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OCCUPATIONAL EXPOSURE TO AIRBORNE ENDOTOXINS DURING POULTRY PROCESSING

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Airborne gram-negative bacterial endotoxin levels were quantified in a live chicken hanging (shackling) room of a poultry processing plant. The mean respirable dust levels at the entrance and exit of the shackling line were 1.13 ± 0.12 and 0.72 ± 0.06 mg/m³, respectively, or approximately 6% of the total dust. Endotoxins constituted 43.3 ± 2.8 µg per gram of respirable dust. Airborne endotoxins were present in the total dust at the mean level of 918.4 ± 159.0 ng/m³ at the room entrance and 634.0 ± 96.9 ng/m³ at the exit, with respirable levels of 44.3 ± 7.8 and 33.6 ± 2.2 ng/m³. Inhalation of gram-negative bacterial endotoxins can result in respiratory and systemic pathophysiology. The potential for adverse health effects in the working environment of the live poultry processing industry is discussed. Medical studies of workers in this area are required to confirm or deny the existence of occupationally related health effects.

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INTRODUCTION

Gram-negative bacteria and their endotoxins are present in many occupationally related organic dusts. Occupational exposures to dusts from animal confinement units (Dutkiewicz, 1978; Thedell et al., 1980), cotton (Pernis et al., 1961; Rylander and Lundholm, 1978), compost (Lundholm and Rylander, 1980), cereal grains (Dutkiewicz, 1978; Olenchock et al., 1980), and sewage (Mattsby and Rylander, 1978) contain large numbers of bacteria and related endotoxins, Predominant clinical signs and symptoms in endotoxin-exposed workers include fever, eye irritation, diarrhea, and fatigue (Mattsby and Rylander, 1978) and headache, nausea, cough, nasal irritation, chest tightness, and phlegm (Donham et al., 1977). Effects of laboratory animal exposures to inhaled endotoxins tend to correlate well with workers' signs and symptoms. Snell (1966) showed that rabbits developed fever, circulating leukopenia, and striking histopathologic changes in the lung after challenge with an aerosol of endotoxin. Rylander et al. (1980) demonstrated significantly increased numbers of leukocytes in the airways of rats after exposure to aerosolized purified Escherichia coli endotoxin, and Hudson et al. (1977) showed granulocyte recruitment to airways after guinea pigs were challenged with aerosolized endotoxin from Salmonella typhosa. As evidence for functional impairment, DeMaria and Burrell (1980) showed that following inhalation of purified endotoxin of endotoxin-containing bacteria, rabbits responded with marked changes in arterial O2 tensions, while studies of the in vitro effects of endotoxins showed that human alveolar macrophages were extremely sensitive to endotoxin effects (Davis et al., 1980). The lung therefore serves not only as a portal of entry and absorbance of endotoxins for systemic alteration of normal functions, but also as a site of localized tissue and cellular damage following inhalation of gram-negative bacterial endotoxins.

Our preliminary study of animal confinement units suggested relatively high endotoxinlike activity in both airborne and settled dusts in swine and poultry units (Thedell et al., 1980). It is our purpose in this paper to examine in depth the occupational environment of workers who hang live chickens in a poultry processing plant. Specifically, gram-negative bacterial endotoxin levels in total and respirable dusts will be quantified.

MATERIALS AND METHODS

Environment

The poultry processing plant that we studied is located in North Carolina. The room in which all general area samples were collected is approximately 2.1 m wide, 9.1 m long, and 2.7 m high. Eleven people work in the room by removing live chickens from crates and hanging the birds

by their feet in overhead, moving shackles. Full crates enter from one side of the room and exit empty at the other side. The shackle line runs parallel with the crate line. Air movement in the roofless hanging room was very turbulent, probably due to 1.2-m-diameter propeller fans above the room and air-exhaust ducts that blow air into the room.

Dust Sampling

The least turbulent air movement was found at the line entrance and exit points. All dust samples were taken at those spots with the intake orifice of each filter holder positioned at the worker breathing zone. Filters, either polyvinyl chloride VM-1 (5 µm pore size) or DM-800 polyvinyl chloride copolymer (0.8 µm pore size), were obtained commercially (Gelman Sciences, Inc., Ann Arbor, Mich.), housed in a standard 37-mm plastic cassette, and used in the closed-face configuration for total dust sampling with a calibrated model G suction pump (Mine Safety Appliances Co., Pittsburgh, Pa.). All samples were collected during a 2-wk period in July in accordance with National Institute for Occupational Safety and Health (NIOSH) Sampling Data Sheet 29.02 (NIOSH, 1977) at a flow rate of 1.5 l/min, and the filters were weighed before and after use on the same Mettler model HL52 balance (Mettler Instrument Corp., Princeton, N.J.). Sampling periods for total dust samples ranged from 150 to 260 min and an entire shift was monitored.

Samples of respirable dust (defined by ACGIH, 1980) were collected for 330-510 min according to the recommended sampling procedures of the Aerosol Technology Committee of the American Industrial Hygiene Association (Anderson et al., 1970). A Dorr-Oliver 10-mm two-piece nylon cyclone (Mine Safety Appliances Co.) was used to separate respirable dust from total dust according to the ACGIH performance criteria for size selectors. A calibrated model G pump (Mine Safety Appliances Co.) was used as a suction source at a flow rate of 1.7 I/min. Three total dust samples and one respirable sample were taken at the entrance and exit each day.

Endotoxin Analyses

After the final weight was determined, each filter was placed in a screw-capped, sterile, nonpyrogenic plastic 50-ml centrifuge tube (Millipore Corp., Bedford, Mass.) and transported to the laboratory. Each filter was extracted with 10 ml sterile non-pyrogenic water (Travenol Laboratories, Inc., Morton Grove, Ill.) by rocking at room temperature for 60 min. Extracts were centrifuged at 1000g for 10 min and supernatant fluids were stored at -88° C until use. Unused filters were treated similarly and used as controls.

Sterile, nonpyrogenic plastic ware was used throughout the study. Endotoxin analyses were performed with a spectrophotometric modification of the *Limulus* amebocyte lysate (LAL) gel test (Pyrostat; Worthing-

342 S. A. OLENCHOCK ET AL.

ton Biochemical Corp., Freehold, N.J.), which is capable of detecting 0.1 ng *Klebsiella* endotoxin equivalents per milliliter. All data were analyzed by linear regression and compared to a standard curve obtained from an *E. coli* reference endotoxin, which was calibrated against the Food and Drug Administration (FDA) reference *Klebsiella* endotoxin. Supernatant fluids from respirable filter samples were assayed at a dilution of 1:25, while those from total dust filters were diluted 1:100 in water.

Statistics

Statistical comparisons of data were done with Student's t-test at the 95% confidence level. Time-weighted averages (TWAs) were calculated as defined by Leidel et al. (1977).

RESULTS

Dust Levels

Twenty-three unused filters were reweighed periodically during the 2-wk sampling period; no consistent weight gain or loss was noted. The mean (\pm SE) error for 12 VM-1 filters was 0.04 \pm 0.01 mg, and 11 DM-800 blanks had a mean (\pm SE) error of 0.03 \pm 0.01 mg. Because the error was relatively insignificant, no correction to the collected sample weights was made. Two different filter types were chosen for this study because it was not known at the start whether airborne endotoxins in collected dusts could be extracted readily or whether the filter type affected the elution of endotoxins. Statistical analyses of the effect of filter type (VM-1 or DM-800) on levels of dust or endotoxins showed no significant differences. In the presentation of results we therefore disregard the filter type.

Total dust concentrations at the entrance and exit of the live chicken hanging (shackling) room are shown in Table 1. The mean (\pm SE) TWA at the entrance was 18.78 ± 1.25 mg/m³ with a range of 10.71-24.20 mg/m³. The mean TWA at the exit was 13.83 ± 1.00 mg/m³ (range, 6.42-17.69 mg/m³). Total dust at the room exit was significantly lower (p < 0.05) than at the entrance, probably due to the greater number of birds at the entrance than at the exit. It should also be noted that it rained on July 23, which is reflected in the distinctly lower values at both sampling points.

Total dust concentrations are useful for evaluating worker exposures if no other information is available. However, respirable dust levels were also determined in this study and are shown in Table 2. The total dust levels are used to calculate the percentage of respirable dust contained in the total dust. Mean respirable dust levels were 1.13 ± 0.12 mg/m³ (0.43-1.60 mg/m³) at the entrance and slightly lower at the exit, 0.72 ± 0.06 mg/m³ (0.43-1.00 mg/m³). The difference, unlike that between the total dust levels, was not statistically significant. The respirable dust levels do reflect,

AIRBORNE ENDOTOXINS 343

TABLE 1. Total Dust Concentrations in Shackling Room

		Entrance		Exit		
Date (July 1980)	Sampling time (min)	Concentration (mg/m³)	TWA ^a (mg/m³)	Concentration (mg/m³)	TWA ^a (mg/m³)	
11	260	13.67	17.12	11.31	14.25	
	210	21.40		17.90		
14	150	17.56		14.58		
	150	24.67	22,12	18.62	17.69	
	210	23.56		16.82		
15	150	14.31		13.96		
	150	16.49	17.48	19.42	15.92	
	190	20.77		14.70		
16	150	14.71		_		
	150	17.29	17.25	8.93	15.30	
	175	19.39		20.76		
17	150	15.38		11.69		
	150	29.82	24.20	14.98	12.04	
	210	26.48		10.19		
18	150	10.13		9.33		
	150	23.16	17.91	15.29	12.38	
	195	19.86		12.48		
21	150	12.80		9.42		
	150	23.29	20.39	14.58	13.10	
	195	24.00		14.80		
22	150	10.18		9.91		
	150	27.16	23.53	13.11	15.40	
	180	31.63		21.89		
23^{b}	150	10.58		5.78		
	150	10.71	10.71	6.44	6.42	
	165	10.83		6.99		
24	150	10.80		-		
	150	17.45	17.07	14.71	15.81	
	175	22.10		16.76		
Mean TWA ^C			18.78		13.83	
			± 1.25		± 1.00	
					(p < 0.05)	

^aTime-weighted average; dashes indicate not done.

however, the effect of rain on July 23. In addition, the mean percentage of respirable dust in the TWA of total dust was similar at the entrance $(6.1 \pm 0.6\%)$ and the exit $(5.3 \pm 0.3\%)$, which indicates that the respirable fraction remained constant in the shackling room.

Endotoxin Concentrations

To evaluate the potential for physiological impairment from the dust exposure, concentrations of gram-negative bacterial endotoxins in the dust

b Rained.

^cMean ± SE. Values were significantly different.

344 S. A. OLENCHOCK ET AL.

TABLE 2. Respirable Dust (Concentrations in	Shackling Room
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Date (July 1980)	Sampling time (min)	Entrar	ice	Exit		
		Concentration (mg/m³)	Total dust TWA ^a (%)	Concentration (mg/m³)	Total dust TWA ^d (%)	
11	470	1.60	9.3	_		
14	510	_	_	1.00	5.6	
15	490	1.06	6.1	0.97	6.1	
16	475	1.32	7.6	0.73	4.8	
17	510	1.10	4.5	0.68	5.6	
18	495	1.09	6.1	0.69	5.6	
21	495	1.16	5.7	0.71	5.4	
22	330	1 . 59	6.8	0.62	4.0	
23^{b}	465	0.43	4.0	0.43	6.7	
24	475	0.80	4.7	0.63	4.0	
Mean concentr	Mean concentration ^C		6.1	0.72	5.3	
		± 0.12	± 0.6	± 0.06 (NS)	± 0.3	

^aTime-weighted average; dashes indicate not done.

were determined. The LAL test was used to quantify endotoxin contamination, and all results are reported as FDA *Klebsiella* endotoxin equivalents since a purified lipopolysaccharide extracted from a *Klebsiella* species is used by the FDA Bureau of Biologics as an endotoxin standard (Selzer, 1970). Blank filters of both types were found to contain <0.2 ng endotoxin per milliliter of extract. All dust samples, however, contained appreciable amounts of endotoxins. Table 3 shows the endotoxin concentrations for the total dust samples at the entrance and exit of the shackling room. The mean (\pm SE) TWAs for total dust endotoxin concentrations were 918.4 \pm 159.0 ng/m³ (414.7-2150.2 ng/m³) at the entrance and 634.0 \pm 96.9 ng/m³ (379.3-1449.8 ng/m³) at the exit. The difference between the two sampling points was not statistically significant.

Endotoxins in respirable dust fractions were, of course, much lower, since the respirable dust fraction was less. Table 4 shows a mean endotoxin concentration of 44.3 ± 7.8 ng/m³ (16.8-85.8 ng/m³) at the entrance and 33.6 ± 2.2 ng/m³ (25.2-44.5 ng/m³) at the exit. As with the endotoxin concentration in the total dust, the difference between entrance and exit levels was not significant. When the endotoxin contamination of the respirable dust is calculated as a percentage of the endotoxin level in the total dust TWA, the percentages are similar at the entrance and exit: 5.8 ± 0.8 and $5.6 \pm 0.5\%$, respectively. Calculation of the overall weight of respirable endotoxin in both total and respirable dusts results in a mean (\pm SE) of $2.5 \pm .0.2$ μ g respirable endotoxin per gram of total dust and 43.3 ± 2.8 μ g respirable endotoxin per gram of respirable dust.

BRained.

^cMean ± SE. NS, not significantly different.

DISCUSSION AND CONCLUSIONS

Exposures to a wide variety of organic dusts in the occupational environment result in the inhalation of many different toxic products of microbial origin. Gram-negative bacteria are ubiquitous contaminants of dusts from agriculture (Dutkiewicz, 1978; Pernis et al., 1961; Rylander

TABLE 3. Airborne $\operatorname{Endotoxin}^a$ Concentrations in Total Dust Samples from Shackling Room

		Entran	ce	Exit		
Date (July 1980)	Sampling time (min)	Concentration (ng/m³)	TWA ^b (ng/m³)	Concentration (ng/m³)	TWA ^b (ng/m³)	
11	260	494.1	524.1	447.7	447.7	
	210	561.3		4		
14	150	_		655,6		
	150	2679.6	2150,2	1612.0	1449.8	
	210	1772.1		1210.8		
15	150	758.2		708.0		
	150	617.8	747.2	703.1	694.1	
	190	840.7		676.1		
16	150	778.7		_		
	150	1574.7	1176,7	595.1	702.4	
	175	_		794,3		
17	150	374.2		444.4		
	150	1343.6	850.9	733,4	588.9	
	210	839.4		_		
18	150	628.0		489.8		
	150	756.0	652.2	409.3	430.7	
	195	591.1		401.7		
21	150	412.0		412.0		
	150	738.2	661.2	615.1	553.2	
	195	793.8		614.3		
22	150	482.2		376.9		
	150	919.1	786.4	456.9	496.8	
	180	929.3		630.0		
23 ^c	150	444.4		-		
	150	447.1	414.7	376.9	379.3	
	165	358.4		381.4		
24	150	_		-		
	150	_	1220.6	406.7	596.7	
	175	1220.6		759.6		
Mean TWA ^d			918.4		634.0	
			± 159.0		± 96.9	
					(NS)	

 $[^]a$ Assayed in duplicate as nanograms of FDA Klebsiella endotoxin equivalents per 10-ml extract of dust; dashes indicate not done.

bTime-weighted average.

CRained

dMean ± SE. NS, not significantly different.

TABLE 4. Airbo	rne Endotoxin ^a	Concentrations	in	Respirable	Dust	Samples	from
Shackling Room							

		Entrance		Exit		
Date (July 1980)	Sampling time (min)	Concentration (ng/m³)	Total endotoxin TWA ^b (%)	Concentration (ng/m³)	Total endotoxin TWA (%)	
11	470	42.8	8.2	_		
14	510	-	_	44.5	3.1	
15	490	-	_	39.6	5.7	
16	475	85.8	7.3	30.2	4.3	
17	510	27.9	3.3	30.7	5.2	
18	495	57.0	8.7	28.9	6.7	
21	495	34.1	5.2	35.9	6.5	
22	330	57.0	7.2	34.0	6.8	
23 ^c	465	16.8	4.0	25.2	6.6	
24	475	33.3	2.7	-	-	
Mean	_	44.3	5.8	33.6	5.6	
concentration ^d		± 7.8	± 0.8	± 2.2 (NS)	± 0.5	

 $^{^{}o}$ Assayed in duplicate as nanograms of FDA *Klebsiella* endotoxin equivalents per 10-ml extract of dust; dashes indicate not done.

and Lundholm, 1978), where their presence is possibly the causative factor in disease production, or at least representative of the "cleanliness" of the airborne dust. Some dusts probably contain larger amounts of gramnegative bacterial endotoxins than others. Specifically, one would expect sewage dust (Mattsby and Rylander, 1978) and animal confinement dust (Thedell et al., 1980) to contain large amounts of fecal matter and therefore significant levels of endotoxins. Our preliminary investigation of endotoxins in animal confinement units (Thedell et al., 1980) showed that settled dust from poultry units contained 11.39 µg bacterial endotoxins per gram of dust, and this was similar to levels found in swine confinement units. Due to the concentrated work force and the redundancy of operation, we focused on the evaluation of airborne endotoxins in the shackling room of a poultry processing unit. The workers in this occupation are but a few of those exposed to similar dusts throughout the poultry processing/growing industry. Baier (1979) estimated that approximately 90,000 workers are employed in poultry dressing plants in the United States. The plant that we studied is typical of others in the area, and approximately 12% of the jobs involve contact with live birds. On a national level, then, the potentially exposed worker population represented by this study is 10,800. This figure is actually an underestimate when one

^bTime-weighted average.

CRained.

dMean ± SE. NS, not significantly different.

considers similar exposures in industries concerned with other birds and products.

It is apparent from the daily total and respirable dust levels that a number of factors affect the dust exposures of individuals. One factor is the effect of atmospheric changes, as seen on July 23. An increase in air moisture or humidity reduced the airborne total and respirable dust levels in the room. The greater number of birds at the shackling line entrance increased the total dust level there compared to that at the exit, but did not alter the respirable fraction. The current federal standard and threshold limit value (TLV) for the respirable fraction of nuisance dusts in the workplace is 5.0 mg/m³ (ACGIH, 1980). Therefore, the respirable dust exposure of workers in this study (approximately 1 mg/m³) was lower than the federal standard. However, the nuisance dust TLV is intended only for application to substances for which no specific threshold limits have been assigned. In order to effectively evaluate potential health risks, one must consider the biologically active fractions of the dusts.

Gram-negative bacterial endotoxins are very active agents (Morrison and Ulevitch, 1978) and the human is one of the most reactive species (Greisman and Hornick, 1969). Endotoxins stimulate a myriad of host metabolic and immunological events, resulting in many of the signs and symptoms seen in exposed workers (Mattsby and Rylander, 1978; Donham et al., 1977). Our study indicates significant levels of gram-negative bacterial endotoxins in the dusts from the chicken shackling room. Washing unused filters in the same way as the dust samples resulted in virtually no detectable endotoxin, which indicates that the filter was not a source of contamination. Daily variations in endotoxin levels no doubt resulted from differences in the source of birds and cages, possible strain variations in the chickens, as well as overall cleanliness in the raising, handling, and transportation of the birds. Respirable levels of endotoxins account for approximately 6% of the TWA of the total dust endotoxin level. Both respirable and total dust contamination, however, may be expected to have biological effects on the host. While respirable particles would be inhaled deeply into the lung, affect the pulmonary macrophages directly (Davis et al., 1980), and be absorbed into the circulation, larger particles would be trapped in the upper respiratory tract and nasopharynx, extracted by the mucous membranes and absorbed as well as transported by the mucociliary escalator and ingested. The lung is well situated to experience large daily doses of endotoxins, providing the potential for a pathophysiologic response.

The levels of endotoxin contamination that we report here $(43.3 \pm 2.8 \mu g/g)$ respirable dust) are higher than those found in settled and airborne dusts in our preliminary study [15.3 ± 6.8 $\mu g/g$ (Thedell et al., 1980)]. This difference is most likely due to variation in sampling techniques and the different occupational setting. In addition, one must be cautious when

interpreting endotoxin levels obtained from the LAL test because substances such as gram-positive cell walls (Kotani et al., 1977), certain proteins and polynucleotides (Elin and Wolff, 1973), and pyrogenic exotoxins (Brunson and Watson, 1976) react nonspecifically in the LAL test. However, the amounts that we observed are of such magnitude that one would be inclined to dismiss the suggestion of nonspecificity as a major component in the reported levels.

In conclusion, we found large levels of airborne gram-negative bacterial endotoxins in the occupational environment of workers in the poultry industry. The potential therefore exists for respiratory and systemic pathophysiology due to the biological activities of endotoxins. Testing of the workers is required to confirm or deny the existence of occupationally related health effects.

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AIRBORNE ENDOTOXINS 349

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