Validity of *in Vitro* Tests on Aqueous Spray Pumps as Surrogates for Nasal Deposition

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Purpose. To determine whether deposition pattern is related to *in vitro* measurements of droplet size, plume geometry, and spray pattern between two different nasal spray pumps believed to have different performance characteristics.

Methods. Ten healthy volunteers inhaled radiolabeled saline from two different spray pumps (pump A and pump B). Deposition pattern was quantified from lateral views of the nose by gamma scintigraphy, expressed as the ratio of anterior to posterior (I:O) and superior to inferior (U:L) deposition. Droplet size was determined by Malvern Mastersizer S. Spray patterns were determined at 2.5 and 5 cm from the tip of the spray nozzle. Two-dimensional images of the emitted plume were captured by high-speed still photography.

Results. There were no significant differences in I:O or U:L ratios for pump A compared to pump B, indicating no significant differences in deposition pattern. The volume diameters, D_{v10} and D_{v50}, were not statistically different for pump A compared to pump B. There was a significant difference in D_{v90} between pump A and pump B, (86.9 ± 5.8 μm and 77.4 ± 2.4 μm, respectively; $P < 0.001$). The ratio of the longest to shortest diameter for the spray pattern with pump A was 1.26 ± 0.06 at 2.5 cm and 1.44 ± 0.08 at 5 cm. The ratio for pump B was 1.13 ± 0.03 at 2.5 cm and 1.19 ± 0.05 at 5 cm. Ratios at both heights were statistically different for pump A compared to pump B ($P < 0.00002$ and $P < 0.000001$, respectively) Plume geometry analysis indicated statistical differences in both the width (17.0 ± 0.97 vs. 18.5 ± 0.56 cm, respectively; $p<0.001$) and the maximum length of the plumes (46.0 ± 1.83 vs. 53.1 ± 4.88 cm, respectively; $p < .002$). The differences in velocity of the plume and spray angle between the two pumps were not statistically different.

Conclusions. Certain *in vitro* tests detected performance differences between the two pumps. However, these differences did not translate into different deposition patterns *in vivo*.

KEY WORDS: deposition pattern; droplet size; nasal spray; plume geometry; regulatory; spray pattern.

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ABBREVIATIONS: BE, bioequivalence; D_{v10}, volume diameter defining 10% of the cumulative volume undersize; D_{v50}, volume defining the median diameter of the cumulative volume undersize; D_{v90}, volume diameter defining 90% of the cumulative volume undersize; FDA, Food and Drug Administration; I:O, inner to outer ratio; L_L, longest chord; L_s, shortest chord; PD, pharmacodynamic; PK, pharmacokinetic; ^{99m}Tc, 99mTechnetium; U:L, upper to lower ratio

INTRODUCTION

According to a recently published US Food and Drug Administration (FDA) draft guidance, it is proposed that the bioavailability and bioequivalence (BE) of nasally administered, locally acting drug solutions may be determined solely using *in vitro* methodology (1). The decision to rely on *in vitro* tests as BE surrogates differs from the traditional BE determinates, which include pharmacokinetic (PK), pharmacodynamic (PD) and clinical studies. This deviation stems from several observations. First, because the dose that is administered nasally is often less than 1 mg, the development of sensitive bioanalytical methods can be a rate-limiting step. And, if it is possible to detect the drug in the bloodstream, there is no established correlation between the concentration of drug in the plasma and the concentration at the receptor site. Finally, it is difficult to define precise clinical endpoints that yield an accurate measure of an individual's response to treatment. Therefore, the rationale for *in vitro* tests is the assumption that, in many cases, *in vitro* studies are more sensitive indicators of the safety and efficacy of nasally administered drugs than clinical endpoints, PD or PK studies (1).

There are a number of *in vitro* tests that are discussed in the draft guidance, for example, emitted dose, priming, and tail off characteristics. In this study, we focused on the validity of *in vitro* tests that quantify the droplet size and shape of the spray plume as it evolves from the spray nozzle. Droplet size tests often involve laser diffraction analysis, and shape tests include spray pattern and plume geometry. To use these *in vitro* tests as BE surrogates, it is important to understand how they relate to *in vivo* performance of aqueous nasal spray products. Currently, there is no proven correlation between the *in vitro* tests proposed by the FDA and biological effect or clinical efficacy (2). It is also important to recognize that most of the proposed tests were initially developed for testing of pressurized metered dose inhaler products intended to achieve pulmonary delivery. Although documented associations between inhaled particle size and biologic effect abound (3–5), the relationship of plume shape to clinical efficacy is undocumented.

Only one study, to date, has investigated the relationship between nasal delivery systems and drug response. In that study, Harris *et al.* (6) demonstrated that the biologic response to a nasally administered drug is a function of where the droplets deposit in the nose, i.e., the deposition pattern. Differences in deposition pattern between the two types of delivery systems investigated, aqueous spray pumps and nasal drops, caused the drug-laden droplets to be removed from the nasal cavity by mucociliary clearance at different rates. The sprayed droplets, which were removed at a slower rate, resulted in greater drug absorption than the nasal drops. A correlation between extent of absorption and biologic response was also proven. These results suggest that changes in deposition pattern that result in changes in the rate of mucociliary drug clearance lead to differences in biologic response.

Based on the assumption that there is a relationship between deposition and drug response, this study was designed to determine whether the deposition pattern of sprayed droplets in the nasal cavity is related to *in vitro* measurements of droplet size, plume geometry, or spray pattern. The droplets were delivered from two different aqueous spray pumps.

Rather than making a comparison of pumps with vastly different *in vitro* properties, the study utilized pumps that might reasonably be selected by a generic manufacturer when copying an innovator's product. Thus, the *in vitro* differences between the two pumps should fall in the "gray" area where documenting equivalence is difficult to justify. This is a scenario that the FDA can expect to encounter.

MATERIALS AND METHODS

Two aqueous spray pumps, designated as pump A and pump B (reference numbers 8PH 4826 and 8PH 4832, respectively, Valois Pharm, Le Vaudreuil, France), were compared. Both pumps are commercially available and deliver 100 μl per actuation. The pumps differ in their mechanical operation. In pump A, liquid starts to be displaced through an exit orifice of a fixed size when sufficient finger pressure is applied to the liquid to displace the internal pump piston. In pump B, a minimum force must be applied to achieve sufficient hydraulic pressure in the liquid before the pump releases any spray.

In Vitro Tests

Spray Pattern

Screw-top polypropylene bottles fitted with either pump A or B were filled with isotonic saline solution containing 0.2% (w/v) dissolved FD&C Blue No. 2 dye. This concentration of dissolved solids is unlikely to influence the emitted droplet size. The pumps were actuated by an automated actuation station (InnovaSystems, Pennsauken, NJ) with an actuation force of 4.5 kg, a dose time of 16.0 ms, a hold time of 2.0 s, and a return time of 65 ms. The resultant spray was captured on a horizontal thin layer chromatography (TLC) plate located at 2.5 or 5 cm above the tip of the spray nozzle. The longest chord (L_L) and shortest chord (L_s) were measured across the spray pattern using a proprietary computer system that utilized optical scanning and image analysis (Valois of America, Greenwich, CT). The ratio of L_L to L_s was also calculated. This ratio, defined as the ovality ratio in the guidance, characterizes the general shape of each pattern. Three units of pump A and three units of pump B were tested. Each unit was tested in triplicate.

Plume Geometry

Images from pump A and pump B were captured in triplicate by high-speed still photography (Lazzaro Studio, Baltimore, MD). All images depict the plume from one plane.[1] Three units of each type of pump were tested. Saline filled pumps were fired by an automated actuation station using the settings described earlier. The emerging spray broke a laser trigger, which initiated frame capture. A calibrated grid, placed behind the spray pump, and a timer were photographed with the resultant spray to aid in making plume geometry measurements. Sequential photographs were taken approximately 0.16 seconds apart (roughly seven frames per second). Maximum plume length and width, along with spray angle were measured from the first photograph in each series. Spray angles were determined by using SigmaScan Pro (Jandel Scientific Software, San Rafael, CA) to delineate the conical shaped spray boundary based on optical density. Velocity of the plume front was calculated by the change in plume front distance per time between the first two to three sequential photographs.

Droplet Size

Emitted droplet size for each pump was determined using a Malvern Mastersizer S (Malvern Instruments, Malvern, UK). Each pump containing isotonic saline solution was fired using an automated actuation station with previously described settings. The spray tip to laser distance was 4.5 cm, whereas the distance between the spray tip and the dectector was 7 cm. The center of the spray tip was located directly below the laser beam and was actuated vertically. The laser passed through the center of the plume. Data collection began upon reaching a beam obscuration of 7% and continued for 100 ms (50 sweeps). The volume diameter of droplets defining 10%, 50% (volume median), and 90% of the cumulative volume undersize (D_{v10}, D_{v50}, and D_{v90}, respectively) was determined for each pump in triplicate. Three units of each type were tested.

In Vivo Tests

Deposition Study Design

A ZLC 370 gamma camera with a large field of view (Siemens Gammasonics, Inc., Des Plaines, IL), equipped with an all-purpose parallel-hole collimator, was used to quantify the regional nasal deposition of ^{99m}technetium (^{99m}Tc)-labeled saline droplets emitted from pump A and pump B in human volunteers. Volunteers also underwent a ^{133}xenon scan to determine the border of their nasal cavities. The nasal area encompassed by the xenon border was divided into smaller regions, which were superimposed on subsequent droplet images for regional deposition analyses. During inhalation of ^{133}xenon, upper regions of the lungs also came into view in the field of the camera. Identification of the upper lung regions made it possible to determine whether droplets penetrated beyond the nasal cavity into the lungs when volunteers inhaled from the spray pumps.

Study Population

Ten healthy volunteers, between the ages of 20 and 50 years, were recruited for this study. Exclusionary criteria included a history of allergic rhinitis, sinusitis, deviated nasal septum, nasal polyps, nasal surgery, and recent cold or influenza infections. Informed consent was obtained from each volunteer, and the research was conducted under the tenets of the Declaration of Helsinki. This study was supported by the Johns Hopkins University Institutional Review Board.

Administration of ^{99m}Tc-Labeled Aerosol

The order of aerosol administration from pump A and pump B was randomized. Dosing with either pump was sepa-

[1] The FDA draft guidance requests that data from the developing plume be collected from "two side views, at 90 degrees to each other". The authors made no attempt to orient the spray pumps in a specific direction such that all orientations could be randomly sampled. Any effects due to orientation should be reflected in the variability of the results.

rated by at least 1 week. Prior to dosing, each volunteer was trained in proper nasal spray inhalation technique as described on a commercial aqueous spray product (Beconase AQ®, Allen & Hansbury, Research Triangle Park, NC). Volunteers inhaled two sprays, one into each nostril, of buffered saline admixed with ^{99m}Tc complexed with DTPA (Syncor, Inc., Baltimore, MD). The average total dose of radioactivity was 20 μCi. The radiolabel was completely dissolved. The total solids concentration of the sprayed product was 1.6% (w/v). A side view of the nasal cavity was obtained immediately following inhalation. The image was acquired for 4 min.

Deposition Study Analyis

Nasal deposition was quantified in terms of radioactivity deposited in inner vs. otuer, and upper vs. lower regions of the nose, as shown in Fig. 1. During computer processing, the spray pump image was registered with the xenon scan. Counts per pixel were calculated in each of the regions.

Inner to outer ratios (I:O) and upper to lower ratios (U:L) were calcualted from the counts per pixel in each of the regions. A larger I:O ratio meant that more droplets deposited at the back of the nasal cavity, whereas a larger U:L ratio meant that a larger portion of droplets deposited in superior regions.

Statistical Analyses

In vitro tests measuring performance characteristics for pump A and pump B were compared using unpaired t-tests. The regional deposition pattern expressed as I:O and U:L ratios for pump A vs. pump B were compared by the Wilcoxon signed-rank test. P values < 0.05 were judged to represent statistical differences.

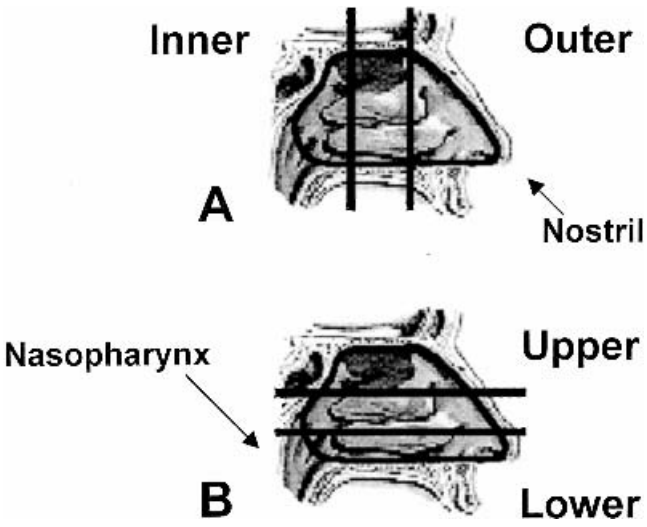

Fig. 1. Lateral view of the nasal cavity is shown illustrating the defined regions of interest. Inner and outer zones represent the anterior and posterior regions of the nasal cavity, respectively (A). The upper zone depicts the superior areas, which includes the olfactory region, and the lower zone denotes the floor of the nasal cavity and inferior turbinate (B). The shaded area represent the olfactory region.

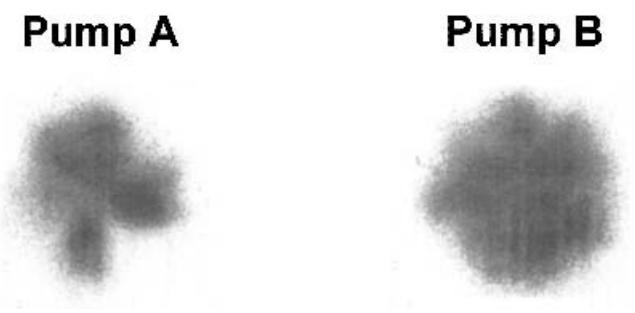

Fig. 2. Representative spray patterns from pump A and pump B, respectively. The distance from the rip of the spray nozzle to the TLC plate was 5 cm.

RESULTS

Spray Pattern

Upon examination of the spray patterns (Fig. 2), it is possible to visualize dissimilarities between pumps A and B. The results are quantified in Table 1 and indicate that the ovality ratios of the L_L and L_s were statistically different at spray tip-to-plate distances of both 2.5 and 5 cm. In addition, the minimum diameter (L_S) was statistically different at 5 cm. These results indicated quantitative differences in plume shape as measured by spray pattern analysis.

Plume Geometry

Images of an evolving plume from pump B captured by high-speed photography are shown in Fig. 3. All images depict plume propagation in one plane. Spray pump characteristics that were derived from these photographs are shown in Table 2. A comparison of plume width and length in the initial photograph indicated statistical differences between pump A and pump B. There were no statistical differences in spray angle or plume front velocity between the two pumps.

Droplet Size

The volume diameter defining 10% of the cumulative volume undersize (D_{v10}) and the volume median diameter (D_{v50}) were not statistically different for pumps A and B (Table 3). However, the D_{v90} values for the two pumps were statistically different. This difference could be due to the "fall back" of large droplets during data collection with the Mal-

Table I. Spray Pattern Data Comparing Pump A and Pump B at 2.5 and 5 cm from the Spray Nozzle[a]

	2.5 cm		5 cm	
Data	Pump A	Pump B	Pump A	Pump B
Ratio[b]	1.26 ± 0.06	1.13 ± 0.03	1.44 ± 0.08	1.19 ± 0.05
Minimum diameter (L_S)[c]	3.33 ± 0.19	3.60 ± 0.36	3.63 ± 0.13	4.42 ± 0.23
Maximum diameter (L_L)[c]	4.18 ± 0.22	4.06 ± 0.43	5.23 ± 0.30	5.28 ± 0.25

Results reported as mean ± SD.

[a] Ratios were statistically different at both 2.5 and 5 cm ($P < 0.00002$, $P < 0.000001$).

[b] Minimum and maximum diameters (cm) determined by imaging software program.

[c] Minimum diameter was statistically significant at 5 cm ($P < 0.0000001$).

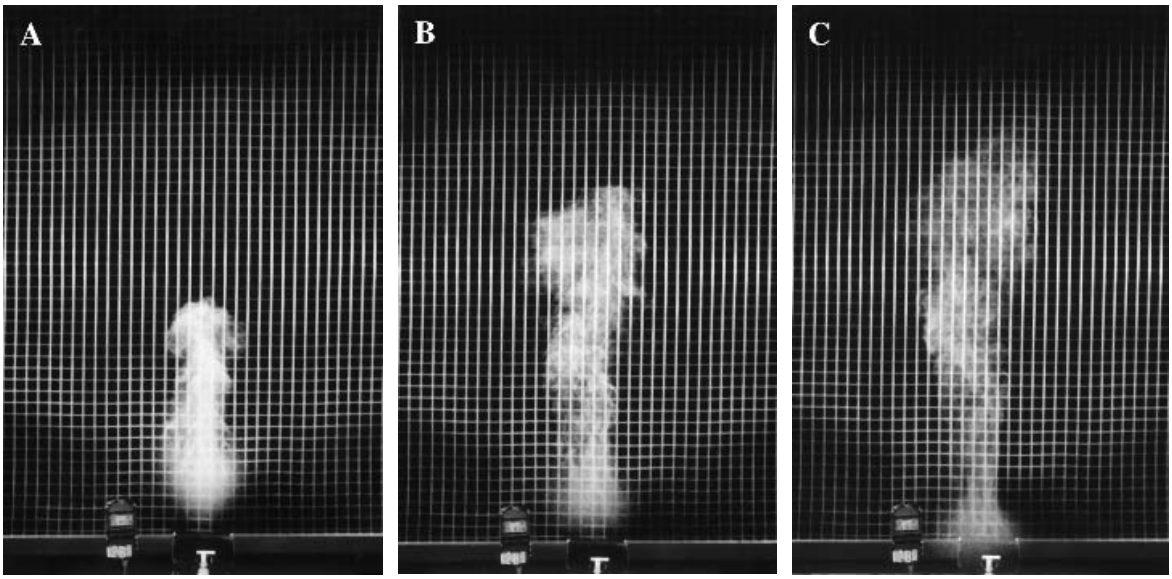

Fig. 3. High-speed photographs of an emitted plume from pump B, demonstrating plume evolution. The beginning (A), middle (B), and end (C) photographs are from the same series from a single spray. Measurements derived from the photographs were taken from the beginning photograph, defined as the first photograph in the series. Each square in the grid behind the spray represents 2.54 cm.

vern Mastersizer. Nasal spray pumps are routinely actuated in an upright position, and the droplets pass through the laser beam from below.

Deposition Study

There were no significant differences in I:O or U:L ratios for pump A compared to pump B (Table 4), indicating that there were no significant differences in deposition pattern for the two spray pumps.

Because the camera simultaneously acquired an image of the nasal cavity and the upper lung regions, droplet deposition ascertained by quantifying activity in those regions previously defined by the ^{133}xenon scan, would have been possible. In fact, no deposition was detected in the upper lung regions of volunteers with either spray pump.

DISCUSSION

Results from this study indicated that certain *in vitro* tests were more sensitive to differences in pump performance. For example, we were able to measure differences in plume shape by ovality ratio from spray pattern and by both the width and length of the plume as measured from high-speed photography. Additionally, we detected differences between pump A and B in the droplet size distribution of the cumulative D_{v90}. However, these differences between the pumps did not translate into differences in deposition pattern in the nose. If one were to apply the draft guidance to these *in vitro* test results, one should conclude that pump A and B are different. However, the deposition data suggests that these two pumps are indistinguishable in terms of where the spray deposits in the nasal cavity.

It is important to note that the two spray pumps tested in this study did not exhibit dramatic differences in pump performance *in vitro*. In other words, they were not two extremes in terms of droplet size and plume shape, but rather they are typical pumps marketed in the United States. Nevertheless, there were statistically significant *in vitro* differences, however small, between pumps A and B, which led to nonsignificant differences in deposition patterns.

Shape Tests

This study suggests that there is a lack of proven clinical relevance in certain *in vitro* tests. It is not surprising that certain shape tests are unable to predict deposition when the anatomy of the nose is considered. The overall length of the nasal cavity from the nostrils to the nasopharynx is approximately 12–14 cm (7). The lengths of the plumes that were measured by high-speed photography were five times the length of the nasal cavity. In addition, the nasal cavity is a

Table II. Plume Geometry Data Comparing Pump A and Pump B from the beginning Photograph[a]

Data	Pump A	Pump B
Width (cm)[b]	17.0 ± 0.97	18.5 ± 0.56
Length (cm)	46.0 ± 1.83	53.1 ± 4.88
Spray Angle (°)	62.0 ± 7.20	58.0 ± 3.81
Velocity (cm/s)	115 ± 33.4	153 ± 66.5

Results reported as mean ± SD.
[a] Plume width statistically different ($P < 0.001$).
[b] Plume length statistically different ($P < 0.002$).

Table III. Emitted Droplet Size Data for Pump A and Pump B[a]

Data	Pump A	Pump B
D_{v10}	21.53 ± 2.06	21.57 ± 1.71
D_{v50}	43.06 ± 2.59	41.17 ± 1.54
D_{v90}[1]	86.66 ± 5.83	77.44 ± 2.35

[a] Mean volume diameters reported as mean (μm) ± SD.
Statistically different ($p < .001$).

Table IV. Regional Analyses Comparing the Deposition Pattern of Droplets Administered by Pump A and Pump B

	I:O ratio		U:L ratio	
Volunteer	Pump A	Pump B	Pump A	Pump B
1	0.357	0.037	0.137	0.303
2	0.220	0.224	0.033	0.032
3	0.009	0.088	0.432	0.356
4	0.137	0.064	0.413	0.233
5	0.011	0.020	0.074	0.019
6	0.009	0.029	0.080	0.096
7	0.064	0.046	0.516	0.267
8	0.300	0.094	0.437	0.350
9	0.226	0.052	0.425	0.430
10	0.161	0.229	0.107	0.099
Mean	0.149	0.088	0.265	0.219
SD	0.126	0.077	0.193	0.147

Counts per pixel were determined for inner, outer, upper, and lower zones in the nasal cavity following each administration. I:O and U:L ratios were calculated.

very narrow passageway with a cross-sectional area of 0.3 cm^2 about 1.5 cm beyond the opening of the nostril (8). In contrast, the shortest cord measured from spray pattern tests at 2.5 cm from the tip of the pumps was 10 times greater than the width of the nasal cavity. Therefore, emitted plumes from spray pumps would never have the opportunity to freely develop in the nasal cavity as they would during spray pattern or plume geometry testing.

In addition, there are variables associated with spray pattern and plume geometry testing that can affect the results. Measurements made from the shape tests depend on how the edges of the spray pattern or plume photograph are defined. For example, the density of droplets in the plume photograph decreases with increasing width and distance from the spray nozzle until a point is reached where individual droplets can be seen and/or the "edges" blur into the background. This creates a dilemma—does the analyst measure the width of the plume across the densest portions or does he continue the measurement to include every droplet that can be visualized? Although imaging software can help eliminate some of the subjectivity, the analyst must still input parameters that determine where the edge begins and ends. Replicating results from analyst to analyst, or laboratory to laboratory, is subjective. Introducing this level of variability can have serious repercussions for a generic product manufacturer when attempting to prove BE with an innovator's product.

Because the shape tests, spray pattern, and plume geometry seem to have limited usage from a BE standpoint, is it really necessary to perform these two *in vitro* tests? Based on methods used to obtain the results from this study, spray pattern is the more robust measurement and is easier to interpret. Spray pattern is more likely to detect differences in plume shape and is, therefore, a more relevant *in vitro* test than plume geometry.

Size Tests

Particles inhaled into the nose primarily deposit by three mechanisms: inertial impaction, gravitational sedimentation, and Brownian diffusion (9–11). Because the nasal passageway narrows at approximately 1.5 cm into the airway, there is an acceleration of the inhaled airstream (Fig. 4) (13). In addition, a bend in the airway exists between the end of the nostrils and the entrance into the main nasal passage (13). The probability of impaction in a bent airway is:

$$\text{Impaction Probability} = Ud^2 \sin\theta/R \quad (1)$$

where θ is the angle of the bend, U is airstream velocity, d is particle aerodynamic diameter, and R is airway radius. Therefore, large and fast-moving droplets, such as those typically emitted from spray pumps, are likely to impact in the anterior regions of the nose. Similarities in droplet sizes found between pump A and B probably best explain why no differences in deposition were detected. In a previous study (14), we found significant differences in deposition pattern from two nasal delivery systems with extreme differences in droplet size. Therefore, based on Eq. 2 and our observations, evaluating the droplet size distribution emitted from nasal sprays is certainly a meaningful BE standard.

The results from the droplet-sizing portion of this study were time averaged rather than at single points in plume evolution. The draft guidance prefers the size distribution to be characterized at three intervals: as the plume begins to form, at an intermediate time, and as it begins to dissipate (1). Another study has evaluated droplet size by laser diffraction using the provisions of the guidance. The average droplet size over the life span of the plume was also determined. The results from that study indicated that the average droplet size was generally similar to the size measured at full plume evolution (intermediate time point), which suggests that there is little significance in evaluating droplet size at different times during the evolution of the plume (15). Based on this observation, time-averaged data are sufficient to characterize the droplet size distribution from an aqueous nasal spray pump.

Deposition Studies

This study found the spray from the two pumps to be statistically different in some aspects of size and shape, yet the mean values were not widely separated. Although pump A and B produced no differences in deposition pattern, at what point will dissimilarities in droplet size or plume shape pro-

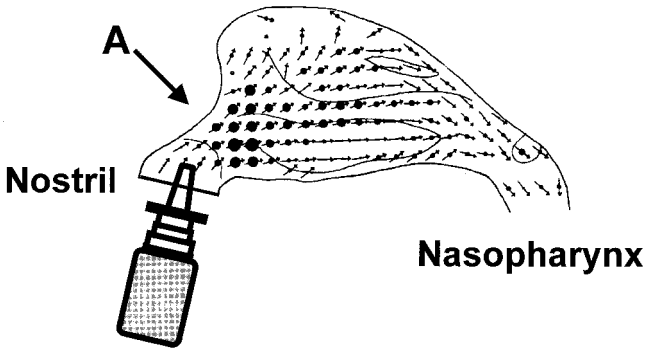

Fig. 4. Lateral schematic of the nasal cavity representing inspiratory nasal airflow derived from models. At approximately point A, the nasal cavity narrows to 0.3 cm^2. Also, at this point, there is a bend in the airway. This results in acceleration of the inhaled air and a change in direction. The arrows represent the direction of inspiratory airflow, whereas the size of each dot indicates the relative air velocity. A spray pump in the nostril shows the proximity of the nozzle to major nasal structures. Drawing adapted from Swift and Proctor (12).

duce differences *in vivo*? Based on what is known about nasal anatomy, it is unlikely that changes in spray angle will alter the distribution of droplets in the nose. However, one study, by Newman *et al.* (16), has investigated the intranasal distribution of a suspension containing ^{99m}Tc-radiolabeled Teflon particles from pumps with spray cone angles of 35° and 60°. In the 13 subjects tested, the results indicated a trend toward a greater area of deposition from the pump with the smaller spray angle. Although the study by Newman *et al.* (16) found no statistical differences in deposition, these results do reinforce the notion that tight specifications on shape tests may be unwarranted.

The effect of droplet size on deposition was investigated in our previous study (14) in which we measured deposition pattern in eight subjects who inhaled radiolabeled saline from a nasal nebulizer (D_{v50}, 6 μm) and a spray pump (D_{v50}, 79 μm). Significant differences in deposition were found between the two delivery systems, which highlights the fact that extremes in droplet size can have a major influence on deposition and are detectable by scintigraphy. Currently, there are no other studies that have investigated the effect of altering droplet size from aqueous spray pumps on deposition in the nose.

CONCLUSIONS

This study supports the contention that *in vitro* spray pattern tests are more sensitive to differences in performance between aqueous nasal spray pumps than deposition patterns determined in the human nasal cavity. In addition, we find no evidence that deposition pattern can be inferred from shape test results. Deposition pattern as measured via gamma scintigraphy has traditionally been used as a delivery system development tool. However, the technique can be utilized to elucidate the effect of varying droplet size and/or shape plume shape on deposition pattern. Because nasal deposition pattern alone has been correlated with biological effect, it is the most appropriate test of BE when PK, PD, and clinical studies are too unreliable or impractical. The authors appreciate the legal and regulatory issues associated with radiolabeling an innovator's product during a BE test but do not see this artificial hurdle as a suitable justification for popularizing an alternative *in vitro* approach that produces erroneous conclusions. Deposition pattern testing by scintigraphy should be accepted as an intermediate step toward the development of appropriate BE standards for nasal solutions.

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