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Advances in experimental and mechanistic computational models to understand pulmonary exposure to inhaled drugs

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Abstract

Prediction of local exposure following inhalation of a locally acting pulmonary drug is central to the successful development of novel inhaled medicines, as well as generic equivalents. This work provides a comprehensive review of the state of the art with respect to multiscale computer models designed to provide a mechanistic prediction of local and systemic drug exposure following inhalation. The availability and quality of underpinning *in vivo* and *in vitro* data informing the computer based models is also considered.

Mechanistic modelling of local exposure has the potential to speed up and improve the chances of successful inhaled API and product development. Although there are examples in the literature where this type of modelling has been used to understand and explain local and systemic exposure, there are two main barriers to more widespread use. There is a lack of generally recognized commercially available computational models that incorporate mechanistic modelling of regional lung particle deposition and drug disposition processes to simulate free tissue drug concentration. There is also a need for physiologically relevant, good quality experimental data to inform such modelling. For example, there are no standardized experimental methods to characterize the dissolution of solid drug in the lungs or measure airway permeability.

Hence, the successful application of mechanistic computer models to understand local exposure after inhalation and support product development and regulatory applications hinges on: (i) establishing reliable, bio-relevant means to acquire experimental data, and (ii) developing proven mechanistic computer models that combine: a mechanistic model of aerosol deposition and post-deposition processes in physiologically-based pharmacokinetic models that predict free local tissue concentrations.

Graphical abstract

Keywords

Drug Delivery, Aerosol, Deposition, Dissolution, Permeation, Respiratory, PBPK

ACCEPTED MANUSCRIPT

1. Lung exposure to inhaled drugs

Successful development of inhaled medicines for the treatment of lung diseases such as asthma and chronic obstructive pulmonary disease (COPD) requires an understanding of local exposure and local target interactions (Cooper et al, 2012). Historically, the development of a novel inhalation drug relied on a series of preclinical and early clinical tests of increasing complexity to progress candidate drugs or terminate those that, for example, were not potent enough, had an unsuitable pharmacokinetic profile or did not possess a significant therapeutic window (i.e. exhibited toxicity at therapeutic doses (Forbes et al, 2011). These experimental methods are necessary to establish the safety and efficacy of a novel medicine. However, they do not necessarily provide a mechanistic understanding of how the drug delivery system, the formulation and the drug molecule interact with lung physiology to provide an optimal balance between the extent and duration of therapeutic effect and unwanted systemic side effects. Hence, relying solely on experimental methods may result in extended development programs and high attrition rates, especially for drugs with novel therapeutic targets. To avoid this, empirical results can be combined with multiscale computer models to provide a mechanistic prediction of drug exposure in target organs (Eissing et al, 2011).

As an example of multiscale computer models, physiological based pharmacokinetic (PBPK) models predict the exposure of drug in a target organ based on absorption, distribution, metabolism and elimination (ADME) in that organ, if such information is available (Zhuang and Lu, 2016). PBPK models of some type are used by most pharmaceutical companies to guide the molecular design of inhaled drugs. However, the combination of PBPK models, which provide understanding of tissue and target interactions, with mechanistic models, which describe key processes governing the rate and extent of local drug exposure, is still in its infancy. For example, there is currently only one commercially available PBPK software with a mechanistic regional deposition, dissolution and permeation model designed for pulmonary drug delivery (Gastroplus™ Nasal-Pulmonary Compartmental Absorption and Transit Model, SimulationsPlus Inc., Rochester, US). Other commercial PBPK software, such as the SimCyp Simulator™ account for pulmonary delivery by reducing dissolution and epithelial permeation into a single first order process in a single pulmonary compartment (<https://www.certara.com/software/physiologically-based-pharmacokinetic-modeling-and-simulation/simcyp-simulator/absorption/>).

Unlike empirical population-based modelling, which can be used to analyse and interpret the clinical pharmacokinetics of inhaled drugs (Borghardt et al 2016a; Borghardt et al 2016b; Bartels et al 2013), mechanistic modelling requires identification of each key step leading up to and controlling rate and extent of absorptive clearance, as well as an understanding of the local and systemic tissue interactions (Borghardt et al 2015). These mechanism-based models are deterministic, but semi empirical in that they rely on robust quantitative data characterizing each of these key processes (Korzekwa et al, 2017). Thus, the development of good experimental model is a mandatory first step for producing the data that informs the mechanistic lung retention/clearance model that underpin any holistic PBPK model that describes these processes and their interactions to predict lung exposure to drugs after inhalation.

This article will review current understanding of key processes governing local pulmonary exposure and our ability to characterise these experimentally as well as the potential of commercial and published computer based mechanistic models to reliably predict local pulmonary exposure after inhaled drug delivery. Potential for further model development, including gaps in supporting *in vitro*, *ex vivo* and *in vivo* data to inform the modelling, will be identified. In section 2 of this article, each of

these key processes is considered critically with respect to influence on pulmonary exposure to drug and the nature of data generated. Key experimental (input) data required to inform the computational models is classified into factors determining aerosol deposition (Table 1) and processes that affect the fate of drug after particle deposition (Table 2). We then describe commercial and published computer based mechanistic models for predicting local and systemic exposure after inhaled drug delivery and consider their pros and cons and potential future developments to improve robustness and quality (Section 3) before reviewing knowledge gaps that may be clinically important and identifying research priorities to address these uncertainties and the limits they impose on current mechanistic models (Section 4).

2. Experimental systems/data for use in modelling exposure after inhalation

The processes that are generally recognised as key determinants of free drug concentration in pulmonary tissue are aerosol deposition, particle dissolution, non-absorptive clearance from lung, absorptive clearance from lung and drug-tissue interactions (Niven 2014; Olsson et al 2011). Each of these processes are considered below.

2.1. Aerosol Deposition

The extent and pattern of drug deposition following inhalation of an aerosolized drug is a function of the total emitted dose, aerodynamic particle size distribution (APSD), patient inhalation manoeuvre, aerosol linear velocity profile and drug bolus profile, as well as airway physiology (see *e.g.* Delvadia et al, 2016). As an obvious critical product attribute, aerosol quality is the focus of much of experimental product characterization. Aspects such as delivered dose and the APSD are studied using standardized filter methods (USP <603>) and impactor type methods (USP <601>) or laser diffraction methods (USP <429>), respectively. The actual clinical impact of aerosol characterization data is less straightforward. Delivered (or emitted) dose can be regarded as the body burden dose and is important as both a quality attribute and as an indicator of product safety. However, for a locally-acting inhaled medicine, a measure reflecting lung deposited dose or lung deposition pattern may be more predictive of therapeutic performance (Hastedt et al, 2016, Olsson et al, 2013, Bäckman et al, 2017).

The most common measure of lung dose is Fine Particle Mass (FPM), either as the calculated mass of the drug aerosol below a fixed aerodynamic size cut-off (*e.g.* $< 5 \mu\text{m}$), or as an actual impactor stage grouping. There are some issues with using FPM as a general measure of lung dose. For example, at high velocities and/or large aerodynamic particle sizes, the standard USP throat model generally underestimates the real throat losses and hence over estimates lung dose (Zhou et al, 2011). Instead, several methods based on assessing mouth-throat (MT) deposition using physiological throat models have been proposed. These methods are generally based on determining aerosol filtration through a patient derived upper airway geometry during a patient-realistic inhalation manoeuvre. Examples include: the OPC throats (Burnell et al, 2007); the VCU throat models (Delvadia et al 2012); and the idealized Alberta throat (DeHaan and Finlay, 2001). Encouragingly, these models generally provide a good prediction of clinical MT deposition suggesting that these methods indeed provide a good empirical model of initial lung deposited dose. For instance, Zhou et al, (2011) and Olsson et al (2013) reported a good correlation between the clinically observed lung dose and the Alberta and OPC throat cast filtration, respectively. Similarly, Longest et al. (2016) reported a very good correlation between computational fluid dynamics (CFD) predictions and experimental deposition data for the VCU model. These throat models can be also used in combination with particle sizing methods, to learn more about the particle size of the aerosol fraction which has passed the throat model (Wei et al 2014). The latter may be important for assessment of lung deposition pattern, as will be discussed below.

CFD models have been proposed as an alternative to physical models for assessing MT deposition, especially as they can predict the deposition within an actual patient geometry (De Backer et al, 2015). CFD based models generally require information with respect to the linear airflow velocity as it leaves the mouthpiece (in addition to the aerodynamic particle size and patient inhalation profile), which makes this approach a bit more complex than a direct measure. However, there are published

data showing excellent correlation between CFD predicted deposition patterns in MT region and the trachea and corresponding experimental measures (cf. Longest et al, 2016) suggesting that the method is robust in its prediction of MT deposition and perhaps deposition in the first few large airway generations.

Unfortunately, neither physical models, nor current state of CFD technology, allow for predictions of deposition pattern beyond the first few generations of the large airways. This lack of methodology to characterize the distribution of the aerosol within the lung is a significant limitation preventing a full understanding of how changes to a product, or patient disease state, may influence clinical performance. As examples where intra pulmonary deposition patterns may influence clinical performance, bronchodilator activity is suggested to be driven mainly by the central airway dose (Usmani et al, 2005), whereas inhaled corticosteroids may be more effective when targeted to the bronchiolar region (Dekhuijzen, 2012). Variations in deposition pattern may also influence pulmonary bioavailability and hence systemic exposure and potential side effects (see e.g. Brutche et al, 2001, Bäckman and Olsson, 2016, Bäckman et al, 2017).

Efforts to quantify aerosol deposition patterns in the lower airways are today based on 1-dimensional typical path models (see. e.g. Schum and Yeh, 1980), alone or in combination with physiological MT models or complex CFD based models to improve predictability of the MT deposition. Examples of 1-dimensional models are the ARLA online calculator from Alberta University (Finlay and Martin 2008), the MPPD software from ARA (Anjivel et al 1995) and the commercially available Mimetikos Preludium™ (Mimetikos/Emmace AB, Lund, Sweden, <http://www.emmace.se/mimetikos-preludium/>). All three models enable the user to predict regional deposition in a Weibel type lung model (Weibel, 1963) based on the APSD and the inhalation flow profile (cf. Table 1). As an example, the Preludium™ model is informed directly by impactor data (APSD), obscuration profiles (drug release) and experimental inhalation profiles.

In summary, experimental techniques to characterize aerosols are well established and so are the computer based models converting the aerosol data into a prediction of drug deposition. However, the deposition models all lack direct clinical validation beyond the first few generations of the large conducting airways since available imaging methods (e.g. gamma scintigraphy) lack the required resolution. This obviously limits full evaluation of deposition model robustness (see also discussion in section 4 and Table 3). Nevertheless, successful development and application of any PBPK model to predict local exposure is likely to require at least an assessment of lung dose, and optimally an assessment of deposition pattern, i.e. distribution of drug in the lungs.

Table 1. *In vitro* and *in silico* techniques for quantitative evaluation of the drug aerosol deposition following inhalation.

Parameter	Clinical Impact	Technique	Status	Supportive data	Comment
Emitted or delivered dose	Total body burden dose	<i>In vitro</i> filter measurement and/or total impactor sized mass	Well established. Pharmacopeial monograph methods for all product types.	None	May be dependent on airflow rate
Total lung dose	Efficacious dose Systemic exposure	Cascade impactor fine particle mass	Well established. Pharmacopeial monograph methods for some product types	Expected patient inhalation flow	May only reflect lung dose in some cases
		Physiological mouth-throat model	Emerging as a clinically relevant <i>in vitro</i> test method	Patient inhalation flow profile	Limited clinical validation, optimal use in combination with breath simulator
		Computational fluid dynamics model	Emerging as a clinically relevant <i>in silico</i> method	Aerodynamic particle size distribution, linear velocity profile, patient geometry, drug bolus	Validated against <i>in vitro</i> measures
Lung deposition pattern	Efficacious dose Systemic exposure	1-dimensional deposition models	Well established computational methods	Total dose, patient inhalation flow profile, lung/airway geometry, mouth-throat dose, aerodynamic particle size distribution, drug bolus	No validation but a requirement for a successful mechanistic model

2.2. Drug release (dissolution) and solubility

Clinical data on compounds with low water solubility suggest a strong relationship between mean absorption time (MAT) from lung and water solubility (Forbes et al, 2015). That would point towards *in situ* dissolution being a critical attribute, i.e. a potential rate limiting step for systemic absorption and thus a determinant of pulmonary exposure. Recent work by Bäckman et al (2017) and Melin et al (2017) also indicated a key role for dissolution in regulating rate of absorption into the systemic circulation for poorly soluble drugs. Given the potential clinical importance, a significant amount of work has been undertaken to develop *in vitro* dissolution tests, ranging from standard USP type test setups to more complex *in vivo* mimicking designs (May et al 2014, Gerde et al, 2017). State of the art regarding dissolution testing of inhaled products has been reviewed in a separate article in this issue (Rossi et al, 2017.). For quality control and regulatory purposes, the most important aspect of dissolution methods is that they should be discriminatory and provide reliable, robust data (Forbes et al, 2015). Since the pioneering work on dissolution methods for inhaled products at the beginning of the century (Davis and Faddah, 2003; Son et al, 2009; reviewed by Riley et al 2012), many experimental variations including some offered as commercial services have evolved and data is being included in regulatory submissions, although to date methods have not been adopted by any pharmacopoeia.

If they are to inform mechanistic modelling, dissolution assays must be predictive of the *in vivo* processes, thus an important consideration is whether the heterogeneity of the lung can be represented in a single assay. There are also challenges of how to introduce relevant doses of appropriate aerosol fractions to the dissolution vessel and the selection of biorelevant medium in terms of composition and volume. At a recent workshop to discuss a proposed inhaled biopharmaceutical classification system (iBCS), dissolution was considered to be of greater

importance than solubility (Hastedt et al 2016) – although the two are clearly linked. For pharmacokinetic modelling and simulation to understand the fate of drugs deposited in the lungs, estimations of lung solubility and dissolution rates in biorelevant media are required. This includes the modelling of any changes to solubility and dissolution that occur in disease conditions (Wang et al 2014).

Drug solubility in the lungs has been measured in a variety of fluids, including (in order of physiological relevance) *in vitro* measurements in water or physiological salt solutions, often supplemented with phospholipids or a surfactant such as sodium dodecyl sulphate, and dilutions of products based on lung surfactant extracts such as Survanta® or Curosurf®. Inhaled particles deposit in a thin film of 12-25 mL of lung lining fluid spread over an area of 100 m² (Frohlich et al 2016). The composition of lung lining fluid is complex but mainly consists of lipids and proteins. The majority of lipid portion is composed of phosphatidylcholines (PC) among which 1, 2 dipalmitoyl PC represent about 40-60%. Other lipids included 1-palmitoyl-2-myristoylPC, 1-palmitoyl-2-oleylPC, phosphatidylglycerols and neutral lipids such as cholesterol. Lung surfactants contain specific proteins termed as surfactant proteins: SP-A, SP-B, SP-C and SP-D. The lipids present in the lung surfactant can self-assemble to form various structures. For mechanistic modelling purposes, inputs for drug solubility in the lungs have included solubility in PBS (Jones and Harrison, 2012, Bäckman et al, 2017) and fasted simulated intestinal fluid (Boger et al, 2016). Liquid crystalline nanostructures in lung surfactant have recently been suggested to have potential effect on respiratory drug delivery, by serving as drug depots thus increasing the residence time of the drug in the lungs and providing a lung retentive mechanism (Das and Stewart, 2016). Investigation of such mechanisms and inclusion of such interactions in mechanistic modelling will help in better prediction of pulmonary exposure to inhaled drugs.

In summary, significant data is accumulating pointing towards dissolution as a key critical product property for inhaled drugs with low water solubility. Several *in vitro* dissolution models have been developed of varied complexity and shown to discriminate between compound or product dissolution in a manner consistent with solubility and particle surface area. Hence, the use of dissolution test methods to understand variability in dissolution within a product, or between an originator product and a generic equivalent, appears to be useful and feasible. However, method robustness and clinical impact of observed changes in dissolution profiles require further attention, especially if method standardization is to be achieved to underpin regulatory use or inclusion into an iBCS. The latter will also require consensus as to selection of dissolution media for use in such assays. For prediction of clinical impact of the rate of absorption from the respiratory tract, it is possible given the complexity of the human lung that the best use of an *in vitro* dissolution method is not as a stand-alone predictive measure of dissolution *in vivo*, but rather as key data informing mechanistic computer based absorption models. Here, the impact of compound dissolution may be addressed in the context of other kinetically competing processes, i.e. absorptive and non-absorptive clearance as discussed below.

2.3. Non-absorptive clearance

Mucociliary clearance (MCC) can be approximated as a first order process (O’Riordan et al, 1992) with capability to remove a significant proportion of the delivered dose of poorly water soluble drug particles from the lungs (Brutche et al, 2001, Bäckman et al, 2017), thus potentially reducing local bioavailability. The same would be expected to apply to highly mucus bound drugs. MCC may be assayed *in vitro* or *ex vivo* by measuring particle transport by cell cultures or explants, or by

measuring ciliary beat frequency as an index of MCC velocity (Donnelley et al 2017). Mucociliary transport rates have also been studied *in vivo* in animals and human volunteers (Donnelley et al, 2014a, Bondesson et al, 2007)). It is recognised that clearance velocity is altered by some inhaled drugs and may be reduced in disease (Donnelley et al, 2014b) and may also vary between species (Hoffman and Asgharian, 2003). A variety of methods for studying drug binding to mucus and particle diffusion in models of respiratory mucus have been developed and this is currently an active area of research (Giorgetti 2016; Griessinger et al 2015). Unfortunately, most available methods can only evaluate total MCC from lung (Bondesson et al, 2007), hence regional variation in MCC is not well understood, especially when considering its potential impact on drug residence time in deep lung. Accelerated mucus clearance by cough is beyond the scope of current mechanistic models.

Alveolar macrophages (AM) protect the lung surface against the inhaled pathogens or dust particles. Lombry et al, (2004) demonstrated the important role played by respiratory macrophages in the disposition of inhaled macromolecules by depleting AM in rats which produced a sevenfold enhancement in pulmonary absorption of IgG and human chorionic gonadotropin after intratracheal instillation. Alveolar macrophages have also been investigated as targets for anti-inflammatory inhaled drugs, capitalizing on their propensity for sequestration of particles. For instance, Axelsson et al (2002) demonstrated AM targeting and prolonged anti-inflammatory effect following administration of a liposomal steroid prodrug. However, AM capture is not generally believed to have a significant impact on the overall rate of absorption of small inhaled drug molecules.

Metabolism contributes to non-absorptive clearance from the lungs and the nature and extent of metabolic activity in freshly isolated human lung parenchymal cells has been reported (Somers et al, 2007). As most measurements are performed in lung homogenates, there are uncertainties regarding regional variation in the lungs and drug access to enzymes in sub-cellular compartments. Species differences, metabolic enzyme polymorphism and expression of different isoforms within a range of ontogeny, populations and disease may also be important factors to consider. Some activities such as esterification of inhaled steroidal drugs are well-known examples of pulmonary metabolism (Miller-Larsson et al, 1998). In an interesting modelling approach for lung metabolism, Campbell et al. (2015) developed a regional PBPK model for lung for 1,3-butadiene and its metabolites taking into account metabolic capabilities specific to sub-divided regions within lungs such as oral/nasal pathways, conducting airways (trachea, bronchi, and anterior bronchioles), transitional airways (terminal bronchioles), and the alveolar region. Results showed that inclusion of differential lung metabolism was important for explaining the observed species differences in the pulmonary metabolism of 1,3-butadiene.

In conclusion, MCC is the predominant non-absorptive clearance process for poorly soluble small molecules inhaled as powders. AM clearance and local metabolism is likely to influence rate and extent of pulmonary absorption only for specific types of API, e.g. macromolecules and prodrugs. Unfortunately, MCC and its variation in rate in different regions of the airway and in disease is not well understood which limits the robustness of any mechanistic model predictions where this mechanism plays a significant role regulating pulmonary bioavailability and local residence time.

2.4. Absorptive clearance

Absorptive clearance removes locally-acting inhaled drugs from their site of action in the lungs. The rate and extent of absorption of inhaled drugs will depend on the relative rates of competing clearance mechanisms that operate in the lungs. Clearance by absorptive transfer from the lung lumen is predominately controlled by the epithelial permeability of free (unbound) drug. *In vitro*

epithelial cell culture (Forbes and Ehrhart, 2005) and ex vivo lung methods (Tronde et al, 2008) are available to screen the permeability of inhaled drugs, and may be configured to avoid or account for the impact of non-absorptive clearance. For example, the summing of BDP & 17-BMP during permeation in cell layers (Grainger et al, 2012) and the contribution of mucociliary clearance has been deconvoluted from absorptive clearance in the isolated perfused lungs (IPL) (Pang et al, 2005).

Methods have been optimized for culturing the most popular respiratory human respiratory epithelial cell lines, 16HBE14o- cells (Ehrhardt et al, 2002 Forbes et al 2003), Calu-3 cells (Grainger et al 2006), such that they exhibit epithelial barrier-like properties to permit the permeability of compounds to be measured. Primary human bronchial epithelial cells have been used, but are less convenient. Despite efforts to develop new human cell lines, only primary epithelial cell cultures produce suitable monolayers to model the alveolar epithelial permeability barrier to drug absorption. There are several methodological variations in IPL which are configured to measure clearance from the airways (Tronde et al, 2008), and the dependency of the absorptive clearance rate on the method for delivering drugs and maintaining the lungs *ex vivo* requires more research.

Apparent permeability coefficients measured in airway cell lines (Mathias et al 2002, Manford et al 2005) and absorption rate in IPL have been explored to predict absorption *in vivo* based on physicochemical molecular descriptors (Tronde et al, 2003a, 2003b, Edwards et al, 2016).

Absorption rate is governed by molecular properties such as lipophilicity, ionization state and target affinity. Several models have attempted to systematize this, for example the QSAR model developed by Cooper et al (2010) to predict efficacious doses of inhaled compounds based on lung plasma partitioning. The QSAR model recently reported by Edwards et al (2016) identified key molecular drivers for pulmonary absorption using a relatively large set of 82 discovery compounds along with 17 marketed compounds which were evaluated for absorptive clearance in IPL. Nine compounds were further evaluated to test the model's predictive ability. Molecular descriptors associated with permeability and hydrophobicity were found to be positively correlated with pulmonary absorption whereas descriptors for charge, ionization and size were negatively correlated. Such QSAR modelling exercises can generate descriptors which can be used as inputs during the mechanistic inhalation modelling during the drug discovery phase where limited experimental data is available. For example, computational, multiscale, cell-based modelling has been used to explore the relationship between the physicochemical properties and absorptive pharmacokinetics of monobasic molecules in the lungs (Yu et al 2010).

A programme to assess the effect of permeability on lung concentration after pulmonary administration is generating an expanding and consistent dataset for antibiotic compounds absorption from rat lungs *in vivo* (Gontijo et al, 2014a, Gontijo et al, 2014b, Marchand et al, 2015, Marchand et al, 2016). In these experiments a standardized protocol was used with compound administration using the Penn Century system and drug concentration determined simultaneously in plasma and epithelial lung fluid (ELF) of healthy rats at various times following intravenous (IV) and pulmonary administration. Plasma and ELF concentrations produced by both routes of administration were used in compartmental analysis and a population PK approach to estimate exposure (AUC) in plasma and ELF. Lung concentrations after pulmonary delivery were highly dependent on epithelial permeability, with major therapeutic advantage in lung exposure for antibiotics with low permeability precluding oral administration. These *in vivo* results correlate relatively well with *in vitro* data using Calu-3 cells and physico-chemical characteristics such as Log D values, suggesting a place for permeability in an iBCS. Interestingly *in vivo* data obtained with moxifloxacin suggested a P-gp mediated efflux transport (Gontijo et al, 2014a).

In general, the impact of transporters has mostly been investigated *in vitro* and *ex vivo* as reviewed recently (Ehrhardt et al, 2017), but active transport mechanisms have yet to be modelled mechanistically to explore their impact on the pharmacokinetics of inhaled drugs. Expression patterns of transporters differs drastically across cell populations in the lungs, including transporters belonging to the family of the solute carriers (SLC) such as OCT, OAT, OATP and PEPT and the ATP-binding cassette (ABC) transporters such as Pgp, MRP and BCRP Nickel et al. (2016). The big question – if and to what extent pulmonary transporters alter the pharmacokinetics of inhaled drugs – still needs to be clarified. In a notable clinical study, Ruparel et al (2008) administered the Pgp substrate ^{99m}Tc -sestamibi as an aerosol to healthy smokers, non-smokers and COPD patients recorded clearance from the lungs scintigraphically for 30 min. The results indicated upregulation of Pgp activity in healthy smokers leading to delayed elimination of administered drug, whereas elimination was not altered in COPD patients and healthy non-smokers. However, there are few quantitative studies demonstrating altered inhaled drug disposition by transporters in humans to validate *in vitro* and *ex vivo* experimental findings and justify incorporation into PBPK models.

To summarize, absorptive clearance of free dissolved drug in ELF is controlled by epithelial permeability. For hydrophilic compounds, experimental evidence suggests epithelial permeability as the rate-limiting step controlling the rate of system absorption, whereas for lipophilic compounds the rate of dissolution is more influential. Several methods have been developed to assess drug permeability, including QSAR methods based on drug physicochemical properties, *in vitro* cell assays and *ex-vivo* methods such as the rat IPL model. These methods provide predictions of the quantitative absorptive clearance from the lung and hence a good predictor of systemic exposure. However, there is a significant increase in epithelial thickness, and a massive reduction in surface area in the alveolar interstitial region compared to the conducting airways. This would suggest that the bulk of systemically absorbed drug is derived from the alveolar interstitial area, thus measurements in IPL and *in vivo* models may not reflect the rate and mechanisms of absorptive clearance from the conducting airway regions and local concentrations in this region (Ehrhardt et al, 2017). Conducting airway tissue are regarded as the main therapeutic target for bronchodilators and inhaled corticosteroids (Usmani et al, 2005 and Dekhuijzen, 2012, respectively). Hence, neither the impact of regional variation passive permeability or the influence of active transport on clinical efficacy are completely understood.

2.5. Tissue retention and lung concentration

Many strategies for the design of lung-retained inhaled API have been based on mechanisms that lower free drug concentrations and reduce absorptive and metabolic clearance. This reduction in free drug may be conferred by ‘tissue affinity’, which includes collectively sequestration in lung lining fluid or tissue compartments through specific and non-specific protein binding, vesicular, lysosomal or cytoplasmic trapping. These mechanisms may provide a depot-effect, thereby retaining drug in the lungs. Non-specific binding is typically low affinity and high capacity; interactions from which dissociation rates are generally rapid and do not favour a “slow release” depot. In contrast, some drugs may be retained in the lung through high affinity receptor binding (Collingwood et al 2012). For dibasic drugs including the β -agonists pH-dependent lysosomal trapping is a mechanism that can retain drug in the lungs (Ufuk et al, 2017; Bäckström et al, 2016b). Poorly water soluble drugs may be retained in structures in lung lining fluid (Das and Stewart, 2016), and may be influenced by drug formulation. Binding to respiratory mucus may also be a retentive mechanism if MCC is slow, e.g. in disease states, or where mucus is the target, e.g. for antibiotic therapy. While drug uptake into macrophages may represent a first step towards non-absorptive clearance by removal from the

lungs or degradation, it may also provide a depot from which drug may be released (Axelsson et al, 2002).

Lung tissue binding is typically measured by equilibrium dialysis or ultrafiltration methods (Cooper et al 2010), and can be measured by direct assessment of partitioning between lung tissue slices and buffer (Bäckström et al, 2016a). Isolated perfused lung models may also be used to establish tissue plasma partitioning coefficients when operated in recirculation mode (Tronde et al, 2003b). Non-lung tissues and lung homogenates are commonly used to study non-specific protein binding. When using lung homogenates, tissue affinity is often assessed in comparison with plasma binding which largely reflects drug binding to albumin (which is also present in the lung lining fluid albeit in lower concentrations). Tissue-binding or more specific retention mechanisms must be included in PBPK models if they are to reflect these important mechanisms of drug retention in the lungs.

Unbound drug concentration in the lungs provides the most relevant measure for target engagement and activity (Cooper et al, 2012), but measurement is problematic. Microdialysis is probably the most elegant technique for on-line determination of unbound drug concentrations in tissue interstitial fluid (ISF) both in animals and human (Marchand et al, 2016). However, it presents several limitations. Compounds with high molecular weight may not diffuse through the membranes and those with high lipophilicity may bind to the probe and tubing components precluding microdialysis studies. Furthermore *in vivo* probe recovery must be determined individually which adds complexity and may considerably extend study duration to such a point that it may become problematic for animal experiments or not compatible with patients-care for human studies. And lung microdialysis is the most challenging since it must be conducted under open chest surgery and therefore general anaesthesia which may also interfere with drug tissue distribution. Therefore although lung microdialysis studies have occasionally been conducted, in particular in rats (Marchand et al, 2008; Zimmerman et al, 2015; Torres et al, 2017), broncho-alveolar lavage (BAL) to estimate drug epithelial lining fluid (ELF) concentrations, remains the most widely used technique to assess “intra-pulmonary” drug concentrations.

ELF concentration is relevant to lumenally-targeted therapies such as antibiotics and is more accessible than tissue concentration. Assessing free concentrations in lung tissue is difficult since the lung is a complex organ and concentrations may vary with the nature of the sample and the sampling site. In all methods, mechanical disruption of the tissue, processing time and dilution may release drug from depots, e.g. lysosomes or undissolved drug in airways. The latter is especially important for some of the more lipophilic drugs, where free drug in tissue may be a very small portion of total drug in lung. Tissue sampling may be conducted both in animals and patients during surgery, but drug concentrations determined in whole tissue homogenates represent a mixture of intra and extracellular concentrations that are difficult to interpret, both for PK (characterization of the transport between plasma and lung) and PD purposes (prediction of drug efficacy).

Considerations related to regional variation in drug concentrations are exemplified by the work of Boger et al (2016) in which overall target occupancy in lung (presumably an indirect measure of free tissue concentration in lung) was very similar to that observed in spleen following inhalation of a lipophilic drug, fluticasone propionate. The authors suggested that the observed clinical lung targeting of this inhaled corticosteroid could be explained by higher free drug concentrations, not in whole lung, but specifically in conducting airway tissue.

Broncho-alveolar lavage (BAL), or micro-BAL, constitutes an interesting although not ideal means of measuring lung concentration. As illustrated above, drug distribution within the lung is probably not homogenous and may differ between systemic administration and inhalation. BAL provides only an average concentration and must be corrected for dilution to get the more relevant ELF

concentration. This is usually done using measured urea concentration within BAL fluid and plasma which could add to the experimental error. Although BAL may be sampled relatively common in ICU patients with severe pulmonary infections, the number of BAL per individual is limited. Conducting BAL in other types of patients as well as in volunteers is seriously limited by ethical concerns. Furthermore, when powder formulations are used for inhalation, there may be some degree of uncertainty as to whether BAL solubilises drug that was undissolved in the lungs. Yet overall ELF concentrations constitute probably the most utilised 'lung concentrations' for compartmental modelling (as described in Section 2.4).

To conclude, a measure of free active drug at relevant target location is the 'holy grail' as it epitomises the true advantage of any topical treatment designed to provide an improved therapeutic ratio. Unfortunately, except for lumenally active inhaled antibiotics where ELF concentrations are accessible, free drug concentrations in lung are very difficult to assess experimentally. Hence, the usefulness of PBPK models to model free drug concentrations based on molecular physiochemical properties as well as experimental measures of lung tissue partitioning. However, the lack of clinical or preclinical data on pulmonary free tissue concentrations and its regional variation means that predictive models can only be indirectly validated with respect to predictions such as total tissue concentrations and total lung retention.

2.6. Systemic PK models

Although not the focus of this review, systemic PK models, preferably based on IV data, are required to convert a mechanistic model prediction of pulmonary and gastrointestinal absorption into a prediction of plasma concentration profiles. This is often the only validated prediction that can be made, thus access to accurate IV data and derived PK models is thus essential for any assessment of mechanistic absorption model robustness (Bäckman et al 2017). Unfortunately, published IV PK data is unavailable for many licensed inhaled medicines which limits model validation. When data is available, the application of compartmental analysis to plasma concentrations obtained after inhalation provides a semi-mechanistic understanding of the local absorption process. Expertise in systemic PK modelling is widespread and several user-friendly software's are available. Plasma drug concentrations versus time profiles may be simulated within peripheral compartments, but this provides limited information since peripheral compartments correspond to virtual compartments with no anatomical meaning. To account for dissolution in lung an absorption compartment can be added, including for example the Weibull equation (Gaspar et al, 2016). If absorption kinetics determine the systemic drug concentration profile ('flip-flop' kinetics), plasma concentrations may reflect the absorption kinetics of a drug, especially if inhalation and IV PK data is obtained in parallel (Melin et al, 2017, Doan et al, 2013). Yet PBPK modelling constitutes a promising alternative and most PK studies of antibiotics in human lungs have been conducted with traditional compartmental analysis (Rodvold et al, 2011, Boisson et al, 2014).

In summary, there are many examples where standard PK models applied to plasma concentration profiles have been helpful in understanding the extent and rate of pulmonary absorption, especially when plasma profiles for inhalation, oral and IV administration are obtained in parallel in same cohort (Melin et al, 2017). Systemic PK models based on IV data is also very useful as they provide one of the few means by which a mechanistic absorption model can be 'validated'. Unfortunately, many inhaled drugs on market lack published IV data limiting the application of mechanistic models.

Table 2. Experimental techniques for quantitative evaluation of the disposition of drugs following aerosol deposition.

Parameter	Potential Impact	Technique	Status	Supportive data required for modelling	Comment
Dissolution rate	Dosing interval (pulmonary residence time), local irritation/toxicity, systemic exposure (C _{max})	Mechanistic computer models	Models well established but lack of consensus on: Solubility media; ELF volumes and Concentration gradients; regional variability	Solubility, pKa, Diffusion rate, particle size distribution, specific surface area, excipients, wettability, surface energy	Criticality depends on kinetic competition with absorptive and non-absorptive clearance
		<i>In Vitro</i> dissolution	Variety of methods published with differences in aerosol capture, dissolution apparatus and medium. No consensus regarding to methodology, <i>in vivo</i> predictivity and media	Solubility, local and regional surface density of doses, relevant PSD-fraction	Emerging commercial options: Unidose (Nanopharm), DissolveIT (ISAB). <i>In vitro- in vivo</i> predictability not firmly established
Non-Absorptive clearance	Dosing interval (pulmonary residence time), pulmonary bioavailability (systemic AUC)	Mechanistic MCC computer models	Several models suggested by limited validation data available	Total MCC, Regional MCC, Impact of disease	MCC significantly impact luminal residence time of solids and mucin bound compounds
		Preclinical MCC models	Measures total MCC or MCC in 1-2 airway generation. Very limited information on regional variability	Patient inhalation flow profile to determine deposition pattern	
		Metabolic degradation in lung homogenates and/or tissue slices	Relatively well established techniques	Chemical structure, reactive groups	Normally not a significant contribution to non-absorptive clearance in lung except for ante- and pro-drugs and macromolecules
Absorptive Clearance	Dose potency and targeting (ELF vs Tissue), Systemic exposure (C _{max})	QSAR models	Well established	Physicochemical molecular properties related to experimental measures	Predictive capability not proven
		<i>In vitro</i> Epithelial cell layers;	Notably Calu-3, 16HBE14o-well established models but methods not validated or standardized	Drug concentrations in ELF	Predictive capability not proven
		<i>Ex-vivo/in vivo</i> models	IPL and PK well established	Lung dose, deposition pattern	Largely reflect AI region and not airways
Local concentration	Dosing interval (pulmonary residence time), Dose potency (local targeting) Therapeutic ratio	Computational PBPK models	Well established in PBPK	Physicochemical properties, target affinity	Not specific to lung tissue or relevant for mechanisms such as lysosomal trapping
		<i>In vitro</i> tissue partitioning (homogenates)	Well established ultrafiltration and/or equilibrium dialysis		Difficult to separate out regions of interest. Not relevant for mechanisms such as lysosomal trapping (disruptive)
		<i>In vitro</i> tissue partitioning (whole tissue)	Emerging: Recirculation IPL and lung tissue slices models in literature. No standardization.		Difficult to separate out regions of interest. More relevant for mechanisms such as lysosomal trapping (non-disruptive)

		Total lung concentrations	Lung tissue homogenates well established method but may not give an accurate estimation of free tissue levels at target due to physiological complexity of compound distribution		Interference from undissolved or trapped compound (disruptive)
		BAL	Well established technique, interference from undissolved compound.	Dilution, extent of solids	Not easily accessible in man but very useful for accessing ELF concentrations for luminally active drugs such as antibiotics.

3. Mechanistic computer based models for simulating pulmonary and systemic exposure after inhalation

3.1. Gastroplus ADRM™

Gastroplus™ (Gastroplus™ Nasal-Pulmonary Compartmental Absorption and Transit Model, SimulationsPlus Inc., Rochester, US) is currently the only commercially available mechanistic computer model that combines a physiological PBPK model with mechanistic models accounting for pulmonary deposition, dissolution, as well as absorptive and non-absorptive clearance (Figure 1). The program considers 3 distinct pulmonary regions (large and small conducting airways and alveolar interstitium, BB, bb and AI, respectively) and one extra thoracic compartment (ET), essentially based on the Weibel lung model (Weibel, 1963). Each region is sub divided into an airway liquid compartment and an epithelial/lung tissue compartment. For each sub-compartment the software allows the user to set relevant physiological parameters such as compartmental dimensions.

Figure 1. Schematic outline of the Gastroplus™ nasal and pulmonary absorption model. Dark blue = mechanistic model of initial aerosol deposition, dissolution, non-absorptive and absorptive clearance in pulmonary and GI lumen; Light amber = PBPK model describing pulmonary tissue free/bound drug concentration and diffusion into system; Dark amber = systemic disposition model. Airway regions defined in model: ET= Extra thoracic; BB= large airways (gen 0-8); bb=small airways (gen 9-16); AI=alveolar interstitial tissue (gen 17-23).

The initial distribution of inhaled drug between these four compartments can be estimated based on a simple built-in IRCP-based 1-dimensional deposition model (IRCP, 1994). The deposition pattern can also be defined by the user, rendering the model compatible with any similar algebraic deposition models (for instance the ARLA and Mimetikos models described in section 2.1). However, the model cannot accommodate the distribution of surface drug concentration in different lung generations that can be obtained from a CFD-type model. Required input data for deposition modelling are listed in Table 1.

Following particle deposition, the software uses Noyes-Whitney principles to model dissolution mechanistically based on actual particle size distribution, solubility and diffusion rate (Table 2), thus providing in theory an accurate reflection of real dissolution disparities imposed by differences in the product, e.g. changes in APSD according to variations in manufacturing processes. However, the software does not allow for the direct use of real dissolution profiles or data derived from such profiles.

Non-absorptive clearance is described by a first order mucociliary transport model simulating the upwards transport of drug in lumen into the extra-thoracic compartment and from there into the gastrointestinal compartments (O’Riordan et al, 1992).

Absorptive clearance of dissolved drug from airway liquid into lung tissue is assumed to be limited by either passive permeability limited diffusion or by active transport processes as regulated by a compartment specific permeability function. The main substance specific variable here is the alveolar interstitial permeability. The software estimates this values based on the molecular weight, (but it can also be entered by the user) and then scales this value to other airway regions based on epithelial thickness. However, regional airway permeability is not easily accessible making this variable subject to some uncertainty (Table 2).

The inclusion of two kinetically competing process of absorptive and non-absorptive clearance, in combination with a mechanistic dissolution model, allow the user to simulate the impact of variations in deposition pattern and dissolution rate on total pulmonary bioavailability and rate of absorption (se e.g. Bäckman et al, 2017, Bäckman and Olsson, 2016, Olsson and Bäckman 2014).

Absorption from lung tissue into the systemic circulation is assumed to be a diffusion limited process and is as such governed by the blood flow through the tissue and the blood tissue partitioning function. The latter is derived from standard blood plasma partitioning and from tissue plasma partitioning, parameters that can be measured experimentally (Table 2) or calculated from physicochemical data using standard PBPK type approaches. More complex tissue interactions, such as sequestration into lysosomes reported to be a main contributor to the retention of di-bases in lung, cannot be simulated.

3.2. PK-SIM™ and SimCyp Simulator™

Although Gastroplus is the only commercially available software to incorporate a mechanistic model of deposition, dissolution, non-absorptive clearance and absorptive clearance, non-mechanistic PBPK models are available. The SimCyp Simulator™ (<https://www.certara.com/software/physiologically-based-pharmacokinetic-modeling-and-simulation/simcyp-simulator/absorption/>) and PK-SIM™ (Computational Systems Biology: Bayer AG, <http://www.systems-biology.com/products/PK-Sim.html>) allow the user to define pulmonary and gastrointestinal (GI) absorption compartments and model PK following pulmonary administration.

For example, Stass et al, (2008, 2013) used PK-SIM to deconvolute PK data obtained in healthy volunteers after inhalation of ciprofloxacin, a locally acting antibiotic, to obtain the relative contribution of oral, tracheobronchial (BB and bb) and alveolar interstitial (AI) deposited drug to the total systemic exposure. The authors did not mechanistically model local absorptive and non-absorptive clearance processes but rather assumed the AI dose to behave as an IV dose and the Bb dose to behave as a delayed oral dose and then used PK-Sim to fit the systemic PK profiles based on these assumptions.

Gauhua et al., (2015) used a multicompartment lung model embedded in the SimCyp PBPK-model to study the local (pulmonary) and systemic pharmacokinetics of anti-tuberculosis (TB) drugs.

Although, the model studied lung exposure following systemic administration of drugs, it is relevant to lung targeting owing to the realistic physiology of the lungs taken into consideration. Regional differences in gas exchange, blood perfusion and transporter expressions were modelled to predict ELF:plasma concentration ratio of administered anti-tuberculosis drugs with reasonable approximation to observed clinical data. Alteration of ELF pH or inclusion of transporter activity suggested significant potential for altering the ELF:plasma concentration ratio of administered drugs. The model provided a framework to optimize dosage regimes in tuberculosis patients to achieve maximum therapeutic efficacy.

These models are of obvious value as PBPK models for predictions of preclinical and early clinical exposures but they reduce dissolution and epithelial permeation into non-mechanistic 1st order processes. This limits the ability of these models to account for real product performance variables and differences between lung regions with respect to the nature of, and balance between, absorptive and non-absorptive clearance.

Figure 2. Schematic outline of pulmonary absorption in SimCyp Simulator™. Dark blue = non-mechanistic model of initial aerosol deposition including a first-order model of absorptive clearance in pulmonary and GI lumen; Light amber = PBPK model describing pulmonary tissue free/bound drug concentration and diffusion into system; Dark amber = systemic disposition model. Airway regions defined in model: ET= Extra thoracic; Lung = (gen 0-23).

3.3. In-house industry models

In addition to the commercially available software programmes, a variety of bespoke mechanistic pulmonary absorption models have also been devised. Although mostly developed and applied to guide the commercial development of inhaled medicines, these have been published to various extents and/or presented at scientific workshops and symposia.

In a recent paper, Boger et al. (2016) predicted the fate of a poorly soluble inhaled drug, fluticasone propionate, in rats. A combined framework of drug and formulation-specific properties along with system-specific inputs were explored using a computer based PBPK model to predict lung selectivity (ratio of local to systemic target occupancy). The time course of glucocorticoid receptor occupancy in lungs was measured *in vivo* following inhalation and intravenous administration of fluticasone propionate. Mechanistic modelling simulations were found to be predictive of the pharmacokinetics and receptor occupancy of FP following intravenous and nose-only inhalation delivery. Key findings of the research were that it is difficult to achieve lung selectivity in well perfused parts of the lungs and that slow drug-receptor dissociation can be the molecule property critical for the lung selectivity.

PulmoSim™, Pfizer's in-house PBPK model, has unfortunately not been fully described in a peer reviewed journal. However, the model has been presented at meetings. For instance, Jones described the development and application of PulmoSim™ at a society of medicines Research Symposium (Collingwood et al, 2012). PulmoSim was claimed to incorporate mechanistic descriptors of drug dissolution, permeation, lung tissue distribution, as well as a systemic distribution and dissipation model. The model was claimed to have been validated against preclinical data on compounds covering a wide physico-chemical space. Jones concluded that the model was useful in predicting human systemic PK but also that validation of local pulmonary drug concentrations were challenging.

Recently, Merck developed an in silico mechanistic model to enable predictions of local pulmonary tissue concentrations during respiratory drug development (Caniga et al, 2016, Cabal et al, 2016). The model integrates a typical lung generation deposition model, dissolution, MCC (large and small airways) and an absorption module with a PD module and a PBPK module. Model predictions have been validated against systemic PK data in rat and humans following local delivery of mometasone furoate, budesonide, salbutamol, and formoterol. The authors concluded that the model provided valuable information regarding lung targeting (pulmonary vs systemic concentration ratio) and how this could be optimized.

4. Knowledge gaps and research priorities to support model development

Historically, novel inhaled drugs, as well as generic equivalents, were developed mainly using *in vitro* and *in vivo* experimental models. As described in preceding sections, significant efforts are now being made to predict or understand key processes that determine pulmonary exposure to inhaled drugs. For example, tissue retention and passive permeability of drugs have been projected using QSAR and PB/PK modelling (Tronde et al 2003a, 2003b; Boger et al 2016)). However, key questions facing the developer of a new drug remain indeterminate, either because of a lack of adequate experimental methods or because the answer depends on how product properties (such as deposition and dissolution) interact with API behaviour conferred by molecular properties (such as permeability, tissue affinity and receptor affinity) and local physiology (including MCC, metabolism, epithelial permeability and target location). Typical questions of this nature may be:

- How can solubility, tissue affinity and potency be best balanced to provide dose potency and duration of effect?
- Does target location impose different requirements of the API and the deposition pattern of the aerosol?
- Can we translate data on non- absorptive clearance from animal models to humans?
- How does smoking or disease type and severity modify aerosol deposition and subsequent pulmonary drug clearance?

Computer based mechanistic modelling provide an opportunity to explore questions like those above by a combination of: (i) understanding the key processes determining local and systemic exposure; (ii) having access to biorelevant experimental data characterizing these processes; and (iii) integrating the experimental data and mechanistic process understanding using computer based mechanistic models. This is obviously recognised by the pharmaceutical industry, as exemplified by recent presentations/publications from Merck (Caniga et al 2016), Pfizer (Collingwood et al, 2012) Bayer (Stass et al 2013) and AstraZeneca (Bäckman et al, 2017, Boger et al 2016)). Unfortunately, as this review identifies, significant gaps in understanding drug uptake by the lungs and available methods/models to study this quantitatively are barriers to a more widespread and successful utilization of the mechanistic modelling approach (Table 3).

Table 3. Identified gaps and potential questions to be addressed in future research

Gap	Clinical impact	Questions and topics for future research
Validated predictions of lung deposition pattern	Efficacious dose Systemic exposure	Several publications have indicated the necessity to understand aerosol deposition pattern, and how this relates to specific products and patients. Currently, pulmonary deposition patterns beyond large airways are only accessible through 1-dimensional computer models based on inhalation manoeuvre, standard Weibel-type lung models and APSD measure. Outstanding questions are: <ul style="list-style-type: none"> • Can a standardized method for biorelevant pulmonary deposition modelling be established? • Can relevant experimental <i>in vivo</i> validation techniques be identified?
Models of dissolution in lung	Duration, Irritation	Dissolution in lungs, as well as its variation between pulmonary regions, is a key predictor of rate and extent of absorption for poorly soluble drugs. Important questions are: <ul style="list-style-type: none"> • For which compounds is dissolution rate limiting and in which lung regions (input to a pulmonary biopharmaceutical classification system?) • Can a pharmacopoeial standard for biorelevant <i>in vitro</i> test methods be established? • How can <i>in vitro</i> dissolution results be linked to <i>in vivo</i> impact? What is the role of computer models? Does regional variation need to be considered?

Variation in mucociliary clearance rate	Bioavailability, Duration	MCC is the predominant non-absorptive clearance process for powder aerosols of poorly soluble inhaled small molecules and as such it influences both bioavailability and duration of therapeutic effect in humans. <ul style="list-style-type: none"> Can regional and disease driven variations in MCC be measured and modelled? How should a biorelevant mechanistic model of MCC be designed – what is the relevance of first order models?
Regional variation in epithelial permeability	Bioavailability, Duration	For very hydrophilic and macromolecular drugs, permeability may be the rate limiting absorption step. It is possible that active transport processes influence bioavailability in airways. Poorly soluble drugs may also show permeability limitations in larger airways due to competition for retention in lung tissue. <ul style="list-style-type: none"> Can experimental models for assessing regional variability in transcellular epithelial permeability be identified and results used to establish more relevant mechanistic models? Is permeability affected by disease, smoking, etc.. What is the impact of transporters (especially in airway regions) – can we measure and model this?
Pulmonary concentrations	Potency and duration of effect	A direct or indirect measure of free drug concentrations (and its regional variation) in lung is challenging using existing methods (except for measuring ELF) but critical for the validation of any computer based model aiming to support compound development. <ul style="list-style-type: none"> Can a usable method be established for direct or indirect measurements of free drug concentrations? Can developments in techniques such as positron emission tomography provide measurement techniques?
Validation of <i>in silico</i> models	Potency and duration of effect	Currently, only one commercially available computer model combines a mechanistic approach to predictions of deposition, dissolution, absorptive and non-absorptive clearance with a PBPK model, and literature data validating this model against experimental observations is scarce. Several models with these features have been published or presented at scientific meetings, but so far are either not publicly available or have no human data validation, or both. <ul style="list-style-type: none"> Can a wider selection of mechanistic models be made available with (preferably) access to key model assumptions to increase transparency? How can <i>in silico</i> models be validated, against each other and against human clinical data? Publications combining clinical data, <i>in vitro</i> product performance data and <i>in silico</i> simulations are required to demonstrate robustness of approach.

Identified gaps and barriers to greater and better use of modelling approaches range from the need for accurate and validated prediction of lung deposition pattern to the accurate assessment of drug concentration in the lungs (Table 3). A concerted effort to address these deficiencies could significantly improve the success rate in bringing novel inhaled drugs to the clinic – thus bringing medical value.

A better understanding of key drivers of local and systemic exposure, and an improved ability to characterize and model these processes could also have an impact on the regulatory landscape. As an example, generic equivalents as well as post approval changes for inhaled proprietary medicines face an elevated regulatory hurdle compared to oral drugs. This is mainly a result of inadequate (or not standardized) means to predict and assess the potential combined impact of API properties and product properties on clinical safety and efficacy. Today, this prohibits for instance the use of a classification system such as the biopharmaceutical classification system which currently guides drug development and provides options for regulatory relief for oral medicine licensing (Hastedt et al, 2016).

Questions related to modelling include, what will be required for modelling to be accepted to support regulatory submissions for original or generic products? Is modelling used for other products for regulatory purposes? Would development of models and their utilisation be helped by greater recognition of the benefits by industry and appropriate expertise in academia and the pharmaceutical research community, plus greater communication and liaison between experimentalists and modellers? At present, the number of different models, their assumptions and the lack of transparency regarding their underlying assumptions present a problem for peer review, accessibility and acceptance.

To extend the scope of current models, factors identified as important by empirical modelling, e.g. disease or smoking could be incorporated and scenarios where *in vitro* and *in vivo* data are at variance (Borghardt et al 2016a; Borghardt et al 2016b; Bartels et al 2013), can be investigated to improve modelling and understanding of inhaled drug delivery. The level of detail that is necessary in PBPK models, such as the number of lung regions modelled and the refinement of region-specific physiological parameters, e.g. epithelial permeability, metabolism, solubility in lung fluid, provides another topic for investigation. Finally, to realise the full potential of modelling, the linking of lung exposure to drug action is required in the form of mechanism-based pharmacokinetic-pharmacodynamic modelling. Such 'systems pharmacology' approaches are beyond the scope of the current article, but provide the key link between drug exposure and drug response, and even the relationship between drug response and disease progression [e.g. Danhof et al 2008].

In conclusion, successful application of transparent mechanistic *in silico* models informed by robust experimental data could benefit discovery of new API's and development of novel inhaled medicines. A better understanding of the science in this area could also impact on the regulatory landscape and potentially provide some science-based regulatory relief facilitating approval of generic equivalents, as well as post approval changes. Beyond the pharmaceutical sector, such models may also prove valuable for risk assessment for environmental air pollutants, occupational inhalation exposures such as crop spraying or aerosol cleaning/healthcare products, and biodefence against airborne agents. Improvements in experimental methods as well as an increased availability (commercial or otherwise) of *in silico* methods as suggested here (Table 3) are likely to benefit such a development. The latter could also result in more wide spread use within both industry and academia, and hopefully, more published scientific studies. Currently, too few examples are available in literature that combine a transparent presentation of key *in vitro* product characteristics, clinical results and mechanistic *in silico* model simulations to provide a sufficient data base for validation and improvement of existing models.

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References

- Anjivel, S., and Asgharian, B., 1995. A multiple path model of particle deposition in the rat lung. *Fundam. Appl. Toxicol.* 28, 41-50.
- Axelsson, B., Bäckman, P., Strandberg, P., and Brattsand, R., 2002. Development of inhaled steroids based upon prodrugs with prolonged intraluminal retention. In: Schleimer, R.P., O'Byrne, P.M., Szefer, S.J., and Brattsand, R. (Eds.). *Inhaled Steroids in asthma: Optimizing effects in the airways* Vol 163, Marcel Dekker, Inc. New York, pp. 576-564.
- Bartels, C., Looby, M., Sechaud, R., Kaiser, G., 2013. Determination of the pharmacokinetics of glycopyrronium in the lung using a population pharmacokinetic modelling approach. *Br. J Clin. Pharmacol.* 76:6, 868-879.
- Boger, E., Evans, N., Chappell, M., Lundqvist, A., Ewing, P., Wigenborg, A., and M Fridén, 2016. Systems Pharmacology Approach for Prediction of Pulmonary and Systemic Pharmacokinetics and Receptor Occupancy of Inhaled Drugs. *CPT Pharmacometrics Syst. Pharmacol.* 5, 201–210.
- Boisson M, Jacobs M, Grégoire N, Gobin P, Marchand S, Couet W, Mimoz O. 2014. Comparison of intrapulmonary and systemic pharmacokinetics of colistin methanesulfonate (CMS) and colistin after aerosol delivery and intravenous administration of CMS in critically ill patients. *Antimicrob. Agents Chemother.* 58 :12, 7331-7339.
- Bondesson, E., Bengtsson, T., Nilsson, L. -E., and Wollmer, P., 2007. Site of deposition and absorption of an inhaled hydrophilic solute. *Br. J. Clin. Pharmacol.* 63 :6, 722-733.
- Borghardt, J.M, Weber, B., Staab, A., Kunz, C., Formella, S., Kloft, C., 2016. Investigating pulmonary and systemic pharmacokinetics of inhaled olodaterol in healthy volunteers using a population pharmacokinetic approach. *Br. J Clin. Pharmacol.* 81:3, 538-552.
- Borghardt, J.M., Weber, B., Staab, A., Kunz, C., Kloft, C., 2016. Model-based evaluation of pulmonary pharmacokinetics in asthmatic and COPD patients after oral olodaterol inhalation. *Br. J Clin. Pharmacol.* 82:3, 739-753. Borghardt, J.M., Weber, B., Staab, A., Kloft, C., 2015. Pharmacometric Models for Characterizing the Pharmacokinetics of Orally Inhaled Drugs. *AAPS J.* 17, 853-870
- Brutche. M.H., Carlen Brutche, I., Munavvar, M., Langley, S.J., Masterson, C.M., Daley-Yates, P.T., Brown, R., Custovic, A., and Woodcock, A., 2001. Comparison of pharmacokinetics and systemic effects of inhaled fluticasone propionate on patients with asthma and healthy volunteers: A random crossover study. *The Lancet.* 356 556-561.
- Burnell, P., Asking, L, Borgström, L., Nichols, S.C., Olsson, B., Prime, D., and Shrubbs, I, 2007. Studies of the Human Oropharyngeal Airspaces Using Magnetic Resonance Imaging IV—The Oropharyngeal Retention Effect for Four Inhalation Delivery Systems. *J. Aerosol Med. Pulm. Drug Del.* 20:3 269-281.
- Bäckman, P., Tehler, U., and Olsson, B., 2017. Predicting exposure after oral Inhalation of the selective glucocorticoid receptor modulator, AZD5423, based on dose, deposition pattern, and mechanistic modelling of pulmonary disposition. *J. Aerosol Med. Pulm. Drug Del.* 30:2, 108-117..

- Bäckman, P., and Olsson, B., 2016. Pitfalls in understanding local exposure. in: Dalby, R.N., Byron, P.R., Peart, J., Farr, S.J., Suman, J.D., Young, P.M., and Traini, D. (Eds.), *Respiratory Drug Delivery 2016*, Volume 1. Davis Healthcare International Publishing LLC., River Grove, pp. 125-132.
- Bäckström, E., Lundqvist, A., Boger, E., Svanberg, P., Ewing, P., Hammarlund-Udenaes, M., and Fridén, M., 2016a. Development of a Novel Lung Slice Methodology for Profiling of Inhaled Compounds. *J. Pharm.Sci.* 105:2, 838-845. DOI: <http://dx.doi.org/10.1002/jps.24575>
- Cabal, A., Jajamovich, G., Mehts, K., Guo, P., and Przekwas, A., 2106. In-silico lung modelling platform for inhaled drug delivery. *Drug delivery to the lungs* 27. pp. 82-86.
- Campbell, J., Van Landingham, C., Crowell, S., Gentry, R., Kaden, D., Fiebelkorn, S., Loccisano, A., Clewell, H., 2015. A preliminary regional PBPK model of lung metabolism for improving species dependent descriptions of 1,3-butadiene and its metabolites. *Chem Biol Interact.* 5:238,102-10.
- Caniga, M., Cabal, A., Mehta, K., Ross, D.S., Gil, M.A., Woodhouse, J.D., Eckman, J., Naber, J.R., Callahan, M.K., Goncalves, L., B.S., Hill, S.E., Mcleod, R.L., McIntosh, F., Freke, M.C., Visser, S.A.G., Johnson, N., Salmon, M., Cicmil, M., 2016. Preclinical Experimental and Mathematical Approaches for Assessing Effective Doses of Inhaled Drugs, Using Mometasone to Support Human Dose Predictions. *J Aerosol Med. Pulm. Drug Del.* 29, 362–377.
- Collingwood, S.P., Coe, D., Pryde, D., and R. Lock, 2012. Respiratory drug discovery, current developments and future challenges: Highlights from the society of medicines research symposium. Held on June 14th 2012 – Horsham, UK. *Drugs of the Future*, 37:8, 619-625.
- Cooper, A.E., Ferguson, D., and Grime, K. 2012. Optimisation of DMPK by the inhaled route: challenges and approaches. *Curr. Drug Metab.* 13, 457-473.
- Cooper, A., Potter, T., and Luker, T., 2010. Prediction of efficacious inhalation lung doses via the use of in silico lung retention quantitative structure-activity relationship models and in vitro potency screens. *Drug Metab. Dispos.* 38, 2218-1125.
- Danhof, M., deLange, E.C.M., Pasqua, O.E.D., Ploeger, B.A., Voskuyl, R.A., 2008. Mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) modelling in translational drug research. *Trends in Pharmacological Sciences* 29, 186-191.
- Das, SC., Stewart, PJ., 2016. The influence of lung surfactant liquid crystalline nanostructures on respiratory drug delivery. *Int J Pharm.* 514:2, 465-474.
- De Backer, J., Vos, W., Vinchurkar, S., Van Holsbeke, C., Poli, G., Claes, R., Salgado, R., and De Backer W., 2015. The effects of extrafine beclomethasone/formoterol (BDP/F) on lung function, dyspnoea, hyperinflation, and airway geometry in COPD patients: novel insight using functional respiratory imaging. *J. Aerosol Med. Pulm. Drug Deliv.* 28, 88-99.
- DeHaan, W.H, Finlay, W.H., 2001. In Vitro Monodisperse Aerosol Deposition in a Mouth and Throat with Six Different Inhalation Devices *J. Aerosol Med. Pulm. Drug Del.* 14:3 361-367.
- Dekhuijzen R.P.N., 2012. Anti-inflammatory drug targeting in asthma. Should inhaled corticosteroids reach the small airways? *Curr. Drug. Ther.* 7:4, 248-254.

Delvadia, R.R., Longest, P.W., and Byron, P.R., 2012. In Vitro Tests for Aerosol Deposition. I: Scaling a Physical Model of the Upper Airways to Predict Drug Deposition Variation in Normal Humans. *J. Aerosol Med. Pulm. Drug Del.* 25:1, 32-40.

Doan, T.V., Grégoire, N., Lamarche, I., Gobin, P., Marchand, S., Couet, W., Olivier, J.C., 2013. A preclinical pharmacokinetic modeling approach to the biopharmaceutical characterization of immediate and microsphere-based sustained release pulmonary formulations of rifampicin. *Eur J Pharm Sci.* 48:1-2, 223-230.

Donnelley, M., Morgan, K.S., Awadalla, M., Farrow, N.R., Chris Hall, and Parsons, D.W., 2017. High-resolution mucociliary transport measurement in live excised large animal trachea using synchrotron X-ray imaging. *Resp. Res.* (2017) 18:95 DOI 10.1186/s12931-017-0573-2

Donnelley, M., Morgan, K.S., Siu, K.K.W., Fouras, A., Farrow, N.G., Carnibella, R.P., and Parsons, D.W., 2014a. Tracking extended mucociliary transport activity of individual deposited particles: longitudinal synchrotron X-ray imaging in live mice. *J Synchrotron Rad.* 21, 768-773.

Donnelley, M., Morgan, K.S., Siu, K.K.W., Farrow, N.G., Stahr, C.S., Boucher, R.C., Fouras, A., and Parsons, D.W., 2014b. Non-invasive airway health assessment: Synchrotron imaging reveals effects of hydrating treatments on mucociliary transit *in vivo*. *Sci. Reports*, 4:3689 DOI: 10.1038 /srep03689

Edwards, C.d., Luscombe, C., Eddershaw, P., and Hessel, E.M., 2016. Development of a novel quantitative structure-activity relationship model to accurately predict pulmonary absorption and replace routine use of the isolated perfused respiring rat lung model *Pharm Res.* 33, 2604-2616.

Ehrhardt, C., Kneuer, C., Fiegel, J., Hanes, J., Schaefer, U., Kim, Kwang-Jin, K., and Lehr, C.-M., 2002. Influence of apical fluid volume on the development of functional intercellular junctions in the human epithelial cell line 16HBE14o-: implications for the use of this cell line as an *in vitro* model for bronchial drug absorption studies. *Cell and Tissue Research* 308, 391-400.

Ehrhardt, C., Bäckman, P., Couet, W., Edwards, C., Forbes, B., Friden, M., Gumpleton, M., Hosoya, K.-I., Kato, Y., Nakanishi, T., Takano, M., Terasaki, T., and Yumoto, R., 2017. Current progress toward a better understanding of drug disposition within the lungs: summary proceedings of the 1st Workshop on Drug Transporters in the Lungs. *J. Pharm. Sci.* (available at <http://dx.doi.org/10.1016/j.xphs.2017.04.011>)

Eissing, T., Kuepfer, L., Becker, C., Block, M., Coboeken, K., Gaub, T., Goerlitz, L., Jaeger, J., Loosen, R., Ludewig, B., Meyer, M., Niederal, C., Sevestre, M., Siegmund, H-U., Solodenko, J., Thelen, K., Telle, U., Weiss, W., Wendl, T., Willmann, S., and Lippert, J., 2011. A Computational Systems Biology Software Platform for Multiscale Modeling and Simulation: Integrating Whole-Body Physiology, Disease Biology, and Molecular Reaction Networks. *Front Physiol.* 2:4, 1-10.

Finlay, W.H., and Martin, R.H. 2008. Recent advances in predictive understanding of respiratory tract deposition. *J. Aerosol Med. Pulm. Drug Deliv.*, 21:2, 189–205

Forbes, B., Asgharian, B., Dailey, L.A., Ferguson, D., Gerde, P., Gumpleton, M., Hardy, C., Hassall, D., Gustavsson, L., Jones, R., Lock, R., Maas, J., McGoverin, T., Pitcairn, G., Somers, G., Wolff, R., 2011. Challenges in inhaled product development and opportunities for open innovation. *Adv. Drug Deliv. Rev.* 63, 69-87.

Forbes, B., Shah, A., Martin, G.P., Lansley, A.B., 2003. The human bronchial epithelial cell line 16HBE140- as a model system of the airways for studying drug transport. *Int. J. Pharm.* 257, 161-167.

Forbes, B., Bäckman, P., Christopher, D., Dolovich, M., Bing, L., Morgan, B., 2015. In vitro testing for orally inhaled products: developments in science-based regulatory approaches. *AAPS J.* 17, 837-852.

Forbes, B., and Ehrhardt, C., 2005. Human respiratory epithelial cell culture for drug delivery applications. *Eur. J. Pharm. and Biopharm.* 60, 193-205.

Frohlich, E., Mercuri, A., Wu, S.Q., Salar-Behzadi, S., 2016. Measurements of Deposition, Lung Surface Area and Lung Fluid for Simulation of Inhaled Compounds. *Frontiers in Pharmacology* 7: Article Number 181.

Gauhua, L., Wedagedera, J., Small, B.G., Almond, L., Romero, K., Hermann, D., Hanna, D., Jamei, M., and Gardener, I., 2015. Development of a multicompartiment permeability-limited lung PBPK model and its application on predicting pulmonary pharmacokinetics of anti-tuberculosis drugs. *CPT Phaermetrics Syst. Pharmacol.* 4, 605-613.

Gaspar, M.C., Grégoire, N., Sousa, J.J., Pais, A.A., Lamarche, I., Gobin, P., Olivier, J.C., Marchand, S., Couet, W., 2016. Pulmonary pharmacokinetics of levofloxacin in rats after aerosolization of immediate-release chitosan or sustained-release PLGA microspheres. *Eur J Pharm Sci.* 93, 184-191.

Gerde, P., Malmlof, M., Havsborn, L., Sjöberg, C.O., Ewing, P., Eirefelt, S., Ekelund, K., 2017. Dissolvt: An In Vitro Method for Simulating the Dissolution and Absorption of Inhaled Dry Powder Drugs in the Lungs. *Assay Drug Dev Technol.* 15:2, 77-88. (ISSN: 1557-8127)

Giorgetti, M., 2016. Studies on the Mucin-binding of Inhaled Drug Molecules. PhD Thesis. University of East Anglia, UK.

Gontijo, A.V., Brillault, J., Grégoire, N., Lamarche, I., Gobin, P., Couet, W., and Marchand S., 2014a. Biopharmaceutical characterization of nebulized antimicrobial agents in rats: 1. Ciprofloxacin, moxifloxacin, and grepafloxacin. *Antimicrob. Agents Chemother.* 58 :7, 3942-3949.

Gontijo, A.V., Grégoire, N., Lamarche, I., Gobin, P., Couet, W., and Marchand S. 2014b. Biopharmaceutical characterization of nebulized antimicrobial agents in rats: 2. Colistin. *Antimicrob. Agents Chemother.* 58:7, 3950-3956.

Grainger, C., Greenwell, L.L., Lockley, D.J., Martin, G.P., and Forbes, B., 2006. Culture of Calu-3 cells at the air-liquid interface provides a representative model of the airway epithelial barrier. *Pharm. Res.* 23, 1482-1490.

Grainger, C., Saunders, M., Buttini, F., Telford, R., Martin, G.P., Jones, S.A., and Forbes, B., 2012. Critical characteristics for corticosteroid solution metered dose inhaler bioequivalence. *Molecular Pharmaceutics* 9, 563-569.

Grießinger, J., Dünhaupt, S., Cattoz, B., Griffiths, P., Oh, S., Borrós i Gómez, S., Wilcox, M., Pearson, J., Gumbleton, M., Abdulkarim, M., Pereira de Sousa, I., and Bernkop-Schnürch, A., 2015. Methods to determine the interactions of micro- and nanoparticles with mucus. *Eur. J Pharm. Biopharm.*, 96 464-476.

Hastedt, J.E., Bäckman, P., Clark, A.R., Doub, W., Hickey, A., Hochhaus, G., Kuehl P.J., Lehr, C.-M., Mauser, P., McConville, J., Niven, R., Sakagimi, M., and Weers, J.G., 2016. Scope and relevance of a pulmonary biopharmaceutical classification system AAPS/FDA/USP Workshop March 16-17th. AAPS Open 2 1–20.

Hoffman, W., and Asgharian, B., 2003 The effect of lung structure on mucociliary clearance and particle retention in human and rat lungs. *Tox. Sci.*, 73, 448-456.

ICRP, 1994. Human respiratory tract model for radiological protection. International Commission on Radiological Protection, ICRP Publication 66, Elsevier Science, Tarrytown, NY

Jones, R.M., and Harrison, A., 2012. A new methodology for predicting human pharmacokinetics for inhaled drugs from orotracheal pharmacokinetic data in rats. *Xenobiotica* 42 75–85

Korzekwa, K., Nagar, S., 2017. On the Nature of Physiologically-Based Pharmacokinetic Models—A Priori or A Posteriori? Mechanistic or Empirical? *Pharm Res* 34, 529–534.

Lombry, C., Edwards, D.A., Prat, V., Vanbever, R., 2004. Alveolar macrophages are a primary barrier to pulmonary absorption of macromolecules. *Am J Physiol Lung Cell Mol Physiol.*, 286:5 L1002-1008.

Melin, J., Prothon, S., Kloft, C., Cleton, A., Amilon, C., Jorup, C., Bckman, P., Olsson, B., and Whlby Hamrn, U., 2017. Pharmacokinetics of the Inhaled Selective Glucocorticoid Receptor Modulator AZD5423 Following Inhalation Using Different Devices. *AAPS J.* DOI: 10.1208/s12248-016-0042-8

Manford, F., Tronde, A., Jeppsson, A.B., Patel, N., Johansson, F., and Forbes, B., 2005. Drug permeability in 16HBE14o- airway cell layers correlates with absorption from the rat lung. *Eur. J. Pharm. Sci.* 26, 215-220.

Marchand, S., Chauzy, A., Dahyot-Fizelier, C., and Couet, W., 2016. Microdialysis as a way to measure antibiotics concentration in tissues. *Pharmacol Res.* 111, 201-207.

Marchand, S., Frasca, D., Dahyot-Fizelier, C., Breheret, C., Mimiz, O., and Couet, W., 2008. Lung microdialysis study of levofloxacin in rats following intravenous infusion at steady state. *Antimicrob. Agents Chemother.* 52:9, 3074-3077.

Marchand, S., Grgoire, N., Brillault, J., Lamarche, I., Gobin, P., and Couet, W. 2015. Biopharmaceutical Characterization of Nebulized Antimicrobial Agents in Rats: 3. Tobramycin. *Antimicrob. Agents Chemother.* 59:10, 6646-6647.

Marchand, S., Grgoire, N., Brillault, J., Lamarche, I., Gobin, P., and Couet W. 2016. Biopharmaceutical Characterization of Nebulized Antimicrobial Agents in Rats. 4. Aztreonam. *Antimicrob. Agents Chemother.* 60:5, 3196-3198.

Mathias, N.R., Timoszyk, J., Stetsko, P.I., Megill, J.R., Smith, R.L., and Wall, D.A., 2002. Permeability characteristics of Calu-3 human bronchial epithelial cells: *In vitro-in vivo* correlation to predict lung absorption in rats. *J. Drug Targeting* 10, 31-40.

May, S., Jensen, B., Weiler, C., Wolkenhauer, M., Schneider, M., Lehr, C.M., 2014. Dissolution testing of powders for inhalation: Influence of particle deposition and modelling of dissolution profiles. *Pharm. Res.* 31, 3211–3224.

Miller-Larson, A., Mattson, H., Hjertberg, E., Dahlbäck, M., and Tunek, A., 1998. Reversible fatty acid conjugation of budesonide. Novel mechanism for prolonged retention of topically applied steroid in airway tissue. *Drug Met and Disp.* 26, 623-630.

Mimetikos AB, Preludium™, <http://www.emmace.se/mimetikos-preludium/> (accessed May 18, 2017)

Niven, R., 2004. Modulating the pharmacokinetics of Inhaled drugs. In: Hickey, A.J., (ed.), *Pharmaceutical Aerosol Technology Edition 2.* Olsson, B., Bondesson, E., Borgstrom, L., Edsbacker, S., Eirefelt, S., Ekelund, K., Gustavsson, L., Hegelung-Myrback., 2011. Pulmonary drug metabolism, clearance and absorption. In: Smyth, H.D.C., and Hickey, A.J. (Eds.), *Controlled Pulmonary Drug Delivery.* Springer.

Olsson, B., Borgström, L., Lundbäck, H., and Svensson, M., 2013. Validation of a general in vitro approach for prediction of total lung deposition in healthy adults for pharmaceutical inhalation products, *J Aerosol Med Pulm Drug Deliv.* 23, 355-369.

Olsson, B., and Bäckman, P., 2014. Mouth-throat models for realistic *in vitro* testing: A proposal for debate. In: Dalby R, Byron P, Peart J, Suman J, Farr S, Young P. (Eds.) *Respiratory Drug Delivery 2014.* Volume 1. DHI Publishing; River Grove, IL: pp. 287-94.

Pang, Y.N., Sakagami, M., and Byron, P.R., 2005. The pharmacokinetics of pulmonary insulin in the in vitro isolated perfused rat lung: Implications of metabolism and regional deposition. *Eur. J. Pharm. Sci.* 25, 369-378.

PK-SIM™ Computational Systems Biology: Bayer AG, <http://www.systems-biology.com/products/PK-Sim.html> accessed on May 30, 2017

Riley, T., Christopher, D., Arp, J., Casazza, A., Colombani, A., Cooper, A., Dey, M., Maas, J., Mitchell, J., Reiners, M., Sogari, N., Tougas, T., and Lyapustina, S., 2012. Challenges with developing in vitro dissolution tests for orally Inhaled Products (OIPs), *AAPS PharmSciTech.* 13:3 978-989.

Rodvold, K.A., Yoo, L., and George, J.M., 2011. Penetration of anti-infective agents into pulmonary epithelial lining fluid: focus on antifungal, antitubercular and miscellaneous anti-infective agents. *Clin Pharmacokinet.* 50 :11, 689-704.

Rossi, A., et al. 2017. *Eur J Pharm Sci.* this issue.

Ruparelia, P., Cheow, HK., Evans, JW., Banney, L., Shankar, S., Szczepura, KR., Swift, AE., Ballinger, JR., Hartman, NG., Chilvers, ER., Peters, AM., 2008. Pulmonary elimination rate of inhaled 99mTc-sestamibi radioaerosol is delayed in healthy cigarette smokers. *Br J Clin Pharmacol.* 65:4, 611-4.

Schum, M., and Yeh, H., 1980. Theoretical evaluation of aerosol deposition in anatomical models of mammalian lung airways. *Bull. Math. Biol.* 42, 1-15.

SimCyp Simulator™ <https://www.certara.com/software/physiologically-based-pharmacokinetic-modeling-and-simulation/simcyp-simulator/absorption/> (accessed May 18 2017).

Somers, G.I., Lindsay N., Lowdon, B.M., Jones, A.E., Freathy, C., Ho, S., Woodroffe, A.J.M., Bayliss M.K., and Manchee, G. R., 2007. A comparison of the expression and metabolizing activities of phase I and II enzymes in freshly isolated human lung parenchymal cells and cryopreserved human hepatocytes. *Drug Metabolism and Disposition* 35, 1797-1805.

- Stass, H., Baumann-Noss, S., Delesen, H., Nagelschmitz, J., Willmann, S., and Edginton, A., 2008. Ciprofloxacin Pulmosphere inhalation powder: A healthy volunteer study. Poster, ATS, Toronto.
- Stass, H., Nagelschmitz, J., Willmann, S., Delesen, H., Gupta, A., and Baumann, S., 2013. Inhalation of a dry powder ciprofloxacin formulation in healthy subjects: A phase I study. Clin. Drug Investig. 33, 419-427.
- Torres, B.G.S., Helfer, V.E., Bernardes, P.M., Macedo, A.J., Nielsen, E.I., Friberg, L.E., and dalla Costa, T., 2017. Population pharmacokinetic modelling as a tool to characterize the decrease in ciprofloxacin free intestinal levels caused by pseudomonas aeruginosa biofilm lung infection in wistar rats, Antimicrob. Agents Chemother. 61:7, doi: 10.1128/AAC.02553-16
- Tronde A., Nordén, B., Marchner, H., Wendel, A.-K., Lennernäs, H., and Bengtsson, U.H, 2003a. Pulmonary absorption rate and bioavailability of drugs *in vivo* in rats: structure-absorption relationships and physicochemical profiling of inhaled drugs. J.Pharm.Sci. 92, 1216-1233.
- Tronde, A., Nordén, B., Jeppsson, A.B., Brunmark, P., Nilsson, E., Lennernäs, H., and Bengtsson, U.H., 2003b. Drug absorption from the isolated perfused rat lung-correlations with drug physico-chemical properties and epithelial permeability, J. Drug. Target.11, 61-74.
- Tronde, A., Bosquillon, C., and Forbes, B., 2008. The isolated perfused lung for drug absorption studies. In: Drug absorption studies: In situ, in vitro and in silico models (Eds.) Ehrhardt, C., and Kim, K.J. Springer, New York, pp. 135-163.
- Ufuk, A, Assmus, F., Francis, I., Plumb, J., Damian, V., Gertz, M., Houston, J.B., and Galetin, A, 2017. In Vitro and in Silico Tools to Assess Extent of Cellular Uptake and Lysosomal Sequestration of Respiratory Drugs in Human Alveolar Macrophages. Mol. Pharm. 14, 1033-1046.
- Usmani, O.S., Biddiscombe, M.F., and Barnes, P.J., 2005. Regional Lung Deposition and Bronchodilator Response as a Function of β_2 -Agonist Particle Size. Am J. Respir. Crit. Care Med. 172, 1497–1504.
- Wang, Y.-B., Watts, A.B., Peters, J.I., and Williams III, R.O., 2014. The impact of pulmonary diseases on the fate of inhaled medicines—a review. Int. J. Pharm. 461, 112-28.
- Wei, X., Byron, P.R., and Longest, W.P., 2014. Predicting variation in lung dose with different mouth-throat Models. in: Dalby, R., Byron, P., Peart, J., Suman, J., Farr, S., and Young, P. (Eds.) Respiratory Drug Delivery 2014, Volume 3. DHI Publishing; River Grove, pp. 773-776.
- Weibel, E.R., 1963 Morphometry of the Human Lung. Springer, Berlin.
- Yu, J.Y., and Rosania, G.R., 2010. Cell-Based Multiscale Computational Modeling of Small Molecule Absorption and Retention in the Lungs. Pharm. Res. 27, 457-467.
- Zhou, Y., Sun, J. J., and Cheng, Y. S., 2011. Comparison of Deposition in the USP and Physical Mouth-Throat Models with Solid and Liquid Particles. J.Aerosol Med. Pulm. Drug Deliv, 24, 277–284.
- Zhuang, X., and Lu C., 2016. PBPK modeling and simulation in drug research and development. Acta Pharm.Sin. B. 6:5, 430–440.
- Zimmermann, e.s., Laureano, J.V., dos Santos, C.N., Schmidt, S., Lagishetty, C.V., de Castro, V.W., and dalla Costa, T., 2015. Simultaneous semimechanistic population analysis of levofloxacin in plasma,

lung and prostate to describe the influence of efflux transporters on drug distribution following intravenous and intratracheal administration. *Antimicrob. Agents Chemother.* 60:2, 946-954.

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