Comparative in vitro evaluation of four corticosteroid metered dose inhalers

de Vries, Tjalling W; Rottier, Bart L; Gjaltema, Doetie; Hagedoorn, Paul; Frijlink, Henderik W; de Boer, Anne H

Published in:
Respiratory Medicine

DOI:
10.1016/j.rmed.2009.02.010

Publication date:
2009

Respiratory Medicine (2009) **103**, 1167–1173

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/rmed

Comparative *in vitro* evaluation of four corticosteroid metered dose inhalers: Consistency of delivered dose and particle size distribution

Tjalling W. de Vries [a,*], Bart L. Rottier [b], Doetie Gjaltema [c], Paul Hagedoorn [c], Henderik W. Frijlink [c], Anne H. de Boer [c]

[a] *Department of Paediatrics, Medical Centre Leeuwarden, P.O. Box 888, 8901 BR Leeuwarden, The Netherlands*
[b] *Beatrix Children's Hospital, Division of Paediatric Pulmonology and Allergology, University Hospital Groningen, University of Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands*
[c] *Department of Pharmaceutical Technology and Biopharmacy, University of Groningen, Ant. Deusinglaan 1, 9713 AV Groningen, The Netherlands*

Received 27 September 2008; accepted 14 February 2009
Available online 9 March 2009

KEYWORDS
Inhaled corticosteroid;
Pressurized metered dose inhaler;
Particle size distribution;
Laser diffraction;
Delivered dose

Summary
Introduction: Recent developments concerning pressurized metered dose inhalers (pMDIs) with inhaled corticosteroids (ICS) are the introduction of ciclesonide and the replacement of propellants. As the results of in vivo studies depend on pMDIperformance, it is necessary to evaluate pMDIs *in vitro* for delivered dose and particle size distributions under different conditions.
Methods: Fluticasone 125 μg, budesonide 200 μg, beclomethasone HFA100 μg, and ciclesonide 160 μg were compared for delivered dose and particle size using laser diffraction analysis with inspiratory flow rates of 10, 20 and 30 l/s.
Results: The volume median diameter of budesonide was 3.5 μm, fluticasone 2.8 μm, beclomethasone and ciclesonide both 1.9 μm. The mouthpiece retention was up to 30% of the nominal dose for beclomethasone and ciclesonide, 11–19% for the other pMDIs. Lifespan, flow rate, and air humidity had no significant influence on particle size distribution. The delivered dose of beclomethasone, budesonide, and ciclesonide remained constant over the lifespan. The delivered dose of fluticasone 125 decreased from 106% to 63%; fluticasone 250 also decreased whereas fluticasone 50 remained constant.
Conclusions: There is a significant difference in median particle size distribution between the different ICS pMDIs. Air humidity and inspiratory flow rate have no significant influence on

Abbreviations: pMDI, pressurized metered dose inhaler; ICS, inhaled corticosteroid; PSD, particle size distribution; CFC, chlorofluorocarbon; HFA, hydrofluoroalkane; LDA, laser diffraction analysis, FP, fluticasoneproprionate, BUD, budesonide; BDP, beclomethasone dipropionate HFA, CIC, ciclesonide; FPF, fine particle fraction; MMAD, mass median aerodynamic diameter; VMD, volume median diameter.

* Corresponding author. Tel.: +31 58 2863385; fax: +31 58 2863390.
E-mail address: tjalling.de.vries@znb.nl (T.W. de Vries).

doi:10.1016/j.rmed.2009.02.010

particle size distribution. Ciclesonide 160 and beclomethasone 100 deliver the largest fine particle fractions of 1.1—3.1 μm. The changes in delivered dose during the lifespan for the fluticasone 125 and 250 may have implications for patient care.

Introduction

For the treatment of asthma the inhalation of medication is preferred and its effectiveness is widely appreciated.[1,2] The preferred size of the inhaled particles for deposition in both central and peripheral airways is claimed to be 1—5 μm for adults,[3] and 1.1—3 μm for children.[4,5] The delivered dose as percentage of the label claim as well as the particle size distribution (PSD) within the delivered dose has major influence on the site and amount of drug deposited in the airways.

Recent developments include the switch from chlorofluorocarbon (CFC) to hydrofluoroalkane (HFA) containing formulations for environmental reasons. This switch resulted in dramatic changes in plume properties[6] and particle size distributions in the aerosol.[7] For instance, for beclomethasone dipropionate (BDP) the same serum levels from a CFC-MDI as from only half the dose with an HFA-MDI after inhalation have been reported.[8] As the results of in vivo studies are largely dependent on pMDI performance, a change in pMDI should be thoroughly investigated to interpret in vivo studies with caution.

Furthermore ciclesonide (CIC), a new inhaled corticosteroid, was introduced. Ciclesonide is a prodrug which is converted into the active metabolite in the airways. Its high protein binding allows its use only once daily. It has been formulated as an aerosol solution for a pMDI with a hydrofluoroalkane as propellant, and most of the particles produced are claimed to be between 1 and 5 μm.[9]

To the best of our knowledge, no studies have been published in which the consistency of delivered dose and physical properties of the aerosol of ciclesonide are compared with other aerosols. Information about delivered dose and PSD is essential for both *in vitro* studies and clinical trials.

This prompted us to compare the ciclesonide 160 μg/dose pMDI with three other widely used pMDIs containing ICS: fluticasone 125 μg/dose, budesonide 200 μg/dose, and beclomethasone 100 μg/dose on their fine particle doses (1—5 μm) and the influence of lifespan of the pMDI, flow rate, and relative air humidity on the aerosol properties.

Materials and methods

Pressurized metered dose inhalators were obtained from the local hospital pharmacy. The pMDIs tested were: fluticasone dipropionate 125 μg/dose (FP, Flixotide®, GlaxoSmithKline); budesonide 200 μg/dose (BUD, Pulmicort®, AstraZeneca); ciclesonide 160 μg/dose (CIC, Alvesco®, Nycomed); and beclomethasone dipropionate 100 μg/dose (BDP, Qvar®, Teva). The CIC and BDP pMDIs are CFC-free and contain clear drug solutions; FP contains a CFC-free suspension whereas BUD contains a suspension in a mixture of CFC propellants. Table 1 compares some properties of the pMDIs studied.

Measurement of delivered dose

pMDIs were connected to a filter system with Gelman glass filters A/E, P/N 61663; diameter 50 mm (Gelman Sciences, Ann Arbor, Michigan, USA). The filter system was supplied with a flow control unit and a solenoid valve with timer to set the flow rate to 20 l/min and suction time to 3 s. The suspension pMDIs were shaken for at least 10 s before doses were fired with the mouthpiece in a coupling flange with seal to prevent suction of false air and loss of aerosol. Time between firing of subsequent doses was at least 60 s to prevent excessive cooling of the pMDIs.

For three different devices (of the same batch) per type of inhaler, 15 individual doses were collected in the filter and analysed subsequently: five doses were taken from the beginning, five from the middle and five towards the end of the lifespan of the pMDI. The remaining doses in between were collected in groups of 5—40 and analysed together. The first ten doses of each new pMDI were wasted. Filter and mouthpiece retentions were dissolved in ethanol (analytical grade) and the drug solutions were analysed with a spectrophotometer (Unicam UV 500, ThermoSpectronic, UK) at 236 nm for fluticasone, 244 nm for budesonide, 243 nm for ciclesonide, and 239 nm for beclomethasone after it was checked with standard drug solutions added to filter depositions, so that the propellants in the drug formulations did not disturb the spectrophotometrical analysis. Checks have also been done on filter adsorption and the release of ethanol soluble components from the plastic mouthpiece parts.

Particle sizing in the aerosols

For measurement of the particle size distributions (PSDs) in the aerosols from the pMDIs we used a laser diffraction apparatus (Sympatec Helos BF Magic) with inhaler adapter (Inhaler 2000, Sympatec, Clausthal-Zellerfeld, Germany). The inhaler adapter has a flow controller and solenoid valve with timer to adjust to the desired inspiratory flow rate and suction time. The flow rate was measured with a venturimeter. Unless indicated otherwise, suction of the aerosol through the laser beam was at a constant flow rate of 10 l/min during 3 s. This was decided after it was checked with time sliced measurements that this time is sufficiently long enough to draw the entire aerosol from the pMDI through the laser beam. All measurements were started on an optical signal on detector channel 30 (for fine particles) of 0.2%. The relative humidity in the laboratory (at a room temperature of 20—22 °C) was varied between low (approx. 30%), median (approx. 55%) and high (approx. 75%) to investigate the effect of ambient conditions on the particle size distribution.

A 100 mm (R3) lens was used with a measuring range of 0.5—175 μm and calculations were made with the Fraunhofer theory after it was checked that no

Table 1 Some physical properties of the metered dose inhalers and actuators studied, measurements in millimetres.

Name	Shape of mouthpiece	Dimensions mouthpiece (mm)	Distance of nozzle to mouthpiece end (mm)	Propellants/solvents	Suspension solution
Fluticasone	Oval, flattened sides	15 × 10	21	Tetrafluorethane	Suspension
Budesonide	Oval	18 × 13	27	Trichlorofluormethane, dichlorodifluoromethane	Suspension
Beclomethasone	Circular	16 diameter	33	Norflurane/ethanol	Solution
Ciclesonide	Circular	16 diameter	33	Norflurane/ethanol	Solution

overestimation of fine particles occurred. Ghost peaks from propellant(s) were removed with forced stability. No sheath or counter flow through the adapter was added to the aerosol cloud and the distance from the exit of the mouthpiece to the laser beam was fixed to 50 mm after it was checked that this yields realistic size distributions. The pMDI was fired manually and the particle size distributions in the aerosol were measured for five doses taken from the beginning, five from the middle and five towards the end of the lifespan of the pMDI. Additionally, the effect of flow rate (10, 20, and 30 l/s) on the PSD was determined. Data per flow rate are the mean of 3 series of 5–10 doses in which each series is for a different pMDIs from the same lot. PSDs are presented as X_{10}-, X_{50}- and X_{90}-values derived from the cumulative volume distribution curves as a function of the particle diameter.

Statistics were performed using SSPS for Windows (version 13.0) and all tests were performed two sided, a $p < 0.05$ was considered statistically significant.

Results

Fig. 1 shows the mean metered dose (with maximum and minimum values obtained) as percent of the label claim from the three different devices of the same batch for all four pMDIs. In Fig. 1 the mean, maximum and minimum per device are for all doses, except for the first ten which were intentionally wasted. As expected there is a spread in metered dose for every individual pMDI. The variation is particularly extreme for at least two of the FP pMDIs, varying from 62% to 158%, respectively from 72% to 162% of the label claim for the devices 1 and 2, this is statistically significant. The mean metered dose is approximately 10% higher for CIC than for the other pMDIs, but the mouthpiece retention in this inhaler is higher too: 30% versus 11–19% for the other pMDIs. As a result, the delivered dose from CIC is more or less the same as that from the FP and BUD pMDI: on average 85% of the label claim. Only the BDP pMDI has a slightly lower delivered dose of 70% of the label claim.

Fig. 2a–d shows the consistency of the delivered doses for all devices within their lifespan. It can be seen that there is a small, not clinically significant spread between the delivered doses of BDP and CIC and that two BUD pMDIs exhibited a low output at the start after having already wasted the first 10 doses, one of them was statistically significant. The delivered dose of FP pMDI decreased statistically significantly with the number of doses taken to less than 70% of the output in the first actuations. Because we were surprised by this result we studied twelve additional FP 125 μg pMDIs taken from different lots, and also measured (in duplo) the delivered dose of FP 50 μg and 250 μg for comparison. Fig. 3 summarises the mean values

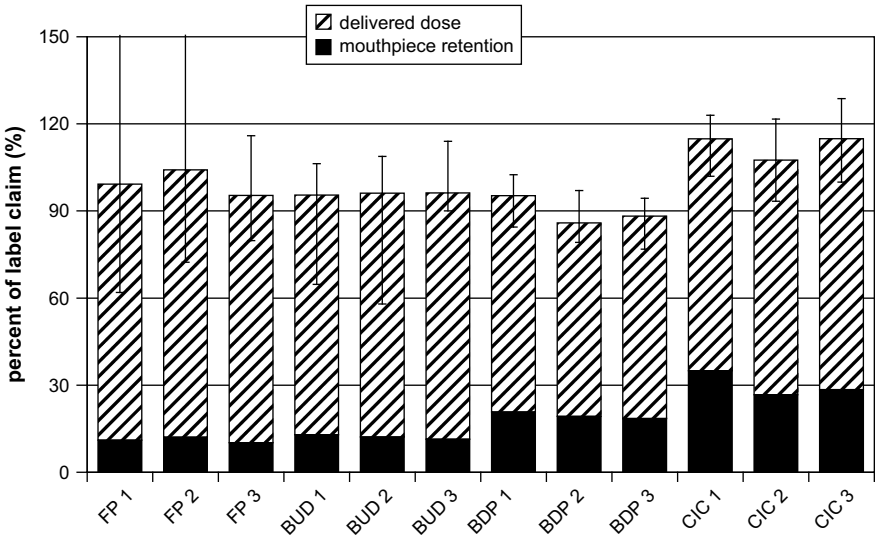

Figure 1 Mean metered dose (for all doses) as percent of label claim for three different devices of the same type taken from the same lot with spread bars indicating the maximum and minimum values obtained. FP = fluticasone 125 μg/dose; BUD = budesonide 200 μg/dose; BDP = beclomethasone 100 μg/dose; CIC = ciclesonide 160 μg/dose.

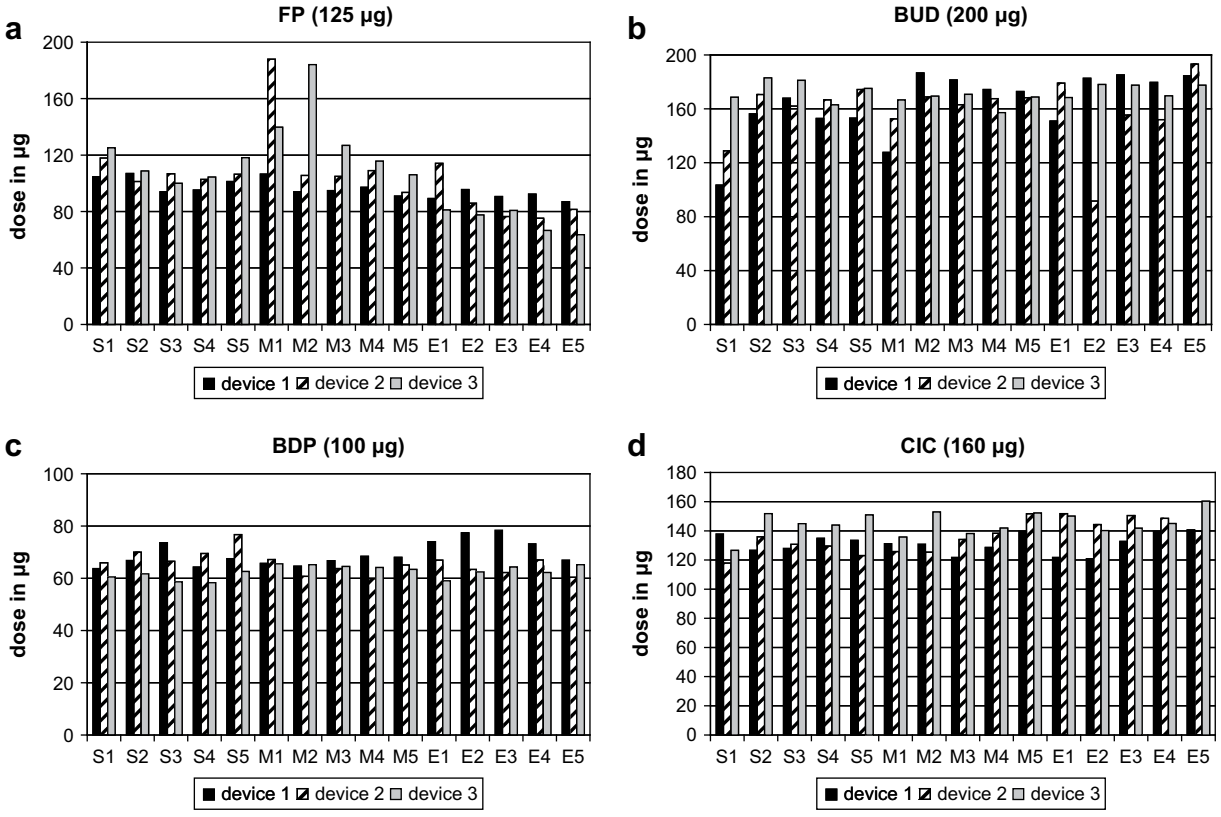

Figure 2 a—d): Delivered dose in µg at the start (S1—S5), the middle (M1—M5) and the end (E1—E5) of the lifespan of the same device. FP = fluticasone 125 µg/dose; BUD = budesonide 200 µg/dose; BDP = beclomethasone 100 µg/dose; CIC = ciclesonide 160 µg/dose.

with spread bars showing maximum and minimum doses obtained. It was found that the output of FP 50 remains constant. However, the delivered dose of FP 250 also decreased statistically significant with the number of doses taken from the device.

Characteristic values (X_{10}, X_{50} and X_{90}) from the cumulative volume distributions in the aerosol are presented in Fig. 4. The X_{50} of FP (3.5 µm) is significantly greater than all others, and the X_{50} of BUD (2.8 µm) is significantly greater than those of BDP and CIC (1.9 µm for both devices). Also the spread in droplet size is much smaller for BDP and CIC. FP 125 µg showed a great inter-device spread in size distribution; mean values for the median droplet diameter varied between 2.1 and 3.8 µm for individual devices from different batches, this is statistically significant. For BDP

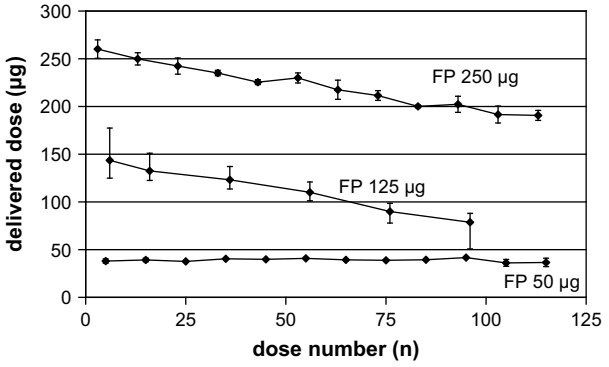

Figure 3 Changes in the delivered dose from fluticasone (FP) 50, 125 and 250 µg with the cumulative number of doses taken from the same device. Data points for FP 125 µg are the mean of 15 devices (from five different batches); those for FP 50 and 250 µg are the mean of two devices (from different batches). Spread bars indicate the maximum and minimum values obtained.

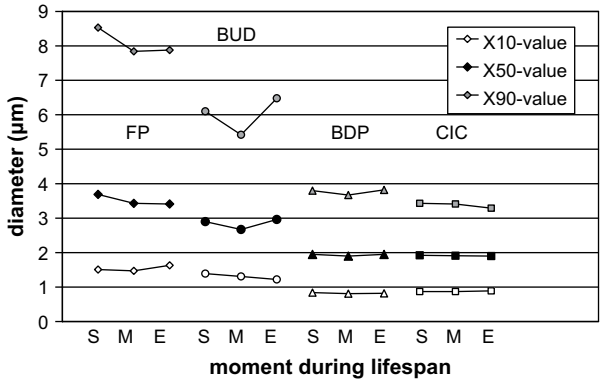

Figure 4 Particle size distribution of within lifespan of the pMDI for fluticasone (FP) 125 µg, budesonide (BUD) 200 µg, HFA beclomethasone (BDP) 100 µg, and ciclesonide (CIC) 160 µg at a suction of 10 l/min through the laser beam. S, M and E refer to doses taken at the start, middle and end of the lifespan respectively.

and CIC the inter-device variation with respect to PSD was negligible, whereas the X_{50}-value for BUD ranged from 2.7 to 3.0 μm which is statistically significant. The data in Fig. 4 are for one particular device producing a representative aerosol and each data point in Fig. 4 is the mean of five doses taken from the start (S), middle (M) or end (E) of the inhaler's lifespan. We also checked the size distributions in the aerosols from the FP 50 and 250 μg pMDIs and found that the FP 50 μg has a much smaller median diameter (X_{50} is 2.7 μm) than the FP 125 μg. In contrast, the FP 250 appeared to deliver significantly larger particles with a median diameter of 4.2 μm (data not shown).

Fig. 4 also shows that the PSDs of all four pMDIs remained rather constant within their lifespan. The relative air humidity within the range from 30 to 75% had no significant influence on particle size distribution. For FP and BUD the inter-device variations were significantly larger than for BDP and CIC.

The influence of the flow rate on the particle size distribution in the aerosol is shown in Fig. 5. This figure demonstrates the small effects of the flow rate on the PSD for all 4 tested devices within the range between 10 and 30 l/min. Due to differences in metered dose, mouthpiece retention and size distribution in the aerosol, different fine particle mass fractions (FPF $<$ 5 μm as percent of label claim) may be expected. This is shown in Fig. 6. All three pMDIs of the same type showed a very high consistency in mean delivered fine particle mass fraction, the fraction $<$ 5.0 μm being highest for the CIC pMDI. The aerosols from the FP and BUD pMDI contained only very few particles $<$1 μm but for the BDP and CIC pMDI nearly 15% of the delivered aerosol was in the particle range of $<$1 μm. There were also considerable differences for the fraction 3.1—5 μm, between FP and BUD on the one hand (respectively 29% and 24% of the label claim) and BDP and CIC on the other (approx. 10% for both). For the fraction 1.1—3.1 μm the CIC pMDI scored best (55%), followed by BDP (44%), BUD (42%) and FP (39%).

Discussion

To our knowledge, this is the first study to compare the *in vitro* performance of four different ICS pMDIs including FP, BUD, BDP, and CIC at three different flow rates and taking lifespan and ambient humidity conditions into account. Most

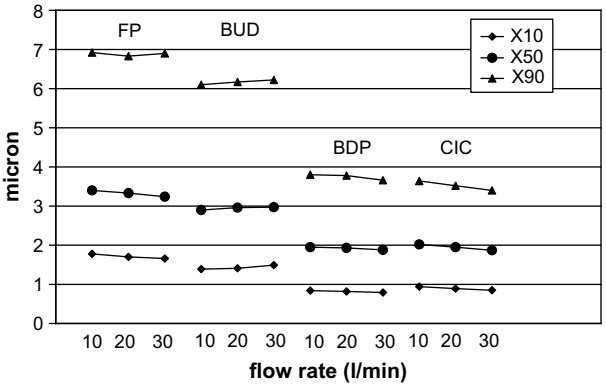

Figure 5 The particle size distribution in the aerosol as function of the flow rate. FP = fluticasone 125 μg/dose; BUD = budesonide 200 μg/dose; BDP = beclomethasone 100 μg/dose; CIC = ciclesonide 160 μg/dose.

previous studies dealt with single pMDIs.[10—15] Terzano studied the particle characteristics of FP, BDP, and fluniso-lide *in vitro* at two different flow rates (30 and 60 l/min).[16] Feddah compared the *in vitro* performance of three ICS DPIs with pMDIs containing the same drugs (FP, BUD and BDP) in an impactor at three different flow rates (30, 60 and 90 l/min) but their study did not include BDP and CIC.[17] Dalby, Barry and Stein also compared the performance of three different pMDIs, but their comparisons included other types of drugs, like salmeterol, salbutamol, and cromolyn sodium.[18—20]

Fig. 1 shows that none of the pMDIs tested delivered a mean dose corresponding with the label claim. The spread found in delivered dose was relatively high for FP and also significant for BUD. For the BUD pMDI this was the result of a random variation, showing a few extremes towards lower values varying between 52% and 79% of the label claim (Fig. 2b). However, for FP a gradual and significant decrease in delivered dose was obtained over the lifespan of the pMDI. This decrease appeared to be consistent for 15 devices taken from five different batches as shown in Fig. 3. On average (for all fifteen devices) the mean of the doses 11—20 from this pMDI was 106% of the label claim versus only 63% for the mean of the doses 92—100. This reduction is not only statistically significant, but also of clinical importance. We did not find an explanation for the inconsistencies found. However, these results point at the importance of knowing which doses from FP and BUD are used for *in vitro* studies as well as for clinical trials.

For all four types of pMDIs the delivered dose is lower than the metered dose due to some retention in the mouthpiece. As summarised in Table 1 mouthpieces have different shapes and dimensions; only those of CIC and BDP are the same. We found a 15% reduction for the mouthpiece retention of BDP and CIC by shortening the distance of the nozzle to the mouthpiece end to 21.5 mm. Shortening also appeared to influence the PSD in the aerosol. For BDP the X_{50}-value decreased from 1.95 to 1.79 μm; for CIC the decrease was from 2.02 to 1.8 μm. Mouthpiece shortening may also have consequences for the deposition in the mouth and valved holding chambers however. The net result of all these effects has to be studied.

Regarding the particle size distributions in the aerosols (Figs. 4—6) previous findings obtained with cascade impactor analysis are confirmed.[10,17,19,20] BDP and CIC pMDIs deliver much finer particles than FP and BUD pMDIs. The data in Fig. 6 suggest that BDP and CIC produce the highest mass fractions within the size rang of $<$3.1 μm and therefore are most appropriate for use in children because of their smaller airways. The relatively high fraction of particles $<$1.1 μm in the aerosols from the BDP and CIC is likely to be exhaled to a large extent again, unless a relatively long breathhold period is established.[21,22]

The PSDs for all four pMDIs do change statistically significant, but we do not think this is clinically relevant within their lifespan, nor when the flow rate or relative humidity is varied between 10 and 30 l/min, respectively 30 and 75%.

In contrast with most previous studies we used a laser diffraction technique for the characterisation of the particle size distribution in the aerosol. With the laser diffraction technique the size distribution of individual doses can be measured more reproducibly than with impactors, which suffer from variable losses in the valve, actuator, and USP throat.[20] Therefore, the laser diffraction

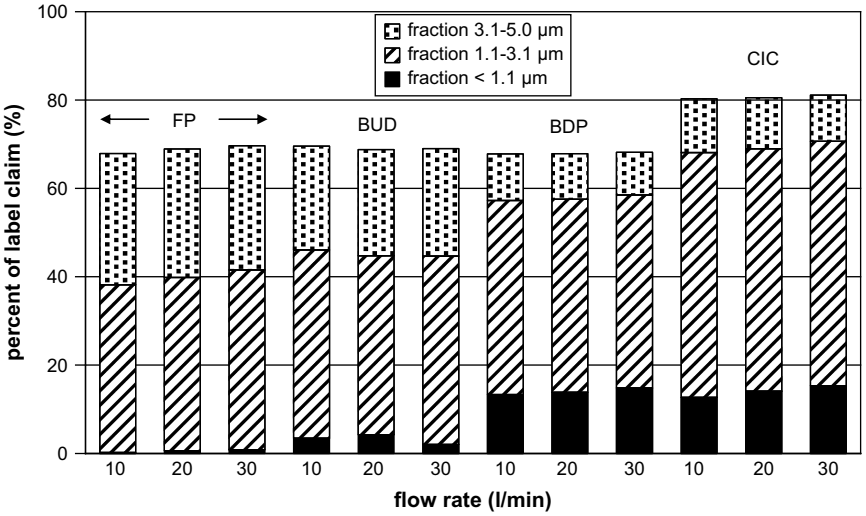

Figure 6 The size distribution within the fine particle fraction as percent of label claim based upon the mean delivered dose from the pMDI at 20 l/min. FP = fluticasone 125 μg/dose; BUD = budesonide 200 μg/dose; BDP = beclomethasone 100 μg/dose; CIC = ciclesonide 160 μg/dose.

technique enables a better assessment of the spread in the fine particle dose in the emitted aerosol. There is a discrepancy between previously published data from cascade impactor analysis and the laser diffraction data in this study for the same pMDIs. For instance, we found a volume median diameter of 1.9 μm for BDP (Fig. 4). Leach and Stein referred for BDP to an average particle size of approximately 1 μm; and from their cascade impactor data mass median aerodynamic diameters (MMADs) of 0.9—1.0 μm can be calculated.[7,20] The reason for the different results may be losses in the induction port. For BDP these losses are within the range from 20 to 30% of the label claim (at approx. 30 l/min). Partly as a result of that, cumulative fine particle fractions collected in the impactor were only between 40 and 50% of the label claim.[20] With the laser diffraction technique there are no losses in an induction port and the mean delivered dose for BDP in our study was more than 70%. Possibly classification occurs in the throat of an impactor, as a result of which the size distribution within the remaining aerosol fraction is changed. Moreover, the evaporation during longer travelling distance in cascade impactors is likely to result in an overestimation of the fine particles. There are more basic differences between cascade impactor and laser diffraction technique, but it is very unlikely that the differences in median diameters obtained with both techniques are the result of that.

Conclusions

In this study we show that the *in vitro* evaluation of ICS pMDIs under well-controlled conditions is mandatory for adequate evaluation of drug delivery studies in patients. We have demonstrated that the performance may vary over the lifespan of the pMDI and the fine particle fraction differs relevantly between different ICS. Design, such as the length of the mouthpiece, significantly influences dose delivery. We found that the delivered dose of FP 125 and 250 decreased significantly during its lifespan, and in some of the BUD pMDIs the first delivered doses were significantly lower. Although our data are consistent, they have to be confirmed by others and the clinical relevance should be investigated. The particles of CIC and BDP are the most appropriate for paediatric use. Our results partly confirmed earlier studies.

Before testing a combination of valved holding chambers and pMDIs, the performance of the pMDI itself should be known in relation to the environmental conditions and the inspiratory flow rate. Furthermore it is important to realise that there may be a significant inter-dose spread as well as inter-device variations regarding delivered dose and PSD. Particularly an inter-device spread makes it necessary that the same pMDI is used for comparison of data obtained with and without holding chamber. Knowledge of *in vitro* performance is essential to value important in vivo studies.

Acknowledgements

Nycomed kindly provided ciclesonide for analytical purposes. Dr. J. Collins reviewed the English.

Conflict of interest

The authors state that they do not have any competing interests.

References

1. GINA. Pocket Guide for asthma management and prevention in children. Available at URL, http://www.ginasthma.org; 2006.
2. British Thoracic Society, Scottish Intercollegiate Guidelines Network. British guideline on the management of asthma. *Thorax* 2008;**63**(Suppl. 4):1—121.
3. Esposito-Festen JE, Zanen P, Tiddens HAWM, Lammers JWJ. Pharmacokinetics of inhaled monodisperse beclomethasone as

a function of particle size. *Br J Clin Pharmacol* 2007;**64**: 328—34.
4. Lindstroem M. Particles in small airways: mechanisms for deposition and clearance. Thesis, Stockholm: Karolinska Institute; 2004.
5. Schuepp KG, Jauernig J, Janssens HM, Tiddens HAWM, Straub DA, Stangl D, et al. In vitro determination of the optimal particle size for nebulised aerosol delivery to infants. *J Aerosol Med* 2005;**18**:225—35.
6. Gabrio BJ, Stein SW, Velasquez DJ. A new method to evaluate plume characteristics of hydrofluoroalkane and chlorofluorocarbon metered dose inhalers. *Int J Pharm* 1999; **186**:3—12.
7. Leach CL. Improved delivery of inhaled steroids to the large and small airways. *Respir Med* 1998;**92**:3—8.
8. Seale JP, Harrison LI. Effect of changing the fine particle mass of inhaled beclomethasone dipropionate on intrapulmonary deposition and pharmacokinetics. *Respir Med* 1998;**92**:9—15.
9. Colice GL. The newly developed inhaled corticosteroid ciclesonide for the treatment of asthma. *Expert Opin Pharmacother* 2006;**7**:2107—17.
10. Leach CL, Davidson PJ, Boudreau RJ. Improved airway targeting with CFC-free HFA-beclomethasone metered-dose inhaler compared with FCF-beclomethasone. *Eur Respir J* 1998;**12**: 1346—53.
11. Asmus MJ, Liang J, Coowanitwong I, Vafadari R, Hochhaus G. In vitro deposition of fluticasone aerosol from a metered-dose inhaler with and without two common valved holding chambers. *Ann Allergy Asthma Immunol* 2002;**88**:204—8.
12. Asmus MJ, Coowanitwong I, Kwon SH, Khorsand N, Hochhaus G. In vitro performance of two common valved holding chambers with a chlorofluorocarbon-free beclomethasone metered-dose inhaler. *Pharmacotherapy* 2003;**23**:1538—44.
13. Asmus MJ, Liang J, Coowanitwong I, Hochhaus G. In vitro performance characteristics of valved holding chamber and spacer devices with a fluticasone metered-dose inhaler. *Pharmacotherapy* 2004;**24**:159—66.
14. Louca E, Leung K, Coates AL, Mitchell JP, Nagel MW. Comparison of three valved holding chambers for the delivery of fluticasone propionate-HFA to an infant face model. *J Aerosol Med* 2006;**19**:160—7.
15. Kamin W, Ehlich H. In vitro comparison of output and particle size distribution of budesonide from metered-dose inhaler with three spacer devices during pediatric tidal breathing. *Treat Respir Med* 2006;**5**:503—8.
16. Terzano C, Mannino F. Aerosol characterization of three corticosteroid metered dose inhalers with volumatic holding chambers and metered dose inhalers alone at two inspiratory flow rates. *J. Aerosol Med* 1999;**12**:249—54.
17. Feddah MR, Brown KF, Gipps EM, Davies NM. In-Vitro characterisation of metered dose inhaler versus dry powder inhaler glucocorticoid products: influence of inspiratory flow rates. *J Pharm Pharm Sci* 2000;**3**:317—24.
18. Dalby RN, Somaraju S, Chavan VS, Jarvis D. Evaluation of aerosol drug output from the OptiChamber and AeroChamber spacers in a model system. *J Asthma* 1998;**35**:173—7.
19. Barry PW, O'Callaghan C. A comparative analysis of the particle size output of beclomethasone dipropionate, salmeterol xinafoate and fluticasone dipropionate metered dose inhalers used with the Babyhaler, Volumatic and Aerochamber spacer. *Br J Clin Pharmacol* 1999;**47**:357—60.
20. Stein SW. Size distribution measurements of metered dose inhalers using Andersen Mark II cascade impactors. *Int J Pharm* 1999;**186**:43—52.
21. Gerrity TR. Pathophysiological and disease constraints on aerosol delivery. In: Byron PR, editor. *Respiratory drug delivery*. Boca Raton, USA: CRC Press Inc.; 1990. p. 1—38.
22. Roller CM, Zhang GI, Troedson RG, Leach CL, LeSoeuef PN, Devadason SG. Spacer inhalation technique and deposition of extrafine aerosol in asthmatic children. *Eur Respir J* 2007;**29**: 299—306.