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Safety assessment of the use of *Bacillus*-based cleaning products

Ninna W. Berg[a,1], Matthew R. Evans[b,2], John Sedivy[c,3], Robert Testman[d], Kimon Acedo[d], Domenic Paone[c,*], David Long[e], Thomas G. Osimitz[f]

[a] Novozymes A/S, Krogshoejvej 36, 2880 Bagsvaerd, Denmark
[b] Novozymes North America, 108 TW Alexander Drive, Building 1A, Durham, NC 27709, USA
[c] Novozymes Biologicals, Inc., 5400 Corporate Circle, Salem, VA 24153, USA
[d] Golden Pacific Laboratories, 4720 West Jennifer Ave, Suite 105, Fresno, CA 93722, USA
[e] Environmental Sustainability Solutions, LLC, 5551 Lake Ridge Trail, Frankfort, MI 49635, USA
[f] Science Strategies, LLC, 1001 East Market Street, Suite 202, Charlottesville, VA 22902, USA

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ABSTRACT

Non-pathogenic *Bacillus* species used in cleaning products produce the appropriate enzymes to degrade stains and soils. However, there is little scientific data regarding the human exposure by inhalation of *Bacillus* spores during or after use of microbial-based cleaning products. Herein, air samples were collected at various locations in a ventilated, carpeted, residential room to determine the air concentration of viable bacteria and spores during and after the application of microbial-based carpet cleaning products containing *Bacillus* spores. The influence of human activities and vacuuming was investigated. Bioaerosol levels associated with use and post-application activities of whole room carpet treatments were elevated during post-application activity, but quickly returned to the indoor background range. Use of trigger spray spot applications generated aerosolized spores in the immediate vicinity, however, their use pattern and the generation of mostly non-respirable particles suggest minimal risks for pulmonary exposure from their use. The aerosol counts associated with use of these microbial-based cleaners were below the recommendation for safe exposure levels to non-pathogenic and non-toxigenic microorganisms except during application of the spot cleaner. The data presented suggest that carpet cleaning products, containing non–pathogenic *Bacillus* spores present a low potential for inhalation exposure and consequently minimal risk of adverse effects.

1. Introduction

1.1. Microbial cleaning applications

Microorganisms have many commercial uses in institutional and consumer products. These include microbial-based cleaning products, which combine traditional cleaning chemistries with microorganisms for an enhanced effect. Established microbial cleaning applications include odor control, hard surface cleaning, stain removal in carpeting and fabric, drain maintenance, and maintenance of grease interceptor and septic systems.

The present work focuses on strains of *Bacillus* species, chosen for inclusion in cleaning products, that are well-characterized, non-pathogenic, non-toxigenic, and meet the generally acknowledged definition of Risk-Group 1 microorganisms. Such organisms are considered to present a low safety risk for people and the environment by US Centers for Disease Control and Prevention guidelines (US CDC, 2009). Several *Bacillus* spp. currently have GRAS (Generally Recognized As Safe) status by the US Food and Drug Administration (FDA) and have a variety of uses as industrial production strains.

1.2. Physiological responses to inhaled bacteria

A concern during the use of microbial-based household cleaners is

Abbreviations: ACH, air changes per hour; ATCC, American Type Culture Collection; BCF, bulked continuous filament; CFU, Colony Forming Units; DMEL, Derived Minimal Effect Level; EFSA, European Food Safety Authority; EU, European Union; GRAS, Generally Recognized As Safe; HEPA, high efficiency particulate air; HP, hypersensitivity pneumonitis; LOQ, limit of quantitation; QPS, Qualified Presumption of Safety; SD, standard deviation; TWA, time weighted average; TLVs, threshold limit values; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Health and Safety Administration; OSH, Occupational Safety and Health

* Corresponding author.
E-mail address: dpao@novozymes.com (D. Paone).
[1] Udbakken 5, DK-2750 Ballerup, Denmark.
[2] 5007 Pomfret Point, Raleigh NC, 27612.
[3] 2028 Hornbeck Ct., Raleigh, NC 27614.

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the potential for inhalation of the microorganisms contained in the products themselves. Although there is a lack of information in the scientific literature regarding the inhalation exposure during use of microbial-based household cleaning products, there is a plethora of data regarding exposure to microorganisms from occupational and environmental sources.

Depending on the susceptibility of the host and a variety of factors, inhalation of microbes or their structural cell wall components can be allergenic, infectious, or can lead to toxic or inflammatory reactions. When considering the potential immune system responses, it is important to remember that exposure to bacteria normally drives a Th1-mediated immune response, while exposure to aerosolized spores from mold and fungi typically produces an allergy-like Th-2 response (Martel et al., 2010). However, inhalation of large amounts of microorganisms may induce inflammatory reactions leading to hypersensitivity pneumonitis (HP), an immunologic disorder, characterized by strong humoral and cellular responses to inhaled antigens, implicating both Type III (IgG mediated) and Type IV (cell mediated) hypersensitivity reactions (Mohr, 2004), rather than the IgE-mediated response that characterizes the Type I hypersensitivity associated with respiratory sensitization.

1.3. Guidelines for acceptable levels of airborne microorganisms

Currently, there are no common internationally accepted threshold limit values (TLVs) for airborne microorganisms in occupational or residential settings. This is likely because it is a complex task and the lack of well-documented dose-response relationships between the exposure to specific levels of microorganisms by inhalation and adverse health effects in humans. However, according to the scientific literature (Eduard et al., 2012; Hansen et al., 2010; Heida et al., 1995) and guidance published from a Canadian research institute focusing on occupational safety (IRSST, 2001, 2007); the general recommendation for non-pathogenic, non-toxigenic microorganisms (total exposure of fungi and bacteria: vegetative cells and spores) during an 8-h time weighted average (TWA) is less than 10,000 CFU/m^3 (= 10^4 CFU/m^3).

The European Agency for Safety and Health at Work has developed an OSHwiki to enable the sharing of occupational safety and health (OSH) knowledge, information, and best practices. It has presented an overview of threshold values for industrial and non-industrial settings in the form of reference values derived from multiple biological agent concentration measurements to provide guidance on the determination of what is typical or acceptable and what is not for a specific type of environment. When it comes to threshold limit values for occupational bioaerosols, the reference values for the total number of bacteria are listed as follows (https://oshwiki.eu/wiki/Biological_agents#Threshold_limit_values_for_occupational_bioaerosols):

- $\leq 1.0 \times 10^3$–7.0×10^3 CFU/m^3 for non-industrial workplaces
- $\leq 7.5 \times 10^2$–1.0×10^7 CFU/m^3 for manufacturing and industrial premises
- for pathogenic microorganisms, there is no safety level (the threshold limit should be 0 CFU/m^3).

The acceptable levels are comparable to the above suggestion, although according to the EU Authority, the bioaerosol exposure to bacteria in the industrial setting might be up to 10^7 CFU/m^3.

1.4. Assessment of exposure to bioaerosols following the use of microbial cleaners

The present study assessed the potential human inhalation exposure to *Bacillus* spores during the use of microbial-based cleaning products. The carpet of a simulated residential exposure room was cleaned and air samples were collected at two heights and various locations to determine the air concentration of viable bacteria and spores during and after application of two microbial-based carpet cleaning products containing selected Bacillus spores. The products were a whole room carpet cleaning product and a spot trigger-spray treatment product. Additionally, the scientific literature was evaluated to determine the normal background airborne bacterial levels in indoor environments.

2. Materials and methods

2.1. Estimation of potential exposure to spores from use of microbial cleaners

The two test products used in this study were:

- Rug Doctor Pet Care Formula, a whole room consumer-use product containing selected *Bacillus* spores that was applied with a standard carpet cleaning machine;
- Bissell Pet Stain and Odor Remover, a spot treatment product containing selected *Bacillus* spores that was applied using a trigger sprayer.

Each of these products are commercially available were formulated with a Novozymes Biologicals commercial *Bacillus* spore blend, were tested simulating the actual recommended application techniques, and were used according to the manufacturer's instructions. Microorganisms contained in these commercially available products include *B, subtilis, B. amyloliquefaciens, B. megaterium* and *B. licheniformis.* Additionally, these products are assessed by quality control and the specific content of viable cells and spores of *Bacillus* spp. and lack of contamination is documented. This study was conducted in a simulated residential exposure room (4.62 m × 4.62 m x 2.43 m or 52 m^3) containing residential plush nylon carpet (Shaw brand 100% BCF nylon, ½" shag) (Fig. 1). The ventilation was set at 0.5 ± 0.1 air changes per hour, a value typical for homes in the United States. The air supplied to the room was pre-filtered through charcoal and HEPA filters and passed through the room on a one-pass basis. Air samples were taken using dry sterilized AGI 30 impingers (Ace Glass, Inc., Vineland, NJ) with air inlets at two heights (one at 1 foot simulating an infant breathing zone, and the second at 5 feet simulating an adult breathing

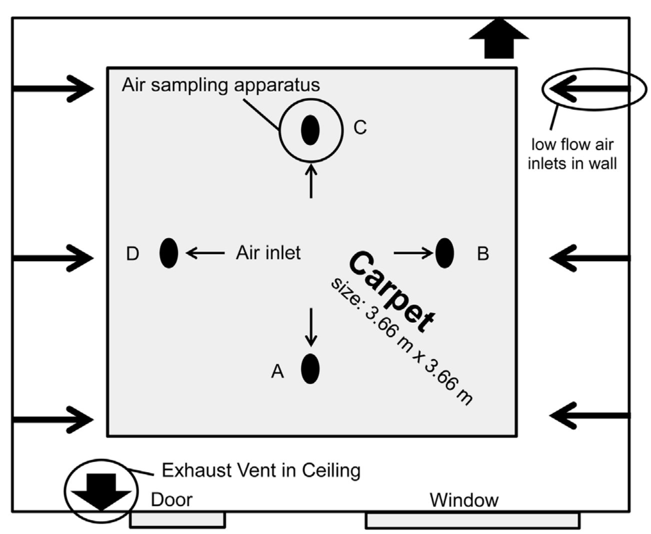

Fig. 1. An illustration of the residential exposure room (4.62 m × 4.62 m x 2.43 m = 52 m^3) with plush nylon carpet. The location of the air impinger apparati for collection of airborne bacteria/spores are illustrated above in locations A through D. The carpet was dried for 48 h after treatment. The trigger spray spot treatment area was 30.5 cm × 30.5 cm (not shown). For the whole room carpet cleaning, four sampling locations were used to collect air samples, while two sampling locations were used for the spot treatment. The air inlet of each impinger faced towards the center of the carpet. For both treatments, the air inlets were at two heights, 1 foot and 5 feet.