

This article was downloaded by: [94.34.8.216]

On: 29 December 2011, At: 03:19

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Grana

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/sgra20>

Aerodynamic versus physical size of spores: Measurement and implication for respiratory deposition

T. Reponen, S. A. Grinshpun, K. L. Conwell, J. Wiest & M. Anderson

Available online: 05 Nov 2010

To cite this article: T. Reponen, S. A. Grinshpun, K. L. Conwell, J. Wiest & M. Anderson (2001): Aerodynamic versus physical size of spores: Measurement and implication for respiratory deposition, Grana, 40:3, 119-125

To link to this article: <http://dx.doi.org/10.1080/00173130152625851>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.tandfonline.com/page/terms-and-conditions>

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Aerodynamic versus physical size of spores: Measurement and implication for respiratory deposition

T. REPONEN, S. A. GRINSHPUN, K. L. CONWELL, J. WIEST and M. ANDERSON

Reponen, T., Grinshpun, S. A., Conwell, K. L., Wiest, J. & Anderson, A. 2001. Aerodynamic versus physical size of spores: Measurement and implication for respiratory deposition. – Grana 40: 119–125. ISSN 0017-3134.

Different methods available for size measurements of fungal and actinomycete spores were compared for four fungal species (*Penicillium brevicompactum*, *Penicillium melinii*, *Cladosporium cladosporioides*, and *Aspergillus versicolor*) and two actinomycete species (*Streptomyces albus* and *Thermoactinomyces vulgaris*). The physical size of spores was measured with three microscopic methods: with an optical microscope from stained (wet) slides, with an optical microscope from unstained (dry) slides and with an environmental scanning electron microscope (SEM) directly from the microbial culture. The aerodynamic diameter, d_a , of airborne spores was measured with an aerodynamic particle sizer. The respiratory deposition of spores was calculated with a computer-based model. The environmental SEM measurements indicated larger size for fungal spores than the optical microscope, whereas for actinomycete spores, both microscopes gave comparable results. Optical microscopic measurements showed that the stained fungal spores were 1.1–1.2 times larger than the unstained ones, which was attributed to the different hydration status of spores. There was no clear trend in the relationship between the d_a and the physical diameter measured with any of three tested microscopic methods. For example, the physical diameter of *Cladosporium cladosporioides* spores was larger than the d_a by a factor ranging from 2.0 to 2.2, whereas the d_a of *Streptomyces albus* spores was larger than the physical diameter by a factor of 1.3–1.5. Thus, the aerodynamic diameter of microbial spores cannot be accurately estimated solely based on the physical diameter but needs information on the density of the spores that may vary considerably. The results on the spore size were utilized to calculate respiratory deposition of spores. The errors in the size measurement were found to result in overestimation of the respiratory deposition of *C. cladosporioides* spores by a factor of 1.2–1.8, and underestimation of the respiratory deposition of *S. albus* spores by a factor of 0.6–0.7. These errors in the size measurement cause bias in the exposure assessment and in the estimation of the efficiency of control devices. More research is needed to standardize the method for particle diameter estimates applicable for airborne spores.

Tiina Reponen (Corresponding Author), Sergey A. Grinshpun, Kenneth L. Conwell, Jonathan Wiest & Marshall Anderson, Department of Environmental Health, University of Cincinnati, P.O. Box 670 056, Cincinnati, OH 45267-0056, USA. E-mail: Tiina.Reponen@uc.edu

(Manuscript received 24 July 2000; accepted 6 July 2001)

Fungal and actinomycete spores are ubiquitous contaminants in both outdoor and indoor environments. Information on the size of spores is needed when assessing respiratory exposure to airborne spores. Spore size also determines their behaviour and transport in the air. The performance characteristics of bioaerosol sampling instruments and air quality control devices are primarily dependent on the particle size of the spores to be sampled or eliminated from the air environment.

There are several definitions of particle size that depend on the size measurement technique. For example, the physical size of a particle refers to its width and length as measured under a microscope, and an optical diameter refers to the diameter measured with an optical particle counter. A commonly used term for non-spherical particles is an equivalent diameter that is a diameter of a sphere having the same value of specific physical property as the non-spherical particle being measured (Baron & Willeke 1993 a). The width and the length can be used to calculate a volume equivalent diameter (= physical diameter), which refers to the diameter

of a sphere that has the same volume as the particle in question. Microscopic counting and sizing, a common analysis method for airborne spores, has recently been facilitated by the development of image analysis systems (Singh et al. 1990, Kildesø & Nielsen 1997). Therefore, the information on physical sizes of fungal and actinomycete spores is available in the literature and is widely used, for example in identification of spores.

However, the aerodynamic equivalent diameter (d_a) is in most cases a more useful and relevant measure of spore size than the physical diameter as it takes into account the shape, surface structure and density of a particle. The aerodynamic diameter of a particle is the diameter of a unit-density sphere with the same settling velocity as the particle in question. The settling velocity here is the velocity of particles due to their gravitational settling. If the physical diameter, shape and density of a particle are known, the d_a can be calculated. It can also be measured with a cascade impactor or an aerodynamic particle sizer, such as APS (TSI, Inc., St. Paul, MN, USA) or Aerosizer (Amherst Process Instruments,

Inc., Hadley, MA, USA, now a division of TSI, Inc.). The above-mentioned aerodynamic particle sizers cannot distinguish biological particles from other particles, and thus, can be used primarily in laboratory-based studies that test spores aerosolized from pure cultures in the absence of other particles. In the future, direct measurement of the d_a in field studies may become possible if appropriate direct-reading techniques are developed to identify bioaerosols. One commercially available, but very expensive device, the Ultraviolet Aerodynamic Particle Size Spectrometer (UV-APS; TSI, INC) simultaneously measures the aerodynamic particle size, the light-scattering intensity, and the fluorescence intensity of the particles passing through the instrument. The device can distinguish if the particle is of biological origin, but cannot provide more detailed identification.

The relationship between the physical and aerodynamic diameter of airborne spores is not well known. Calculation of the d_a from the physical diameter may not be accurate, because of the lack of information on the densities of spores that are most common in indoor or outdoor air. However, it has been shown that neither the physical nor the aerodynamic diameter is constant but varies with the hydration status of the spore (Pasanen et al. 1991, Madelin & Johnson 1992, Reponen et al. 1996). If the air humidity is low, spores lose water, may decrease in physical size, partially collapse, change shape or develop internal gas bubbles (Lacey 1991, Madelin & Johnson 1992). Although the surface of many types of spores is known to be hydrophobic due to the protein-containing rodlet-layer (Cole et al. 1979), the dyes commonly used for staining spores for microscopic analysis can cause spores to inflate to their physical maximum within less than one minute (Beckett et al. 1984). When determining microorganism sizes under electron microscope dimensional changes have been shown to depend on the preparation techniques (King & Brown 1983, Montesinos et al. 1983, Beckett et al. 1984).

In this study, different methods for size measurements of fungal and actinomycete spores were compared. The effect of the variation in the size measurement data on the exposure assessment was demonstrated by calculating the respiratory deposition of spores of different sizes by a computer-based model (International Commission on Radiological Protection, ICRP 1994).

MATERIALS AND METHODS

Microbial species and their preparation for measurements

Four fungal species (*Penicillium brevicompactum*, *Penicillium melinii*, *Cladosporium cladosporioides*, and *Aspergillus versicolor*) and two actinomycete species (*Streptomyces albus* and *Thermoactinomyces vulgaris*) were selected for the experiments. As seen in Table I, the shape of these spores varied from spherical to ellipsoidal, the surface structure varied from smooth to echinulate and the volume equivalent diameter ranged from 0.9 to 5.5 μm .

All the fungal cultures were grown on malt agar (Difco Laboratories, Detroit, MI, USA) at 25°C for 7 days. *S. albus* was grown on ISP-medium-2 (ISP2=International Streptomyces Project agar 2; Difco Laboratories, Detroit, MI, USA) at 37°C for 7 days and *T. vulgaris* on trypticase soy agar (TSA; Becton Dickinson Microbiological System, Cockeysville, MD, USA) at 50°C for 3 weeks.

Microscopic size measurements

The physical size of spores was measured with three microscopic methods: an optical microscope from stained slides, an optical microscope from unstained slides and an environmental scanning electron microscope (SEM) directly from the microbial culture.

When preparing a slide for the optical microscope, a piece of fungal or actinomycete culture was taken from an agar plate with a sterilized knife and placed on a microscopic slide (Superfrost® /Plus, Fisher Scientific, Pittsburgh, PA, USA). To study the effect of slide preparation technique on the spore size, two types of slides were prepared: stained and unstained. Staining was done immediately after the samples were prepared by placing a drop of lactophenol cotton blue (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) on the slide. This water-based stain is commonly used when preparing microscopic slides for fungal identification (Samson et al. 1995). When preparing unstained slides, the samples on slides were first air-dried for one hour. After that, a drop of permount medium was added on each slide (Fisher Scientific, Pittsburgh, PA, USA). Permout medium consists of a synthetic resin dissolved in toluene and is commonly used in cyto- and histochemistry to create a permanent bond between the slide and the coverslip and to improve the optical quality of the slides (Keebler & Somrak 1993). Without any mounting medium, the spore images had a diffraction halo around them, which interfered with the size measurements. The stained slides are referred as "wet", and the unstained slides as "dry" later in the text.

All the optical microscope samples were digitally imaged by a color video camera (Sony DXC-760MD 3CCD, Sony Electronics, Inc., Tokyo, Japan) attached to the trinocular head of a phase-contrast microscope (Nikon Eclipse E800, Nikon Corp., Tokyo, Japan). The phase-contrast optical system consisted of a phase-contrast condenser (0.9 N.A.) and an oil immersion objective (CFI DL 100X, Plan Achromat, 1.25 N.A.). The video camera control unit was connected to a Matrox Millennium video board.

Environmental SEM analysis was done for spores of four microorganisms with an Electroscan E-3 equipped with an image acquisition and archiving system (Philips ElectroScan, Wilmington, MD, USA). A piece of agar, spores and mycelium growing on it, was cut out of the microbial culture and placed on the sample tray. During the imaging, the pressure in the Environmental SEM was 7–10 mm Hg and the magnification 5000–10000 X.

The digitally acquired image files from the optical microscope and the environmental SEM were analyzed by an Image-1/MetaMorph® imaging software system (Universal Imaging Corporation, West Chester, PA, USA) running on a Pentium® 166 MHz CPU platform. Interactive morphometric analysis of the samples by Image-1/MetaMorph® consisted of decoding the color image, configuring the decoded image "Lookup table" as a monochrome and then applying a threshold-based boundary detection algorithm. A calibration scale was then activated and the "Show Regional Statistics" tool was engaged to display the resulting morphological measurements. Prior to measuring the size of spores, the microscope and image analysis system was calibrated with standard polystyrene latex particles (PSL) of two sizes: 1.02 and 2.43 μm (Bangs Laboratories, Inc., Carmel, IN, USA).

When determining the physical size of PSL particles and spores, the width and length of at least 100 particles were measured per particle type. Because all the spores were considerably or slightly ellipsoidal, the volume equivalent diameter (d_{volume}) was calculated from the physical size as follows:

$$d_{\text{volume}} = \sqrt[3]{W^2 L} \quad (1)$$

where W is the width and L is the length of the spore. The average and standard deviation of d_{volume} was then calculated for each spore type. The aspect ratio of spores was calculated by dividing the length of the spores by their width (L/W). The following equation was

used to evaluate the effect of the shape and the density of spores on their aerodynamic diameter, d_a :

$$d_a = \sqrt{\frac{\rho_p}{\rho_o \chi}} d_{\text{volume}} \quad (2)$$

where ρ_p is the density of the particle, ρ_o is the unit density (1 g/cm^3) and χ is the dynamic shape factor. Dynamic shape factor is defined as the ratio of the drag force on a non-spherical particle to that on a sphere having the same volume and settling velocity as the non-spherical particle (Baron & Willeke 1993 b). For example, for an ellipsoidal particle having an aspect ratio of 2, the dynamic shape factor varies between 0.95 and 1.14 depending on the orientation of particles in the air flow (Fuchs 1964).

Aerosolization and measurement of the aerodynamic diameter of spores

The d_a of spores was determined in the experimental setup described in detail elsewhere (Reponen et al. 1996). Fungal and actinomycete spores were first aerosolized using our recently developed agar-tube and swirling-flow dispersers, respectively (Reponen et al. 1997). In these dispersers, dry spores are released directly from the microbial growth on agar simulating the dry release of fungal and actinomycete spores typically occurring in the nature. After aerosolization of the spores, the aerosol flow was adjusted to the relative humidity of the $30 \pm 5\%$ by mixing appropriate portions of desiccated and humidified air.

The effect of moisture conditions on spore size was studied in more detail with *Cladosporium cladosporioides*. These spores were aerosolized both as dry and wet spores, and their aerodynamic diameter after dry and wet dispersion, respectively, was measured at two relative humidities: 30% and 100%. Dry *C. cladosporioides* spores were aerosolized with the agar-tube disperser as described above. For the wet dispersion, the spores were harvested from the agar plates into sterilized water and the suspension was aerosolized with a Collison nebulizer as described e.g., by Lin et al. (2000). The aim of these additional experiments with *C. cladosporioides* was to explore how the intake of water inside the spores and condensation of water on the spore surface affects their aerodynamic diameter.

The size distributions of aerosolized spores were measured with an Aerosizer (API Mach II, Amherst Process Instruments, Inc., now TSI Inc.). The Aerosizer measures the concentration of particles and their health-related aerodynamic equivalent diameter. For each size distribution, at least 10 particles per size channel were measured and each size distribution measurement was repeated three times. To prevent the release of spores outside the experimental system, all components were housed inside a biosafety cabinet (Model 6TX; Baker Company, Inc., Sanford, ME, USA).

Geometric mean size and geometric standard deviation were calculated from the aerodynamic size distributions.

Respiratory deposition

The deposition of spores in the human respiratory tract was calculated for two species with distinctly different sizes: *Cladosporium cladosporioides* and *Streptomyces albus* (Table I). The calculations were performed by a computer-based model, LUDEP 1.0 (ICRP 1994) using the measured physical and aerodynamic diameters of particles and their standard deviations. The model calculates regional deposition of particles into five different separate regions of the airways: 1) anterior nasal passages, 2) naso- and oropharynx, and the larynx, 3) bronchi, 4) bronchioles, and 5) alveoli. The deposition efficiency of particles into each respiratory region is represented by two components: aerodynamic deposition due to impaction and gravitational settling, and thermodynamic deposition due to diffusion. The efficiency of aerodynamic deposition is an exponential function of d_a^2 and the efficiency of thermodynamic deposition is an inverse function of d_a . Other parameters that affect the deposition

are airway dimension, flow rate, and breathing habit (mouth or nose breathing). Detailed formulas for calculating deposition in each of the region are presented in ICRP (1994). As these calculations are intended to give an appreciation for the effect of different size measurement results on the changes in respiratory deposition, the deposition was calculated only for the case of an adult male. Four different mean respiratory flow rates were included in the calculations: $0.45 \text{ m}^3/\text{h}$ (sleeping), $0.54 \text{ m}^3/\text{h}$ (sitting), $1.5 \text{ m}^3/\text{h}$ (light exercise), and $3.0 \text{ m}^3/\text{h}$ (heavy exercise).

RESULTS AND DISCUSSION

Physical and aerodynamic diameter of spores

The results on the calibration of the optical microscope and the image analysis system with PSL particles are presented in Table II. The manufacturer has measured the size with a transmission electron microscope. The results show that the error in measuring PSL particles of known size is less than 3%. Thus, we are able to measure accurately the physical size of particles in the size range of about $1\text{--}2.5 \mu\text{m}$.

Table III presents the results on d_{volume} and aspect ratio of five different spore types using three microscopic techniques: an environmental SEM, an optical microscope with unstained spores (dry) and an optical microscope with stained spores (wet). *Penicillium melinii* has two d_{volume} values obtained from the environmental SEM measurements due to difficulties in measuring these spiny spores, see Fig. 1. With the high magnification of environmental SEM, the spines became more visible than what was possible to obtain with the optical microscope. The largest diameter was measured from the apex of one spine to the apex of another spine on the opposite side of the spore, and smallest diameter from a hollow between the spines to another hollow on the opposite side of the spore.

The results show that the environmental SEM measurements of fungal spores indicate larger size for fungal spores than the optical microscope measurements. For actinomycete spores, the environmental SEM and optical microscope gave comparable results. For fungal spores, the environmental SEM measurements are closer to the optical microscopic measurements of wet spores than those of dry spores. Optical microscopic measurements showed that stained fungal spores were larger than the unstained fungal spores by a factor of 1.1 to 1.2. This can be attributed to the water contained in the stain as described below.

The aspect ratios determined for most of the spores were close to unity as these spores are close to spherical. The highest aspect ratios were measured for *C. cladosporioides* and *S. albus* spores, which are ellipsoidal.

When observing the shape of the spores under the optical microscope, it was noted that unstained spores were partly collapsed, whereas stained spores were more spherical, see Fig. 1. Typically the difference in the width of stained versus unstained spores was higher than the difference in their length. This contributes to the slightly higher aspect ratios of dry spores than of wet spores, see Table III. The results indicate that the spores absorb water from the stain and thus expand to their maximum width. The shape and aspect ratio of spores observed under the environmental SEM was similar to those observed under the optical microscope from wet slides. This can be explained by the sample preparation

Table I. Physical characteristics of tested spores.

^aVolume equivalent diameter was calculated from the physical size; ^bSamson et al. 1995; ^cRamirez 1982; ^dLocci 1989; ^eMadelin and Johnson 1992; ^fLacey 1989.

Species	Shape	Surface structure	Physical size	Volume equivalent diameter ^a
Fungi				
<i>Penicillium brevicompactum</i>	Spherical or slightly ellipsoidal ^b	Smooth or slightly rough ^b	3–4.5 ^b	3.8 ± 0.8
<i>P. melinii</i>	Spherical ^c	Echinulate ^c	5–6 ^c	5.5 ± 0.5
<i>Cladosporium cladosporioides</i>	Ellipsoidal to lemon-shaped ^b	Smooth ^b	3–7 × 2–4 ^b	3.6 ± 1.3
<i>Aspergillus versicolor</i>	Spherical ^b	Echinulate ^b	2–3.5 ^b	2.8 ± 0.8
Actinomycetes				
<i>Streptomyces albus</i>	Slightly ellipsoidal	Smooth ^d	0.7–1 ^e	0.9 ± 0.2
<i>Thermoactinomyces vulgaris</i>	Spherical or slightly ellipsoidal ^f	Ridged ^f	0.5–1.5 ^f	1.0 ± 0.5

Table II. Calibration of the size measurements with an optical microscope and image analysis system with PSL (polystyrene latex) particles.

^ad_{volume} = volume equivalent diameter.

Size given by the manufacturer, μm		Size measured in this study, d _{volume} ^a , μm	
Average	Standard deviation	Average	Standard deviation
1.02	0.02	1.05	0.09
2.43	0.14	2.43	0.15

A. Stained (wet) B. Unstained (dry)

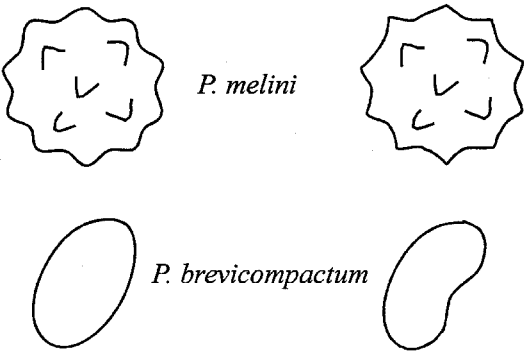


Fig. 1. The effect of staining on the shape of *P. melinii* and *P. brevicompactum* spores.

technique for environmental SEM: the spores were still attached to the mycelium and agar, having the source for moisture available. In contrast to traditional SEM, the environmental SEM does not require dehydration of the sample and is therefore expected to give information on spore size and shape on their natural state.

Table III presents also the aerodynamic diameters of spores. It shows that the aerodynamic diameters of *P. brevicompactum* and *S. albus* were larger than their respective physical sizes measured with any of the three microscopic methods. The situation was opposite for *C. cladosporioides* and *T. vulgaris*. The largest relative difference between physical and aerodynamic diameter was observed for *C. cladosporioides*. In this case, the physical diameter was larger than

the aerodynamic diameter by a factor ranging from 2.0 to 2.2. The second largest difference was observed for *S. albus*. In this case, the aerodynamic diameter was larger than the physical diameter by a factor of 1.3 to 1.5. It should be noted that these two spore types also had the highest aspect ratios. With *P. melinii* spores, the aerodynamic size is in between the results obtained with the three different microscopic methods. Thus, the results indicate that there is no clear trend in the relationship between the aerodynamic diameter and the physical diameter measured with any of three tested microscopic methods. The aerodynamic behaviour of particles depends on the shape and density of the particles in addition to their physical diameter. All of these parameters can vary depending on the moisture conditions of the spores during the measurements.

Table III also presents the standard deviations for the physical and aerodynamic sizes of spores. Thus, it is possible to compare the variation within the methods versus the variation between the methods. The largest standard deviation was observed for *C. cladosporioides* spores: 3.6 ± 0.7 μm (dry optical size measurement), 4.0 ± 0.8 μm (wet optical size measurement), and d_a = 1.8 μm with geometric standard deviation = 1.1 (aerodynamic size measurement). Based on these data, the size values ranged as follows: 2.9–4.3 μm (dry optical measurement), 3.2–4.8 μm (wet optical measurement), and 1.6–2.0 μm (aerodynamic measurement). Thus, even taking into account variation within the method, the physical size measurement gives larger particle size than the aerodynamic size measurement. This indicates that the between-methods variation was larger than the within-methods variation.

The effect of moisture conditions on the aerodynamic size of spores were explored in more detail with *C. cladosporium* spores, which had the largest discrepancy between the physical and aerodynamic diameter. These spores were aerosolized using both the dry and wet dispersion methods and the air humidity was adjusted either to 30% or 100% right after the dispersion. The spores had about 2 seconds to adjust to the air humidity before their aerodynamic size was measured. The results shown in Table IV indicate that the aerodynamic size of *C. cladosporium* released as dry spores increased by a factor of about 1.3 when the relative humidity was increased from 30% to 100%. This confirms the earlier reports on the hygroscopic increase in spore size, i.e., the tendency of spores

Table III. Comparison of equivalent volume diameters (μm) of spores measured with an environmental scanning electron microscope (SEM) and an optical microscope and aerodynamic diameters (μm) measured with an aerodynamic particle sizer.

^aArithmetic mean $\pm$ standard deviation of 100 measurements; ^blargest diameter; ^csmallest diameter; ^ddata not available.

Microbial species	Microscopic measurements of physical size						Aerodynamic diameter
	Environmental SEM		Optical microscope (dry)		Optical microscope (wet)		(geometric standard deviation)
	Diameter ^a	Aspect ratio	Diameter ^a	Aspect ratio	Diameter ^a	Aspect ratio	
Fungal spores							
<i>Penicillium brevicompactum</i>	2.1 ± 0.2	1.1	1.8 ± 0.2	1.3	2.2 ± 0.2	1.1	2.3 (1.1)
<i>Penicillium melinii</i>	3.4 ± 0.3^b	1.0	2.3 ± 0.2	1.1	2.7 ± 0.2	1.1	3.0 (1.1)
	3.0 ± 0.3^c	1.1					
<i>Cladosporium cladosporioides</i>	N/A ^d	N/A ^d	3.6 ± 0.7	1.9	4.0 ± 0.8	1.7	1.8 (1.1)
Actinomycete spores							
<i>Streptomyces albus</i>	0.6 ± 0.1	1.6	0.7 ± 0.1	1.1	N/A ^d	N/A ^d	0.9 (1.3)
<i>Thermoactinomyces vulgaris</i>	0.8 ± 0.2	1.1	0.7 ± 0.1	1.1	N/A ^d	N/A ^d	0.6 (1.5)

Table IV. The aerodynamic diameter of *C. cladosporioides* spores at two relative humidities (RH) of the air when dispersed as dry spores with an agar-tube disperser and as wet spores with Collison nebulizer.

Dispersion method	Aerodynamic diameter (geometric standard deviation), μm	
	RH=30%	RH=100%
Agar-tube disperser (dry)	1.8 (1.1)	2.3 (1.2)
Collison (wet)	2.0 (1.2)	2.4 (1.6)

to absorb water from the air and grow in their size (Pasanen et al. 1991, Madelin & Johnson 1992, Reponen et al. 1996).

Previous studies on the hygroscopic increase in spore size did not discuss if water from the air is condensed on the spore surface or absorbed inside the spore. To address this issue, we conducted an additional experiment by aerosolizing the spores from water with a wet disperser. We hypothesized that water would be absorbed inside the spores while the spores are in the suspension before the aerosolization. This would cause the spores to inflate to their maximum size. It was further postulated that the dry air (RH=30%) would then cause the evaporation of water from the spore surface but the 2 second residence time in the experimental system may not be enough to evaporate water that is inside the spores. The spore aerosolization with a wet disperser into the air with 100% relative humidity represents the extreme of having water both absorbed inside the spores and condensed on the spore surface.

When *C. cladosporioides* spores were aerosolized with the wet disperser into dry air, the aerodynamic size of spores was about larger (by a factor of about 1.1) than the corresponding particle size when the spores were aerosolized with a dry disperser. Thus, we observed 10% size increase due to absorption of water inside the spore. The d_a of *C. cladosporioides* spores measured immediately after placing them in the suspension and after they had stayed in the suspension for one hour was the same. This indicates that water was absorbed inside the spores immediately after

placing the spores in the suspension. When the spores were aerosolized with the wet disperser into humid air, the d_a was larger than with the wet dispersion into dry air by a factor of 1.2. Thus, the condensation of water on the spore surface caused 20% increase in the spore size. However, the d_a of spores that were aerosolized as dry spores into humid air was only slightly smaller than the d_a of spores that were aerosolized with the wet disperser into humid air. The results indicate that hygroscopic growth of *C. cladosporioides* is caused both by the condensation of water on the spore surface and by the absorption of water inside the spore.

Comparison of the d_a of wet and dry *C. cladosporium* spores with the physical diameters measured under a microscope (see Tables III and IV) shows that even the smallest physical diameter ($3.6 \mu\text{m}$) is still 1.5 times larger than the largest measured d_a of spores ($2.4 \mu\text{m}$) by a factor of 1.5. It becomes evident that the difference between the d_a and physical diameter cannot be explained by different hydration status of the spores. The ellipsoidal shape of *C. cladosporium* spores explains only a part of this difference. The aspect ratios of *C. cladosporium* spores were measured to be 1.9 with dry spores and 1.7 with wet spores. The dynamic shape factor of particles with an aspect ratio of 2 varies between 0.95 and 1.14 depending the orientation of particles in the air flow (Fuchs 1964). Thus, spores with a physical diameter of $3.6 \mu\text{m}$, an aspect ratio of 2, density of 1 g/cm^3 would have $d_a = 3.4\text{--}3.7 \mu\text{m}$ as calculated from Equation 2. This calculated d_a is much larger than the measured d_a that may be attributed to the density of spores if it is well below 1 g/cm^3 . This could be caused by empty spaces inside the spores that cannot be wetted even when the spores stay in the water for about an hour. Another possibility could be that the measurements with the aerodynamic particle sizer are not accurate for *C. cladosporium* spores due to their non-spherical shape or dark color.

Respiratory deposition of spores

Respiratory deposition was calculated for two spore types, which had the largest discrepancies between the physical and

Table V. Respiratory deposition of *Cladosporium cladosporioides* spores^a.

^aCalculations were conducted for an adult male; ^bmeasured with a phase-contrast microscope from stained samples (wet); ^c60% mouth breathing and 40% nose breathing.

Activity level and breathing type	Total Deposition, %		
	Calculated for d = 1.8 µm (aerodynamic)	Calculated for d = 4.0 µm (physical) ^b	$\left(\frac{Dep_{d=4.0}}{Dep_{d=1.8}}\right)$
Sleeping			
Nose breathing	55	88	1.6
Mouth breathing ^c	45	79	1.8
Sitting			
Nose breathing	60	91	1.5
Mouth breathing ^c	48	82	1.7
Light exercise			
Nose breathing	82	97	1.2
Mouth breathing ^c	46	84	1.8
Heavy exercise			
Nose breathing	61	92	1.5
Mouth breathing ^c	50	90	1.8

aerodynamic diameters: *C. cladosporium*, (aerodynamic size smaller than the physical size) and *S. albus* (aerodynamic size larger than the physical size). The results presented in Tables V and VI have been calculated using the two spore size values (aerodynamic and physical diameters). All the other factors affecting deposition have been kept constant. The deposition is expressed as a total deposition, i.e., sum of the fractional depositions in different parts of the airways. The calculations were done for four different activity levels (sleeping, sitting, light exercise, heavy exercise) and two breathing types (nose and mouth breathing). The last column in Tables V and VI presents the ratio of the total deposition calculated using the physical diameter to the one calculated using the aerodynamic diameter ($Dep_{physical}/Dep_{aerodynamic}$).

Table VI. Respiratory deposition of *Streptomyces albus* spores^a.

^aCalculations were conducted for an adult male; ^bmeasured with an environmental scanning electron microscope; ^c60% mouth breathing and 40% nose breathing.

Activity level and breathing type	Total Deposition, %		
	Calculated for d = 0.9 µm (aerodynamic)	Calculated for d = 0.6 µm (physical) ^b	$\left(\frac{Dep_{d=0.6}}{Dep_{d=0.9}}\right)$
Sleeping			
Nose breathing	24	15	0.6
Mouth breathing ^c	20	13	0.7
Sitting			
Nose breathing	27	16	0.6
Mouth breathing ^c	21	14	0.7
Light exercise			
Nose breathing	42	24	0.6
Mouth breathing ^c	19	12	0.6
Heavy exercise			
Nose breathing	28	16	0.6
Mouth breathing ^c	20	12	0.6

The total respiratory deposition of *C. cladosporium* spores varied from 45 to 82% when calculated from the aerodynamic diameter, and from 82 to 97% when calculated from the physical diameter. The ratio $Dep_{physical}/Dep_{aerodynamic}$ varied from 1.2 to 1.8. Thus, the respiratory deposition of *C. cladosporioides* is overestimated by a factor of 1.2 to 1.8 when calculated using the physical size instead of the aerodynamic size. The total respiratory deposition of *S. albus* varied from 19 to 42% when calculated from the aerodynamic diameter, and from 12 to 24% when calculated from the physical diameter. The ratio $Dep_{physical}/Dep_{aerodynamic}$ varied from 0.6 to 0.7. Thus, the respiratory deposition of *S. albus* is underestimated by a factor of 0.6–0.7 when calculated using the physical size instead of the aerodynamic size.

Tables V and VI also show that the total deposition is consistently higher for nose breathing than for mouth breathing. This is attributed to the deposition of particles in the nose and nasopharynx during nose breathing.

CONCLUSIONS

The results indicate that the aerodynamic diameter of microbial spores cannot accurately be estimated solely based on the physical diameter (without information on the density of the spores). Depending on the microbial species, the physical diameter may be either smaller or larger than the aerodynamic diameter. Both types of diameter measurements are affected by the humidity of the air and the moisture condition of their source. These kinds of errors in the size measurement cause errors in the exposure assessment as well as in the estimation of the efficiency of control devices. More research is needed to standardize the method for particle size determination applicable to airborne spores.

ACKNOWLEDGEMENTS

The environmental SEM imaging was performed by Dr. Sudeep M. Rao of Environmental Microscopy Laboratory in the University of New Mexico. We are thankful for his assistance.

REFERENCES

Baron, P. A. & Willeke, K. 1993 *a*. Aerosol fundamentals. – In: Aerosol measurement, principles, techniques and applications (ed. K. Willeke & P. A. Baron), pp. 8–22. – Van Nostrand Reinhold, New York.

Baron, P. A. & Willeke, K. 1993 *b*. Gas and particle motion. – In: Aerosol measurement, principles, techniques and applications (ed. K. Willeke & P. A. Baron), pp. 23–40. – Van Nostrand Reinhold, New York.

Beckett, A., Read, N. D. & Porter, R. 1984. Variations in fungal spore dimensions in relation to preparatory techniques for light microscopy and scanning electron microscopy. – *Journal of Microscopy* 136: 87–95.

Cole, G.T., Sekiya, T., Kasai, R., Yokoyama, T. & Nozawa, Y. 1979. Surface ultrastructure and chemical composition of the cell walls of conidial fungi. – *Experimental Mycology* 3: 132–156.

Fuchs, N. A. 1964. The mechanics of aerosols. – Pergamon Press, Oxford.

ICRP (International Commission on Radiological Protection). 1994. Human respiratory tract model for radiological protection. – *Annals of ICRP* 24, Numbers 1–3.

- Keebler, C. M. & Somrak, T. M. 1993. The manual of cytotechnology. 7th ed. – American Society of Clinical Pathologists, Chicago IL.
- Kildesø, J. & Nielsen, B. H. 1997. Exposure assessment of airborne microorganisms by fluorescence microscopy and image processing. – *Annals of Occupational Hygiene* 41: 201–216.
- King, E. J. & Brown, M. F. 1983. A technique preserving aerial fungal structures for scanning electron microscopy. – *Canadian Journal of Microbiology* 29: 653–658.
- Lacey, J. 1989. Thermoactinomycetes. – In: *Bergey's Manual of systematic bacteriology*, vol. 4 (ed. S. T. Williams, M. E. Sharpe & J. G. Holt), pp. 2573–2585. – William & Wilkins, Baltimore, MD.
- Lacey, J. 1991. Aggregation of spores and its effect on aerodynamic behavior. – *Grana* 30: 437–445.
- Lin, X., Reponen, T. A., Willeke, K., Wang, Z., Grinshpun, S. A. & Trunov, M. 2000. Survival of airborne microorganisms during swirling aerosol collection. – *Aerosol Science and Technology* 32: 184–196.
- Locci, R. 1989. Streptomyces and related genera. – In: *Bergey's manual of systematic bacteriology*, vol. 4 (ed. S. T. Williams, M. E. Sharpe & J. G. Holt), pp. 2451–2493. – William & Wilkins, Baltimore MD.
- Madelin, T. M. & Johnson, H. E. 1992. Fungal and actinomycete spore aerosols measured at different humidities with an aerodynamic particle sizer. – *Journal of Applied Bacteriology* 72: 400–409.
- Montesinos, E., Esteve, I. & Guerrero, R. 1983. Comparison between direct methods for determination of microbial cell volume: electron microscopy and electronic particle sizing. – *Applied and Environmental Microbiology* 45: 1651–1658.
- Pasanen, A.-L., Pasanen, P., Jantunen, M. J. & Kalliokoski, P. 1991. Significance of air humidity and air velocity for fungal spore release into the air. – *Atmospheric Environment* 25A: 459–462.
- Ramirez, C. 1982. Manual and atlas of the *Penicillia*, pp. 303–306. – Elsevier Biomedical Press, Amsterdam.
- Reponen, T., Willeke, K., Ulevicius, V., Reponen, A. & Grinshpun, S. 1996. Effect of relative humidity on aerodynamic size and respiratory deposition of fungal spores. – *Atmospheric Environment* 30: 3967–3974.
- Reponen, T., Willeke, K., Ulevicius, V., Grinshpun, S. A. & Donnelly, J. 1997. Techniques for dispersion of microorganisms into air. – *Aerosol Science and Technology* 27: 405–421.
- Samson, R. A., Hoekstra, E. S., Frisvald, J. C. & Filtenborg, O. 1995. Introduction to food-borne fungi. 4th ed. – Centraalbureau voor Schimmelcultures, Baarn-Delft.
- Singh, A., Yu, F. P. & McPeters, A. 1990. Rapid detection of chlorine-induced bacterial injury by the direct count method using image analysis. – *Applied and Environmental Microbiology* 56: 389–394.