

EVALUATION OF AIRBORNE AND ENDOTOXIN IN CORN STORAGE AND PROCESSING FACILITIES IN COLORADO

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Abstract. The main objectives of this research were to determine what aerosols were present by taking total dust (TD) samples and thoracic particulate mass samples (TPM) on farms and in grain elevators. Cascade impactors were used to characterize size distribution of dust and endotoxins at each site. Total dust concentrations on farms had a geometric mean 3.4 mg/m^3 and 3.3 mg/m^3 in elevators. The geometric mean (GM) concentrations for the TPM were 2.4 mg/m^3 on farms and 1.0 mg/m^3 in elevators. Endotoxin concentrations as geometric means were alarming at $3,175 \text{ EU/m}^3$ total dust and 983 EU/m^3 by TPM on farms. In elevators, the GM concentrations for endotoxins were $2,534 \text{ EU/m}^3$ total dust and 526 EU/m^3 by TPM. The mass median aerodynamic diameter (MMAD) for endotoxins on farms was $8.0 \mu\text{m}$ and $6.5 \mu\text{m}$ in elevators. The paired t-test was applied to the log ratios of endotoxin concentrations (EU/m^3) and dust concentrations (mg/m^3), for paired samples of the TD and TPM. A higher content of endotoxin was associated with TPM for farms but not elevators. It was concluded that although the TPM fraction (dust) may represent a small part of the total mass, the aerosol size is optimum for deposition in the lung's tubular airways, and might cause airway inflammation due to the endotoxins. The TPM fraction of corn dust represents the best measure of exposure with regard to the potential development of long-term airways inflammation, and the potential of chronic obstructive pulmonary disease among chronically exposed workers. All endotoxin concentrations were well above recommended exposure levels of several researchers familiar with endotoxin health effects.

Key words: respiratory hazards, aerosols, agriculture, corn dust

Rezumat. Principalele obiective ale acestui studiu au fost determinarea tipurilor de aerosoli prezenți la silozurile și elevatoarele de cereale, utilizând ca indicatori cantitățile de pulberi totale (TD) și fracția masică toracică de pulberi (TPM). Pentru caracterizarea distribuției dimensionale și a cantităților de endotoxine s-au utilizat impactoare tip cascadă. La silozuri, media geometrică a concentrațiilor de pulberi totale a fost de $3,4 \text{ mg/m}^3$, iar la elevatoare a fost de $3,3 \text{ mg/m}^3$. La silozuri, media geometrică (GM) a concentrațiilor pentru TPM a fost de $2,4 \text{ mg/m}^3$, iar la elevatoare a fost de $1,0 \text{ mg/m}^3$. Media geometrică a concentrațiilor de endotoxine a înregistrat valori alarmante de 3.175 EU/m^3 pentru pulberile totale și 983 EU/m^3 pentru TPM în cazul silozurilor. La elevatoare, media geometrică a concentrațiilor de endotoxine a fost de 2.534 EU/m^3 pentru pulberi totale și 526 EU/m^3 pentru TPM. La silozuri, diametrul median al fracției masice (MMAD) corespunzător concentrațiilor de endotoxine a fost de $8,0 \mu$, iar la elevatoare a fost de $6,5 \mu$. Pentru compararea fracțiilor perechi de TD și TPM s-a utilizat testul t aplicat la logaritmul rapoartelor dintre concentrațiile de endotoxine

(EU/m³) și concentrațiile de pulberi (mg/m³). La silozuri s-a evidențiat o asociere dintre concentrațiile mari de endotoxine și TPM, dar nu și la elevatoare. S-a concluzionat că, în pofida faptului că fracția TPM poate reprezenta o mică parte din masa totală a pulberii, dimensiunea aerosolilor este optimă pentru depunerea în căile respiratorii și poate cauza inflamații datorită conținutului de endotoxine. Fracția TPM reprezintă cel mai bun indice de expunere la pulberi de porumb ce pot induce inflamații de lungă durată a căilor respiratorii precum și afecțiuni pulmonare de tip obstructiv. Toate concentrațiile de endotoxine s-au situat cu mult sub limitele de expunere recomandate de cercetători cu experiență în domeniul efectelor exercitate de endotoxine.

Cuvinte cheie: toxice respiratorii, aerosoli, agricultură, pulbere de porumb

INTRODUCTION

The National Institute for Occupational Safety and Health (NIOSH) estimates that over 500,000 workers in the U.S.A. and 100,000 workers in Canada, are exposed to grain dust (1). Research has shown that the prevalence of respiratory symptoms among grain industry personnel is higher than that among the general population (2). Epidemiological studies conducted over the past few decades have demonstrated that overexposure to grain dust likely causes “grain fever”, Organ Dust Toxic Syndrome (ODTS), wheezing, chest tightness, productive cough, eye and nasal irritation, and symptoms of chronic obstructive respiratory disease. Grain dust may also induce asthmatic reactions via allergic mechanisms, particularly in individuals who are predisposed to developing allergies (i.e., atopic individuals) (1,2).

The Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for total dust is 15 mg/m³ for Particulates Not Otherwise Classified (PNOC) (3). The American Conference of Governmental Industrial Hygienists

(ACGIH), has a threshold limit value/time-weighted average (TLV-TWA) of 10 mg/m³ for total dust for particulates having a quartz content of less than 1% (4). There is a TLV of 4.0 mg/m³ for grain dust that only includes grain dusts from oats, wheat and barley (4). However, whether or not these standards mimic human lung deposition and consequently the health effects caused strictly by total grain dust, is a topic of concern. Therefore, several studies have recommended that “total dust PELs be established in the United States, be converted to thoracic particulate mass TLVs, as proposed by the ACGIH, and the particle size selective TLV documentation” for those dusts that may cause damage to the thoracic region of the pulmonary system (4).

The short introduction will not attempt to discuss every research paper ever written on grain dust. However, several investigators have suggested that the organic grain dust component that is inhaled by workers may not be the root cause of acute and chronic respiratory health effects (5-8). It has been demonstrated that exposures to wheat dust with endotoxins have a statistically significant positive

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correlation with reduced pulmonary function among wheat harvest workers even at moderate concentrations of contaminant exposure (5). Other research has suggested that the component of wheat dust that might be of most concern is the endotoxin associated with the airborne dust (7). Subsequent work relied upon wheat dust air samples which were exposed to cell cultures of alveolar macrophages. This research demonstrated that endotoxins were responsible for 75% of the inflammatory response based upon the release of cytokine biomarkers $\text{TNF}\alpha$ (8).

Endotoxins are heat-stable lipopolysaccharide complexes which are integral to the outer membrane of gram-negative bacteria which are ubiquitous contaminants of soils, water, and other living organisms. Reviews of the chemical composition of endotoxins provide exhaustive descriptions of the molecular nature of these materials and indicate a tremendous variety of molecular structures (9-12). This variability in composition is of importance to the differences in the strength of toxicities found with different endotoxins (13,14). Endotoxins are released into the environment after bacterial cell lysis or during active cell growth (15). Release also occurs when intact bacterial cells are phagocytized by macrophages, in which case the liberated endotoxins contain increased toxicity (16). Ultrastructural analyses of gram-negative bacteria associated with environmental exposures demonstrate shedding of large amounts of endotoxin-

containing membrane vesicles (2.0 μm to 4.0 μm in diameter) (17).

Numerous studies have been conducted of endotoxin exposures in agricultural operations ranging from confined housing of animals to fruit and vegetable production and handling (18-25). These all papers demonstrated significant exposures to endotoxins, however this literature review will discuss only in detail those studies associated with grain dust, and more specifically to corn dust exposure. Up until 1998, almost all research on the environmental exposures and potential health effects of grain dust, dealt primarily with wheat. A very small number of studies addressed air concentrations and exposures to barley and oats (1,2). Two studies from New York did document dust and endotoxin levels in corn storage silos where total dust levels ranged from 0.2 mg/m^3 to 113 mg/m^3 . The mean endotoxin concentration by total dust sampling was 885 EU/m^3 (26,27). It must be noted that these exposures followed storage and microbial action in the creation of silage for animal feed.

At the request of the Colorado Corn Growers Association in 1998, research was undertaken by the High-Plains Intermountain Center for Agricultural Health and Safety in corn storage bins and corn in grain elevators. Average concentrations on farms was 365,291 EU/m^3 with one concentration over 1.7 million EU/m^3 . Grain elevators handling corn had mean concentrations of 94,235 EU/m^3 (6). Thus, this follow-up research was

prompted to replicate and verify these alarming results.

METHODS AND MATERIALS

Total dust and thoracic particulate mass (TPM) corn dust samples were collected at four farms and four elevators in Colorado during the months of July and August of 1999. Four total dust and four TPM samplers were positioned in pairs, and one cascade impactor was used at each site. Five sets of samples were collected at each site.

Total dust is often assumed to collect particles of all sizes. Total dust sampling is the legally recognized method in the United States. The authors realize this is not the best scientific method for dust sampling. However, total dust was collected in closed face 37 mm sampling cassettes with mixed cellulose ester filters with 0.8 μm in pore size. Escort ELF pumps were calibrated by the bubble burette method at 2.0 liters per minute (lpm). TPM refers to particles collected on a membrane filter after a cyclone separator with a cut size of 10.0 μm MMAD. TPM corn dust samples were collected by using the BGI thoracic cyclone followed by a 37 mm cassette with mixed cellulose ester filters that were 0.8 μm in pore size. A six-staged personal cascade impactor (Sierra Instruments) was used with mylar substrate in conjunction with 0.8 μm mixed cellulose ester backup filters for particle size classification (7). The Escort ELF pumps were also calibrated using the bubble burette method at 1.0 lpm in order to collect

the predominate large particles in the aerosol. The cascade impactor, when operated at 1.0 lpm, produced the cutoff diameters (d_{50}) of 15 μm , 8.7 μm , 5.1 μm , 2.1 μm , 1.4 μm and 0.78 μm from stages three to eight respectively. Data reduction of impactor data followed routine occupational hygiene methods (28). The mass of dust or endotoxin found on each impactor stage was plotted on log-probability paper, cumulative percent of particles less than given size versus the log of particle size. A linear plot represented a log-normal distribution of particle size. The mass median aerodynamic diameter (MMAD) was the particle size at the 50% point of the particle size distribution. The geometric standard deviation (GSD) was determined by dividing the MMAD into the particle size at the 84.14% particle size (one standard deviation from the geometric mean).

Samples were collected as long as the activities were undertaken by workers. Pre-desiccation and post-desiccation of the filters was done before and after samples were taken. Pre- and post-weighing of filters was done using a Mettler analytical balance with a sensitivity of 0.05 mg. After the gravimetric analysis of the filters, the samples were sent to the NIOSH laboratory in Morgantown, West Virginia for endotoxin analysis by the Limulus Amoebocyte Lysate (LAL) method.

It must be recognized that there is no internationally accepted and standardized method for analysis of gram-negative bacterial endotoxins from

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environmental samples. NIOSH analyses for airborne dusts undergo an aqueous extraction, and the supernatant fluids are analyzed by the LAL assay in accordance with the test-system manufacturer's procedure (29). The pre-weighed dust was rocked at room temperature for an hour, and an aliquot of the supernatant fluid was mixed with reagent. Endotoxin present in the fluid, where, in the kinetic chromogenic assay, it catalyzed the activation of a pro-enzyme present in the amoebocyte lysate. The initial rate of activation was related to the concentration of endotoxin, and the activated enzyme catalyzed the split of p-nitroaniline from the substrate. That release was then measured at 405 nm continuously during incubation, and the endotoxin concentration was calculated by the comparison of the sample's reaction time with that of known standards.

ETHICS

The Human Subjects Research Committee of the Office of Regulatory Compliance at Colorado State University, oversees all human subject research at the University. This research protocol was reviewed by this Committee and found to be in compliance with all rules and regulations pertinent to Human Subjects Research.

STATISTICS

Descriptive statistics, such as range, geometric means, and geometric standard deviations, were calculated for total dust and TPM concentrations at

each farm and elevator site, as well as for endotoxin concentrations for both the total dust and TPM fraction. Regression analysis was used to estimate both the relationship between corn dust concentrations (both total and thoracic) and endotoxin concentrations, and the linear relationship, between total dust and TPM. Linear regression was also used to calculate the MMAD and the GSD for the cascade impactor data as a method of double checking the validity of hand made plots on the log-probability paper described previously under methods and materials for cascade impactor data reduction. The paired t-test was applied to the log ratio of endotoxin and dust concentrations for paired samples of total and TPM fraction to determine which sampling method best represented endotoxin exposures.

RESULTS

The range for the total dust concentrations and TPM concentrations was moderate to high (Table 1). On farms, total corn dust ranged from 0.4 mg/m³ to 53.6 mg/m³ (GM = 3.4 mg/m³), and in elevators, total corn dust ranged from 0.1 mg/m³ to 160.9 mg/m³ (GM = 3.3 mg/m³). TPM concentrations on farms ranged from 0.2 mg/m³ to 15.3 mg/m³ (GM = 2.4 mg/m³) and in corn elevators, dust concentrations by TPM ranged from 0.1 mg/m³ to 4.7 mg/m³ (GM = 1.0 mg/m³).

Endotoxin concentrations were of great concern. On farms, the endotoxin concentrations ranged from 499 EU/m³ to 54,653 EU/m³ (GM =

3,175 EU/m³) as measured by total dust sampling (Table 1). In corn elevators, the range was from 58 EU/m³ to 77,006 EU/m³ (GM = 983 EU/m³). By TPM measurement, the concentration ranged from 284 EU/m³

to 29,266 EU/m³ (GM = 2,534 EU/m³) in the corn bins on farms. TPM concentrations of endotoxins in corn elevators ranged from 55 EU/m³ to 3,733 EU/m³ (GM = 526 EU/m³).

Table 1. Geometric Mean (GM) and range of dust concentration and endotoxin concentration for both the Total Dust and Thoracic Particulate Mass for Farms and Elevators

| | Total Dust | | Thoracic Particulate Mass | |
|--------------|---|--|---|--|
| | Dust concentration (mg/m ³) | Endotoxin concentration (mg/m ³) | Dust concentration (mg/m ³) | Endotoxin concentration (mg/m ³) |
| Sites | | | | |
| Farms* | GM = 3.4 0.4 - 53.6 | GM = 3.175 499 - 54,653 | GM = 2.4 0.2 - 15.5 | GM = 2,534 284 - 29,266 |
| Elevators** | GM = 3.3 0.1 - 160.9 | GM = 983 58 - 77,006 | GM = 1.0 0.1 - 4.7 | GM = 526 55 - 3,733 |

* N = 14 for total dust; N = 16 for thoracic particulate mass

** N = 15 for total dust; N = 14 for thoracic particulate mass

As regards acceptable exposure levels, different investigators have recommended different thresholds of effect for acute or chronic health effects. For example, one author has stated that at 90 EU/m³ there should be no change in pulmonary function for smokers (30). In a much larger study, 330 EU/m³ could be tolerated with no pulmonary function change (31). This is further exacerbated by the fact that 75% of epithelial inflammation experienced from grain dust exposure is caused by endotoxins (8). Thus endotoxins exposures in corn bins and corn elevators should be of great concern as regards the respiratory health of workers exposed to corn dust on farms and in elevators.

One objective of this study was to investigate the relationship between corn dust particles and of endotoxin-

bearing particles in order to determine what aerosol sampling method is most appropriate to assess corn dust exposures, total dust or TPM. In order to evaluate this, the paired t-test was applied to the log ratio of endotoxin concentrations (EU/m³) and dust concentrations (mg/m³) to paired samples for both the total dust and TPM fraction.

For all farms and elevators, for the ratios for total dust sampling, the p-values were 0.06 on farms and 0.08 in elevators. For TPM, p-values were 0.02 on farms and 0.05 in elevators which indicated that the ratio of endotoxin concentration (EU/m³) and dust concentration (mg/m³) and also the ratio of endotoxin units (EU) and mass of the dust (mg), was significantly higher in TPM than in the total dust. Therefore, one might

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conclude that although the TPM fraction may represent a small portion of total mass, because of endotoxin particle aerodynamic size and behavioral properties, the TPM fraction may be a better indicator of possible tissue inflammation and subsequent injury due to the endotoxin deposition in the tubular airways. Therefore, the TPM fraction may represent a worse case potential exposure with regard to the potential development of chronic obstructive pulmonary disease initiated by endotoxin exposures.

CASCADE IMPACTOR DATA

On each site, a six-staged Marple personal cascade impactor (32) was

used to characterize the size distribution of the dust particles in the work environments that were surveyed. The data obtained from the cascade impactor was used to calculate Mass Median Aerodynamic Diameter (MMAD) and Geometric Standard Deviation (GSD) for both the dust particles and for particles bearing endotoxins. Dust and endotoxins were found on all stages of the impactor, thus lending reliability to the fact that the particle size distributions were log normal following regression analysis and the plotting of results on log-probability paper. Results are summarized in Table 2.

Table 2. The cascade impactor data for all the Farms and Elevators

| Sites* | Dust | | Endotoxin | |
|------------------------|----------------|----------|----------------|----------|
| | Mean MMAD (µm) | Mean GSD | Mean MMAD (µm) | Mean GSD |
| Farm 1 | 7.5 | 8.67 | 7.4 | 3.38 |
| Farm 2 | 14 | 3.64 | 9 | 3.11 |
| Farm 3 | 23 | 4.35 | 13 | 2.56 |
| Farm 4 | 12 | 5 | 5.5 | 3.45 |
| MEAN for All Farms | 13 | 3.5 | 8 | 2.9 |
| Elevator 1 | 12 | 3.34 | 9.5 | 2.95 |
| Elevator 2 | 10 | 2.09 | 6.8 | 2.94 |
| Elevator 3 | 30 | 3.33 | 6.8 | 3.75 |
| Elevator 4 | 10 | 8 | 4 | 3.25 |
| MEAN for All Elevators | 10 | 3.1 | 6.5 | 3.1 |

* N for each farm and elevator was 5.0 impactor samples

The mean MMAD on farms for dust was 13 µm. For endotoxin-bearing dust, the mean size was 8.0 µm. These are optimal sizes for deposition in the tubular airways of the lung. In grain elevators, the mean MMAD was 10 µm for corn dust and 6.5 µm for

endotoxin bearing dusts. In addition, if the GSDs are taken into consideration, particle size ranges are of great significance for deposition in the thoracic region of the lungs. This is very significant regarding the potential for causing bronchitis and the

potential for development of chronic obstructive pulmonary disease. Figures 1 and 2 show the relationship

between endotoxin and dust particle size distribution for both the farms and the elevators.

Fig. 1. Cascade Impactor Data for Endotoxin Dust Size Distribution for all the Farms

Fig. 2. Cascade Impactor Data for Endotoxin Dust Size Distribution for all the Elevators

DISCUSSION

Fortunately this research did not detect the alarmingly high concentrations of endotoxins found in our initial research (1.7 million EU/m³). Endotoxins found in this research ranged from 58 EU/m³ to 77,006 EU/m³, concentrations that could still cause serious health effects among workers processing corn. These differing concentrations of endotoxins of the two studies might be explained by sampling in different facilities, and weather conditions may have differed so that the growth of gram negative bacteria was reduced prior to this research effort. Another possible explanation may have been the length of corn storage and growth time for gram-negative bacteria. We had no data on corn storage time.

Our data suggests that significant quantities of particles should be deposited in the thoracic region of the

lungs of workers in corn handling operations. Although these particles may represent a small total dust mass, they may cause severe damage because of the high concentration of endotoxins in corn dust. Measuring only total dust concentration for exposure to the corn dust might not always correlate well with the incidence of the specific respiratory disease, especially diseases related to the tubular airways. This research demonstrated that measuring TPM fraction may be a better measure of exposure to endotoxins.

Furthermore, endotoxins associated with dry corn dust are concentrated in the particle size ranges where particles deposit in the tubular airways of the lungs. Thus, great concern for the possible development for chronic obstructive pulmonary disease exists in corn handling facilities where workers experience long-term

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exposures.

During sampling, it was noted that grain handlers were exposed to corn dust in greater amounts during two conditions:

A) If the amount of corn in the bins is very low (farms); and

B) when compressed air is used to clean the settled dust from the floor, greater aerosol concentrations were created in the elevators.

It was also observed that the use of proper respirators was not being enforced in any of the facilities, and there was little concern for adverse respiratory reactions experienced by workers. Many workers appeared to have great resistance to the effects of the dust exposures, sometimes referred to as "healthy worker effect". This is supported by the fact that there was a high turnover rate for the sensitive workers as stated by supervisors at all sites.

Researchers and other investigators should take note that the endotoxin sizes encountered in this research only applies to dry untreated corn dust in corn storage bins and in country grain elevators in Colorado. The aerodynamic endotoxin particle size found in these environments had a mean size of 7.2 μm MMAD (combined elevators and farms). Endotoxins found in other environments may be of very different sizes. For example, in research yet to be published from our group, endotoxins with an MMAD range of 2.0 μm to 3.0 μm were found in equestrian riding arenas. Other researchers have found small

endotoxin particle sizes as well in the size range of 2.0 μm to 4.0 μm (17). In other words, the environments and source of dust will dictate endotoxin particle size.

RECOMMENDATIONS

Taking into account the general methods of cleaning and handling of grain by the grain handlers, routes of exposure in corn bin and elevator work environments, the following steps are recommended to reduce the worker exposure to the grain dust:

- General ventilation

Construct an opening on the roof of the bin that can be opened and closed as desired so that the dust can escape when dust is generated. General ventilation is highly recommended when emptying the last portion of the grain.

- The Use of Respirators

The use of NIOSH approved respirators should be enforced while emptying, cleaning or processing the corn.

- Use of Compressed Air

Compressed air for cleaning should be limited as much as possible, so the settled dust will not be blown into the air.

- Construction of the Bins

It was observed that on-site shed storage seems to generate lesser amounts of dust than the metal storage bins, since there is a larger opening at the front of the structure and workers can operate from the outside of the shed most of the time.

- Use of Dust Cyclone air cleaner

The use of a dust cyclone was used in

one instance to exhaust fine dust particles in the open air, and the use of cyclones required forced air ventilation.

• Administrative Controls

Frequent breaks and training and education regarding the use of respirators and recognizing signs and symptoms of respiratory disease should help workers, farmers and elevator managers to reduce exposures.

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