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Journal of Toxicology and Environmental Health, Part A Current Issues

ISSN: 0098-4108 (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/uteh19

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Stephen A. Olenchock , David C. Christiani , Judith C. Mull , Shen Yi-e & Lu Pei-lian

To cite this article: Stephen A. Olenchock , David C. Christiani , Judith C. Mull , Shen Yi-e & Lu Pei-lian (1984) Airborne endotoxins in a rice production commune in the people's republic of China, Journal of Toxicology and Environmental Health, Part A Current Issues, 13:4-6, 545-551, DOI: 10.1080/15287398409530519

To link to this article: https://doi.org/10.1080/15287398409530519

AIRBORNE ENDOTOXINS IN A RICE PRODUCTION COMMUNE IN THE PEOPLE'S REPUBLIC OF CHINA

Stephen A. Olenchock

Immunology Section, Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Morgantown, West Virginia

David C. Christiani

Occupational Health Program, Harvard School of Public Health, Boston, Massachusetts, and Norfolk County Hospital, South Braintree, Massachusetts

Judith C. Mull

Immunology Section, Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Morgantown, West Virginia

Shen Yi-e and Lu Pei-lian

Pneumonoconioses Section, School of Public Health, Shanghai First Medical College, Shanghai, People's Republic of China

Concentrations of total and vertical elutriated airborne dusts were quantified for five work areas within a rice production commune near Shanghai, the People's Republic of China. Mean (± SE) commune levels of 15.69 ± 1.91 mg/m³ and 6.00 ± 1.47 mg/m³ were found for total and vertical elutriated dusts, respectively. Analysis of the airborne dusts for the presence of gram-negative bacterial endotoxins resulted in mean levels of 21.23 ± 8.63 ng/mg total dust and 14.57 ± 7.97 ng/mg elutriated dust, with respective airborne concentrations of 492.12 and 100.22 ng endotoxin/m³. These data show that rice production in the People's Republic of China is associated with a dust burden that is contaminated with relatively high levels of endotoxins. The long-term respiratory effects of inhalation of these dusts should be studied.

INTRODUCTION

Respiratory exposures to potentially immunotoxic dusts occur in a variety of occupations, especially in those that generate airborne vegetable dusts. Workers from farms to port grain terminals can experience dust exposures and subsequent lung disease from handling grain. Acute and

The authors thank Beverly J. Wilhelm for help in preparing this manuscript and the workers and staff of the rice commune for their cooperation in this study.

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Requests for reprints should be sent to Stephen A. Olenchock, Chief, Immunology Section, DHHS, NIOSH, 944 Chestnut Ridge Road, Morgantown, West Virginia 26505.

Journal of Toxicology and Environmental Health, 13:545-551, 1984

chronic respiratory effects of grain dust exposures can include such responses as farmer's lung (Pepys, 1969), grain fever syndrome (doPico et al., 1982), chronic bronchitis (Dosman et al., 1980), or asthma (Chan-Yeung et al., 1979). Environmental assessment of grain facilities showed the presence of gram-negative bacteria in the airborne dusts (Dutkiewicz, 1978), and our laboratories quantified the presence of biologically active endotoxins (Olenchock et al., 1980). Gram-negative bacterial endotoxins are lipopolysaccharide-protein complexes that comprise an integral part of the bacterial cell walls (Windholz et al., 1976). They can elicit profound immunotoxic and immunomodulating effects *in vitro* and *in vivo* (Morrison and Ulevitch, 1978; Bradley, 1979), and therefore can exacerbate adverse pulmonary reactions to grain dusts. Furthermore, pulmonary function decrements were shown to correlate better with airborne endotoxin levels than with gravimetric dust sampling during experimental exposures to airborne cotton dusts (Olenchock et al., 1983a). It is therefore of interest to evaluate the occupational environment for the presence of endotoxins in airborne vegetable dusts, not only because of the inherent toxicity of endotoxins but also because of the potential use of endotoxin levels as measures of "toxicity" or "cleanliness" of airborne dusts (Olenchock et al., 1983a).

The purpose of this paper is to report the airborne levels of gram-negative bacterial endotoxins in a rice production commune in which the processes and dust control technologies differ from those found in the United States. We report also the gravimetric dust concentrations in various areas of the plant.

MATERIALS AND METHODS

Environment

The commune that we studied is located in the countryside outside of Shanghai and has approximately 40 workers involved in various aspects of rice production and processing. Crude grain is sifted to remove debris, then processed to remove the coat and skin. The coat is taken to a warehouse to be used as feed for swine, while the skin is powdered and used as medicine. The remaining grain is processed further and used as a major food source. The rice production plant consists of four floors, each 20 m X 8 m X 3.6 m in dimension. There was little air turbulence, and air was exhausted into cyclone collectors.

The average temperature during the sampling months of March and April was 16°C, and the average relative humidity was 73.4%. No sampling was performed on rainy days because work was reduced or ceased.

Dust Sampling

Total dust samples were collected at the breathing zone on 37-mm, 5-μm pore size polyvinyl chloride filters (Millipore Corp., Bedford, Mass.).

Sample time was 10 min at a flow rate of 18 l/min. Vertical elutriated dusts of approximately 15 μm and smaller aerodynamic size were collected in areas of least air turbulence on the same filter type, but the sampling time was 180 min and the flow rate was 7.3 l/min. The filters were wrapped individually, dust-face down, in Parafilm M laboratory film (American Can Co., Greenwich, Conn.) and mailed to our laboratories in the United States.

Endotoxin Analyses

Filters and the respective Parafilm M wrappers were extracted separately by rocking in 10 ml sterile, nonpyrogenic water (Travenol Laboratories, Inc., Morton Grove, Ill.) for 60 min at room temperature. Extracts were centrifuged at 1000 X g for 10 min, and the supernatant fluids were stored at −80°C until assayed. Sterile, nonpyrogenic plastic ware was used throughout these analyses. Gram-negative bacterial endotoxin levels in the supernatant fluids were quantified in duplicate by a spectrophotometric modification of the *Limulus* amebocyte lysate gel test (Pyrostat; Millipore Corp.). The results were analyzed by linear regression, compared to a standard curve, and reported in terms of nanograms of United States reference endotoxin. Endotoxin levels for each filter and its respective Parafilm M wrapper were combined and reported as a single value for that sample. Unused filters and Parafilm M were treated similarly and used as negative controls.

RESULTS

Total dust concentrations collected at the breathing zone ranged from a low of 11.39 mg/m^3 in the grinding operations to a high of 25.00 mg/m^3 for waste collection (Table 1). An overall plant mean and standard error

TABLE 1. Concentration of Total Dust and Endotoxins in Air in a Rice Production Commune

Area (*N*)	Dust concentration (mg/m^3)	Endotoxin[a]	
		Dust (ng/mg)	Air (ng/m^3)
Coarse sifting (2)	18.34 ± 3.35[b]	3.20 ± 0.11[b]	58.23 ± 8.76[b]
Grinding (4)	11.39 ± 1.68	4.03 ± 1.15	48.56 ± 15.95
Hulling (4)	19.03 ± 4.78	51.19 ± 25.67	1341.28 ± 757.06
Fine screening (2)	13.33 ± 0	22.92 ± 2.06	305.46 ± 24.48
Waste collection (2)	25.00 ± 0	12.06 ± 1.67	301.50 ± 59.04
Overall (14)	15.69 ± 1.91	21.23 ± 8.63	492.12 ± 246.80

[a]U. S. reference endotoxin concentrations presented in terms of dust weight (ng/mg) and air volume (ng/m^3).
[b]Mean ± SE. Each sample (*N*) assayed in duplicate.

TABLE 2. Concentration of Vertical Elutriated Dust and Endotoxins in Air and Comparison with Levels of Total Dust

	Dust		Endotoxin		
Area	Concentration (mg/m^3)	% Of total dust (%)[a]	Dust (ng/mg)	Air (ng/m^3)	% Endotoxin in total dust (%)[b]
Coarse sifting	10.33[c]	56.3	2.70[c]	27.89[c]	47.9
Grinding	3.72	32.7	5.60	20.83	42.9
Hulling	8.44	44.4	53.33	450.11	33.6
Fine screening	3.75	28.1	15.63	58.61	19.2
Waste collection	4.89[d]	19.6	5.08[d]	21.95[d]	7.3
Overall	6.00 ± 1.47[e]	38.2	14.57 ± 7.97[e]	100.22 ± 70.33[e]	20.4

[a]Percent of total dust that is vertical elutriated dust.
[b]Percent of endotoxin in total dust that is vertical elutriated endotoxin.
[c]Average value from duplicate assays.
[d]Average of two filters assayed in duplicate.
[e]Mean ± SE ($N = 6$).

of 15.69 ± 1.91 mg/m^3 was calculated for all operations. All total dust samples contained quantifiable endotoxins. The dusts varied from 3.20 ng endotoxin/mg dust from the sifting operations, to 51.19 ng/mg in the hulling process, with an overall plant mean (± SE) of 21.23 ± 8.63 ng/mg. Calculation of the airborne concentration of gram-negative bacterial endotoxins resulted in levels that ranged from 48.56 ng/m^3 in the grinding area to 1341.28 ng/m^3 in the hulling area. The overall plant mean (± SE) for airborne endotoxins was 492.12 ± 246.80 ng/m^3. Ranking of the different processes by dust concentration resulted in waste collection as the most dusty, followed by hulling, coarse sifting, fine screening, and grinding, the least dusty. By comparison, ranking of the dust or air by endotoxin contamination resulted in hulling as the most contaminated area, followed by screening and waste collection. Grinding and sifting areas were of similar magnitude of endotoxin contamination.

Vertical elutriated dust concentrations and endotoxin contents of the dusts and air are presented in Table 2. The lowest levels of vertical elutriated dusts were found in the grinding and fine screening operations (3.72 and 3.75 mg/m^3, respectively), and the highest level was found in the coarse sifting area (10.33 mg/m^3). The most highly endotoxin-contaminated dust was found in the vertical elutriator filter from the hulling area (53.33 ng/mg), and the least contaminated was from coarse sifting operations (2.70 ng/mg). Airborne levels of endotoxins ranged from 20.83 ng/m^3 in the grinding area to 450.11 ng/m^3 in the hulling area. The overall plant mean vertical elutriated dust concentration of 6.00 mg/m^3 was 38.2% of the mean total dust level, while the vertical elutriated endotoxin concentration of 100 ng/m^3 was 20.4% of the total dust endotoxin level.

DISCUSSION AND CONCLUSIONS

Grain processing and storage workers are exposed to airborne dusts that are heterogeneous in composition (Cotton and Dosman, 1978), and levels of the dusts vary with the work area. A study of grain-elevator work areas in the Canadian Prairie Provinces found mean total airborne dust concentrations that ranged from 1.36 to 109.0 mg/m^3 (Farant and Moore, 1980). Respirable dust levels in that study were reported as 0.34–13.7 mg/m^3. Mean airborne total dust in grain elevators in the Port of Vancouver, British Columbia, ranged from 5.15–17.62 mg/m^3 as measured by area samplers (Chan-Yeung et al., 1981), while personal samplers collected 3.68–17.20 mg total dust/m^3 in that same study. Personal dust samples were taken also during a study of grain handlers in Saskatchewan, Canada. Corey et al. (1982) found nonrespirable dust levels of 3.8–8.6 mg/m^3 and respirable dust levels of 0.6–1.3 mg/m^3. Grain handlers in port grain terminals in the Superior-Duluth areas of the United States were exposed to mean total dust concentrations of 3.29 mg/m^3 as observed by doPico et al. (1983). Health evaluations of the workers lead doPico et al. (1983) to conclude that the dust concentrations that they observed were related to adverse health effects.

Mean total dust levels that we report herein for the rice production commune in the People's Republic of China ranged from 13.33 to 25.00 mg/m^3, which were higher than those reported in the United States (doPico et al., 1983). They were likewise generally at the higher end of the ranges reported for area or personal sampler total dust in the Port of Vancouver (Chan-Yeung et al., 1981), and they were lower than some found in the Canadian Prairie Provinces (Farant and Moore, 1980). By comparison, the vertical elutriated dust levels in the People's Republic of China were higher (3.72–10.33 mg/m^3) than the respirable samples that were found in Saskatchewan (Corey et al., 1982) and were higher than most areas in the study of grain elevators in the Canadian Prairie Provinces (Farant and Moore, 1980). It should be noted, however, that the processes that were performed in the rice production commune differed markedly from those in the other studies. The latter represent predominantly storage facilities, while the rice commune processed and stored the rice and related materials.

Contamination of airborne dust with gram-negative bacterial endotoxins was markedly greater in the total dust from the rice commune in China (21.23 ng/mg) than in total grain dust (0.33–0.44 ng/mg) that was collected in port grain terminals in the northern United States (Olenchock et al., 1980). In fact, aged settled grain dust contained only 0.43 ng/mg (Olenchock et al., 1978). Likewise, vertical elutriated rice dust contained a high level of endotoxins (14.57 ng/mg). Calculation of the airborne burden of endotoxins in the rice commune resulted in means of 492.12 ng/m^3 for total dust and 100.22 ng/m^3 for vertical elutriated dust. While no data are available to compare these concentrations directly with those found in other grain dust exposures, other occupational dust exposures can be examined. The air of a poultry processing plant in the United States contained from 634 to 918 ng endotoxins/m^3 air (Olenchock et al., 1982). In that same study, respirable dust samples contained 33–44 ng/m^3. Air in swine-confinement or poultry-confinement units in Sweden contained endotoxin levels in total dust of 120 or 310 ng/m^3, respectively (Clark et al., 1983). Experimental generation of dusts from carded cottons resulted in airborne endotoxin levels of 23–202 ng/m^3 when standard cottons were used (Olenchock et al., 1983c). By more direct comparison to another industry in the Shanghai area, cotton textile manufacturing, the airborne endotoxins in the vertical elutriated rice dusts were comparable to those in one textile mill (100.5 ng/m^3); however, they were threefold less than those (331.5 ng/m^3) in another textile mill (Olenchock et al., 1983b).

The data presented in this study show that total and vertical elutriated dust levels in a rice production commune in the People's Republic of China are, for the most part, as high as or higher than levels reported for grain storage facilities in other parts of the world. Quantification of the presence of biologically active endotoxins showed that the air in the rice commune contained considerable amounts of endotoxins when compared to airborne concentrations from animal confinement and processing plants, as well as from cotton carding. The respiratory effects of long-term inhalation of rice dusts and their associated endotoxins by workers should be examined. Inhalation of rice-husk dust by laboratory rats resulted in changes in the lung architecture, such as bronchiectasis, bronchiolectasis, bronchiolitis, interstitial pneumonitis, and focal emphysema (Sood and Prabhakar, 1978). Likewise, rice has been shown to contain allergenic and lymphocyte-stimulating materials (Shibasaki et al., 1979) and was associated with serum complement depressions *in vivo* (Strunk et al., 1978). The biological actions of endotoxins in the rice dusts would be expected to exacerbate these rice dust-related effects, and the potential for respiratory pathophysiology in the workers exists. Rice processing in the People's Republic of China is associated with a dust burden that is contaminated with relatively high amounts of gram-negative bacterial endotoxins.

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Received January 1, 1984
Accepted January 23, 1984