International Journal of Occupational Medicine and Environmental Health Vol. 7, No 2, 125–134, 1994

ENDOTOXIN IN THE OCCUPATIONAL ENVIRONMENT OF BAKERS: METHOD OF DETECTION*

ANNA DOMAŃSKA[1] and GRAŻYNA STROSZEJN-MROWCA[2]

[1]Clinic of Occupational Diseases
[2]Department of Aerosols
The Nofer Institute of Occupational Medicine
Lodz, Poland

Key words: Endotoxin, Limulus test, Flour dust, Gram-negative bacteria, Bakery work sites, Area sampling

Abstract. The present study was conducted in a large mechanical city bakery. The AS-50 aspirators equipped with cellulose membrane filters were used for dust sampling. Airborne microbial content was assessed by means of sedimentation and aspiration using an Andersen sampler on Petri plates containing McConkey's medium. The following Gram-negative rods were detected in the bakery atmosphere: *Erwinia herbicola, Acinetobacter Lwoffi* and *Klebsiella oxytoca*, in concentrations ranging from 1.4 10^4 to 3.5 10^5 colony forming units per cubic meter (cfu/m^3). Endotoxin concentration in flour dust sampled in selected work areas of the bakery ranged from 6.7 micrograms of endotoxin per gram of dust (μg/g) to 20.3 μg/g. Endotoxin level in the air was 0.04–0.05 micrograms of endotoxin per cubic meter (μg/m^3) The results of our study show that aspiration sampling is necessary for evaluation of airborne bacterial content and demonstrate the efficacy of the Limulus test of varying sensitivity to assay endotoxin level in the airborne dust. The advantage of this method is the possibility of assessing endotoxin in crude dust ecxtracts.

INTRODUCTION

Endotoxin, a biologically active lipopolysaccharide (LPS) (20) present in the cell wall of Gram-negative bacteria is a well-known inflammatory agent in the induction of so-called toxic pyrexia (ODTS) and an aggravating stimulus in the course of several allergic diseases such as bronchial asthma, a common work-related condition among bakers (1, 2, 6, 17, 31). It is responsible for impaired respiratory function (lowered ventilation index FEV_1) (3) and circulatory disorders in occupationally exposed persons (8).

*The presented study was conducted and financed as a part of the research programme IMP 19.4. "Introduction of endotoxin assessment by means of LAL tube test-efficacy and sensitivity of the assay".

Address reprint reguests to A. Domańska, Ph.D. Clinic of Occupational Diseases, The Nofer Institute of Occupational Medicine, P.O. Box 199, 90-950 Lodz, Poland.

INSTYTUT MEDYCYNY PRACY IM. PROF. J. NOFERA

The pathogenic role of endotoxin consists in the activation of alveolar macrophages to release pyrogens such as interleukin 1, 6, 8 and other mediators of inflammation such as cachectin, prostaglandins, leukotrienes and others (9, 32). Occupational exposure to endotoxin occurs most frequently in the following branches of industry: textile (12, 15, 22, 23, 27), food processing (16), timber (7, 25), agriculture (21) and agricultural processing (13, 14, 24, 29, 33), including the baking industry (6, 31).

Studies of environmental endotoxin content have been introduced only recently. Endotoxin concentrations in the atmosphere of different work areas vary over a wide range of values. For example, 0.2–1870 μg/m³ in the animal feed industry in Holland (29); 54.93 μg/m³ in grain mills in Poland (8). In the poultry slaughter house endotoxin concentration in the air was 0.02–1.5 μg/m³ (14). In the house dust endotoxin concentration measured with the Limulus test was found to be in the range of 0.12–20 μg/mg (19). In the presence of domestic air humidification systems, the level of endotoxin varies between 0.13 and 0.39 μg/m³ (11, 26).

Flour dust is the main source of endotoxin in bakeries. Surveys conducted during the 1980s in the Polish baking industry showed that the concentrations of flour dust frequently exceeded admissible levels. Respiratory and skin diseases attributed to fluor dust allergens were found to occur in 8–44% of all currently employed bakers. According to what was then a generally accepted point of view (6), the respiratory disorder in bakers was essentially of allergic nature and the characteristic disease syndrome was called “bakers’ asthma”. The presumed causal agents of the disease were allergens present in fluor dust, mainly bacteria, spores of Actinomycetes and molds, as well as storage mites (e.g. Tyroglyphus and others) (6).

The investigations conducted in Britain in 1992 confirmed that the increase in immunological reactivity and airway responsiveness in bakers correlate with increased concentrations of flour dust (31).

The pathogenic properties of flour dust can be, at least, partly explained, by the presence of Gram-negative endotoxic bacteria often found in grain from which flour is obtained. It has been shown recently that plant enzymes such as alpha-amylase can act as pathogenes, thus contributing to the allergenic properties of fluor dust. These enzymes proved to be an important factor in the pathogenesis of allergy in bakers (1, 2).

Some countries have adopted a hygienic standard of exposure to endotoxin-containing organic dust (the Occupational Exposure Limit = OEL). For example, in Denmark, OEL is equal to 5 mg/m³ (33), in the USA and Canada 3 mg/m³ and in Holland 4 mg/m³ (29); in Poland, according to the updated OEL documentation for dust of plant and animal origin (12), the proposed limit is 4 mg/m³ for total dust and 2.0 mg/m³ for respirable dust containing less than 10% of free silica.

Gram-negative bacteria found on the surface of vegetal raw materials such as grain, herbs, or cotton are the sources of endotoxin in organic dust (such as fluor dust) (5). The most commonly identified bacterial species occurring in organic dust are: *Erwinia herbicola* and *Alcaligenes faecalis.*

Erwinia herbicola or (synonimously), *Enterobacter aglomerans,* occurs in its natural environment on the surface of fruit, seed, and leaves of various plants, particularly on cereal leaves and stipules of cotton bushes. In grain mills and storehouses, the total number of these bacteria in airborne dust varies from

INSTYTUT MEDYCYNY PRACY IM. PROF. J. NOFERA

100 – 5000 thousand/m^3 air. In grain dust, the concentration of *Erwinia herbicola* is about 3×10^8 cfu/1g dust, and in flour dust – 1.4×10^5 cfu/1 g dust (8).

Environmental determination of bacterial content based on culture methods is insufficient in evaluating exposure, because only live forms of microorganisms, capable of growing, are detected. Although non-viable forms preserve some of their endogenic properties, it is useful to introduce endotoxin concentration as a new parameter for a more accurate estimation of exposure to that particular pathogen in the environment.

The Limulus Amoebocyte Lysate (LAL) Endotoxin Test is commonly used to assay endotoxin concentration (18, 32). Airborne endotoxin concentration can also be determined by means of gas chromatography, but the correlation between these two methods is rather poor (28). According to Swedish investigators, the Limulus test detects only the fraction of lipopolysaccharides dissociating through the cell wall. Gas chromatography allows to measure the total lipopolysaccharide concentration, including the fraction that remains inactive in the Limulus method, as well as other non-endotoxic bacteria and various cellular artefacts – e.g. polynucleotides, proteins (10).

The purpose of the present study was to examine the usefulness of the Limulus test of varying sensitivity for the estimation of endotoxin level in airborne flour dust in selected work areas in the bakery. Air contamination with Gram-negative bacteria was evaluated in parallel to the measurement of endotoxin level.

MATERIALS AND METHODS

1. Air sampling

Air samples were collected in the main facilities of a large mechanical bakery at the following work sites:

a. at the kneading trough covering the process of adding flour and kneading

b. at the set of machines where bread or rolls are formed, during the process of dough dividing and portioning.

Air samples were collected to estimate the content of Gram-negative bacteria in the bakery atmosphere and as a source of flour dust in which endotoxin concentration was measured. Bacterial concentration was determined using Koch's sedimentation method of passive air sampling (exposure of Petri plates containing Mc Conkey's medium for 20 minutes), and by aspiration using a 6-level Andersen sampler with the following pore sizes:

1st level – 1.81 mm
2nd level – 0.91 mm
3rd level – 0.71 mm
4th level* – 0.53 mm
5th level* – 0.34 mm
6th level* – 0.25 mm

*These are levels at which the respirable fraction of the aerosol is collected only.

INSTYTUT MEDYCYNY PRACY IM. PROF. J. NOFERA

Air samples were collected by an AS-50 aspirator at a flow rate of 28.3 1/min. for 20 minutes on Mc Conkey's medium (Diagnostic Merc) (4); two plates were provided for each type of measurement. The plates were then incubated for three days at the temperature of 27°C followed by one day in the refrigerator (optimal growth conditions for saprophytic bacteria).

Airborne dust was assessed in the same work areas in which samples were taken for microbiological analysis. Air was aspirated with an AS-50 aspirator for 30 minutes through cellulose membrane filtres of 0.8 μm pore size. Dust content was measured by weighing the filters before and after aspiration; the difference in weight was equal to the mass of the aspirated dust.

2. Bacterial concentration and identification of species

Air samples containing bacteria were collected on Mc Conkey's medium (Diagnostic Merc). Species identification was based on the analysis of biochemical properties of the cultured strains, using the Epl 21 HTL Warszawa diagnostic test for Gram-negative rod identification. Following incubaion, the number of colonies on the plates was counted and bacterial concentration was calculated from the number of colonies and the volume of air passing through the impactor according to the formula quoted by Dutkiewicz (8).

a) in the case of sedimentation

$$x = \frac{a \cdot 10000}{\pi r^2 \cdot K}$$

where: x – number of bacteria in 1 m^3 of air, expressed as (cfu)
a – average number of colonies on 1 plate
r – plate radius (cm)
πr^2 – plate area (cm^2)
K – exposure time in minutes/5

b) in the case of aspiration:

$$x = \frac{a \cdot 1000}{V}$$

where: x – number of bacteria in 1 m^3 of air, expressed as (cfu)
a – average number of colonies on 1 plate
V – volume of air sampled during time t (in m^3)

3. Obtaining dust extract and endotoxin assay

Dust containing filters were placed in 50 ml sterile Erlenmayer flasks and immersed in 10 ml of sterile Coca's solution (30) at pH 8.2 to obtain endotoxin extract. Thus prepared, the samples were frozen and defrozen twice according to the procedure described by Dutkiewicz (8). Endotoxin was assayed in crude dust extract (at dilutions 1:10, 1:100, 1:1000) by means of the Limulus – Endotoxin-Test (LAL) manufactured by α-DiaLab of sensitivity 0.125 EU/ml and 0.06 EU/ml containing reference *E. coli* 055:B5 at titre 100 ng = 1000 EU (Endotoxin Units) and calculated from the formula presented in the updated OEL documentation (9).

Endotoxin content in 1 g of flour dust according to the formula (1):

INSTYTUT MEDYCYNY PRACY IM. PROF. J. NOFERA

$$B = \frac{ASE \cdot ORP}{SPW} \quad (1)$$

where: B – concentration of endotoxin in studied dust, expressed as (μg/g)
ASE – minimal dose of standard endotoxin capable of producing coagulation of test reagent
ORP – inverse of the highest sample dilution capable of producing coagulation of test reagent
SPW – initial concentration of dust sample in distilled water (g/ml).

The concentration of endotoxin in the air samples collected through a filter was calculated according to the formula (2):

$$Y = \frac{B \cdot A}{1000} \quad (2)$$

where: Y – concentration of airborne endotoxin, expressed as (μg/g)
B – concentration of endotoxin in airborne dust, expressed as (μg/g)
A – concentration of airborne dust, expressed as (mg/m^3) (see: next formula (3)).

Concentration of airborne dust was calculated according to (3):

$$A = \frac{G}{V \cdot 1000}$$

where: A – concentration of dust in air, expressed as (mg/m^3)
G – mass of dust collected on a filter, expressed as (mg)
V – volume of air passing through a filter, expressed as (liters).

Dust extract collected on membrane filtres was diluted in the following proportions: 1:10, 1:100, 1:1000, using nonpyrogenic water and nonpyrogenic test tubes included in the Limulus reagent set (α-DiaLab). A parallel dilution series of *E. coli* B5:055 reference endotoxin was prepared to assess the reactivity of the Limulus

Table 1. Dilution series of standard *E. coli* – 055:B5 endotoxin used as a positive control in the Limulus test (1 EU = 0.1 ng)

	Concentration of reference endotoxin in EU/ml (in endotoxic units EU/ml)	Reactivity to the Limulus reagent of varying sensitivity	
		0.125	0.06
1	500 000	+	+
2	10 000	+	+
3	5 500	+	+
4	2 500	+	+
5	1.250	+	+
6	0.620	+	+
7	0.310	–	+
8	0.150	–	+
9	0.075	–	+
10	0.037	–	–
11	0.016	–	–

INSTYTUT MEDYCYNY PRACY IM. PROF. J. NOFERA

assay of varying sensitivity. The values of reactivity for the reagents used throughout the test are shown in Table 1. Reactivity assessment is recommended before the use of each new lot. In the case of Limulus reagent of sensitivity 0.62 EU/ml maximum dilution of dust extract, still producing coagulation, was 1:100 (with negative result for 1:1000 dilution). Coagulation was obtained in the 1:1000 dilution after addition of the Limulus reagent of sensitivity 0.075 EU/ml to a similar dilution series of dust extract. The more sensitive reagent produced coagulation in all the dilutions of the series, i.e., 1:10, 1:100, 1:1000.

RESULTS

a) Bacteria

The content of Gram-negative bacteria in the atmosphere of the bakery facilities is shown in Table 2. The content of Gram-negative bacteria in the studied work areas was measured in parallel to the estimation of dustiness. The results show

Table 2. Gram-negative bacterial content in the atmosphere of the bakery facilities measured by: a) Koch's sedimentation method, and b) Andersen's aspiration method

Work site	Work shift	Bacterial Content in dusty air contaminants in cfu/m^3 Total no.of Gram-negative bacteria	Respirable fraction
1. Kneading trough	night	280 000(b) 250 (a)	88 000
–preparation of dough	day	14 000 (b) 9 (a)	0
2. Dough dividing machine	night	350 000 (b) 4 400 (a)	304 000
–portioning of bread and rolls		14 000 (b) 9 (a)	0

*– arithmetic mean of 9 measurements

considerable variation dependent on the method of measurement, work shift and the fraction of airborne dust. In Koch's sedimentation method, the levels of airborne bacterial concentration were significantly lower in comparison with those obtained by aspiration sampling. Possible explanations of this are: a smaller volume of air in close contact with the culture medium in the case of Koch's method and the growth of only those particles which can freely settle on the surface of the medium. In the case where Andersen sampler cannot be used (no available electric power supply), Koch's method allows only an approximate estimation of the presence of bacteria in the studied environment. The discrepancy between the results obtained by Koch's method and those obtained by aspiration sampling (Andersen's) can reach two ranges of magnitude.

In the air samples collected during the night shift, bacterial content was found to be higher in comparison with the day shift. This was true for both sampling methods.

INSTYTUT MEDYCYNY PRACY IM. PROF. J. NOFERA

In the dough portioning area, where several persons work in constant movement at sliding production chains, airborne bacterial content assessed by Koch's sedimentation method was higher than in the proximity of the kneading trough where only one worker was employed. Aspiration sampling by means of the Andersen sampler showed no such difference in bacterial content between the two investigated work areas. This may be due to a relative proximity of the two sites, and because the Andersen sampler collects large quantities of air over wide production spaces, the two environments were assessed as a whole. The use of the Andersen sampler permitted to evaluate bacterial content in respirable dust, i.e. the fraction of the aerosol reaching pulmonary alveoli of particle size less than 3.5 μm.

As shown in Table 2, bacterial content in the respirable fraction, sampled at the kneading trough during the night shift, was 8×10^4 cfu, i.e. 28.5% of total bacterial content in total dust, whereas in the dough portioning area the respirable fraction constituted as much as 86% of total bacterial content.

During the day shift total bacterial content, at both locations, was lower than that of the night shift, which was equal to 1.4×10^4 cfu. Also, no respirable fraction was detected in the day shift samples. The discrepancy might be due to a lower production output, especially of small assortment, as well as to variable micro-climatic conditions in the production facilities.

Examination of colonies and biochemical analysis permitted to identify the species of Gram-negative bacteria detected in the bakery atmosphere. The following species of Gram-negative bacteria were found:

Erwinia herbicola – Gram-negative endotoxic rods originating (= *Enterobacter aglomerans*) mainly from grain.

Actinetobacter Lwoffi – Gram-negative endotoxic bacteria, widely distributed in the environment, occurring in soil, water, on the surface of skin and mucosa of humans and animals, on plant and animal products. Present in the atmosphere of farming facilities.

Klebsiella oxytoca – Gram-negative rod occurring in the intestinal tract of humans and animals.

b) Airborne endotoxin

Endotoxin concentrations in the bakery atmosphere measured at the studied work locations are shown in Table 3. At the kneading trough endotoxin concentration in airborne dust, measured in reference to standard endotoxin of sensitivity 0.62 EU/ml. was 6.7 μg/g, and 8.1μg/g if using the standard of sensitivity 0.075 EU/ml.

In the area of dough portioning, assessed similarly endotoxin concentrations were 16.7 μg/g and 20.3 μg/g, respectively. Higher endotoxin content in airborne dust in the environment of dough portioning machine was due to higher concentration of Gram-negative bacteria in work area, which may be due to larger number of persons employed as well as to the type of flour used (in this case, wheat flour). However, because airborne dust concentration in the environment of dough was much lower than in the proximity of the kneading trough (Table 4), endotoxin concentration at both locations was in the same range of values (0.04 – 0.05 $\mu g/m^3$).

INSTYTUT MEDYCYNY PRACY IM. PROF. J. NOFERA

Table 3. Endotoxin concentration in dust (µg/g) and endotoxin concentration in air (µg/m³) in the studied work areas of the bakery

Work site	Mass of dust collected on a filter	Endotoxin concentration in dust	Endotoxin concentration in air
	mg	µg/g	µg/m³
1. Kneading trough preparation of dough	9.2	6.7 (a)	0.04 (a)
		8.1 (b)	0.05 (b)
2. Dough dividing machine – dividing and forming bread and rolls	3.7	16.7 (a)	0.04 (a)
		20.3 (b)	0.05 (b)

The assessed endotoxin concentrations in dust and air are lower than corresponding levels in grain elevators and storehouses due to high degree of dustiness from fresh raw material.

Table 4. Total dust concentration in the studied work areas of the bakery (determined by stationary air sampling using the AS-50 aspirators)

Work site	Sampling time	Air filtration rate	Total dust concentration
	min.	1/min.	mg/m³
1. Kneading trough – perparation of dough	30	50	27.5; 16.3; 11.6; 6.1
2. Dough dividing machine – dividing and forming bread and rolls	30	50	2.1; 1.9; 2.5; 2.0

CONCLUSIONS

1. In the atmosphere of a large mechanical bakery we detected Gram-negative endotoxic bacteria of the following species:
   - *Erwinia herbicola*
   - *Acinetobacter Lwoffi*
   - *Klebsiella oxytoca*
2. The concentrations of Gram-negative bacteria in the investigated work areas were higher than the proposed hygienic standard value of OEL = 20 000 cfu/m³ (9).
3. Endotoxin was detected in airborne dust and air in the selected work areas of the bakery.
4. Endotoxin concentrations measured in the work areas were lower than the proposed standard value of OEL = 0.2 µg/m³ (9).

INSTYTUT MEDYCYNY PRACY IM. PROF. J. NOFERA

REFERENCES

1. Baux X, Fruhmann G, Hang B, Rasche B, Reiher W, Weiss W. Role of Aspergillus amylase in bakers asthma. Lancet 43: 505—508, 1986.
2. Brisman J, Belin L. Clinical and immunological responses to occupational exposure to α-amylase in the baking industry. Br J Ind Med 48: 604—608, 1991.
3. Castellan R, Olenchock S, Kinsley K, Ankinson J. Inhaled endotoxin and decreased spirometric values. An exposure — response relation for cotton dust. N Engl J Med 317: 605—610, 1987.
4. Cormier Y, Tremblay G, Meriaux A, Brochn G. Airborne microbial contents in two types of swine confinement buildings in Quebec. Am Ind Hyg Assoc J 51 (6): 304—309, 1990.
5. Dutkiewicz J. Studies on endotoxins of *Erwinia herbicola* and their biological activity. Zbl Bact Hyg A 236: 487—508, 1976.
6. Dutkiewicz J. Airborne microbial flora as a noxious agent in the bakery work environment. Med Wiejska 18 (2): 119—127, 1983 (in Polish).
7. Dutkiewicz J. Bacteria, fungi and endotoxin in stored timber logs airborne sawdust in Poland In: C. O'Rear G, Liewelyn (ed.). Biodeter Res 2: Plenum press N. York, 1989.
8. Dutkiewicz J, Jabłoński L. Biological noxious agents in occupational exposure. PZWL, Warsaw, 1989 (in Polish).
9. Dutkiewicz J, Mołocznik A. Revised OEL documentation for dust of plant and animal origin, OEL Office, The Nofer Institute of Occupational Medicine, Lodz, 1993 (in Polish).
10. Elin R, Wolff S. Non-specifity of the limulus amoebocyte lysate test: positive reactions with polynucleotides and proteins. J Infect Dis 128: 349—352, 1973.
11. Flaherty D et al. Bacterial endotoxin isolated from a water spray air humidification system as a putative agent of occupation-related lung dissease. Infect Immun 43:206—212, 1984.
12. Gościcki J, Włodarczyk L, Bielichowska G. Evaluation of work environment in the linen textile mills. I. Airborne microbial flora in the work sites of the linen spinning mills. Med Pr XXXI, 2: 91, 1980 (in Polish).
13. Heederik D, Brouwer R, Bierstekert, Boleij J. Relationship of airborne endotoxin and bacteria levels in pig farms with the lung function and respiratory symptoms of farmers. Int Arch Occup Env H 62 (8): 595—601, 1991.
14. Hagmar L, Schultz A, Hallberg T, Sjondm A. Health effects of exposure to endotoxins and organic dust in poultry slaughter house workers. Int Arch Occup Env H 62 (2): 159—164, 1990.
15. Ikonomowa Z et al. Microbial contamination of the work area air at the tricot knitting mills. Gig Tr Prof Zabol 1: 16—19, 1989.
16. Masalin K, Murtomaa H, Maurman J. Oral health of workers in the modern Finnish confectionery industry. Comm Dent Oral Epid 18 (3): 126—130, 1990.
17. Michel O, Duchatean J, Sergysels R. Effect of inhaled endotoxin on bronchial reactivity in asthmatic and normal subjects. Am Phys Soc 6: 1059—1064, 1989.
18. Milton D, Gere R, Feldman H, Greaves I. Endotoxin in measurement; aerosol sampling and application of a new Limulus method. Am Ind Hyg Assoc J 51 (6): 331—337, 1990.
19. Michael D, Ginamni R, Duchatean J, Vertougen F, Le-Bon B, Sergysels R. Domestic endotoxin exposure and clinical severity of asthma. Clin Exp Allergy 21 (4): 441—448, 1991.
20. Morrisson D, Razinddin A. Lipopolysaccharides and endotoxin. Amsterdam, Elsevier 1982.
21. Olenchock S, May J, Pratt D, Piacitelli L, Parker J. Presence of endotoxins in different agricultural environments. Am J Ind Med 18 (3): 279—284, 1990.
22. Olenchock S, Cristiani D, Mull J, Ye T. Lu P. Airborne endotoxin concentrations in various work areas within two cotton textile mills in the People's Republic of China. Biomed Environ Sci 3 (4): 443—451, 1990.
23. Ozesmi M, Aslan H, Hillerdal G, Rylander R, Ozezmi C, Baris Y. Byssinosis in carpet weavers exposed to wool contaminated with endotoxin. Br J Ind Med 44 (7): 479—483, 1987.
24. Poulsen K, Nielsen H. Health problems among persons exposed to grain dust. Ugeskr-Laeger 8 (28): 1986—1990, 1991.

INSTYTUT MEDYCYNY PRACY IM. PROF. J. NOFERA

25. Rastogi S, Gupta B, Husain I, Mathur N. Respiratory health effects from occupational exposure to wood dust in sawmill. Am Ind Hyg Assoc J 50: 574–578, 1989.
26. Rylander R, Haglind P. Airborne endotoxins and humidifier disease. Clinical Allergy 14: 109–112, 1984.
27. Rylander R. The role of endotoxin from reactions after exposure to cotton dust. Am J Ind Med 12: 687–697, 1987.
28. Sanesson A, Larsson L, Schultz A, Hagmer L, Hallberg T. Comparison of the Limulus amoebocyte lysate test and gas chromatography – mass spectrometry for measuring lipopolysaccharides (endotoxin) in airborne dust from poultry – processing industries. Appl Environ Microbiol 56 (5): 1271–1278, 1990.
29. Smid T, Heederik D, Mensink G, Houba R, Boleij J. Exposure to dust, endotoxins, and fungi in the animal feed industry. Am Ind Hyg Assoc J 53 (6): 362–368, 1992.
30. Strauss M. The preparation of allergenic extracts for testing and treatment. In: Fundamentals of Modern Allergy. J Samuel ed. Prigal. N. York 1960.
31. Tee R, Gordon D, Gordon S et al. Immune response to flour and dust mites in a United Kingdom Bakery. Br J Ind Med 49 (8): 581–587, 1992.
32. Wildfeuer A, Heymer B, Schleifer K, Haferkamp O. Investigations on the specificity of the Limulus test for the detection of endotoxin. Appl Microbiol 28: 867–870, 1974.
33. Vinzents P, Nielsen B. Variations in exposure to dust and endotoxin in Danish piggeries. Am Ind Hyg Assoc J 53 (4): 237–241, 1992.

Received for publication: March 30, 1994
Approved for publication: April 28, 1994

INSTYTUT MEDYCYNY PRACY IM. PROF. J. NOFERA