 16 or 50 kg patient, the largest dose for both patient sizes was delivered by tracheal tube/non-valved spacer combination, median 1 or 0.6 µg kg^{-1}, respectively, followed by the second generation #2 SAD/direct method combination, at 0.7 or 0.5 µg kg^{-1} per actuation respectively, Figure C, B, Appendix 1.

Respirable fraction was greater for the non-valved spacer than the direct method for both 5 and 7 mm tracheal tubes (5mm with spacer 100%, direct 0%, CI = −100 to −83, 7 mm with spacer 79%, direct 52%, CI = 16.8 to 38.9), but not for a 9 mm size (72% with spacer, 69% direct, CI = −4.5 to 8.4), Figure A, Appendix 1.

5 | DISCUSSION

We demonstrated that some commonly used methods for delivering aerosolized salbutamol in anesthetic settings do not deliver the intended salbutamol dose, and we highlight effective methods for drug delivery comprehensively for tracheal tubes, and more simply for SADs and TTs (Infographic, Figure 5). We compare effectiveness of salbutamol delivery via these types of artificial airways within certain patient sizes. Our findings should be of particular interest to anesthetists who typically administer aerosolized salbutamol in emergency situations.

The effectiveness of each method is summarized in Figure 5, where methods five, six and seven delivered less drug than what ambulant children without artificial airways receive,[11-14] likely a result of lower than labeled-claim dose per actuation. Methods one, two and three (Figure 5) actuated at least the labeled-claim dose or more, and all gave a delivered dose of at least what an ambulant child without artificial airways is reported to receive.[11-14] For example, delivery of drug through a 3 mm tracheal tube artificial airway when combined with a non-valved spacer in our model was 5.7 µg (5.6% of total-emitted (measured) dose per actuation), which is comparable to awake neonates using a spacer and facemask in a radiolabeled *in vivo* study (−4.5% of total-emitted dose).[11] Similarities to awake delivery can be explained because the loss of drug available for inhalation with in-line spacer methods (ie, loss to set-up, −60% spacer, and 10–15% tracheal tube) is comparable to that deposited in the facemask and spacer when ambulant children without artificial airways use a spacer and pMDI, thus giving a comparable inhaled dose.[13] Therefore, to ensure that the labeled-claim dose is actuated, and the child will receive at least the delivered dose that an ambulant child without artificial airways would, per actuation, a valved or non-valved spacer method, or direct method of administration should be employed routinely.

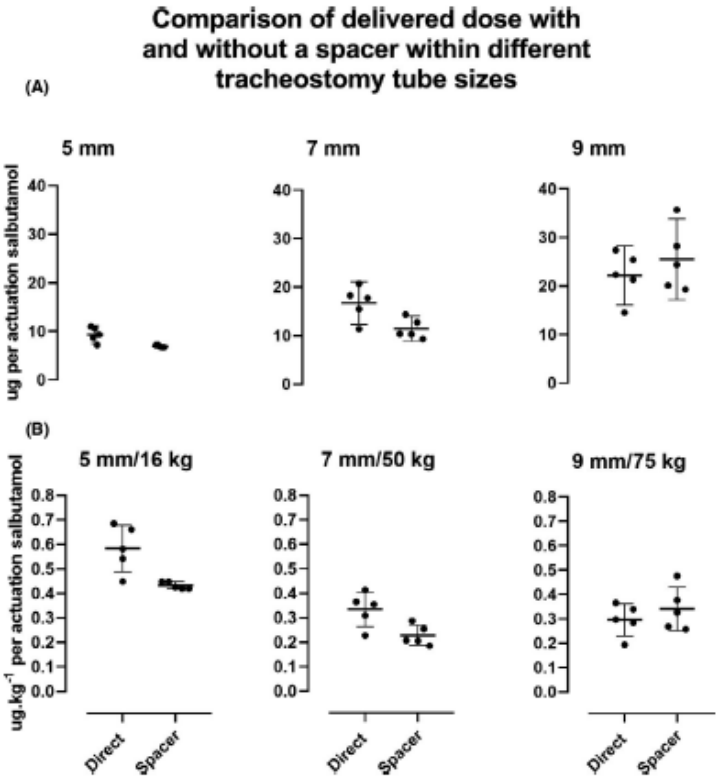

FIGURE 4 Median (95 % CI) salbutamol µg/actuation (A) or µg kg^{-1} per actuation (B) exiting TT airway. TT = tracheostomy tube. Airway sizes of 5, 7, and 9 mm were divided by representative patient sizes of 16, 50 and 75 kg respectively. Delivery was via pressurized metered dose inhaler either via the direct administration/interrupted circuit method: direct insertion of pMDI actuator (Ventolin®, GlaxoSmithKline, Boronia, VIC, Australia) into artificial airway 15 mm port, as with method in Figure 1D for tracheal tube but with TT instead (Pediatric Tracheostomy tube, cuffed, Shiley™, Covidien™, Mansfield, MA, USA), or with additional valved spacer (AeroTrach Plus®, Trudell Medical, London, Ontario, Canada), Figure 1H. There was no evidence of a difference in delivered-dose with the addition of a spacer for any size TT. The direct method of administration, delivered more drug for both 5 and 7 mm tracheostomy tubes

The non-valved spacer delivered a larger dose than any other method, for both 5 and 9 mm tracheal tubes, and 100 and 72% of this respectively was respirable (<5 µm diameter). Similar delivered doses were achieved via a 5mm ETT by Garner & Wiest,[15] and with an 8 mm (comparable to 9 mm) ETT by Ran & Harwood[16] when combined with a non-valved spacer (AeroVent Plus®, 155 mL). While there was a clear trend indicating use of the non-valved spacer resulted in an increased delivered dose when compared to the direct method—sometimes there was no evidence for this difference; that is, the direct method was as effective. For example, our results showed no evidence of a difference between the non-valved spacer and the direct method, when tested with three out of four types of SAD in a size two, no difference with or without a spacer for a 9 mm cuffed tracheostomy tube, and no difference for a 3 or 7 mm tracheal tube. Regardless, with tracheal tubes we show that respirable fraction is greater with a spacer than the direct method. So considering either the spacer was more effective, or there were no differences in delivered dose with or without a spacer for tracheal tubes and SADs, we recommend the use of an in-line non-valved spacer (~140 mL) to maximize the respirable fraction of aerosolized drug via any size tracheal tube or SAD type tested here.

A few findings were contrary to our initial assumptions that a spacer would improve delivery with all artificial airways. In fact, a tracheostomy tube (size 5 or 7 mm) used with the direct method increased delivered dose compared to a spacer, however with a 9 mm tube there was no difference with or without a spacer, indicating that any effect of small tube size had reached its maximum.

Filter studies that utilize breathing simulators to mimic inhalation patterns, have long been used to assess total drug delivery of an inhalation device. Filter studies are often coupled with aerosol particle sizing methods to characterize the particle size distribution of the delivered aerosol, and results from both types of studies are used in attempts to predict total, and regional aerosol lung deposition.[17,18] The discrepancy between the results from these *in vitro* studies and their *in vivo* counterpart studies is minimal when assessing total lung dose (usually via radiolabeled lung deposition studies). For example, a lung deposition study in mechanically ventilated infants reported ~1% of emitted dose deposited in the lungs when using a pMDI with non-valved spacer (AeroChamber® MV, 145 mL),[19] and a simulated mechanically ventilated infant study, under similar ventilatory conditions, reported deposition of 2.3% of emitted dose.[15]

There are three key contributors to variance in our study: 1) expected variance is ±10% just from the canister as reported by the manufacturer, 2) slight timing errors have a minor effect that will increase variation.[20] The likelihood of timing error therefore increases with the number of actuations used. For example, Piccuito and Hess[21] took the average of four actuations per experiment and had low variance, however we took the average of ten doses, 3) variance was also

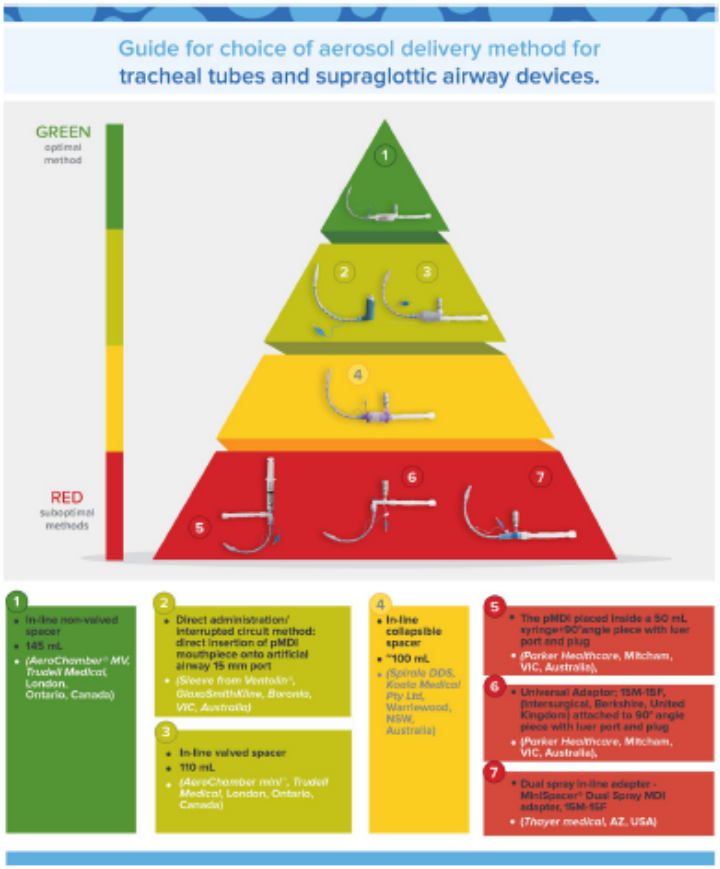

FIGURE 5 Guide for choice of aerosol delivery method for tracheal tubes and supraglottic airway devices. Best choice for aerosol delivery is #1, the non-valved spacer, in decreasing order through to #7. *supraglottic airway device types = probreathe (first generation), supreme (second generation #1), flexible, or Ambu (second generation #2) [Colour figure can be viewed at wileyonlinelibrary.com]

introduced by the way we used the canister, that is, 10 puffs at a time, without shaking in between—only beforehand—as would happen in an emergency situation but not what the manufacturer recommends. In the clinical scenario, to shake in between every dose would mean disconnection after every puff administered, which is unlikely to happen in the emergency situation for a ventilated patient.

Our study was novel in several ways: Firstly, we simulated use of the pMDI in an emergency situation, without shaking between 10 consecutive actuations. This is not how the manufacturer specifies the device to be used, thus there is a paucity of evidence using this technique. Secondly, we report drug delivery through artificial airway via the commonly used "direct administration" or "interrupted circuit" method. The direct administration method requires interrupting the ventilation circuit, which is not possible for mechanically ventilated patients—whom are reported on more frequently than spontaneously breathing patients. Finally, we report delivery through a SAD, where a paucity of evidence exists for the optimal method with this device—yet it is the most commonly used airway device in modern anesthesia.

In conclusion, in a simulated clinical emergency in spontaneously breathing children, the in-line non-valved spacer delivery method was the most effective method at delivering aerosolized salbutamol through artificial airways and Figure 5 can be used as a guide for choosing administration methods for tracheal tubes and SADs. If a spacer is not available, and the breathing circuit can be interrupted for a brief period, then the direct method is the next best delivery method for all artificial airway types and should be used in favor of the syringe and adapter methods. If the syringe and/or adapter methods are the only options available, then the dose required to achieve a therapeutic effect will be greater than our following dose estimates for a non-valved spacer coupled with a tracheal tube. Approximately 2 $\mu g\ kg^{-1}$ per actuation for a 3 kg neonate, ~1 $\mu g\ kg^{-1}$ per actuation for a 16 kg child, and ~ 0.5 $\mu g\ kg^{-1}$ per actuation for a 50–75 kg child/adult. When a non-valved spacer is coupled with the second-generation#2 SAD, 16 and 50 kg patients would receive 0.7 or 0.5 $\mu g\ kg^{-1}$ respectively. All other SAD types and sizes, and TT sizes received less: ~0.3–0.5 $\mu g\ kg^{-1}$ per actuation.

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design, data collection, data analysis, data interpretation, or writing of the article.

CONFLICT OF INTEREST

BvUS is a section editor for Pediatric Anesthesia. Otherwise, the authors declare that they have no conflict of interest.

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APPENDIX 1

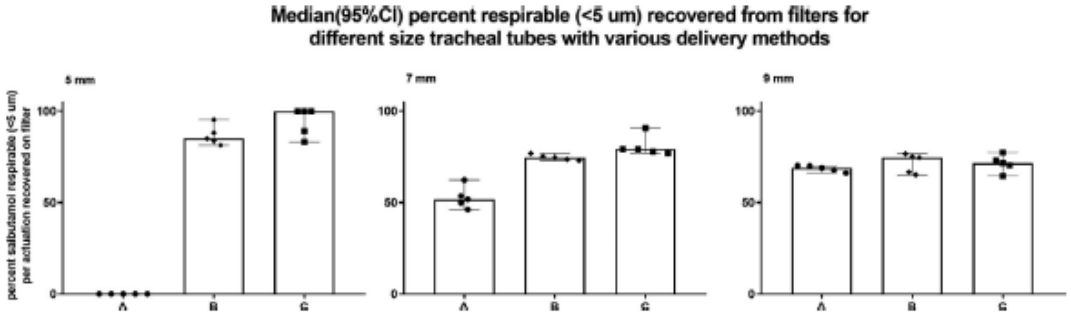
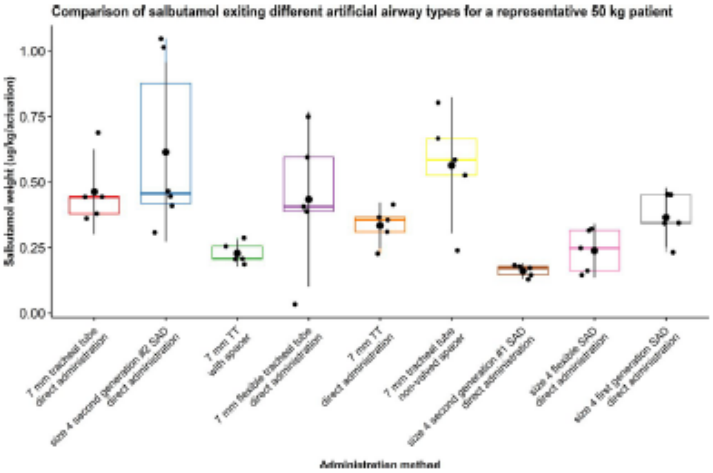


FIGURE A Median (95% CI) percent exiting tracheal tube under 5 µm mass median aerodynamic diameter (respirable). A = Direct administration method, B = Valved spacer method, C = Non-valved spacer method. Approximately 70% of aerosol that will exit the tracheal tube is respirable for all methods with a 9 mm tracheal tube, and for the valved and non-valved spacer methods with a 7 mm tracheal tube. Approximately half of the drug exiting the tracheal tube with the direct method for a 7 mm tracheal tube was respirable, and none for a 5 mm tracheal tube. When using a 5 mm tracheal tube with a valved or non-valved spacer, nearly all of the drug exiting the tracheal tube will be respirable

FIGURE B Mean (95% CI), black star, blackline, with boxplot (range) salbutamol µg kg^{-1} per actuation exiting various artificial airways types for a 50 kg patient. The top three delivery methods were: the 7 mm tracheal tube/non-valved spacer method, the size 4 second generation #2 SAD/direct administration both with mean ~0.6 µg kg^{-1} per actuation, and the 7 mm tracheal tube (flexible or standard)/direct administration with ~0.5 µg kg^{-1} per actuation. The TT with or without a spacer and all other SAD types delivered ~0.3 µg kg^{-1} per actuation or less. TT, tracheostomy tube [Colour figure can be viewed at wileyonlinelibrary.com]



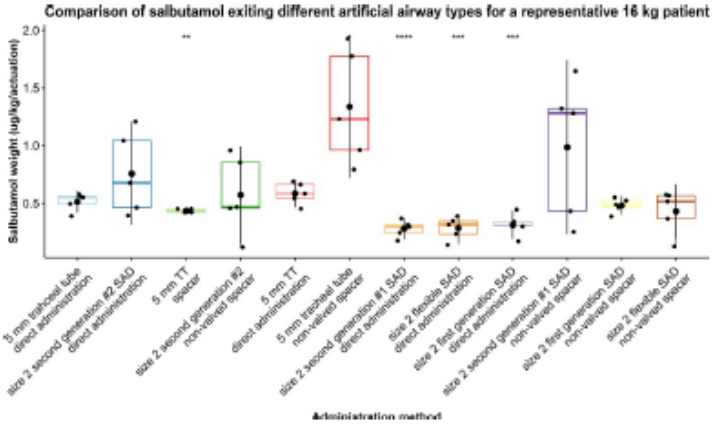


FIGURE C Mean (95 % CI), black star, blackline, with boxplot (range) salbutamol μg kg^{-1} per actuation exiting various artificial airways types for a 16 kg patient. The top three delivery methods were; the 5 mm tracheal tube/non-valved spacer with mean –1.5 μg kg^{-1} per actuation, the size 2 second generation #1 SAD/non-valved spacer with mean –1 μg kg^{-1} per actuation, and the size 2 second generation #2 SAD/direct administration with mean –0.75 μg kg^{-1} per actuation. The tracheal tube direct, TT with or without a spacer, and all other SAD types and methods delivered ≥ –0.5 μg kg^{-1} per actuation. TT, Tracheostomy Tube, SAD, Supraglottic Airway Device [Colour figure can be viewed at wileyonlinelibrary.com]

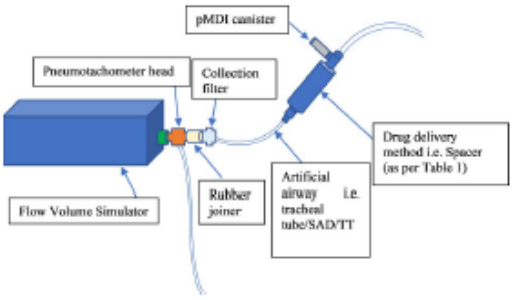


FIGURE D In vitro flow domain simulating a spontaneously breathing child. Breathing simulated by a flow-volume simulator passes through a: tracheal tube or, supraglottic airway device (SAD) or, tracheostomy tube (TT), that is connected to a drug delivery device method for a pressurized metered dose inhaler (in-line spacer demonstrated here, see Figure 1). Drug is captured on collection filter. Flow measurements were constantly taken with the pneumotachometer to confirm breathing parameter accuracy and assess inhalation timing for timing of pressurized metered dose inhaler (pMDI) actuation to coincide with the beginning of inhalation. During experimentation, the pMDI device was actuated while in the upright position [Colour figure can be viewed at wileyonlinelibrary.com]

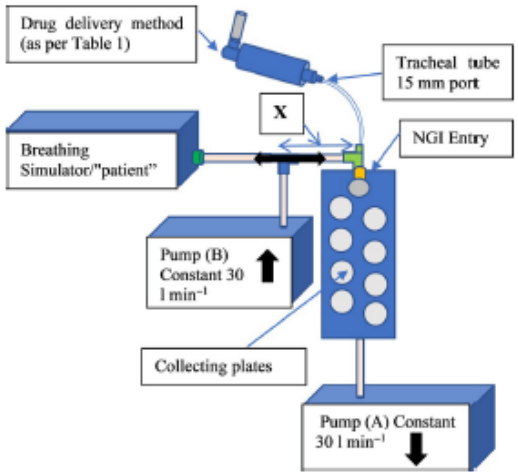


FIGURE E Flow domain set-up to assess particle size distribution after simulated breathing based on a spontaneously breathing child on ventilatory support. Airflow direction is indicated by the thick black arrows. A pneumotachometer was used in place of pMDI canister and spacer while validating the system. It was confirmed that the breathing pattern measured at the breathing simulator port was the same as that at the port of the tracheal tube while pumps were running. Pump A pulls air through at a rate of either 30 or 60 L min^{-1}. Pump B introduces replacement air at the equivalent rate to that exiting via pump A, thereby ensuring zero flow at point X in between breaths. Breathing pattern inspiration and expiration runs from spacer and pMDI to breathing simulator. The "breath" exiting the tracheal tube is preferentially drawn into the 30 L min^{-1} flow of the NGI. During experimentation, the pMDI device was actuated while in the upright position.
NGI, Next Generation Impactor [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE A Comparison of delivered dose with various delivery methods within different size tracheal tubes

	Median salbutamol delivered to filter, μg per actuation, median n = 5 (% of median total-emitted)				
	Tracheal tube size (internal diameter)				
Administration device	3 mm	5 mm	7 mm	9 mm	Average μg(% of median total-emitted)
a) Syringe(*)	0.6 (1.3)	2.6 (4.9)	6.3 (15.2)	5.4 (12)	3.7 (8.35)
b) Adapter(*)	0.4 (0.5)	1.8 (2.5)	5.1 (7.9)	14 (19)	5.3 (7.5)
c) Dual Spray	0 (--)	1.2 (--)	6.4 (--)	11 (--)	4.65 (--)
d) Direct Administration	5.7 (4.3)	8.8 (10)	22 (19.4)	28 (26.3)	16 (15)
e) Flexi Direct	--	--	20 (--)	--	(--)
f) Collapsible Spacer	--	--	--	17 (--)	(--)
g) Valved Spacer	4.3 (5.2)	9.2 (12.4)	21 (20.3)	26 (26.3)	15 (16.1)
h) Non-valved Spacer	5.8 (5.7)	20 (17.3)	29 (24.5)	55 (44.1)	27.5 (27.4)

(*)Please note total measured is low for these methods, see Table B.

TABLE B Comparison of total-emitted dose with various delivery methods within different size tracheal tubes

	Measured total-emitted salbutamol, μg per actuation, median (range), rinsed from testing components and identified with HPLC, (median n = 5 runs) shake only before first actuation, 10 actuations per run. (Labeled-claim dose = 100 μg+/- 10%)				
	Tracheal tube size (internal diameter)				
Administration device	3 mm	5 mm	7 mm	9 mm	Average, all sizes
a) Syringe(*)	44.2 (44.3–37.7)	53 (58.2–42.3)	41.6 (46.1–18.9)	45.1 (63.2–37.9)	46
b) Adapter(*)	84.4 (95.9–46.9)	72.2 (94.1–58.6)	64.6 (77–52.5)	73.7 (86.1–56.5)	72.2
c) Dual Spray	--	--	--	--	--
d) Direct Administration	132.9 (214.3–75.7)	88.4 (145.3–78.7)	113.2 (188.1–99.4)	106.5 (194–90.9)	110.25
e) Flexi Direct	--	--	--	--	--
f) Collapsible Spacer	--	--	--	--	--
g) Valved Spacer	82 (94.5–75.9)	74.3 (90.3–70.2)	103.3 (209.5–68.8)	99.1 (130.8–69.9)	90
h) Non-valved Spacer	102.2 (121.6–84.9)	115.9 (305.8–102.3)	118.2 (152.6–66.2)	124.6 (238.3–107.5)	115

TABLE C Comparison of delivered dose with various supraglottic airway device types within size 2 or 4

	Median salbutamol delivered to filter, μg per actuation, (% of median total-emitted, n = 5)		
	Methods of administration in size 2 or 4		
	2		4
Supraglottic airway device types	Direct Administration	Non-valved spacer	Direct Administration
a) Second generation #2 (Ambu®)	10.8 (10.5)	7.4 (7.3)	23.3 (16.6)
b) Flexible LMA	5 (4.2)	7.8 (9.1)	12.4 (11.5)
c) First generation LMA (Probreathe)	4.9 (4.4)	8.3 (7.7)	17.2 (12.3)
d) Second generation #1 (Supreme™)	4.7 (5)	20.5 (16.6)	13.3 (8.6)

TABLE D Comparison of total-emitted (measured) dose with various supraglottic airway device types within size 2 or 4

	Measured total-emitted salbutamol, μg per actuation, median (range), rinsed from testing components and identified with HPLC, (n = 5 runs) shake only before first actuation, 10 actuations per run. (Claim dose = 100 μg+/- 10%)		
	Methods of administration in size 2 or 4		
	2		4
Supraglottic airway device types	Direct Administration	Non-valved spacer	Direct Administration
a) Second generation #2 (Ambu®)	102.7 (107.5–72.8)	101.7 (112.9–76.1)	140 (106–147)
b) Flexible LMA	118.8 (129.2–69.9)	86 (117.8–72.74)	108 (74.1–125)
c) First generation LMA (Probreathe)	112.6 (129.8–94.8)	107.6 (143.1–81.9)	140 (106–159)
d) Second generation #1 (Supreme™)	93.3 (101.2–44)	123.1 (139.9–86)	155 (139–252)

TABLE E Percent measured on device components for the top #3 performing methods via a tracheal tube

	Percent measured on device components for the direct administration method, non-valved and valved spacer methods.				
	Tracheal tube sizes				
	9 mm	7 mm	5 mm	3 mm	Average (SD)
Direct Administration Method					
Filter	24	17	8	5	14 (8)
Tracheal tube	67	77	88	82	79 (8)
Actuator	9	5	5	14	8 (4)
No end-filter	0	0	0	0	0
Non-valved spacer					
Filter	39	24	14	6	21 (12)
Tracheal tube	7	21	4	4	9 (7)
Spacer	51	44	69	80	61 (14)
End-filter	6	11	12	10	10 (2)
Valved spacer					
Filter	28	18	11	5	16 (9)
Tracheal tube	13	26	15	6	15 (7)
Spacer	58	48	66	83	64 (13)
End-filter	2	5	9	6	6 (3)

TABLE F Ventilator settings for each representative patient's size of artificial airway, used to program the flow volume simulator. Breath parameters were chosen to simulate the breath of the average patient in which the artificial airway would be used. TT, Tracheostomy Tube; SAD, Supraglottic Airway Device

Tracheal tube size, TT size, SAD size (internal diameter, mm/length, cm)	Assumed Patient Weight (approximate) (kg)	Age (approximate) (yrs)	Breaths per minute	Peak volume flow rate for tracheal tube ($m.s^{-1}$)	Breath volume (ml)
Tracheal tube = 9 mm/32 cm, TT = 9 mm/8.5 cm, SAD = N/A	75	18+	12	4.2	500
Tracheal tube = 7 mm/30 cm, TT = 7 mm/7.5cm, SAD = differs by type, see Table 1	50	16	15	6.1	300
Tracheal tube = 5 mm/24 cm, TT = 5 mm/4.5 cm, SAD = differs by type, see Table 1	16	4	25	8.7	96
Tracheal tube = 3 mm/16 cm, TT = N/A, SAD = N/A	3	0–1	30	7.9	18

Chapter 4: Laboratory simulation of nebulised tobramycin through tracheostomy to spontaneous breathing children

Plain language introduction to Chapter 4:

Chapter 4 was designed to obtain laboratory experimental results for nebulised tobramycin (liquid) aerosol delivery, to ensure that both a solid and a liquid particle had been measured experimentally as these represent both types of medicinal aerosol on the market. Additionally, Inhaled tobramycin is an antibiotic – which must be delivered responsibly in alignment with antibiotic resistance protocols. Results of this chapter indicate that tobramycin delivery was severely reduced to children with tracheostomy airways. Aerosol delivery was reduced due to liquid-film build-up inside the tracheostomy tube which then led to droplet formation and partial or complete blockage of the airway until the droplet was released. The information obtained in this chapter will help to build an evidence base for which to form guidelines from, as currently guidelines do not exist for aerosol delivery of tobramycin to children who breathe through a tracheostomy airway. Furthermore, experimental data obtained in this chapter are required to build a future in silico, liquid particle, model of aerosol delivery that can be used to support experimental data.

Laboratory simulation of nebulised tobramycin through tracheostomy to spontaneously breathing children

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What's Known on This Subject

Tracheostomy-associated respiratory infections (TRAINs) are common and treated with inhaled, nebulised tobramycin, yet current dosing guidelines refer only to children without tracheostomies. Aerosolized medicine delivery through artificial airways, like a tracheostomy tube, is usually less efficient than through native airways.

What This Study Adds

This study shows that the delivered dose of tobramycin through a tracheostomy airway is between 1–15% of the dose administered and is received predominantly via droplets from the tracheostomy tube to the child's upper airway, rather than as an aerosol. Consequently, only a small percentage of the drug will reach the lungs during aerosolized drug delivery which has to be incorporated in any dosing regimen.

Contributors Statement Page

CRediT statement: Natalie Anderson: conceptualization, data curation, formal analysis, investigation, methodology, project administration, visualization, writing – review and editing, writing – original draft.: **Dr William Ditcham**: conceptualization, formal analysis, funding acquisition, methodology, resources, supervision, visualization, writing – review and editing, writing – original draft.: **Associate Professor Barry Clements**: writing – formal analysis, review and editing, writing – original draft: **Professor Britta S. von Ungern-Sternberg:** conceptualization, formal analysis, funding acquisition, resources, supervision, visualization, writing – review and editing, writing – original draft.

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Abstract

Objective: TRacheostomy-Associated respiratory INfections (TRAINs) are common and treated with inhaled tobramycin but lack guidelines to standardize diagnosis and care. This study aimed to investigate the in vitro dose of nebulised tobramycin delivered to simulated spontaneously breathing children with tracheostomy airways. **Methods**: A breathing simulator was programmed for volume-controlled ventilation at 6 mL/kg, for a 3 and 16 kg child representing a child under or over 6 years of age. Tobramycin, 80 mg, or 300 mg for under and over 6yrs respectively, was delivered as per the standard hospital protocol and collected on filters. High-Performance Liquid-Chromatography Mass-Spectrometry was used to assay for tobramycin antibiotic collected on filters and confirm delivered dose. **Results:** The jet nebuliser delivered more tobramycin than the vibrating mesh nebuliser from an 80 mg (ages <6yrs) dose for both a 3 kg child: 2.1 vs 0.7 mg (3 mm, p=0.047) and a 16 kg child: 8.7 vs 3.5 mg (5 mm size, p =0.022), 11.4 vs 10 mg (4 mm size, p=0.43). The jet nebuliser delivered more tobramycin than the vibrating mesh nebuliser from a 300 mg dose for 3 and 5 mm tube sizes: 8.4 vs 3.7 mg (3 mm, p=0.00076), 33.2 vs 25 mg (5mm, p=0.2) but not for the 4 mm size (39.4 vs 46.5 mg, p=0.1). **Conclusion**: When administering inhaled tobramycin to patients via tracheostomy, the low availability of drug must be incorporated into the appropriate dosing regime.

INTRODUCTION

Tracheostomy is performed in children with long term need for invasive ventilatory support who are often living with complex chronic medical conditions (Chawler et al., 2021; Chia et al., 2020; de Araujo, 2022; de Trey, 2013; Resen, 2018; Watters, 2017). TRacheostomy-Associated respiratory INfections (TRAINs), including pneumonia and bacterial tracheitis, are common as tracheostomies bypass many of the first-line barriers to infection provided by the upper respiratory tract. Incidence of TRAINs in children in the pediatric intensive care unit, is 1.8–8.3 per 1000 ventilator days and in children who are chronically tracheostomy dependent, 30–40 percent will be readmitted to hospital within 12 months of tracheostomy placement with a lower respiratory tract infection (Gipsman 2022; Woods, 2022). The most common pathogen causing respiratory infection in children with TRAINs is Pseudomonas aeruginosa (P. aeruginosa) which is commonly treated with the inhaled (nebulised) antibiotic tobramycin (Gipsmann, 2022; Morrison, 2022), as an "off label" treatment, as inhaled tobramycin is only approved by therapeutic administrations (US FDA, EMA and TGA) as a treatment in cystic fibrosis. Further standardized guidelines only exist for "off-label" use for people with non-cystic fibrosis bronchiectasis and not patients with tracheostomy (Chawla, 2021; Elborn 2022; Willis 2012; Volsko, 2021).

Inhaled tobramycin is preferred to intravenous administration due to the significant risk of oto- and nephro-toxicity, which are of particular concern in children with tracheostomy due to their high concomitant use of other drugs potentially toxic to these organs (Gipsman, 2022; Simon 2021). Normally, the advantage of inhaled drug delivery is that large doses can be delivered directly to the lung with reduced risk of systemic toxicity. However, the presence of a narrow caliber tracheostomy tube impacts significantly on the amount of aerosol (drug) reaching the lung and research into developing means for overcoming this issue and standardizing delivery techniques and dosing is limited (Atag et al., 2021; Gipsman, 2022; Morrison, 2022; Willis 2012; Volsko, 2021 Zhu, 2015). Current recommended doses in spontaneously breathing or mechanically ventilated tracheostomized children under 6 years of age, based on institutional guidelines are: 2 mL of 40 mg per mL (under 6 years of age) or 5 ml of 60 mg/mL tobramycin inhalation solution, nebulised and inhaled twice daily for 14–28 days, followed by a 28 day "drug holiday", or antibiotic-free period, to reduce the risk of Pseudomonas aeruginosa developing antimicrobial resistance (Ramsey, 1999; Simon, 2021). Despite evidence of sub-optimal dose

delivery in tracheostomized children, inhaled antibiotic treatment for TRAINs has been demonstrated to decrease: 1) the number of hospitalizations, 2) the length of stay in the intensive care unit, and 3) the bacterial load, without systemic side effects (Atag et al., 2021). Since the presence of a tracheostomy tube may significantly reduce aerosol delivery and there is only data available on delivery of tobramycin to adults during mechanical ventilation (Dhanani et al., 2018) it is essential to develop an evidence base for which to standardize treatment and care for children.

AIM

Considering the lack of an evidence base on which to develop guidelines standardizing delivery and dosing (Dhanani et al., 2018, Gipsman, 2022; Willis 2012), this study used laboratory experimentation to estimate the potential amount of tobramycin that would be delivered to the lungs from two different doses (300 mg (60 mg/mL) for a 16 kg child (> 6years) or 80 mg (20 mg/mL) for a 3 kg child (< 6yrs old)), when delivered by either a jet or vibrating mesh nebuliser, to (simulated) spontaneously-breathing tracheostomized children, unassisted, using a tracheostomy mask through different-sized tracheostomy tubes (3 mm for the < 6 year old child, and 4 and 5 mm for the > 6 year old).

METHODS

Delivery Set-up and Analysis

A breathing simulator (Flow Volume Simulator, Series 1120, Hans Rudolph inc., Missouri, U.S.A) was programmed with a pediatric breathing pattern to replicate ventilation parameters for each of three child delivery scenarios (Table 1, appendix). Tobramycin (80 mg in 4 mL (80 mg in 2 mL diluted in 2 mL saline 0.9%) or 300 mg in 5 mL total dose) was delivered as detailed in the standard dosing and administration protocol (Simon, 2021) with two different nebulisers: 1) a jet nebuliser, the PARI® LC SPRINT (MMD: 3.5 um) with PARI BOY® SX compressor (Figure 1 a) or 2) a vibrating mesh nebuliser, the Aerogen® Solo nebuliser with Aerogen® Pro X controller (Figure 1 b). A filter (glass microfibre, Whatman, 90 mm, Sigma-Aldrich, Australia) was placed in a filter holder at the end of the tracheostomy tube to capture the aerosolised drug solution. After delivery, both the tracheostomy tube and the filter were rinsed with 50 mL of ultra-pure water and sonicated for 2 mins, before being transferred into a 50 mL volumetric vial for High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) analysis. Six replicate measurements were

taken for each combination of tracheostomy tube size, dose, and nebuliser type. T-tests were conducted in R Studio (Version 1.1.463 – © 2009–2018 RStudio, Inc) between delivered dose with each nebuliser type at both high and low starting dose, within each tracheostomy size.

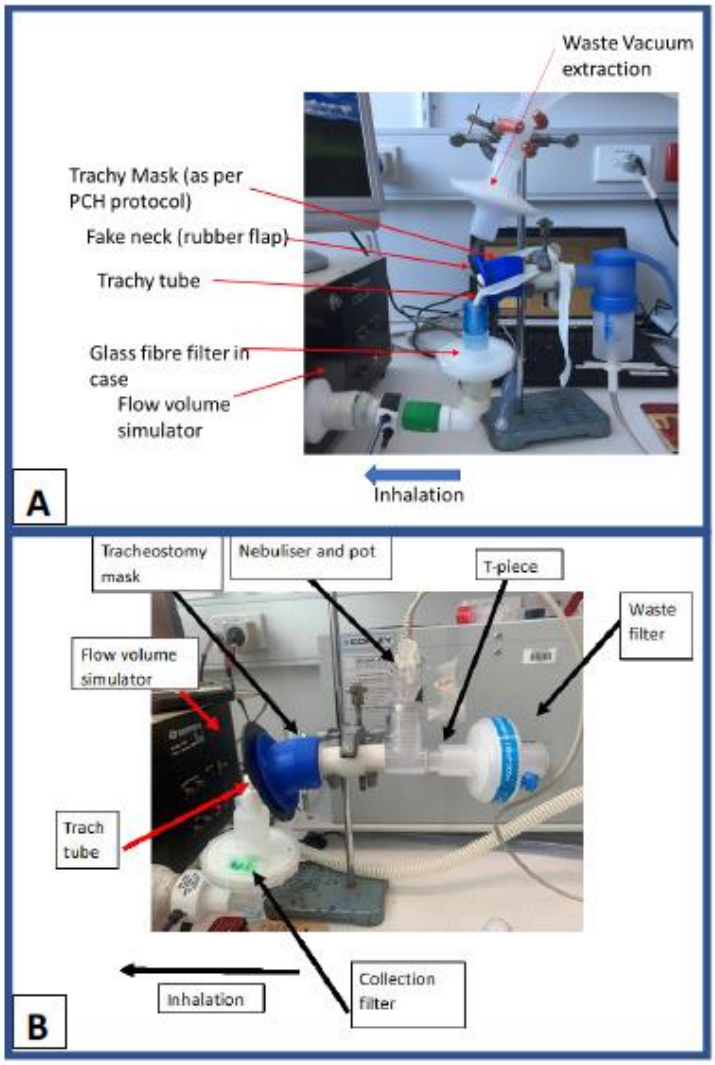

Figure 1. (A) Aerosol delivery set-up of nebulised tobramycin, using jet nebuliser delivered through tracheostomy tube using simulated breathing. (B) Aerosol delivery set-up of nebulised tobramycin, using vibrating mesh nebuliser delivered through tracheostomy tube using simulated breathing * please note with set-up B the waste filter was not used with a high dose and instead was left open to air. Inhalation was in the direction of (toward) the flow volume simulator.

High Performance Liquid Chromatography with Mass Spectrometry (HPLC-MS) with Electrospray Ionization (ESI) detection of Tobramycin

The full method is described in Appendix A. Tobramycin standard (Pharmaceutical Secondary Standard, Certified Reference Material, Sigma-Aldrich Product No. PHR1079, 94.2 wt.%) was diluted with LC-MS grade water (Merck, LiChrosolv, catalogue number 1.15333.4000) to create standards of 0.8, 0.6, 0.4, 0.2, 0.1, and 0.01 mg/mL. Samples were run through an Agilent Poroshell 120 HILIC column (4.6x100 mm, with 2.7 µm particle size) on an Agilent 6120 Quadrupole LCMS, with Agilent 1260 Infinity HPLC System (G6120B Single Quadrupole LC/MS; G1311B Pump; G1329B Autosampler; G1314F Variable Wavelength Detector). An isocratic mobile phase was used with 15% water (+0.1% trifluoroacetic acid), 85% acetonitrile (+0.1% trifluoroacetic acid), pumped at 1 mL/min. ESI-MS conditions included positive ion mode, scan 220–475 amu. Extracted Ion Chromatograms (EIC) with mass range 467–471 amu were used for quantitation of Tobramycin. UV data was collected at 210 nm. Tobramycin peak retention time for standard samples ranged from ~2.4–2.5 minutes and in samples ranged from ~2.7–2.9 minutes.

Variance from labelled-claim due to measurement technique (HPLC-MS)

The variance from labelled-claim due to measurement technique was obtained each day of HPLC processing and averaged 15% variance (increase) from labelled-claim (range + 4–26%). Variance was attributed to an increase in electrospray response due to the presence of dissolved salts in the pharmaceutical formulations which varied between the chosen formulations (MYLAN™ and SUN Pharmaceuticals™) (Constantopoulos, 1999). Delivered (mg) dose measured with HPLC-MS was therefore correspondingly normalised based on observed daily variance.

Confirmation of elution of complete dose of tobramycin from filters

To confirm complete recovery of tobramycin from filters, 500 µL of 40 mg/mL tobramycin solution (containing 20 mg tobramycin) (MYLAN™) was pipetted on a glass fibre filter, (Whatman™, Sigma-Aldrich, Australia). Subsequently, the filter was placed in 100 mL of ultra-pure water in a small sonicator for two minutes before transferring to a glass vial. The rinse water was assayed as above, for tobramycin and detection of 20 mg of tobramycin confirmed.

Terminology

Delivered dose (DD) is the dose delivered to the filters, as quantified by HPLC analysis. Starting dose is the dose loaded into the nebuliser pot, also known as the loaded dose. Administered dose is the dose remaining after retention in the nebuliser.

RESULTS

Comparison between nebulisers for each child delivery scenario

80 mg starting dose (ages <6yrs)

The delivered dose (DD) from a jet nebuliser was greater than from the vibrating mesh nebuliser for scenario one (3 kg child, 3 mm tube); 2.1 vs 0.7 mg (p=0.047), scenario two (16 kg, 4 mm tube) 11.4 vs 10 mg (p=0.43) (Figure 2) and scenario three (16 kg, 5 mm tube) 8.7 vs 3.5 mg (p =0.022).

300 mg starting dose (ages > 6 years)

The DD from a jet nebuliser was greater than the vibrating mesh nebuliser for scenario one (3 kg child, 3 mm tube), 8.4 vs 3.7 mg (p=0.00076) and scenario three (16 kg, 5 mm tube) 33.2 vs 25 mg (p=0.2) but not for scenario two (16 kg, 4 mm tube) (39.4 vs 46.5 mg, p=0.1) (Figure 2).

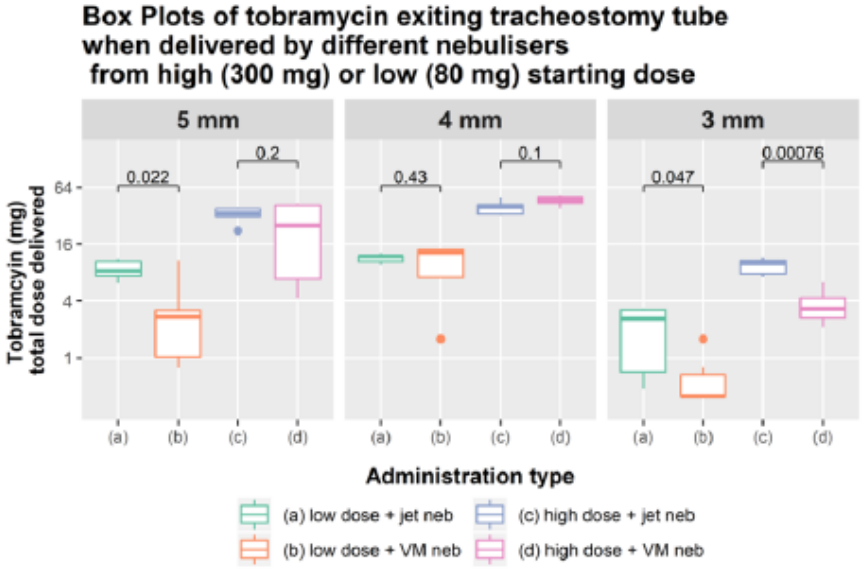

Figure 2. Tobramycin delivered to children by nebulization through different tracheostomy tube sizes from either an 80 mg (low), or 300 mg (high) starting dose of tobramycin via a 3, 4 and 5 mm diameter tracheostomy tube. Box plots indicate median and range from six repeat measurements (an outlier was excluded with 4 mm high dose vibrating mesh neb and low dose 4 mm vibrating mesh neb)

Dose overview and mg.kg^{-1} dose

Jet nebuliser

When using a jet nebuliser, approximately 10% of the loaded (labelled claim) doses (either 80 or 300 mg) were delivered to a 16 kg child using a 5 mm tracheostomy airway and approximately 15% with a 4 mm tracheostomy airway. With the 5 mm tube the mean delivered dose was 8.7 mg or 0.5 mg.kg^{-1} from an 80 mg starting dose and 33.2 mg or 1.9 mg.kg^{-1} from a 300 mg starting dose (Table 1, Figure 3). With the 4 mm tube the mean delivered dose was 11.4 mg or 0.7 mg.kg^{-1} from an 80 mg starting dose and 39.4 mg or 2.1 mg.kg^{-1} from a 300 mg starting dose (Table 1).

Approximately 3% of the loaded doses (80 or 300 mg) were delivered to a 3 kg child using a 3 mm tracheostomy airway. The mean delivered dose was 2.1 mg or 0.7 mg.kg^{-1} from an 80 mg starting dose and 9.5 mg or 3.2 mg.kg^{-1} from a 300 mg starting dose (Table 1).

Table 1. Dose reporting for administration of inhaled tobramycin by different methods from different starting doses. Child weights were 16 kg for a 5 mm and 4 mm tracheostomy airway, or 3 kg for a 3 mm tracheostomy airway. Delivered dose = drug weight recovered from the filter, measured with analytical chemistry (HPLC-MS) (also called "inhaled dose" or "absolute dose" or "deposited dose" or "deposited drug" or "dose recovered from filters"). Administered dose = drug (weight) assumed to be in mass of measured (weighed) fill that has left the nebuliser based on known concentration (also called "ex-nebuliser dose" or "nebuliser output" or "loaded dose delivered" or "total administered dose").

		Delivered dose (mg (% of starting))		Delivered dose as percent of Administered dose (%)*		Delivered mg.kg^{-1} dose		Administered dose (%)	
Tube size (mm, ID)/child weight	From starting dose:	(80mg)	(300mg)	(80mg)	(300mg)	(80mg)	(300mg)	(80mg)	(300mg)
	Nebuliser type								
3 /3 kg	Jet nebuliser	2.1(2.6)	9.5(3.2)	4	10.3	0.7	2.8	72	73
4/16 kg	Jet nebuliser	11.4(14.3)	39.4(13)	20.1	31.1	0.7	2.1	72	73
5 /16 kg	Jet nebuliser	8.7(11)	33.2(11)	16	33.4	0.5	1.9	72	73
3 /3 kg	Vibrating mesh nebuliser	0.7(0.8)	3.7(1.2)	0.2	1	0.2	0.4	99	99
4/16 kg	Vibrating mesh nebuliser	10(13)	46.5(16)	5.4	14.1	0.5	2.9	99	99
5 /16 kg	Vibrating mesh nebuliser	3.5(4.4)	25(8.3)	1.2	5.8	0.2	1.6	99	99

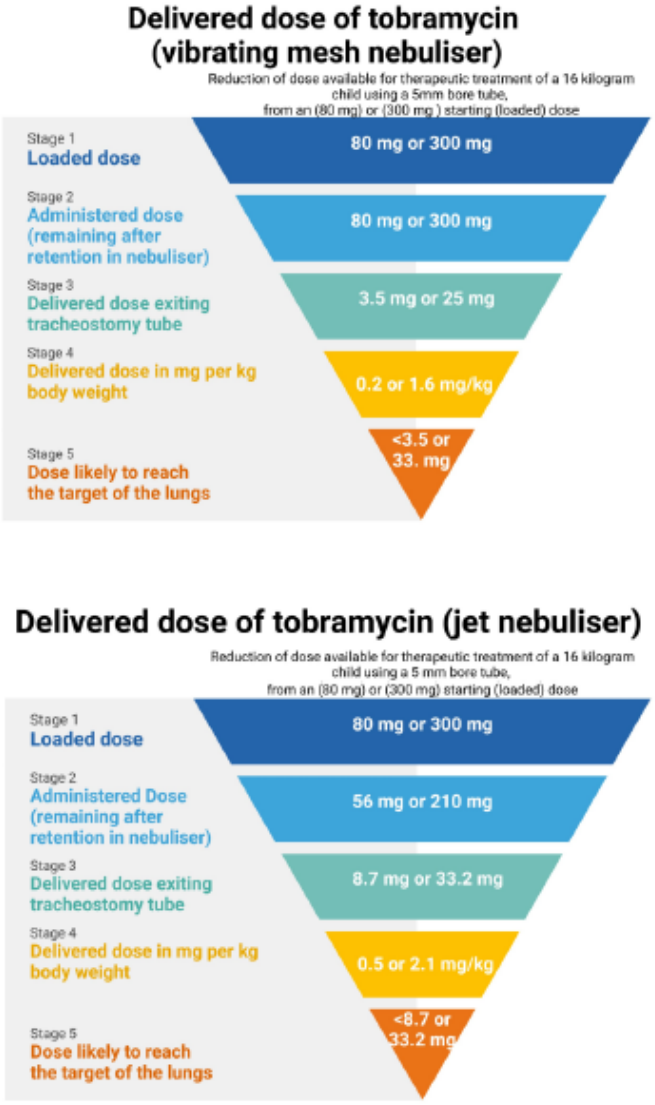

Figure 3. Comparison of Delivered dose (DD) and mg.kg-1 dose by nebuliser type for a 16 kilogram child at two different starting doses. The administered dose of tobramycin decreased from the loaded dose either due to retention in the nebuliser (Stage 2) or to fugitive losses to atmosphere where the simulated breathing rate permitted less than half the total nebulised dose to be inhaled (Stage 3). The dose to the lungs is likely to be significantly reduced since droplet formation occurred when liquid film built up in the airway (Stage 5).

Vibrating mesh nebuliser

When using a vibrating mesh nebuliser, between 4–8% of the loaded doses (either 80 or 300 mg) were delivered to a 16 kg child with a 5 mm tracheostomy airway and approximately 15% with a 4 mm tracheostomy airway. With a 5 mm tube the mean delivered dose was 3.5 mg or 0.2 $mg.kg^{-1}$ from an 80 mg starting dose and 25 mg or 1.6 $mg.kg^{-1}$ from a 300 mg starting dose (Table 1, Figure 3). With a 4 mm tube the mean delivered dose was 10 mg or 0.5 $mg.kg^{-1}$ from an 80 mg starting dose and 46.5 mg or 2.9 $mg.kg^{-1}$ from a 300 mg starting dose (Table 1).

Approximately 1% of the loaded doses (80 or 300 mg) were delivered to a 3 kg child with a 3 mm tracheostomy airway, where the mean delivered dose was 0.7 mg or 0.2 $mg.kg^{-1}$ from an 80 mg starting dose and 3.7 mg or 0.4 $mg.kg^{-1}$ from a 300 mg starting dose (Table 1).

DISCUSSION

To improve the empirical evidence-base to develop guidelines for children using tracheostomy airways to breathe, this study assessed the delivered dose of inhaled (nebulised) antibiotic tobramycin from two starting doses, to simulated spontaneously breathing children using a tracheostomy airway via two types of nebulisers. Although a similar quantitative study has been conducted in adults (Dhanani et al., 2018), to the best of the authors knowledge tobramycin aerosol delivery has not been investigated quantitatively in a delivery setting to spontaneously breathing children using tracheostomy.

We found that 15% (or less) of the administered dose exited the tracheostomy tube with either a jet or a vibrating mesh nebuliser. The jet nebuliser delivered slightly more drug overall, when considering it nebulized less of the starting dose (~70%) compared to the vibrating mesh nebuliser (~100%) which we attribute to the increased airflow generated with the device (5.1 L/min) compared to the vibrating mesh nebuliser. Depending on the airway size (3,4 or 5 mm), with a jet nebuliser, ~3–15% of the administered dose was delivered with either a low (80 mg) or high (300 mg) starting dose and with a vibrating mesh nebuliser, ~1–15%. Although not directly comparable – as delivery was using adult breathing pattern and 8 mm tracheostomy airway bore – our results are similar to the doses previously reported in laboratory simulated (experimental) study; 6.9% (with tracheostomy collar) or 13.8% (t-piece) of administered albuterol dose via jet nebulization (Ari, 2012). In a separate study delivering salbutamol to simulated adults with tracheostomy (8

mm ID) and jet nebuliser, 12.9% of the total administered dose was recovered with a mask interface or 15.3% with a t-piece (Piccuito, 2005). More recently, a study by Dhanani et al., 2018, showed delivery of tobramcyin with two difference doses using a vibrating mesh nebuliser (AeroNeb Pro), yielding recoveries of 117 mg from a 500 mg dose (100 mg/mL) or ~30% of the loaded dose and 34 mg from an 80 mg dose (40 mg/mL) or ~24% of loaded dose, with an adult inhalation parameter (500 ml breaths) and a 7 mm tracheostomy airway. We recovered a maximum of 15% of the loaded dose with a 5 mm tube size, compared to their recoveries of ~30% with a 7 mm tube size. We suspect our reduced (by half) dose is both due to our reduced breathing parameters – 100 mL breaths compared to 500 mL breaths and that we used a mask set-up for delivery which would have increased dose-waste. Changes in ventilation parameters are known to alter drug delivery (Dhanani et al., 2018, J. Dhanani et al., 2016). Considering these differences, our results were comparable to all previously reported in vitro experimental set ups. In vivo, in awake children, nebulised delivery of budesonide with face mask and a jet nebuliser producing 4.2 µm MMD particles (versus ours producing 3.5 µm MMD particles) was between 5–8% (radiolabelled lung deposition) (Schueepp, 2004). Comparatively, in small samples of awake children inhaling a smaller particle size aerosol (2.5 µm) with a vibrating mesh nebuliser, radiolabelled lung deposition of budesonide was between 36–38% (Schueepp, 2004) or 34% in a slightly larger group with a similar particle size (2.6 µm) (Schueepp, 2009).

Limitations

Although the study was not designed to measure aerosol particle size of the exiting aerosol, it was observed that aerosolization was in fact not the primary mode of delivery throughout the duration of nebulisation and that droplet formation occurred with both 5 and 4 mm airway sizes with both nebuliser types. We noted higher amounts of drug were received through a 4 mm airway than a 5 mm airway with the same breathing pattern (for a 16 kg child) and we suspect this is partly because the breathing tube is narrower and shorter, which would affect droplet size and formation (Appendix table 2), with droplets likely cleared more quickly from the 4 mm tube than the 5 mm given the same breathing rate. Since the smaller 3 mm tube was used with only an 18 mL breath drug delivery was reduced compared to both 4 and 5 mm tubes. Droplet formation occurred at 1–2 minutes into nebulisation when surface liquid film (due to coagulation and "rain-out") had built up inside the tracheostomy tube, although some primary aerosol delivery occurred via nebulization

ventilation settings (500 mL breaths), since particle sizing has been previously reported for this scenario (Dhanani et al., 2018). However, for delivery of tobramycin to children, in future, in accordance with US Pharmacopeia (Pharmacopeia, 2015), or relevant ISO standards (International Organisation for Standardisation, 2023) particle size and characteristics of the exiting drug should be measured under realistic conditions to ensure the filter dose accurately estimates the lung dose. A novel method to measure aerosol particle size from nebulisers has also been validated (Niazi et al., 2021).

We noted inconsistent significance with results: although the jet nebuliser delivered more over all (the administered dose was higher), the delivered dose was not significantly different to the vibrating mesh nebuliser in several comparisons. We suspect variation could be due to differences in formulation parameters between differing concentrations of tobramycin and their effect on the reuse of the vibrating mesh nebuliser particularly. The manufacturer states that the Aerogen Solo® can be used in a single patient for up to 28 days without cleaning, but previously it has been shown that repeated use of the Aerogen Solo® nebuliser without rinsing, can clog the membrane with a salbutamol solution (Lin et al., 2020). Although clogging the membrane did not affect aerosol drug output with salbutamol solution (Lin et al., 2020), tobramycin has different properties and is notoriously "sticky" making it difficult to rinse. In this study both nebulisers were rinsed with deionised water in between each use and only three new vibrating nebulisers were used for the entirety of 36 tests (roughly 12 tests per new nebuliser) however this may have been insufficient to remove build-up. Additionally, electrolyte (salt) content effects vibrating mesh nebuliser aerosol particle size more than jet nebulisers, and differences in tobramycin solution viscosity between solution concentrations (i.e. increased viscosity with increased concentration) may also effect the particle size output (Dhanani et al., 2018) or also clog the vibrating mesh more rapidly (Lin et al., 2020). Therefore, future studies should investigate tobramycin solutions of different concentrations for effects of viscosity on particle size output and whether clogging of the membrane with repeated use may reduce delivered dose. Although wasteful, an interim solution to reduce variabiliy would be to use each vibrating mesh nebuliser only once.

Future directions

Overall, delivered dose was generally low – at best 15% of the starting dose, which may not even reach the child if nebulization is not the primary mode of delivery due to droplet formation. These

drawbacks to nebulised delivery should be noted, particularly when delivering antibiotics to children in a hospital setting. For example, fugitive waste of 50% is known to occur with nebulised medical aerosol delivery with either jet nebulisers or vibrating mesh nebulisers (Saeed, et al., 2018). Fugitive waste is not surprising given that the inhalable dose per minute for a child is far less than the delivered dose per minute by the nebuliser at constant flow rate. Although fugitive waste is not bioaerosol waste, in the context of an antibiotic it should be minimized or eliminated to not contribute to antibiotic resistance in the hospital environment.

Despite nebulised drug delivery allowing passive aerosol delivery (no input required by the patient), lung site specificity, and having a variety of antibiotics formulated for use, there are known limitations of delivering liquid aerosols compared to solid particles (Tiddens, 2014). Therefore, it is important to consider new methodologies and developments that could enhance liquid aerosol delivery. For example, although spacer devices ("secondary devices") are commonly known to be used with pressurised metered dose inhalers, less commonly known is that secondary devices like spacers can be used for improving administration of nebulised drug (Longest, 2019) or that heated dryer systems are in development (Spence, 2022). Additionally, there are increasingly better ways to deliver dry powders to patients with artificial airways, such as with excipient enhanced dry powder formulation through nasal cannula, however this aerosol formulation has not been adapted for inhalation via the oral route (Howe, 2021).

Assisted ventilation

A limitation in our study is that we did not consider scenarios with high-flow oxygen supplemental to spontaneous breathing. Because in our study the jet nebuliser delivered more and it had a driving flow of ~5.1 LPM as opposed to the vibrating mesh nebuliser having very low flow, it is possible this extra force assisted re-nebulization and deterred rainout within the delivery set-up, thereby increasing aerosol delivery. Therefore, it is suspected that high-flow in combination with a vibrating mesh nebuliser, may "re-aerosolise" liquid rainout in the t-piece or tubing, thereby assisting nebulization and perhaps increasing delivered dose. However, future studies should investigate both droplet formation and high flow scenarios to determine any positive effect.

Patient comfort

We used a tracheostomy mask, and not a direct connection to the tracheostomy tube to allow

simulated patient comfort. Although the mask is known to decrease the delivered dose (Ari, 2012) it is required with children (and preferred by adults) to minimise the discomfort of the aerosol set-up. A mask permits a looser fit, which allows coughing and distance from the device – especially helpful in the case of a noisy or sputtering devices. Additionally, we studied the delivery of drug with the patient in an upright position, however we are aware that very young babies may be lying down during delivery and some patients may be incompletely upright. In these cases, a swivel piece would be used and likely reduce aerosol delivery – the addition of a 90 degree angle would trap aerosol as it attempts (and fails) to follow the diverging flow. Sometimes a nurse technician will allow the end of the t-piece to be open to air when a vibrating mesh nebuliser is used to allow for increased patient comfort, instead of using a ventilation filter or valved t-piece. We tested an open-air set up with the 4 mm high-dose only and found that although the dose was higher with a vibrating mesh nebuliser it was not significantly different. The open-end may allow slightly less liquid film build up in the tube, however, also permits fugitive waste and so its use may not be suitable depending on the setting.

Covid-19 protocols

We did not consider Covid-19 protocols which may still be in place to varying degrees depending on the hospital location. During the Covid-19 pandemic, tracheostomy was indicated as an aerosol generating procedure as was use of a nebulization with a jet nebuliser, however these procedures are often necessary and aerosol generation can be easily mitigated with practical recommendations (Ari, 2022). Such recommendations include giving preference to devices that do not require breaking the ventilation circuit such as use of a vibrating mesh nebuliser, avoiding use of a tracheostomy mask, and preference instead for a t-piece, or using a valved t-piece with a jet nebuliser (Ari, 2022).

Conclusion

When administering inhaled tobramycin to patients via tracheostomy, the low availability of drug must be incorporated into the appropriate dosing regimen. It is essential to use antibiotics appropriately to reduce antibiotic resistance, particularly in vulnerable populations. The evidence obtained in this study can be used to contribute to the formation of standardized guidelines for aerosol delivery to children breathing through tracheostomies.

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Appendix A.

High Performance Liquid Chromatography with Mass Spectrometry (HPLC-MS) with Electrospray Ionization (ESI) detection of Tobramycin:

Tobramycin is a very polar analyte and has very little retention (and hence less chance of separation) on the commonly used C18 and C8 based reverse-phase HPLC columns. The HPLC column used with success to obtain retention of Tobramycin in this work was an Agilent Poroshell 120 HILIC column (4.6x100 mm, with 2.7 micron particle size). This column has a bare silica gel type HILIC stationary phase. Electrospray Ionisation (ESI) LC/MS was used with a volatile mobile phase modifier (0.1% trifluoroacetic acid, Merck, catalogue number 8082600101) to provide complete protonation (the ionised form) of the analyte for entry into the mass spectrometer. Tobramycin standard (Pharmaceutical Secondary Standard, Certified Reference Material, Sigma-Aldrich Product No. PHR1079, 94.2 wt.%) was diluted with LC-MS grade water (Merck, LiChrosolv, catalogue number 1.15333.4000) to create standards of 0.8, 0.6, 0.4, 0.2, 0.1, and 0.01 mg/mL. Samples (800 µL, accurately weighed) of each of these standard solutions were diluted (1:1) with acetonitrile (Merck, LiChrosolv, catalogue number 1.00030.4000) + 0.1% trifluoroacetic acid (800 microlitres, accurately weighed) in HPLC vials for LC/MS analysis in duplicate. Trifluoroacetic acid was not added to the standard samples in water prior to the acetonitrile addition step to prevent the chance of acid catalysed degradation.

Samples were run on an Agilent 6120 Quadrupole LCMS, with Agilent 1260 Infinity HPLC System (G6120B Single Quadrupole LC/MS; G1311B Pump; G1329B Autosampler; G1314F Variable Wavelength Detector) with column at ambient temperature. Injection volume 5 µL (with needle wash). An isocratic mobile phase was used with 15% water (+0.1% trifluoroacetic acid), 85% acetonitrile (+0.1% trifluoroacetic acid), pumped at 1 mL/min flow rate. ESI-MS conditions included positive ion mode, scan 220–475 amu (additional settings: Fragmentor 70, Gain EMV 1.0, Threshold 150, Step size 0.10). Extracted Ion Chromatograms (EIC) with mass range 467–471 amu were used for quantitation of Tobramycin. UV data was collected at 210 nm. Tobramycin peak retention time for standard samples ranged from ~2.4–2.5 minutes. Tobramycin retention time in samples ranged from ~2.7–2.9 minutes due to presence of buffers in the MYLAN formulated tobramycin pharmaceutical product. It is known that the presence of salts can affect the retention of analytes in HILIC chromatography (8). Total run time was 6 minutes. Peak areas

for integration were obtained from Extracted Ion Chromatograms (EIC) with mass range 467–471 amu. Blank samples showed zero peak area for mass range 467–471 amu.

Appendix Table 1. Details of child drug delivery scenarios simulated. Each tracheostomy tube was positioned to mimic the child in a seated/upright position.

	Child weight	**Tube bore size**	**Dose**	**Breathing parameters (6 ml/kg)**	**Nebuliser type**
Child scenario 1.	3 kg	3 mm	80 and 300 mg	18 ml, 30 bpm	Jet and VM
Child scenario 2.	16 kg	4 mm	80 and 300 mg	96 ml, 25 bpm	Jet and VM
Child scenario 3.	16 kg	5 mm	80 and 300 mg	96 ml, 25 bpm	Jet and VM

Appendix Table 2. Tracheostomy size and length for paediatric tracheostomy tubes used (Shiley, covidien).

Length (mm)	**Internal diameter (mm)**
39	**3**
41	**4**
50	**5**

Chapter 5: Progress towards a multiphase *in silico* model for assessment of solid particle aerosols to supplement experimental studies of aerosolised drug delivery to children through artificial airways

Plain language introduction to Chapter 5.

Chapter 5 builds a computational model to describe the physical and mechanistic phenomena underlying the aerosol delivery measured experimentally in Chapter 3. Understanding aerosol delivery in a manner sufficient to build a model and mimic the real-life findings ensures a thorough understanding of the principles involved and contributes towards a theoretical framework – in this case for aerosol delivery to patients with artificial airways. Further, the studies described in Chapter five were designed to not only computationally replicate the laboratory experimental studies conducted in chapter 3, but to provide supplemental information that was not (is not) able to be obtained experimentally. The computational investigations described in this chapter, thus support, and extend the results in Chapter 3. The investigation described here made progress towards a theoretical framework by uncovering that limitations of the model involved the inability to simulate individual particle-particle interaction and the importance of doing so in a closed system with a heavy particle load, rather than only calculating equations based on particle-clouds. It also highlighted the importance of accurately accounting for turbulence (deterministic chaos), which requires a different type of modeling, and "heavier" computer use than completed here. Generally speaking, "models", attempt to explain complex phenomena using theoretical frameworks as simply as possible, which can then conserve computing resources and time, however in this instance complexity has proven an essential component of the model. Future iterations of the model described here are expected to be used to obtain particle size exiting the airway, an example of data which are not possible to obtain experimentally and investigate ways to further improve aerosol delivery in the off-label setting.

Progress towards a multiphase in silico model for assessment of solid particle aerosols to supplement experimental studies of aerosolised drug delivery to children through artificial airways

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Introduction

Delivery of aerosolised drugs through artificial airways is often the quickest and most effective way to deliver therapeutics during a perioperative emergency, such as with bronchospasm (1). There are various methods currently used in practice to connect the aerosol generation device to the artificial airways of a spontaneously breathing child, however many do not utilize best practice methods for awake children (2). Additionally, the optimal method and device to deliver aerosolised drugs to children remains debated in both operative and non-operative emergency settings (3-6). Previous evidence (2) suggests however, that using current best practice for mechanically ventilated adults (7), and ambulant children (8), which is a pressurised metered dose inhaler (pMDI) with a spacer, may be better than many currently used methods to deliver aerosolised salbutamol (2). Best clinical practice must be utilised as aerosolised delivery of salbutamol to intubated patients is an off-label use and the responsibility of clinical outcomes therefore rests with the administering physician. Currently, with even the best delivery methods, 20% of a single dose (2) or less (9) will be delivered to the child which can clearly be improved upon with further study.

The current gold standard experimental method to assess aerosol lung deposition *in vivo* in humans is a radiolabeled aerosol study (10). However, the method is limited as the minimal possible radiation dose should be given if complying with International Committee on Radiation Protection and therefore a scanner with a short acquisition time, and lesser quality spatial resolution must be used, such as 2D gamma scintigraphy (11). Three dimensional methods, including 3D Single Photon Emission Computed Tomography (or SPECT), have longer acquisition times, typically about 20 mins or more, and therefore require higher doses of radiation which can be harmful to children or adults. Further, within a 20-minute time frame aerosol has begun clearance from the lung by the mucocillary escalator, making meaning of the total dose and deposition location less accurate. Other promising imaging methods, such as propagation-based phase-contrast x-ray (12), hierarchical phase contrast computed tomography (13), or contrast enhanced Magnetic Resonance Imaging (14) are still being investigated for use in human participants. Laboratory experimental methods such as filter-capture studies are an alternative to radiolabeled studies (2, 15) and can be used where it is unethical to use radiation such as in a perioperative

setting or in children. Filter-capture studies are where aerosol delivery is simulated through a model of an upper airway geometry and the dose that would enter the bronchus is captured on either a single (i.e. one at the end of the upper airway/mouth-throat model) or sometimes multiple filters if the mouth throat model extended past the bronchus (i.e. one at each bronchi) under simulated breathing conditions. The dose delivered to the filter is assumed to reach the lung (the upper airway is assumed to remove all the large particles), however as particle size is not measured in these studies it cannot be certain – and only particles under 5 µm will reach the lung. The presence of a filter also alters the flow field (adds resistance) and therefore will influence particle deposition. Methods that assess particle size of medical aerosols (with high particle-load) require a constant flow of at least 15 LPM and therefore filter studies that simulate breathing are unable to, by design, measure particle size simultaneously (16). There is no known experimental study or method that can (without diluting the airflow) assess the particle size on the filter and the total dose simultaneously. Therefore, to improve assessment of aerosol lung deposition, 3D methods capable of precisely tracking aerosol particles in time and space should be employed to accurately estimate aerosol particle size, and identify the underlying physical phenomena involved in aerosol delivery and aerosol lung deposition.

Computational fluid dynamics (CFD) offers an *in silico* alternative to *in vivo* lung deposition studies, providing detailed predictions of 3D flow patterns by solving numerically the equations of motion for fluids (17). CFD simulations allow accurate characterization and visualization of flow properties, such as turbulence formation or pressure distribution, that cannot be assessed with laboratory or *in vivo* methods. *In silico* analysis with CFD gives a mechanistic understanding of aerosol delivery and can be used to identify limitations of an aerosol delivery system and determine improvements through efficient computational investigation and avoiding challenges encountered in practical experiments such as human error.

Computational Fluid-Particle Dynamics (CFPD) has been more recently developed to simulate the motion of discrete particles in a flow field and is particularly relevant to the study of aerosols (18). However, as with all computational models, direct validation within a context of use is required (18,19) e.g. with *in vivo* radiolabeled lung deposition data (20), although indirect validation of the model is acceptable where *in vivo* radiolabeling data are unavailable (such as in children) (21,22). The indirect validation approach, which utilizes laboratory experimentation, is supported by best practice guides, such as the European Research Community on Flow Turbulence and Combustion (ERCOFTAC) standard for airflow in biomedical flows in the lungs (21) or the American Society of Mechanical Engineers (ASME) 2018 "Validation verification and credibility guideline for Computer Modelling and Simulation of medical devices" (18). Increasingly, computational models of aerosol lung deposition are being used within the pharmaceutical context (22,23). For example, since the "21st Century Cures Act" and the "Fit-For-Purpose Initiative" at the United States Food and Drug Administration (24,25), drug companies have been allowed to submit human-biology-based *in vitro* and *in silico* data in lieu of *in vivo* animal studies, for consideration as part of a weight of evidence approach which has led to the development of an

increasing number of *in silico* models to study aerosol delivery (20, 26, 27). *In silico* CFPD models for delivery of solid particle medical aerosols to children have been previously studied (28–34), however models rarely investigate spontaneously-breathing patients (or children) with artificial airways.

Therefore, this work develops a computational fluid-particle dynamics model that can be used to investigate solid-particle aerosolised medicine delivery through artificial airways, as measured by matching aerosol mass exiting each artificial airway in both laboratory experimental and *in silico* studies.

Methods

Three artificial airways were chosen to investigate pressurised metered dose inhaler (pMDI) generated aerosol (Ventolin®, GlaxosmithKline, Australia) deposition through: 1) a standard tracheal tube, 2) a pre-formed tracheal tube (RAE), and 3) a tracheostomy tube, all in the paediatric size of 5 mm (internal diameter). To indirectly validate the computational simulation, two complimentary studies were performed for simulated, spontaneously-breathing children: first, simulated aerosol deposition data was acquired for two of the chosen artificial airways from studies previously published by our laboratory (2) and data for the third was acquired by laboratory investigation. Secondly, a computational fluid-particle dynamic model was employed for a solid particle aerosol with an identical set-up to the laboratory study parameters. Finally, optimisation studies were performed to determine accurate input details for the computational study.

The criterion for validation of the computational model was that aerosol capture *in silico*, matched that captured in laboratory data within the standard deviation for all three representative artificial airways (Figure 1); a standard tracheal tube (A), a pre-formed tracheal tube (B) and a tracheostomy tube (C).

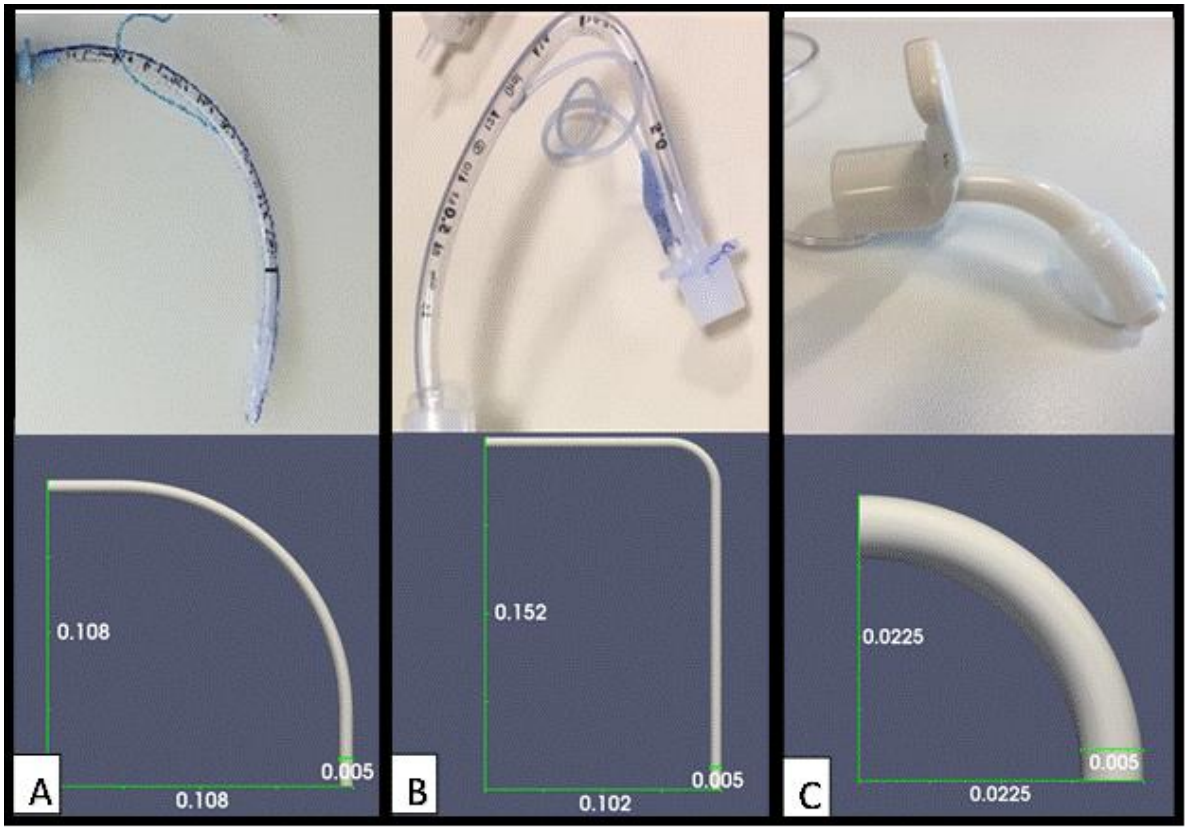

Figure 1: Artificial airway types tested. A= standard tracheal tube, B=pre-formed tracheal tube, C=tracheostomy tube. Top is actual device and bottom is geometry created for computational simulation. Top images not to scale. Bottom measurements are in meters.

Laboratory component

A set-up was devised where the flow was created by a flow volume simulator (Series 1120, Hans Rudolph, Missouri, USA, Appendix Figure A) programmed with a paediatric inhalation parameter, (Appendix, Table A). The aerosol administered exits the pMDI canister, travels through the tracheal tube to where it was trapped on a collection filter (Unifilter, Carefusion, Australia), with aerosol also being trapped along the way in the tracheal tube. Aerosol was introduced into the flow by actuation of the pMDI ten times, in time with each inhalation, without shaking in between actuations, as would occur during in emergency setting. Three repeat measurements were taken, all in a temperature-controlled environment.

Inhalation parameters

The ventilatory guidelines used at Perth Children's Hospital were taken for a representative, spontaneously breathing, 16 kg patient (Appendix, Table B) to create a breathing pattern file using the flow volume simulator software. The breathing pattern was then run using the flow volume simulator and recorded with a pneumotachometer which measured pressure differences within 0–160 lpm flow range, using Research

Pneumotach System Software, (version 4.9.6, Hans Rudolph, Missouri, USA). These recorded data were then used to create the velocity parameters used for the final in silico simulations (Appendix, Table A).

Mass detection using high-performance liquid chromatography (HPLC)

A method previously used to detect salbutamol was employed to measure salbutamol aerosol rinsed from components of the set-up (35). Briefly, each component of the set-up (tracheal tube and filter) was rinsed with ~50 mL of methanol (HPLC Grade, Thermofisher Scientific, Australia). The tracheal tube was cut into segments prior to rinsing to determine regional deposition. Rinse was assayed using High Performance Liquid Chromatography to detect salbutamol. Salbutamol standards in the range of 40 μg.ml-1 to 0.3125 $\mu g.ml^{-1}$ were prepared from stock of 100 $\mu g.ml^{-1}$ salbutamol (salbutamol hemi-sulphate analytical standard, Sigma Aldrich, Australia) in methanol, to create a standard curve to interpolate drug concentrations. The mobile phase consisted of methanol to potassium phosphate buffer (20 mM, pH 8.0) 35:65. The column was a reverse phase C18, 5 μm, 250 x 4.6 mm (Pinacle DB, restek International, Bellafonte, PA, USA). The quaternary solvent delivery system (Agilent Technologies, 1200 Series, Model G1311A), was used to generate a flow rate of 1 $ml.min^{-1}$. Retention time of salbutamol was 10 minutes. Detection was at 227 nm for ultraviolet (UV) detection with diode-array detector (Agilent Technologies 1200 series, Model 1206 DAD, G4212B).

Characterisation of aerosol spray velocity

To determine aerosol velocity when the salbutamol (Ventolin) inhaler was actuated, a high-speed camera and lens with magnification ratio of 0.13 (Fast Cam SA3 Photron, 120 K M2 with a Nikon AF-S 105 mm, micro lens) was used. The photographic set-up included illumination from below and directly in front of the Ventolin canister. The camera was operated at 2000 frames per second. The first frame showing particles was used for analysis (Appendix, Figure B). Analysis was completed in Microsoft Paint with grid squares showing and contrast enhanced to ensure the velocity head could be seen. The height of the pMDI mouthpiece was measured for determination of grid-square in mm which was then used to determine the distance travelled per single frame. Horizontal offset-error was determined as three grid squares and equated to 4.3 $m.s^{-1}$. Duration of a single frame was 0.5 milliseconds. The calculated injection speed of the salbutamol pMDI was used as to verify the computational study.

In silico component

Creation of in silico geometries

To mimic all artificial airway tubes, numerical geometric models were created (Figure 1) in the CFD software OpenFOAM (v 7.0, and v 8.0, The Open Foam Foundation, available at openfoam.org) using a python file (Python programming language, version 3.7.3, available at python.org) and discretised using hexahedral cells (Appendix, Table C).

In silico model final parameters

The final model used a multi-phase particle-in-cell model, and PIMPLE algorithm – the Lagrangian "multi-phase particle in cell" solver (MPPICFoam), to solve for conservation of momentum and mass (Navier-Stokes equations), coupled with discrete Lagrangian particle tracking. The air was treated as incompressible and isothermal and flash evaporation was assumed for the hydrofluoroalkane propellant (HFA-134a) (36). Therefore, the air density and dynamic viscosity were set to 1.225 $kg.m^{-3}$ and 17.89 $\times 10^{-6}$ Pa.s, respectively, to correspond to the temperature condition recorded (15°C) in the artificial airway during an actuation with the pMDI using a thermocouple (Pico Technology TC-08). The Flow-Reynolds (Re) number for a 5 mm diameter tube with an average velocity of 6.25 $m.s^{-1}$ was calculated to be in the critical zone, ~2800 in air, where flow is considered laminar with Re <2000,turbulent with Re>4000 and 2000<Re<4000 indicating a transitional flow model. Therefore, for all geometries under test, the cases were simulated with an entirely laminar model (i.e. turbulence model was turned off), without taking into account any turbulent features that might appear in the flow, and with a turbulent model (RAS, k-epsilon) firstly with both 1 and 2% turbulent intensity at the inlet (both average and peak velocity), with 2% (at average velocity) being used in the final case (Appendix, Table E). Gravity was specified in the same direction as the laboratory testing (-9.81 $m.s^{-2}$ in the z direction) and applied to both discrete (aerosol) and continuous (air) phases. To represent the pMDI being actuated, a cone injection model (inner diameter of 0, outer diameter of 0.5 mm, outer cone angle of 8.5°) was employed at the inlet centre with an injection speed of 40 $m.s^{-1}$ (37,38) and duration of 0.156 seconds (38). Initially, two different inlet velocity boundary conditions were investigated as laboratory optimisation indicated an average speed of 78 $m.s^{-1}$ ± 4.3 $m.s^{-1}$, with peak velocity at 88 $m.s^{-1}$ ± 4.3 $m.s^{-1}$. However, it was observed that the change of injection speed had very limited impact on the simulations results. The total simulation time was 0.3, 0.32 or 0.4 seconds, allowing for aerosol clearance (post injection), and an adjustable time-step (maximum value of 10^{-5} seconds) was used. Since it was observed that all particles did not clear from the larger geometries in the allocated 0.3, 0.32 or 0.4 s, those incomplete simulations were attempted to run past the allocated finish time. A mass median aerodynamic diameter (MMAD) of 3.75 µm, range 0.1–20 µm, with a mass Rosin-Rammler distribution, n value 1.5, was chosen to define the salbutamol aerosol particles (39). The total number of particles (13827570) injected was calculated for a 95 µg mass injection (100 µg, minus losses to the actuator), based on the density of salbutamol (1300 $kg.m^{3}$) (40) and the volume of a spherical particle of MMAD 3.75 µm. A gradient dispersion RAS model was used on the particles to perturb the velocity in the direction opposite to the gradient of turbulent kinetic energy (k), with a random Gaussian distribution. MPPICFoam, only accounts for collisions with walls without resolving particle-particle interactions and is a transient solver for the coupled transport of a single kinematic particle-cloud, including the effect of the volume fraction of particles on the continuous phase (41). Boundary conditions were applied on the outer patches defined as inlet, outlet, and solid wall (Appendix, Table D). Particles were defined as captured when they came into contact (or "stick") with the tube's solid wall and both "escape" and "stick"

boundary condition trialled at the outlet. This allowed for mass exiting and depositing in the geometry to be tracked and reported for easy post-simulation analysis.

Results

Overview

Laminar and turbulent simulations were reported, where possible, given that the calculated Reynolds number was in the transition zone: both were attempted, however several simulations did not converge at the specified finishing time under a particular model (i.e. tracheostomy tube: turbulent model, pre-formed tracheal tube: turbulent model) (Table 1). Therefore, results are shown in full (i.e. pressure distribution and velocity, and particle deposition) only for those that converged (Table 1): (i.e. the tracheostomy tube–laminar model and standard tracheal tube–laminar and turbulent models). Only pressure distribution and velocity results were taken at time 0.15 s for all cases including those that did not converge to allow comparison, except for the tracheostomy tube (turbulent model) which did not run past 0.128 s (Table 1). Additionally, pressure and velocity results were shown at the end time for those cases that did converge for both the turbulent and laminar model (i.e. the standard tracheal tube only). It should be noted that for the cases that converged, some mass remained active at the specified finish time (0.3, 0.32 or 0.4 s), i.e. mass remining in system (µg) (Table 1). The total mass injected (95 µg) either deposited in the tube (83.7 µg) or exited the tube (11.3 µg) for the tracheostomy airway only (laminar model). For all other tubes and models some small amount of mass remained active (Table 1). The standard tracheal tube with turbulent model ran until 0.32 seconds however the sum-total mass depositing in or exiting the tube was only 72 µg (with 22.6 µg remaining active in system) and attempts to run past this time failed to converge, as did attempts to run the tracheostomy tube, turbulent model (37 µg remaining active in system).

Table 1. Overview of simulations.

geometry	flow model	total mass injected (ug)	mass in system at end time (ug)	mass depositing in tube (ug)	mass exiting tube (ug)	sum total mass depositing or exiting tube (ug)	mass remaining active in system at end time (ug)	end time	attempted additional end time	converged at endtime
standard tracheal tube (A)	laminar	95.0	95.0	32.5	60.1	92.6	2.4	0.32	N/A	Y
	turbulent	95.0	94.2	82.5	0.8	83.3	11.7	0.3	0.34	Y
preformed tracheal tube (B)	laminar	95.0	94.1	26.0	68.1	94.1	0.9	0.4	N/A	Y
	turbulent	95.0	95.0	70.5	1.9	72.4	22.6	0.3	0.32	N
tracheosotmy tube (C)	laminar	95.0	95.0	83.7	11.3	95.0	0.0	0.3	0.3	Y
	turbulent	95.0	78.5	57.4	0.0	57.4	37.6	0.128	0.3	N

Pressure distribution and velocity during spray injection

For all tubes using the laminar model, the spray injection (jet) location (spray speed 40 $m.s^{-1}$) was centered on the inlet, as would be the case in real life after the pMDI was joined to the artificial airway port. However, because of the large difference in velocity between the particle spray jet and the continuous phase flow, the spray jet created a low-pressure region near the inlet and flow recirculation around the spray jet (Figure 2,4). A high-pressure region is observed where the spray jet impinges on the tube wall (Figure 2 D). The velocity of the spray jet decreased to ~13 $m.s^{-1}$ from the initial 40 $m.s^{-1}$ (Figures 2,3, C) with a laminar model, and to between 5–8 $m.s^{-1}$ with a turbulent model (Figures 4,5 C). When comparing the turbulent and laminar model for the standard tracheal tube (Figures 3 and 4) the velocity of the spray jet dropped from the inlet value by roughly 2 $m.s^{-1}$, from 5.6 to 3.8 $m.s^{-1}$ (Figure 4, C and A), which does not happen with the laminar case which is maintained at 12 $m.s^{-1}$ (Figure 3, C and A).

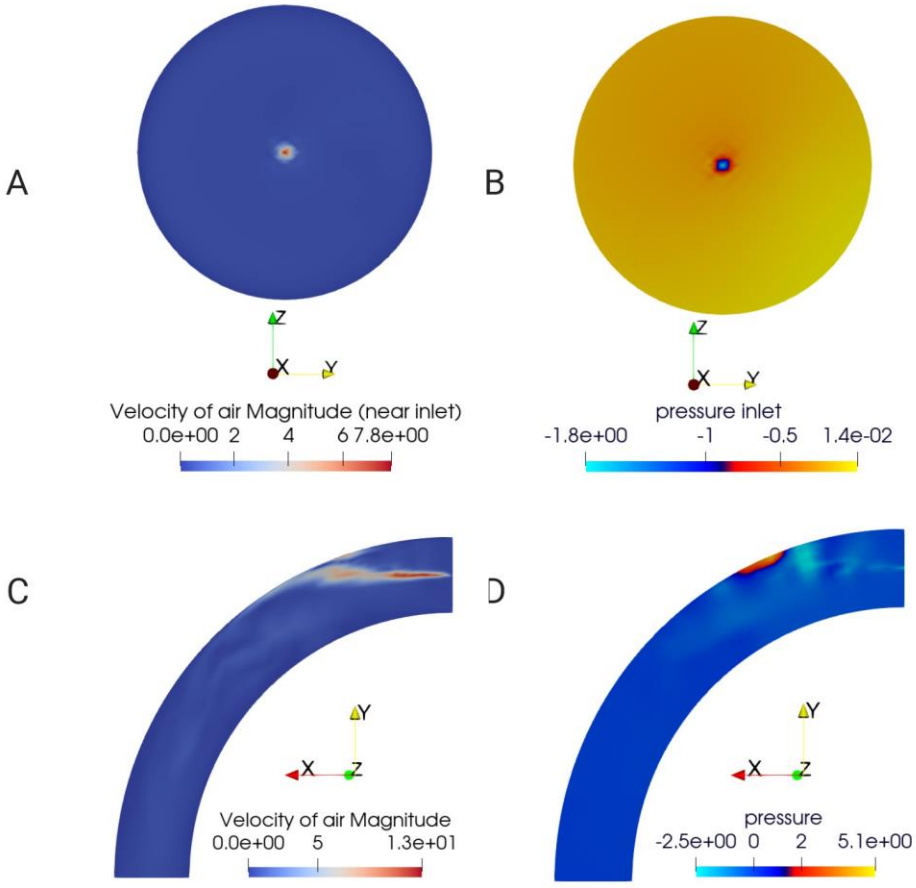

Figure 2. Velocity and pressure fields for the tracheostomy tube, laminar model, time 0.15 s. A: Velocity magnitude (m/s) on inlet plane, B: pressure (Pa) on inlet plane, C: velocity magnitude ($m.s^{-1}$) on cutting plane (z=0), D: pressure

on cutting plane (z=0). Please note that A & B are taken ~ 1 mm inside the inlet, so the peak value for the inlet velocity (when considering the effect of the particle injection) is displayed only in C & D.

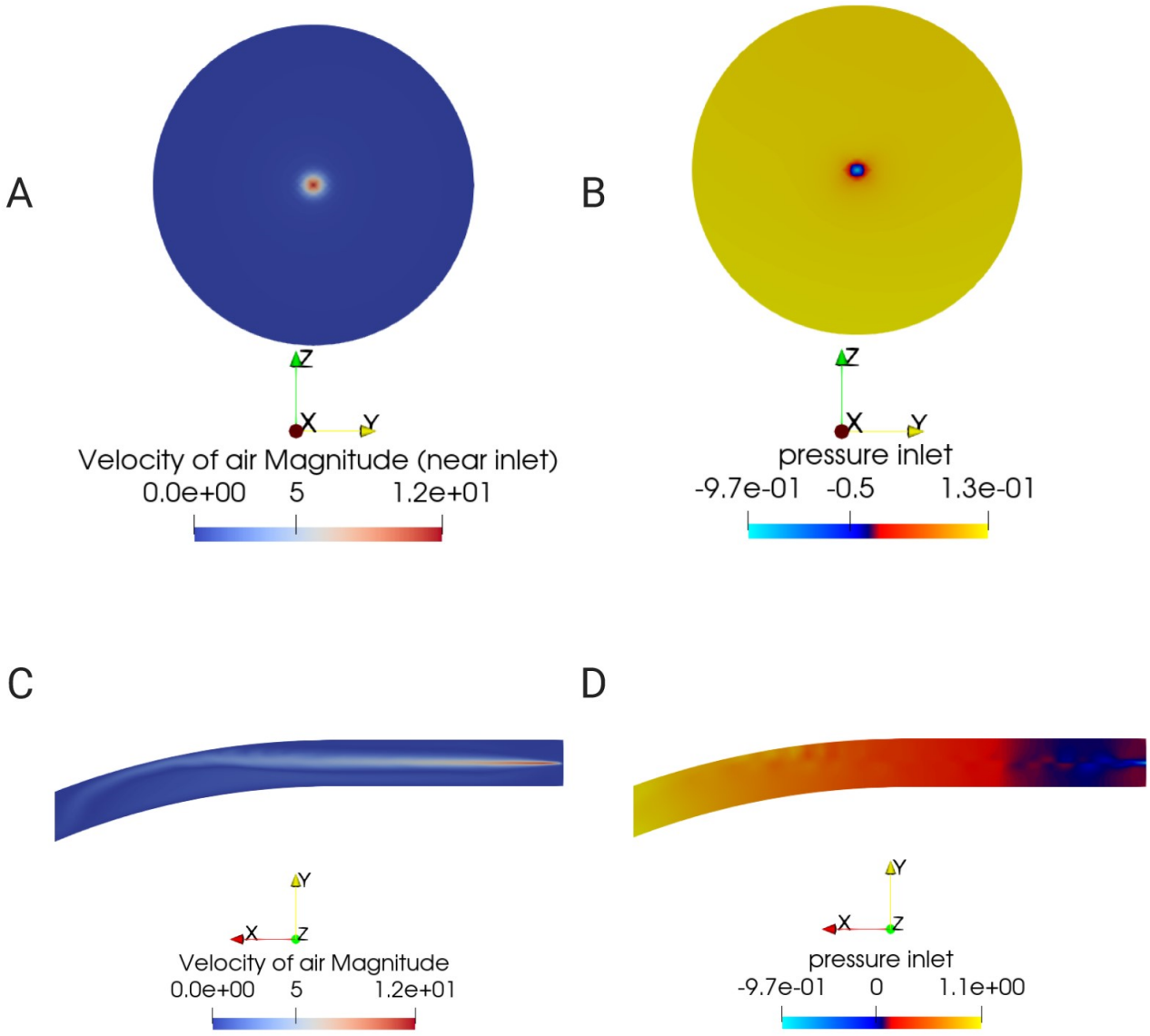

Figure 3. Standard tracheal tube–laminar model, time 0.15 s. Velocity and Pressure at inlet: A: Velocity magnitude ($m.s^{-1}$) on inlet plane, B: pressure (Pa) on inlet plane, C: velocity magnitude ($m.s^{-1}$) on cutting plane (z=0), D: pressure on cutting plane (z=0). Please note that A & B are taken ~ 1 mm inside the inlet, so the peak value for the inlet velocity (when considering the effect of the particle injection) is displayed only in C & D.

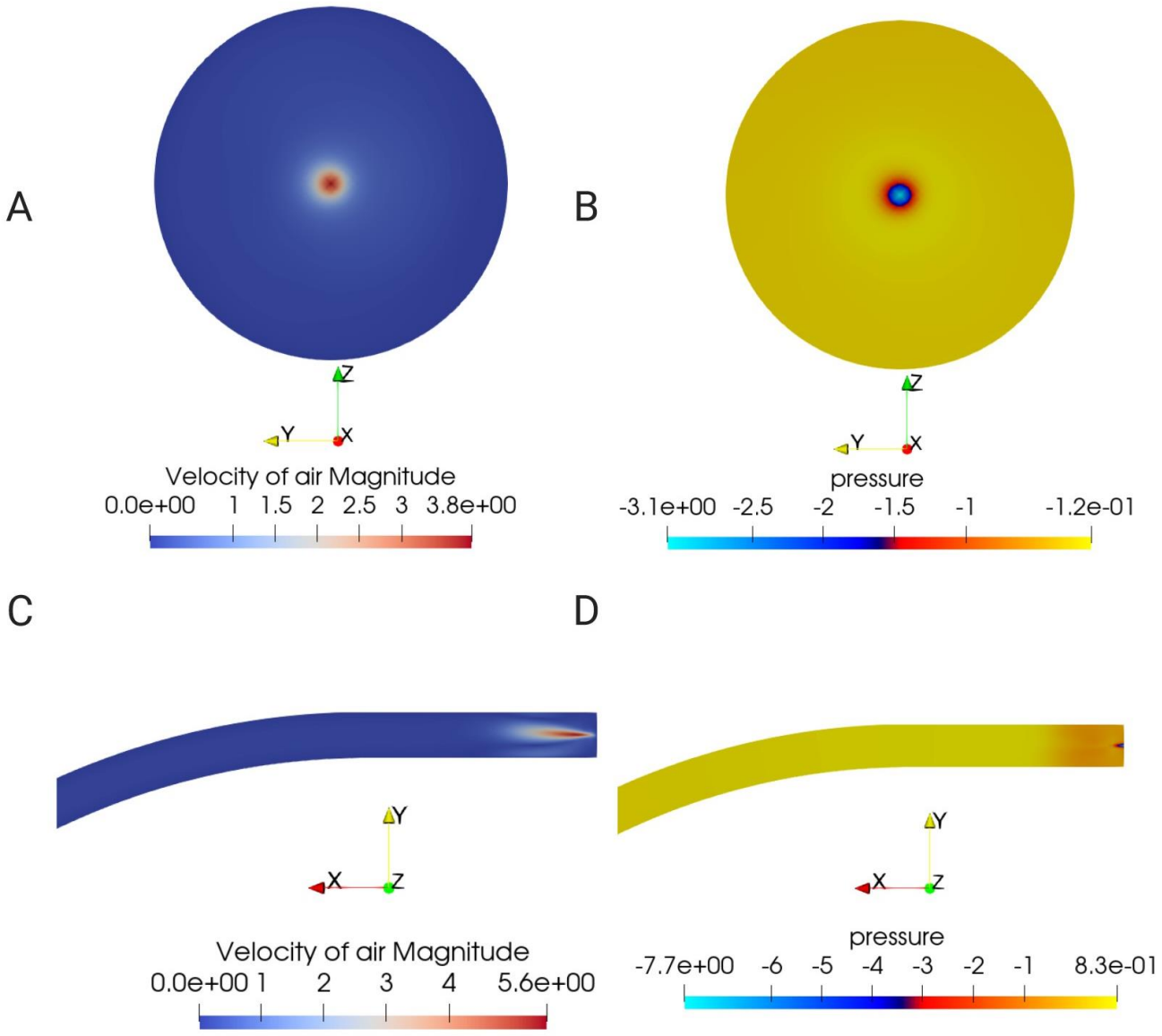

Figure 4. Standard tracheal tube, turbulent model, time 0.15 s. Velocity and Pressure at inlet: A: Velocity magnitude ($m.s^{-1}$) near inlet plane, B: pressure (Pa) on inlet plane, C: velocity magnitude ($m.s^{-1}$) on cutting plane (z=0), D: pressure on cutting plane (z=0). Please note that A & B are taken ~ 1 mm inside the inlet, so the peak value for the inlet velocity (when considering the effect of the particle injection) is displayed only in C & D

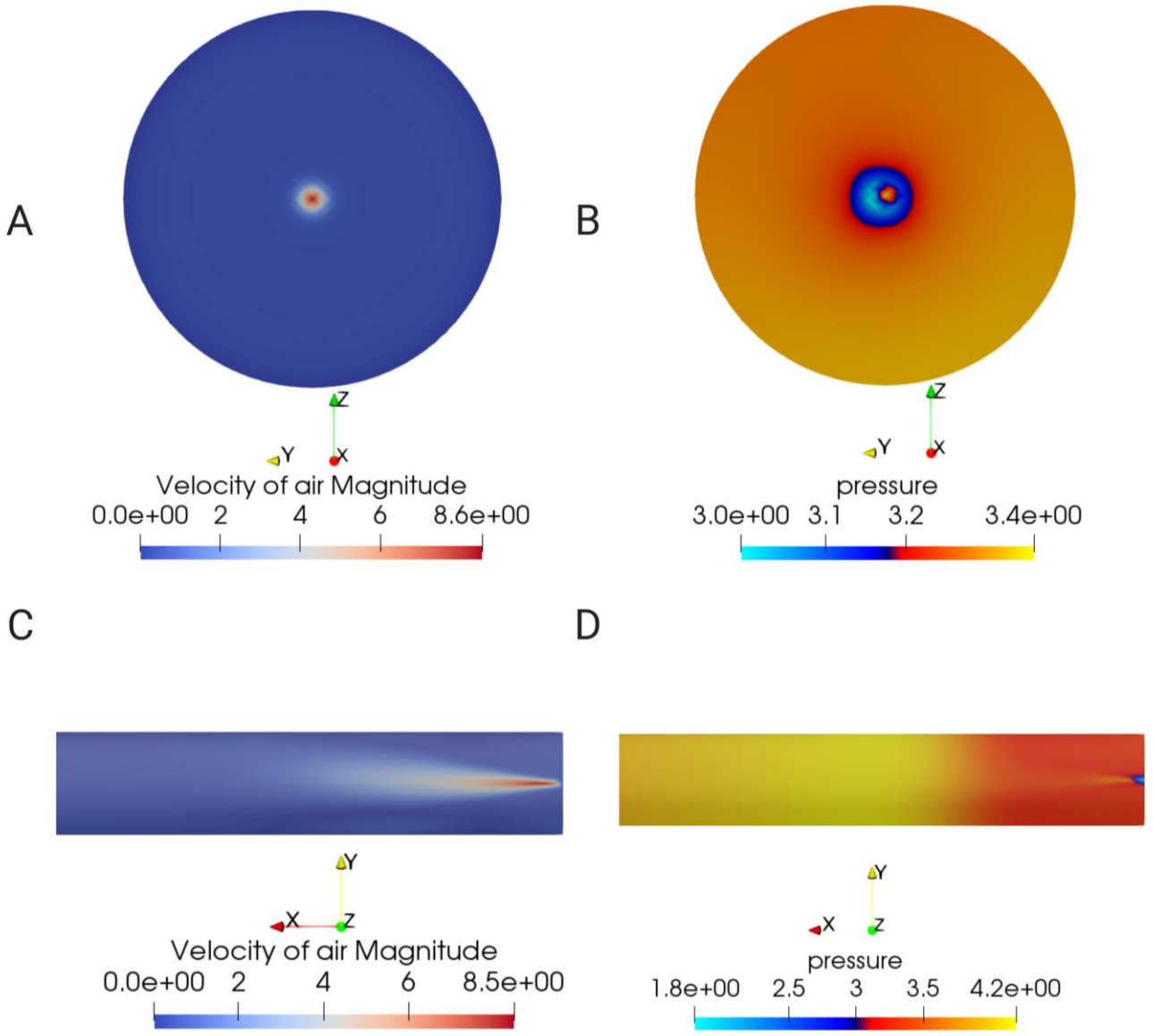

Figure 5. Preformed tracheal tube, turbulent model, time 0.15 s. Velocity and Pressure at inlet: A: Velocity magnitude ($m.s^{-1}$) on inlet plane, B: pressure (Pa) on inlet plane, C: velocity magnitude ($m.s^{-1}$) on cutting plane (z=0), D: pressure on cutting plane (z=0). Please note that A & B are taken ~ 1 mm inside the inlet, so the peak value for the inlet velocity (when considering the effect of the particle injection) is displayed only in C & D.

Particle deposition

The goal of the analysis of particle deposition was threefold: 1) to identify the temporal and spatial deposition patterns (Figures, 6–10), 2) to identify differences between the turbulence and laminar models (Figure 11) and 3) to identify differences between the particle deposition in the experimental versus in silico studies (Table 2). Results are shown for particle deposition only for those that simulations that converged (Table 1): (i.e. the tracheostomy tube–laminar model and standard tracheal tube–laminar and turbulent models). Particle deposition is reported for those particles that deposited in the tube in Figures 8, 9 and 10. Particles that exited the tube are reported in Table 1 (full results) and Table 2 (with relevance to the laboratory experimental results). Particle mass deposition in space (in the tube) between the inlet and outlet is shown in Figure 6, and particle number deposition over time is shown in Figure 7.

Particle deposition on the tube wall reached a peak closer to the inlet (normalised tube length 0–0.15) (Figure 6, B) with the turbulent model than with the laminar model.

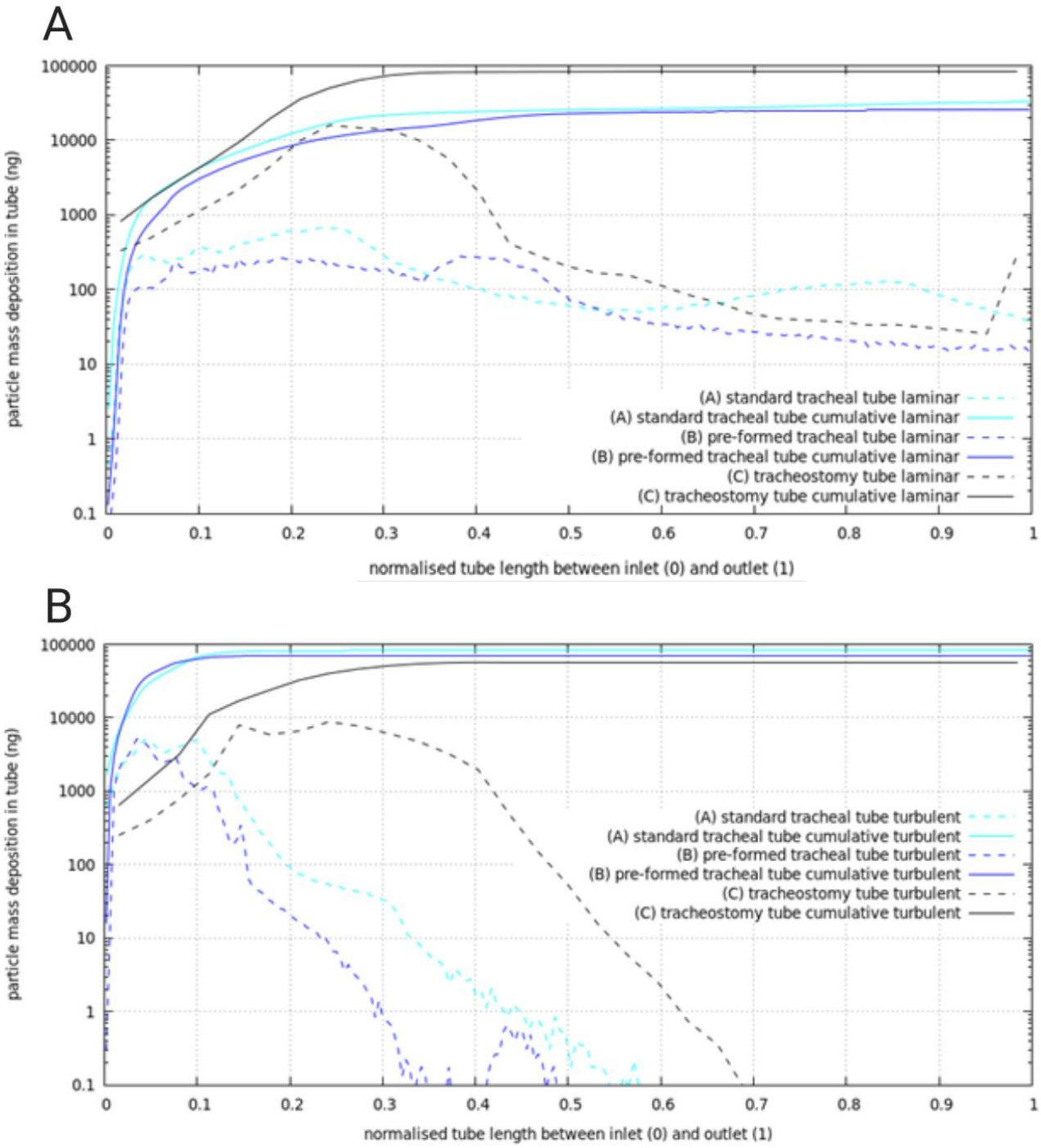

Figure 6. Particle mass (ng) deposition in each tube, normalised by tube length, where the value of 0 is the inlet and 1 is the outlet. A = laminar model, B = turbulent model. Note: the uptick (281 ng increase) for panel A; tracheostomy tube laminar (black dotted line), is present but not visible in the corresponding cumulative graph (solid black line).

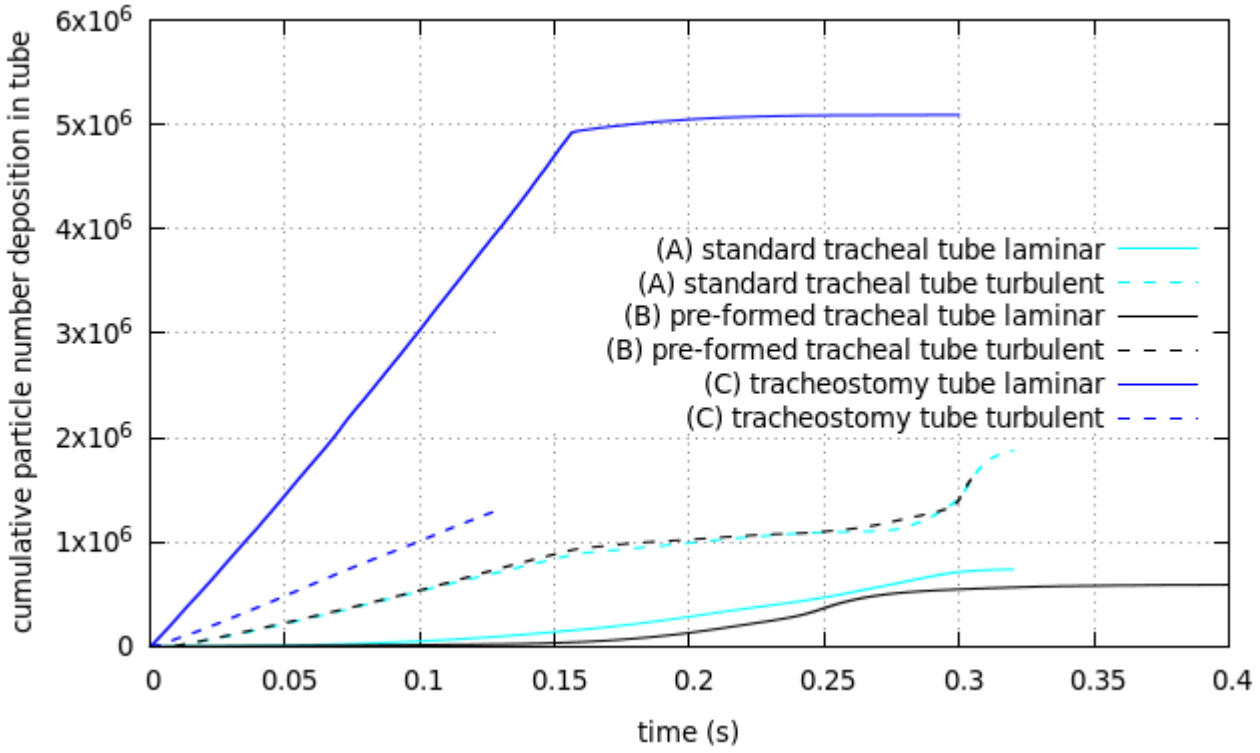

Figure 7. Cumulative particle number deposition in tube, over time. Straight line=laminar model, dotted line=turbulent model. Injection time is from 0–0.156 s.

Particle deposition increased steadily until the injection ceased at 0.156 s for the tracheostomy tube (laminar and turbulent models) and the standard and pre-formed tracheal tubes (turbulent model only). For the turbulent model deposition increased again after approximately 0.3 s, which corresponded to rapid increase in continuous phase velocity from 3.3 to 6.1 $m.s^{-1}$ (during time 0.285–0.35 s, Appendix Table A) and resulted in less mass exiting the tube (Table 1).

For the tracheostomy tube–laminar model–mostly all particles of 0.1–1 µm in size exit the airway (Figure 8, G) and particles above 11 µm all deposit in the airway (Figure 8, J, K, L). Increased deposition was seen due to gravity (-9.81 $m.s^{-1}$ Z direction) with particles sized 2–6 µm (Figure 8, H, I) but not larger particles 15–20 µm (Figure 8, J, K). The high-pressure region observed where the spray jet impinges on the tube wall (Figure 2 D) coincided with larger particle deposition, particularly with the tracheostomy tube (Figure 8, K&L)

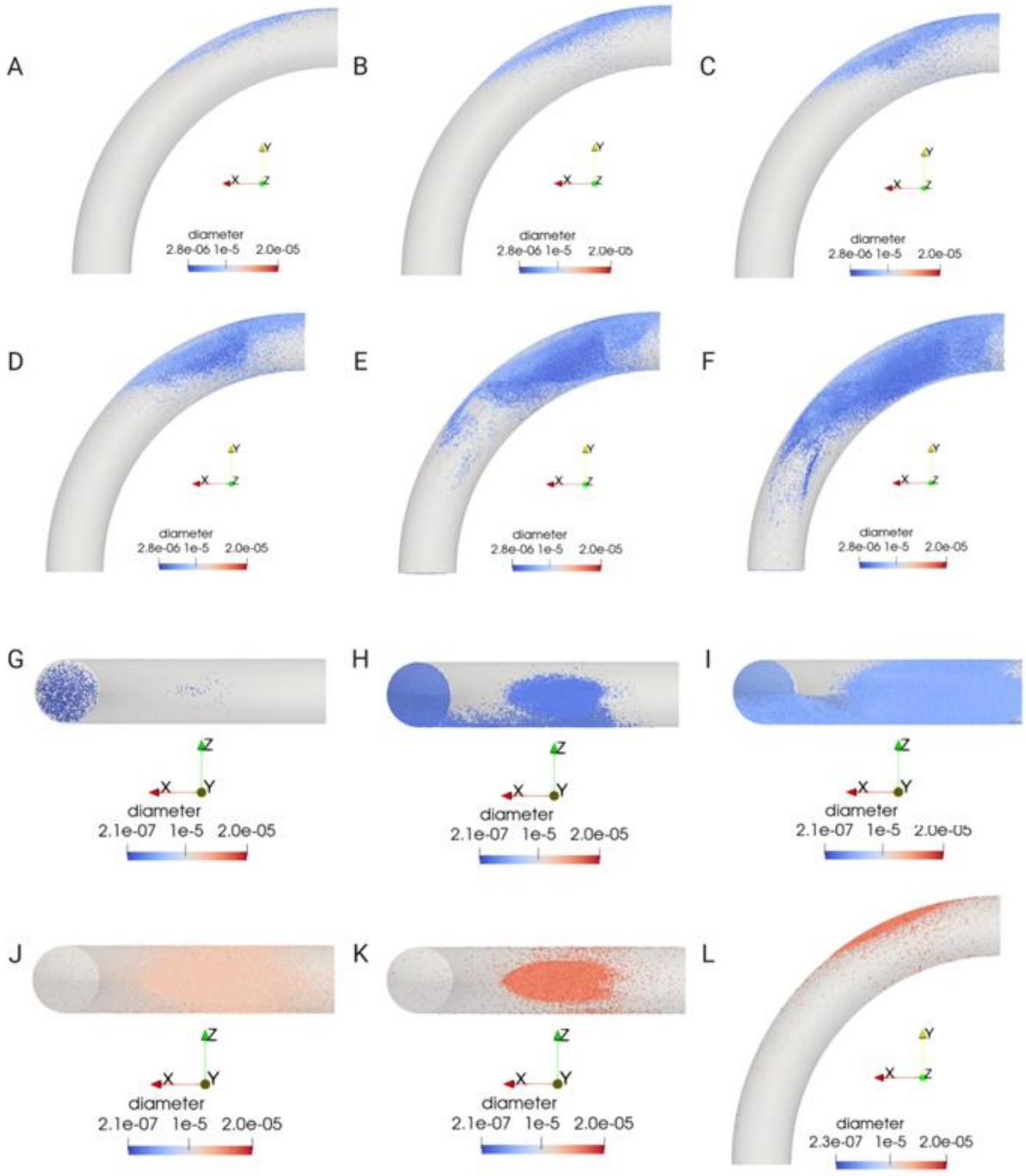

Figure 8. Tracheostomy tube, particle deposition laminar model. A–F, particle deposition at times 0.002, 0.01, 0.02, 0.04, 0.1 and 0.15 (i.e. during spray injection) and G–L, breakdown of particles by particle size ranges, at end-time (0.3 s): G: particles sized 0.1–1 µm, H: 2–3 µm, I: 4–6 µm, J: 11–14 µm, K&L: 15–20 µm. Please note that gravity is -9.81 m.s^{-2} in the Z direction to mimic the laboratory situation. Diameter scale for all figures is in m.

For the standard tracheal tube–laminar model–particle deposition due to spray-injection proximity to the tube walls comprised of mostly larger particles during 0.002–0.02 s (Figure 9, A–C), with some smaller particles

deposited between 0.04 and 0.15 timepoints (Figure 9, D–F). Almost no particles of size 0.1–1 µm are present (Figure 9 G) and all particles above 11 µm deposit in the airway (Figure 9, J, K). Most particles that remained active at the end time (Figure 9 L) are between 3–7 µm diameter.

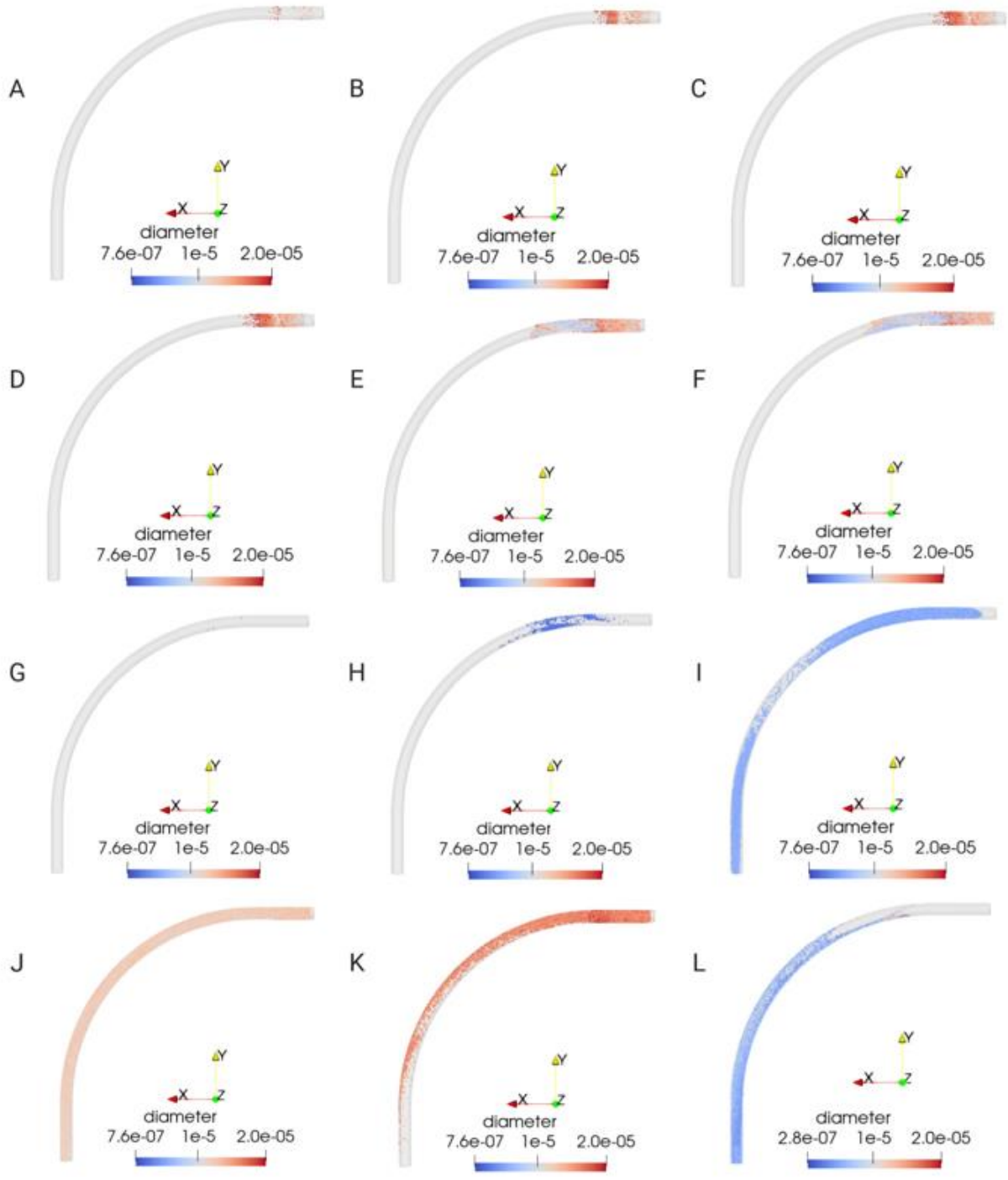

Figure 9. Standard tracheal tube, particle deposition, laminar model. A–F, particle deposition at times 0.002, 0.01, 0.02, 0.04, 0.1 and 0.15 (i.e. during spray injection) and G–L, breakdown of particles by particle size ranges, at end-

time (0.32 s): G: particles sized 0.1–1 µm, H: 2–3 µm, I: 4–6 µm, J: 11–14 µm, K: 15–20 µm, L: active particles at end time. Diameter scale for all figures is in m.

For the standard tracheal tube–turbulent model–particles above 11 µm in size deposit in the recirculation region created by the spray injection (Figure 10, D) and particles 2–6µm in size deposit preferentially in the negative pressure region (Figure 4, D, Figure 10, B–F), close to the inlet. Most of the particle deposition is restricted to the first third of the tube geometry (0–0.3 normalised tube length), close to the injection location at the inlet (Figure 6, A). Particle sizes 0.1–1 µm and 2–3 µm, 4–6 µm, 11–14 µm, 15–20 µm and active particles at end time are displayed in Figure 10 (G–L), where almost no particles of size 0.1–1 µm remain active. Only smaller particles are deposited immediately close to the inlet region (by backflow) and larger particle (15–20 µm) deposition begins a short distance from the inlet, likely due to impaction from the injection spray (Figure 10 C–F).

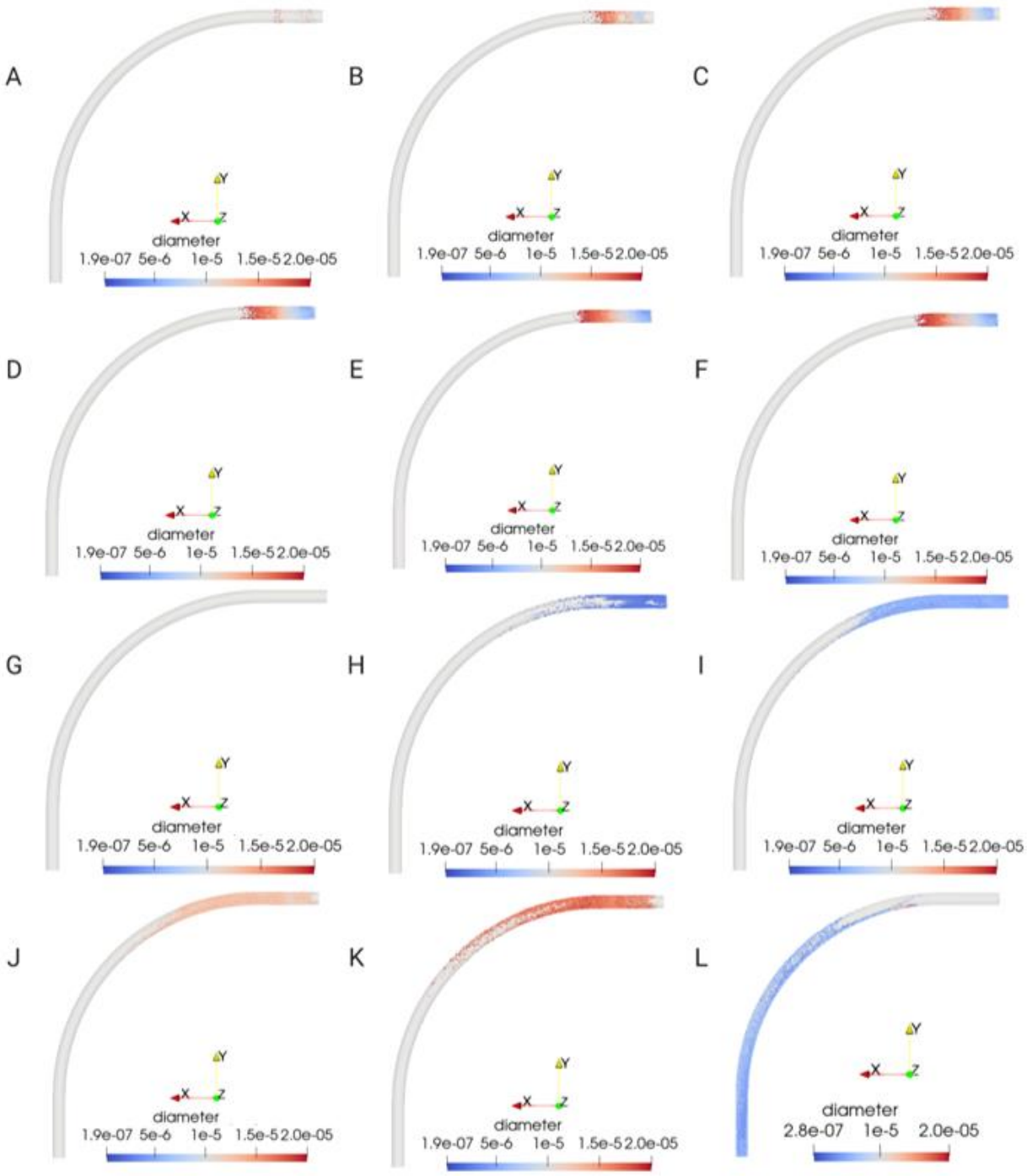

Figure 10. Standard tracheal tube, particle deposition, turbulent model. A–F, particle deposition at times 0.002, 0.01, 0.02, 0.04, 0.1 and 0.15 (i.e. during spray injection) and G–L, breakdown of particles by particle size ranges, at end-time (0.32 s): G: particles sized 0.1–1 µm, H: 2–3 µm, I: 4–6 µm, , J: 11–14 µm, K: 15–20 µm, L: active particles at end time. Diameter scale for all figures is in m.

Laminar and turbulent model comparison–standard tracheal tube only

The peak velocity at the simulation final timestep was higher with the laminar model than with the turbulent model (Figure 11, C, D) which allowed particles to move further through the flow domain for the laminar model during the same time-period (Figure 11 A vs B).

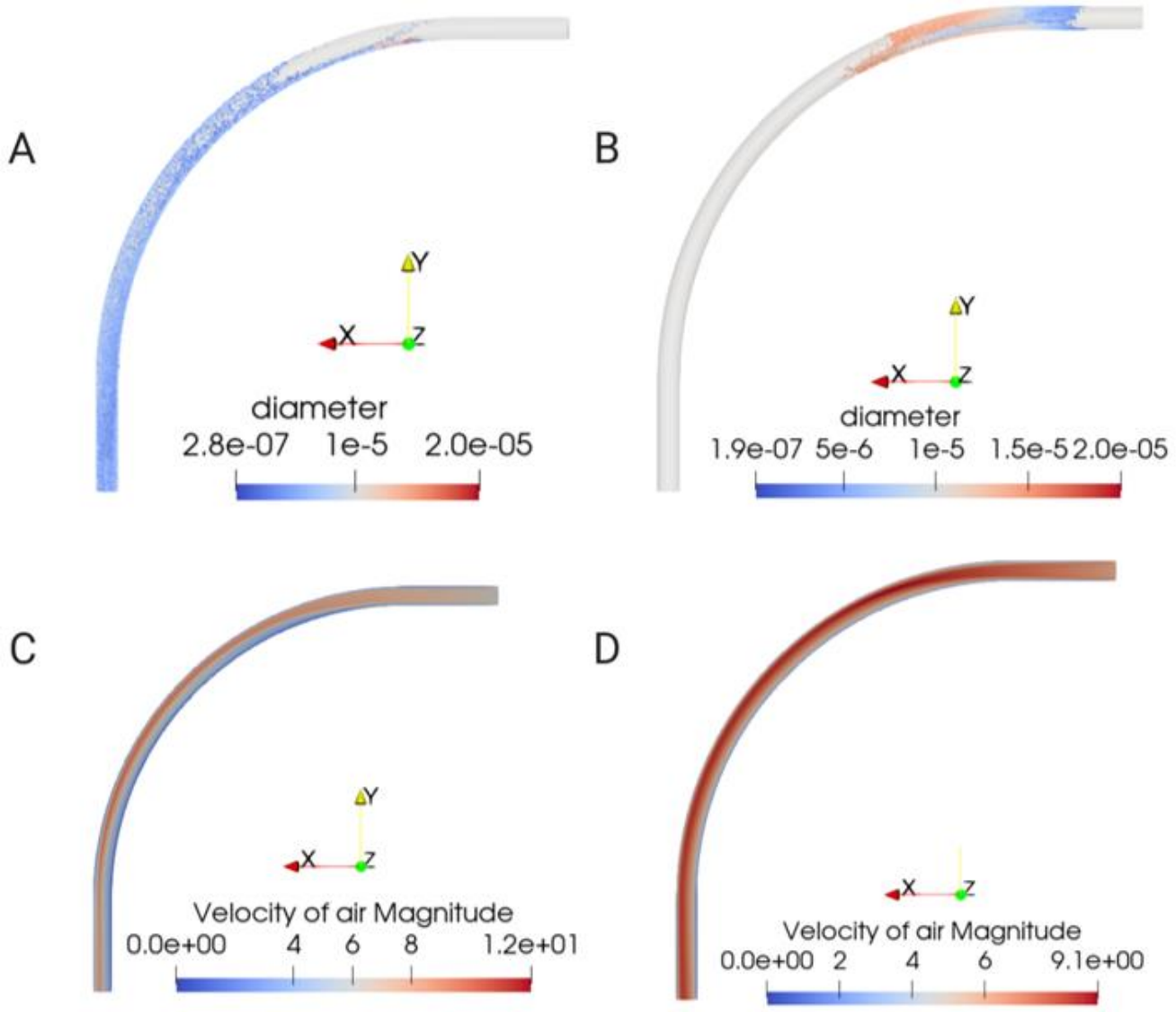

Figure 11. Comparison between laminar and turbulent models for the standard tracheal tube. A: laminar model, active particles at end-time (0.32 s), B: turbulent model, active particles at end-time (0.32 s), C: laminar model, velocity at end-time (0.32 s), turbulent model, velocity at end-time (0.32 s). Diameter scale is in m.

Table 2. Salbutamol mass exiting each tube*: In silico* and laboratory experimental comparison. Injected mass total is 95 µg salbutamol, from one actuation of a pMDI (Ventolin™). Mean from laboratory result is from three repeat measurements or as cited. *In silico* estimates are also shown as percent of total mass (95 µg). Underlined values indicate the model that most closely represented the laboratory result for that geometry. *Reported from (1). [a]The sum total

mass exiting or depositing in the tube was 93 µg for this simulation (~2 µg active) , [b]The sum total mass exiting or depositing in the tube was 83 µg for this simulation (~12 µg active), [c]The sum total mass exiting or depositing in the tube was 94 µg for this simulation (~1 µg active), [d]The sum total mass exiting or depositing in the tube was 72 µg for this simulation (~23 µg active), [e]The sum total mass exiting or depositing in the tube was 95 µg for this simulation (Table 1). Results are 0 for the tracheostomy tube with the turbulent model since no mass exited the airway during this time.

Tube type	***In silico* estimated mass exiting each tube with different models**		**Laboratory measured mean mass exiting the tube, n=3 or reported***
	Laminar (µg (% of total mass injected))	**Turbulent (µg (% of total mass injected))**	**% of total**
A (standard tracheal tube)	60 (60%)[a]	0.78 (1%)[b]	10 %*
B (pre-formed tracheal tube)	68 (%)[c]	1.86 (2%)[d]	4.7 %
C (tracheostomy tube)	11.3 (11%)[e]	0	13.6 % *

Discussion

Medicinal aerosol delivery via artificial airways requires off-label use of approved inhaled medicines and is therefore an understudied area. This work attempts to progress a model of medicinal (salbutamol) aerosol delivery via pressurised metered dose inhaler through three commonly used artificial airways that are used during surgery in children. For the simulations that converged, and where close-to-full mass was recovered (approximately 95 µg), it was identified that: 1) a laminar model best represented the experimental drug delivery data for a tracheostomy tube, 2) a turbulent model better represented the preformed tracheal tube, and 3) neither a laminar or turbulent model was representative of the standard tracheal tube. It was expected that two different models would be required, as the flow Reynolds number was originally calculated to be in the transition zone.

There were clear differences between turbulent and laminar models for the cases that ran to completion. When using turbulent models, a greater number of particles deposited earlier in both time and space, compared to the laminar model. However, for the particles that remained in the system, with both laminar and turbulent models there was an increase in deposition between the 0.28–0.32 time point, which corresponded to rapid increase in velocity from 3.3 (at 0.285 s) to 6.1 $m.s^{-1}$ (at 0.35 s). Turbulent modelling approaches also resulted in smaller particles depositing later (in time), generally not moving as far (in space), and a backflow of smaller particles depositing near the inlet boundary. Particle deposition was spread more evenly over time and throughout the (spatial) length of the artificial airway with a laminar model. Particles decelerate quickly after the injection due to

viscous effects (i.e. drag) in the continuous phase (i.e. air), and the motion of the particles then becomes dominated by the entrainment from the flow of the continuous phase. By using the RAS *k–ε* turbulence model, the velocity of the spray injection also appears to drop more rapidly, in comparison to the laminar case.

Low pressures (relative to atmospheric pressure set at the outlet) were observed around the spray region with both the tracheostomy tube and standard tracheal tubes, where the particles are injected at high velocity (40 $m.s^{-1}$). Flow recirculation was also observed around the spray region, resulting in smaller particles depositing close to the inlet boundary. Deposition of particles near the inlet is consistent with experimentally reported "losses to actuator", the small amount of drug (~5 µg) commonly found in mouth pieces after injection (42). Since the injection region (injection speed 40 $m.s^{-1}$) was placed on the inlet (required to accurately model the real-life setting) backflow toward the inlet was not unexpected.

A key aspect of this simulation was the time delay required to accurately model the real life setting – the particle injection begun at time 0 and went until 0.156 seconds, correspondingly, the velocity of air at this time is only at 0.3 $m.s^{-1}$. Having a high-speed jet entering the air at rest created a back flow toward the inlet, which is suspected to have led to small calculation errors accumulating over time and in some cases not running to completion.

Comparison with experimental results

For the first two geometries (i.e. the standard tracheal tube and the preformed tracheal tube), using a laminar model greatly overestimated particle deposition and a turbulent model underestimated deposition. Largely, the discrepancy between experimental and computational result could be due to inability to sufficiently model turbulence, which could be improved with a better-quality mesh. It has been previously shown that hexahedral and tetrahedral meshes can be used to model aerosol deposition with similar accuracy, however a more detailed investigation into mesh type and how the cells near the surface effect turbulence properties could determine the best quality mesh to use (43).

Limitations and Future Directions

Modelling of turbulence

Here, an eddy-interaction model (EIM) – the standard *k–ε* was used to simulate the continuous flow which affects the motion of the particles and their trajectories – the only option built in with the MPPICFoam solver – the standard model based on Launder and Spalding and with a rapid distortion compression term by El Hanry (44, 45). Previously the standard *k–ε* model has come under criticism for being inaccurate for prediction of re-circulating flows and complex shear layers, such as in flows subjected to curvature, like seem with the preformed tracheal tube (geometry B) particularly (44, 46,47,48). It has previously been shown that EIM *k–ε models* can over predict particle deposition by 200% (compared to experimental values) in simulated mouth-throat models, which may be attributed to the model's dissipative nature (49, 50, 51). Whilst the geometries modelled here were simple,

the calculated Reynolds number indicated transitional flow, and all geometries were curved, so a similar principle would apply, especially considering the ~30-fold increase in deposition seen between laminar and turbulent models of the same geometry here (i.e. both the standard and pre-formed tracheal tubes). The effect of turbulence modelling was likely to be less pronounced with the tracheostomy geometry due to particles depositing in the tube earlier (in time) compared to the other two geometries, which was due to the injection location being closer to a wall. The standard k-ε models have previously failed in capturing the laminar-transitional-turbulent airflows when compared with the present LRN k-ω model (51). Alternatively, numerous model validations demonstrated that the low-Reynolds-number k-ω model, plus near-wall correction EIM, produce good accuracies when simulating micron-particle deposition in human upper airways, and is more computationally efficient (52, 53, 54). The LRN k-ω model with near wall corrections has been shown to predict within 10% of experimental data in other complex (polydisperse) pharmaceutical aerosols in airflow models of the mouth and throat (55). The LRN k-ω model has also demonstrated good agreement with experimental data in 90ºbends and has good agreement with historical data (55, 56). It is indicated that Large Eddy Simulations (LES) may predict particle deposition better when compared with standard k-ε and standard k-ω models or the shear-stress-transport model (50). LES can directly predict the instantaneous fluctuating velocity to calculate the particle trajectories and is a better choice than the RANS plus EIM modelling.

Accounting for particle-particle collision

Although this study was only designed to look at similarities between experimental and *in silico* models, the particle deposition patterns indicate that adhesive forces would occur due to particle-particle contact . High particle load is present during the injection spray, or as part of either thermodynamic or hydrodynamic cloud phenomenon (50,58) which will alter the particle size distribution. The collision exchange model used by the OpenFOAM (version 8) solver (MPPICFoam), is a transient solver for the coupled transport of a single kinematic particle cloud including the effect of the volume fraction on the continuous phase (41). The collision model is based on the work of O'Rourke (2009) and multi-phase particle in cell (MPPIC) modelling is used to represent collisions without resolving particle-particle interactions (4158). Whilst particle-particle interactions may only present to a small degree, adhesive properties of the particles and the flow rate have an enormous impact on the dynamics of the entire system, and more complex models or complete resolution should be considered in future models (59). Particle-particle interactions can be resolved with a discrete element method, however it is more computationally intense to directly solve collision calculations with high particle load (59, 60,61). Combined continuum-based and discrete-element-based models (termed CFD-DEM models) are increasingly being used to consider multiphase flows and solve particle-particle interactions (61,62). Further, when aerosol particles contact one another, they form agglomerates (63) and whilst agglomeration (and resultant altered particle size distribution) is difficult to calculate for poly-disperse aerosols, any particle-particle interaction resulting in agglomeration would mean that more larger particles were present and therefore the particle distribution would be skewed towards a larger

MMAD. The effect of agglomeration and increased particle size distribution has been seen previously with inhaled corticosteroids produced by pMDI where the measured fraction of particles less than 5μm (MMAD) was reduced with a larger dose per actuation (250 μg) compared to the smaller dose (125 μg) of the same aerosol drug formulation (64). If particle-particle interaction could be captured accurately the current model can be used to simulate particle flows with higher concentrations such as are found with the use of pMDI aerosol generators in confined geometries like artificial airways.

Conclusion

The work described here adds progress towards a multiphase model to assess high particle-load in air under transitional flow conditions such as is present during an inhalation when administering a dose of aerosolised medication by a pressurised metered dose inhaler through an artificial airway. Having identified key limitations of the model, such as use of a *k–ε model for turbulence*, future models using LRN k-ω model with near wall corrections should be applied. Considerations should also be made for particle-particle interactions, such as the use of combined CFD-DEM models. Future models can be used to supplement experimental aerosol studies providing data unobtainable experimentally, such as accurate particle size data exiting the artificial airway, which would be indicative of the expected therapeutic effect delivered to the child and could potentially replace difficult experimental studies.

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APPENDIX

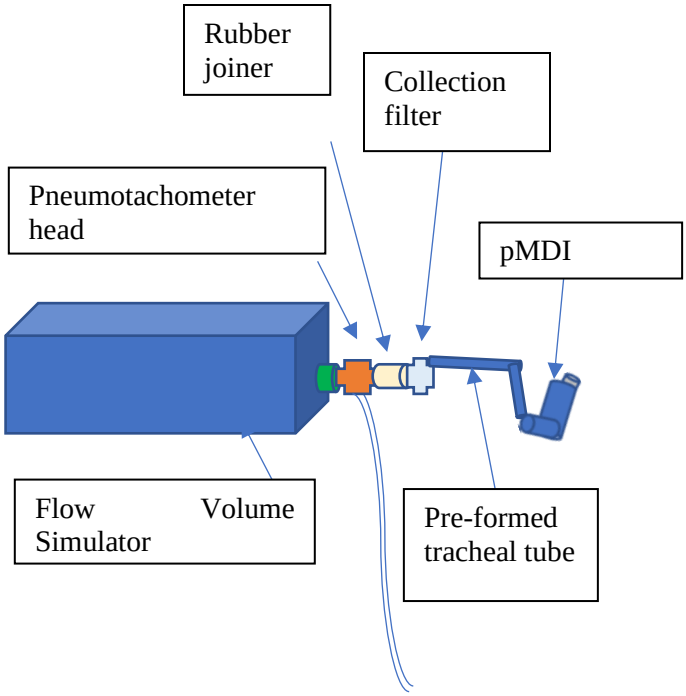

Figure A. pMDI = pressurised metered dose inhaler. The pMDI is actuated into a flow generated by the flow volume simulator programmed with a paediatric sinewave. Drug gets trapped on the collection filter which can then be rinsed and assayed to quantify drug.

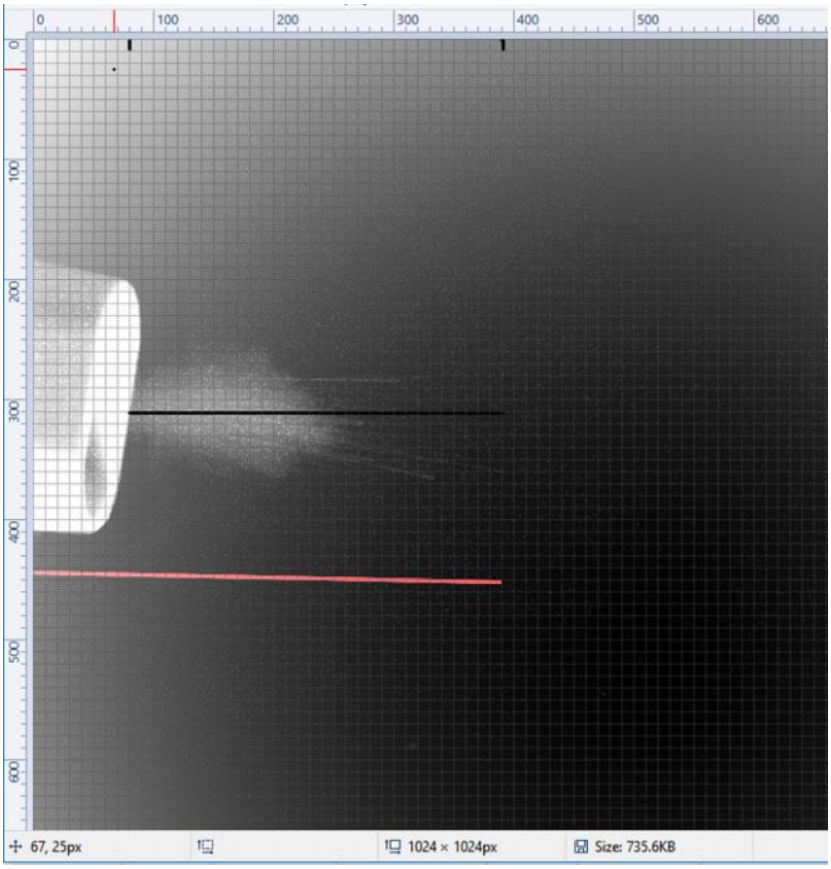

Figure B. High speed camera image taken to determine aerosol velocity when the salbutamol (Ventolin) inhaler was actuated, a high-speed camera. The velocity determined from the image was used to optimise injection spray velocity and verify computational results.

Table A. Inlet velocity Each time step shows a ramped increase in the velocity up to 0.8 second, the peak of the inhalation.

Time	Velocity (x)
0	0
0.05	0.1
0.1	0.2
0.15	0.3
0.2	0.4
0.21	0.4
0.225	1.0
0.235	1.2
0.245	1.5
0.25	1.7
0.265	2.2
0.275	2.7
0.285	3.3
0.295	4.3
0.3	5.3
0.35	6.1
0.4	6.8
0.45	7.6
0.5	7.7
0.55	8.1
0.6	8.5
0.65	8.5
0.7	8.6
0.75	8.9
0.8	9

Table B. Ventilation guidelines for children at Perth Childrens Hospital.

waveform settings, and ETTs appropriate for the age of the child being modelled.
Ventilator settings, Volume controlled ventilation 6 ml/Kg:

ETT 3.0 LMA 1	Neonate 3 kg = 18 mls
ETT 3.5 LMA 1.5	1 year old 10 kg = 60 mls
ETT 4.0 LMA 2	2 year old 12 kg = 72 mls
ETT 5.0	4 year old 16 kg = 96 mls
ETT 6.0 LMA 3	10 year old 30 kg =180 mls
ETT 7.0 LMA 4	16 year old 50 kg = 300 mls

AGE	Ventilator SETTINGS
<1yr	RR = 30 : inspiratory time 0.6
1-6yr	RR = 25 : inspiratory time 0.8
>6-12yr	RR = 20 : inspiratory time 0.9
>12-16yr	RR = 15 : inspiratory time 1.0

Table C. Number of cells in each geometry.

Geometry	**Internal diameter**	**Number of cells**
Pre-formed tracheal tube	5 mm	1064391
Standard Tracheal tube	5 mm	1093897
Tracheostomy tube	5 mm	109861

Table D. Boundary conditions

	inlet	**outlet**	**solid wall (tube)**
Velocity	uniform Value Table (see appendix Table B)	Zero gradient	Fixed value (0 0 0)
pressure	Zero Gradient	Fixed value uniform 0	Zero gradient
Wall condition	escape	Escape or stick	stick

Table E. Conditions in air (kinematic viscosity, 1.5e-05 $m^2.s^{-1}$) for k and epsilon with varying turbulence values as calculated from https://www.cfd-online.com/Tools/turbulence.php using option: Turbulence intensity from turbulence kinetic energy (k) and freestream velocity U infinity.

Freestream Velocity ($m.s^{-1}$)	**Turbulence intensity (%)**	**Length scale (m)**	**Turbulent kinetic energy value $(m^2.s^2)^{-1}$**	**Turbulence dissipation (epsilon) value $(m^2.s^3)^{-1}$**
6.25	1	0.00019	0.006	0.212
6.25	2	0.00019	0.023	1.69
12.5	1	0.00019	0.023	1.69
12.5	2	0.00019	0.09	13.6

Chapter 6: A rapid semi-quantitative screening method to assess chemicals present in heated e-liquids and e-cigarette aerosols

Plain language introductory statement to Chapter 6.

Electronic cigarettes are an unapproved therapeutic good worldwide, and as such rigorous safety and efficacy testing has not been conducted to ensure that the product does not pose a threat to human health. Therefore, Chapter 6 sought to test the chemical composition of various e-liquids that are heated by the e-cigarette device to produce an aerosol. The complex aerosol mixture produced by the electronic cigarette presents difficulties to measure and thus to determine any health effects in humans and a simplified testing method is suggested here which can reduce testing time and difficulties obtaining enough sample to measure. The results from this work were used to advise regulators and consequently, a modest list of certain ingredients were banned in Australia by the TGA in October 2021.

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A rapid semi-quantitative screening method to assess chemicals present in heated e-liquids and e-cigarette aerosols

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Abstract. Electronic cigarettes (e-cigarettes) lack regulatory status as therapeutic products in all jurisdictions worldwide. They are potentially unsafe consumer products, with significant evidence they pose a risk to human health. Therefore, developing rapid, economical test methods to assess the chemical composition of e-liquids in heated and unheated forms and the aerosols produced by e-cigarettes is crucial. Four different e-liquids were heated using two different methods: (1) "typical" vaping using an e-cigarette device, by cycling "on" for 3 s every minute for 2 h (e-liquid obtained from remainder in the tank and aerosol collected in an impinger), and (2) "accelerated" heating, using an e-cigarette coil submerged in e-liquid and heating in short 20 s bursts on then 20 s off for 2 min only (liquid traps aerosol produced). All e-liquids were then analysed to test for the presence and quantity of 13 chemicals by gas chromatography–mass spectrometry and compared to an unheated sample. E-liquids heated with the accelerated method showed a comparable trend to the typical heating method, i.e. increase or decrease in chemical compound quantity, for more than two-thirds of the detected compounds analysed over all e-liquids. Six chemicals were detected as aerosol from the impinger fluid with the typical heating method at negligible levels. We propose that this accelerated version of the typical vaping method could form the basis of a standardized screening tool to test heated e-liquids (and e-cigarette aerosols) for harmful or banned substances. This will ensure that only approved products reach the consumer and reduce potential e-cigarette harm.

1 Introduction

Chemicals are present in the aerosol produced by electronic cigarettes (e-cigarette) when the e-liquid it contains is heated and aerosolized (Goniewicz et al., 2014; European Parliament, 2014). The e-cigarette refers to the aerosol-generating device, which uses the "e-liquid" to create aerosol by an evaporation–condensation method. If in sufficient quantities, and dependent on the hazard type, chemicals present in an e-cigarette-produced aerosol have the potential to negatively impact health when inhaled (European Association for the Co-ordination of Consumer Representation in Standardisation, 2021). For example, group 1 carcinogens acetaldehyde and formaldehyde have been found in e-cigarette

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aerosols and are degradation products of the e-liquid base components, propylene glycol and glycerol (Goniewicz et al., 2014). Other chemical ingredients found in unheated e-liquids that pose a risk to human health (e.g. respiratory irritants, sensitizers) may include flavours or solvents added to the e-liquids. These chemicals can be present in unheated e-liquid, heated e-liquid, aerosolized e-liquid, or any combination of the three forms. Yet, as recently as 2015, no country in the world regulated e-liquid ingredients beyond nicotine levels.

As more evidence emerges suggesting that e-cigarette aerosols negatively impact health, regulation is rapidly evolving in this area (European Parliament, 2014; Budzyńska et a., 2020; Therapeutic Goods Administration, 2021). For example, the European Union (EU) Tobacco Product Directive (TPD) states that only ingredients in nicotine-containing e-liquids that do not pose a risk to human health in heated or unheated forms can be used (European Parliament, 2014), and many countries (including United Kingdom, Germany, and France) banned some ingredients as a result (European Parliament, 2014; European Association for the Coordination of Consumer Representation in Standardisation, 2021; Budzyńska et al., 2020). A modest ingredient ban for nicotine-containing e-liquids came into effect in October 2021 in Australia, prior to a complete ban on non-prescription e-cigarette (and e-liquid) importation and sale in Australia being announced in May 2023 (Nogrady, 2023; Therapeutic Goods Administration, 2021). Known health effects of banned ingredients include e-cigarette- or vaping-associated pulmonary (or lung) injury (a.k.a VAPI or VALI/EVALI). There is emerging evidence of self-reported lung conditions associated with e-cigarette use (Winnall et al., 2023; Osei et al., 2020; Bircan et al., 2021). Health concerns are amplified by a multibillion-dollar market driving product sales and increasing use in young people. Consequently, pre-market approvals (including unheated ingredient listing), which allow independent determination of product safety (Australian Government, 2023; United States Food and Drug Administration, 2023), are preferred by regulators.

Whilst chemical testing of unheated e-liquids is relatively common, the safety of e-liquids remains largely unassessed due to the sheer scale of the market. Additionally, except for the EU TPD guidance (to the best of our knowledge), testing heated e-liquids for inclusion in pre-market product approval is not required in Australia or elsewhere (European Parliament, 2014; Winnall et al., 2023; Scientific Committee on Health Environmental and Emerging Risks, 2021; Larcombe et al., 2022). Methods to assess chemical content generally involve testing of unheated e-liquids or the e-cigarette aerosol generated, but not the heated e-liquid (Scientific Committee on Health Environmental and Emerging Risks, 2021). However, the heated e-liquid is more representative of what the user inhales compared to the unheated e-liquid and easier to assess when compared to e-cigarette aerosol (Larcombe et al., 2022; Erythropel et al., 2019). Importantly, chemicals present in e-liquids are known to degrade due to heating, by either boiling or evaporative convection (depending on wetted-wick temperature), and this can be exacerbated by the presence of catalytic surfaces such as Kanthal. The secondary products formed, at high ($> 200\,°C$) or low temperatures ($< 200\,°C$), may have increased or decreased toxicity compared to the parent compound (Erythropel et al., 2019; Floyd et al., 2019; Goniewicz et al., 2014; Jaegers et al., 2021; Li et al., 2021; Zhao et al., 2016; Saliba et al., 2018).

Many approaches have been trialled to address the difficulties in directly assessing the chemical composition of e-cigarette-produced aerosol. Such challenges include collecting enough aerosol to perform an assay (and therefore detect potentially toxic compounds) and overload of the main excipients (propylene glycol and vegetable glycerin). These difficulties induce increasingly complicated test methods, yet current methods have been shown to be outdated or limited (Floyd et al., 2019; Herrington and Myers, 2015; Scientific Committee on Health Environmental and Emerging Risks, 2021). Despite the expansive range of tests, a simple test capable of assessing heated and unheated e-liquids, and the aerosol produced all at once, is yet to be established, but vital.

In this study we aimed to (1) establish and validate a simple "accelerated", method of heating and aerosolizing e-liquids (i.e. "vaping") that would be comparable to, but quicker than, the "typical" method of heating and aerosolizing e-liquid and (2) assess the chemical composition of the heated e-liquid and the aerosol produced all at once. We hypothesized that the accelerated method and the commonly used typical vaping method would result in similar heating-induced chemical changes in the e-liquids. The two heating methods were compared by measuring the presence and concentration of a range of chemicals. We hope that this accelerated test methodology can form the critical first step in establishing a rapid test for screening of e-liquids for banned substances.

2 Materials and methods

2.1 Typical vaping process

A set-up was designed to replicate the heating–cooling process an e-liquid would undergo when an e-cigarette is used in a typical way (Fig. 1a) (Etter and Bullen, 2014; St Helen et al., 2016; CORESTA, 2015). The method allowed sample collection at two points for analysis of heated e-liquid and aerosol, respectively: (1) from the remainder in the e-liquid tank (atomizer) and (2) from the impinger (Fig. 1a). To begin, the e-cigarette (MVP4, Innokin, Shenzhen, China, operating wattage range of 6–100 W, temperature range of 150–315 °C, maximum current of 35.5 A) atomizer (the e-liquid tank, containing e-cigarette coil) was filled with ~ 3.5 mL of e-liquid, and the impinger was filled with 5 mL of e-liquid

excipient (50 : 50 glycerin–propylene glycol (v/v), Sigma Aldrich, Milwaukee WI, USA). A flow of $\sim 3\,\mathrm{L\,min^{-1}}$ ambient filtered air was drawn through the system via a laboratory bench vacuum and kept stable through monitoring with a flow meter (max $5\,\mathrm{L\,min^{-1}}$, TSI, 800669, Shoreview, MN). New coils (Kanthal BVC, 100–200 W, 0.28 Ω, Innokin Scion) were used each time to avoid cross-contamination of chemical species and to control for coil ageing effects. The e-cigarette device was set to 80 W (reading 0.28–0.35 Ω) each time the device was connected to the atomizer. To vape the device in line with recommendations by the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA), the ignition button was held for ∼ 3 s, and the aerosol was drawn from the device into a 60 mL syringe and then expelled through two ∼ 4 mm ID, 15 cm tubing lengths into the custom-made (27 L, 30 × 30 × 30 cm) chamber using a three-way tap (Dispoflex™, Disposafe Health and Lifecare Ltd, Haryana, India) (CORESTA, 2015). This process was repeated every minute for 2 h (with the atomizer tank refilled after ∼ 60 min), for 120 puffs total. While we acknowledge that vaping topography is extremely variable, 120 puffs over a 2 h period (120 puffs × 60 mL, therefore 7.2 L of inhaled aerosol-containing air) was chosen to be representative of what a typical vaper might use (Etter and Bullen, 2014). After heating, the liquid was transferred to glass sample vials and kept at 4 °C to minimize the loss of volatiles.

2.2 Accelerated vaping process

An accelerated vaping process was developed, based on standard tests for oxidation of oils (Fig. 1b) (American Society for Testing Materials, 2022). Our premise for collection of e-cigarette aerosols in the liquid was as follows:

1. An e-cigarette is an evaporation–condensation aerosol generator, intended to modify the e-liquid as little as possible during aerosolization; however, it does thermo-oxidize, hence the need for this research.
2. Our accelerated method of heating the e-liquid via a submerged coil creates a "bubbling aerosol generator" (Vidmantas et al., 1997). Like an evaporation aerosol condensation generator, a bubbling generator will modify the e-liquid minimally; however, it may allow more volatile compounds to preferentially aerosolize.
3. The creation of an aerosol via bubbling can allow aerosol capture whilst bubbling either through the bulk liquid (when cooling) or at the gas–liquid surface (Ghiaassiaan and Yao, 1997; Koch and Weber, 2012).
4. Surface bubbles can generate aerosol by either jet or film droplets when they burst, and based on combinations of surface tension and bubble size, aerosol will recombine with the liquid the bubble arises from when it bursts (Koch and Weber, 2012; Mead-Hunter et al., 2018).
5. Thereby, through a combination of these processes it is reasonable to assume we retain a representative sample of the same material that is aerosolized, as well as possibly more of the thermo-oxidized (aged) material we are interested in.

To create the bubbler, an identical Kanthal BVC coil, as used in the typical vaping process, was connected to a power supply (MP3090, PowerTech, China) by means of solid coper wires connected to each end of the coil (end cap removed). The power supply was set at 7.4 V and 27 A (0.274 Ω) to stay within the maximum power (200 W) of the coil used for the typical process and to ensure that the resistance matched that of the typical vaping process. The coil was placed in a 100 mL beaker, which was open to air and held at a 45° angle with a clamp stand, and ∼ 30 mL of e-liquid was poured into the beaker, enough to completely submerge the coil and ensure the full volume of liquid would not heat to boiling temperature within the 1 min total heating period (20 s on 20 s off × 3), and limited planar surface evaporation would occur (Fig. 1b). The 45° angle was used both to minimize the liquid volume needed to immerse the coil and to ensure any aerosol (or vapour) produced would recondense on the wall of the beaker, allowing it to be collected for sampling. The power supply was then turned on to operate the coil for 5 × 20 s "burst" intervals, with 20 s pauses interspersed for a total "on" time of 1 min, mimicking a "short cluster" vaping pattern for a user (St Helen et al., 2016). After heating, the liquid was transferred to glass sample vials and kept at 4 °C to minimize the loss of volatiles.

2.3 Sample and chemical selection

Four flavoured e-liquids, labelled "nicotine-free", were assessed – "butterscotch tobacco", "menthol", "choc caramel", and "tiramisu" – which were purchased from online suppliers and analysed as 50 : 50 propylene glycol–glycerin (v/v) ratios. The propylene glycol–glycerol mixture was selected as 50 : 50 since it is a commonly sold ratio. Each e-liquid chemical composition was assessed using both methods to quantify 13 chemicals – 4-(4-methoxyphenyl)-2-butanone, ethyl vanillin, eugenol, nicotyrine, nicotine, menthol, thymol, ethyl maltol, trans-cinnamaldehyde, 2-chlorophenol, benzyl alcohol, benzaldehyde, and furfural – with a molecular weight range from 178.23 to 96.09 $\mathrm{g\,mol^{-1}}$. The 13 chemicals were chosen based on (i) being previously identified, known ingredients in e-liquids and (ii) the availability of a standard for the chemical (Larcombe et al., 2022).

2.4 Chemical analysis method of accelerated and typical vaping process

Thirteen chemicals were tested for, in four different e-liquids, using gas chromatography–mass spectrometry. For each of the four e-liquids, we tested for chemicals in three forms –

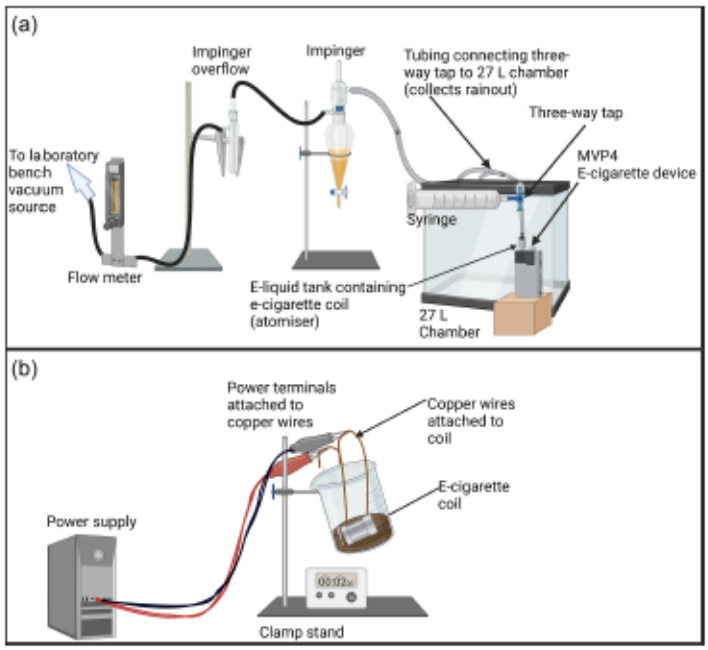

Figure 1. Vaping set-ups. **(a)** Typical vaping set-up. A vacuum drew air through the system at $\sim 3\,L\,min^{-1}$. The aerosol was drawn into a 60 mL syringe, and a three-way tap was turned to allow the syringe to push the aerosol through two ~ 4 mm i.d. and ~ 15 cm tubing lengths and into the 27 L chamber for mixing. Air containing aerosol was drawn first into an impinger with 50 : 50 propylene glycol–glycerin base liquid. **(b)** Accelerated vaping set-up. The power supply was attached to copper wires, which were attached to an e-cigarette coil (Kanthal BVC), which was always submerged in e-liquid within a 100 mL beaker. Key differences between methods: (1) aerosol is allowed to mix in air before capture rather than impinged immediately in liquid, and (2) volume of liquid is different; 3.5 mL is present in the atomizer with the typical method compared to ~ 30 mL in the beaker with the accelerated method. Key similarities between methods: both methods apply the same amperage to the coil (and therefore heat coil to the same temperature). Created with https://www.biorender.com/ (last access: 21 October 2023).

(i) "unheated" e-liquid (i.e. straight out of the bottle), (ii) remainder of e-liquid in the atomizer and collected from the impinger after typical vaping, and (iii) e-liquid remaining in the beaker after accelerated vaping. The latter sample (iii) was taken in order to detect aerosols, assuming that aerosols (not vapour) would be captured in the e-liquid during the accelerated method. The aerosol generated from the typical method was captured in an impinger containing 50 : 50 (v/v) glycerin–propylene glycol. Our intention was for this collected aerosol in the impinger to be added to the atomizer tank sample for equivalent comparison to the accelerated sample; however negligible values for the impinger result meant that they were excluded from the final analysis.

Samples obtained from both methods used to heat e-liquids were compared to unheated e-liquids, both within e-liquid type and within chemical compound, with the purpose of the comparison being to identify trends of increase or decrease from unheated e-liquid.

2.5 Chemical detection and analysis

Chemical analysis of accelerated and typical vaping process e-liquids, including sample and chemical detection, has been previously described in detail elsewhere (Larcombe et al., 2022). The samples (0.25 g) were accurately weighed and placed into amber vials with 4.75 mL ultrapure water. Thereafter, 10 µL of a $1\,g\,L^{-1}$ 4-bromophenol-d4 stock solution was added as an internal standard. Prior to the analysis, 1.6 g of analytical-grade sodium chloride was added to increase volatilization and the vials tightly capped. To facilitate adsorption, the samples were incubated at 90 °C for 15 min prior to solid-phase micro-extraction using a divinylbenzene/carboxen/polydimethylsiloxane fibre from Supelco®, allowing for 13.6 min adsorption of the analytes on the fibre. The fibre was then desorbed at 250 °C in the injector in spitless mode for 5 min followed by 15 min in split mode. A Gerstel MPS2 multifunction autosampler was used to perform automated solid-phase micro-extraction injections. Analysis was carried out with an Agilent 6890N gas chromato-

graph interfaced with an Agilent 5973 network mass selective detector, fitted with a HP-INNOWax polyethylene glycol stationary-phase capillary column (30 m, 0.25 mm, 0.25 µm, Agilent J&W, Australia), to separate polar compounds. A constant flow (1.2 mL min^{-1}) of helium (99.999 % pure, BGC, Australia) was used as a carrier gas. Optimal gas chromatography–mass spectrometry conditions were determined, as measured by maximum sensitivity, baseline separation of analytes, and Gaussian peak shapes. In order to ensure a good separation of the different analytes, the oven was held isothermal at 37 °C (2 min), then heated to 260 °C at 5 °C min^{-1} and held at the final temperature for 10 min. Detection of analytes was carried out using a mass spectrometer in electron impact ionization mode at 70 eV. The mass spectrometer quadrupole temperature was set at 150 °C and the mass spectrometer source at 230 °C. The compounds were identified using a combination of their retention times and comparison of the mass spectra data of pure compounds and the specific diagnostic ion fragments of each component, with the National Institute of Standards and Technology Mass Spectral search programme from the NIST (National Institute of Standards and Technology)/EPA (Environmental Protection Agency)/NIH EI (National Institutes of Health Electron Ionization Library) and NIST Tandem Spectral Library, which came integrated with the analysis software.

3 Results

Over all e-liquids, 3 of the 13 compounds tested for were not detected in any e-liquid form (4-(4-methoxyphenyl)-2-butanone, thymol, 2-chlorophenol) (Table 1).

3.1 Inclusion and exclusion criteria for analysis

There were 16 instances where a chemical was detected in unheated and both heated forms (Table 1), and all were included in analysis. Analysis involved (1) simple comparison in table format of the heated (two methods) and unheated form of an e-liquid sample and (2) comparison via fold change compared to unheated ($(Y - X)/X$, where X is the unheated sample (mg L^{-1} concentration), and Y is the heated sample (mg L^{-1} concentration)) for both typical and accelerated heating methods.

Fold-change analyses were not possible on the following: one chemical was undetected in unheated form but detected in both heated forms (menthol in butterscotch tobacco); one chemical was undetected in unheated form and detected in only one heated form (trans-cinnamaldehyde in choc caramel). A further three chemicals were detected in unheated form and only one heated form (benzyl alcohol (tiramisu), eugenol (tiramisu), and furfural (tiramisu)) (Table 1, represented by italicized values).

3.2 Behaviour of the different chemicals detected in e-liquids

To compare the effect of heating, results are displayed as fold change compared to unheated ($(Y - X)/X$, where X is the unheated sample (mg L^{-1} concentration), and Y is the heated sample (mg L^{-1} concentration)) for both typical and accelerated heating methods (Fig. 2). Specific chemicals (benzaldehyde, benzyl alcohol, ethyl vanillin, ethyl maltol, furfural, menthol, nicotine, and nicotyrine) were present in unheated form and both heated forms in 16 instances.

Over all e-liquids, in these 16 instances, 70 % (11/16) demonstrated a consistent trend within chemical type; i.e. both methods of heating either increased or decreased in concentration compared to the unheated sample. Ethyl vanillin (choc caramel and tiramisu), furfural (butterscotch tobacco), ethyl maltol (tiramisu), and benzaldehyde (choc caramel) are the five exceptions.

3.3 Chemical characterization by e-liquid type

In the menthol e-liquid, of the 13 chemicals tested, 9 were not detected in the heated or unheated sample (Table 1). Of the four that were detected (nicotine, nicotyrine, menthol, and benzaldehyde), all (4/4, 100 %) exhibited the same trend (increasing concentration) after heating when compared to the unheated sample for both typical and accelerated heating methods (Table 1, Fig. 2).

In the butterscotch tobacco e-liquid, of 13 chemicals tested, 8 were not detected in heated or unheated form, and an additional 1 (menthol) was undetected in unheated form (Table 1). Of the four chemicals detected in each sample, three (3/4, 75 %) (ethyl vanillin, benzyl alcohol, and benzaldehyde) exhibited the same trend for both typical and accelerated heating methods when compared with the unheated e-liquid (Table 1, Fig. 2). However, furfural increased after typical heating but decreased with the accelerated method when compared to the unheated sample.

In the tiramisu e-liquid, of 13 chemicals tested, 7 were not detected in heated or unheated form, and an additional 3 were undetected in 1 form of heating (Table 1). Of the three detected in each sample, one (1/3, 33 %) (benzaldehyde) exhibited the same trend after both typical and accelerated heating (Table 1, Fig. 2). The remaining two chemicals detected (ethyl vanillin and ethyl maltol) both decreased after typical heating but increased after accelerated heating when compared to the unheated sample.

In the choc caramel e-liquid, of 13 chemicals tested, 7 were not detected in heated or unheated form, and an additional 1 (trans-cinnamaldehyde) was undetected in both unheated and heated form (Table 1). Of the five chemicals detected in each sample, three (3/5, 60 %) (benzyl alcohol, ethyl maltol, and furfural) exhibited the same trend after both typical and accelerated heating methods (Table 1, Fig. 2). Benzaldehyde increased after typical heating but decreased

Table 1. Assessment of 13 different chemical compounds from four e-liquids in both unheated and heated samples (accelerated and typical vaping methods). The unheated sample is comprised of only e-liquid in "fresh" or un-vaped form. The heated sample for the typical method assessed the leftover from the atomizer tank, and the accelerated method assessed heated e-liquid produced from a coil submerged in e-liquid. Chemicals are listed in alphabetical order. Bold font: increase from unheated sample. Acc: accelerated or aged. U: undetected. Italicized results indicate that the compound was not present in one of the heating method samples, and therefore fold change analysis was not possible for that chemical in that e-liquid flavour. Numbers are accurate to three significant figures (digits), and values less than zero are displayed with logarithmic scale for ease of reading.

Chemical tested (mg L^{-1})	Menthol unheated	Menthol heated (acc)	Menthol heated (typical)	Butterscotch	Butterscotch (acc)	Butterscotch (typical)	Tiramisu unheated	Tiramisu heated (acc)	Tiramisu heated (typical)	Choc caramel unheated	Choc caramel heated (acc)	Choc caramel heated (typical)
Benzaldehyde	9.87×10^{-3}	**1.29×10^{-1}**	**4.51×10^{-2}**	1.49	**2.33**	**2.16**	6.24×10^{-1}	**2.48**	**1.06**	1.49	2.81×10^{-1}	**2.05**
Benzyl alcohol	u	u	u	13.40	**49.53**	**53.46**	345	*u*	285	2.38	49.53	231
Ethyl maltol	u	u	u	u	u	u	339	**723**	225	891	693	669
Ethyl vanillin	u	u	u	45.65	1.46	1.63	747	**3250**	2373	**1660**	**1740**	76.98
Eugenol	u	u	u	u	u	u	*1.89×10^{-1}*	*u*	*1.25×10^{-1}*	u	u	u
Furfural	u	u	u	1.73×10^{-1}	9.38×10^{-2}	**7.21×10^{-1}**	*6.57×10^{-1}*	*u*	***8.68×10^{-1}***	5.16×10^{-1}	**4.78**	**2.25**
Menthol	32.54	**932**	**230**	*u*	***30.18***	***11.83***	u	u	u	u	u	u
Nicotine	4.77×10^{-1}	**8.53**	**8.60**	u	u	u	u	u	u	u	u	u
Nicotyrine	2.70×10^{-4}	**271×10^{-3}**	**1.92×10^{-3}**	u	u	u	u	u	u	u	u	u
Thymol	u	u	u	u	u	u	u	u	u	u	u	u
Trans-cinnamaldehyde	u	u	u	u	u	u	u	u	u	*u*	**13.39**	*u*
2-chlorophenol	u	u	u	u	u	u	u	u	u	*u*	u	u
4-(4-methoxyphenyl)-2-butanone	u	u	u	u	u	u	u	u	u	u	u	u

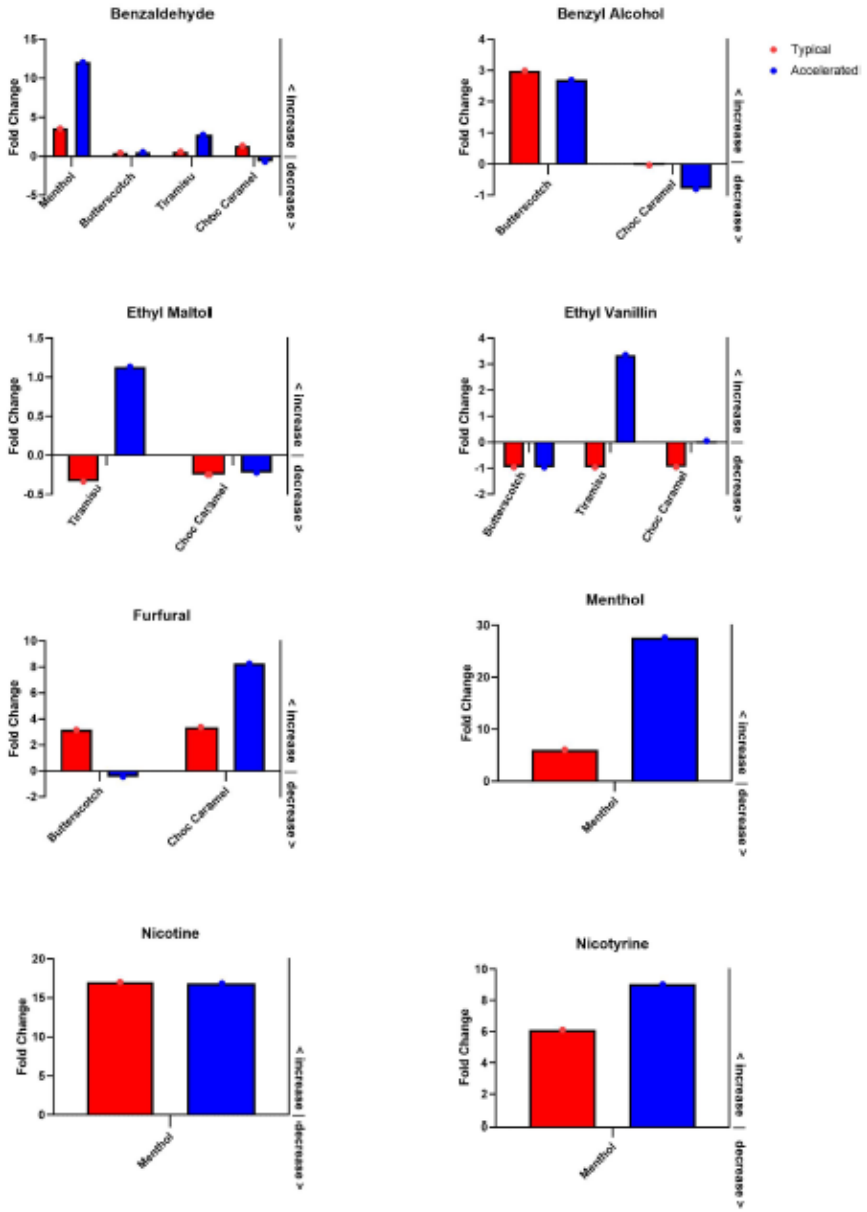

Figure 2. Fold change comparison between heating methods. E-liquid flavours are described on the x axis. Accelerated and typical methods are indicated by the blue and red, respectively. The y axis indicates the fold change compared to unheated, i.e. fold change $= (Y - X)/X$, where X is the unheated sample (mg L^{-1} concentration), and Y is the heated sample (mg L^{-1} concentration). Note different scales. Values that fall below the horizontal line at zero indicate a decrease in concentration from the unheated sample; values above zero are an increase from unheated. If any chemical was not detected in a particular flavour then those flavours are not shown (e.g. there was no ethyl maltol detected in the menthol or butterscotch flavour). Graph created with GraphPad Prism 8.

after accelerated heating compared to the unheated sample. Conversely, ethyl vanillin increased after accelerated heating but decreased after typical heating when compared to the unheated sample.

3.4 Impinger results from typical vaping heating method

Only 6 of the 13 chemicals tested were detected at negligible quantities in the impinger fluid – furfural, benzaldehyde, menthol, benzyl alcohol, ethyl maltol, and ethyl vanillin – and as such they were unable to be included in final analysis.

4 Discussion

The accelerated method used here is simple and cost-effective and has the potential to produce heated e-liquid and aerosol in a similar manner to an e-cigarette but on an accelerated time frame, allowing chemical assessment in a single experiment. Due to the prohibitive costs of commercially available vaping machines, many "in-house" simplified methods and set-ups (e.g. e-cigarette puffing machines) have been developed. However, to the best of our knowledge, they all focus on generation of e-cigarette aerosol and not on assessment of the heated e-liquid (Palazzolo et al., 2021).

Comparison of the accelerated and typical heated samples with their unheated counterpart showed that in ~70 % of the e-liquids tested the heating methods demonstrated a similar trend, i.e. increase or decrease in chemical concentration. The four chemicals implicated in the five differing comparisons were mostly aldehydes (ethyl vanillin (2/11), furfural (1/11), and benzaldehyde (1/11)) except for ethyl maltol (1/11), being an alcohol. In two chemicals – ethyl vanillin (2/11) and ethyl maltol (1/11) – an increase in chemical concentration with the accelerated heating method compared to the typical method was detected. The observed "increase" with the accelerated method can be attributed to a loss of aerosol with the typical vaping experimental method. Rainout (recondensation of the aerosol as it cools) of the liquid aerosol was observed in the three-way tap system and the very thin tubing (ID ~4 mm) connecting the tap system to the 27 L chamber (Fig. 1a). A modification of the design (e.g. larger tubing) would reduce the rainout losses, thus increasing the yield from the impinger with the typical method and improving reproducibility between methods. It is also possible that the flavour aldehydes were present in their propylene glycol acetal form instead of their aldehyde form, as aldehydes are known to form acetals readily (Erythropel et al., 2019). The inability to fully capture the aerosol from the typical heating method due to rainout meant we are unable to confirm the suitability of our accelerated heating method as an impinger for aerosol, but only its validity to compare heating methods.

The remaining two discrepancies involved furfural (1/11) and benzaldehyde (1/11), and these compounds were found in increased quantities with the typical method compared to the accelerated method. Because these two discrepancies contain low-molecular-weight and low-boiling-point products we suspect they may have evaporated more readily (compared to the typical method) and that our accelerated method was simply unable to capture compounds with low boiling point that volatilize easily (Erythropel et al., 2019). For example, furfural has the lowest boiling point and molecular weight of all chemicals detected (162 °C, 96.09 g mol^{-1}) and was detected to have decreased from the unheated sample in both butterscotch and tiramisu flavours. While our study design angled the beaker at 45° to allow re-condensation of any vapour on the beaker wall, the experiment was carried out in a ventilated fume hood, which may have increased the loss of highly volatile compounds. In future studies, the addition of a lid on the angled beaker as well as monitoring the liquid temperature on the wick (or surrounding the wick) may help to reduce discrepancies to allow full validation of the method for detection of aerosols (Li et al., 2021; Palazzolo et al., 2021; Bitzer et al., 2018). Further studies should also be designed to consider the solubility of the flavouring compound in the base excipients (i.e. propylene glycol or glycerol) and not the boiling point, as this is suggested to be a major indicator of whether a compound will be detected in and therefore carried over into an aerosol (Erythropel et al., 2019).

Undetected chemicals included 4-(4-methoxyphenyl)-2-butanone, thymol, and 2-chlorophenol. Considering that no "fruity" flavours were assessed, it is less surprising that 4-(4-methoxyphenyl)-2-butanone was undetected, as it is a raspberry ketone methyl ether – a common flavouring in "berry"-flavoured e-liquids. Although we were looking for thymol because of its use as a precursor for racemic menthol (produced from m-cresol), its absence may be explained because pulegone and other terpenoids are also used as the precursor (Dylong et al., 2022). Additionally, menthol was not detected in either tiramisu or choc caramel flavours, so perhaps synthetic analogues were present (where menthol was not detected) such as N-ethyl 2-isopropyl-5-methylcyclohexanecarboxamide (trade name WS-3). As the demand for menthol increases, alternative methods to produce L-menthol are on the rise, such as from citronellal (Dylong et al., 2022).

Chlorophenols like 2-chlorophenol have previously been detected in e-liquids probably because they are notorious environmental contaminants. Particularly, 2-chlorophenol is a priority contaminant in both the US and EU and has previously been found in e-liquids (Larcombe et al., 2022; Chivers et al., 2019; Igbinosa et al., 2013); 2-chlorophenol is used for many applications, predominantly as a detergent but also as an intermediate in the manufacturing of agricultural chemicals, pharmaceuticals, biocides, and dyes. Therefore, it is commonly detected in environmental water samples after being discharged from industrial effluents (Igbinosa et al., 2013; Yahaya et al., 2019). It has previously been suggested that 2-chlorophenol may be a contaminant from the glyc-

erin excipient for two reasons: (1) vegetable glycerin is made from plant crops such as canola, and 2-chlorophenol has been found in canola as a pesticide residue, and (2) glycerin (not from plants) is a by-product of biodiesel production, and biodiesel can be made with canola (Abdel-Gawad and Hegazi, 2010; Yahaya et al., 2019). It is possible that derivatives of 2-chlorophenol or other phenolic derivatives known to be priority contaminants were present. However, it was not within the scope of the study to assess these compounds as they are not commonly reported to be found and/or tested for in e-liquids. Eugenol and trans-cinnamaldehyde were the least detected compounds; they were found in only one flavour e-liquid. Cinnamaldehyde might not be detected because it is known to form propylene glycol acetals ($\alpha\beta$, unsaturated aldehyde), and this form is known to be relatively reactive (Erythropel et al., 2019).

Limitations and future directions

A limitation of this study is that we tested a pre-determined list of chemicals, based on our knowledge of known e-liquid ingredients, available standards, and available analytical methods, rather than looking for a complete chemical characterization. This approach allowed us to test a larger range of e-liquids and demonstrate the utility of the accelerated ageing technique, as per the overarching goal of the study. However, an "open-ended" approach may be useful for future studies if this method is to become standardized. An open-ended approach would allow a more complete comprehension of the ageing process and oxidation reactions occurring but would require a broader range of analytical techniques than demonstrated here. Furthermore, whilst we assessed for some ingredients which are now banned (i.e. benzaldehyde and cinnamaldehyde), this study was designed and conducted prior to the enactment of banned ingredients by the Therapeutic Goods Administration in 2021 (Therapeutic Goods Administration, 2021), and expanding future analysis to include the full range of banned chemical products would be helpful. The same comment applies for chemicals banned in other jurisdictions.

The method we describe in this study has many advantages over current methods for testing e-liquids. It is a rapid and inexpensive set-up allowing assessment of the chemical composition of heated e-liquids and, with minor modifications, their resultant aerosols. It could be used with any available coil that can be modified and powered as described. Furthermore, the accelerated method is likely to capture aerosol generated from a heated e-liquid in a manner comparable to the "typical user" vaping method as described in CORESTA in terms of both type and quantity of chemicals produced. Our submerged, rapid heating–cooling method can economically sample heated liquid and aerosols (but not vapour) within a single sample in 2 min, which may have advantages over some other methods. This method is more representative of what the user inhales as it is testing a heated liquid during exposure to the coil (potentially) catalytic surface rather than only an unheated e-liquid.

5 Conclusion

In summary, the accelerated method described here is a suitable screening tool for rapid chemical assessment of heated e-liquids and their aerosols that mimics typical e-cigarette vaping on an accelerated time frame. The EU TPD recommendation is to assay heated e-liquids; however (to the best our knowledge) there has only been one previously published study on the effects of ageing and/or heating on e-liquids. We propose that this method (with our recommended improvements) can be used as a standardized screening tool for e-liquids and their aerosols to identify potentially harmful chemicals, such as those recently banned in Australia or previously banned in Europe and the United Kingdom. With minor modification, this test could be used prior to importation or sale to ensure that only tested products, containing approved ingredients, reach the consumer. In the absence of an approved therapeutic goods status for e-cigarettes, the type of high-throughput testing described here is necessary as a minimal precaution to assess and reduce the potential harms of a consumer product that is generally accepted in the public to be a less harmful alternative to smoking.

Data availability. Data can be obtained upon request by contacting the author.

Author contributions. NA: data curation, formal analysis, investigation, methodology, project administration, visualization, writing (review and editing), writing (original draft). PP: data curation, formal analysis, investigation, methodology, validation, writing (review and editing). RMH: formal analysis, investigation, writing (review and editing). BM: conceptualization, methodology, resources, writing (review and editing), funding acquisition. AL: conceptualization, methodology, project administration, resources, supervision, visualization, writing (review and editing), funding acquisition. SA: data curation, formal analysis, investigation, methodology, resources, supervision, writing (review and editing), validation, visualization.

Competing interests. The contact author has declared that none of the authors has any competing interests.

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Chapter 7: General discussion: Limitations and future directions

The overarching vision for the work presented in this thesis was to use laboratory experimentation (Chapter 3,4,6) supplemented with an *in silico* study (Chapter 5), to assess the safety and/or efficacy of various off-label uses of inhaled medicines to help inform clinical outcomes and practice. Off-label use is particularly notable in susceptible populations such as patients with artificial airways and young children, where much of this thesis was focused (Fink, 2012). The other focus of this thesis, and of considerable public health concern, was the inhaled aerosol produced by the unapproved therapeutic the e-cigarette – used unapproved for the purpose of smoking cessation. While part of the original plan for this thesis was to further assess the safety of the unapproved e-cigarette aerosol with computational studies after preliminary experimental assessment, the regulatory environment in this area has evolved rapidly in Australia over the last few years, making such a study less of a priority. For example, government consultation processes informed by scientists (including our research team) and peak bodies, fueled by concerns for e-cigarette use in young people, led to a ban of several e-liquid ingredients in 2021 and a complete ban on importations for non-prescription use in 2023 (Australian Government, 2023). However, the use of computer modelling and simulation to assess inhaled drugs during device design and pre-clinical (pre-market) testing is of increasing relevance and may indeed form part of the pre-market testing process itself, since it can potentially replace *in vivo* radiolabeled deposition studies.

Chapter Two review and summary

Since best practice must be employed when using medications in an off-label manner, the educational review article published in *Pediatric Anesthesia* (Chapter 2) (Anderson et al., 2022) reviewed and summarized current best practice for relieving bronchospasm caused by acute asthma exacerbation for both awake and anesthetized and/or ventilated (intubated) children. Although limited evidence was available, best practice was determined to be with the short-acting beta agonist salbutamol (an example of a solid particle aerosol), delivered by a pMDI and spacer, assuming patient (child) compliance with treatment if awake. Nebulized aerosol delivery via a facemask occurred in the awake setting but was not common practice in the anesthetized and/or ventilated setting. Nebulised delivery involved a longer treatment time compared to pMDI and spacer but offered the benefit of a continuous delivery of multiple medications. There was little evidence to suggest that nebulised therapy to treat bronchospasm was useful for anesthetized and/or ventilated children. There were a variety of methods used commonly to deliver aerosol produced by a pMDI to anesthetized children and it was unclear which methods would be most effective, and spacer methods were used infrequently, warranting further investigation.

Regardless that the best aerosol generation device was with a pMDI and spacer, it was clear from this educational review that both liquid and solid particle inhaled medication delivery systems have benefits in a pediatric emergency setting. Having the ability to choose the patient interface in an awake and upright-seated setting give the option to choose from both solid and liquid aerosol delivery, which given a distressed child, is advantageous.

Closing the gap between aerosol delivery science and clinical practice is essential to minimize morbidity and mortality by facilitating a safe recovery and identify potential areas for technological or clinical improvements.

Chapter Three review and summary

Since there was a lack of data available to inform aerosolised pMDI salbutamol delivery to anesthetized spontaneously breathing children, the Chapter 3 study published in *Pediatric Anesthesia* in 2020 (Anderson et al., 2020) was designed to gather experimental laboratory data comparing the effectiveness of simulated pMDI salbutamol delivery. This was done for different types of artificial airways within certain patient (child and adult) sizes, when using a variety of commonly used delivery methods (ways to attach the pMDI to the artificial airway device). The measured outcome was mass of salbutamol dose exiting the artificial airway. Results demonstrated several key things: 1) that commonly used methods for delivering aerosolised salbutamol in anaesthetic settings do not deliver the manufacturer-intended salbutamol (100 µg) dose per actuation (or "puff"), 2) some methods do not allow the pMDI device to function as intended and should be discontinued (i.e. taking a pMDI canister out of the mouth piece and placing it in a syringe to actuate the dose delivery) and 3) some methods (like spacer methods) are more effective for drug delivery than others. Those methods identified as most effective delivered at least the same dose that an awake child would receive, and all child sizes/ages would receive an equivalent dose, when considering µg or $mg.kg^{-1}$ dose received. This study summarised that when aerosolised delivery is via standard tracheal tubes and supraglottic airway devices, the best method to deliver aerosol is by using a valved or non-valved spacer which will maximise delivered dose and respirable fraction of the inhaled dose. If a spacer device is not available (e.g. in lower income settings) the direct method of administration – attaching a pMDI with mouthpiece directly to the artificial airway port – could be employed with similar effectiveness to a spacer method, but slightly reduced respirable fraction. For a tracheostomy airway, the direct method is as effective as using a spacer.

This chapter provided experimental results of mass exiting the artificial airways under a variety of delivery methods and matched experimental *in vivo* data where it was available. Further, this work highlighted that the manufacturer intended dose per actuation (or puff) was not delivered to the child with any airway type or method and thus could be improved. Results from this study can be used to inform clinical best practice and future guidelines for aerosol delivery to anesthetized children breathing spontaneously using an artificial airway.

Chapter Four review and summary

To improve the empirical evidence-base with which to develop aerosol delivery guidelines for children using artificial airways, the original study in Chapter 4 assessed the delivered dose of the inhaled (nebulised) antibiotic tobramycin (a liquid aerosol) from two starting doses, to simulated spontaneously breathing children using a tracheostomy airway, via two types of nebulisers. Although a similar quantitative study has been conducted in mechanically ventilated adults (Dhanani et al., 2018), to the best of the authors knowledge tobramycin aerosol

delivery had not been investigated quantitatively in a delivery setting to children (either intubated and spontaneously breathing or mechanically ventilated). In this study, when tobramycin aerosol was delivered to simulated children, 15% (or less) of the nominal dose exited the tracheostomy tube with either a jet or a vibrating mesh nebuliser. Depending on the airway size (3, 4 or 5 mm), with a jet nebuliser, only ~3–15% of the nominal dose was delivered with either a low (80 mg) or high (300 mg) starting dose. With a vibrating mesh nebuliser, ~1–15% was delivered. Such a low delivery of drug has important ramifications, particularly as an antibiotic, and emphasises the need for delivery optimisation. We found that the ventilation parameter of breath volume had a greater effect on the aerosol delivery than the size of the airway – there was less aerosol delivery through the 3 mm airway (18 mL breath) than either 4 or 5 mm airway (96 mL breaths). This study also found that due to the proximity of the nebuliser to the tracheostomy tube, aerosolization was not the primary mode of delivery and build-up of the aerosol in the tracheostomy tube caused a liquid film on the surface of the tracheostomy tube, which then coagulated to form larger droplets which formed a partial mechanism of delivery.

It was identified that: 1) aerosolised delivery was between 1–3% of the nominal dose when delivered via a 3 mm ID, and between 10–15% when delivered by either a 4 or 5 mm ID tube, and therefore breath volume rather than tube size influenced drug delivery, a finding consistent with previous studies in adults (Dhanani et al., 2018), 2) when the nebuliser is placed at close proximity to the tracheostomy airway, the primary mechanism of inhaled delivery is not solely aerosolization, but may be primarily delivery via large droplets. Therefore, to improve clinical practice and likelihood of a respirable fraction of aerosol reaching the patient (and decrease surface film build up in the tracheostomy tube) increasing the distance between the nebuliser and the tracheostomy tube should be investigated in future studies.

In summary, inhaled antibiotic delivery via nebuliser should be improved. Future studies should be conducted in accordance with US Pharmacopeia (Pharmacopeia, 2015), or relevant ISO standards (International Organisation for Standardisation, 2023) to assess particle size and characteristics of the exiting drug and completed under realistic (i.e. gravitationally correct) conditions to ensure secondary droplet/condesate phenomena is captured, and therefore both filter dose and lung dose estimate is accureate. Special consideration should be made when choosing measurement equipment to ensure that a potentially high particle load of aerosol can be assessed (i.e. high concentration of particles per cubic centimetre), along with properties such as surface tension, viscosity of the drug formulation, and if possible, hygroscopic effects once inhaled. Computational fluid dynamics studies should be employed in combination with experimental studies to explain physical phenomena contributing to poor delivery and optimise future delivery.

Chapter Five review and summary

To improve aerosol delivery – which was demonstrated to be poor for both solid and liquid aerosols in Chapter 3 and 4, Chapter 5, sought to replicate the experimental set-up of Chapter 3 *in silico*, using computational fluid dynamics studies. *In silico* studies allowed a comprehensive and detailed analysis of the physical conditions under which the aerosol was delivered and could elucidate areas for improvement. Chapter 5 investigated aerosol delivery via three common types of artificial airways used to intubate children during surgery and investigated aerosol generation via pressurised metered dose inhaler for the solid particle aerosol, salbutamol used to treat perioperative bronchospasm. The simulations were conducted to replicate (as best as possible) the real life scenario. The computational results were then compared to earlier obtained experimental data from Chapter 3.

While the comparison with experimental results was not in complete agreement, it was possible to obtain several key findings from the study and make suggestions for model improvements. A key finding was that the high speed jet of the particle injection entering the air at rest (during actuation of the pMDI) created a low pressure (relative to atmospheric pressure set at the outlet) region near the inlet (or the artificial airway port). This occurred with both the tracheostomy tube and the standard tracheal tube but not the preformed tracheal tube which was due to differences in the tube lengths (i.e. the shorter tubes (tracheostomy and standard tracheal tube) created greater negative pressure). This also resulted in smaller particles depositing close to the inlet boundary, which is consistent with the loss of a small component of the dose into the actuator mouthpiece – usually referred to as "losses to actuator" in laboratory experimental studies. It was found that the eddy-interaction model (EIM) – k–ε, which came built-in with the open-source solver used, was not able to sufficiently resolve turbulence. The deposition when turbulence modelling was turned on was greater compared to the laminar models, with up to 98% deposition in one geometry, whereas the maximum mass deposited with any laminar case was 40%. Over-prediction of particle deposition has been seen previously with EIM k–ε modelling, with an up to 200% increase compared to experimental values, which was attributed to the model's dissipative nature. Alternatively, numerous model validations demonstrated that the low-Reynolds-number k-ω model, plus near-wall correction EIM, produce good accuracies (within 10% of experimental values) when simulating micron-particle deposition in human upper airways, and are still computationally efficient.

In summary, Chapter 5 made progress towards a multiphase model to assess high particle-load in air under transitional flow conditions such as is present during an inhalation when administering a dose of aerosolised medication, by a pressurised metered dose inhaler, through an artificial airway. Key model improvements were identified to include: 1) use of the low-Reynolds-number k-ω model, plus near-wall correction EIM to resolve turbulence, 2) use of a collision exchange model that can resolve collision with walls and particles. If computational resources are available, a combined continuum-based and discrete-element-based model (termed CFD-DEM) could be used to solve particle-particle interactions.

Chapter Six

Since electronic cigarettes are used as an unapproved therapeutic good, and therefore their safety and efficacy is undetermined, Chapter 6 sought to determine the presence of a range of chemicals in the liquid (e-liquid) used in e-cigarettes. The intention was to use these chemical data to inform future computational models of aerosol delivery, and be combined with toxicological computational models, to inform the absorption, distribution, metabolism, excretion, and toxicity of the electronic cigarette aerosol. Since current methods to sample and test e-cigarette aerosols were known to be inefficient, this Chapter sought to develop a novel method to produce e-cigarette aerosol and heated e-liquid, on a decreased timeframe in addition to quantitatively testing for chemicals. To develop the method, the regular method of generating e-cigarette aerosol in the laboratory was used and compared to the novel method, for both heated e-liquid samples and their unheated counterpart for several different flavours of e-liquid. Results showed that in ~70% of the e-liquids tested, the heating methods demonstrated a similar trend, i.e. increase, or decrease of chemical concentration. However, aerosol loss was visible with both the regular method (due to liquid rainout in the experimental setup) and the novel method (due to vapour loss) and determined to account for some of the 30 % discrepancy. As such, minor modifications to both laboratory set-ups were proposed, such as using wider bore tubing to reduce liquid capture (rainout) in the tube in the regular set up and putting a lid on the beaker in the novel set up to reduce vapour loss. Further suggestions were made to the testing set-up design: 1) to improve the range of chemicals for detection (e.g. synthetic analogues of menthol) 2) to consider solubility of the chemical in the parent compound, and 3) to measure the wetted wick temperature of the coil. A key finding of this study was also the identification of two flavour chemicals that should not be inhaled in large or small quantities: furfural and benzaldehyde, the latter which is now a banned ingredient as per the Therapeutic Goods Order 110.

This study, published in *Aerosol Research* in 2023 (Anderson et al., 2023), provided a quick and simple testing method for both producing e-cigarette aerosol and for measuring semi-quantitatively the chemical constituents of the aerosol produced. This method could form the basis of a rapid method that could be used in a regulatory setting. By using this method with a larger repertoire of chemicals, chemicals which are known to be dangerous to health can be identified and used in computational modelling and simulation to determine health effects.

Future Directions

All the chapters of this Thesis have involved the testing of various devices and therapeutics that are used to deliver inhaled substances in an off-label or unapproved manner, with the goal of improving the evidence base for clinical practice, improving guidelines or regulatory guidance, or improving industry or regulatory standards. Experimental (laboratory) testing was largely used to provide real-life evidence of efficacy or safety of the therapeutic set up. However, there are limitations to experimentally obtained data, and therefore computer modelling and simulation, such as was used here in Chapter five, is increasingly being used to test the safety, quality, and the effectiveness of medical products. Computer modelling and simulation using computational fluid-

particle (CFPD) dynamics has the potential to model the aerosol-flow system under study with "perfect" accuracy, if: 1) the boundary conditions are understood entirely, and 2) all particles/fluids are fully resolved (e.g. with direct numerical simulation). CFPD models have advanced rapidly over the last 20 years for assessment of medical aerosols and lung inhalation, culminating in an international best practice advice case for static lungs (Koullapis et al., 2018). However, the best practice advice case is only relevant for solid particle aerosol modelling (Poorbahrami et al., 2021) in static adult lungs and assessment of liquid medical aerosol particles has been used in fewer applications and mainly outside the therapeutics field (Abishek et al., 2019, Mead-Hunter et al., 2013, Mead-Hunter et al., 2017). This is because there are difficulties inherent with liquid modelling due to differences in their physical behaviors compared to solid particle aerosols, such as evaporation and condensation which require gas-liquid phase change dynamics to be considered (Sperry et al., 2023). Furthermore, a best practice advice case relevant to inhaled medication deposition in child lungs has not yet been developed. Significant progress, however, has been made in pediatric CFPD models in the last five years (Poorbahrami et al., 2021). Due to the relatively new use of these models in the pharmaceutical industry, guidelines require further development to support these types of technologies and methods in regulatory applications. Examples of guidelines for use of computer modelling and simulation in drug development include the Avicenna Strategy for *in silico* trials (Viceconti et al., 2021) and the American Society for Mechanical Engineers guide for validation of medical devices (The American Society of Mechanical Engineers, 2018), however specific regulatory guideline development (e.g. CFPD use for study of inhaled medicines) is yet to be established. As such, the use of CFPD models is currently considered able to reduce, but not eliminate, the need for extensive experimental studies, or alternatively, it may supplement other data types. Better technologies and methods are particularly relevant for susceptible populations such as children or patients with artificial airways, and progress with computational model development is essential to build credibility (Poorbahrami et al., 2021).

The goal of any model is that it explains as simply and accurately as possible a complex physical phenomenon, and that simplicity is only sacrificed for the benefit of accuracy. Despite explaining complex phenomena more simply than they occur in "real-life", current CFPD models are still extremely complex, computationally-resource intensive and can only be used by people with a comprehensive understanding of the software, physics and the phenomena involved or within a multidisciplinary team. Due to the intense computational resource requirement, models of the whole lung are usually processed in sections. For example, the upper airway is processed first, and results used for input to the lower airway. Additionally, the lower airways do not usually progress beyond the 8th generation and most models only compute the inhalation and not exhalation, unless combined with lower dimensional MPPD models (Kuprat et al., 2023). The most significant progress to date, has been a lung model which includes both upper airway, and lower airway down to terminal bronchiole (Khoa et al., 2023). To further progress of the model described in Chapter 5, the artificial airway geometry could be joined to a model of a child's lung.

7.1.2 Human lung models in health and disease

Since the commencement of this work, and the publication of the ERCOFTAC case (in 2019), more complex models of the lung have been created and aerosol lung deposition simulated down to the terminal bronchioles (Khoa et al., 2023). Additionally, the model provided by the Environmental Protection Agency in 2023 (United States Environmental Protection Agency, 2023) – which is able to be generated simply with access to an internet browser with a high speed internet connection – has considerably reduced the time and skill required to generate these models compared to just a few years ago. However, models are still static and the ability to simulate movement and the extracellular matrix, both which change in disease, is an important component of any lung model that is still under development (Iravani et al., 2020, Mead-Hunter et al., 2013). Movement of the lung should be considered in both health and disease particularly since ventilation is impaired in certain disease conditions and aerosol will resultantly flow to certain areas of the lung (Tiddens et al., 2014). There has been some progress in assessment of imaging data from patients with disease conditions and simulation with CFD and these new approaches may help improve aerosol lung deposition in patients with disease conditions that impair ventilation (Oakes et al., 2018).

7.1.3 The future of aerosol delivery: new aerosol generation and delivery technologies and environmental impact of inhaled medicines

Nebulization was the first method to produce medicinal aerosol over 60 years ago as historically drugs were easier to formulate as liquids. However, as chemical engineering and drug formulation technologies and methods improve (like excipient enhanced growth or spray-drying), more drugs can be formulated as solid-particles. Solid particles are typically more desirable than liquid aerosols – they are less hygroscopic and can therefore penetrate further into the humidified lung environment, simultaneously making them more respirable. Additionally, nebulised drug delivery takes longer than either dry powder inhaler (DPIs) or pressurised metered dose inhaler (pMDI) delivery (minutes as opposed to seconds), and the pMDI remains the most attractive and "easy to use" delivery device compared to DPIs, for all patient ages. However, problems that previously existed with DPIs are increasingly being resolved, such as de-aggregation and a strong inhalation force being required to activate the device, making them as attractive as pMDIs. Additionally, as pharmaceutical companies work towards changing inhaler devices in alignment with the Kigali amendment (2017) to the Montreal protocol (1987) inhaled drug formulations containing propellants (e.g. pMDIs) will change to use lower global warming potential propellants or reformulate to DPIs (that have lower global warming potential compared to DPIs) (Montgomery & Blakey, 2022, Pritchard, 2020, United Nations, 2016). To increase use of these new technologies, barriers must be overcome, such as that in Australia DPIs are only allowed to be prescribed under the benefits scheme by a general practitioner if a patient cannot use a pMDI (Montgomery & Blakey, 2022). Regardless of barriers, it seems likely that modelling of solid particle aerosols may be increasingly important.

7.1.4 Computational safety assessment of electronic cigarettes

Electronic cigarettes are a problem worldwide for respiratory health, with an estimate of over 68 million vapers globally (Jerzyński et al., 2021). Ideally, these devices should have been designed and approved as therapeutic products, since they were originally intended as a smoking cessation tool (Hon, 2004). However, as they are currently designed and used, e-cigarettes are unsafe for human use, and contain many constituents that cigarettes produce, albeit often in lesser quantities (Banks et al., 2022). With increasingly stricter regulations and manufacturing requirements, testing for banned chemical ingredients in heated e-liquids and their aerosols will become increasingly important, and should ideally be incorporated as part of the manufacturing/product development process. CFD has already become an important tool in this space to characterize the dose of nicotine to the lungs and systemic circulation to determine therapeutic efficacy, particularly with new formulations such as protonated nicotine-salt forms (Sperry et al., 2023). The use of CFD to study e-cigarettes is particularly of benefit as there is a huge variety of devices, over 7500 e-liquids (or more) on some markets (National Center for Chronic Disease Prevention and Health Promotion (US) Office on Smoking and Health, 2016) and a variety of ways a user can program (or use) their device. Computational study has already been useful in identifying that the acidity of a formulation has the largest impact on nicotine evaporation – where higher levels of acidity in the formulation reduce evaporation rates and increase nicotine delivery to the lung (Sperry et al., 2023).

7.1.5 Decreasing product development times for children's medicines

The development of a medical device or therapeutic good is a long process, taking 7–12 years for development in adults (Van Norman, 2016) and then up to an additional 7–8 years for the same product to be approved for use in children or other population groups (Bi et al., 2019, Mehrotra et al., 2016). Partly, this is because there has been an unclear or absent direct regulatory pathway for development of therapeutics for children, but also because studies in children are more complex than studies in healthy adults. Additionally, there has been a reliance on animal testing, which often fails to accurately produce the results seen in humans either because of safety or efficacy, leading to products failing to reach market or dropping off the market shortly after launch. Introduction of new laws and regulations, such as the United States FDA Modernization Act 2.0 (Han, 2023) will allow the requirement for animal testing to decrease and a corresponding increase in the uptake and use of non-animal models, tests, technologies and methods (such as CFD) which promise to decrease the time taken for a product to reach the market.

Within inhalation medicine, the new product situation is as bad, if not worse than for other therapeutics as they are considered a "complex" therapeutic in both the EU and the US (but not in AU), meaning there are multiple additional steps to ensure a correct dose is first administered before pharmacokinetics and safety testing can be assessed in the body. This is far more complex than an intravenous medication or an oral tablet. The inhaled medicine field has responded to this by the introduction of the inhaled bioequivalence classification system, like the gastrointestinal bioequivalence classification system which was introduced over 20 years ago (Amidon et al.,

1995, Hastedt et al., 2022). The new iBCS, will help products be “de-risked” meaning that it will (hopefully) take less time for an inhaled therapeutic to come to market (Hastedt et al., 2022).

7.1.5. New imaging methods could allow direct validation of CFPD–PBPK models

Clinical validation of computational methods in humans is important for their use in orally-inhaled drug product development. Efforts should be made to develop safe, high-resolution medical imaging methods to assess the lung deposition of orally inhaled products such as phase-contrast compact synchrotron light source x-ray and phase-contrast magnetic resonance imaging. New imaging methods can also be used to help build more human relevant models of the lung, which can be used in computational assessment. Ideally the clinically validated CFPD model can then be used in combination with physiologically-based pharmacokinetics models (Haghnegahdar et al., 2019) to ensure that the entire process from drug delivery to clearance (mass balance) can be assessed in a “virtual” human, further aiding progress toward the human digital twin (Viceconti et al., 2023).

Conclusion

This body of research has contributed empirical evidence towards improving drug delivery to children in a hospital setting and improved our knowledge about the safety and efficacy of off-label use of approved and unapproved therapeutics, including salbutamol, tobramycin, and the electronic cigarette. Through computational assessment, it has contributed to the body of knowledge on solid particle aerosol behavior when produced by a pMDI and delivered through artificial airways and further outlined direction for future work that can build upon this progress. Results from this Thesis have helped to inform clinical practice and regulatory standards and could further be used to inform consensus statements, or standardized drug delivery guidelines for children breathing through artificial airways.

References

Introduction and discussion

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Statement

Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.

Appendix

Chemical analysis of fresh and aged Australian e-cigarette liquids

Alexander Larcombe[1,2,*], Sebastien Allard[3,*], Paul Pringle[3], Ryan Mead-Hunter[2,4], Natalie Anderson[1,2], Benjamin Mullins[2,4]

The known: E-cigarettes are increasingly popular in Australia, but little is known about the chemicals inhaled by users. Evidence is mounting that e-cigarettes are not benign and can pose significant health risks.

The new: Our comprehensive chemical assessment of Australian e-liquids identified a wide range of potentially harmful chemicals in these liquids, both in their purchased forms and after simulated vaping.

The implications: Australian e-liquids include potentially toxic chemicals, for many of which no information on inhalation health effects are available. Despite the sale of nicotine-containing e-liquids without prescription being illegal, trace amounts were detected in some samples, with implications for their effects on health and addiction.

Abstract

Objectives: To assess the chemical composition of electronic cigarette liquids (e-liquids) sold in Australia, in both their fresh and aged forms.

Design, setting: Gas chromatography–mass spectrometry analysis of commercial e-liquids sold in Australia (online and physical stores).

Main outcome measures: Chemical composition of 65 Australian e-liquids — excipients/solvents, flavouring chemicals, other known e-liquid constituents (including nicotine), and polycyclic aromatic hydrocarbons — before and after an accelerated ageing process that simulated the effects of vaping.

Results: The measured levels of propylene glycol and glycerol often diverged from those recorded on the e-liquid label. All e-liquids contained one or more potentially harmful chemicals, including benzaldehyde, menthol, *trans*-cinnamaldehyde, and polycyclic aromatic hydrocarbons. Nicotine or nicotyrine were detected in a small proportion of e-liquids at extremely low concentrations.

Conclusions: Australian e-liquids contain a wide variety of chemicals for which information on inhalation toxicity is not available. Further analyses are required to assess the potential long term effects of e-cigarette use on health.

Electronic cigarettes (e-cigarettes) are a relatively recent development, and are often marketed as alternative nicotine delivery systems for tobacco smokers.[1] Research into e-cigarettes has increased substantially in recent years, together with debate about their role in tobacco harm reduction and in normalising cigarette smoking. Central to the discussion are the potential health effects of e-cigarettes, many of which are related to the chemical composition of the "e-liquid" used.[2]

E-liquids typically consist of a glycerol and propylene glycol base, flavourings, nicotine, and other chemicals that are heated, aerosolised, and inhaled. An emerging body of evidence suggests that e-liquids often contain a range of potentially toxic chemicals.[2-10] However, information about the composition of e-liquids used in Australia is limited. This is important, as the Australian regulatory status of e-cigarettes and e-liquids is complex, differentiating the market from that of many other countries. E-cigarettes and e-liquids that do not contain nicotine are largely unregulated in most states, and no devices have been approved for therapeutic use.[11] However, we have previously reported that e-liquids labelled "nicotine-free" often contain nicotine.[6] Further, there is evidence that some common e-liquid ingredients react with each other[9] or with components of the e-cigarette device,[12] or may be converted into other chemical species when heated.

Analysis of e-liquids that have been "aged" by repeated heating and cooling may therefore provide additional information about the potential effects on health of e-cigarette use. We employed gas chromatography–mass spectrometry to analyse 65 Australian e-liquids for common excipients, flavourings, nicotine, polycyclic aromatic hydrocarbons, and other chemicals, both in their fresh state and after subjecting the e-liquids to an accelerated ageing process that simulates the effect of "vaping".

Methods

We purchased a range of e-liquids from Australian online and brick-and-mortar stores. Online suppliers of e-liquids were deemed eligible sources if they were operational, were based in Australia, sold e-liquids (some stores sold only e-cigarette devices), and sold their own brand of e-liquids. Online suppliers were identified in a Google search (February 2020) for the term "Australian e-liquid". Of the first 25 search results, ten were for eligible online e-liquid suppliers; we purchased the five best selling e-liquids from each supplier, as reported on their websites. We also purchased 15 e-liquids from brick-and-mortar stores in our home state, Western Australia, selected as a cross-section of local brands and manufacturers at the time of purchase; three were manufactured in New South Wales, 12 in Western Australia. All 65 e-liquids were described as being nicotine-free, and they encompassed a wide range of flavours, including ice/menthol, fruit, dessert, and tobacco (Supporting Information 1).

Selection of chemicals for analysis

On the basis of similar studies,[2,6,8] we selected chemicals for analysis that we expected to find in e-liquids or were known toxins associated with e-cigarettes, including common excipients and solvents (glycerol, propylene glycol, benzyl alcohol), flavouring chemicals (menthol, eugenol, thymol, benzaldehyde, *trans*-cinnamaldehyde, ethyl vanillin, ethyl maltol, furfural, 4-(4-methoxyphenyl)-2-butanone), and other e-liquid constituents (nicotine, nicotyrine, 2-chlorophenol, phenol). We also measured the levels of ten polycyclic aromatic hydrocarbons (PAHs) — large hydrocarbons associated with thermal degradation — for which certified reference material standards were available.

E-liquid analyses

Our analytical methods are fully described in online Supporting Information 2. Briefly, analytical standards for each chemical

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were purchased from Sigma-Aldrich Australia, Rowe Scientific, or Cayman Chemical. As a simple solvent does not reflect the sample matrix of commercial e-liquids, standards were prepared for analysis in 50% propylene glycol/50% glycerol.

Analytes were extracted from the sample matrix by solid phase micro-extraction, which enables solventless extraction using a fused silica or stainless steel fibre coated with a thin film polymer. Headspace (gas phase) analysis using solid phase micro-extraction removes a large fraction of the interference by the propylene glycol/glycerol matrix (> 90% of the sample by mass), and has previously been used for the analysis of volatile compounds, additives, and flavours in e-liquids.[13] We used a divinylbenzene/carboxen/polydimethylsiloxane fibre for all analyses (Supelco). Polar compounds were separated on a 30 m × 0.25 mm HP-INNOWax (polyethylene glycol) analytical column (Agilent J&W), with a film thickness of 0.25 µm and 4-bromophenol-2,3,5,6-d4 as internal standard. Non-polar compounds and PAHs were analysed on a 30 m × 0.25 mm Zebron ZB-5MS analytical column (Phenomenex) with a film thickness of 1 µm and biphenyl-d10 as internal standard. Analytes were detected by mass spectrometry (Agilent 6890N GC/Agilent 5973 network mass selective detector) in electron impact ionisation mode (70 eV); to optimise sensitivity, compounds were quantified in selected ion monitoring mode.

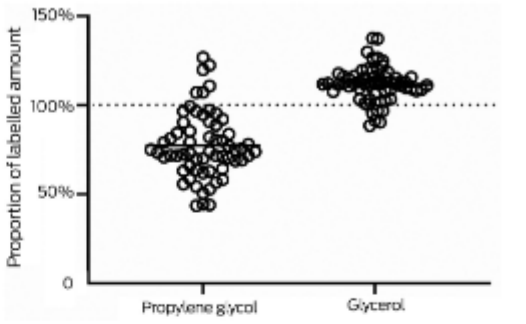
E-liquid ageing

To evaluate chemical changes resulting from vaping (aerosol generation from evaporation and condensation), which can cause thermal decomposition, oxidation, and polymerisation of e-liquid components, we developed a method for simulating the ageing of e-liquids (Supporting Information 2). After accelerated ageing, e-liquids were re-analysed using the same techniques as for fresh e-liquids.

Ethics approval

Ethics approval was not required for this study.

1 Propylene glycol and glycerol content of 62 e-liquids, as proportions of the labelled amount*



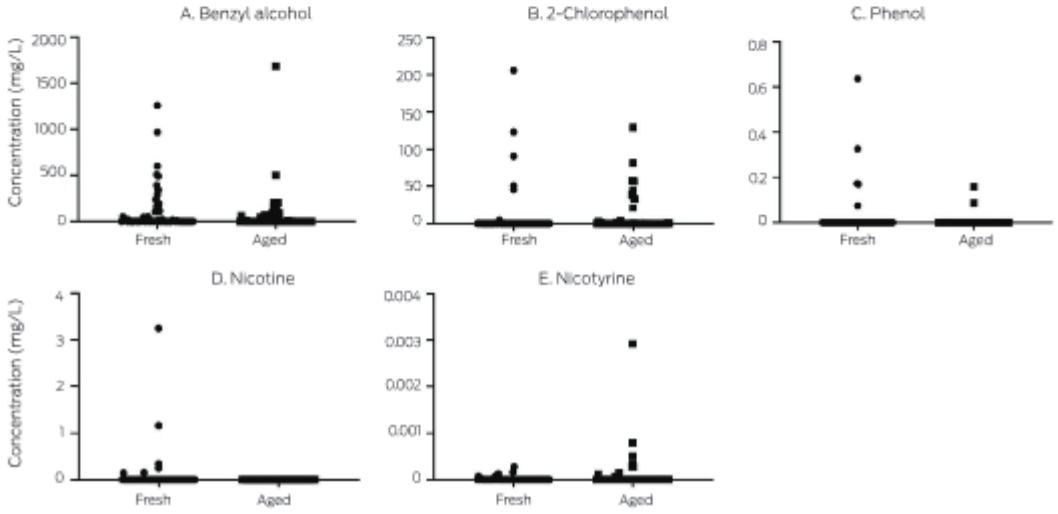
* The solid horizontal lines indicate the respective mean levels of propylene glycol or glycerol.

Results and discussion

Excipients and solvents

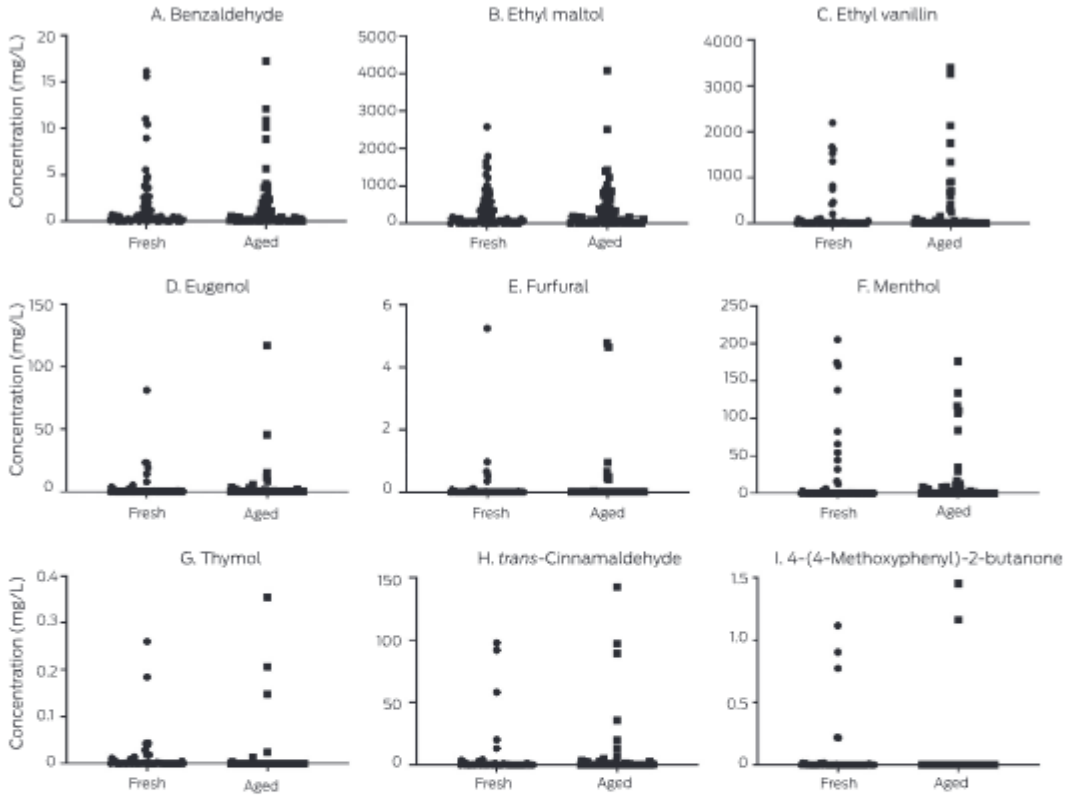
Propylene glycol and glycerol were the main ingredients by proportion in each e-liquid. The propylene glycol/glycerol ratio was not specified for three e-liquids. Most e-liquids were labelled as including 30% propylene glycol/70% glycerol, but the actual value was within 10 percentage points of the labelled amount for only 11 (propylene glycol) or 21 (glycerol) of these 62 e-liquids; in one case, the propylene glycol/glycerol ratio was the reverse of that indicated on the label. The mean propylene glycol content was 77.4% of the label value (standard deviation [SD], 18.5%; range 43.7–126.8%), the mean glycerol content was 111.8% of the label value (SD, 9.7%; range, 88.5–137.5%) (Box 1). The magnitude of these differences was consistent with manufacturers mixing propylene glycol and glycerol on a volume-for-volume rather than a weight-for-weight basis; as the density of propylene glycol is 1.04 g/cm³ and that

2 Concentrations of non-flavouring chemicals in 65 Australian e-liquids, fresh and after ageing



The values for individual products are included in the online Supporting Information 1.

3 Concentration of flavouring chemicals in 65 Australian e-liquids, fresh and after ageing



The values for individual products are included in the online Supporting Information 1.

of glycerol is 1.26 g/cm³, volume-based mixing will increase the amount of glycerol by about 21% of the intended level.

Benzyl alcohol, a solvent/flavour enhancer, was found in 42 of 65 e-liquids and 32 of 65 aged e-liquids, at levels of up to 1687 mg/L (Supporting Information 1). Benzyl alcohol, also found in e-liquids by other studies,[8,14,15] is a dermal sensitising agent and skin allergen that elicits severe reactions in some people.[16]

Nicotine

Nicotine was found in trace amounts in six fresh e-liquids (maximum, 3.25 mg/L) but not in aged e-liquids (Box 2; Supporting Information 1). In our earlier study, six of ten e-liquids contained nicotine (maximum, 2900 mg/L).[6] The results for our more recent samples may indicate cleaner manufacturing processes, or that nicotine was present as nicotine salts rather than as free-base nicotine.[17] Nicotine is relatively common in "nicotine-free" e-liquids,[18] with implications for health and addiction. Nicotine in Australian "nicotine-free" e-liquids could be the result of accidental contamination or poor quality control during manufacture.

Nicotyrine, formed by the dehydrogenation and oxidation of nicotine,[5] is not usually detected in e-liquids unless they have been exposed to air, in which case it accumulates over time. We detected it in seven fresh (including two that also contained nicotine) and nine aged e-liquids (maximum, 2.9 µg/L), indicating that they had previously contained nicotine.

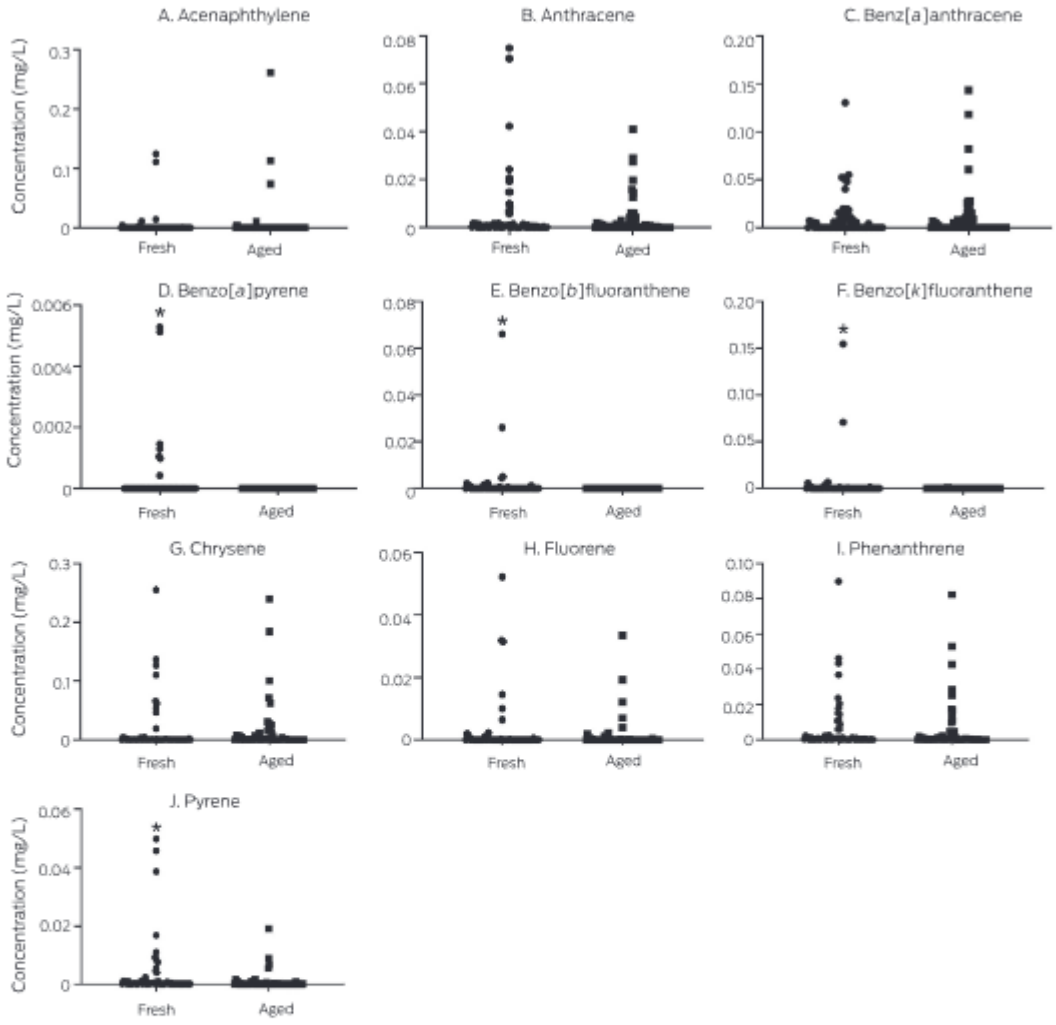
Flavouring chemicals

A range of flavouring chemicals were detected in fresh and aged e-liquids (Box 3; Supporting Information 1). Some, including furfural, thymol, and 4-(4-methoxyphenyl)-2-butanone were found only infrequently or at very low levels. Benzaldehyde, added to e-liquids for its almond-like flavour, was detected in 60 fresh and 61 aged e-liquids at concentrations ranging from 11.4 µg/L to 17.3 mg/L. Benzaldehyde inhibits microsomal cytochrome P450 2A6 (CYP2A6)[19] — increasing systemic nicotine exposure and blood nicotine concentrations in smokers[20] — reduces phagocytosis,[10] and is an inhalation irritant.[21] Benzaldehyde can also react with propylene glycol in e-liquids, producing aldehyde propylene glycol acetals[9] that activate airway irritant receptors.[22]

Other flavouring chemicals we found frequently or at high concentrations were:

- menthol: 44 fresh (maximum, 205 mg/L) and 50 aged e-liquids (maximum, 176 mg/L);
- ethyl maltol: 58 fresh (maximum, 2583 mg/L) and 52 aged e-liquids (maximum, 4084 mg/L);

4 Concentration of polycyclic aromatic hydrocarbons in 65 Australian e-liquids, fresh and after ageing



The values for individual products are included in the online Supporting Information 1. * Statistically significantly higher median concentration in fresh than aged samples (Mann–Whitney test; $P < 0.05$). ◆

- *trans*-cinnamaldehyde: 48 fresh (maximum, 97.9 mg/L) and 38 aged e-liquids (maximum, 142.5 mg/L); and
- ethyl vanillin: 59 fresh (maximum, 2192 mg/L) and 50 aged e-liquids (maximum, 3393 mg/L).

The high frequency of detection and the high concentrations of these chemicals have concerning health implications. Menthol enhances the addictive properties of nicotine[23] and inhibits nicotine metabolism.[20] Menthol was detected in most e-liquids, but only a small proportion were labelled as being "menthol"- or "ice"-flavoured. Conversely, one "menthol" e-liquid contained no menthol, and may have instead contained potentially carcinogenic analogues such as pulegone, or synthetic "coolants" such as *N*-ethyl-p-menthane-3-carboxamide.

Ethyl maltol is added to e-liquids as a sweetener. The effects of heating and inhaling it are largely unknown, but it increases free radical formation in e-cigarette aerosols.[4] Free radicals induce oxidative stress, which affect cell survival and proliferation, and inflammation. Ethyl maltol reacts with iron and copper (potentially present in e-liquids as coil residue) to produce toxic hydroxypyranone complexes.[12]

trans-Cinnamaldehyde impairs innate immune cell function in the lung,[3] suppresses bronchial airway epithelial cell ciliary motility and mitochondrial function,[7] inhibits microsomal CYP2A6, impairs neutrophil, macrophage and natural killer cell function, and reduces oxidative burst when heated and inhaled.[10]

Ethyl vanillin is used widely in foods, beverages, cosmetics, and drugs for its potent vanilla odour and flavour. In e-liquids, it

reduces oxidative burst[10] and inhibits *in vitro* free radical formation.[4] Like benzaldehyde, both *trans*-cinnamaldehyde and ethyl vanillin react with propylene glycol in e-liquids to produce aldehyde propylene glycol acetals.[9]

Other chemicals

We found 2-chlorophenol in 27 fresh and 30 aged samples, at concentrations of up to 206 mg/L (Box 2; Supporting Information 1). Similar chemicals have been identified as pesticide or herbicide residues or decomposition by-products in canola oil,[24] from which glycerol is derived. While not as ubiquitous as in our earlier study,[6] this acutely toxic chemical, used in disinfectants and insecticides, remains a problem for the e-liquid manufacturing process.

Polycyclic aromatic hydrocarbons

PAHs are produced during the thermal decomposition of organic material, including tobacco and fossil fuels. Lower temperature thermal decomposition in e-cigarettes generally produces a greater proportion of low molecular weight PAHs, such as acenaphthylene, fluorene, and anthracene. Most PAHs are known or suspected carcinogens, and exposure has been linked with a range of adverse health effects in humans.[25] The health effects of inhaling specific individual PAHs, however, have not been studied in detail. We generally detected PAHs at very low levels (Box 4; Supporting Information 1). The accelerated ageing process did not increase the levels of any PAH; those of benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and pyrene were all markedly lower in aged than in fresh e-liquids; indeed, benzo[*a*]pyrene and benzo[*b*]fluoranthene were not detected in aged e-liquids. These high molecular weight PAHs may have been chemically modified to low molecular weight PAHs (which we did not analyse), or PAHs formed during the ageing process may have been preferentially volatilised and not recovered.

Health effects of inhaled e-liquid aerosols

To estimate the potential effect on health of inhaling aerosols generated from e-liquids, some assumptions must be made. As acute inhalation toxicity levels (median lethal concentrations that kill 50% of a test animal population [LC_{50}]) have not been established for most of the chemicals we detected, we cannot calculate no observed adverse effect levels (NOAELs). Of the chemicals with established inhalation LC_{50} values, we found that four (benzaldehyde, menthol, 2-chlorophenol, benzyl alcohol) were frequently detected in e-liquids at concentrations that exceeded the inhalation LC_{50}. However, the inhalation LC_{50} is measured in air, and it is not possible to directly convert concentrations in e-liquids to concentrations in air without assumptions about puff volume and rate of use. Further research is needed to determine whether aerosols generated from these e-liquids contain these chemicals at relevant concentrations.

Conclusion

Our findings support and build upon earlier reports on the chemical composition of e-liquids. We found that a range of harmful chemicals are present, and that the heating/cooling/ageing process can affect e-liquid chemical composition. We acknowledge some limitations to our investigation, including the fact that we measured only a pre-determined selection of chemical analytes. Future studies would benefit from a discovery approach to identifying novel chemicals in e-liquids. Further, we acknowledge that the chemical composition of e-liquids is not entirely representative of the aerosol inhaled by e-cigarette users. Nevertheless, our finding that every e-liquid tested contained one or more chemicals potentially harmful to health provides a clear motivation for further investigations.

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Supporting Information

Additional Supporting Information is included with the online version of this article.