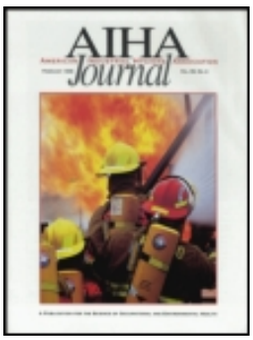

American Industrial Hygiene Association Journal

ISSN: 0002-8894 (Print) (Online) Journal homepage: http://www.tandfonline.com/loi/aiha20

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To cite this article: G.J. Kullman , P.S. Thorne , P.F. Waldron , J.J. Marx , B. Ault , D.M. Lewis , P.D. Siegel , S.A. Olenchock & J.A. Merchant (1998) Organic Dust Exposures from Work in Dairy Barns, American Industrial Hygiene Association Journal, 59:6, 403-413, DOI: 10.1080/15428119891010668

To link to this article: http://dx.doi.org/10.1080/15428119891010668

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Organic Dust Exposures from Work in Dairy Barns

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Environmental surveys were conducted in 85 barns, predominantly dairy, in central Wisconsin to characterize exposures to organic dusts and dust constituents from routine barn work. Environmental analytes included airborne dusts (total, inhalable inlet, and respirable), particle size distributions, endotoxins, total spore and bacteria counts, viable bacteria and fungi, histamine, cow urine antigen, mite antigen, ammonia, carbon dioxide, and hydrogen sulfide. The geometric mean (GM) concentration of airborne dusts include area total, 0.74 mg/m^3; personal inhalable inlet, 1.78 mg/m^3; and area respirable, 0.07 mg/m^3. Viable bacteria and fungi, spores, endotoxins, histamine, cow urine antigen, and mite antigen were quantifiable constituents of these organic dusts and potential respiratory exposure hazards from routine dairy barn work. Endotoxin concentrations from the inhalable inlet samples ranged from 25.4 endotoxin units per cubic meter of air (EU/m^3) to 34,800 EU/m^3. The GM endotoxin concentration from these samples, 647 EU/m^3, exceeds estimated threshold exposure levels for respiratory health effects. Ammonia was a common irritant quantified in most dairy barns. There were significant correlations between the concentrations of organic dusts and certain dust constituents, although in most instances these correlations were not strong. These sampling results demonstrate the complex nature of organic dusts and provide quantitative description of the exposures to toxic and immunogenic dust constituents during routine barn work.

Keywords: ammonia, bioaerosol, dairy barns, endotoxin, histamine, organic dust

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Dairy farming is one of the agricultural industries where farmers are at increased risk for respiratory health problems.[1–6] Exposure to complex organic dusts containing toxic and immunogenic constituents can often occur during common, daily operations in dairy barns. Operations leading to exposure to such dusts include feeding and feed handling, application of bedding materials, barn cleaning and maintenance, manure handling, milking, and general animal confinement.[2,7–10] Dusts from these activities are largely organic and can be comprised of many toxic and immunogenic constituents.[2,10–12] This study describes the exposure assessment completed in 85 Wisconsin barns, predominantly dairy, during routine barn work and is a part of a larger research effort designed to test hypotheses on relationships between respiratory disease and exposure to organic dust and dust constituents. Presented here is the industrial hygiene exposure assessment.

METHODS

Measurements were taken to characterize occupational exposures and environmental conditions inside the barns during daily routine farming activities. Air sampling was done to measure organic dusts, organic dust constituents, and certain gases/vapors.

Barns Sampled

The study cohort included a random sample of farmers and barns drawn from a larger prospective cohort study; the sample included 101 farmers in 85 different barns from a three-county area in central Wisconsin. Eighty-four of the 85 barns (99%) had cattle. Eighty (94%) of these were active dairy barns. All 85 barns were sampled once in random order, over one to two days during the period January 1992 to March 1993. Sampling started in the morning, when the farmer first entered the barn, and environmental measurements

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TABLE I. Environmental Sampling Methods

Analytes	Media/Sampler	L/min	Analytical Methods
Total dust			
Cow urine and mite antigen: *T. putrescentiae*	37-mm DM-800 filter in an open-face filter cassette	18	gravimetric analysis[13–15] 1) RAST inhibition[17–19] 2) RAST inhibition[17–19]
Total dust histamine	37-mm DM-800 filter in an open-face filter cassette	4.0	gravimetric analysis[13–15] radioimmunoassay[20–21]
Total dust real-time monitoring[B]	MiniRAM aerosol monitor (passive sampler)	—	direct reading sampler[15]
Inhalable inlet dust[A,C]			
endotoxins	25-mm DM-800 filter in a cassette with a 15-mm diameter opening	2.0	gravimetric analysis[13–16] kinetic chromogenic *Limulus* amobocyte lysate test (KLAL)[22]
Respirable dust			
endotoxins	37-mm DM-800 filter with a nylon cyclone	1.7	gravimetric analysis[13–15] KLAL[22]
Particle size distributions	Grease-coated Mylar media in a cascade impactor (Marple personal sampler, Graseby Andersen Inc.)	2.0	gravimetric analysis[13–15,23]
Microorganisms			
Viable bacteria/fungi[B] Total organisms	25-mm polycarbonate filter in an open-face filter cassette (NFE method)	2.0	dilution plating on agar[D(24–30)] epifluorescence microscopy[24–25]
Dust constituents in air	25-mm polycarbonate filters in an open-face cassette	2.0	Light and scanning electron microscopy[26,31–35]
Viable bacteria and fungi in air[B]	all-glass impinger with peptone water	12.5	enumeration of bacteria + fungi by dilution plating on nutrient agar[D(24–30)]
Gas concentrations in air: CO, CO_2, H_2S, NH_3	indicator tubes—short-term, direct reading tubes	—	direct reading[15,31]
Gas concentrations in air: CO_2, NH_3, H_2S[B]	long-term samples—diffusion tubes	—	direct reading[15,31]
Barn/silo ventilation	rotating vane anemometer	—	direct measure[15]
Temperature and relative humidity	psychrometer	—	direct measure[15]

[A]These samplers were manufactured from 25-mm cassettes to include the features of the commercial inhalable dust samplers.[16] Key differences are that these samplers did not have a lip and the authors weighed only the filter, not the entire filter housing/cassette. Dust visibly adhered to internal cassette surfaces was brushed onto the filter at the time of gravimetric analysis.
[B]Measurements taken only during detailed surveys
[C]Both personal and area samples taken
[D]Tryptic soy agar was used for bacteria: (1 liter deionized water, 40 g tryptic soy powder, and 10 mL of 1% cyclohexamide). Malt extract agar (MEA) was used for fungi: (1 liter deionized water and 33.6 g MEA).[24–30]

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and samples were collected during the principal daily barn activities and chores including milking, feeding, barn cleaning, manure removal, and application of bedding.

Environmental Measurements

Environmental analytes in air included total, inhalable, and respirable dusts, aerodynamic particle size distributions, endotoxins, total spore and bacteria counts, viable microorganisms, histamine, cow urine antigen, mite antigen, carbon dioxide, ammonia, and hydrogen sulfide. Table I describes the sampling and analytical methods that were used. All sampling pumps were calibrated in the field prior to use with a primary standard. Bioaerosol sampling and analysis was performed as previously described.[26] Physical measurements (temperature, relative humidity, air movements, and ambient conditions) were taken to describe both barn and ambient conditions during each survey.

Sampling Strategy

Both personal and area samples were used to assess occupational exposures and environmental conditions related to dairy barn operation. Personal breathing zone samples were taken to measure exposures to inhalable inlet dusts and endotoxins. These samples were collected by attaching a sampler to the farmer and positioning the sampling orifice in the breathing zone. The personal exposure measurements were collected using a sampler with a 15-mm sampling inlet operated at 2.0 L/min and patterned after the inhalable dust sampler. Area samples were collected in the barn by positioning sampling locations to best reflect worker exposures for various barn activities. Most sampling equipment was positioned in wire baskets that could be easily and quickly positioned for sampling at various locations in the barn. Two sampling stations were used inside each barn to be representative of barn exposures and concentrations. A third area sampling station was positioned outside the barn to measure ambient dust concentrations. The ambient station was positioned upwind from the dairy barn and in a position that reflected the background conditions. (Note: Some area samples were collected using the 15 mm diameter inlet sampling train; these area samples would not be comparable to inhalable dust measures.)

Two types of field survey designs were used: a general survey, and a more detailed survey. General environmental surveys were done at 60 barns and completed during either summer or winter operating conditions. One day was spent at each of the general survey farms and sampling was completed during a typical sampling period of 4 to 6 hours. More detailed environmental surveys

TABLE II. Airborne Dust Concentrations in mg/m³

	Samples	GM	GSD	MIN	MAX
Total Area:					
Within barns	211	0.74	3.05	0.007[A]	6.5
Ambient outside barns	99	0.03	3.87	0.007[A]	0.95
Inhalable inlet[B]					
Personal	159	1.78	2.90	0.007	53.6
Area	252	0.74	2.67	0.007	6.93
Respirable					
Area	217	0.07	4.09	0.007	8.03

[A]The lower limit of analytical detection divided by the square root of 2.
[B]Collected using a 15 mm diameter inlet operated at 2.0 L/min.

were done at 25 dairy barns. The detailed surveys included all elements of the general survey plus additional sampling for viable microorganisms, inorganic gases/vapors, and real-time measures for airborne dusts. Each of the detailed survey barns was sampled twice, once during summer (June through August) operating conditions and again during winter (January to March) operating conditions. Site selection and the ordering of site visits for the detailed surveys were randomized using a random number generator. The detailed survey sites by design included only dairy barns.

Statistical Analysis

General descriptive statistics (totals, means, and frequencies) were calculated using the Statistical Analysis System (SAS, version 6.08) means and univariate procedures. Regression analysis was used to evaluate the correlation between simple measures of dust concentration and the concentration of the more specific toxic or immunogenic dust constituents.[36] Spearman's rank correlation coefficients were used to assess the association between general measures of dust concentration inside the barn with concentrations of specific toxic or immunogenic dust constituents. (A nonparametric measure of correlation was used since the airborne concentrations of some environmental analytes were not normally or lognormally distributed.)

RESULTS

Table II presents barn dust concentrations as time-weighted averages (TWAs) presented in milligrams of dust per cubic meter of air (mg/m³). Sampling results, provided for three dust size fractions, include number of samples, geometric mean (GM), geometric standard deviation (GSD), minimum (MIN), and maximum (MAX) values.

Figure 1 presents the airborne dust concentrations related to barn activity as determined by real-time monitoring with the MiniRAM aerosol monitor (MIE, Inc., Billerica, Mass.). The sample provides a typical pattern of dust concentration by dairy barn activity. (Note: The ordinate scale shows concentration in mg/m³; this dust concentration should be considered approximate since particle measurement is based on the light scattering, rather than a true measure of mass.)

Figure 2 describes the composite particle size distribution from 95 air samples collected inside dairy barns. The data appeared to have a unimodal distribution with a mass median aerodynamic diameter (MMAD) of approximately 13.5 μm and a GSD of 2.1.

Table III presents the concentrations of the organic dust constituents measured in barn air samples. Those organic dust constituents quantified include histamine, total spores and bacteria,

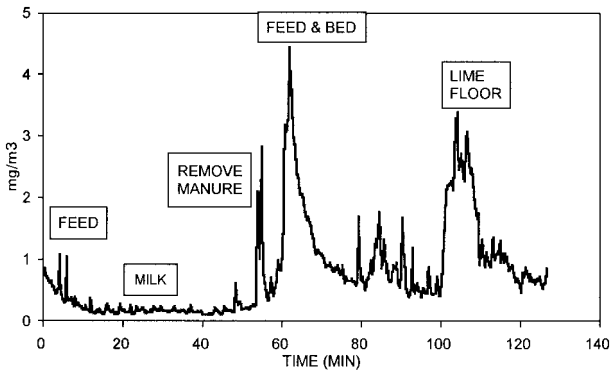

FIGURE 1. Airborne dust concentrations by time and activity

cow urine antigen, mite antigen (*Tyrophagus putrescentiae*), and endotoxins. All of these analytes were present at quantifiable levels in barn air.

Table IV presents concentrations of viable microorganisms collected from the detailed survey farms. Geometric mean concentrations of mesophilic fungi (yeasts and molds), mesophilic bacteria, and thermophilic bacteria are reported in colony forming units per cubic meter of air (CFU/m³). Sample results are reported as TWAs according to the two sampling methods used, Nucleopore filtration and elution (NFE) methods and all-glass impinger (AGI) methods.

Figures 3 and 4, polarized light micrographs of airborne particulate, demonstrate the variable nature of airborne dusts in the dairy barn by different barn tasks. Spores and fungal hyphae are abundant during the application of straw or hay bedding materials (Figure 3). Starch particles are predominant during the feeding of grains, and birefringent mineral particles are predominant during the application of lime (Figure 4). The complex microbiological nature of organic dusts from dairy barns is evidenced in the numerous types of spores present in the air. Figure 3c shows fungal hyphae and spores consistent with those of *Drechslera* and *Cladosporium*. Figure 3d shows urediniospores consistent with

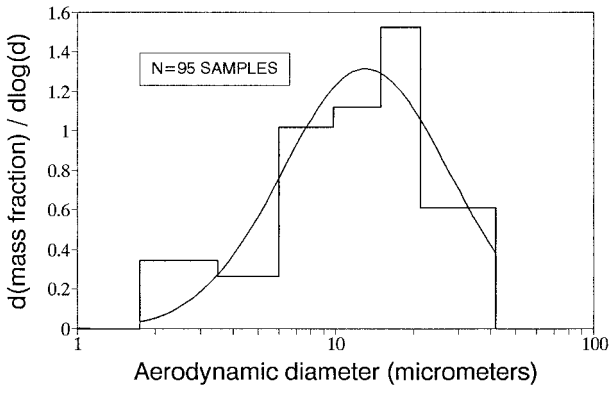

FIGURE 2. Particle size distribution of airborne dusts by cascade impaction

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TABLE III. Organic Dust Constituents in Air

	Samples	GM	GSD	MIN	MAX
Total histamine (picomoles/m³)	106	11.8	5.94	0.71	224
Total spores/bacteria (organisms/m³)	181	1.2×10^7	2.97	1.5×10^4	2.6×10^8
Total cow urine antigen (micrograms/m³)	102	167	7.43	0.007	4580
Total mite antigen— *T. putrescentiae* (micrograms/m³)	97	0.01	7.9	0.0007	2.17
Endotoxins (EU/m³)					
Inhalable	194	647	4.31	25.4	34800
Respirable	216	16.8	5.84	0.16	1380

Note: All area samples except inhalable endotoxins which included largely personal samples.

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Puccinia (rust) as well as spores consistent with those of *Aspergillus* or *Penicillium*. Figure 5 shows several scanning electron micrographs of spores collected from air samples. Based on size, shape, and morphology, the spores in Figure 5c are similar to those of thermophilic actinomycete bacteria; the spores inFigure 5b are similar to those produced by fungi of the *Aspergillus* genus.

Table V presents TWA concentrations of ammonia and carbon dioxide measured inside the barns. Hydrogen sulfide concentrations in air were all below the lower range of quantification (less than approximately 1 ppm). The table presents GM statistics for the mean gas concentrations measured inside each barn as determined from two samples positioned at separate barn locations.

Tables VI to VIII show the correlation results for the mean barn concentrations of total, inhalable inlet (personal and area samples combined), and respirable dusts with mean barn concentrations for the significant organic dust constituents. All of the analytes presented in these tables had significant correlation with the reported dust fraction although the correlation coefficients were generally moderate to poor. Table VI lists those organic dust constituents showing significant correlation with airborne total dust. The correlation between total dust and inhalable inlet dust was good with a Spearman's R value of 0.761. Of the organic dust constituents, histamine and total spore and bacteria concentrations had moderate correlation with total dusts (coefficients of 0.431 and 0.408, respectively).

Table VII lists those organic dust constituents showing significant correlation with airborne inhalable inlet dust concentrations. Inhalable inlet endotoxin and total spore and bacteria concentrations had moderate correlation to inhalable inlet dusts; correlation coefficients were 0.618 and 0.443, respectively.

Table VIII lists those organic dusts and dust constituents with significant correlation to airborne respirable dust. The correlation between respirable dust and the other measures of airborne dust was poor but significant.

DISCUSSION

Work in dairy barns involves exposures to complex organic dusts generated by various barn activities. These dust exposures are often cited in the etiology of respiratory disease. Dairy farmers are at risk for those respiratory diseases common to organic dust exposure including asthma and rhinitis, bronchitis, hypersensitivity pneumonitis (HP) and organic dust toxic syndrome (ODTS). Dairy farmers are described to be at increased risk, compared with other farmers, for HP and ODTS. An overview of the clinical literature suggests that these respiratory health problems are significant and affect many dairy farmers.[1,2]

The environmental sampling results from this study describe the concentrations of organic dusts and dust constituents inside barns during routine livestock farming. The 85 barns surveyed were used to house cattle (99%), predominantly dairy cows (94%). Total dust concentrations from 211 area samples ranged from below 0.01 mg/m³ to a high of 6.5 mg/m³. The GM total dust concentration, 0.74 mg/m³ (GSD, 3.05), was similar to those reported in separate studies by Louhelaine and Virtanen in Finnish barns.[37,38] Background (ambient) total dust concentrations (N=99) taken upwind of the barns sampled in the present study had a GM concentration of 0.03 mg/m³ (GSD, 3.87).

These barn dust concentrations are substantially lower than those reported for specific worst case events such as the uncapping of silos or the operation of a bedding chopper.[39–42] The total dust

TABLE IV. Viable Microorganisms in Air

	Method	Samples	GM	GSD	MIN	MAX
Mesophilic fungi						
Yeast	NFE	65	9.7×10^3	5.6	2.1×10^2	2.9×10^5
	AGI	45	1.7×10^4	3.8	1.3×10^3	2.5×10^5
Molds	NFE	65	1.9×10^4	4.2	1.7×10^3	1.6×10^6
	AGI	45	1.1×10^4	6.1	2.9×10^2	1.2×10^6
Mesophilic bacteria	NFE	67	3.4×10^5	3.9	8.9×10^3	5.2×10^6
	AGI	46	5.8×10^5	3.0	6.1×10^4	4.1×10^6
Thermophilic bacteria	NFE	63	3.4×10^3	4.2	1.5×10^2	7.3×10^5
	AGI	42	3.6×10^3	2.9	3.6×10^2	3.4×10^4

Note: Concentrations in colony-forming units per cubic meter of air (CFU/m³).
AGI = all-glass impinger methods; NFE = Nucleopore filtration and elution method.

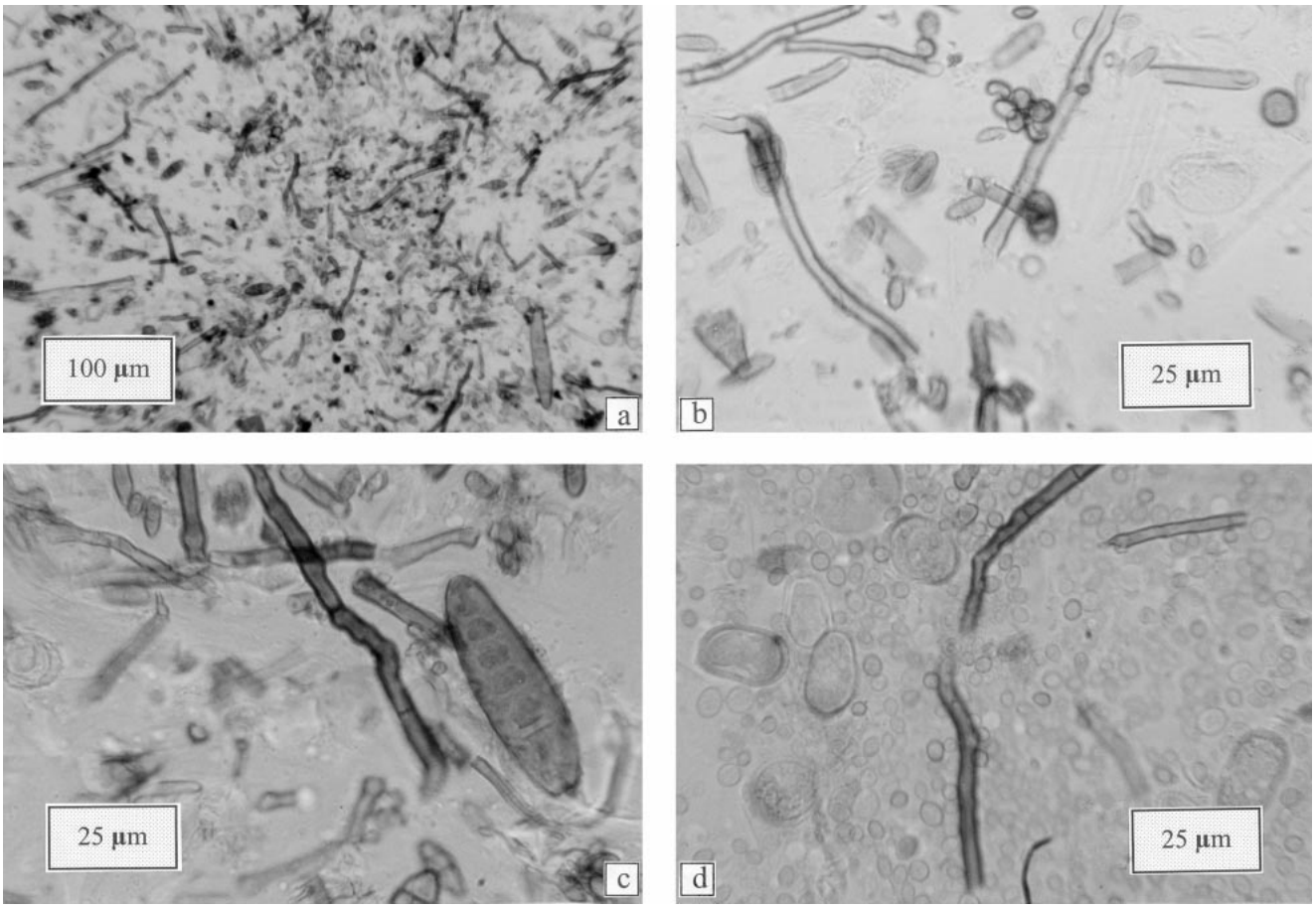

FIGURE 3. Polarized light micrographs of airborne particulate from bedding

concentrations from dairy barns were also lower than total dust concentrations (range 1.4 to 8.3 mg/m³) reported by Donham et al. in swine confinement buildings.[43]

Louhelaine and Virtanen both noted that personal dust exposure measurements were higher than measurements collected by area sampling in dairy barns.[37,38] This is consistent with sampling results from the present study. The personal breathing zone samples collected with the inhalable inlet samplers had a GM of 1.78 mg/m³ (GSD, 2.9) exceeding the area measurements for all other dust fractions including total dust. Respirable dust concentrations were much lower, with a GM concentration of 0.07 mg/m³ (GSD, 4.09). Bedding, feeding, and the application of lime were barn activities associated with increased dust concentrations in air as indicated from the real-time measurements for dust concentration. Milking was associated with lower dust concentrations. Baruah, Lacey, and others also have shown that farm activities involving the aerosolization of materials from hay or straw during bedding or feeding contribute significantly to barn exposures.[8–10,41,44,45]

The airborne dusts from area barn samples had a MMAD of 13.5 µm with a GSD of 2.1 The particle size data suggest that over 60% of the mass of airborne particulate generated from routine barn operations was in a size range exceeding 10 µm in aerodynamic diameter and was nonrespirable. Approximately 26% of the dust was in an optimal respirable size range (between 1 and 10 µm) for penetration to the lower airways and gas exchange regions of the lung.[14] The particle size distribution data reported from studies from silo uncapping were smaller (approximately 7 µm, with GSDs of 3.8 or lower) than those measured during routine barn activity.[39,40] The particle size distributions reported for the chopping of bedding in dairy barns were consistent with those measured during this study.[41,42]

The complex nature of organic dusts from dairy barns is evidenced in the multiplicity of toxic and immunogenic dust constituents identified. The specific organic dust constituents quantified during this study included histamine, endotoxin, cow urine antigen, *Tyrophagus putrescentiae* mite antigen, total spore and bacteria counts, and viable microorganisms including yeasts, mesophilic molds, mesophilic bacteria, and thermophilic bacteria. Histamine, a potent vasodilator, bronchoconstrictor, and immunomodulator, can cause inflammatory lung response directly and modulate the response from other agents such as endotoxins. Histamine was present at detectable levels in approximately 82% of the airborne dust samples at a GM concentration of 11.8 picomoles/m³ (GSD, 5.94). Potential sources for the histamine detected in these samples included animal excreta, insects, and microorganisms.[20,46] The importance of histamine in the pathologic sequelae of organic dust lung disease is a focus of ongoing research.[20,42,46] The histamine concentrations measured in the dairy

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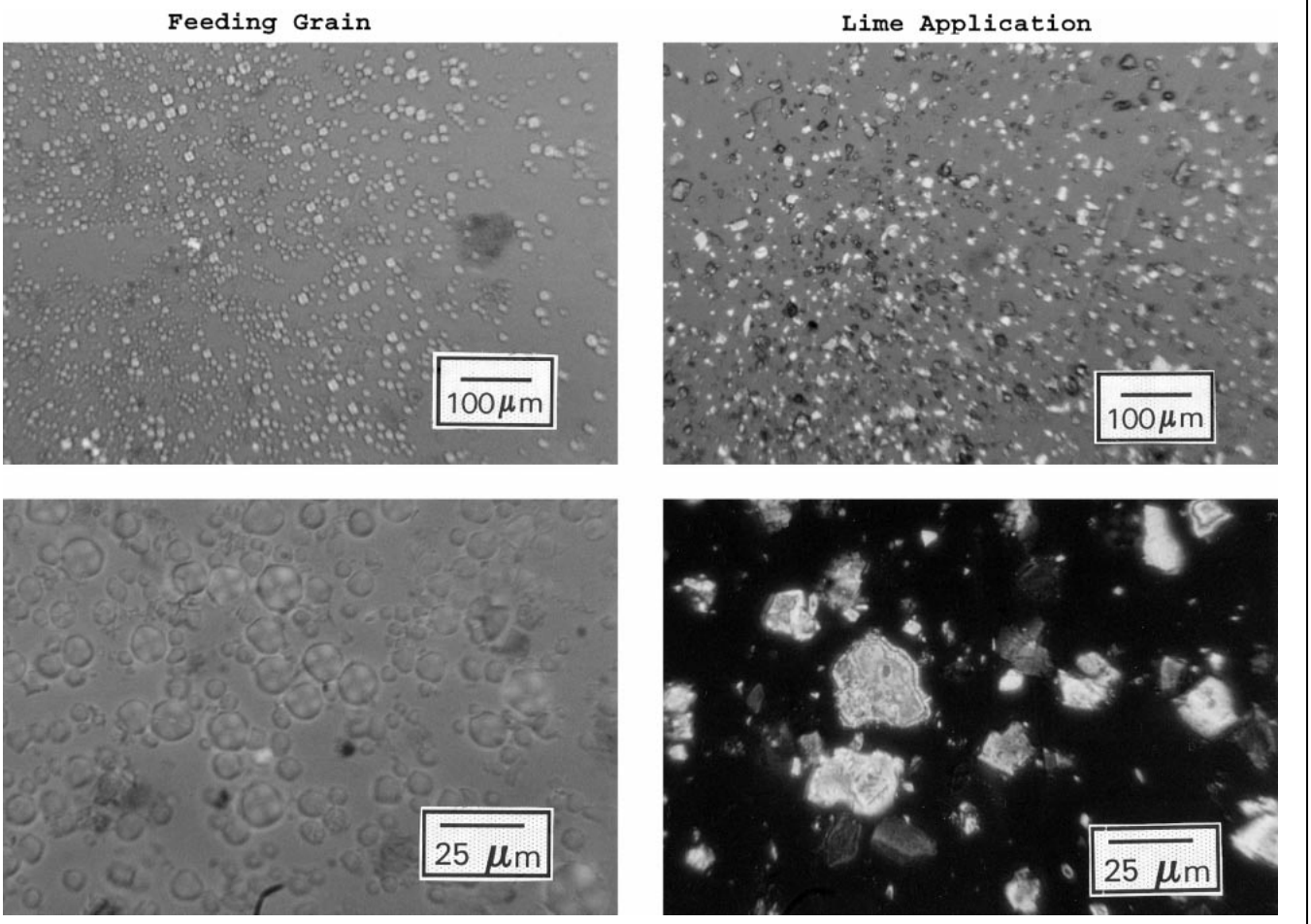

FIGURE 4. Polarized light micrographs of airborne particulate from feeding grain (starch particles) and lime application

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barns were far lower than those given to patients in clinical challenge settings (typically in the milligram per cubic meter range).[46] The GM concentration from the present study (11.8 picomoles/m³) would be equivalent to 1.3×10^{-6} mg/m³. However, the histamine exposure regimen in dairy farming is more frequent (normally daily) and increased in exposure duration (hours) as contrasted with a clinical histamine challenge.[20]

Endotoxins, lipopolysaccharides contained in the cell wall of Gram-negative bacteria, can induce a variety of biological responses including inflammatory, immunological, and hemodynamic activity. The pulmonary macrophage is extremely sensitive to the effects of endotoxins and a primary target cell for endotoxin-induced pulmonary injury following respiratory exposure. Exposures to endotoxins have been reported to cause acute fever, dyspnea, chest tightness, coughing, and decreases in pulmonary function.[47–52] Recent human and animal studies suggest that inhaled endotoxins play a primary role in the etiology of pulmonary inflammation and lung disease associated with exposures in the grain industry.[53,54] The highest endotoxin exposure predicted to cause no adverse pulmonary response was measured in exposure studies among subjects sensitive to cotton dusts, 9 nanograms of elutriated endotoxin per cubic meter of air; this concentration is equivalent to approximately 90 EU/m³.[49,51] Threshold endotoxin exposures among healthy human subjects exposed to cotton dusts are reported by Rylander as approximately 1000 to 2000 EU/m³ for an across shift acute pulmonary response (decline in FEV1), 3000 to 5000 EU/m³ for chest tightness, and 5000 to 10,000 EU/m³ for fever.[50] Endotoxins were detected in over 94% of the respirable dust samples and in all inhalable inlet dust samples taken during the present study. The GM inhalable inlet endotoxin concentration measured during routine barn activities was 647 EU/m³ (GSD, 4.31); inhalable inlet endotoxin concentrations ranged from 25.4 EU/m³ to 34,800 EU/m³. Approximately 93% of personal inhalable dust samples exceeded 90 EU/m³. Area respirable endotoxin concentrations were lower, with a GM of 16.8 EU/m³ (GSD, 5.84) and ranged from 0.16 EU/m³ to 1380 EU/m³. These data show that routine daily farm activities in the dairy barn involve exposures to endotoxins at concentrations exceeding minimum human thresholds. Andersen et al. measured a GM respirable endotoxin concentration of 40 EU/m³ during routine farm activities in 28 Swedish dairy barns during March and April.[55] Total endotoxin concentrations from the Andersen study had a GM of approximately 740 EU/m³. These endotoxin concentrations are in a range similar to those measured during the present study. Endotoxin concentrations measured during routine barn activity were less than those associated with worst case events such as uncapping silos or use of a bedding chopper inside a barn.[39–42] Donham et al. describe slightly higher respirable endotoxin concentrations, ranging

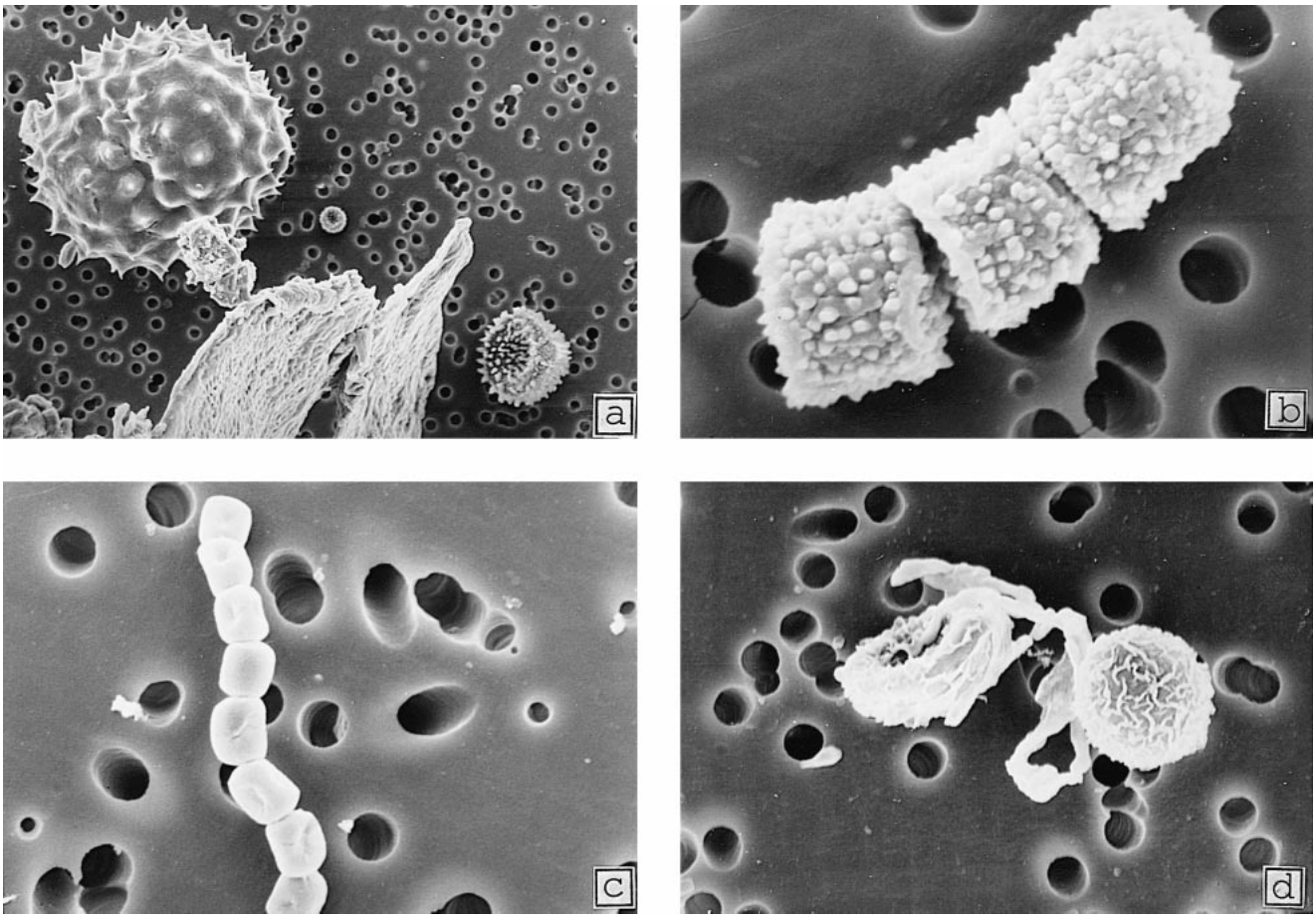

FIGURE 5. Scanning electron micrographs of airborne spores from dairy barns. For size reference, the holes in the filter media are approximately 0.8 μm in diameter.

from approximately 100 to 5600 EU/m³, from area samples collected in 30 Swedish swine confinement barns.[43] Data from the present study show that Gram-negative bacteria and endotoxins are normal constituents in airborne dusts from dairy barns, and routine barn activities can produce air concentrations that exceed suggested human thresholds for respiratory response.

Cow urine antigen, another immunogenic constituent of airborne dusts, was measured inside dairy barns. Human sensitization to bovine (cow) antigen has been reported in the literature and quantification of this analyte is reported in several studies. Cow urine antigen measurement yielded a GM concentration of 167 μg/m³ (GSD, 7.43) in the 85 barns surveyed during this

TABLE V. Ammonia and Carbon Dioxide Concentrations in PPM

	Barns Sampled[A]	GM	GSD	MIN	MAX
Ammonia	83	6.4	2.6	0.1	26.1
Carbon dioxide	83	1700	1.7	ambient (≃300)	5300

Exposure Standards[B]	Ammonia (ppm)	Carbon Dioxide (ppm)
NIOSH	25	5000
ACGIH	25	5000
OSHA	50	5000

[A]Two passive indicator tube samples were collected from each barn and averaged to find the barn concentration. The statistics reported above are for the average barn concentrations of ammonia and carbon dioxide.
[B]Exposure standards: time-weighted average exposure standards and criteria by NIOSH, recommended exposure limits; ACGIH, threshold limit values; and OSHA, permissible exposure limits.[60–62]

TABLE VI. Correlation Between Airborne Total Dust Concentrations and Other Environmental Analytes

Analyte	Samples	Spearman's R Value	p-Value
Inhalable dust	85	0.761	0.0001
Respirable dust	85	0.315	0.0033
Total histamine	79	0.431	0.0001
Respirable endotoxin	85	0.397	0.0002
Inhalable endotoxin	85	0.391	0.0002
Total spore count	77	0.408	0.0002
Total mite antigen	75	0.259	0.0251

Note: Correlation results were based on mean barn concentrations for environmental analytes.

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TABLE VII. Correlation Between Airborne Inhalable Inlet Dust Concentrations and Other Environmental Analytes

Analyte	Samples	Spearman's R Value	p-Value
Total dust	85	0.761	0.0001
Respirable dust	85	0.305	0.0045
Total histamine	79	0.394	0.0003
Respirable endotoxin	85	0.361	0.0007
Inhalable endotoxin	85	0.618	0.0001
Total spore count	77	0.443	0.0001

Note: Correlation results were based on mean barn concentrations for environmental analytes.

study. Several other studies reference concentrations of cow antigen, although cow epithelial antigen was quantified in those studies. Campbell et al. report results from quantification of bovine epithelial antigen by RAST methods in a New York dairy barn at a concentration of 16 $\mu g/m^3$.[19] Virtanen et al. report concentrations of bovine epithelial antigen measured by ELISA in 18 Finnish cow barns; concentrations ranged from 180 $\mu g/m^3$ to 1600 $\mu g/m^3$ with a GM of 460 $\mu g/m^3$.[38] Cow urine and epithelial antigen would be expected constituents of airborne dusts in dairy barns.[6,19,38]

Tyrophagus putrescentiae mite antigen was present at quantifiable levels in 80 of the 98 area total dust samples collected, at a GM concentration of 0.01 $\mu g/m^3$ (GSD, 7.90); the maximum concentration measured during the study was 2.17 $\mu g/m^3$. *T. putrescentiae* is a feed storage mite common to many agricultural settings where hays and feeds are used. It is one of the more common mites identified in Wisconsin dairy barns. There have been several studies that implicate feed storage mite exposure in the etiology of allergic rhinitis and asthma.[3,6,56] Only one previous study reports mite concentrations in dairy barn air. Campbell et al. quantified the presence of mite antigen in dairy barn air; they reported maximal *Lepidoglylyphus destructor* mite concentrations of 12 $\mu g/m^3$ by RAST inhibition methods.[19] These data show that mite antigen is one of the common immunogenic constituents of dairy barn air.

Fungi and bacteria have been a focus of many exposure assessment studies in dairy barns. Certain bacteria and fungi have been identified as agents in the etiology of HP; these organisms and associated metabolites are potential etiologic agents for other respiratory health problems related to organic dust exposures such as asthma and ODTS. Airborne concentrations of microorganisms were evaluated in this study using two measures: a total microscopic spore and bacteria count that is not dependent on cultureability, and viable measures of yeast, molds, mesophilic bacteria, and thermophilic bacteria. Total spore and bacteria concentrations

TABLE VIII. Correlation Between Airborne Respirable Dust Concentrations and Other Environmental Analytes

Analyte	Samples	Spearman's R Value	p-Value
Total dust	85	0.315	0.0033
Inhalable dust	85	0.305	0.0045
Respirable endotoxin	85	0.232	0.0323
Inhalable endotoxin	85	0.233	0.0314
Total spore count	77	0.309	0.0063

Note: Correlation results were based on mean barn concentrations for environmental analytes.

in dairy barns ranged from below detectable limits, approximately 2×10^4 organisms/m^3, to a high of 2.6×10^8 organisms/m^3. The GM spore and bacteria count was 1.2×10^7 organisms/m^3 (GSD, 2.97). Since many of the toxic and immunogenic effects of microorganisms are not directly a result of their viability, a measure of total spore and bacteria counts is potentially more relevant to pulmonary responses. Total spore and bacteria concentrations have been measured in other studies of dairy farming. Baruah measured airborne spore concentrations in a dairy barn during routine farming activities. Spore concentrations ranged from 9.5×10^4 spores/m^3 to 1.6×10^7 spores/m^3. These spore concentrations were in a range approximately one log order lower than those measured in the present study. However, the sampling and analytical methods used by Baruah had poor efficiency for quantifying smaller spores, such as thermophilic actinomycetes.[44] Larson et al., with methods similar to those used here, quantified total spore and bacteria concentrations in dairy barns during work with organic materials. Concentrations ranged from 6.6×10^6 organisms/m^3 to 6.5×10^8 organisms/m^3, with a mean of 2.35×10^8 organisms/m^3. The mean spore and bacteria concentration measured in the barns as background was 1.51×10^7 organisms/m^3.[57] Andersen et al. report mean spore and bacteria concentrations of 5.6×10^9 organisms/m^3 from 14 dairy farms with symptomatic farmers (ODTS or HP) and concentrations of 2.8×10^8 organisms/m^3 from 11 control farms. These concentrations, measured during worst case conditions involving the handling of feed or bedding materials, not surprisingly were higher than the concentrations measured during routine activities from the present study. Similar sampling and analytical methods were used in both studies.[55] Other measures of total spore and bacteria counts from worst case activities show potential for peak exposures well above the time-weighted concentrations measured during routine activities.

Viable microorganisms are a subset of the total airborne microorganisms. Viable sampling methods identify only those microorganisms that are both alive and cultureable by the sampling and analytical methods used. Consequently, concentrations of viable microorganisms are usually lower than measures of total microorganisms. Of the types of viable microorganisms measured, mesophilic bacterial concentrations were highest in the dairy barns, followed by mesophilic fungi (yeasts and molds) and thermophilic bacteria. GM concentrations of mesophilic bacteria inside dairy barns during routine activities were 3.4×10^5 CFU/m^3 by NFE methods and 5.8×10^5 CFU/m^3 by AGI methods. Mesophilic fungi including molds and yeasts were the next most abundant viable microorganisms in air at GM concentrations of approximately 1×10^4 to 2×10^4 CFU/m^3. Geometric mean thermophilic bacterial concentrations were quite similar by both NFE and AGI methods, approximately 3.5×10^3 CFU/m^3. These measurements describe the concentrations of viable microorganisms inside dairy barns during routine activities of milking, feeding, bedding, and manure handling.

Viable bacteria and fungi are among the most frequent analytes quantified in other studies of dairy barn exposures. Kotimaa et al. found that mesophilic bacteria were the most abundant microorganisms liberated from grain substrates, while fungi and actinomycetes were the more common organisms liberated from hay and straw.[10] Specific bacterial and fungal genera were not identified in the samples from the present study, although this type of characterization has been done in other aerobiological studies of dairy barns.[9,10,44,45,58,59]

Most of the concentrations of viable microorganisms reported in the literature are for specific worst case exposure events and are

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usually higher than the concentrations measured by time-weighting over periods of both low and high exposure, as in this study. Lacey et al. reported viable fungal concentrations ranging from 2.4×10^7 to 2.4×10^9 CFU/m³ from feeding and application of bedding.[9] The highest concentrations were measured during the shaking of hay. Fungal concentrations were lower during milking, at 4.1×10^5 CFU/m³. The thermophilic actinomycete concentrations reported during these worst case exposure activities ranged from 6.2×10^6 to 7.6×10^8 CFU/m³, higher than those measured during the present survey.[9] Wardrop et al. report mesophilic fungal concentrations of 1.0×10^5 to 5.4×10^6 CFU/m³ inside dairy barns during the unbaling of hay. Concentrations of thermophilic actinomycetes were lower at 1.0×10^3 to 8.0×10^5 CFU/m³.[45]

NIOSH, in collaborative research with the New York Center for Agricultural Medicine and Health, found high levels of viable microorganisms associated with the uncapping of silos and during the application of bedding with a bedding chopper. Mesophilic fungal concentrations measured by impinger samples during silo uncapping had a GM of 1.9×10^6 CFU/m³; thermophilic bacterial concentrations were higher, with a GM of 2.2×10^7 CFU/m³. Viable fungal and mesophilic bacterial concentrations measured by NFE methods during bedding chopping were also higher than those measured during routine barn operation.[39–42] These sampling results demonstrate the abundant airborne microflora to which dairy farmers are exposed daily during barn work.

At present, there are no occupational exposure standards or criteria specifically for organic dusts from dairy or other animal confinement barns. However, the numerous toxic and immunogenic constituents present in organic dust from dairy barns would indicate that these dusts should not be considered nuisance particulates.[60–62]

Carbon dioxide and ammonia were present at quantifiable concentrations inside dairy barns. Hydrogen sulfide concentrations in air were all below quantifiable levels (less than 1 ppm). Carbon dioxide, a product of cellular respiration and a natural constituent of ambient air (approximately 325 ppm), was measured as a surrogate of barn ventilation.[63–65] Increased CO_2 concentrations inside dairy barns result from respiration of the cows and other barn animals. Combustion sources (tractors, bedding choppers, or feed carts) could also contribute to barn CO_2 concentrations. Increased ventilation involving outside air intake and barn air mixing reduces barn CO_2 concentrations. The CO_2 concentrations measured during this study were increased above normal ambient concentrations at a GM of 1700 ppm (GSD, 1.70), but well below existing occupational exposure criteria (see Table V).[60–62]

Concentrations of CO_2 are not referenced in any of the other dairy barn studies. Donham et al. report similar CO_2 concentrations in Swedish swine confinement barns.[43]

Ammonia was detected in most of the barns sampled, at a GM concentration of 6.4 ppm. Ammonia is a recognized irritant of the eyes, respiratory tract, and skin,[63–65] and is an excretion product of animal metabolism that can be evolved from urine, the primary source of ammonia in a dairy barn. The GM concentration of ammonia was low by comparison with existing occupational exposure standards and criteria, although the TWA concentration measured inside one of the dairy barns (26.1 ppm) exceeded occupational exposure criteria (see Table V). The ammonia concentrations measured inside some of the barns suggest potential for exposure-related symptoms of respiratory irritation.

Ammonia concentrations were not reported in any of the other environmental studies of dairy barns referenced. Donham et al. reported similar concentrations of ammonia in Swedish swine confinement barns.[43]

There were significant, positive correlations between concentrations of organic dusts and certain dust constituents. Histamine, respirable endotoxin, and total spore and bacteria concentrations were significantly correlated with all measures of airborne dust in total, inhalable inlet, and respirable size fractions. Mite antigen was significantly correlated with only total dust. In most instances correlation was highly significant, although the strength of the correlation was generally low to moderate. The highest correlation between dust and dust constituents was seen between inhalable inlet dusts and inhalable inlet endotoxins (Spearman's R value 0.618). Of specific dust constituents, histamine had the highest correlation with total dust (Spearman's R value 0.431). Total dusts and inhalable inlet dust concentrations were highly correlated (Spearman's R value 0.761); this was the highest correlation observed among any of the environmental analytes. The correlations between respirable dust and either total or inhalable inlet dust concentrations were significant but with much lower coefficients.

These findings on correlation among environmental analyte concentrations are consistent with sampling results from other dairy exposure assessments. Andersen et al. found poor but significant correlation between total dust and total endotoxin concentrations in a survey of 14 dairy farms.[55] Virtanen et al. compared correlation between total dust concentrations and cow epithelial antigen concentrations. Consistent with the present findings for cow urine antigen, no significant correlation was found for airborne dust and cow antigen concentrations.[38]

CONCLUSIONS

These data show that dairy farmers are exposed to organic dusts containing many toxic and immunogenic constituents during daily routine barn work. Environmental agents present at quantifiable levels in barn air included total dust; inhalable inlet dust; respirable dust; total histamine; inhalable endotoxins; respirable endotoxins; *Tyrophagus putrescentiae* mite antigen; cow urine antigen; and microorganisms including fungi (yeasts and molds), mesophilic bacteria, and thermophilic bacteria. Ammonia was present at elevated levels but hydrogen sulfide was not routinely detected. Exposures to most of these organic dust constituents are believed to be risk factors for respiratory disease. Significant correlation was seen between concentrations of organic dusts and certain dust constituents. These correlations, while statistically significant, were only moderate to poor in strength.

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