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Quantitation of airborne endotoxin levels in various occupational environments

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Endotoxins are heat-stable, lipopolysaccharide-protein complexes that are integral parts of the cell walls of gram-negative bacteria (12). They are released into the environment after cell lysis and are known to exert profound effects on both humoral and cellular components of host mediation systems (1, 8). Complement and coagulation systems are affected by endotoxins, and direct interactions with basophils, mast cells, endothelial cells, macrophages, platelets, polymorphonuclear leukocytes, and T and B lymphocytes have been reported (1, 8). The primary target cell for endotoxin-induced damage by inhalation is the pulmonary macrophage (11), and human macrophages in particular are extremely sensitive to the effects of endotoxins in vitro (3).

Respiratory and other systemic signs and symptoms that are suggestive of exposure to airborne endotoxins have been reported for workers in swine confinement buildings (4, 7) and sewage treatment plants (6). Chest tightness, cough, shortness of breath, fever, and wheezing are only a few of the reported reactions. Functional changes after exposure to endotoxin-containing cotton dusts have been reported as well. In one study of card-generated cotton dusts, there was a high correlation between the acute pulmonary function change in humans and the endotoxins in the elutriated dusts (2).

The laboratories of the National Institute for Occupational Safety and Health have quantitated the levels of endotoxins in a variety of industrial and, most notably, agricultural settings. Exposure was assessed in such environments as farming (including silage unloading and bedding production), cotton textile and nontextile operations, animal confinement and processing, grain storage, peanut shelling, mushroom growing, and work with biotechnology in the United States, as well as in rice production and cotton textile mills in the Peoples Republic of China. Airborne dusts and bulk samples were collected by a variety of standard techniques. The type of filter used for collecting dusts did not seem to affect the analyses for endotoxin levels (10), although proper control filters needed to be analyzed in parallel with those that contained the sample. Sterile plasticware was used throughout the assays. The standard procedure for extracting the filters began with rocking the filter in sterile, nonpyrogenic water (Travenol Laboratories, Deerfield, Illinois) at room temperature for 60 min. Samples of dusts or bulk materials were extracted with a similar procedure, but the relative volumes were changed to approximately 250 mg in 10 to 25 ml of water. The extracts were then decanted and centrifuged at 1 000 *g* for 10 min. The supernatant fluids were frozen at −80°C or assayed immediately in duplicate. The endotoxin concentration was quantitated with the *Limulus* amebocyte lysate assay, first with the spectrophotometric modification, and more recently with the quantitative chromogenic method currently recommended for organic dusts (10). The data were compared to standard endotoxins, and the results reported in terms of nanograms or endotoxin units (EU) per unit of dust or air.

Endotoxin concentrations in bulk samples from selected industrial environments are shown in table 1. The oat and mixture (oat, soybean, and cotton) samples were obtained from a storage bin that was being unloaded. The corn silage data demonstrate the changes in endotoxin contamination from the surface (spoiled or moldy) to the deeper (good) feed silage. Since surface silage is removed by hand prior to the feeding of animals, it involves the greatest exposure to airborne endotoxins (9). The snow inducer listed in table 1 was made from the gram-negative bacterium *Pseudomonas syringae* and was obtained in pellets. The remarkably high level of endotoxin would be expected, but it points also to the potential hazard to workers and end-users of the agent. The mushroom farm samples show the unique potential for endotoxin exposure due to the variety of materials used in the growing of mushrooms.

Table 2 presents data from airborne dusts that were generated acoustically in the laboratory (5) or collected at the workplace. For comparisons with table 1, the data are presented in terms of endotoxin units per milligram of airborne dust. The oat and mixture samples show a greater endotoxin contamination of airborne dusts than the bulk material samples in table 1. The removal of the top layer generated airborne dusts with a relatively high endotoxin content. The levels of endotoxins per volume of air was 13 219.30 EU/m^3 in the respirable dust and 88 502.50 EU/m^3 in the total dust sample. The endotoxin level in the respirable dust far exceeded the calculated threshold of 90 EU/m^3 (converted from 9 ng/m^3) in vertical elutriated cotton dusts which was described for a zero mean response of the forced expiratory volume in 1 s (no change in

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acute pulmonary response) after exposure. Finally, the samples from a pilot peanut shelling operation also demonstrated the presence of airborne endotoxins in fractionated dusts.

The presented results illustrate the ubiquitous presence of endotoxins and demonstrate the potential for respiratory impairment in agricultural workers exposed to dusts containing these agents. The quantitation of endotoxin levels in the air and in bulk materials from various occupational environments and the subsequent assessment of their effect on the respiratory health of the workers should lead to a definition of the mechanism of disease production and also to the development of appropriate dust control and protective measures.

Table 1. Endotoxin concentrations in bulk samples from selected industrial environments.

Environment and location	Endotoxin (EU/mg)
Grain storage bin, Alabama	
Oats	122.66
Mixture (oat, soybean, cotton)	173.87
Tower silo, New York	
Corn silage	
Surface	104.07
15—20 cm under surface	6.79
46 cm under surface	1.09
Biotechnology, California	
Snow inducer	50 304.84
Mushroom farm, Florida	
Preflush	611.28
Chicken manure	220.37
Spawn	31.08
Spawn mate	1.12

Table 2. Endotoxin concentrations in airborne samples from selected industrial environments.

Environment and location	Endotoxin (EU/mg)
Grain storage bin, Alabama	
Oats[a]	325.71
Mixture (oat, soybean, cotton)[a]	313.01
Tower silo, New York	
Hay silage	
Respirable	463.70[b]
Total	872.70[b]
Peanut shelling, Georgia	
Exhaust dust	
>10 mm	226.10[b]
<10 mm	251.80[b]

[a] Dust acoustically generated in the laboratory.
[b] Mathematically converted to endotoxin units from nanograms.

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