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Research article

Safety of workers exposed to harmful airborne bioaerosols – legal status and innovations

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Abstract: *Filtering respiratory protective equipment is commonly used for protection against bioaerosols in industrial workplaces. However, the EU legislation does not specify the requirements for its antimicrobial properties. The result is that in the market there is no equipment available that would ensure complete safety of workers exposed to inhalation of harmful bioaerosols. This is particularly important in the case of equipment intended for multiple and long-term use. The aim of this study was to develop an innovative filtering half mask for the protection of respiratory tract that would have confirmed antimicrobial properties. The half mask was tested for compliance with EN 149:2001+A1:2009, and the survival rate of microorganisms in nonwovens was determined (E. coli bacteria and A. niger moulds). The reduction in the number of E. coli bacteria was 98.69% and the reduction in the number of A. niger moulds was 67.68% after 32 h of storage under conditions simulating the conditions of use of the equipment. It was found that the biocidal activity of the half mask is sufficient to ensure a significant reduction in the number of microorganisms during its long-term use in the workplace. This in turn means that continuous disinfection of respiratory protective equipment would be maintained even without employees' intervention, which is not possible in case of standard respiratory protective equipment.*

Keywords: *biosafety, bioaerosols, bioactive filtering nonwovens, respiratory protective devices, filtering half masks.*

Introduction

Respiratory protective devices (RPDs) against bioaerosol are classified as personal protective equipment (PPE) that is applicable where there is no possibility of preventing occupational risk by eliminating it at source or minimizing it by organizational solutions or technical protection measures. In Poland, the basic legal act regulating the provisions on the use of PPE is The Labour Low (*pol. Kodeks Pracy*) [1]. According to art. 2376, p. 1 of The Labour

Low, the employer is obliged to provide the worker with (free of charge) PPE protecting him/her from dangerous and/or harmful factors in the working environment and to inform him/her about the proper means of using this PPE. At the same time, according to the regulation of the Minister of Labor and Social Policy (MPiPS) [2] PPEs provided to employees should be appropriate to the existing threats. They should also correspond to workplace conditions and at any time they themselves should not cause or increase existing risks.

In order to meet these requirements, an employer employing workers exposed to biological agents must apply the risk assessment recommendations contained in the Regulation of the Minister of Health [3]. The employer should also take into account the fact that the respiratory protection against harmful bioaerosols, in addition to high ability to capture fine particles, should also have confirmed antimicrobial properties. This is due to the fact that, with long-term use in an environment where microorganisms are transported by air, they can proliferate and form a biofilm in the nonwoven structure, which may constitute a secondary source of danger to the user [4-8]. This problem is particularly important for multiple and long-term use of RPE, which is accepted in many workplaces.

Moreover, according to the provisions of the Regulation of the Ministry of Labor and Social Policy [2], the employer is responsible for establishing safe conditions for the use of PPE. In particular, the time period after which the PPE should be replaced with a new one should be determined [9, 10]. The service life of filtering RPDs used to protect workers against airborne biological factors strongly depends on the working environment [11]. Therefore, it should be established taking into account such factors as the type the RPD used by employees, their occupational activities and working environment conditions, i.e. humidity, temperature, dust concentration and the nature of the biological agents in the working environment (i.e. classification of microorganism into the hazard category).

For example, in case of agricultural and industrial workplaces, where organic matter is the carrier of biological factors, a rapid clogging of filtering surface occurs. As a result breathing resistance quickly increases, which hinders the performance of normal occupational activities and reduces the service life of RPDs [12]. Moreover, This the clogging of the filtering material leads to increased humidity inside the respirator during intensive work, which together with the presence of organic dust further favors the development of microorganisms on the filtering material [13].

On the other hand, for RPDs used in health care facilities and diagnostic laboratories, the clogging of the filtering surface is usually less problematic than a greater variability of biological agents and frequent breaks in the use of equipment related to occupational activities [12,14]. Of particular importance here is the aspect related to the safety of use of RPD, which results from the varied ability of microorganisms to survive and proliferate in filtering nonwovens [15]. The degree of sensitivity of different microorganisms to environmental factors is varied. Some, such as influenza viruses, die very quickly in the external environment, while others, such as tuberculosis mycobacteria or microorganisms having a protein shell (e.g. *Klebsiella*), can maintain their ability to infect new organisms for months [16].

The microclimate of the working environment can also significantly contribute to the development of microorganisms inside the filtering material of the RPD [8, 15]. Therefore, in the case of occupational activities in environments where there are long periods of elevated temperature and humidity or the processed organic material is moist (e.g. forest wood chips at the heat and power plant processing biomass), the long-term use of standard RPDs may cause the development of microorganisms on the material. This is supported by the latest research results of Majchrzycka et al. [13, 15]. Authors of the study confirmed on the basis of conducted breathing simulations that during use under the facepiece of RPD there are favorable conditions for the continuous accumulation of moisture from exhaled air. Moreover, high humidity persisted throughout the whole use of half masks, even when breaks in use occurred. It has also been shown that these conditions affect the survival of some species of microorganisms.

To address the abovementioned issues research institutes and manufacturers of respiratory protective equipment have attempted to develop high-performance RPDs with antimicrobial properties [17-20]. The works described in the literature focused mainly on solutions designed for short-term use. So far, however, reusable RPDs with time-dependent biocidal activity haven't been developed. Therefore, the aim of this study was to develop an innovative reusable filtering half mask for the protection of respiratory tract under exposure to harmful bioaerosols that would have confirmed time dependent biocidal activity.

Experimental

Materials

The model of the half mask consisted of a set of 3 nonwovens interconnected in a durable manner using ultrasonic bonding. The construction of the filtering half mask is shown in Figure 1.

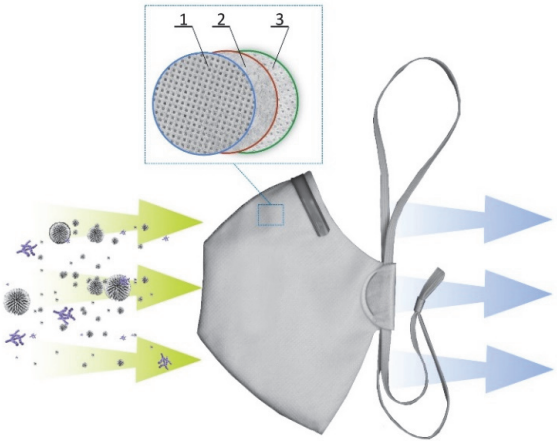

Figure 1. Arrangement of filtering layers in the construction of the bioactive half mask: 1) spun-bonded nonwoven (90 g/m^2), 2) electret PP melt-blown nonwoven with SPBS (120 g/m^2), 3) spun-bonded nonwoven (40 g/m^2)

The outer and inner layers of the half mask were made of commercial spun-bonded nonwovens with surface weights of 90 g/m^2 and 40 g/m^2. Electret PP melt-blown nonwoven with a set of porous biocidal structures (SPBS) with surface weight of 120 g/m^2 constituted a middle filtering layer. To produce the bioactive filtering nonwoven polypropylene granulate (PP) HL 508 FB (Borealis, Austria) having a melting point of 158°C and a melt flow index of 800 g/10 min was used. Antimicrobial properties were obtained by introducing 7% of SPBS into nonwoven fabric during its production.

To prepare the SPBS five different types biocidal structures were prepared and then mixed in equal amounts. Each type of structures was prepared by embedding specific amount (from 2 to 10% by mass) of hexamethylene-1,6-bis (N, N-dimethyl-N-dodecylammonium) dibromide (a gemini surfactant compound (GS)) on the halloysite nanocrystals. The biocidal activity of a given type of structures depended on GS concentration on halloysite nanocrystals. Time-dependent biocidal activity was obtained by introducing predetermined amount (from 0 to 5% by weight) of 1,2-propanediol into the structures. Detailed description of the preparation procedure of SPBS can be found in [21, 22]. The resultant concentration of GS in the bioactive nonwoven fabric was 0.336%.

Methods

Filtering efficiency of bioactive half masks

The testing procedures for determination of filtering and utility properties of nonwovens were based on the European standards relating to the performance of filtering RPDs [23].

The filtration efficiency of liquid aerosol was determined by measuring the penetration index of paraffin oil mist. Aerosol was passed at a predetermined linear velocity of 95 l/min through a nonwoven sample mounted in a sample holder of 100 mm diameter. Particle size distribution was log-normal with a median of Stokes diameter of 0.4 μm. Concentration of paraffin oil mist was measured with laser photometer AP2E (Lorenz, Germany) upstream and downstream of the sample.

The filtration efficiency of aerosol with solid dispersion phase was determined by measuring the penetration index of sodium chloride aerosol (NaCl). Aerosol with a median diameter of particles of 0.6 μm was passed at a predetermined linear velocity of 95 l/min through a nonwoven sample mounted in a sample holder of 100 mm diameter.

The penetration measurements were performed after 3 min from the beginning of each test (in the initial stage of filtration). In accordance with the requirements of EN 149:2001+A1:2009 [23] tests were carried out on a total of 9 samples: 3 new samples (N), 3 samples after the test for simulated wearing in which air was passed bi-directionally through the half mask for 200 min (SWU), 3 samples after mechanical strength test and thermal conditioning in (70±3)°C for 24 h and (-30±3)°C for 24 h (WMKT).

Breathing resistance of bioactive half masks

Breathing resistance tests were carried out on Sheffield dummy head. Exhalation resistance was measured using continuous flow of 160 l/min. During the tests the Sheffield dummy head was successively placed in 5 different positions: facing directly ahead, facing vertically upwards, facing vertically downwards, lying on the left side and lying on the right side. Inhalation resistance was measured at 2 levels of volume flow rate, i.e.: 30 dm^3/min and 95 dm^3/min.

In accordance with the requirements of EN 149:2001+A1:2009 [23] tests were carried out on a total of 12 samples: 3 new samples (N), 3 samples after the test for simulated wearing (SWU), 3 samples after thermal conditioning in (70±3)°C for 24 h and (-30±3)°C for 24 h (KT), 2 samples after thermal conditioning and continuous flow test in which air with volume flow rate 300 dm^3/min of was passed one-directionally through the half mask for 20 s (KTKP) and 1 sample after continuous flow test (KP).

Antimicrobial properties of bioactive half masks

The antimicrobial properties of functional filtering nonwovens used in the construction of the half mask were analyzed using gram-negative bacteria *Escherichia coli* (ATCC 10536) and *Aspergillus niger* moulds (ATCC 16404). A microorganism inoculum was prepared. Bacteria colonies were transferred into 20 mL of TSB medium (Tryptic Soy Broth, Merck, Germany) and incubated in 37±2°C for 24 h. In the case of mould, colonies from slants were washed using MEB medium (Malt Extract Broth, Merck, Germany). The average density of the suspension of microorganisms ranged from $1{,}2\times10^9 – 5{,}5\times10^9$ CFU/mL for *E. coli* and $2{,}0\times10^8 – 3{,}1\times10^8$ CFU/mL for *A. niger*. A 10 µL of inoculum and 15 µL of sterile saline (0.85% NaCl) were applied onto bioactive and control (unmodified) nonwoven samples with surface area of 4 cm^2 to obtain a mass humidity of 200%. Next, the samples were placed in sterile Petri dishes and incubated. To simulate working conditions, samples were initially incubated for 8 h in a climatic chamber (T = 28±2°C, RH = 80%), which corresponded to one working shift, then they were stored for another 16 h under room temperature (T = 24°C; RH = 45%), and then incubated for another 8 h. The 16 h storage period corresponded to the user's break in operation during which the half mask should be stored under microclimate conditions in accordance with the manufacturer's standard recommendations for such equipment, i.e. T < 25 C; RH < 60%.

The samples were collected immediately after inoculum application (at 0 h) and after 8, 24, 32 h of incubation. The viability of microorganisms on the tested nonwovens was measured using quantitative static method AATCC 100-2014 [24]. The samples of nonwoven were placed in 50 ml of sterile saline (0.85% NaCl) and shaken for 5 minutes to wash the microorganisms from the tested samples. The dilutions of samples in 0.85% NaCl (from 10^{-1} to 10^{-6} in 2 repetitions) were then made. 1 mL and 0.1 mL of dilutions were placed on sterile Petri plates and covered with TSA medium (Tryptic Soy Agar, Merck, Germany) for bacteria and MEA medium (Malt Extract Agar, Merck, Germany) for moulds. Then the plates were

incubated at a temperature of 37±2°C for 24 h (bacteria) and at a temperature of 27±2°C for 72 h (moulds), and then grown colonies were counted (the result is given in CFU/sample). The experiment was carried out in three independent repetitions for both selected nonwovens (control and bioactive).

The reduction of the number of microorganisms (R) after contact with the biocidal nonwoven was calculated using the following formula:

$$R = \frac{K-B}{K} \cdot 100\% \quad (1)$$

where K denotes number of microorganisms on the control nonwoven in subsequent incubation times [CFU/sample], and B denotes number of microorganisms on the bioactive nonwoven in subsequent incubation times [CFU/sample].

Results and discussion

Results of penetration tests of model aerosols and breathing resistance are presented in Table 1.

Table 1. Penetration and breathing resistance of bioactive half masks

Type of sample	NaCl aerosol penetration, [%]	Paraffin oil mist penetration, [%]	Inhalation resistance at 30 dm^3/min, [Pa]	Inhalation resistance at 90 dm^3/min, [Pa]	Exhalation resistance at 160 dm^3/min, [Pa]
N	M: 0.27 SD: 0.11	M: 0.19 SD: 0.04	M: 67.6 SD: 4.1	M: 281.8 SD: 6.8	M: 116.4 SD: 3.6
SWU	M: 0.37 SD: 0.44	M: 0.22 SD: 0.02	M: 69.0 SD: 2.8	M: 273.6 SD:6.7	M: 123.3 SD: 4.6
WMKT	M: 0.76 SD: 0.61	M: 0.29 SD: 0.01	-	-	-
KT	-	-	M: 66.1 SD: 3.7	M: 266.7 SD: 9.8	M: 121.3 SD: 4.2
KP	-	-	65.3*	278.9*	124.6*
KTKP	-	-	M: 69.3 SD: 2.3	M: 277.0 SD: 6.6	M: 126.2 SD: 2.6

N – sample as received (new), SWU – after the test for simulated wearing, WMKT – sample after mechanical strength test and thermal conditioning, KT – sample after thermal conditioning, KP – sample after continuous flow test, KTKP — sample after thermal conditioning and continuous flow test; M: mean, SD: standard deviation,
* one sample was tested

It has been found that the bioactive filtering half-mask has very good filtration properties. This was confirmed by the results of penetration measurements obtained for sodium chloride aerosol and paraffin oil mist. As a result of the SWU penetration increased by 0.10% for NaCl aerosol and by 0.03% for paraffin oil mist in relation to the new samples. This could be associated with slight changes in the porosity of the nonwoven material caused by continuous loosening of the fibrous structure in exhalation and inhalation phase. A slightly higher increase in penetration in relation to the new samples was noted for half masks subjected to WMKT (0.49% for NaCl and 0.10% for paraffin oil mist). This result indicates the

loss of electrostatic charge of the nonwoven fabric under the influence of high temperature, which is typical of electret materials. It should be emphasized, however, that these changes do not disqualify the half mask in terms of high protective efficacy. The results of the model aerosol penetration measurements have confirmed that the bioactive half mask meets the requirements of EN 149:2001+A1:2009 for the highest protection class marked with the FFP3 symbol.

The inhalation resistance of the tested half masks at air flow of 30 dm^3/min ranged from 65.3 to 69.3 Pa and at air flow 95 dm^3/min in the range of 266.7 to 281.8 Pa. No significant changes in inhalation and exhalation resistance were observed for samples subjected to SWU, KT and KP. This means that the pre-treatment does not impair the breathing comfort and the half mask can be still classified as the highest protection class (FFP3) according to EN 149:2001+A1: 2009 standard.

Average number of microorganisms surviving on control and bioactive nonwovens is shown in Table 2.

Table 2. Number of microorganisms on control and bioactive nonwovens**

Microorganisms	Type of nonwoven	Number of microorganisms during incubation, CFU/sample			
		0	8	24	32
E. coli	control	M:7.77×10^5 SD:7.12×10^4	M:2.83×10^6 SD:5.89×10^5	M:2.83×10^5 SD:1.30×10^5	M:6.10×10^4 SD:1.84×10^4
	bioactive	M:6.82×10^5 SD:1.37×10^5	M:6.47×10^5* SD:1.10×10^5	M:7.20×10^3* SD:8.63×10^2	M:7.98×10^2* SD:1.59×10^2
	R,%	no	12.22	77.14	97.46
A. niger	control	M:1.07×10^4 SD:1.21×10^3	M:1.52×10^4 SD:5.31×10^3	M:3.38×10^3 SD:1.32×10^3	M:1.60×10^3 SD:5.25×10^2
	bioactive	M:1.37×10^4 SD:4.32×10^3	M:3.02×10^3* SD:5.74×10^2	M:1.85×10^2* SD:1.17×10^2	M:5.17×10^2* SD:1.94×10^2
	R,%	no	no	80.14	94.53

M: mean; SD: standard deviation; *: means for control and bioactive nonwovens (among groups of microorganisms at the same incubation time) are significantly different (t-student test, $p < 0.05$); no- not observed; **: table contains preliminary results, further analysis has been presented in [25]

The number of *E. coli* bacteria on the control and bioactive nonwovens at t = 0 h ranged from 6.82×10^5 CFU/sample to 7.77×10^5 CFU/sample and did not differ significantly. On the control nonwoven after the first 8 h of incubation at elevated humidity, a statistically significant increase in the number of *E. coli* (2.83×10^6 CFU/sample) was observed in relation to the biocidal nonwoven (6.47×10^5 CFU/sample). Then, the number of bacteria on both nonwovens (control and bioactive), decreased with the incubation time. However, the reduction of the number of bacteria for bioactive nonwovens was greater than for the control sample and it increased from 12.22% (t = 8 h) to 97.4669% (t = 32 h).

The number of *A. niger* moulds on the control and bioactive nonwovens at t = 0 h ranged from 1.07×10^5 CFU/sample to 1.37×10^5 CFU/sample and did not differ significantly between both types of samples. After first *A. niger* moulds on the control nonwoven reached 1.52×10^4 CFU/sample and on bioactive one only 3.0×10^3 CFU/sample. The difference in the number of microorganisms on both nonwovens was statistically significant. During the subsequent incubation times, the number of *A. niger* moulds on the biocidal and control nonwovens decreased. The reduction was much higher for the bioactive samples and it varied from 80.11% after t = 24 h of incubation, trough 94.53% after t = 32 h.

Survival of microorganisms on antimicrobial filters have been extensively described in the literature. Various materials have been used to prevent microbial growth in nonwoven filters such as e.g. nanosilver, silver nitrate, zinc oxides, phosphated quaternary amine complexes or quaternary ammonium salts [26-29]. Similarly to our studies, a significantly greater reduction in the number of microorganisms during incubation was observed for nonwoven fabrics containing biocidal agents. Previous studies, however, did not consider the possibility of time-dependent activation of such substances. Because of the lack of gradual release function of the biocidal agent over time, the incorporated biocidal compounds could adversely affect the skin of the user when used in the construction of RPDs. By using bioactive nonwovens with time-dependent bioactivity this problem can be significantly reduced.

Conclusions

The functional nonwoven fabric used in the construction of bioactive filtering half mask exhibited antimicrobial activity against the selected bacteria and moulds. Greater antimicrobial activity was observed for *E. coli* bacteria (R = 97.46% after 32 h of incubation), lower for *A. niger* moulds (R = 94.53% after 32 h of incubation). At the same time, the half mask showed very good filtration properties both for solid and liquid aerosols.

The results of this study indicate that it is reasonable to use biocidal filtering nonwovens to produce reusable RPD, as this ensures that hygienic conditions will not be compromised during use and storage of the equipment even without the employee's intervention. In order to implement this type of equipment for universal use, it is necessary to popularize knowledge about the legal issues concerning the examination of its antimicrobial properties and develop appropriate attitudes towards health and safety at work among employees.

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