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ANTIMICROBIAL EFFICIENCY OF IODINATED INDIVIDUAL PROTECTION FILTERS

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The Air Force Research Laboratory (AFRL) has a broad interest in oxidizing-agent and photoactivated oxidizing agents that, when applied to textiles and other materials, will act as broad spectrum antimicrobials. For this project, poly(styrene-trimethylammonium triiodide) was incorporated into filtration devices to augment filtration. The overall goal of the project is to replace existing HEPA media with an antimicrobial membrane for both individual protection and collective protection devices. In this report we investigate the microbial efficacy of an iodinated Clip-On prototype and a commercial-off-the-shelf (COTS) carbon-HEPA aerosol canister. Our data indicate that the Clip-On, in series with a COTS canister provides an additional 2 logs of attenuation vs. a standard COTS canister when challenged with MS2 coliphage.

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ANTIMICROBIAL EFFICIENCY OF IODINATED INDIVIDUAL PROTECTION FILTERS

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ABSTRACT

The Air Force Research Laboratory (AFRL) has a broad interest in oxidizing agent and photo activated oxidizing agents that when applied to textiles and other materials will act as broad spectrum antimicrobials. For this project poly(styrene–trimethylammonium triiodide) was incorporated into filtration devices to augment filtration. The overall goal of the project is to replace existing high efficiency particulate air (HEPA) media with the antimicrobial membrane for both individual protection and collective protection devices. In this report we investigate the antimicrobial efficacy of an iodinated clip-on prototype and a commercial-off-the-shelf carbon HEPA aerosol canister (COTS). Our data indicate that the clip-on, in conjunction with a COTS canister, provide an additional three logs of protection versus a standard COTS canister when challenged with MS2 coliphage.

INTRODUCTION

Biological weapons are not new, and have been used as warfare agents for thousands of years (5). The United States, the Soviet Union, and many other countries had active programs aimed at developing bio-warfare agents (3, 6, 7). It is widely accepted that many bacterial and viral agents were weaponized (2,4,7). The extraordinary events of terror carried out in the United States and overseas in the last decade, in conjunction with the instability in the former Soviet Union and the Middle East, has lead to speculation that biological weapons could be used to inflict mass casualties on either military or civilian installations (3,10). The anthrax attacks carried out in the United States after 9/11 demonstrated how easy it is to carry out bio-attacks. If an attempt had been made to aerosolize the biological agent the casualties may have been much higher.

The devices currently used for protection from a biological attack utilize HEPA filters. HEPA filters are 99.97% efficient at removal of 0.3µm particles such as potassium chloride (KCl) or dioctyl phthalate (DOP) (1,8). While 99.97% is very good, a challenge of 10^6 particles should yield a penetration of 300 particles. This is especially significant when dealing with biologicals that have a minimum infectious dose (MID) of well below 300 particles. It is thought that for many of the bio-weapons viruses
(0.05 µm to 0.3 µm in size), the minimum infectious dose is <100 plaque-forming units (PFU) (3). Therefore the HEPA filter may not be adequate to protect the user from infection when challenged with high loads of viruses. In addition HEPA filters have large pressure drops that are exhausting to the user in individual protection (IP) and are costly to run in collective protection (CP).

In an attempt to enhance the capability of IP filters, AFRL is evaluating filters that contain antimicrobial agents. For this study, prototypes that incorporate poly(styrene–trimethylammonium triiodide) as the antimicrobial were evaluated (Triosyn Corp., Williston, Vt.). The iodinated filters are thought to kill the organisms as they pass through the filter, which

augments mechanical filtration. The goals of the project are to reduce penetration of biologicals below the MID of the pathogens potentially used as bio-weapons and to reduce the pressure drops of the canisters. The product evolution for IP was designed to go through three phases: 1) a clip-on device, as a proof on concept, that augment existing COTS canisters; 2) a COTS canister with the antimicrobial integrated into the canister; 3) a canister that replaces the HEPA filter with the antimicrobial filter. The design of the final prototype aims to provide all the current specifications of COTS canisters but also adds the enhanced antimicrobial protection and a lower pressure drop. This paper covers the initial work in which COTS canisters and the iodinated clip-on prototypes were challenged with aerosolized MS2 coliphage.

EXPERIMENTAL METHODS

Escherichia coli (ATCC 15597) was maintained on trypticase soy agar (Difco 236950) at 37°C. MS2 coliphage (ATCC 15597-B1) stock solutions were produced according to standard protocols (9).

A customized Bio-Aerosol Testing System (BATS) (Triosyn Corp, Williston, Vt.) was used to generate the MS2 aerosol and to challenge the test articles. The bio-aerosol was generated using Colison nebulizers and was then drawn through the filters for eight hours at a rate of 85 L/min. One port did not contain a canister so that the challenge load to the filters could be determined. Viable viruses in the effluent were captured in all glass impingers (AGI) that contained 100 ml of 1X PBS (pH 7.4). Standard phage counts were done to determine the amount of viable MS2 that penetrated each filter. The specific protocols for challenging canisters with MS2 were as follows:

1. Challenge canisters were glued onto retaining plates and attached to the BATS. The canisters were suspended in the BATS with only the threaded connection not exposed to the viral challenge. 2.AGIs (Chem. Glass CG-1822-04) containing 100 ml of buffer (1X PBS pH 7.4 + 0.001% antifoam A (Sigma A6457) were plumbed to the BATS system (each port/filter used 2 AGIs). 3.Flow rates for each filter/port were calibrated to 85 L/min.
4. 1.5 ml of the MS2 stock (~10^{11} PFU/ml) was added to 150 ml of sterile water. The solution was divided equally into three Collison nebulizers (BGI Inc.,Waltham, Mass.). The nebulizers were then attached to the BATS.
5. The run was started by pressurizing the nebulizers to 20 psi and starting the vacuum to draw the aerosol through the canisters.
6. The instrument was stopped at 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hr time points. At each time point the impingers were replaced with impingers containing fresh buffer. At hours 3 and 6 the nebulizers were replenished with more MS2 stock solution.
7. At each sampling point 10 ml were removed from each impinger and placed in sterile test tubes. For the positive control the solution was serially diluted 1/10 out to 10^{-6}.
8. Phage plating was done in triplicate for each impinger and for the 10^{-4}, 10^{-5}, and 10^{-6} dilution of the positive control. One ml of the sample was mixed with one ml of mid log phase *Escherichia coli* (grown in special MS2 media (1% tryptone, 1% NaCl, 0.5% yeast extract) for 2.5 hours at 37°C with shaking at 200rpm) and 9 ml of 50°C MS2 media (1% tryptone, 0.8% NaCl, 0.1% yeast extract, 0.1% dextrose, 4mM $CaCl{\cdot}2H_2O$, 0.001% thiamine, 1% agar). The mixture was inverted three times then poured into 100X15mm Petri dishes. The plates were incubated overnight at 37°C then the plaques were counted the following day.
9. The total plaque-forming units for each filter was determined by multiplying to the average number of the countable plaques from both impingers (2 impingers per port/filter) X dilution X

180 ml (the impingers have ~10% loss of liquid during the run). The detection limit for the method was 30 PFU/sampling period.

RESULTS AND DISCUSSION

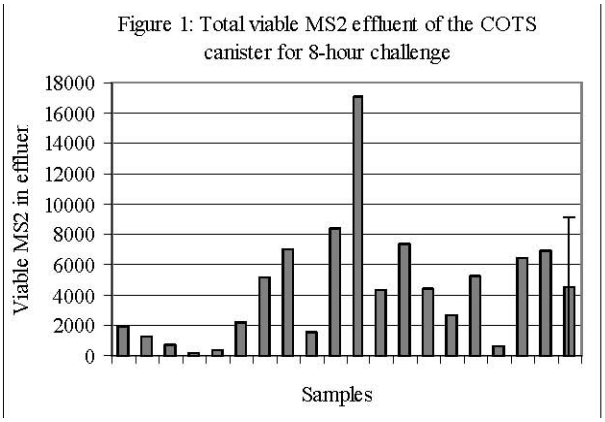
All eighteen COTS canisters were penetrated by MS2 coliphage (fig. 1, table 1). The average penetration was 4,424 PFU; however, the penetration was highly variable with a standard deviation equivalent to the mean. Analysis of the penetration on an hourly basis (fig 2) indicates that the penetration was constant yielding a linear response over the 8-hour test period. The MS2 penetration is not a surprise because the HEPA is only 99.97% efficient and thus penetration would be expected. In fact, given the challenge loads of ~10^{10} PFU the penetration was expected to be greater than what was observed. This suggests that the COTS canisters are actually performing at greater than 99.97% efficiency. However, the test measures only viable particles and there may be mechanical forces that are killing some of the MS2 as it penetrates the filters. There are likely to be many forces that determine that actual viable MS2 penetration. However, it is clear that viable viral particles penetrate the filter in amount well in excess of the MID for the bio-warfare viruses.

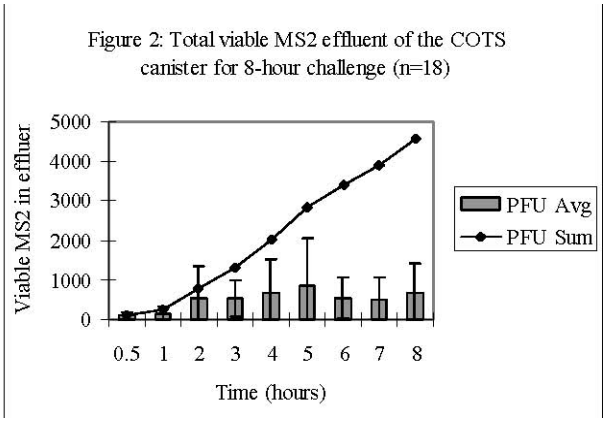
Table 1: PFU counts of MS2 penetration and total challenge for eight-hour test

	COTS		Iodinated Clip-On + COTS	
Samp. num.	MS2 in effluent*	MS2 pos. cont. challenge	MS2 in effluent*	MS2 pos. cont. challenge
1	1920	1.09E+09	180	2.45E+10
2	1312	2.69E+08	450	2.45E+10
3	737	2.69E+08	180	2.45E+10
4	178	2.69E+08	120	5.69E+09
5	380	2.69E+08	30	5.69E+09
6	2190	3.40E+09	60	4.50E+11
7	5130	3.40E+09	0	4.50E+11
8	7050	3.40E+09	0	2.80E+09
9	1530	3.40E+09	0	2.80E+09
10	8370	3.40E+09	60	7.53E+10
11	17100	2.45E+10	60	7.53E+10
12	4350	2.45E+10		
13	7410	6.50E+09		
14	4410	6.50E+09		
15	2700	5.70E+09		
16	5280	4.50E+11		
17	660	2.80E+09		
18	6420	7.50E+10		
19	6960	5.78E+09		
Average	**4424**	**3.27E+10**	**104**	**1.04E+11**
Stdev	**4283**	**1.03E+11**	**137**	**1.80E+11**

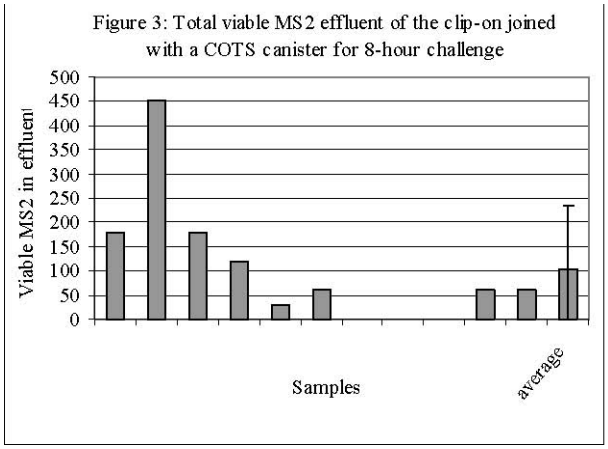
* detection limit is 30 PFU for each sampling period

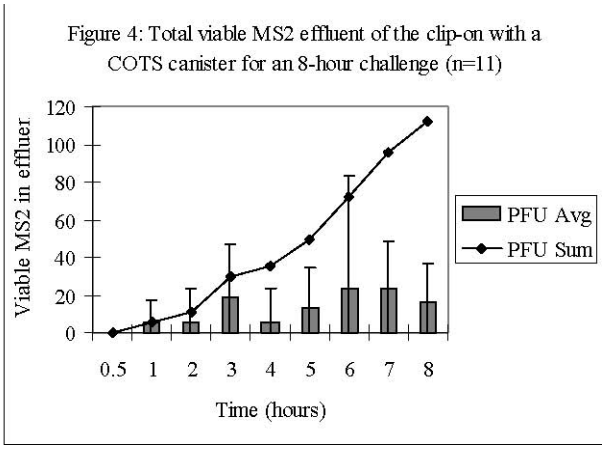
The iodinated clip-on, when used with the COTS canister provided a three log increase in bio efficacy versus the COTS canister alone (figs 5, 6). The average penetration through the clip-on + COTS canister was 104 PFU with a standard deviation equivalent to the mean (fig 3, 4). The actual variability of the MS2 penetration varied from undetectable levels for three of the canisters up to 450 PFU for one of the canisters. The variability may be due to seal leaks and inconsistent manufacturing processes. All the clip-on units were individually made and thus were subject to individual variations. The seal issue in





question was the junction between the clip-on unit and the COTS canister. The specification of the outer ring diameter for the COTS canister used for this study was variable and thus in some instances there may not have been a good seal between the clip-on and the COTS canister. From a very practical stand point we do not expect that the clip-on unit will ever be fielded. It adds weight to the canister and is bulky and impairs the vision of the user. However, it allowed us a first test to determine the feasibility of the iodinated membrane.





The mechanism by which the clip-on is reducing viral particles is thought to take place by the viral particles coming in contact with the iodine. As organisms pass through the filter, iodine is also selectively released and kills the organisms. Based on small-scale media tests, the iodinated membrane attenuated viral penetration by 4 logs versus an untreated membrane (Trioysn - unpublished). Also, the iodinated media was proven to be biocidal against various bacteria, spore formers, and MS2 (Trioysn - unpublished). We have yet to run the control experiment in which the non-iodinated clip-on is used in conjunction with the COTS canisters.

Figure 5: Average viable MS2 effluent for clip-on (n=11) & COTS canister (n=18) for 8-hour challenge

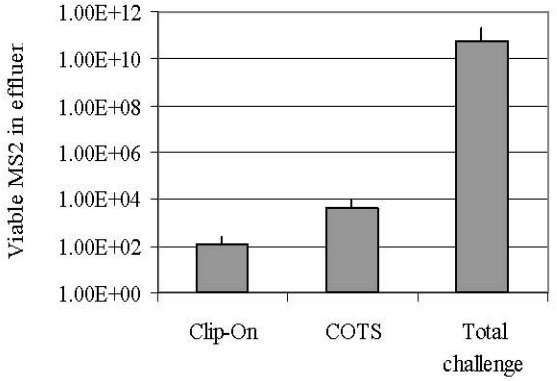
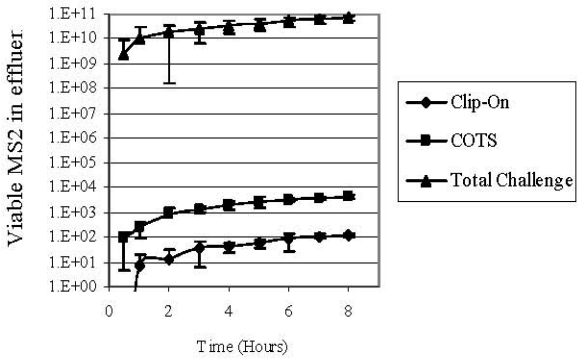


Figure 6: Average viable MS2 effluent for clip-on (n=11) & COTS canister (n=18) for 8-hour challenge



The use of MS2 for the initial testing of the canisters is appropriate because it allows for a relatively uncomplicated and quantifiable challenge. However, MS2 coliphage is much smaller (20 nm) than the bio-warfare viruses of interest (50 nm – 300 nm), therefore it may not be the ideal challenge organism. To fully demonstrate the concerns with the existing COTS canisters and the mitigating effects of the iodinated canisters, a mammalian virus, using tissue culture, needs to be used to challenge the filters. Ultimately we would like to challenge animals with effluents from the canisters to determine the bio-efficacy of the canisters.

CONCLUSIONS

MS2 coliphage penetrates COTS canisters at levels well above the MID for the bio-warfare viruses. This is not surprising given that HEPA filtration is only 99.97% efficient for filtering 300nm particles. We have shown that the addition of the iodinated clip-on prototype to existing COTS canisters offers added protection from MS2 penetration. For three of the prototypes the MS2 penetration was reduced to undetectable levels. MS2 coliphage, being very small may not be the most appropriate organism for challenging test articles. A mammalian virus challenge using tissue culture and live animals is being developed. This will allow for a more appropriate challenge of all the test articles.

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