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PROJECT REPORT

RISK OF EXPOSURE TO PHMB FROM THE LIVINGUARD™ FACE MASK

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BACKGROUND

Contact-killing microbicidal agents have been proven to be the most advantageous for controlling transmission of air and surface borne pathogens. Some of most common agents in this category include quaternary ammonium compounds, antibiotics, antimicrobial peptides, and non-leaching physically adsorbed polycationic molecules. One such positively charged molecule is poly(hexamethylene biguanide) hydrochloride (PHMB). It is a broad-spectrum antimicrobial agent which has proven efficacious against Gram-positive and Gram-negative bacteria, fungi, yeast, and viruses.

There have been no reports of genotoxic, epigenetic effects or acquired resistance to PHMB in any bacteria. It is principally due to the complex mode of action of PHMB which involve selective condensation of bacterial chromosomes. Studies investigating the mammalian toxicity and environmental impact of PHMB have provided variable data, which is reflective of testing procedures. The applicable and relevance of these data need to be evaluated based on the use application.

PHMB was originally introduced as swimming pool sanitizer more than six decades ago. Since then has been approved / used for areas of public health, agriculture, food processing, healthcare and biomedical industry. In the context of these broad applications many studies have been performed to assess its toxicity profiles for ascertaining its safety and risk assessment for animal exposure and ecological and environment impacts. These findings have been extensively reviewed and evaluated by regulatory agencies and stakeholders.

The U.S. EPA has reviewed the use of PHMB as disinfectant and reported 550 ppm as acceptable residue level (EPA 2004). According to a study submitted to EPA (MRID# 451877-06) extraction of PHMB from treated fabric using artificial perspiration simulant method (ISO-105-E04) the leachate can be 7.67 $\mu\text{g}/\text{cm}^2$ and 1 $\mu\text{g}/\text{cm}^2$ from pre-washed and post washed cotton (100%) fabric. These results are not applicable to Livinguard face mask because of difference in the use application and consequently exposure prospects.

Similarly, inhalation toxicity of the PHMB has been studied using actively aerosolized PHMB aqueous solutions. For example, in one study Alpk:APfSC rats were exposed by nose to formulation containing 20.6 (% w/w) PHMB for 4 hours to a single dose of 1.76 mg/L of the formulation, which corresponded to 0.36 mg/l of PHMB. Based on the results of that study, the LC50 for the formulation could not be established; however, it was deemed to be higher than 0.36 mg/l of PHMB. In another study, similar results were obtained when Wistar CRL:(WI) rats were exposed nose-only to an aerosol of PHMB in aqueous solution.

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PERSPECTIVE

Risk assessment studies are conducted using a specific exposure scenario considering the parameters most appropriate / pertinent to the technology use practice. New technologies and applications are being constantly introduced for controlling transmission of airborne infections. Livinguard Technologies AG has introduced a novel design of face mask, made of fabric treated with PHMB using a proprietary technology. The treatment technology claim to un-leachably bind the PHMB molecule to fabric article. The use of PHMB treated face mask presents the potential exposure routes including, a) dermal contact, b) inhalation of PHMB molecules released from the fabric under the drag force of breathing air on face mask. There is plenty of data on the dermal toxicity of PHMB; whereas few studies have reported inhalation toxicity of aerosolized PHMB molecules. In these studies, the aqueous stocks of PHMB are actively aerosolized using different types of nebulizers. However, no information is available on the release of PHMB from fabric under normal breathing condition and the associated inhalation toxicity, therefore.

EXPERIMENTAL DETAILS

Experiments were performed to assess the potential release of PHMB molecules from Livinguard face mask made of PHMB treated fabric. The experimental system consisted of a Livinguard face mask mounted on funnel (artificial breathing mouth) which was connected to the liquid impinger containing phosphate saline buffer (PBS) or cell culture medium (EMEM) (Figure 1). A peristaltic pump was used to filter air through the face mask and the filtered air was sparged the liquid in impinger. For each type of face mask, the system was continuously operated at air flow rate of 6 L/minute for 12 hours.

The PBS collected from the impinger connected to different types of mask were spiked with *E. coli* (ATCC 25922) at the concentration of 10×10^6 cfu per mL. As a control 100 mL of PBS was inoculated similarly. After one-hour contact time, all the samples were neutralized using D/E neutralizing buffer (Fisher Scientific). All the samples were serially diluted and analyzed on Brilliant selective agar plates. Plates were read after 48-hour incubation at 37°C. Similarly, the cell culture medium collected from the impinger were placed on human lungs fibroblast cells (ATCC CCL-171). Cells were microscopically observed daily for 5 days to note any elicitation of toxic morphology. No difference was noted in the *E. coli* concentration in the control PBS and the PBS retrieved from impinger collecting air passed through different masks (Table 1). The human lungs cells exposed to the cell culture medium sparged with air filtered through Livinguard face mask for 12-hour did not exhibit any toxicity (Figure 2).

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Figure 1. Experimental setup to for collecting the PHMB molecules released from the Livinguard Face Mask under artificial breathing condition

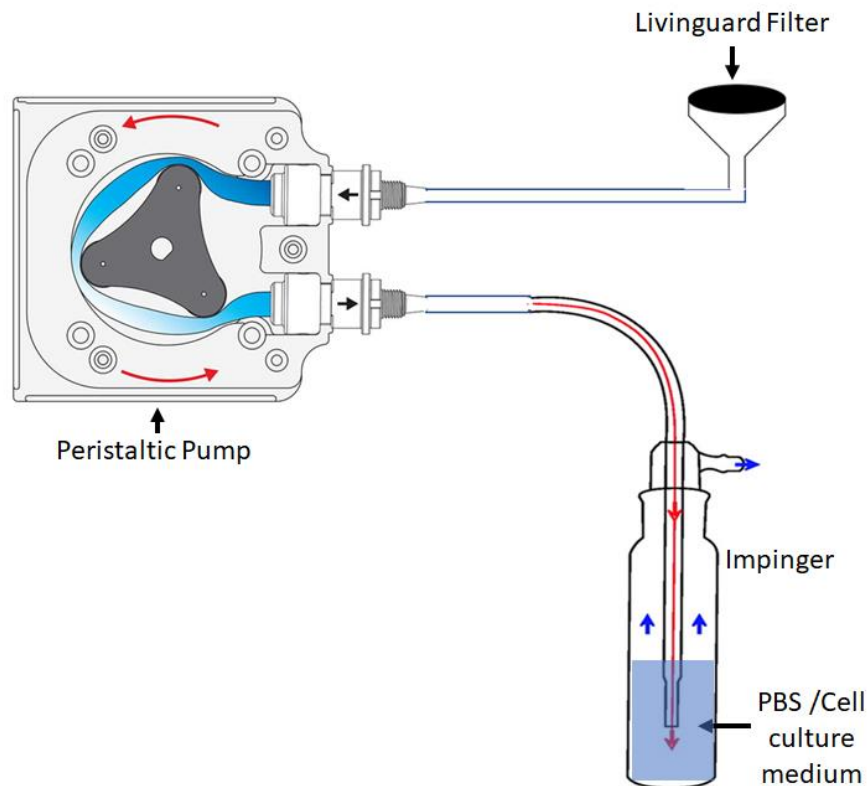


Table 1. Reduction in Bacterial Counts after 2-hour Contact with the PBS Sparged with Air Filtered Through Livinguard Mask

Sample	Initial Concentration CFUs /mL	Reduction in PBS sparged for air filtered through Livinguard Mask
Unwashed mask	3×10^6	0%
30 times washed mask	3×10^6	0%
150 times washed mask	3×10^6	0%
Control	3×10^6	0%

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Figure 2. Human Lung Fibroblast Cells Exposed to Culture Medium Sparged with Air Filtered Through Livinguard Mask



CONCLUSION

These results suggest that air passed through the Livinguard face mask at a rate of 6 L/min for 12 hours did not release PHMB molecule to the concentration which can elicit toxicity in human lung cells. In addition, the air extract from Livinguard face mask did not show and biocidal efficacy against *E. coli*. The data from these experiments suggest that Livinguard face mask does not pose any significant risk of PHMB inhalation during 12-hour continuous use, because PHMB were below detection level.