



Bioavailability of Therapeutic Proteins by Inhalation—Worker Safety Aspects

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ABSTRACT

A literature review and analysis of inhalation bioavailability data for large therapeutic proteins was conducted in order to develop a practical estimate of the inhalation bioavailability of these drugs. This value is incorporated into equations used to derive occupational exposure limits (OELs) to protect biopharmaceutical manufacturing workers from systemic effects. Descriptive statistics implies that a value of 0.05, or 5% is an accurate estimate for large therapeutic proteins (molecular weight ≥ 40 kDa). This estimate is confirmed by pharmacokinetic modeling of data from a human daily repeat-dose inhalation study of immunoglobulin G. In conclusion, we recommend using 5% bioavailability by inhalation when developing OELs for large therapeutic proteins.

KEYWORDS: inhalation bioavailability; occupational exposure limit; OEL; therapeutic proteins

INTRODUCTION

Biologics constituted >30% of approved pharmaceuticals, with 179 new biologics (that are not vaccines or blood products) approved between 1993 and early 2013 (Sathish *et al.*, 2013; U. S. FDA, 2013). These drugs have offered novel and effective treatments for immune-mediated inflammatory diseases, infection, hematology, and a variety of cancers. This class of molecules includes various recombinant proteins, fusion proteins, and monoclonal antibodies that are being developed to treat these various diseases. They

are large chains of amino acids that are produced through expression in biological organisms (typically Chinese hamster ovary cells, or *Escherichia coli* bacteria) instead of synthetic organic chemical manufacturing. Because amino acids are naturally occurring, mostly in dilute solution, and the studies to date demonstrate poor inhalation bioavailability as a drug delivery route, there is thought that proteins pose a much lower risk to workers handling them in the workplace. However, these are still very potent, pharmacological agents when delivered by the therapeutic

route of administration (e.g. intravenous or subcutaneous injection). Any pharmacologic effects that may occur following protein inhalation are relevant to setting occupational exposure limits (OELs). In a review, pharmacologically relevant absorption through the airways was reported for the relatively small proteins and peptides like insulin, calcitonin, growth factors, alpha-1-antitrypsin, luteinizing hormone-releasing agonists and antagonists, vasopressin analog, interferons, and granulocyte colony-stimulating factor, and also for larger proteins, namely immunoglobulins (Ig), in animals and limited clinical studies (Agu *et al.*, 2001). Thus, the risk from inhaled proteins may not be presumed to be zero. Biopharmaceutical workers are involved with large manufacturing scale production of these proteins. Consequently, occupational health professionals need to ensure that both the pharmacological and toxicological effects of these drugs are prevented among workers handling these materials. Companies and government agencies accomplish this by setting workplace OELs.

To develop an OEL, nonclinical toxicological, pharmacological, and pharmacokinetic (PK) data along with clinical pharmacodynamic, PK, and safety data are reviewed. The risk assessment models for OELs use multiple uncertainty factors applied to a Point of Departure (POD), a No Observed Adverse Effect Level (NOAEL), or Lowest Observed Adverse Effect Level (LOAEL) for the most sensitive endpoint (the critical effect) in the most relevant species (Naumann and Sargent, 1997; EPA, 2011; ICH, 2011). Due to the extensive datasets required for pharmaceuticals, chemical-specific adjustment factors (uncertainty and PK factors) to the POD may be used (Sargent and Kirk, 1988; Silverman *et al.*, 1999; IPCS, 2001).

There is some suggestion that using the traditional small molecule approach may be overly conservative for protein therapeutics. First, the preclinical and clinical study designs for patient safety are *via* parenteral injection (typically intravenous or subcutaneous administration) with intermittent dosing. This gives a clear blood exposure that equates to a pharmacological and/or toxicological effect. In contrast, the usual route-to-route adjustment in the OEL calculation is generally assumed to be 100% bioavailability for small molecule pharmaceuticals when inhalation bioavailability or toxicity data are not available. This inhalation bioavailability assumption would significantly

overestimate the exposure for a therapeutic protein. There are biological reasons to expect that the ability of an inhaled protein to enter the systemic circulation is limited. Therefore, an adjustment to the route-to-route bioavailability factor in the calculation may be appropriate.

We have performed an analysis of published data related to the inhalation bioavailability of therapeutic proteins in order to estimate a more accurate correction factor for use when calculating OEL for biopharmaceutical manufacturing.

Absorption and facilitation of proteins through airways and alveoli

The lung is naturally permeable to a number of therapeutic peptides and proteins, and far more permeable to proteins than any other portal of entry into the body. The large [80–120 m², up to 140 m² (Witschi and Last, 2001)] absorptive surface of the lung is covered by an extremely thin layer of fluid (volume 10–30 ml) (Patton *et al.*, 2010). Thus, an inhaled aerosol can be dispersed and deposited in quite high concentrations in close proximity to the bloodstream. Moreover, the surface fluids of the lung contain antiproteases that inhibit the enzymatic breakdown of proteins. Unlike the nasal passages and gastrointestinal tract, where lateral movement of bulk fluid occurs, the alveoli of the deep lung are cul-de-sacs where residence times of molecules at the absorptive surface may be prolonged (Patton *et al.*, 2004). ‘Blocked’ peptides like cyclosporin, peptides that have been chemically altered to protect them from degradation by peptidase enzymes exhibit very high bioavailability by the pulmonary route compared to the oral and dermal routes (*ibid*). In general, proteins with molecular weights (MW) between 6 and 50 kDa are relatively resistant to most peptidases and have a relatively high bioavailability following inhalation when compared to larger proteins. However, aggregation of inhaled proteins stimulates opsonization (coating) by surfactant and by proteins suspended in the lung fluids. This way, the aggregated proteins become marked for phagocytosis and intracellular enzymatic destruction (Möller *et al.*, 2008).

Facilitation of absorption of proteins through airways and alveoli

For most proteins to reach the circulation, the material must first reach the deep lung. This is primarily

dependent on particle size. The nasal passages and lungs are designed to filter out large particles that may interfere with lung function and gas exchange. Particles $>10\ \mu\text{m}$ will be deposited *via* sedimentation or impaction in the nasopharynx and tracheobronchial passages. In these regions, the epithelium is thicker and coated with a mucus layer, both of which act to limit systemic absorption. In addition, the ciliated epithelia move the particle-containing mucus toward the pharynx where it is swallowed. This fraction of the inhaled protein particles is expected to be degraded in the gastrointestinal tract and systemic exposure is negligible. Efficient pulmonary drug delivery for systemic exposure requires particles with aerodynamic diameters of $1\text{--}3\ \mu\text{m}$ (Patton *et al.*, 2004). Particles $1\text{--}5\ \mu\text{m}$ in diameter are deposited in the small airways and alveoli with $>50\%$ of the $3\text{-}\mu\text{m}$ diameter particles being deposited in the alveolar region (Labiris and Dolovich, 2003). For instance, inhalation of $0.8\text{-}\mu\text{m}$ insulin particles resulted in similar absorption relative to subcutaneous administration, while that of $4\text{-}\mu\text{m}$ particles was only 38% , and inhalation of $8\text{-}\mu\text{m}$ particles resulted in $<1\%$ absorption (Wolff, 1998).

The toxicological concern is for particles with an aerodynamic diameter $<10\ \mu\text{m}$ as these can be deposited in the alveolar region where the epithelium, the most significant barrier to absorption of inhaled drugs, is thinnest ($0.2\ \mu\text{m}$) (*ibid*). Once reaching the deep lung, there are many factors limiting the ability of a protein to be systemically absorbed. First, the alveoli epithelial cell layer, interstitium and finally, the capillary endothelial cell layer must be crossed to reach the circulation. Second, the alveolar space contains many enzymes which may degrade proteins and peptides and thus neutralize their biological activity. Proteins have varying degrees of enzymatic stability or susceptibility to protease inactivation in the fluid lining the lung epithelial layer. Macrophages and other inflammatory cells contribute to degradation by releasing peroxides and other proteinases. These macrophages also have the capability to engulf and digest proteins and other debris. The mucociliary escalator clears macrophages and inhaled particles deposited in the mucus lining upward and ultimately leads to swallowing of a fraction of the dose. Physicochemical characteristics influence absorption. Proteins must be soluble in order to pass through a thin mucus and surfactant layer. Molecules that are hydrophilic, charged

(i.e. anionic or cationic) (Patton *et al.*, 2010), or of the wrong size are limited in their ability to cross this barrier. The cutoff for tight junctions between alveolar type 1 cells is $0.6\ \text{nm}$, whereas only particles in the size range of $4\text{--}6\ \text{nm}$ can cross endothelial junctions (Agu *et al.*, 2001).

However, there are biological reasons for systemic exposure potentially to occur after inhalation to proteins. Mechanisms for transport exist as there are reports of proteins $>0.6\ \text{nm}$ crossing into the circulation. Alveolar epithelial cells have pores and vesicles which allow passive diffusion. Receptor-mediated processes and vesicular transport exist to transport larger molecules across cellular layers to the blood. Nonpassive transport processes, such as active transcytosis, has been reported for a series of peptides and proteins *in vitro* using primary human alveolar epithelial cell monolayers (Bur *et al.*, 2006). For larger proteins ($>40\ \text{kDa}$), transcytosis (vesicular transport of macromolecules across the interior of a cell) may be the dominant mechanism, whereas for smaller proteins, both transcytosis and paracellular mechanisms may be important (Mobley and Hochhaus, 2001). The high MW of IgG class Igs ($\sim 150\ \text{kDa}$), from which many therapeutic monoclonal antibodies are derived, have demonstrated evidence of low absorption in rats (Sweeney *et al.*, 2002). Research has suggested that this transport occurs by receptor-mediated transcytosis *via* the neonatal constant region fragment receptor (FcRn) (Sweeney *et al.*, 2002; Kim *et al.*, 2004; Ober *et al.*, 2004; Sakagami, 2006; Baker *et al.*, 2009; Maillet *et al.*, 2011). The FcRn plays an important role in IgG transport in other tissues. Recently, it has become clear that the FcRn is expressed in the upper airways of primates and bronchial and alveolar epithelial cells of rats (Spiekermann *et al.*, 2002; Patton *et al.*, 2004; Sakagami, 2006; Roopenian and Akilesh, 2007). The role of the FcRn in systemic absorption was further demonstrated by Bitonti and colleagues who fused erythropoietin, a protein that had minimal inhalation bioavailability and does not bind to the Fc region, to the Fc-domain of IgG₁ (EpoFc) allowing it to bind with high affinity to FcRn. From inhalation studies in monkeys it has been reported that systemic absorption of EpoFc deposited in the lung was similar to subcutaneous injection in nonhuman primates and humans (Bitonti *et al.*, 2004; Bitonti and Dumont 2006; Dumont *et al.*, 2006). The bronchial epithelial cells

of the human, the nonhuman primate, and the mouse express the neonatal Fc receptor (FcRn) in adult life (Patton *et al.*, 2004). Based on this discovery, attempts were made to utilize the FcRn to aid the absorption for pulmonary delivery of a bioactive Fc-fusion protein across the respiratory epithelium. While the principle of FcRn-mediated pulmonary absorption has been demonstrated with EpoFc and some other molecules in nonhuman primates and humans (Bitonti *et al.*, 2004; Bitonti and Dumont 2006; Dumont *et al.*, 2006), the total (effective) inhaled bioavailability was still low. In contrast to rats, humans and nonhuman primates have no detectable functional FcRn in alveolar cells (Kuo *et al.*, 2010). With EpoFc, even under optimized experimental conditions (aerosolized formulation, optimized administration technique), the fraction of the inhaled dose deposited in the lung was 13–15% of which 6–35% was bioavailable, resulting in a total (effective) inhaled bioavailability in the range of only 1–5% (Table 1). Nonetheless, no macromolecule using FcRn-dependent absorption has been commercialized underscoring the difficulty of translating laboratory findings into real-world drug products.

Other intrinsic properties of the lung may enhance systemic absorption of proteins. Small amounts of inhaled protein may enter the pulmonary lymphatic system as it removes fluid, lipoproteins, plasma proteins, and phagocytized particles. Once entering the lymph and lymph nodes, the fluid is returned to the venous blood circulation (Tronde, 2002). Lastly, the sheer size of the alveolar epithelium surface area [~ 80 – 120 m^2 , up to 140 m^2 (Witschi and Last, 2001)] and the close contact with the extensive capillary network allow for increased opportunity for systemic exposure (Patton *et al.*, 2004).

Finally, the type of breathing maneuver affects pulmonary deposition of particles (Patton *et al.*, 1999). The optimal breathing technique is known to vary considerably depending on the inhaler technology and the particle size used. For instance, patients are instructed to hold their breath after the inhalation of small particles, but not necessarily after inhaling larger particles, and patients using dry powder inhalers are instructed to inhale fast, whereas those using jet nebulizers are instructed to inhale as slowly as possible to achieve optimal deposition (Heinemann *et al.*, 2008). Deep breathing of a large protein (erythropoietin fused to the Fc domain of an IgG₁) was poorly

absorbed under forced deep breathing with breath holding to maximize deposition in the alveolar region of the lung, when compared to shallow breathing. This was attributed to FcRn expression being significantly greater in the epithelial cells of the upper and central airways (Bitonti and Dumont 2006). Under regular breathing patterns of moderate exertion in the workplace, it is not clear where aerosols would be deposited.

There are many examples of macromolecules where systemic exposure is a fraction of the calculated inhaled dose. Although many studies have been conducted with a wide range of high MW proteins, varying exposure conditions and experimental methods for verifying systemic exposures make it difficult to generalize on the inhalation bioavailability for proteins. First, many of these were not ‘naïve’ experiments. Rather, they were conducted specifically to optimize therapeutic pulmonary delivery by targeting increased deposition and enhanced absorption to optimize protein uptake over a short exposure period. The methods used included selected inhalation devices, the form of the protein administered (as a nebulized liquid or dry powder), and the inclusion of absorption enhancement additives (Ali, 2010); utilization of intratracheal instillation underestimates the extent of absorption that can be achieved through inhalation delivery (Patton *et al.*, 1998). Most of these factors would be expected to exaggerate the inhalation exposure compared to regular breathing conditions in the workplace.

In addition, it is difficult to reconcile and interpret the inhalation bioavailability values derived in these disparate studies. Some experiments measured pharmacological activity in order to demonstrate systemic exposure. Other reports give blood concentrations of the inhaled protein of interest but data from intravenous exposure is not available for comparison. Still other researchers compare the inhalation bioavailability to the subcutaneous exposure with no information on the intravenous exposure. Consequently, the inhalation bioavailability values presented in the literature cannot be compared except in a more qualitative sense.

For calculating an OEL, a key difference between small molecules and proteins is inhalation bioavailability. However, data on pulmonary bioavailability for therapeutic proteins in an occupational setting is limited or not available. In an earlier research by Effros and Mason (1983) it was found that the clearance

Table 1. Bioavailability of selected peptides and proteins administered by inhalation or intratracheal instillation (sorted by increasing molecular weight)

Molecule	MW (kDa)	Application	Bioavailability % (range)	Test system	Reference
RGD peptide (Arg, Gly, Asp)	0.7	i.t.	23 (20–25.8)	Rat	Smith <i>et al.</i> (1994)
dDAVP (Desmopressin)	1.1	i.t.	20	Adult rat	Folkesson <i>et al.</i> (1991)
		i.t.	45	Juvenile rat	Folkesson <i>et al.</i> (1991)
Cyclosporine	1.2	i.t.	<25	Dog	Unpublished (Novartis)
Leuprolide	1.2	i.t.	50 (5–95)	Dog	Patton <i>et al.</i> (2004)
Leuprolide acetate	1.2	a.i.	100	Rat	Adjei <i>et al.</i> (1992)
		a.i.	12.3 (4.3–18.3)	Human	Adjei and Garren (1990)
Detirelix	1.5	i.t.	29	Dog	Bennett (1994)
		i.t.	9.8	Sheep	Schreier <i>et al.</i> (1994)
Calcitonin	3.4	i.t.	11.5	Rat	Komada <i>et al.</i> (1994)
		i.t.	17	Rat	Patton <i>et al.</i> (1994)
Parathyroid hormone (1–34)	4.1	i.t.	40	Rat	Patton <i>et al.</i> (1994)
		i.t.	13 (10–16)	Rat	Codrons <i>et al.</i> (2004)
Tesamorelin (TH9507)	5.1	i.t.	13	Dog	Jansen <i>et al.</i> (2004)
Insulin	5.8	i.t.	11.9	Rat	Yamamoto <i>et al.</i> (1994)
		i.t.	3.1	Rat	Lombry <i>et al.</i> (2004)
		i.t.	6.5 ^a	Rat	Komada <i>et al.</i> (1994)
		a.i.	57	Rabbit	Colthorpe <i>et al.</i> (1992)
Parathyroid hormone (1–84)	9.4	i.t.	23	Rat	Patton <i>et al.</i> (1994)
Interferon- α	19	a.i.	11 (10–12)	Human	Gonda (2006)
		i.t.	37.5 (19–56)	Rat	Patton <i>et al.</i> (2004)
		i.t.	>56 ^a	Rat	Patton <i>et al.</i> (1994)
Granulocyte colony-stimulating factor–GCSF	19	i.t.	12	Rat	Patton <i>et al.</i> (2004)
		i.t.	62	Hamster	Patton <i>et al.</i> (1994)
Heparin	20	i.t.	44.5 (43–46)	Rat	Schanker and Burton (1976)
Epoetin–unglycosylated	21	i.t.	<5	Sheep	Unpublished (F. Hoffmann-La Roche)
Somatotropin–hGH	22	i.t.	10	Rat	Patton (1990)
		i.t.	36	Rat	Patton <i>et al.</i> (1994)

Table 1. Continued

Molecule	MW (kDa)	Application	Bioavailability % (range)	Test system	Reference
Pulmozyme (rhDNase)	37	a.i./i.t.	<15	Rat	Green (1994)
		a.i./i.t.	<2	Monkey	
Epoetin beta	40 (36–45)	i.t.	<5	Sheep	Unpublished (F. Hoffmann-La Roche)
Alpha 1-antitrypsin–glycosylated (natural)	45	i.t.	<1	Sheep	Hubbard <i>et al.</i> (1989)
Alpha 1-antitrypsin–glycosylated (<i>Escherichia coli</i>)	51	i.t.	<1	Sheep, dog	Smith <i>et al.</i> (1989)
Bovine serum albumin	67	i.t.	5 (4.3–5.6)	Rat	Folkesson <i>et al.</i> (1991)
Albumin	68	i.t.	4.5	Rat	Patton <i>et al.</i> (2004)
Epoetin-Fc-monomer	72	i.t.	3 (1–5) ^b	Monkey	Bitonti <i>et al.</i> (2006)
Epoetin-FC-dimer	112	i.t.	1 ^a	Monkey	
IgG	150	i.t.	1.7	Rat	Patton <i>et al.</i> (2004)
		i.t.	4.7 ^c	Rat	Lombry <i>et al.</i> (2004)
Bovine IgG	150	i.t.	1.5	Adult rat	Folkesson <i>et al.</i> (1991)
		i.t.	0.7	Juvenile rat	Folkesson <i>et al.</i> (1991)

a.i., aerosol inhalation; i.t., intratracheal instillation.

^aFor the statistical analysis a value of 60% was used.

^bTotal bioavailability estimated by correction for reported dose fraction deposited in the lung (i.e. percentage absorbed from deposited dose fraction × percentage of dose deposited in the lung).

^cDepending on the doses; 4.7% was achieved with 500 µg, 10.9% with 50 µg, and 38% bioavailability was achieved with doses of 5 µg. Only the result of the high dose level was given credence due to analytical limitations (see text).

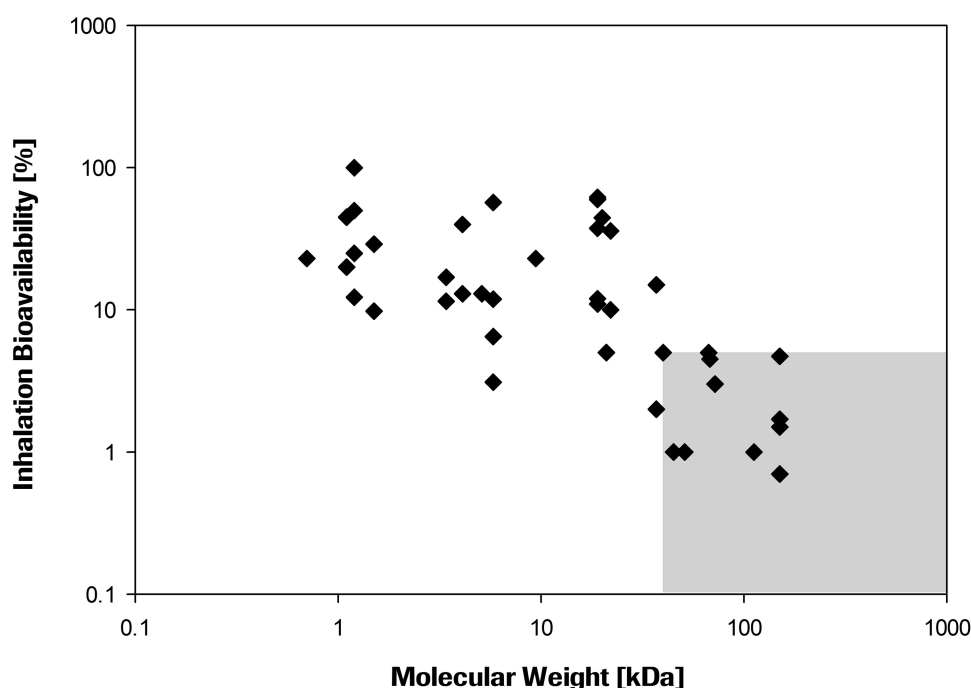
rate of lipophobic solutes including proteins from the lung is inversely correlated with the MW (Effros and Mason, 1983). This suggested that an estimate of the inhalation bioavailability based on MW may be a reasonable approach. Therefore an analysis of literature data was conducted of the relationship between the inhalation bioavailability of proteins under similar exposure conditions (e.g. aerosolized instillation), and MW (Fig. 1), to derive a conservative estimate of systemic bioavailability for OEL calculations.

ANALYSIS OF PUBLISHED DATA

In 2007 and 2008, a consortium of pharmaceutical industry occupational toxicologists sought to identify any driving or predictive factors for the assessment of inhalation bioavailability of proteins. A team

of scientists from Amgen, BMS, Eli Lilly, Novartis, Roche, and other experts performed a comprehensive literature search on this topic, collecting well over 100 articles and carefully reviewing each in an attempt to extract the following parameters:

- MW
- Number of amino acids
- Xlog P/Log P
- Hydrophilicity/lipophilicity
- pKa
- Isoelectric point (pI)
- Particle size
- Exposure details
- Estimated lung deposition
- Systemic/local effects



1 Relationship between molecular weight and inhalation bioavailability. The graph includes published data listed in Table 1. Grey rectangle denotes the area of substances ≥ 40 kDa and assumed inhalation bioavailability $\leq 5\%$. Note that some of the points represent upper limits, rather than measured values.

A total of 77 articles met the quality criteria for review, representing a total of 310 individual experiments on 86 unique proteins. For each substance, the parameters of interest, as far as available, were collected. Various statistical methods were used in an attempt to develop a robust model for fitting the observed inhalation bioavailability data. Details on the methods applied are not presented here. However, due to the limited number of parameters captured consistently in these experiments, none of the statistical models, other than the one using MW alone, was found to be a useful predictor for inhalation bioavailability.

As a follow-up activity of this previous work, the inhalation bioavailability data (from studies with aerosol inhalation and intratracheal installation) already collected from literature and complemented by recent published data were sorted by MW, divided into three groups containing at least 11 values (group 1: MW < 10 kDa; group 2: $10 \text{ kDa} < \text{MW} < 40 \text{ kDa}$; group 3: MW $\geq 40 \text{ kDa}$) and analyzed using explorative descriptive statistics (shown as boxplot diagram). To reduce the bias due to heterogeneity in the experimental methods, and ensure the quality and consistency of

data entered into the analysis, the following evaluation criteria were applied to the experimental data:

- 1) Data were excluded if the calculation of the bioavailability values was not clearly described.
- 2) Extreme values were excluded if the publication provides good reason to assume special circumstances leading to the outlying results.
- 3) If a range of results was published for a protein with the same experimental setup, the mean value was used.
- 4) The values from different experimental animals were not merged.
- 5) If there was evidence or suspicion that the same data were referred to in different publications (e.g. an original publication and a review article), the data were included only once.
- 6) If identical or similar data were published by the same author, they were included only once.

- 7) If an inhalation bioavailability value was censored (i.e. reported as ' $> x$ ' or ' $< x$ ') the value x was used.
- 8) Inhalation bioavailability data from experimental disease models with inflammation or other lung lesions were disregarded.
- 9) If results with a formulated (solution) and an unformulated substance (dry powder) were published, the data from the unformulated substance experiment was used. Experiments with absorption enhancers were disregarded.
- 10) Results from intranasal administration were disregarded.
- 11) If a range of MWs exists for a substance, the MW stated in the publication was used (e.g. heparin). If not specified in the publication, the middle of the range was used (e.g. epoetin beta).

For example, rule 2 was applied to a few peptides (glucagon, somatostatin, vasoactive intestinal peptide) with MWs of 3–3.5 kDa and comparatively low inhalation bioavailability ($<1\%$) (Patton *et al.*, 1994). These were considered to be outliers as these peptides appear to be cleaved effectively by lung peptidases such as neutral endopeptidases (Wolff, 1998). Rule 8 was applied to inhalation data from animal disease models where the lung was damaged by pathogens or tumors. Such data were also excluded from the analysis since they are irrelevant to the occupational exposure situation, and pathologically altered lung tissue may result in increased pulmonary epithelial permeability (Folkesson *et al.*, 1991; Adjei and Gupta, 1994). The low-dose PKs results described by Lombry *et al.* (2004) for IgG are suspect. They used a total IgG enzyme-linked immunosorbent assay (ELISA) for measuring the plasma level excursion after administration of 5 and 50 μg IgG to rats. Given the facts, that the normal range of endogenous IgG plasma concentration in the rat are on the order of several mg ml^{-1} (orders of magnitude higher than the administered dose) and the precision of an ELISA is typically in the range of $\pm$ several percent (Salauze *et al.*, 1994; Alpha Diagnostic International, 2011), the changes in total IgG plasma concentration expected due to administration of 5 and 50 μg are well within the range of variability of the ELISA. Only the results of the high dose

of 0.5 mg appear to be plausible and were therefore used for the data analysis.

A total of 25 unique molecules were included in the final dataset, which provided 41 data points for the boxplot analysis using Microsoft Excel Version 14.0.6106.5005.

The published values may often represent the best results of a test series with optimization of the experimental conditions (e.g. administration technique, formulation, test species) to achieve maximum lung deposition. Moreover, the majority of the data are from animal experiments using intratracheal instillation or powder insufflation with forced deposition of substance in the lung which does not mimic human exposure *via* normal breathing. Therefore, it is reasonable to consider the inhalation bioavailability values achieved in these animal models as rather overpredictive of a human exposure occurring as inadvertent uptake of 'crude' substance by normal breathing.

In a complementary approach, PK modeling of simulated serum omalizumab concentration–time profiles following human subcutaneous dose administration were compared with existing human inhalation data. These results, shown as [Supplementary material](#) is available at *Annals of Occupational Hygiene* online, support the conclusion drawn from the regression analysis by an independent methodology that inhalation bioavailability of large therapeutic proteins was $<5\%$.

RESULTS

The proteins and values included in the descriptive analysis are listed in [Table 1](#). When presented graphically ([Fig. 1](#)), there is clear evidence of decreasing bioavailability with increasing MW. However, the low number of values in the high MW range along with high variability at the mid and lower MW range does not allow the application of predictive statistical analysis across all data with a meaningful level of confidence.

Dividing the data into a low MW group ($\text{MW} < 10 \text{ kDa}$), mid MW group ($10 \text{ kDa} < \text{MW} < 40 \text{ kDa}$), and high MW group ($\text{MW} \geq 40 \text{ kDa}$) shows to a great extent overlapping values between the low and mid MW group but distinctly lower and less variable values in the high MW group ([Table 2](#), [Fig. 2](#)). In this group, all experimental inhalation bioavailability values were $\leq 5\%$. Despite the limited number of values, it appears reasonable to assume that the inhaled bioavailability

Table 2. Descriptive statistics of inhalation bioavailability data

	Low MW group N = 19	Mid MW group N = 11	High MW group N = 11
Minimum	3.1	2.0	0.7
25th Percentile	11.9	10.0	1.0
Median	20.0	15.0	1.7
75th Percentile	40.0	44.5	4.7
Maximum	100.0	62.0	5.0

Descriptive statistics of inhalation bioavailability data (% values) from Table 1 divided into the following groups: Low MW group = MW < 10 kDa, mid MW group = 10 kDa < MW < 40 kDa, high MW group = MW ≥ 40 kDa. N = number of values per group.

for large proteins with MWs ≥ 40 kDa are likely in the range of ~5% or below.

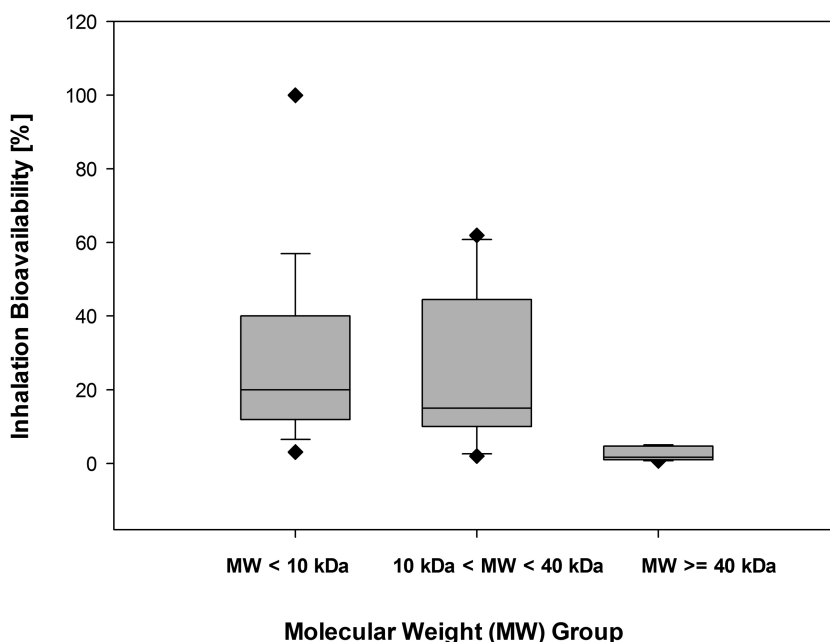
DISCUSSION

The purpose of this manuscript was to evaluate the known inhalation bioavailability for a range of proteins in order to make a general estimation of the inhalation bioavailability of proteins. A clear inverse linear relationship between molecule weight and inhalation bioavailability was observed for proteins. For MW ≥ 40 kDa all experimental inhalation bioavailability values were ≤5%, suggesting the low likelihood that an inhaled protein would cross into the systemic circulation to potentially cause pharmacological effects. Typically, parenteral (e.g. intravenous or subcutaneous) preclinical and clinical studies are available to derive the OEL for large proteins. Instead of using a default factor of 1.00 for 100% systemic absorption after inhalation, this PK parameter of 0.05 (5% for proteins with MW ≥ 40 kDa), results in a 20-fold higher OEL for those proteins. This is a reasonable approach and can even be considered a conservative default assumption for use in the derivation of OELs of large proteins based on the reasons cited above.

The best independent support for the credibility of this conclusion comes from a repeat dose study in humans with a 10-min nebulizer exposure to an IgG (i.e. E25 or omalizumab) (Fahy *et al.*, 1999; Sweeney *et al.*, 2002). This clinical study serves as a good model for occupational exposure to biological monoclonal antibodies as it: (i) was a human inhalation study of IgG; (ii) measured both deposited (bronchoalveolar lavage) and systemic (serum) levels; and (iii) the

dose was administered daily over 56 days. Only a small fraction of omalizumab was absorbed systemically compared to the subcutaneous route. Based on PK modeling we conducted to compare serum levels from both intravenous and inhalation administration, the resulting bioavailability from deposited omalizumab was 1.6–4.3%. In addition, ~15% of the administered dose was actually deposited in the alveoli, thus reducing the absolute bioavailability from inhalation to 0.2–0.6%. Thus, the 5% inhalation bioavailability assumption for large molecules is clearly confirmed and may even be considered as an overestimate for monoclonal antibodies such as IgG.

This bioavailability factor is additionally conservative in that it assumes sufficiently small particle size, mass median aerodynamic diameter, and small variability in the size distribution of particles, all of which influence deposition and clearance (and thus overall bioavailability) (Musante *et al.*, 2002; Kim and Malik 2003). The target particle size for the proteins in the dataset was in the low micron range, to optimize deep lung deposition and increase the potential for absorption. In the workplace, the particle size in situations where product may be accidentally released into the air is unknown, but it is unlikely to be a monodispersed aerosol optimized for the respirable particle size range like those generated in the laboratory experiments from which we abstracted and analyzed the data to derive the bioavailability factor for large proteins estimated in the present study. Based on simulations, realistic, polydispersed particle distributions resulted in reduced human exposure concentrations compared to estimates derived with the experimental



2 Boxplot analysis of inhalation bioavailability data by group. The bottom and top edges of the box indicate the intra-quartile range (IQR). The line inside the box indicates the median value. The whiskers that extend from each box indicate the range of values that are outside of the IQR, but are close enough not to be considered outliers (a distance less than or equal to 1.5 x IQR). Diamonds indicate the minimum and maximum values.

particle distribution used in laboratory animal studies (Jarabek *et al.*, 2005).

Finally, unlike most small molecule drugs, protein therapeutics are much more unstable if not stored under precise conditions, resulting in the loss of their complex spatial conformation and biological potency. Proteins like antibodies can degrade due to physical or chemical instabilities (Wang *et al.*, 2007). Physical instability can progress down two major pathways: denaturation and aggregation (*ibid*). Denaturation is the loss of secondary, tertiary, and/or quaternary structures of proteins, usually accompanied by a loss of biological function (Martinez, 2004). It can have result from protein exposure to high temperatures, shearing from mechanical agitation, detergents disrupting side-chain hydrophobic interactions, and pH extremes (Martinez, 2004; Wang *et al.*, 2007). Aggregation of proteins can occur from shaking, long-term storage, freeze-thaw process, lyophilization process (Wang *et al.*, 2007). Chemical instability can be initiated through a variety of conditions, and can occur through a wide variety of pathways, such as disulfide bond formation/exchange, nonreducible cross-linking, deamidation, isomerization, and oxidation (*ibid*). Droplets or spills

on surfaces would expose the drug product solution to air-liquid interfaces that result in large shear forces that can unfold proteins resulting in a loss of biological potency (Hawe *et al.*, 2012). Consequently, therapeutic proteins residues would not be expected to persist and pose a biological hazard to workers.

There were a few assumptions made to estimate the inhalation bioavailability factor. First, most studies were single short bolus inhalation or intratracheal instillation of protein, which is not so similar to the workplace where exposure is defaulted to an 8-h continuous exposure but is more likely a 1-h task in biologics manufacturing. Also, workers are generally healthy but their activity level may change exposure. Breathing patterns may change deposition and thus absorption. Deposition with breath holding compared to without breath holding approximately doubles when particles are $<5\ \mu$ (Imai *et al.*, 2012). High energy tasks that require rapid breathing may also increase bioavailability.

As with other substances, the 5% bioavailability for molecules > 40 kDa refers to the inhalable fraction of the protein. There are safety-relevant concerns for which the 5% bioavailability factor should not be

used for certain molecules with a MW $\geq$ 40 kDa such as antibody drug conjugates (proteins with highly toxic drug covalently bound), enzymes or other proteins with a special concern. This factor should only be used to evaluate and protect from systemic effects. If there is concern for direct effects on the lung (e.g. respiratory irritation or immunological response/sensitization) or receptor-mediated pharmacology or toxicology (e.g. endotoxin), then this PK factor should not be applied. For example, reductions in forced expiratory volume in 1 s and carbon monoxide diffusing capacity were observed during treatment with Exubera® and Afrezza® inhaled insulin that were consistent with a reversible, nonprogressive, and nonstructural, pathologic effect on lung function in adults with type 2 diabetes (Rosenstock *et al.*, 2009; Raskin *et al.*, 2012). There are numerous cases of occupational asthma from inhalation exposure during the industrial large scale use of enzymes, e.g., from the food, feed, detergent, and paper industry. Enzymes are considered to be potent respiratory sensitizers and have OELs in the ng m⁻³ range (A.I.S.E., 2002). However, there have been no reports in the literature of anaphylaxis or other direct pulmonary effects attributed to workplace inhalation exposure to therapeutic proteins. In addition, medical surveillance of employees working with protein therapeutics in a manufacturing setting by the authoring companies has not revealed any symptoms or documented cases of respiratory sensitization. Recently, some have extrapolated the hazard potential of parenterally administered monoclonal antibodies to an actual risk to healthcare employees based on the assumptions that 'intake by inhalation is significant' and that 'there is a risk that the respiratory tract will be sensitized' (Halsen and Krämer, 2011). Our findings refute the assumption that inhalation exposure to large protein therapeutics is significant.

Conclusion

Establishing OEL for monoclonal antibodies and other therapeutic proteins is generally hampered by the lack of reliable information on their bioavailability by the inhalation route. Data from animal inhalation studies, if available, are often difficult to translate quantitatively to humans due to highly variable results and the differences in methods (e.g. intratracheal instillation and use of absorption enhancers). Based

on critical evaluation and statistical analysis of published animal data, a default value of 5% inhalation bioavailability is proposed for proteins $\geq$ 40 kDa.

SUPPLEMENTARY DATA

Supplementary data can be found at <http://annhyg.oxfordjournals.org/>

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Supplementary Material for Bioavailability of Therapeutic Proteins by Inhalation - Worker Safety Aspects

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Determination of Absolute Systemic Bioavailability of Omalizumab Following Daily Inhalation - a Modeling Approach

INTRODUCTION

Absolute systemic bioavailability of monoclonal antibodies following inhalation is one of the important factors to calculate occupational exposure limits. However, this subject is not well studied and values are not readily available in the literatures. Fahy *et al* (1999) explored the inhalation route of delivery of a recombinant humanized monoclonal antibody (IgG), omalizumab (Xolair®) in asthmatic subjects using a nebulizer. The serum omalizumab concentrations were detectable over 8 weeks after daily inhalation exposure. However, neither relative nor absolute inhalation bioavailability of omalizumab can be derived from the existing data due to the lack of relevant serum omalizumab concentration data in humans after intravenous (IV) or subcutaneous (SC) dosing. In current study, we used a pharmacokinetic (PK) modeling approach to simulate serum omalizumab concentration-time profiles following SC dose administration. The inhalation bioavailability following lung deposition (referred herein as IT) was derived by comparison of the simulated subcutaneous data and existing inhalation data.

MATERIALS AND METHODS

Model Development

The pharmacokinetic software Phoenix Version 1.2 (Pharsight[®], A Certara Company[™], St. Louis, MO) was used for model fitting, parameter estimation and model simulation.

The omalizumab concentration vs. time data following single SC doses (75-375 mg) in patients were extracted from Hayashi *et al.* 2006 using Plot Digitizer (by J. A. Huwaldt). One- and two-compartment models with first-order absorption and first-order elimination were fitted to each data set. The best-fitting curve was selected based upon goodness-of-fit criteria, including Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC), an estimation

criterion value for the maximum likelihood method, coefficient variation of the estimates (CV%), visual inspection of the model fitting, and diagnostic plots. The absorption rate constant (K_a) was fixed at 0.5 day^{-1} based on previously published data for omalizumab (Hayashi *et al.*, 2007).

The best-fit PK model was verified with two sets of data: 1) the concentration vs time data following single doses of CMAB007 (a biosimilar of omalizumab) at 150 and 300 mg in healthy subjects (extracted from Zhou *et al.* 2012); 2) the concentration vs time data following multiple doses of omalizumab at 225-300 mg/2-4 weeks in patients (extracted from Hayashi *et al.* 2006).

IT Bioavailability

The serum omalizumab concentration vs. time profiles following daily SC doses at 0.15 and 1.5 mg (i.e., theoretical deposited daily doses in lung following daily inhalation at 1 and 10 mg as stated by Fahy *et al.*, 1999) for 8 weeks were simulated (Fahy *et al.*, 1999). The simulated concentrations were compared with the observed mean concentrations following daily inhalation at 1 and 10 mg in patients using nebulizers (data obtained from Fahy *et al.* 1999). The IT bioavailability ($F\%$) relative to IV injection was calculated by comparison to SC dosing and subsequent correction for the SC bioavailability (62 % relative to IV injection, Xolair package insert, 2010) as follows

$$F\% = \frac{\text{Serum Omalizumab Concentration (IT)}}{\text{Serum Omalizumab Concentration (SC)}} \times 100 \times 62\%$$

RESULTS AND DISCUSSION

The one-compartmental model with first-order absorption and first-order elimination was selected as the best-fit model to describe the pharmacokinetics of omalizumab (Figure 1). The model parameters obtained from the compartmental analysis are listed in Table 1 and were in excellent agreement with those from the noncompartmental analysis of Xolair (Genentech 2010) and CMAB007 (Zhou *et al.*, 2012). In addition, the model was verified with the external data (Figure 2) and the predicted values were close to the observed values.

This model was used to simulate serum omalizumab concentrations following daily SC dose administration and the predicted values are shown in Table 2. Following SC dose administration, the shapes of the predicted concentration vs time curves were similar to the observed concentrations following daily inhalation, thus the IT bioavailability at different time points was averaged to give a mean value for the respective dose (Table 2). The IT bioavailability relative to IV injection was 4.3% and 1.6%, respectively, following lung-deposited doses at 0.15 and 1.5 mg/day (theoretically calculated doses following inhalation at 1 and 10 mg/day as stated by Fahy *et al.*, 1999).

CONCLUSIONS

Overall, the one-compartment model with first-order absorption and first-order elimination provided reasonable description of the omalizumab concentration data after single and multiple SC dosing. Based on the simulated SC data, the inhalation bioavailability following lung deposition (ie, IT) of omalizumab relative to IV injection was < 5% in humans.

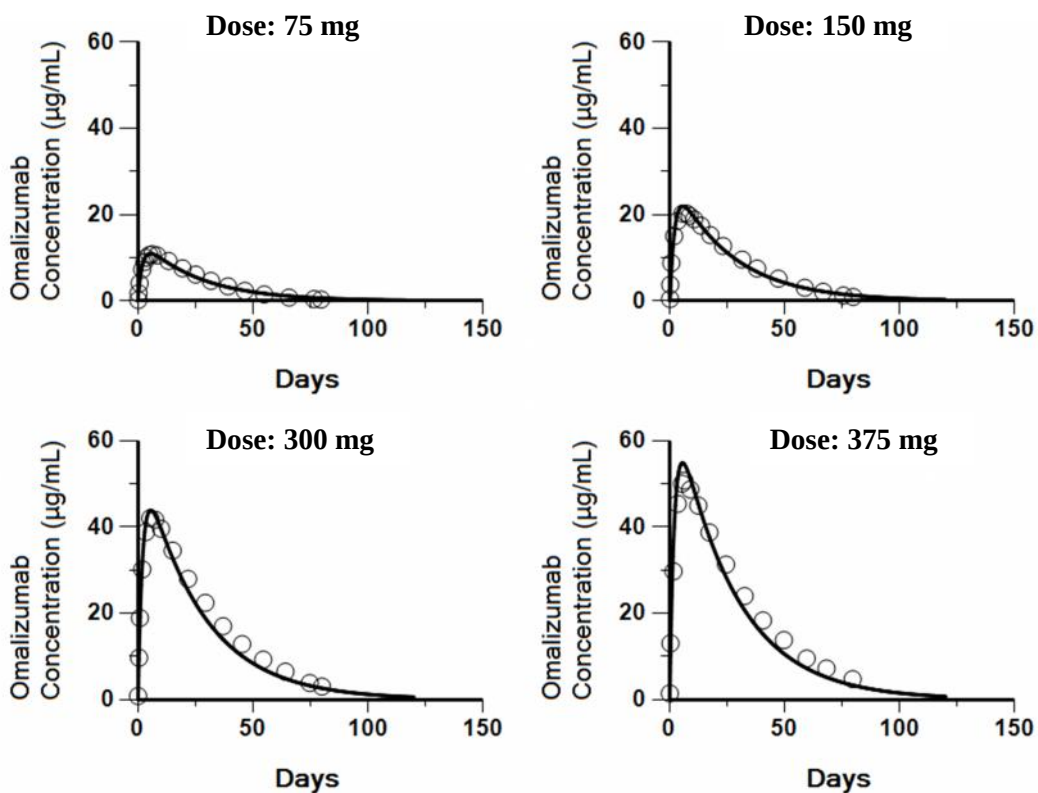
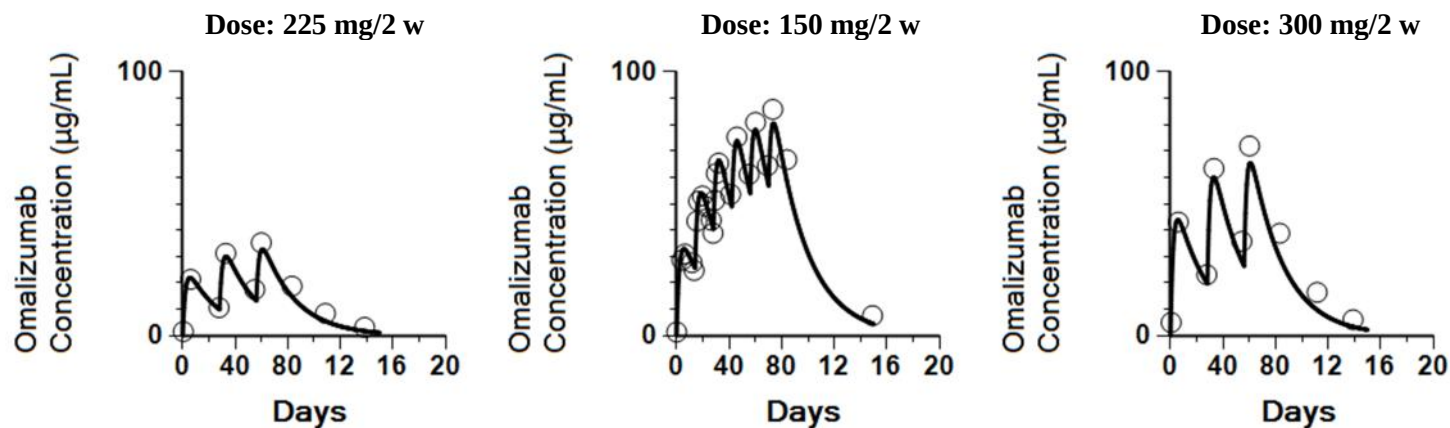


Figure 1

Mean serum omalizumab concentrations (open circles) after single SC dosing in patients (data obtained from Hayashi *et al.* 2006). The lines are the best-fit model simulation to the concentration vs. time data.

a)



b)

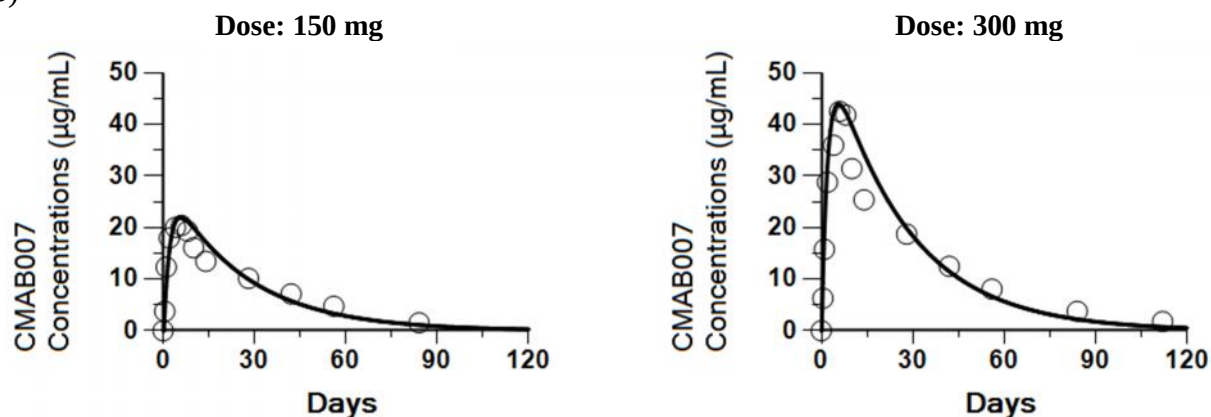


Figure 2

Mean serum omalizumab and CMAB007 concentrations (open circles) in healthy subjects after dosing with omalizumab or CMAB007 (data obtained from Hayashi *et al.* 2006 and Zhou *et al.*, 2012). The line is the model simulation based upon best-fit to single SC doses. The simulated data well predicted the observed omalizumab and CMAB007 concentrations following repeated and single doses, respectively.

Table 1

Final Pharmacokinetic Parameter Values Obtained after Fitting a 1-Compartment Pharmacokinetic Model to Data Obtained after SC Dosing of Omalizumab

Parameter	Description	Estimate	SD ^a	CV% ^a
V/F	Apparent Volume of Central Compartment (mL/kg)	78.7	4.24	5.39
CL/F	Apparent Clearance (mL/day/kg)	3.1	0.12	3.98
Ka	Absorption Rate Constant (1/day)	0.5	NA	NA

^aSD and CV% values are estimates of parameter errors that were based on the model fitting of the data. These values do not reflect the inter-individual variability.

NA: not applicable.

Table 2

Comparison of Serum Omalizumab Concentrations after Daily Inhalation and Subcutaneous Dosing

Dose (mg)	Day	Serum Omalizumab Concentration (µg/mL)		Absolute Inhalation Bioavailability ^c (%)
		IT ^a	SC ^b	
0.15	14	20	267	4.7
	28	23	447	3.2
	42	33	552	3.7
	56	37	612	3.7
	70	35	397	5.5
	83	20	239	5.2
Mean				4.3
SD				0.9
1.5	14	73	2668	1.7
	28	105	4474	1.4
	42	143	5518	1.6
	56	143	6122	1.4
	70	98	3974	1.6
	83	61	2391	1.6
Mean				1.6
SD				0.1

^aObserved data were obtained from Table 4 in Fahy *et al.* 1999.

^bThe data were simulated using a one-compartment model with first order absorption and first order elimination.

^cInhalation Bioavailability (%) = $\frac{\text{Serum Omalizumab Concentration (IT)}}{\text{Serum Omalizumab Concentration (SC)}} \times 100 \times 62\%$ (SC bioavailability)

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