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Release of Simulated Anthrax Particles from Disposable Respirators

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A preliminary study was undertaken to evaluate the potential for a disposable respirator that has been contaminated with anthrax spores to release spores in handling after use. The release of inert particles from disposable respirators was measured for masks dropped 3 feet onto a hard surface. Ten experimental runs were conducted for each of two N95 mask types, the Moldex 2200N95 and the 3M 8210. Anthrax spores were simulated with a test aerosol of single and double 1- μ m polystyrene spheres. For the Moldex mask loaded with approximately 20 million spheres on it, an average of 0.16% was released; for the 3M mask an average of 0.29% was released.

Keywords disposable respirators, particle release, anthrax particles, resuspension, N95

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As a result of U.S. postal workers' exposure to anthrax spores in late 2001, increased attention has been directed to the use and performance of respirators in an anthrax exposure situation. One aspect of use is the handling of used respirators that become contaminated with anthrax spores as a result of use in an anthrax spore-contaminated environment. The concern is that if anthrax spores become dislodged from disposable respirators with handling, then special consideration must be given to their disposal to prevent subsequent anthrax exposure to the wearer or others handling the respirator. Exposure may be by the cutaneous, ingestion, or inhalation route. A simple test apparatus was devised to provide a near worst-case test of the fractional release of simulated anthrax spores from a loaded disposable respirator.

Previous Work

There are no previous studies that attempt to characterize the fractional release of particles from an N95 disposable respirator when it is dropped onto a hard surface. Based on current

understanding of adhesion and resuspension, the expectation would be that micrometer-sized particles deposited in a fibrous filter would adhere to the fibers and not easily be dislodged by air currents or moderate shaking.⁽¹⁾ There are few investigations that provide information related to the current study. John et al.⁽²⁾ and Madler and Koch⁽³⁾ published their findings on the measurements of the release of adhered particles by impacting other particles or objects on a particle-laden surface. Two studies^(4,5) found significant release of aerosol from contaminated fabric that was subject to agitation. Montalvo et al.⁽⁶⁾ found that particles smaller than 20 μ m were released from cotton fibers by high velocity airflow. An air drag force 80,000 times that of gravity was required to release 90% of the particles.

In 1997 Qian et al.⁽⁷⁾ published their study of the reaerosolization, due to high reverse airflow (sneeze simulation), of particles and tuberculosis bacteria from N95 respirators. They found that air velocity, particle size, and humidity all affect particle release from respirators. They found that for relative humidity less than 22%, a back flow through a disposable respirator of 3.0 m/sec caused the release of less than 0.025% of *Mycobacterium tuberculosis* bacteria. About 6% of 5- μ m test particles were aerosolized under these conditions.

The objective of this preliminary study was to determine the fractional release of anthrax simulating particles from a disposable respirator when it is dropped onto a surface.

EXPERIMENTAL

Each test consisted of three phases: (1) particle loading, (2) mask drop test (particle release and resuspended particle collection), and (3) microscopic analysis. Two brands of N95 disposable respirators, Moldex 2200N95 (Moldex-Metric, Inc., Culver City, Calif.) and 3M 8210 (3M, St. Paul, Minn.), were tested. Polystyrene latex (PSL) microspheres (Duke Scientific, Palo Alto, Calif.) were used to generate a test aerosol. They are 1 μ m in diameter with a coefficient of variation in size of < 5%. They were generated as an aerosol by nebulizing a liquid suspension of the spheres with a Devilbiss D40 nebulizer and drying the resulting aerosol droplets to obtain an aerosol consisting of single, double, and triple (or more) spheres. Such

clusters of spheres are referred to as singlets, doublets, triplets, and so on.

The concentration of spheres in the liquid suspension was adjusted to give an aerosol with a high concentration of singlets and doublets without excessive numbers of large clusters of four or more. The suspension was nebulized at 35 kPa (5 psig) to give a loading concentration of 300 to 650 particles/cm³ in the loading chamber.

Loading of test respirators with the test aerosol particles was done in a small loading chamber used for this purpose in previous studies.⁽⁸⁾ The test aerosol is passed through a Kr-85 neutralizer to remove static charge from the particles before entering the chamber. The mask is sealed to a face form with a contoured clamping device. The face form is connected to an external, mechanical breathing machine.⁽⁹⁾ The breathing machine was operated at a light work rate (35 W) with a minute volume of 20.8 L/min. During each run a sample of aerosol particles from the chamber was collected on a membrane filter and counted by optical microscopy to determine particle number concentration in the loading chamber. Mask loading was calculated from the average loading chamber concentration, minute volume of the breathing machine, and duration assuming 100% collection efficiency.

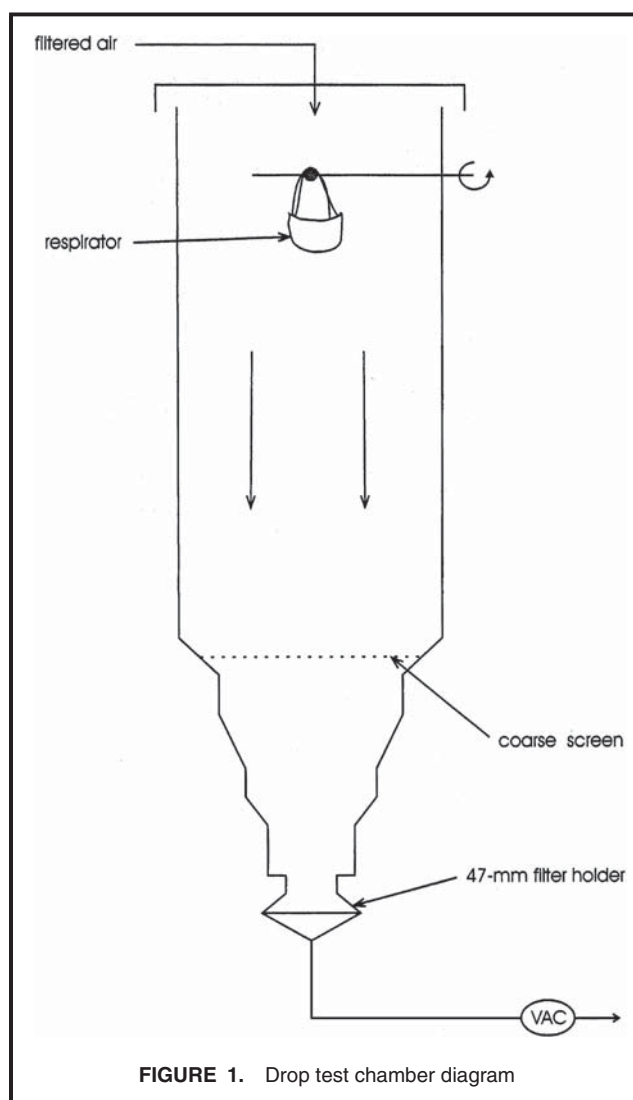
When the mask was loaded to approximately its target concentration, it was carefully removed from the chamber and from the face form and subject to the drop test described below. Each respirator was loaded with about 20 million (range: 18 to 29 million) 1- μ m polystyrene latex spheres. This corresponds to the amount of loading that would occur after 15 minutes of use in an environment containing 0.04 mg/m³ of particles with a similar size and density to anthrax spores.

The drop test was conducted using an apparatus built specifically for this series of tests. A diagram of the apparatus is shown in Figure 1. The drop chamber was a vertical duct, 12 inches in diameter, with a converging section and a collection filter (open-face) at the bottom. A rigid coarse screen (inverted 8-mm standard sieve) was the landing surface. Air was continuously drawn down the duct, through the coarse screen, and through the collection filter during the test so that any resuspended particles were captured on the filter. To minimize any contamination from room air, the top of the 12-inch duct was flooded with an excess of HEPA-filtered air.

The mask was removed from the loading chamber and hung by its straps in the top of the drop chamber. While the sampling filter (Millipore HA, 0.45 μ m pore size) was sampling at 45 L/min, the mask was released, causing it to drop 0.91 m (3 ft) and land on the screen. Particles released on impact were carried by the flow of filtered air to the sampling filter at the bottom of the chamber.

Particles collected on the sampling filter were counted by optical microscopy. The percent released was calculated from the original number on the mask and the number captured on the sampling filter. Blank runs followed the same procedure except that the masks were not loaded with the test aerosol.

To determine the falling velocity at impact, a clean 3M mask was videotaped as it fell in front of a calibrated grid. Images



of the falling mask were captured to a computer with an All-In-Wonder Pro video board and software (ATI Technologies, Inc., Thornhill, Ontario, Canada). The images were replayed frame by frame to determine the position and velocity of the mask as a function of time.

Analysis

Particles were counted by optical microscopy at 1000 \times (100 \times objective [oil immersion] with a 10 \times eyepiece) magnification. At this magnification the spherical shape of the test aerosol particles could be clearly observed. This served to distinguish test particles from other similar-sized particles in the environment and from mask fragments. The drop test filters were cleared with immersion oil and particles counted in randomly selected fields. Singlets, doublets, and triplets were counted separately.

The total number of particles on the filter was calculated based on the fraction of the active area counted. Release

TABLE I. Results of Drop Tests for Moldex 2200 and 3M 8210 Particulate Respirators

Mask	Percent of Singlet Particles Released	Percent of Doublet Released Particles	Percent of Doublet Particles Released (mean [std. dev.])
Moldex 2200	0.17 ^A (0.13) ^B	0.13 ^A (0.21) ^B	0.16 ^A (0.13) ^B
3M 8210	0.29 ^A (0.07) ^B	0.22 ^A (0.32) ^B	0.29 ^A (0.09) ^B

^AMean.

^BStandard deviation.

fractions were calculated as the ratio of released particles divided by the calculated total number of particles on the mask, assuming 100% collection by the mask.

One blank run was conducted before each regular run. The landing screen was washed before each set of runs on a given day. Ten replications were conducted for each mask type.

RESULTS

Based on an average of three videotaped runs, the mask takes 0.51 sec to travel 0.91 m (3 ft) and reaches a maximum velocity of 2.8 m/sec at the point of impact.

Particle release results are summarized in Table I. Values given are the percentages of singlets, doublets, and total particles loaded on the mask that were released and captured on the drop test sample filter. For the 10 measurements of the Moldex 2200 respirator, the mean percent released, corrected for blanks, was 0.16%, with a range from 0.0 to 0.38% and standard deviation of 0.13%. The 95% confidence interval of the mean is from 0.07 to 0.25%. For the case of a mask with 20 million particles on it this corresponds to a mean release of 32,000 particles with a 95% confidence interval of 14,500 to 52,000.

For the 10 measurements of the 3M 8210 respirator, the mean percent released, corrected for blanks, was 0.29% with a range from 0.17 to 0.48% and standard deviation of 0.09%. The 95% confidence interval of the mean is from 0.23 to 0.35%. For the case of a mask with 20 million particles on it this corresponds to a mean release of 58,000 particles with a 95% confidence interval of 45,000 to 70,000.

DISCUSSION

For both mask brands the average percent released (total particles) is significantly greater than zero ($p < 0.01$) for both Moldex and 3M masks. This was also true for singlets ($p < 0.01$), and doublets ($p < 0.05$).

The difference in the percent release (total particles) for the two mask types was also statistically significant ($p = 0.02$). This was also true for singlets. For both masks there are far fewer counts for released doublets and greater variability, consequently the difference in percent of doublets released by the two masks was not statistically significant. There were insufficient numbers of triplets to test their significance.

Anthrax spores are about 1 μm in diameter and ovoid or rod-shaped.⁽¹⁰⁾ One-micrometer PSL spheres were chosen because when generated at moderately high concentration they have a significant proportion of doublets and thus are similar in size and in geometry. The authors assume that longer rods would be more likely to be released because their mass is greater, but their adhesive force to a filter fiber is about the same as a sphere. It is likely that particles that are smaller than and/or wetter than the test particles will exhibit a lower fractional release than the test particles.

The PSL particles used were neutralized to Boltzmann's equilibrium charge distribution before being collected on the respirator filter. Anthrax spores might be carrying some charge depending on how they were mechanically dispersed. This would reduce the likelihood of their release on impact compared to the PSL particles.

The authors were unable to find information on the density of anthrax spores. The PSL particles have a density of 1050 kg/m^3 , somewhat less than *Bacillus globigi* spores of 1400 kg/m^3 , which may be analogous to anthrax spores.⁽¹¹⁾

Anthrax spores are believed to have a sticky coating, but those that are easy to disperse will not have a sticky coating on their surface. The PSL particles used in this study have a surfactant surface coating about 1 nm thick and may be considered roughly comparable to anthrax spores.

With a particle load in the range of 20 million on a respirator, it is expected that the fractional release will remain constant and it follows that absolute release will be directly proportional to loading. This is based on calculations that indicate the total surface area for collection—that is, the surface area of the fibers in the filter—is approximately 25,000 cm^2 . On average, each particle has 127,000 μm^2 of clean fiber around it. Thus, collected particles are isolated and not influenced by other collected particles. While it is true that a collected particle is a site of preferential deposition, the probability of that happening is low compared to collection on a clean fiber.

From the videotaped measurements, the average downward velocity of the mask was 1.8 m/sec, so the downward flow of clean air, which has a velocity of about 0.01 m/sec, had a negligible effect on mask velocity during the drop test.

The energy available for releasing the particles comes from the kinetic energy of the falling mask and is proportional to velocity of impact squared. It is anticipated that the fractional release would increase significantly with increasing impact velocity.

There was no statistically significant difference between the fractional release of singlets and doublets for either mask ($p = 0.58$ and $p = 0.37$).

CONCLUSIONS

A small, but consistent, fraction of 1- μ m particles captured by a disposable respirator can be released into the air and carried away by air currents when the respirator is dropped 3 ft onto a hard surface. For these tests the fraction released ranged from 0 to 0.5%. Further studies are planned to characterize the phenomenon. These preliminary tests suggest caution in handling and disposing of respirators that maybe contaminated with anthrax spores.

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