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Review

Endotoxins Affecting Human Health during Agricultural Practices: An Overview

B. Surya Kumar Chhetry [1], Krishna Narayan Dewangan [1,*], Dipendra Kumar Mahato [2,*] and Pradeep Kumar [3,4,*]

[1] Department of Agriculture Engineering, North Eastern Regional Institute of Science and Technology, Nirjuli 791109, India
[2] CASS Food Research Centre, School of Exercise and Nutrition Sciences, Deakin University, Burwood, VIC 3125, Australia
[3] Department of Botany, University of Lucknow, Lucknow 226007, India
[4] Department of Forestry, North Eastern Regional Institute of Science and Technology, Nirjuli 791109, India
* Correspondence: kndewangan2001@yahoo.co.in (K.N.D.); dmahato@deakin.edu.au (D.K.M.); pkbiotech@gmail.com (P.K.)

Abstract: Agricultural operations and the processing sector generate dust laden with endotoxin in the workplace. Endotoxin, a pro-inflammatory agent, has adverse effects on health, especially in the lungs, as exposure to endotoxin reduces lung function capacity. Endotoxin exposure to workers and its harmful impact on the health of agricultural workers needs to be studied in detail for future interventions to reduce exposure to endotoxin. The review can help to identify the analytical methods used to determine endotoxin exposure in agriculture. A detailed study of the research articles published in the last two decades related to agriculture and allied fields was carried out. In the agricultural sector, *Pantoea agglomerans*, a Gram-negative bacterium, was predominantly present. The filters were stored at a temperature of −20 °C, and *E. coli* 055: B5 was the predominately used standard to analyze the endotoxin. The quantitative kinetic Limulus Amebocyte Lysate test was the most common detection method for quantifying endotoxin. Control strategies to reduce endotoxin exposure are also emphasized in this review.

Keywords: dust; endotoxin; lung function; gram-negative bacteria; health; occupational exposure

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1. Introduction

Agricultural operations engender dust in the atmosphere. Dust may be generated naturally or by human activities. Dust is generated during different operations in crop production due to interactions between machines and soil and between machines and plants and post-harvest processing. Dust is a heterogeneous mixture of organic and inorganic components based on its composition. Workers are exposed to different levels of dust in various occupational workplaces. Dust is categorized as inhalable, thoracic and respirable dust based on its particle size [1–3]. Airborne dust is usually inhaled through the nose or mouth.

According to the American Conference of Governmental Industrial Hygienists (ACGIH), the threshold limit values for respirable and inhalable dust are 3.0 and 10 mg m^{-3}, respectively, and the occupational safety and health administration (OSHA) has permissible exposure limits of respirable and total dust of 5.0 and 15 mg m^{-3} [4]. The National Board of Occupational Safety and Health (NBOSH), Sweden, has recommended dust exposure limits for normal and organic dust of 10 mg m^{-3} and 5 mg m^{-3}, respectively [5]. The Ministry of Labour, Government of India, recommends a time-weighted average (TWA) limit, over 8 h of dust exposure, of 5 and 10 mg m^{-3} for respirable and total dust, respectively [3]. Different organizations around the world namely, ACGIH, OSHA, NBOSH and the Ministry of Labour, India, have different exposure limits for exposure to dust, and there

is a need for a general standard for dust exposure that can be accepted internationally by all countries. Agriculture is highly assorted with various respiratory hazards from organic or inorganic particulates, chemicals, gas and other contagious agents [1]. During the cultivation of crops, dust is generated during field preparation, sowing/planting, plant protection activities, harvesting, threshing and post-harvest processing. Mołocznik & Zagórski [6] reported that the mean total dust concentration during field preparation and harvesting was higher than the recommended value of 10 mg m^{-3} [6]. Molocznik [2] testified that the harvesting of cereals using a combine contributes to the highest level of dust concentrations in agricultural operations, ranging from 31.7 to 72.9 mg m^{-3}, with respirable dust constituting 3.9–8.8 mg m^{-3} [2]. Dewangan and Patil [3] measured the dust concentration in rice mills, oil mills, flour mills and tea industries and found that oil mills generate maximum dust compared to others. The mean values of respirable, thoracic, inhalable and total dust were 5.76, 35.65, 68.71 and 111.02 mg m^{-3}, respectively [3]. The median personal inhalable cotton dust concentration in the textile industry in Shanghai, China, was 1.74 mg m^{-3} [7]. Dust exposure to workers in most agricultural operations and industry is above the recommended exposure limit. Thus, agricultural operation can either be a single or a combination source of air pollution to the atmosphere.

Endotoxin is present in organic dust generated during the crop cultivation [8], swine, poultry and grain industries [9]. Endotoxin is ubiquitous and represents significant components of bioaerosols [10]. Endotoxin is an essential biological component of airborne particulate matter and consists of active lipopolysaccharides (LPS) comprising cell wall components of the outer membranes of Gram-negative bacteria (GNB). The GNB cell envelope has two parts: the inner membrane surrounding the cytoplasm and the outer membrane providing a protective barrier to the external environment [11]. The outermost layer consisting of LPS of GNB is termed endotoxin. The general structure of endotoxin consists of lipid A, which is covalently attached to the molecule of the outer membrane via a substitution of the saccharide portion, an O-specific side chain linked in smooth LPS (S-form LPS) and an oligosaccharide containing up to fifteen monosaccharides [12]. LPS is the indicator for active infection in exposed workers. The O-specific side chain is the receptor for many bacteriophages, responsible for serological specificity [13]. The oligosaccharide helps to connect the O-specific side chain and lipid A. Lipid A is the most pyrogenic component of LPS [14], and it is hydrophobic in nature. Lipid A is the innermost part of LPS, and it is an acylated β-1′-6-linked glucosamine disaccharide [15]. There are high variations in structure within bacterial species [16]. There is a positive correlation between exposure to dust and endotoxin exposure [4,17]. According to the National Health Council of the Netherlands, the recommended threshold value of endotoxin exposure is 90 EU/m^3 for 8 h of the working day [18,19]. The conversion of endotoxin concentration from ng/m^3 to EU/m^3 depends on the endotoxin standards used (*E. coli*), manufacturer and laboratories. The most common conversion factor for *E. coli* 055: B5 is 10 EU/m^3 equals 1 ng/m^3. Many studies have been performed for assessments of endotoxin in agriculture [5,19–25]. There are some review papers on endotoxin exposure that have focused on different areas, such as agriculture work, animal housing and agricultural industries. These review papers have not focused on areas of sampling, the flow rate, types of dust and management and control strategies. An attempt is being made here to critically review the literature published in the last two decades to identify the source, occurrence of endotoxin in agriculture and cereal/fruit crops and detection techniques used to examine the health effects on the workers. This review also focuses on management and control strategies adopted in an agricultural setting to reduce endotoxin exposure for the safety and security of agricultural workers to improve their quality of life.

Effect of Dust and Endotoxin on Lungs

During agricultural operations, an enormous quantity of dust is generated and released into the environment, and this can be inhaled by the workers [20]. Dust is the heterogeneous mixture of organic and inorganic materials, which can contain endotoxin.

The inhalation of dust can damage workers' lungs, which may be termed occupational lung disease [26,27]. Respirable dust particles with an aerodynamic diameter below 5 µm may reach the tracheobronchial and alveolar regions of the lungs [8]. In India, the incidence of occupational lung disease ranges from 15% to 30% [28]. Agricultural workers usually work for at least 8 h in a dusty environment. With an increase in the duration of exposure to dust, the chances of respiratory morbidity increase [29–31]. A smaller fraction of dust enters the lungs via the windpipe and reaches the bronchi and bronchiole region via the windpipe and is discharged in the form of mucous generated by the goblet cells. The basic physiology of the respiratory system of humans includes attempts to expel the dust from the lungs [32]. The most common symptoms of the inhalation of dust are cough, dyspnea, wheezing, nasal irritation or wetting, irritation and redness of the eyes [28,32], chest tightness, morning phlegm, shortness of breath and morning cough [33]. These symptoms can be acute or chronic. Coughing is a part of natural respiratory physiology, a predictor and pioneer symptom of all respiratory diseases, and dyspnea is associated with cough, wheezing and rhinitis [33]. Dyspnea and wheeze indicate the severity of respiratory symptoms [34]. Due to the inhalation of dust in various occupational settings, spirometry measurements, such as the forced expiratory volume in the first second (FEV1), forced vital capacity (FVC) and percentage of forced expiratory volume in the first second (FEV1%), decrease significantly [28,33,35–39]. Rice mill workers have considerably higher incidences of chronic cough, wheezing with shortness of breath, and asthma than control volunteers [36]. The duration of dust exposure is proportional to the reduction in the peak expiratory flow rate [33,40]. Due to the inhalation of dust in rice mills, the ratio of forced expiratory volume in the first second (FEV1) to forced vital capacity (FVC) of workers is more than 70%, showing a restrictive type of abnormality in the lung [38,41]. When finer dust particles are inhaled, they may bypass the lung defense mechanisms and get stuck in the alveoli, which causes a localized inflammatory response [29,42]. The enzymes secreted during a localized inflammatory reaction disintegrate the alveolar septum, weaken lung defense systems and alter lung tissue repair mechanisms, causing significant lung deformities [29,42].

The organic dust fraction combines microorganisms like bacteria, fungi, viruses and protozoa and compounds like endotoxins, glucans, mycotoxins, peptidoglycans and enzymes [43–45]. Endotoxin is produced during bacterium disintegration to develop its biological activities [46]. The endotoxin is deposited in the lungs during the inhalation of airborne finer particles (dust) [47]. Airborne endotoxin is highly associated with adverse respiratory outcomes in exposed agricultural workers [48,49], and the concentration of endotoxin varies in agricultural environments [10,50]. Exposure to endotoxin exacerbates asthma, wheezing, a decline in lung function [51], shortness of breath, chest tightness [52], chronic bronchitis, bronchial hyper-responsiveness and cough [44,52]. Acute endotoxin inhalation induces flu-like symptoms, such as chills, coughing, mild fever and bronchoconstriction [53]. Exposure to endotoxin can also significantly change the body's white blood cell count, resulting in immune function problems [41,54,55]. Endotoxin exposure may alter circulating levels of inflammatory and immunologic response markers that may be implicated in lung carcinogenesis [17,56]. Male poultry farmers have the highest rate of lung cancer when compared with that in farmers who do not rear animals [17]. The effect of endotoxin on allergic reactions may depend on the age of the workers [54]. Long-term exposure to endotoxin decreases the FEV1 and FVC [20,52,57] and reduces the FEV1 by up to 25% [53].

In agricultural settings, the inorganic portion of dust may contain crystalline and amorphous silica. Crystalline silica is the second most abundant element in the earth's crust. Crystalline silica in agricultural settings can be present in the soil and husk of paddy and can be produced during the burning of plant residues like rice husk and sugarcane [58]. Exposure to airborne respirable crystalline silica causes pulmonary diseases, lung cancer and silicosis [59,60]. Silicosis is characterized by shortness of breath, cough, fever and bluish skin. Due to exposure to crystalline silica, there is a loss in lung function capacity,

resulting in chronic obstructive pulmonary disease (COPD) [61]. The clinical symptoms caused by endotoxin exposure to the workers in different workplaces is shown in Figure 1.

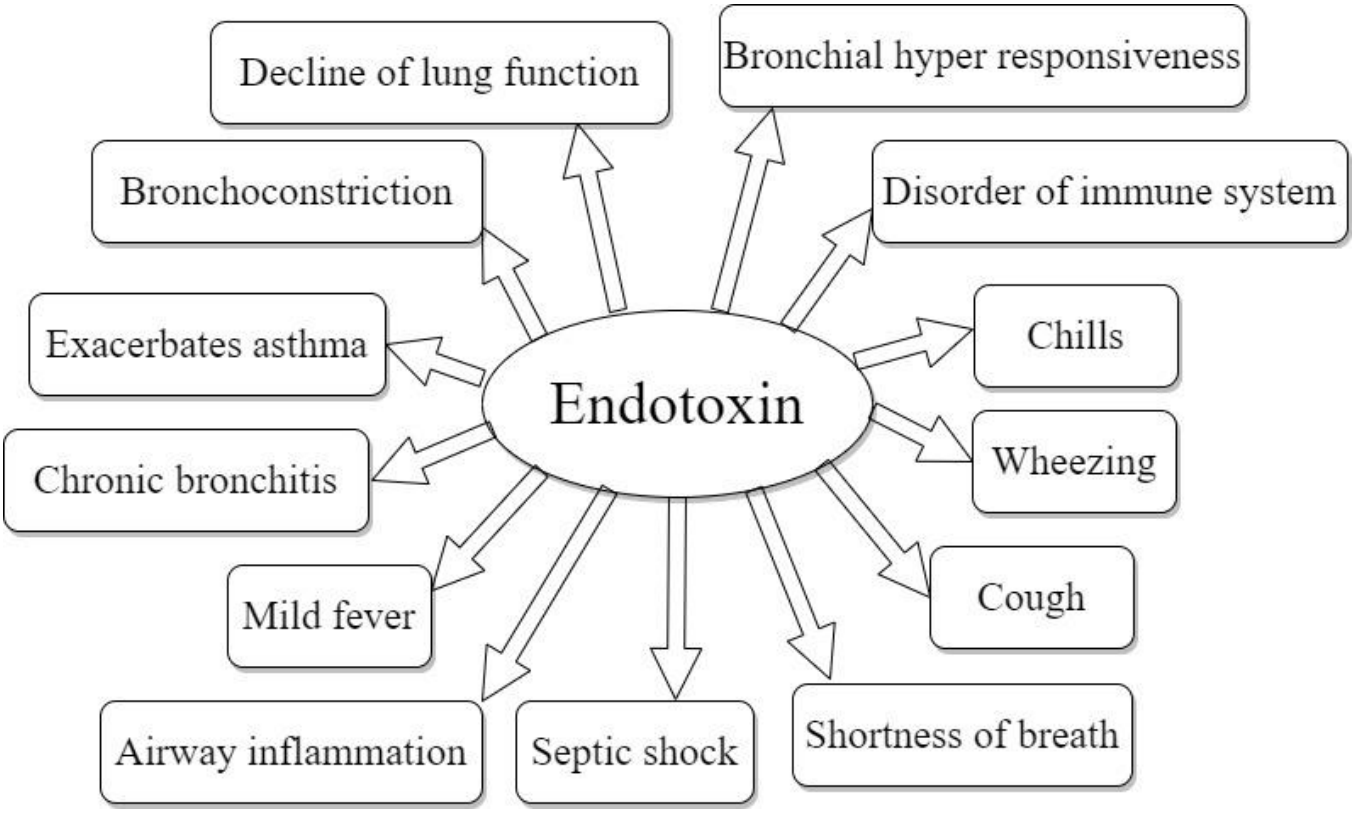

Figure 1. Clinical symptoms caused by exposure to endotoxin.

2. Major Sources of Endotoxin

Endotoxin is produced by bacteria and contains lipopolysaccharide (LPS). The long-term inhalation of organic dust and bacteria and their endotoxins can trigger acute and chronic respiratory disorders. Agricultural activities generate bioaerosols that contain bacteria and fungi. The common available bacteria were isolated from different farms and settings are presented in Table 1.

Table 1. Microorganisms in dust of different animal housing and industries.

Location	Geographical Location	Microorganism Species Present	Reference
Swine and poultry houses	Europe	*Bacillus*, *Streptomyces*, and thermophilic bacteria	[21]
Poultry farms	Egypt	*Bacillus* spp., *Micrococcus* spp., *Proteus* spp., *Pseudomonas* spp., *Staphylococcus* spp., *E. coli* and *Clostridia* spp.	[62]
Pea moss factory	Canada	*Penicillium* and *Torulomyces*	[63]
Potato processing plants	Poland	*Corynebacteria*, *Pseudomonas* spp, and *Acinetobacter calcoaceticus*	[64]
Settle dust	India	*Pantoea agglomerans*	[65]
Animal building	USA	*Acinetobacter*, *Bacteroides*, *Enterobacter*, *Moraxella*, *Pasteurella*, *Pseudomonas* and *Vibrio*	[66]
Settle grain dust	USA	*Pseudomonas*, *Serratia*, *Acinetobacter*, *Klebsiella* and *P. agglomerans*	[67]
Poultry farms	Poland	*Acinetobacter*, *Enterobacter*, *Escherichia*, *Pantoea* species and *Klebsiella genera*	[68]
Gin houses	India	*Enterobacter agglomerans*, *E. cloacae* and *E. aerogenes species*	[69]

P. agglomerans has the most endotoxic and allergenic properties [64] and is significant for work-related diseases [70,71]. *P. agglomerans* inhabits plants, soil, air and dust [72]

and is abundantly present in onions, causing the rotting of onions in the center [73] and rotting of seeds and bolls of cotton [74–76]. Kullman et al. [77] reported that mesophilic and thermophilic bacteria were present in dairy barns and comprise Gram-positive bacteria (GPB) and GNB [77]. The mean personal exposure to bacteria and fungi in flower greenhouses in Denmark was 5.3×10^3 and 1.9×10^5 CFU/m^3 [22]. The dominating GNB species was *P. agglomerans*, and the highest concentration of GNB was in flax farms (median: 112.2 thousand CFU/m^3) and grain handling elevators (median value of measurement: 20.45 thousand CFU/m^3) [78]. The threshing of pearl millet in outdoor farms was associated with the highest reported concentration of GNB, and the total microorganisms present were 108.75×10^5 CFU/m^3 [23]. Dust collected from agricultural plants (gram, amaranth, rice, millet, sorghum, wheat and maize) showed a mean value of the measured concentration of GNB of 13.44 million CFU/m^3, which accounted for 11.12% of total microorganisms present. *Pantoea agglomerans* species are the most dominating, producing strong endotoxin in India [65]. Agricultural workers exposed to mesophilic GNB can experience harmful occupational health effects. There is a need to establish an exposure limit for microflora accepted internationally [79,80].

3. Occurrences of Endotoxin in Agriculture

Agriculture can be related to the generation of enormous amounts of dust, of which organic dust from agricultural activities is the primary source of endotoxin. The endotoxin concentration is a significant concern in agricultural operations and sectors because the exposure value exceeds the recommended threshold value. The occurrence of endotoxin in different animal housing, food processing and other agricultural industries, such as hemp and cotton, are presented in Tables 2–4. The location of dust measurements was inside the farms or industries.

There is a variation in the occurrence of endotoxin in agricultural settings. This variation may be due to different sources of dust in agricultural settings. The harvesting of crops (nuts) using a mechanical harvester results in a higher concentration of dust [81,82]. Endotoxin has a significant correlation with the temperature [60,82,83]. The endotoxin concentration increases with an increase in the temperature [82] and relative humidity [60,84,85]. The endotoxin concentration is significantly higher on warmer days than on cold days [86,87]. In poultry and swine buildings, there is an increase in the dust and endotoxin concentration on winter days as ventilation is reduced to conserve heat [88]. The concentration of endotoxin in dust decreases due to the growth of fungi and bacteria in the livestock feed in poultry and swine farms [66]. The animal feeds may contribute to endotoxin contamination, but the major source of endotoxin inside animal houses is animal feces [88,89].

Endotoxin assessments depend on different sampling, extraction and analysis procedures followed [24]. The endotoxin concentration is higher during livestock farming than during crop farming [81]. The endotoxin concentration increases with an increase in the density of animals that are reared (pig) and a reduction in the frequency of cleaning in swine houses [88]. The concentration of endotoxin depends on the duration of exposure [88] and the type of workplace [90].

Table 2. Occurrence of endotoxin in animal housing around the world.

Location of Sampling	Country	No. of Samples	Dust Concentration, mg/m^3	Fraction of Dust	Analytical Method	Endotoxin Concentration	Range of Endotoxin Concentration	Affected Population	Reference
Poultry farm	Switzerland	36	7.01 (0.42–21.75)	Total Dust (PM10)	Kinetic-turbidimetric (KT) Limulus assay test	257.58 ng/m^3	18.99–1634.8 ng/m^3	Farmers	[21]
Pig production	Denmark	40	3.95 (1.11–13.75)	Total Dust (PM10)	KT Limulus assay test	58.01 ng/m^3	1.30–1101.7 ng/m^3	Farmers	[21]
Pig production	Germany	100	5.00 (<0.09–76.7)	Total Dust (PM10)	KT Limulus assay test	76.3 ng/m^3	0.01–2090.1 ng/m^3	Farmers	[21]
Floor-housed poultry	Canada	181	9.56	Total Dust	Chromogenic-end point (CEP) LAL assay test	1106.40 EU/m^3	Nil	Poultry workers	[48]
Cage-housed poultry	Canada	122	7.57	Total Dust		1291.47 EU/m^3	Nil	Poultry workers	[48]
Poultry farm	Denmark	14	AM: 5.7; GM: 3.5	Inhalable	Quantitative kinetic chromogenic (QKC) LAL test	AM: 1960 EU/m^3; GM: 805 EU/m^3	61–7090 EU/m^3	-	[91]
Pig production	Taiwan, Republic of China	95	-	Respirable	Kinetic Limulus assay test	47 EU/m^3	0.02–1643 EU/m^3	Worker	[88]
Swine farms	South Korea	36	0.505	Inhalable	LAL Kinetic QCL	812 EU/m^3	Nil	Workers	[92]
Swine farms	South Korea	36	0.128	Respirable	LAL Kinetic QCL kit	38.6 EU/m^3	Nil	Workers	[92]
Pig farm	Italy	18	-	PM10 ($\leq$0.49 µm)	Endpoint chromogenic LAL	16.261 EU/m^3	-	-	[93]
Pig rearing farmers	Denmark	354	AM: 4.9; GM: 3.4	Inhalable Dust	QKC LAL test	AM: 6200; GM: 1500 EU/m^3	13.69–370,000 EU/m^3	Pig farm workers	[94]
Pig farm	Denmark	354	AM: 4.9; GM: 3.4	Inhalable	QKC LAL test	AM: 6240; GM: 1490 EU/m^3	13.69–374,000 EU/m^3	Worker	[95]
Mixed farms (cattle and pigs)	Denmark	8	AM: 2.9; GM: 1.9	Inhalable	QKC LAL test	AM: 900; GM: 448 EU/m^3	13.69–2910 EU/m^3	Worker	[95]
Cattle farms	Denmark	124	AM: 1.6; GM: 1.0	Inhalable	QKC LAL test	AM: 759; GM: 358 EU/m^3	13.69–5890 EU/m^3	Worker	[95]
Dairy	Denmark	124	AM: 1.6; GM: 1.0	Inhalable	QKC LAL test	AM: 760; GM: 360 EU/m^3	13.69–5900 EU/m^3	Worker	[95]
Dairy farms	France	112	AM: 0.42; GM: 0.24	Thoracic	Kinetic LAL test	AM: 318; GM: 128 EU/m^3	2–8672 EU/m^3	Dairy farmers	[96]
Dairy farms	Ireland	38	AM: 1.7; GM: 1.5	Inhalable	QKC LAL test	AM: 197; GM: 128 EU/m^3	26–900 EU/m^3	Worker	[97]
Cattle feeding section	USA	15	AM: 1.272; GM: 0.9148	Inhalable	Recombinant Factor C assay	AM: 237.9; GM: 163.3 EU/m^3	-	Worker	[98]
Cattle miking section	USA	91	AM: 0.9304; GM: 0.7856	Inhalable	Recombinant Factor C assay	AM: 419.5; GM: 320.2 EU/m^3	-	Worker	[98]
Free stall dairy	USA	4	-	Inhalable (<100 µm)	Kinetic LAL	129.3 EU/m^3		-	[99]

Table 2. *Cont.*

Location of Sampling	Country	No. of Samples	Dust Concentration, mg/m^3	Fraction of Dust	Analytical Method	Endotoxin Concentration	Range of Endotoxin Concentration	Affected Population	Reference
Dairy farms	USA	114	GM: 0.67	Inhalable	Recombinant Factor C (rfc) assay	GM: 438 EU/m^3	0.05–4430 EU/m^3	Dairy farm worker	[100]
Minks	Denmark	7	AM: 1.4; GM: 1.3	Inhalable	QKC LAL test	AM: 301; GM: 214 EU/m^3	93–1050 EU/m^3	-	[95]
Equine farms	USA	58	-	Respirable	Kinetic chromogenic (KC) LAL test	-	1.72–19.0 EU/m^3	-	[101]
Equine farms	USA	58	-	Inhalable	KC LAL test	-	50.2–1024 EU/m^3	-	[101]
Horse stables	The Netherlands	95	AM: 2.4; GM:1.2	Inhalable	Quantitative kinetic LAL method	Am: 2073; GM: 555 EU/m^3	<22.19–48,484 EU/m^3	Workers	[102]
Livestock farms	The Netherlands	211	AM: 0.023; GM: 0.0215	PM10	QKC LAL test	0.657 EU/m^3	GM: 0.46–0.66 EU/m^3	Outside animal farms	[103]

Table 3. Occurrence of endotoxin during different agricultural operations around the world.

Location of Sampling	Country	No. of Samples	Dust Concentration, mg/m^3	Fraction of Dust	Analytical Method	Endotoxin Concentration	Range of Endotoxin Concentration	Reference
Rice mills	Malaysia	79	79	Inhalable	Chromogenic Endpoint LAL	AM: 0.29 EU/m^3	-	[20]
Grain, seed and legume production sector	The Netherlands	15	GM: 2.5	Inhalable	QKC LAL	GM: 2700 EU/m^3	96–41,200 EU/m^3	[24]
Grain, seed and legume processing industries	The Netherlands	173	GM: 1.4	Inhalable	QKC LAL test	GM: 500 EU/m^3	2.3–149,060 EU/m^3	[24]
Animal processing industries	The Netherlands	81	GM: 0.4	Inhalable	QKC LAL test	GM: 51 EU/m^3	2–6230 EU/m^3	[24]
Seed processing industry	The Netherlands	101	GM: 1.6	Inhalable	LAL assay	GM: 1800 EU/m^3	10–274,000 EU/m^3	[25]
Animal production sector (11 companies)	The Netherlands	27	GM: 2.4	Inhalable	QKC LAL test	GM: 1190 EU/m^3	62–8120 EU/m^3	[24]
Coffee processing factories	Tanzania	193	AM: 3.69; GM: 2.50	Total Dust	KC LAL test	AM: 8200; GM: 3500 EU/m^3	42–75,083 EU/m^3	[104]
Hog load-out task	USA	19	7.14 (2.01–31.06)	Inhalable	KC LAL assay test	12,150 EU/m^3	3497–84,357 EU/m^3	[105]
Swine building power washing		13	-	Impinger	KC LAL assay test	40,353 EU/m^3	5401–180,864 EU/m^3	[105]

Table 4. Occurrence of endotoxin in other different agricultural industries around the world.

Location of Sampling	Country	No. of Samples	Dust Concentration, mg/m^3	Fraction of Dust	Analytical Method	Endotoxin Concentration	Range of Endotoxin Concentration	Reference
Textile Mills	Shanghai, China	56	1.74	Inhalable	KC LAL assay test	2226.83 EU/m^3	Nil	[5]
Licensed private pesticide applicators	USA	204	0.90	Inhalable	KC LAL assay test	163 EU/m^3	-	[17]
Textile sectors	Nepal	24	AM: 2.34; GM: 0.81	Inhalable	LAL assay	AM: 4460; GM: 2160 EU/m^3	86–26,300 EU/m^3	[19]
Greenhouse	Denmark	75	AM: 0.36; GM: 0.25	Inhalable	Kinetic LAL	Am: 96.9; GM: 44.4 EU/m^3	95% CI: 32.4–60.8	[21]
Ornament plant or flower production	Spain	37	<0.09 (<0.09–0.88)	Total Dust (PM10)	KT Limulus assay test	0.36 ng/m^3	0.05–12.68 ng/m^3	[21]
Cotton mill (threshing of cotton)	India	2	25	-	LAL gel clot test	0.625 µg/m^3	-	[23]
Outdoor sickle harvesting of maize in farm	India	2	2.5	-	LAL gel clot test	0.0625 µg/m^3	-	[23]
Outdoor sickle harvesting of sorghum in farm	India	2	2.5	-	LAL gel clot test	0.0625 µg/m^3	-	[23]
Outdoor sickle harvesting of pearl millet in farm	India	2	7.5	-	LAL gel clot test	0.625 µg/m^3	-	[23]
Outdoor threshing of pearl millet	India	2	55	-	LAL gel clot test	31.25 µg/m^3	-	[23]
Outdoor threshing of maize	India	2	92.5	-	LAL gel clot test	31.25 µg/m^3	-	[23]
Cotton mill (carding and yarning)	India	2	2.5	-	LAL gel clot test	0.0625 µg/m^3	-	[23]
Cleaning of Bengal gram in godown	India	2	115	-	LAL gel clot test	62.5 µg/m^3	-	[23]
Cleaning of sorghum in godown	India	2	70	-	LAL gel clot test	6.25 µg/m^3	-	[23]
Cleaning of wheat in godown	India	2	50	-	LAL gel clot test	62.5 µg/m^3	-	[23]
Cleaning of red gram in godown	India	2	77.5	-	LAL gel clot test	62.5 µg/m^3	-	[23]
Grinding of grain for flour	India	2	150	-	LAL gel clot test	1.25 µg/m^3	-	[23]
Cleaning of rice in godown	India	2	257.5	-	LAL gel clot test	124.9 µg/m^3	-	[23]
Horticulture sector (21 companies)	The Netherlands	291	GM: 0.6	Inhalable	QKC LAL test	GM: 170 EU/m^3	1.6–191,430 EU/m^3	[24]
Gin house	India		2.11	-	LAL technique	2.77 µg/m^3	2.16–3.38 µg/m^3	[69]
Grain handling companies	Norway	166	GM: 1	Inhalable	QKC LAL test	GM: 628	11–64,250	[90]
Soil tillage	USA	4	-	Inhalable	Kinetic LAL	34.3 EU/m^3	-	[99]
Bean threshing	USA	4	-	Inhalable	Kinetic LAL	220.3 EU/m^3	-	[99]

Table 4. *Cont.*

Location of Sampling	Country	No. of Samples	Dust Concentration, mg/m^3	Fraction of Dust	Analytical Method	Endotoxin Concentration	Range of Endotoxin Concentration	Reference
Hemp processing plant	UK	-	-	Inhalable	Kinetic QCL test	AM: 19,569.4; GM: 14,345 EU/m^3	2177–36,962 EU/m^3	[106]
Textile and garment factories	Ethiopia	95	AM: 1.3; GM: 0.75	Inhalable	QKC LAL test	AM: 2647; GM: 831 EU/m^3	12–30,801 EU/m^3	[107]
Wheat harvesting	Colorado, USA	-	GM: 0.83, AM: 1.32	Total dust	Quantitative chromogenic modification of LAL	GM: 54.24; Mean: 104.6 EU/m^3	4.4–744.4 EU/m^3	[108]
Sawmill industries	Norway	481	GM: 0.09	Thoracic	Kinetic LAL assay	GM: 3 EU/m^3	-	[109]
Sawmills	Croatia	-	-	Respirable	Quantitative chromogenic end-point LAL	336.5 EU/m^3	-	[110]

AM: arithmetic means; GM: geometric mean.

3.1. Occurrence of Endotoxin in Animal Housing

Any structure that houses livestock is certain to produce dust. Dust can be generated from feed, excreta, feathers, fur etc. The occurrence of endotoxin in animal housing around the world is presented in Table 2. The animals and workers are exposed to different levels of dust in animal housing. The floor-housed poultry rearing system in Canada has the highest level of total dust (9.56 mg/m^3) [48]. The cage-housed poultry system has lower dust concentrations due to the fact that the poultry is kept in the layer system and the poultry cannot perform dust bathing on the floor, which reduces the dust. The endotoxin concentration was found to be lower in the floor-housed poultry rearing system than in the cage system. In a floor-housed poultry rearing system, the excreta are collected in the floor and the moisture is absorbed by the bedding material, which is not the case with the cage-house poultry rearing system. The inhalable dust concentration in poultry farms of Denmark has a dust concentration of 5.7 mg/m^3, which is above the permissible exposure limit [91]. In the case of the swine/pig farms, the dust generated was found to be in the range of 0.128 [92] to 5.0 mg/m^3 [21] and the geometric mean of endotoxin concentration varies from 16.261 [93] to 1500 EU/m^3 [94] The reason for higher endotoxin levels is due to concentrated animal feeds.

In dairy farms, the dust and endotoxin concentrations were measured at the milking section and feeding sections and in free stall dairy farms. The geometric mean of the dust concentration varies from 0.2 [96] to 1.7 mg/m^3 [97], and the endotoxin concentration varies from 128 [98] to 448 EU/m^3 [91]. The lowest concentration of dust was observed in a mink farm (7 mg/m^3) [95], and the highest concentration of dust was reported in horse stables of the Netherlands (95 mg/m^3) [102], and the endotoxin concentration was 214 [95] to 555 EU/m^3 [102]. The endotoxin and dust concentrations were higher in pig farms than in cattle and poultry farms due to the higher density of animals and use of concentrated feeds in pig farms. Environmental factors, such as temperature, relative humidity and wind velocity, affect the levels of airborne endotoxins at the farms [82,90,103]. Endotoxin exposure is higher for persons living in rural regions with intense livestock production [10].

3.2. Occurrence of Endotoxin in Food Processing Industries

In food processing industries, water is used while processing the food. Endotoxin varies with the pre-processing methods used in food processing plants. Dry pre-processed methods (sun dried cherries of coffee) result in higher concentrations of endotoxin than wet pre-processed food processing methods by using water to depulp coffee cherries [104]. The occurrence of endotoxin in food processing industries around the world is presented in Table 3. The presence of water helps to reduce dust and endotoxin exposure to the workers, as the dust gets absorbed in the water [24]. Yang, 2013, reported that the total suspended dust increases on colder days [66].

3.3. Occurrence of Endotoxin in Other Industries

Byssinosis is commonly developed in workers who are exposed to cotton dust [5]. In rice mills, dust can be generated due to abrasion of the paddy. The occurrence of endotoxin in other agricultural industries around the world is presented in Table 4. During the post-harvest handling of cereal/fruit crops, dust is generated due to the breaking of plant materials. The workers are exposed to this contaminant and are exposed to dust, which can reduce the forced vital capacity of the workers [28,33,35–39]. Many researchers have collected data on inhalable, thoracic and total dust, but very few researchers have sampled the respirable fraction of dust samples [88,92,101,110]. The respirable dust fraction can penetrate beyond the terminal bronchioles of the lungs [28,41]. Respirable dust laden with endotoxin can cause serious health issues for the workers.

4. Dose of Endotoxin Exposure and Health Effects

The health effect of endotoxin is related to the dose of endotoxin exposure in the workers [111]. Endotoxin present in a respirable fraction of dust may cause cellular reactions

in the alveoli of the workers [112]. Exposure to bacterial endotoxin for a long time increases the risk to respiratory health, and the most common problem associated is endotoxic shock [113–115]. Due to endotoxin exposure in the workers, there is a significant across-shift decrease in the lung function when the endotoxin exposure limit exceeds 53 EU/m^3 [116]. There is an across-shift decrease in maximal mid-expiratory flow of the worker in animal feed industries due to exposure to endotoxin when the limit exceeds 15 ng/m^3 [117].

Most grass seed extracts can produce the proinflammatory cytokines IL1, IL6, IL8 and TNF, which is comparable to that with LPS, and this is a probable cause of organic dust toxic syndrome in workers [118]. In vitro, LPS induces cytokines, which has heritability effects on the workers, and it is associated with cytokine SNPs, which cause clinical disorders [119]. TLR-4299 (A/G) and TLR-4399 (C/T) increase the risk of septic shock as well. IFN-6-174 (G/C) is linked to tuberculosis, and IL-6-174 (G/C) is associated with the development of the metabolic syndrome and ischemic heart disease [120].

The maximum exposure limit to LPS (50 µg/m^3) can induce acute symptoms and fever, and the change in lung function with dyspnea and the inhalation of LPS can modify eosinophilic inflammation [120]. The increase in endotoxin concentrations in the house can be linked to a higher serum level of total allergen-specific immunoglobulin E (IgE) [54]. Mononuclear phagocytes (monocytes and macrophages) are the primary cells that respond initially to inhaled endotoxin via the quick release of tumor necrosis factor (TNF). Inflammatory mediators produced from monocytes/macrophages can cause pyrexia, neutrophil recruitment, activation of airway epithelial cells and direct bronchial hyper-reactivity [44]. Endotoxin exposure can cause respiratory disorders, and a decrease in lung function occurs when the endotoxin exposure level ranges from 0.2 to 470 ng/m^3 [121]. In healthy youth, blood pressure of workers is increased with a short duration (130 min) of exposure to endotoxin β-1,3-D-Glucan [122]. Latza et al. [123] and Oldenburg et al. [124] reported that there is a significant dose-dependent effect on bronchial symptoms and that bronchial symptoms increase as the exposure limit exceeds 450 EU/m^3.

With an increase in concentration, endotoxin increases respiratory symptoms, such as wheezing, wheezing with shortness of breath and cough [118]. Exposure to higher concentrations of endotoxin exacerbates the risk of lung function, causes mortality in male workers in textile industries [125], lowers pulmonary functions in patients [126–129], causes dust toxic syndrome and chronic bronchitis [91,130], and results in fever, chest tightness and bronchoconstriction in cotton workers [130]. Long-term and high-level exposure to endotoxin reduces the risk of lung cancer [129] and decreases pulmonary function after 16–20 h of exposure when endotoxin exposure exceeds 4 ng/m^3 [131]. In vitro, endotoxin increases the oxidative stress induced by amorphous silica-engineered nanoparticles in lung epithelial cells [132]. Eighty-nine per cent of dairy workers were found to be exposed to endotoxin above the recommended exposure limit in dairies located in Colorado and Wyoming, USA, which resulted in a significant risk to workers' health [100]. A proper dose for endotoxin exposure that can be accepted worldwide has not been formulated.

5. Detection Techniques

The assessment of endotoxin differs with different sampling methods, extraction methods, sample storage temperatures and procedures employed [82,133]. Dust of different fractions is collected on different samplers. The most common filter used for dust collection is a 37 mm glass fiber filter. The gravimetric dust sampling method is a common method to quantify the dust concentration in agricultural operations and settings. The dust fractions that are used for endotoxin analysis are inhalable [19,92,95,99,100,107], thoracic [96] and respirable dust [92,110]. Some authors also quantify endotoxins in total dust [48] and PM10 [21,93]. There is no recommended temperature for storing the dust samples before endotoxin analysis. The common storage temperatures are −80 °C [22], −20 °C [17,95] and 4 °C [96,105]. Other temperatures for storing the dust samples are dry ice temperatures [109] and −70 °C [100]. During filter extraction, the most common pyrogen-free water (PFW) used for media extraction is 0.05% Tween 20 (also known as Polysorbate 20). Other PFWs

that are used are 0.01% Tween 80, sterile nonpyrogenic water (Travenol Laboratories, INC., Morton Grove, III, Deerfield, IL, USA) and TAP Buffer (0.05 M potassium phosphate, 0.01% triethylamine, pH 7.5). The quantity of PFW used is either 5 or 10 mL.

Centrifugation of the solution is carried out with the help of a centrifuge, and the supernatant obtained is used for the analysis of endotoxin concentration. There is no standard frequency and time for centrifugation. The most common frequency and time for supernatant extraction is 1000× *g* for 10 min. Other frequencies and times that are employed for centrifugation are 600× *g* for 5 min, 600× *g* for 20 min, 350 rpm for 60 min, 2000× *g* for 10 min, 1420 × *g* for 30 min, 5000 rpm for 10 min, 2000 rpm for 10 min and 900× *g* for 5 min.

The concentration of endotoxin is estimated from the endotoxin of standard bacteria. The standard bacteria that are used to estimate the endotoxin concentration in agricultural settings and operations are *E. coli* (Endosafe; CSE lot no. ET 84092, Wilmington, MA, USA); *E. coli* 0111: B4, *E. coli* O55:B5, *E. coli* 6 standards, *E. coli* 0113:H10 (Difco, Franklin Lakes, NJ, USA) and U.S. endotoxin standard EC-5. *E. coli* O55:B5 is the most commonly used standard. Internationally, endotoxin is measured in endotoxin units (EU) per unit volume of air. The unit for endotoxin varies largely from laboratory to laboratory and based on the standard bacteria and analytical procedure used. The conversion value of endotoxin from ng/m^3 to EU/m^3 for *E. coli* O55:B5 and *E. coli* 0113:H10 (Difco) is 10 EU/m^3 equals 1 ng/ m^3, with 8 EU/m^3 equals 1 ng/m^3 for the *E. coli* 6 standard.

The different analytical methods used for the analysis of endotoxin are the Limulus Amebocyte Lysate (LAL) assay, gel clot assay, chromogenic-end point LAL assay, spectrophotometric modification of the LAL gel test, quantitative chromogenic modification of LAL gel test, kinetic-turbidimetric Limulus assay, quantitative kinetic chromogenic LAL test and recombinant factor C assay. The most common analytic method is the LAL assay. There are different types of LAL assays, such as the gel clot assay, turbidity assay and chromogenic assay [134]. The most frequently followed analytical method is the kinetic chromogenic assay, which is the quantitative method for detecting the presence of endotoxin. In the kinetic chromogenic assay, the supernatant of the samples is mixed with LAL reagent and placed in a microplate reader and monitored at a wavelength of 405 nm over time to detect the color change. The kinetic chromogenic LAL assay is the most widely used test to quantify endotoxin present in environmental samples [43]. Other LAL methods used to detect the endotoxin of GNB include a spectrophotometric modification of the LAL gel test, quantitative chromogenic modification of the LAL gel test, endpoint chromogenic LAL, and LAL gel clot test. Another alternative method for the quantification of bacterial endotoxin is the recombinant Factor C (rFC) assay. The rFC assay is a recently developed method for analyzing bacterial endotoxin, and it uses the rFC reagent produced from the cDNA of the Mangrove horseshoe crab (*Carcinoscorpius rotundicauda*) [9,135]. Different detection techniques followed are presented in Table 5.

Table 5. Different endotoxin detection techniques and filter conditions.

Sampler Type	Sampling Rate, L/min	Filter Type	Standard Used	Storage Temperature for Samples	Absorbance	Reference
Button aerosol samplers	4	25 mm binder-free glass fiber	*E. coli* 055: B5	−20	405 nm	[17]
IOM sampler	2	25 mm Glass filters,	-	Room temperature	405 nm	[19]
IOM sampler	2	25 mm glass fiber	-	−20	405 nm	[20]
Technischer Überwachungsverein (TÜV)	3.5	37 mm glass fiber filters	*E. coli* 6 standard	-	405 nm	[21]

Table 5. *Cont.*

Sampler Type	Sampling Rate, L/min	Filter Type	Standard Used	Storage Temperature for Samples	Absorbance	Reference
AP-2A personal sampler	2	37 mm glass fiber	*E. coli* 0113:H10	-	-	[23]
Plastic conical inhalable samplers	3.5	37 mm glass fiber filters	-	−20	-	[24]
PAS6 sampling heads	2	25 mm glass fiber filters	-	−20	-	[25]
-	2	37 mm glass fiber filter	*E. coli* O55:B5	-	-	[48]
Vertical elutriators	7.4	37 mm Glass fiber filters	*E. coli*	-	-	[69]
GSP samplers	3.5	Teflon filter	*E. coli* O55:B5	−80	-	[72]
Gilian nylon cyclone	1.7	37 mm polycarbonate membrane filters	-	4	405 nm	[88]
Three-stage cassette	1.7	PVC membrane	-	-	-	[92]
Sierra-Anderson High volume cascade impactor	1270	Glass microfiber	*E. coli* 0111: B4	-	-	[93]
Conical inhalable sampler	3.5	37 mm glass fiber	-	-	-	[94,134]
Plastic inhalable conical sampler	3.5	37 mm glass fiber	*E. coli* (O55:B5)	−20	-	[95]
Thoracic parallel particle impactor (PPI)-T	2	37 mm glass fiber filter	-	4	-	[96]
IOM sampler	2	25 mm glass microfiber	-	-	-	[97]
SKC button sampler	4	Teflon 25 mm	*E. coli* 055: B5	-	440 nm	[98]
SKC Button samplers	4	25 mm PVC filters	United States Reference Standard EC-6	−70	-	[100]
Button Aerosol sampler	2.5	37 mm glass fiber	*E. coli* 055: B5	-	405 nm	[101]
PAS-6 inhalable dust sampler	2	25 mm glass fiber filters	*E. coli*	−20	-	[102]
Harvard impactors	10	37 mm Teflon filters	-	−20	-	[103]
25 mm three-piece conductive cassettes	2	Glass fiber filters	-	-	-	[104]
Inhalable dust sampler and Impinger	2	-	-	4	-	[105]
Gillian gilair 5	2.1	Polycarbonate	-	-	-	[106]
Conductive plastic inhalable conical sampler	3.5	37 mm glass-fiber (GFA) filter	-	−20	-	[107]
-	1.5–2	37 mm glass fiber	-	Dry ice	-	[108]
BGI GK2.69 cyclones	1.6	Glass fiber filters	-	−20	-	[109]
Casella Bedford	-	Cellular	*E. coli*	−20	405 nm	[110]
Button aerosol samplers	4	-	*E. coli* O55:B5	-	-	[136]

6. Management and Control Strategies

Reducing endotoxin exposure to workers in an agricultural setting is very important to reduce health hazards. The Occupational Safety and Health Administration (OSHA) recommends training the workers on recognizing the health effect of endotoxin exposure from bioaerosols [137]. Four management and control strategies can be implemented to reduce endotoxin exposure. The first strategy is the reduction of endotoxin at the source. Intercepting the travel of endotoxin to the workers could be the second strategy.

Administrative control methods could be used if the first two strategies fail. The last strategy is the use of personal protective equipment (PPE).

The endotoxin concentration depends on dust concentration and the amount of dust exposure [107,138]. Dust generation needs to be reduced to minimize endotoxin exposure in an agricultural setting. Operations involving size reduction and grinding in livestock houses should be avoided or isolated, as these operations generate dust (animal fat or vegetable oil) [139]. Dust generation is reduced by spraying finer droplets of water or other liquid over the source of dust generation. Poultry breeding houses [140–144] and pig buildings [145] have used the liquid spraying technique to suspend the dust generated. This control method sprays the liquid in small-scale spice grinding mills [146]. Artificial intelligence, such as animal activity tracking sensors and image sensing sensors, can be employed to track the activity of animals to predict the dust concentration and provide suitable mechanisms, including the spraying of water or liquid to reduce dust and endotoxin [147]. In livestock buildings, dust is generated from the feed, and it is reduced with the addition of animal fat or vegetable oil in the feed [139]. The mixing of fat or oil into the feed suspends the dust available or generated with the feed. Mechanical interventions are used to reduce dust emissions where water spraying is not feasible. Interventions are used to facilitate dust concentration in rice mills [7], poultry housing [139], pig buildings [147–149] and bakeries [149,150]. Dust generation in poultry housing is reduced by reducing the relative humidity [140].

The endotoxin concentration is high in the production process due to rotten fresh vegetables, plant material and other waste material. Exposure can be reduced by removing rotten parts, soil and plant parts at the early stages of processing and cleaning seeds [151]. The use of water in the processing units results in the emission of more endotoxin than processing without the use of water [148] as the growth of microorganisms increases with an increase in moisture. Dust and endotoxin exposure is reduced by using a barrier or an enclosure for isolation so that the dust generated at the source is trapped and accumulated. This strategy is used in food processing plants [152–154]. Airborne endotoxin concentrations may be affected by the ventilation and hygiene of the building [82]. If isolation is not effective, ventilation is performed in the buildings [155–157] to reduce dust propagation to the workers. Animal housing and processing plants have ventilation to reduce dust exposure. Dust and endotoxin can be decreased effectively using ventilation and an exhaust system. However, ventilation at a higher velocity may spread the settled dust and keep it suspended in the workplace [155]. Job rotation, which reduces exposure time, can be practiced to further reduce dust and endotoxin exposure. Spaan et al. [158] reported that workers who are performing the same task at the same location have exposure to endotoxin similar to that for workers who are performing different tasks at the same location [158].

PPE is used to reduce dust and endotoxin exposure, as other strategies are ineffective. OSHA recommended using NIOSH-certified N95 masks/respirators for respiratory protection for workers exposed to dust and endotoxin [137]. There is a significant reduction in respirable symptoms experienced by workers after using N95 masks [159]. Woodworkers were found to have a positive view of the importance of using PPE, although workers did not use it frequently [160]. Regular workers are more likely to use PPE than temporary workers because temporary workers are not generally provided PPE [161]. However, the use of PPE has many limitations. PPE in a hot climate or workplace can induce dehydration and heat exhaustion. Dehydration and heat exhaustion affect the overall productivity and safety of the workers, and it can lead to acute or chronic diseases and in extreme circumstances, death. Young male agricultural workers use more respirators or masks to protect them from dust than older female workers [162]. Since male workers participate in hazardous and heavy work periodically, they are more likely to be exposed to the hazardous environment than female workers [163]. PPE should be made accessible to the workers. Training could be provided to the workers regarding the use of PPE, and awareness should

be encouraged [164]. Dust settles on workers' clothes in the workplace; therefore, the same clothes should not be used before laundry to reduce endotoxin exposure.

7. Conclusions

Agricultural operations generate a sizeable amount of dust during various operations. This dust is inhaled through the nose and mouth of the workers who are exposed to dust. Endotoxin is present in the dust to which the workers are exposed. The inhalation of dust with endotoxin causes many respiratory disorders in the workers. In the agricultural sector, *Pantoea agglomerans* of GNB are predominantly present. Dust samples are collected using different dust samplers at different flow rates using different filters. The dust samples are analyzed gravimetrically to determine the concentration of dust. The filters are stored at a temperature of −20 °C in most research articles. *E. coli* 055: B5 is the predominately used standard to quantify endotoxin. Quantitative kinetic LAL is the most common detection method for quantifying endotoxin. It was observed that the concentration of endotoxin is above the threshold recommended limit in major agricultural operations and industries. The presence of causative GNB and other microorganisms is relatively high in the dust. Dust containing bacterial endotoxin has a significant impact on health. This paper does not analyze the effect of temperature and relative humidity on the working environment of the workers. The use of personal protective equipment was found to be an effective strategy to reduce exposure to endotoxin and dust in agricultural farms and settings.

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