# 86. PARTICLE SIZE AND ORGANISM NUMBER: IMPACT ON BIOAEROSOLS

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## INTRODUCTION

There exists a global concern regarding the potential for an attack with a biological weapon (BW) within the next several years. This is amply demonstrated by efforts to develop highly advanced detection instruments and warning systems, produce innovative clothing to protect against weapons of mass destruction (WMD), determine methods to mitigate possible effects, and provide effective consequence management. Obviously, it is most important to properly assess the threat of a biological weapon and to understand the potential impact to apply the best defensive and protective measures to thwart a BW attack.

Although there are several potential dissemination scenarios to describe an attack with a biological weapon, the greatest concern is directed at the potential release of a bioaerosol. Aerosol dissemination, stemming from the development of large military-affiliated offensive BW programs, has been recognized as the most efficient method for spreading a biological agent. The 1995 incident on the Tokyo subway by the Aum Shinrikyo in their use of aerosolized sarin, albeit crudely designed, demonstrated clearly that terrorists would now achieve enough technical sophistication to employ recognized methods with WMD use.

To defend against a bioaerosol attack, there is much to be gained in understanding and predicting the anticipated effects of an aerosolized agent on an exposed population. Such knowledge will provide greater insight into the design of a variety of materials and protocols under development to respond to such an attack. Detectors, rapid response teams, predictive dispersion codes, protective clothing, and decontamination equipment are just a few items in the growing arsenal to protect against a WMD attack. The widely recognized proliferation of biological weapons mandates, in particular, the necessity for protection against bioaerosols.

## PARTICLE SIZE EFFECTS

Particle size is likely the single most important element in the design of a bioaerosol. Humans are seriously affected by bioaerosols with a characteristic particle size range (i.e. mass median diameter or MMD) of one to five microns because these particles are most likely to reach and be retained in the deep tissues (i.e., the alveolar region) of the lungs during respiration. Bioaerosols with larger particle sizes (i.e., 5 to 20 microns MMD) are generally much less likely to cause serious infection because the upper respiratory tract easily clears such particles. Depending on the agent such large particles, however, might infect the trachea, pharynx, or eye. Figure 1 shows the essential characteristics of particle deposition – not retention – in the respiratory system. Aerosolized particles with a MMD up to 10 microns are deposited in the respiratory system, but only those particles with an approximate MMD of one to five microns reach the pulmonary spaces. Although deposition of particles up to 10 microns in diameter in the respiratory system is highly effective, removal of the larger particles in this range is also effective. The upper respiratory tract removes essentially 100% of the particles with diameters $\geq$10 microns, and this decreases to approximately 80% for particles with diameters of five microns. As the particle diameter is reduced to one or two microns, virtually none of the particles are removed from the respiratory tract (1)

## PARTICLE SIZE AND INFECTIVITY

The parameters for description of a bioaerosol include the particle size range or MMD and microbial infectivity described as the lethal dose ($LD_{50}$) or infective dose ($ID_{50}$) that causes a probable clinical response in 50% of the exposed, unprotected subjects. Based on the human respiratory response to aerosolized particles of a particular size range as described above, it is critical to assess the relationship between the MMD of aerosolized particles with the infectivity of the agent contained within the particles. Certain aspects of the relationship are not obvious. These might include atmospheric effects on the viability of aerosolized organisms as varying particle sizes, the effects of different MMDs on long-range dispersion and rates of settling of aerosols, and the potential for infection outside of the lung (i.e. the upper respiratory tract or eye). Although it is not possible to perform such an assessment for every microbial threat, defending against and effectively managing an attack imposes an obligation of understanding as to how to characterize the aerosolized agent.

Characterizing a particle aerosol using MMD dictates variation in absolute particle size. The range of particle sizes will provide a basis for susceptibility based on the human respiratory response. Ultimately, this will, of course, depend on both the viability and infectivity of the microbe, as well as the finite range of particle sizes. Knowing the stability and pathogenic character of an organism, the relation between particle size and infectivity is dependent in large part on the number of organisms within a given particle, a value difficult to determine.

It is important to note that exposure to a bioaerosol is often expressed as a function of mass within a given volume. Providing that the particle size is within a finite range and the infectivity of the agent is known, the units (i.e., mg • $min/m^3$) commonly employed for exposure are acceptable. However, as described above, particle size contributes significantly to the clinical response during exposure to a bioaerosol. Using the value of mass, alone, does define particle size and, subsequently, the likely distribution within the respiratory system.

Table 1 shows the number of small spheres of unit radius that will occupy the space of a large sphere. Figure 2 shows conceptually the organizational structure of many small spheres packed into a large sphere or particle. There are two possible packing arrangements, face-centered-cubic (FCC) and hexagonal-close-packed (HCP), depending on whether a unit sphere is placed at the center of the large sphere or is tangential to the center point of the large sphere. Fortunately, as it concerns this discussion, there is little difference between the two arrangements. As the size of the large sphere increases, filling fractions show a considerable increase reaching a maximum of more than 70%, and show little difference in the filling fraction between the FCC and HCP packing arrangements (see Table 1 and Figure 3). Although the radius sizes for the large sphere shown in Table 1 are well above those sizes of concern with respect to bioaerosols, it should be realized that these are idealized calculations. When attempting to apply these principles to microbes, organism size will vary within a finite range providing a significant difference in filling fraction. It should also be appreciated that the filling fraction will change depending on the relative size of the small particle and also if there are minor size and shape variations in the small particles filling the large sphere.

## PARTICLE $LD_{50}$: *Bacillus anthracis*

For the purposes of this discussion, spores of *Bacillus anthracis* will be used to describe concepts associated with particle size and organism number. The spores, known to be resistant to many of the effects likely to impact a bioaersol, are assumed to be rigid spheres.

One of the important concepts to characterize a bioaerosol is the relationship of organism number to particle size. This helps to reconcile the potential number of particles required to establish an infection. The $LD_{50}$ for spores of *Bacillus anthracis* is estimated at 8,000 to 10,000 spores. Providing there is one spore per particle, this $LD_{50}$ would be considered accurate. However, an agent preparation will contain substances to promote agent dispersal and will likely consist of particles that contain more than one spore. The number of spores per particle in a dry agent aerosol will yield the number of particles that contain enough spores to account for the $LD_{50}$. The number of spores per particle will obviously vary based on particle size.

By means of an ultrastructural morphometric analysis, the mean diameter for spores of *B. anthracis* was found to be $0.966 \pm 0.205$ microns.(2) The spores varied in shape from spherical to slightly ovoid. Allowing for a spore to be a sphere, spore volume based on the mean diameter is 0.472 microns$^3$. The volume of a spherical particle with a diameter of five microns is approximately 65 microns$^3$. The fractional filling volume is required to determine the number of spores that could occupy the volume of a given particle size. This is necessitated by the fact that spores, as spheres, are considered rigid and will not deform as packed within a spherical particle. Considering variation in spore size and shape, a reasonable estimate for the fractional fill is approximately 0.4 (i.e., 40% of the volume of the particle). Without the fractional fill parameter, about 137 spores would occupy the space in a given particle with an MMD of five microns. With the fractional fill, this number is reduced to an average of 55 spores per particle.

Because microbes as dry agents will often clump because of attractive surface effects (i.e., electrostatic charge, "hairy" endosporium, etc.), it is most important to add hydrophobic materials or other dry diluents to retain a uniform particle size. Although some of these chemical additives might occupy some of the space resulting from the fractional fill, the number of spores in a given mass will be reduced, although not necessarily in direct proportion to volume. Assuming that 50% of the mass of a dry agent preparation consists of additives to promote effective dispersion and 10% of these materials occupy the void volume created by the fill fraction within a five-micron particle, five-micron particles might contain an average of 33 spores per particle. In this case, the "particle $LD_{50}$", as opposed to the infectivity of *B. anthracis*, in terms of five-micron particles that would contain a total of 8,000 to 10,000 spores is estimated to be between 242 and 303 particles. Using similar calculations for three-micron particles, the "particle $LD_{50}$" would be from 1111 to 1388 particles. Thus, the $LD_{50}$ value based on particles would be less than the typical $LD_{50}$ value by one or two orders of magnitude depending on particle size. These types of calculations to characterize bioaerosols provide valuable tools for defensive measures, especially for the development of items such as detectors and dispersion codes.

## PARTICLE INFECTIVITY AND VIRUSES

Although the use of *B. anthracis* spores in a five-micron particle demonstrates the principles that govern particle size as it relates to infectivity, it does not show adequately the increase in efficiency of packing small particles into a relatively large spherical volume. The increase in efficiency is based on the fill fraction as shown in Table 1. Viruses are typically 100 to 1000x smaller than bacteria and are often characterized with infectivity values of 100 virions or less. Although subject to many of the same bioaerosol parameters described previously, virus infectivity can often be reduced to a single particle within a bioaerosol.

Figure 2 shows a large sphere or particle with a diameter 16x greater than that of the individual small spheres. If the large sphere were a particle with a MMD of three microns, the volume of the large sphere would be approximately 14.1 microns$^3$. The MMD of each

small sphere would be approximately 0.19 microns, much larger than that of most viruses. With a fill fraction of 0.6, the three-micron particle would contain approximately 2450 virions. Therefore, for most viruses, this would easily yield a clinical response by inhalation of a single particle from an aerosolized agent.

## SUMMARY

The effects of particle size on retention within the respiratory system and the relationship between infectivity and particle size are important concepts with respect to bioaerosols. Microbial infectivity measured as particles within a bioaerosol as opposed to the typical infectious or lethal dose may be reduced by one or two orders of magnitude to that of a single particle, depending on the agent. These considerations should be useful in the development of detection and protection devices needed to predict the occurrence or limit the effects of a BW attack.

## REFERENCES

1. Hatch, T.F. (1961) Bacteriol. Rev. 25:237-240.
2. Geisbert, T.W., et al. (1993) Report: "Ultrastructural and morphometric comparison of *Bacillus anthracis* spores with spores of other *Bacillus* species."

## KEY WORDS

Bioaerosol, infectivity, microbe, particle size

## FIGURES AND TABLE

Figure 1. Total and regional deposition of inhaled particles in relation to the aerodynamic particle size. (*from Hatch, 1961*).

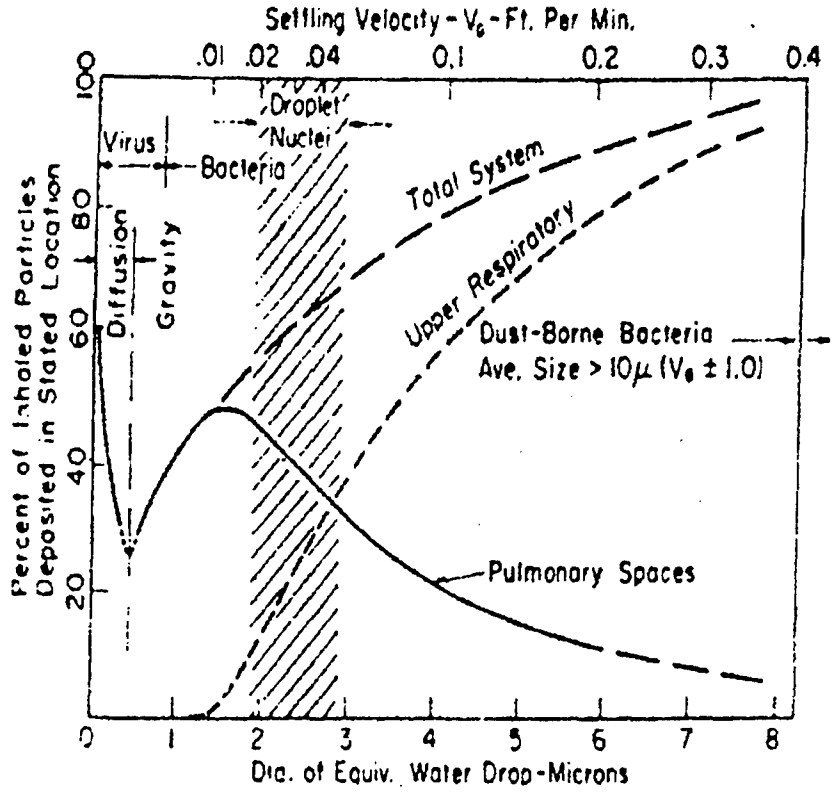

Figure 2. FCC lattice with outer sphere 16x diameter of small spheres

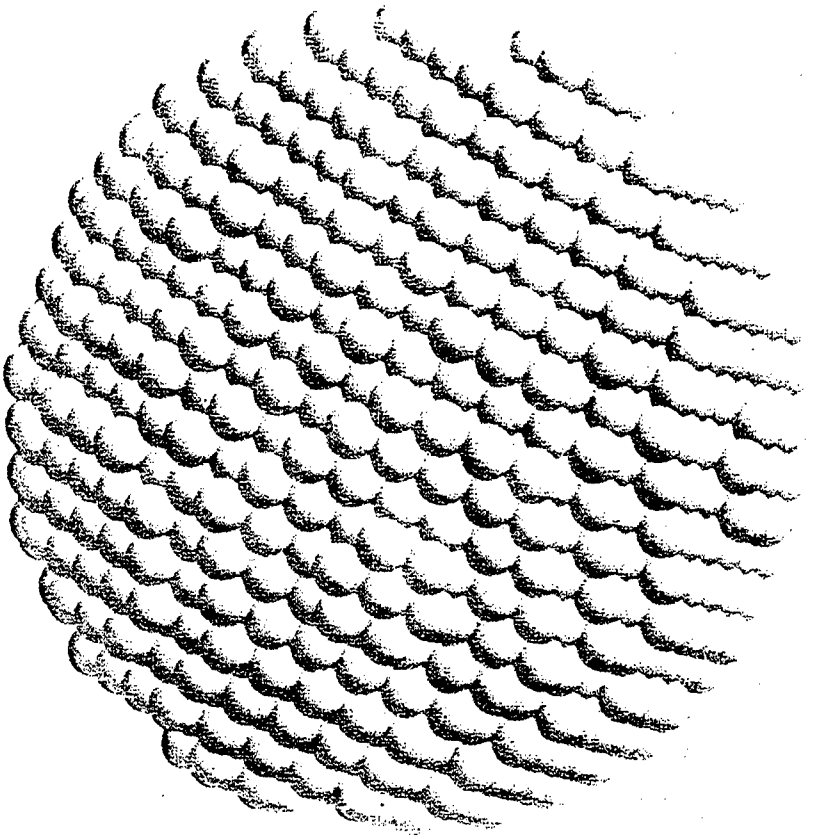

Figure 3. Filling fraction of the hcp and fcc lattices as a function of the relative radius of the outer sphere.

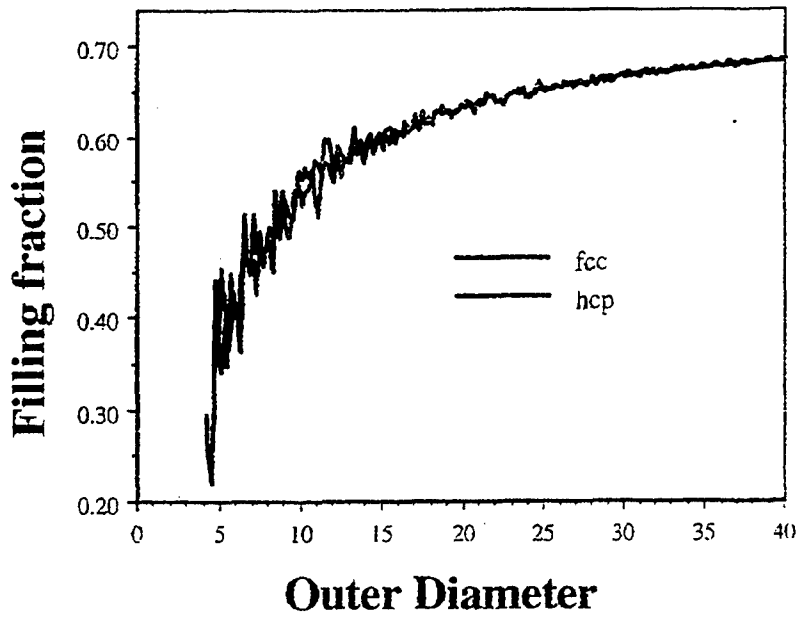


Table 1. Number of spheres of unit radius placed inside a larger sphere of given radius. Filling fraction is the total volume of small spheres divided by the volume of the large sphere.

| Large Sphere Radius | FCC Number | HCP Number | FCC Fraction | HCP Fraction |
|---|---|---|---|---|
| 4 | 19 | 19 | 0.2969 | 0.2969 |
| 5 | 43 | 57 | 0.3440 | 0.4560 |
| 6 | 87 | 87 | 0.4028 | 0.4028 |
| 7 | 177 | 159 | 0.5160 | 0.4636 |
| 8 | 249 | 257 | 0.4863 | 0.5020 |
| 9 | 369 | 389 | 0.5062 | 0.5336 |
| 10 | 555 | 527 | 0.5550 | 0.5270 |
| 11 | 683 | 763 | 0.5131 | 0.5733 |
| 12 | 959 | 955 | 0.5550 | 0.5527 |
| 13 | 1289 | 1261 | 0.5867 | 0.5740 |
| 14 | 1601 | 1639 | 0.5835 | 0.5973 |
| 15 | 1985 | 2037 | 0.5881 | 0.6036 |
| 16 | 2491 | 2493 | 0.6082 | 0.6086 |