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Microbial growth on respirator filters from improper storage

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PASANEN A-L, KEINÄNEN J, KALLIOKOSKI P, MARTIKAINEN PI, RUUSKANEN J. Microbial growth on respirator filters from improper storage. *Scand J Work Environ Health* 1993;19: 421—5. Microbiological contamination and particle penetration were studied in two respirator filters with high efficiency. Microbial growth in filter materials during storage under humid conditions and the passing of microorganisms through the filters were particularly examined. Filters with different fiberglass and cellulose proportions were loaded in environments containing high microbial levels and incubated at a relative humidity of 98%. Particle penetration through loaded and incubated filters and carbon, nitrogen and microbial content were measured. After incubation, considerable particle penetration and the passing of fungal spores were observed for filters composed mainly of cellulose, probably because of humid conditions, which stimulated fungi to grow and extend mycelia and spores through the filter. Microbial activity, microorganism concentrations, and the chemical properties of the filter materials also supported this hypothesis. Storing used respirators in humid environments may result in heavy microbial contamination of the filters, especially if the filter material is biodegradable by microorganisms.

Key terms: bacteria, fungi, humid conditions, microbiological contamination, penetration.

Many studies have reported the effects of particle size, air flow rates, test conditions, and face-seal leakage of respirators on the filtering efficiency of particle filters (1—4). In most cases, the penetration of artificial test aerosols through the unused respirator filters has been studied. However, Lacey et al (5) tested the penetration of actinomycete spores (0.6—1.0 μm in diameter) through more than 20 different types of unused respirator filters. Before the penetration experiments, the filters were stored in the laboratory for six months to 20 years. The authors observed that actinomycete spores penetrated all of the filters tested with a wide variety of penetration percentages (0.1—44%), depending on type and material of the filter. A mean spore penetration value of 0.3% was measured through the respirator filter, the use of which has been recommended for protection against farmer's lung.

Besides spore penetration through the respirator filter, microorganisms can grow in the filter material under suitable conditions. Under humid conditions, actinomycetes and fungi can utilize and decompose such materials as cellulose, which is also used as a component of respirator filters (6, 7). In some studies, significant fungal and bacterial growth has been described in moist ventilation filters composed of fiberglass and synthetic fibers (8, 9).

In this study we have examined the effect of microbial accumulation on particle penetration through two P3 respirator filters composed of fiberglass and cellulose. These filters were developed to protect against agricultural and biological dusts. In a simulation of actual conditions during the use of respirators, the respirator filters were first loaded in their typical places of use and then stored under humid conditions which favor microbial growth.

Materials and methods

Two P3 respirator filters, each with a different composition, were selected for the experiments. Filter A was suited for a half-face mask (two filters per mask) and filter B to a full-face mask (one filter per mask). According to a manufacturer the filters differ in their composition, filter A containing about 85% fiberglass and 15% cellulose and filter B containing the opposite amounts of the two materials.

Two filters of both types were first artificially loaded in a cow barn, and a similar set of filters was loaded in a waste water treatment plant. When the respirator filters were loaded, air was filtered through them with a pump at a constant airflow rate of 10 (filter A in half-face mask) or 20 (filter B in full-face mask) $l \cdot min^{-1}$, values which correspond to an average inspiratory flow rate of an adult [12—30 $l \cdot min^{-1}$ (10, 11)]. The filters were loaded 8 $h \cdot d^{-1}$ for two weeks of active work periods in the barn and continuously for one week in the treatment plant.

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Background concentrations of microorganisms were measured in the barn and in the treatment plant at the height of 70—120 cm. The samples were collected with six-stage impactors (Andersen Inc, United States) on plate count agar (PCA) and R2A agar for bacteria, caseinate-propionate agar (CPA) for actinomycetes, malt extract agar (MEA) for fungi, and Eggins-Puch agar (EPA) for cellulolytic microorganisms. Cycloheximide was used as an antibiotic against fungal growth, and streptomycin sulfate against bacterial growth. The plates were incubated at 20—23°C for 7 d. The air temperature and relative humidity were measured in the loading environments in 3-h periods.

After the loading of the respirator filters, particle penetration, microbial concentrations, water content, and the amounts of dissolved organic carbon, total carbon and total nitrogen were determined from the unused filters and one of the loaded filters of both types. The remaining filter of both types was placed in a 1.2-l (filter A) or 2.7-l (filter B) airtight glass chamber where the relative humidity of the air was regulated at 98% with saturated aqueous potassium sulfate. During 35 d of incubation at 20°C, microbial activity was followed in the filters by measuring carbon dioxide (CO_2) accumulation every 4—7 d with an infrared analyzer (model 555, Ionics, United States). After incubation, the particle and spore penetration through the filters was measured, and microbial and chemical analyses were also made from the filter materials.

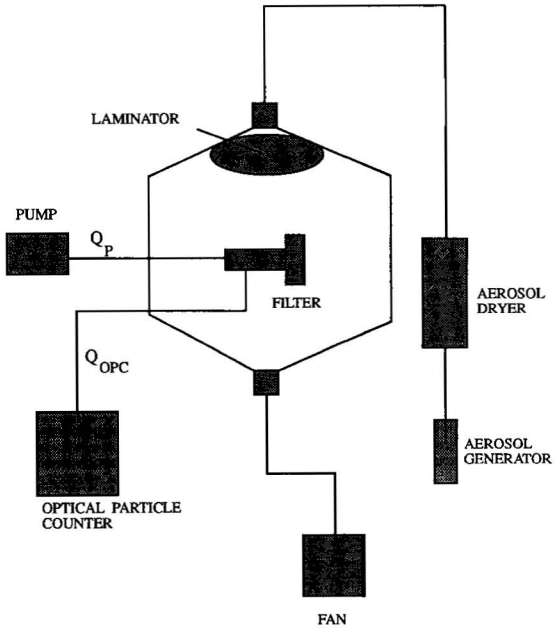

Figure 1. Experimental set-up for measuring particle or fungal spore penetration through respirator filters.[Q_P = airflow rate (10 or 20 l · min^{-1}), pump; Q_{OPC} = airflow rate (2.8 l · min^{-1}), optical particle counter]

The particle penetration measurements (figure 1) were made by mounting the respirator filter on a filter holder and measuring the outside and inside particle number concentrations. Inert polystyrene latex (PSL) particles (Sigma Chemical, United States) were used as test aerosols with the following particle size distribution: 45.9% of particles in the size range of 0.45—0.70 µm, 51.5% in the range of 0.70—1.65 µm, 2.5% in the range of 1.65—3.80 µm, and 0.1% in the range of 3.80—10.0 µm. A water suspension of PSL particles was aerosolized by controlled airflow to a standard nebulizer (TSI 3076, TSI, United States), and the sampling was designed to produce isokinetic flow at the entrance of the optical particle counter. The pump airflow rate (Q_P) was 10 l · min^{-1} for filter A and 20 l · min^{-1} for filter B. Particle counts were classified into five particle size ranges of 0.45—10.0 µm with an optical particle counter (HIAC/ROYCO 4100 with a 1200 sensor, Pacific Scientific, United States) using the airflow rate (Q_{OPC}) of 2.8 l · min^{-1}. The penetration was calculated from the number of counts taken consecutively with and without the respirator filter in line.

The passing of fungal spores through incubated filters B was also measured. The spore samples were taken on MEA plates with the six-stage impactor placed between the tested filter and the pump.

Microorganisms were analyzed by cultivation methods. Filter material samples (2—3 g) were homogenized, suspended into buffered saline solution, homogenized, and shaken for 30 min. Dilutions were plated on PCA, R2A, CPA, MEA and EPA. The plates were incubated at 20—23°C for 7 d.

For the dissolved organic carbon analyses filter material samples (1 g) were suspended in 0.9% sodium chloride, homogenized, and shaken for 30 min. The mixtures were filtered, and the pH of the filtrates was adjusted to 2 with phosphoric acid. The suspensions were analyzed with a total carbon analyzer (model 555, Ionics). Amounts of total carbon and total nitrogen were measured directly from the filter material with a carbon-hydrogen-nitrogen analyzer (CHN 600, LECO Corporation, United States). The water content of the filter material was calculated from the weight loss after overnight incubation at 105°C.

Results

In the barn, the airborne mesophilic fungal spore counts were 1.3—1.6 · 10^3 cfu · m^{-3} (cfu = colony-forming units), and the bacterial counts were 1.5—4.8 · 10^4 cfu · m^{-3}. The actinomycete spore counts remained below 40 cfu · m^{-3}, and the counts of cellulolytic microorganisms were 20—420 cfu · m^{-3}. Spores of *Aspergillus* species comprised 53—81% of the fungal spores in the air samples. The other common fungi were yeasts and *Penicillium* species. The air temperature averaged 12—16°C, and the relative humidity was 93—96%. In the treatment plant, air-

borne fungal spore counts ranged between $9.5 \cdot 10^2$ and $1.2 \cdot 10^3$ cfu · m^{-3}, and the bacterial counts between $4.2 \cdot 10^3$ and $3.0 \cdot 10^4$ cfu · m^{-3}. Counts of actinomycete spores and cellulolytic microorganisms were below 10 cfu · m^{-3}. Over 90% of the airborne fungal spores belonged to the *Botrytis* species. The other fungi found in the samples were *Penicillium* species, *Cladosporium* species, and yeasts. The air temperature was 13°C, and the relative humidity 49%.

No particle penetration was detected through either filter A in the particle size range of 0.45—10.0 µm. Neither did particles penetrate through the unused filter B and the filter loaded in the treatment plant, but 5.5% of the large particles (5.7—10.0 µm) penetrated the filter loaded in the barn. In addition, a considerable number of particles penetrated the incubated B filters. The particle or fungal spore size distributions in the air passed through the incubated B filters are presented in figure 2. In the case of the incubated B filter loaded in the treatment plant, the percentages of particle penetration were 0.33, 13.5, and 41.3% in the size ranges of 1.65—3.8, 3.8—6.0 and 5.7—10.0 µm, respectively. However, the particle concentrations passing through the filter were lower than those of test aerosols outside the filter. Particle penetration through the incubated B filter loaded in the barn was the following: 0.04% in the size range of 0.45—0.7 µm, 0.08% in the range of 0.7—1.65 µm, 18.8% in the range of 1.65—3.8 µm, 288.5% in the range of 3.8—6.0 µm, and 371.4% in the range of 5.7—10.0 µm. Thus, in the range of 3.8—10.0 µm, the particle concentrations passing through the filter were three to four times higher than test aerosol concentrations outside the filter.

The fungal spore counts passing through the incubated B filter varied from 10 to 10^5 cfu · m^{-3} in each size range. The fungal spore distributions resembled those of penetrated particles. *Penicillium* and *Aspergillus* were the most dominant fungal genera of penetrated fungal spores.

The production of carbon dioxide in the filters during incubation is illustrated in figure 3. The carbon dioxide yields were higher in the filters loaded in the barn than in those loaded in the treatment plant. In both cases, the carbon dioxide production rate was highest for filter B.

The microbial concentrations in the unused filters were all below the detection limit (10 cfu · g^{-1}). The concentrations of microorganisms in the filters after the loading and incubation are presented in table 1. In general, the concentrations of microorganisms were higher in the filters loaded in the barn than in those loaded in the treatment plant. Species of *Torula, Aspergillus, Penicillium, Cladosporium, Botrytis*, and yeasts were identified the most frequently. Actinomycete spores and cellulolytic microorganisms occurred in low concentrations in the filters.

The bacterial and actinomycete spore concentrations in the A and B filters were one to three orders of magnitude higher after the incubation. Fungal

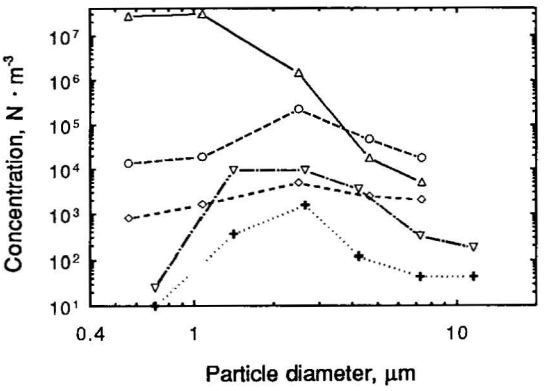

Figure 2. Concentrations of particles or fungal spores penetrating though loaded and incubated respirator filters (filter B type). (△ = test aerosol concentration outside the respirator filter, O = particle concentration passing through the incubated respirator filter loaded in the barn, ◊ = particle concentration passing through the incubated respirator filter loaded in the waste water treatment plant, ▽ = fungal spore counts passing through the incubated respirator filter loaded in the barn, + = fungal spore counts passing through the incubated respirator filter loaded in the waste water treatment plant)

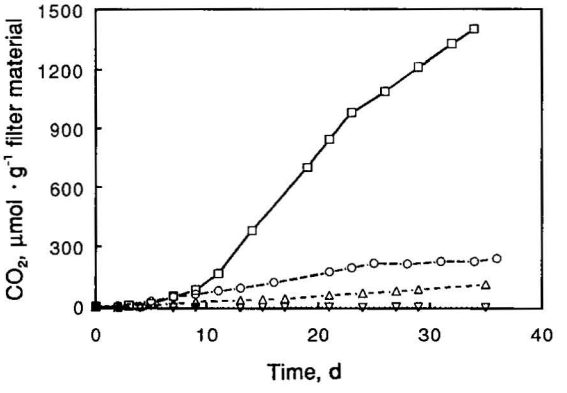

Figure 3. Production of carbon dioxide (CO_2)during incubation in the loaded respirator filters. (O = filter A loaded in the barn, □ = filter B loaded in the barn, ▽ = filter A loaded in the waste water treatment plant, △ = filter B loaded in the waste water treatment plant)

spore concentrations increased 10-fold in the B filter loaded in the barn, but decreased in the A filter during incubation. The concentrations of cellulolytic microorganisms remained unchanged in all of the incubated filters. After incubation, the fungal spore concentrations were one to two orders of magnitude higher in the B filters than in the A filters regardless of the loading environment. *Penicillium, Aspergillus*, and yeast were the most frequent fungi in the incubated filters.

No difference in dissolved organic carbon and total carbon was found between the unused, loaded, and incubated respirator filters. The dissolved organic carbon ranged from 0.6 to 0.8 mg · g^{-1} in filter A and from 0.9 to 1.3 mg · g^{-1} in filter B. The percentages

Table 1. Microbial concentrations in filters A and B after the loading and the incubation at a relative humidity of 98% for 35 d. Filter A was 85% fiberglass and 15% cellulose, whereas the proportion of fiberglass and cellulose was the opposite in filter B. (cfu = colony-forming units)

Respirator filter	Microorganisms in filter material (cfu · g^{-1})			
	Bacteria	Fungi	*Actinomycetes*	Cellulolytic
Filters loaded in barn				
After loading				
Filter A	$0.2—0.3 \cdot 10^6$	$18 \cdot 10^3$	30	90
Filter B	$0.2—0.4 \cdot 10^6$	$59 \cdot 10^3$	30	<10
After incubation				
Filter A	$28—43 \cdot 10^6$	$2.0 \cdot 10^3$	$22 \cdot 10^3$	100
Filter B	$25—57 \cdot 10^6$	$600 \cdot 10^3$	$11 \cdot 10^3$	130
Filters loaded in waste water treatment plant				
After loading				
Filter A	$1.0—1.4 \cdot 10^3$	130	450	<10
Filter B	$3.3—3.4 \cdot 10^3$	$1.1 \cdot 10^3$	20	<10
After incubation				
Filter A	$10—400 \cdot 10^3$	$3.2 \cdot 10^3$	$2.0 \cdot 10^3$	<10
Filter B	$53—79 \cdot 10^3$	$95 \cdot 10^3$	120	<10

of total carbon were 3 and 15%, respectively. Total nitrogen was 0.08% in the unused A filter and 0.05% in the unused B filter. After the loading and incubation, the corresponding percentages were 0.17 and 0.1%. Water content was 1.0% in the loaded A filter and 2.8% in the loaded B filter; after incubation, it was 7.8% in filter A and 6.0% in filter B.

Discussion

Loading respirator filters in environments with high airborne microbial counts did not cause particle penetration through the filters although the bacterial and fungal spore concentrations in the filters were high after the loading. During the incubation at a high relative humidity, the increase in carbon dioxide production indicated microbial growth, particularly in the B filters and in the filters loaded in the barn.

Significant particle penetration through the loaded B filters after the incubation was also detected. The size distribution of penetrated particles resembled the size range of airborne fungal spores (12). In fact, the air samples collected with an impactor revealed that part of the penetrated particles were fungal spores and other fragments of *Penicillium* species and *Aspergillus* species. The same fungal genera were also found in the air samples collected in the loading environment. Spore counts were about one order of magnitude lower than the penetrated particle concentrations because only viable spores were measured in this study. This result agrees with previously reported data, according to which airborne viable fungal spores comprise 1—10% of the total spores in agricultural environments (13, 14). *Penicillium* and *Aspergillus* belong to the fast growing fungi which are unpretentious in their moisture and nutritional requirements (6, 15). Their spores are also easily released into the air from cultures (16). This fact might explain the dominance of spores of *Penicillium* species and *Aspergillus* species in the incubated filter materials, as well as in the air samples.

The most probable explanation for the penetration of fungal spores through the incubated B filters is that the fungi grew through the filter material. Microbiological and chemical analyses from the incubated filters also supported this possibility. Although microbial concentrations increased during the incubation in both filter materials, fungal spore concentrations were one to two orders of magnitude higher in the B filters than in the A filters, whereas bacterial concentrations were at the same level in both filters. This finding also explained the differences in carbon dioxide production between the filter materials. On the other hand, the concentration of cellulolytic microorganisms was not particularly high. However, microorganisms did not necessarily decompose the filter material (cellulose); instead they utilized organic compounds in dust accumulated on the filters. The dissolved organic carbon and total carbon were also higher in filter B than in filter A, a finding which indicates that filter B contained more organic compounds (eg, cellulose) for energy sources of microorganisms. The result agrees well with the manufacturer's information about the proportions of cellulose and fiberglass in the filter materials. The amount of total carbon in the filters increased during the loading, particularly in the barn, and this increase also improved the conditions for microbial growth.

The guidelines of the European Committee for Standardization suggest that respiratory protective devices should be stored to protect against excessive moisture (17). The results of the present study show

that microbiological growth in respirator filters can start rapidly if respirators are kept at a very high relative humidity (>90%). In this study, the relative humidity of the air in the barn was at this level. The minimum relative humidity of a material for fungal growth in building materials containing cellulose and in ventilation filters composed of fiberglass has been reported to be in the range of 75—96% (9, 18). Thus, at the equal relative humidity range, the risk of microbial growth in respirator filters and also of the passing of fungal spores into the breathing air is possible.

The results of the present study indicate that storing used respirators in humid environments can rapidly cause microbial growth in respirator filters, especially if the filter material contains suitable nutrients for microorganisms. During the use of contaminated respirators, microbial propagula could enter the user's respiratory tract and cause considerable health risk. Thus the respirators should be stored in dry areas after use, and attention should be paid to the interval between filter changes.

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