DISSERTATION

COMPARISON OF OCCUPATIONAL AND ENVIRONMENTAL EXPOSURES AT COLORADO DAIRIES

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY BRADLEY RAYMOND LESTER ENTITLED COMPARISON OF OCCUPATIONAL AND ENVIRONMENTAL EXPOSURES AT COLORADO DAIRIES BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

COMPARISON OF OCCUPATIONAL AND ENVIRONMENTAL EXPOSURES AT COLORADO DAIRIES

Occupational contaminant levels and environmental emissions were compared at two Colorado dairies. Along with meteorological conditions, analytes measured included odor, hydrogen sulfide, ammonia, total and inhalable particulate and endotoxin, and bioaerosols including fungi, mesophilic bacteria, and gram-negative bacteria. Meteorologic conditions varied widely in temperature (range: -12.5 - 41.1°C), relative humidity (range: 0.6 - 92.3%) and wind speeds during sampling (range: 0.48 - 8.66 m/s). Geometric mean bioaerosol concentrations for the Anderson sampler and SKC Biosampler include: mesophilic bacteria, 1282 and 383 CFU/m^3, gram-negative bacteria, 667 and 265 CFU/m^3, and fungi, 781 and 252 CFU/m^3. The Anderson sampler collected significantly ($p<0.001$) higher bioaerosol concentrations for all three categories. Peak ammonia levels at the study and control dairies ranged from 2.0 - 142 and 2.0 - 23 ppm. Peak ammonia was significantly ($p<0.05$) higher at the study dairy. Mean hydrogen sulfide levels at the study and control dairies ranged from 4.0 - 394 and 4.0 - 890 ppb. Peak hydrogen sulfide levels at the study and control dairies ranged from 37 - 17,000 and 210 - 5,200 ppb. Mean peak hydrogen sulfide was significantly ($p<0.05$) higher at the control dairy. Odor measures ranged from 0 – 15 D/T at both dairies. Inhalable particulate at study and control dairy lagoons ranged from < LOD - 2.3 mg/m3. Inhalable endotoxin at study and control dairy lagoons ranged from 2.1 - 487.2 EU/m^3. Total particulate at study and control dairy lagoons ranged from < LOD to 2.4 mg/m^3. Total

endotoxin at study and control lagoons ranged from 2.5 - 6587 EU/m^3. Inhalable particulate for tasks at both dairies ranged from 0.06 - 8.0 mg/m^3. Total particulate for tasks at both dairies ranged from 0.03 - 6.9 mg/m^3. Inhalable endotoxin for tasks at both dairies ranged from 2.0 - 11096 EU/m^3. Total endotoxin for tasks at both dairies ranged from 5.9 - 6758 EU/m^3.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Introduction and Literature Review

Objectives

This project evaluated the effectiveness of a unique lagoon algae system that is intended to shift the anaerobic process to an aerobic process with very little energy cost. Two Colorado dairies agreed to participate in this study. The study dairy has total land area of approximately 60 acres with a single lagoon of approximately 8 acres. The study dairy milks approximately 1350 cows, with approximately 125 cows being kept dry and not milked. The study dairy raises the female calves on site. The waste treatment system is as follows: The milking parlor is rinsed with fresh water, approximately 20,000 gallons per day; recycled water, approximately 300,000 to 400,000 gallons per day, is used to rinse the other areas. Waste flows by gravity into a leaky dam separation and settling basin and then into a primary lagoon. Dry lots are and settling basins are scraped and the solids are composted.

The lagoon is treated with a novel proprietary algae intervention system developed by Agsmart called the O2Solution®. A proprietary blend of single cell algae is grown in large water tanks in an environmentally controlled greenhouse, which was constructed on site next to the lagoon. The number of 1,000-gallon growth tanks is determined by the volume of the waste stream and the biological oxygen demand (BOD)

loading. These tanks are actual biological air compressors constantly producing super-saturated oxygenated liquid. The tanks receive a specific diet of nutrients that is metered into each tank to assure a consistent and productive algae population. Temperature and light are also controlled for optimum algae growth. A patented blend of microbials, including specific cold-water microbials, are also added and dispersed through the waste material to supplement activity of naturally occurring organisms and optimize the breakdown of solids. The algae-water contains large amounts of high purity, super-saturated (10 - 40 mg/L) oxygenated water. It is crucial that oxygenated water be equally dispersed throughout the waste material to assure uniform oxygen levels. The fresh super-saturated water containing the oxygen produced by the algae is then pumped into the wastewater lagoon through micro diffusers. Low energy compressors (1/3 horsepower) are matched to the micro diffuser plates to assure proper vertical mixing and dispersal of the O2Solution®. Agsmart claims that this oxygen energizes microbials that can then quickly and easily digest waste in a lagoon.

The algae are designed to increase the percent of dissolved oxygen in the lagoon to above 1 mg/L, therefore transforming the anaerobic environment into an aerobic environment. Agsmart claims that the O2 Solution® treatment produces high purity, super-saturated O_2 with proper mixing and the seeding of productive microbials the O2Solution® results in a waste lagoon that generates little odor or noxious gas and is devoid of solids build-up at any level. Agsmart markets the O2Solution® towards agriculture, municipal waste treatment, and industry to be a simple, economical, and effective solution to the problems of wastewater lagoon odor, sludge buildup, ammonia, nitrogen, and phosphorous levels (Agsmart 2007).

The control dairy has total land area of approximately 340 acres with two lagoons of approximately 3 acres each. The control dairy milks approximately 3000 cows, with approximately 350 cows being kept dry and not milked. The control dairy does not raise the female calves on site. The waste treatment system is as follows: The milking parlor is rinsed with fresh water, approximately 180,000 gallons per day, including drinking water while recycled water, approximately 80,000 gallons per day, is used to rinse the other areas. Waste flows by gravity into a leaky dam separation and an earthen basin separation then into a primary and secondary lagoon. Dry lots and settling basins are scraped and the solids are composted.

The overall objective of this research was to compare the study and control dairies to determine if the novel algae intervention utilized at the study dairy was able to reduce emissions from the study lagoon. There are three more specific objectives to this study. First, we compared the SKC Biosampler and the Anderson two-stage viable particle sampler for measuring culturable airborne bacteria and fungi. Second, we compared the environmental emissions from the two dairy lagoons. Third, we characterized and compared the occupational exposures for a variety of tasks at each dairy. These specific objectives are examined further in the following three chapters: 2) *Comparison of SKC Biosampler and Anderson Two Stage Viable Particle Sampler for Measuring Airborne Bacteria and Fungi at Colorado Dairies*; 3) *Comparison of Environmental Emissions from Colorado Dairies*; 4) *Characterization of Occupational Exposures for Various Tasks at Colorado Dairies.*

In the first study, *Comparison of SKC Biosampler and Anderson Two-Stage Viable Particle Sampler for Measuring Airborne Bacteria and Fungi at Colorado*

Dairies, we compared two viable bioaerosol samplers, the Anderson two-stage viable particle sampler (ThermoAnderson, Smyrna, GA.) and the SKC Biosampler (SKC Inc., Eighty Four, PA.), at the lagoons of the two Colorado dairies using three selective media, R2A for mesophilic bacteria, eosin methylene blue (EMB) for Gram-negative bacteria, and Malt Extract Agar (MEA) for fungi.

The Anderson two stage aluminum viable particle sampler can be used whenever a size distribution is not needed and only respirable and non-respirable segregation or total counts are needed. Ninety-five to one hundred percent of the viable particles above 0.8 μm in an aerosol can be collected on a variety of general purpose solid bacteriological agar (ThermoAnderson 2007). This sampler separates viable particles into two size ranges with the 50% cut-off diameter of Stage 1 at 8.0 μm for spherical particles of unit density or their aerodynamic equivalent (Hatch 1955).

In the SKC Biosampler, the airborne microorganisms are drawn into three nozzles through which they are projected at an angle toward a curved surface where they are collected by the combined forces of impaction and centrifugation. During normal operation, the sampler is used with a liquid that swirls upward on the sampler's inner wall and removes collected particles. The swirling motion of the collection liquid generates very few bubbles thus producing minimal aerosolization of collection particles (SKC 2007, Lin 1999). When 20 milliliters of water is used as the collection fluid, the physical collection efficiency of the Biosampler has been shown to be about 79% for 0.3 μm particles, 89% for 0.5 μm particles, 96% for 1.0 μm particles, 100% for 2.0 μm particles (Willeke 1998).

In the second study, *Comparison of Environmental Emissions from Colorado Dairies*, we compared the environmental emissions from the lagoons at the two Colorado dairies to determine if the novel algae intervention by Agsmart has any effect. We measured total and inhalable particulate and endotoxin, carbon dioxide, ammonia, hydrogen sulfide, and odor.

In the third study, *Characterization of Occupational Exposures for Various Tasks at Colorado Dairies*, we characterized and compared the different tasks at the two Colorado dairies. We measured total and inhalable particulate and endotoxin for six tasks including: milking, feed loading, feed distribution, calf feeding, maintenance, and veterinary care. There were only three common tasks between the two dairies including: milking, feed loading, and feed distribution.

Literature Review

Agriculture provides jobs to over 2 million Americans (Donham 2000a) and is one of Colorado's top industries. Dairy and livestock production are among the top five commodities in Federal Region VIII. One of the top priorities of the High Plains Intermountain Center for Agricultural Health and Safety (HICAHS) is to reduce injury and illness among the livestock and dairy producers in this region.

Colorado's dairy industry makes a significant contribution to the state's agricultural economy, totaling over $223,000,000 annually (Colorado 2001). The state of Colorado defines a dairy concentrated animal feeding operation (CAFO) as one which contains 700 head or 800,000 pounds live weight (Kress 2007). In 2006, the state of Colorado ranked 16th in the United States in total milk production with 2,547,050,000

pounds of milk for an 8.5% increase over 2005. Colorado had 170 licensed dairy herds each with an average of 647 cows for a total of 110,000 cows. In total, the United States' 9,112,000 cows produced 181,798,000,000 pounds of milk in 2006. Increase in milk production and larger herd sizes are trends that U.S. dairy producers can expect to see for years to come (Cooley 2007).

Productivity has been driven by advances in technology with a concomitant increase in the size of the average farm. Using technologically advanced modern buildings and machinery, farmers are able to farm more land and raise more animals in less time and with less effort.

The industrialization of livestock production has led to concern over occupational and public health impacts from air and water emissions. Market forces have further driven the concentration of livestock. Feed and water are served on a continuous basis as needed to increase animal growth and milk and egg production. Manure slurry (mixture of feces and urine) covers the feedlots until tractors and trucks carry it away for field application or until it is washed away with high powered water spray and pumped to storage containers or lagoons for later use as fertilizer. It is estimated that, per year in the United States, there is 3 times more confined animal waste produced compared to human waste (EPA 2003).

Along with environmental concerns, there has been an increasing concern for the health of livestock, workers, and the communities in the vicinity of large confined animal feeding operations. Current real estate trends along the Front Range in Colorado have led land developers to encroach on dairy operations and other agricultural entities. Property near city centers comes with a considerable price. Therefore, development moves away

from cities towards less expensive real estate, usually agricultural land. Houses, shopping centers, restaurants, and schools are being constructed next to dairies and other CAFOs and farms.

The study dairy sits just east of a small Colorado town approximately 12 miles from one of Colorado's largest cities. The study dairy once sat more than a mile from the outskirts of this small agricultural town. Today, hundreds of new houses sit directly across the street in the predominately downwind direction. Since 2003, 763 new home construction permits were granted in this town. In 2005, the median house value in Colorado was $223,300, while it was $176,700 in this small town (City 2007a). Families who could not afford to purchase a home in the large city where they work and play can purchase a home that is twice the size with twice the land near this small town, ignoring the fact that a large dairy farm sits directly across the street. The 2000 U.S. Census reports that this small town had 2,672 people. Today, it is estimated that 4,128 people live there (City 2007a). The dairy owner felt pressure from the odor complaint calls that had been placed to the mayor's office. This dairy owner solicited the help from a new company with a novel lagoon treatment designed to change lagoon microbiology and reduce lagoon solids and odor.

The control dairy sits approximately 10 miles east of a small agricultural town and approximately 10 miles north of another. The 2000 U.S. Census reported that these small towns had increased in size from 2,370 to 2,611 and from 1,565 to 1582 people (City 2007b, 2007c). A large upscale housing development was being built approximately 2 miles north of the control dairy with acreages and houses ranging from $70,000 to $800,000 (Pelican 2007).

The majority of the contaminants in and around dairies and feedlots originate from the manure storage piles and lagoons. Air emissions from locations contain numerous toxic compounds including ammonia, hydrogen sulfide, dimethyl sulfide, particulates, bacteria, fungi, endotoxin, acetaldehyde, acetone, benzene, chloroform, hexane, methanol, phenol, toluene, and xylene. Workers spend an increasing amount of time indoors and around animals, experiencing greater exposure than in the past. Workers also suffer increased rates of respiratory disease – up to 30% are affected by chronic obstructive pulmonary disease (COPD) (Cathomas 2002, Donham 1995, Kullman 1998). The workforce in dairies and feedlots has changed significantly, with Hispanics now predominating. In the Midwest and Western regions of the United States, 90 percent of migrant farm workers are Hispanic (Von Essen 1998). Many of these farm workers do not have a farm background and their employment in agriculture tends to be entry-level and temporary (Kirkhorn 2002). Minority status and work involving dairy cattle have been associated with a significantly increased risk of injury (Schenker 1995, McGwin 2000, Nordstrom 1995). Within the dairy industry, most injuries occur while milking or treating cows for lameness (Boyle 1997).

A small number of studies have evaluated occupational exposures to contaminants from dairies and feedlots (Cathomas 2002, Kullman 1998). Fewer studies have been performed to evaluate how engineering interventions can reduce the airborne concentrations in the vicinity of feedlots and dairies and reduce occupational exposures.

Evidence for Human Health Effects

Respiratory diseases, including chronic obstructive pulmonary disease (COPD) and asthma, has been well documented for livestock workers in swine and poultry production industries. Confinement air is made up of a complex mixture of gases including ammonia, carbon monoxide, carbon dioxide, and hydrogen sulfide; dusts composed of feed particles, insect parts, pollen, grains, mineral ash, animal dander, dried feces and urine; biological components including bacteria, viruses, fungi, endotoxin, proteins, and proteolytic enzymes. Some contaminants emit strong odors that can be a nuisance to workers and the general public (Donham 1985, 1986, Clark 1983, Crook 1991). Workers are routinely exposed to many biological, chemical, and physical agents through continued close contact with animals. Gases such as carbon dioxide, carbon monoxide, and hydrogen sulfide cause a range of acute effects from impaired consciousness to death (Donham 1977, 1990). Ammonia, dust, and endotoxin are related to airway irritation, decreased lung function, organic dust toxic syndrome, chronic obstructive pulmonary disease, chronic mucous membrane irritation, hypersensitivity pneumonitis, hyper-reactive airways disease, wheeze, chest tightness, chronic cough, chronic sinusitis, and chronic bronchitis (Clark 1983, Donham 1995, 2000b, Kullman 1998, Reynolds 1993, 1994, 1996).

Fewer studies have been conducted in the dairy and feedlot industries, but they have found similar exposures and similar concerns for high risk of pulmonary disease (Cathomas 2002, Kullman 1998). Multiple studies have examined the respiratory health of dairy farmers in the Doubs region of France. The authors conducted a twelve-year

longitudinal study with a total cohort of 500 dairy farmers and controls. They found that the prevalence of chronic bronchitis was higher in dairy farmers. In a cross-sectional analysis, they found that all respiratory function parameters and blood oxygen saturation were significantly lower in dairy farmers compared to non-farming controls (Dalphin 1989, Dalphin 1993, Dalphin 1998a, Dalphin 1998b, Chaudemanche 2003, Mauny 1997, Gainet 2007).

Most studies have focused on measurement of gases and vapors. There is less information concerning dusts and bioaerosols. Several studies focused on odor, rather than individual constituents contributing to that odor. Chemical known to contribute to odor from dairy and cattle CAFOs include: acetate, ammonia, butyrate, carbon dioxide, dimethyl sulfide, hydrogen sulfide, L-lactate, methane, methylmercaptan, nitrous oxide, propionate (Barnwart 1975, Braam 1997, Dewes 1999, Groot Koerkamp 1998, Jeppsson 1999, Kullman 1998, McCrory 2001, Svensson 1994, Swierstra 2001, Varel 2001, 2002). A variety of volatile fatty acids, amines, and other sulfur-containing compounds also contribute to odor (Hartung 1994, Mackie 1998, Zhu 2000).

Concentrations of ammonia inside U.S. dairy barns has ranged from 0.1 to 26 parts per million (ppm), and carbon dioxide averaged 1700 ppm with a maximum of 5300 ppm. (Kullman 1998). Swierstra et al. measured much higher levels of ammonia concentrated in the exhaust from Swedish dairy farms (up to about 11,000 ppm), but did not report more diluted ambient concentrations (Swierstra 2001). Groot Koerkamp (1998) reported ammonia levels of 0.3 – to 1.3 ppm on site for cattle, slightly lower than levels emitted from swine and poultry CAFOs. Svensson (1994) studied emissions of ammonia during land applications and reported levels ranging from 0.8 ppm to 53 ppm.

Hydrogen sulfide inside dairy barns was below levels of quantification (1 ppm) (Kullman 1998). Barnwart and Bremmer found that hydrogen sulfide and methylmercaptan accounted for 70 – 97% of sulfur compounds volatized from manure samples (Barnwart 1975).

In one of the few comprehensive studies of aerosol exposures at dairy farms Kullman et al. evaluated airborne dusts (total, inhalable, and respirable size fractions) bacteria, fungi, spores, endotoxin, histamine, cow urine antigen, and mite antigen in 85 Wisconsin dairy barns. Personal breathing zone dust levels averaged: 1.78 mg/m^3 for inhalable fractions and 0.07 mg/m^3 for respirable fractions. Inhalable endotoxin ranged from 25 to 35,000 endotoxin units per cubic meter of air (EU/m^3). Airborne concentrations of culturable fungi and bacteria ranged from 10^2 to 10^6 colony forming units per cubic meter of air (CFU/m^3). Takai et al. also measured dusts in European cattle barns, but it is not known if these data are comparable to U.S. operations. Dust levels were all less than 1 mg/m^3.

Pell reviewed bacteria and protozoans present in dairy manure and their potential for human health effects, although no air sampling was conducted. Organisms identified as components of the manure included: *Cryptosporidium parvum, Giardia* spp. (protozoans) and *Listeria monocytogenes, Escherichia coli* O157:H7, *Salmonella spp.*, and *Mycobacterium paratuberculosis* (bacteria). Bovine viruses that might be excreted include: adenovirus, entervirus, parvovirus, papillomavirus, rhinovirus, and respiratory sincytial virus. The likelihood that these organisms can survive in an aerosol to reach communities at some distance from a CAFO has not been evaluated and may be quite variable depending on the specific organism.

In general, concentrations of dusts, bacterial endotoxin, ammonia, and hydrogen sulfide reported for workers in diary and cattle CAFOs are similar or lower than concentrations associated with adverse respiratory effects in swine and poultry CAFOs (Kullman 1998, Groot Koerkamp 1998, Swierstra 2001, Takai 1998). Pathogens that cause disease in humans are found in dairy and cattle manure. Pell (1997) discussed the potential health effects of *Cryptosporidium parvum, Giardia* spp. (protozoans) and *Listeria monocytogenes, Escherichia coli* O157:H7, *Salmonella spp.*, *Mycobacterium paratuberculosis* (bacteria), and a variety of viruses. Most of these have been associated with gastrointestinal problems among consumers. There is no documentation of transmission to workers in these environments. The effects of cell wall components, particularly Gram-negative bacterial endotoxins may be much more important agents of disease and certainly play a key role in respiratory disease experienced by workers in swine and poultry CAFOs. Schiffman et al. (1998) has also documented psychological effects in communities near a variety of livestock facilities.

Multiple agencies have recommended exposure limits for several of the contaminants found at dairy farms (Table I).

The Occupational Safety and Health Administration (OSHA) promulgate 8-hour time weighted average (TWA) enforceable occupational standards called Permissible Exposure Limits (PELs). The American Conference of Governmental Industrial Hygienists (ACGIH) and The National Institute for Occupational Safety and Health (NIOSH) also recommend 8-hour TWAs and short term exposure limit (STEL) occupational standards called Threshold Limit Values (TLVs) and Recommended Exposure Limits (RELs).

Table 1. Current 8-hour agricultural and animal confinement exposure recommendations

Hazard	OSHA PEL	NIOSH REL	ACGIH TLV
PNOC[a] Total	15 mg/m^3	NE	NE
PNOC[a] Respirable	5 mg/m^3	NE	3 mg/m^3
PNOC[a] Inhalable	NE	NE	10 mg/m^3
Grain Dust[b]	10 mg/m^3	4 mg/m^3	4 mg/m^3
Organic Dust	NE	NE	NE
Ammonia	50 ppm	25 ppm	25 ppm
Hydrogen Sulfide	2 mg/m^3	10 ppm[c]	10 ppm
Endotoxin	NE	NE	NE

NE = not established; [a] = Particulate not otherwise classified; [b] - Grain dust consists of 60-75% organic materials (cereal grains) & 25-40% inorganic materials (soil), and includes fertilizers, pesticides & microorganisms; c = 10 minute exposure

These agencies recommend standards for both total and respirable particulate not otherwise classified (PNOC), grain dust, ammonia, and hydrogen sulfide. Concentrations of total and respirable PNOC and grain dust range from 3 to 15 mg/m^3, ammonia from 25 to 50 ppm, and hydrogen sulfide from 1.4 to 10 ppm (ACGIH 2007, NIOSH 2007, OSHA 2007).

Specific knowledge of the toxicant concentrations in ambient air in and around large CAFOs is essential for understanding how the emissions from CAFOs affect the health and quality of life of workers and those living and working near CAFOs. A brief review of the hazards imposed by several of the compounds that were studied is provided below.

Particulate

Confinement dust is primarily composed of organic compounds. Organic dust is a complex mixture of components including vegetable products, insect fragments, animal dander, feed and fecal particles, pesticides, microorganisms, endotoxins, and pollen.

Ammonia, molds, bacteria, and endotoxin can attach to dust particles and become deposited in the lung (Donham 1986, Tripp 1999).

Dust particles can settle at different levels of the respiratory system depending on size. Particles greater than 10 microns are generally deposited in the upper respiratory tract. Particles from 3 to 10 microns are most often deposited in the major airways of the lower respiratory tract, and particles smaller than 3 microns are respirable and can reach deep into the lung parenchyma (Tripp 1999). Adverse health effects associated with confinement workers include cough, wheeze, shortness of breath, chronic bronchitis, decrease in lung function, asthma-like syndrome, and organic dust toxic syndrome (Clark 1983, Crook 1991, Donham 1989, Thorne 1999).

Donham and Reynolds have found that a high proportion of disease occurs in workers at dust levels above 2.5 mg/m^3 total and 0.23 mg/m^3 respirable for swine confinement operations and poultry house operations. Both have recommended that these levels serve as occupational limits for CAFO workers (Donham 1988, 1995, 2000b, Reynolds 1996). Kullman et al. found personal breathing zone dust levels averaged: 1.78 mg/m^3 for inhalable fractions and 0.07 mg/m^3 for respirable fractions on 85 Wisconsin dairy farms (Kullman 1998). Respirable dust concentrations swine confinements may reach as high as 40% of the total dust. Dust levels are highest in the finishing buildings (up to 15 mg/m^3) and from 3 to 5 mg/m^3 in the farrowing and nursery buildings (American 1998). Poultry workers were exposed to a median dust level of 11.53 mg/m^3 based on personal sampling (Simpson 1999).

Reynolds et al. measured area and personal samples in turkey barns in winter and summer and found area total dust levels ranging from 4.7 to 7.6 mg/m^3, area respirable

dust samples from 1.0 to 1.5 mg/m^3, and personal respirable dust samples from 0.3 to 0.7 mg/m^3 in winter; in summer they found area total dust levels ranging from 1.2 to 4.4 mg/m^3, area respirable dust samples from 0.3 to 0.4 mg/m^3, and personal respirable dust samples from 0.5 to 1.1 mg/m^3 (Reynolds 1994). Simpson et al. found the highest grain dust exposures to be associated with grain cleaning, with a median level of 72.5 mg/m^3 (Simpson 1999). Firth et al. measured personal inhalable dust levels at New Zealand dairy, sheep, arable, and mixed farms. They found median inhalable levels of 0.60, 0.70, 1.71, and 0.54 mg/m^3, respectively. Interquartile ranges were from 0.22 to 2.45 mg/m^3, overall (Firth 2006).

Nieuwenhuijsen et al. measured inhalable and respirable dust for a variety of tasks on California farms including milking, moving, handling, and feeding cows and feeding, moving, and handling poultry. Inhalable values ranged from 0.30 to 6.67 mg/m^3. The highest inhalable values were associated with feeding poultry and scraping poultry houses. Respiratory values ranged from 0.08 to 0.25 mg/m^3. The highest respirable values were associated with handling poultry and scraping cow stalls (Nieuwenhuijsen 1999). Cathomas et al. measured total dust concentrations and organic dust concentrations on 6 Swiss dairy farms in winter and summer months. In winter, total dust ranged from 0.15 to 3.5 mg/m^3 and organic dust ranged from 0.11 to 2.21 mg/m^3. In summer, during the hay storage process, total dust ranged from 0.23 to 7.3 mg/m^3 and organic dust ranged from 0.76 to 4.9 mg/m^3. Organic dust was classified as PM_{10} (Cathomas 2002).

Endotoxin

Exposure to endotoxin can cause cough, chest tightness, mucous membrane irritation, decrease in lung function, chronic airways obstruction, chronic bronchitis, byssinosis, bronchial hyperreactivity, dyspnea, fever, rigors, myalgia, arthralgia, and other influenza-like symptoms (Merchant 1986, Reynolds 1996, Ross 2000, Donham 1989, Thorne 1999). Workers in cotton and flax mills, wool carpet workers, swine confinement workers, and animal feed workers have reported symptoms linked to endotoxin exposure (Castellan 1995, Ozesmi 1987, Thorne 1999).

Endotoxin is a lipopolysaccharide protein component of the outer wall of Gram-negative bacteria. Endotoxin is a potent inflammatory agent that produces systemic effects and lung obstruction even at low levels of exposure (Heederik 1991). Animal feces and plant materials contaminated with bacteria are major contributors of endotoxin to organic dust. Exposure to such dust is prevalent in livestock farming (Thorne 2000). CAFOs produce large airborne concentrations (900-24,000 EU/m^3) of endotoxin (Thorne 1997, Thorne 2000).

Currently, in the United States, there are no established occupational exposure limits or ambient air standards for endotoxin. Donham et al. have recommended 100 EU/m^3 as an occupational limit (Donham 1988). In the Netherlands, the Dutch Expert Committee on Occupational Standards (DECOS) has proposed an occupational exposure limit of 50 EU/m^3 (4.5 ng/m^3) over an 8-hour exposure period (DECOS 1996, Duchaine 2001). A limit of 200 EU/m^3 is currently enforced.

Kullman et al. found inhalable endotoxin ranging from 25 to 35,000 EU/m^3 with a geometric mean (GM) of 647 EU/m^3 and respirable endotoxin ranging from 0.16 to 1380

EU/m^3 with a GM of 16.8 EU/m^3 on 85 Wisconsin dairy farms during routine barn activities (Kullman 1998). Nieuwenhuijsen et al. found inhalable endotoxin levels ranging from 23.58 to 120.38 EU/m^3 for cow activities and from 222.25 to 1861.18 EU/m^3 for poultry activities on California farms (Nieuwenhuijsen 1999). The highest values were associated with feeding poultry and scraping poultry houses. Respirable endotoxin levels were generally low with the majority of samples below the limit of detection. The highest value was 18.93 EU/m^3 measured during poultry house cleaning (Nieuwenhuijsen 1999). Anderson et al. measured a GM respirable endotoxin concentration of 40 EU/m^3 and a GM total endotoxin concentration of 740 EU/m^3 during routine activities in 28 Swedish dairy barns in March and April (Anderson 1989). Reynolds et al. measured area total and respirable and personal total and respirable endotoxin concentrations at turkey barns in winter and summer. Total area concentrations ranged from 208 to 10960 EU/m^3 and respirable area concentrations ranged from 59 to 2547 EU/m^3. Personal respirable concentrations ranged from 91 to 568 EU/m^3. Winter values were consistently higher than summer values (Reynolds 1994). Donham et al. found similar respirable endotoxin concentrations from area samples collected in 30 Swedish swine confinement barns. Values ranged from 100 to 5600 EU/m^3 (Donham 1989). In comparison, Mueller-Anneling et al. found ambient endotoxin concentrations in Southern California to range from 0.19 to 1.85 EU/m^3 at 13 sampling sites (Mueller-Anneling 2004). Park et al. reported indoor airborne endotoxin concentrations ranging from 0.02 to 19.8 EU/m^3 in the homes of 20 employees of the Harvard School of Public Health (Park 2000).

Hydrogen Sulfide

Hydrogen sulfide (H_2S) is one of the most significant gases emitted from CAFOs. Arising from the storage, handling, and anaerobic digestion and decomposition of animal wastes, H_2S is recognized as both a pulmonary irritant and an asphyxiant (Donham 1977). Exposure in workers has been linked to increased respiratory symptoms, irritation and cough, as well as increased incidence of headache and migraine (Tripp 1999). Community exposures ranging from 70 to 300 parts per billion (ppb) were reported to cause adverse health effects including shortness of breath, eye irritation, nausea, loss of sleep, and increased prevalence of asthma and chronic bronchitis (Partti-Pellinen 1996). Chronic low-level exposure is associated with the loss of ability to detect odors, irritation to mucus membranes, and ocular and airway irritation. At higher levels, H_2S exposure causes a loss of consciousness, shock, pulmonary edema, coma, and death (Tripp 1999).

Ammonia

Ammonia (NH_3) is a major constituent of animal waste and is released from CAFOs, manure storage vessels, and land application of manure. Ammonia is highly water-soluble and is rapidly absorbed in the upper airways, resulting in damage to the airway epithelia. Respirable dust can act as a vehicle for ammonia deposition by carrying it deep into the lung (Donham 1986). Acute exposure at high concentrations (50-150 ppm) can lead to severe cough, mucus production, nasal irritation, and an increase in nasal airway resistance; exposure to even higher (>150 ppm) concentrations may cause scarring of the upper and lower airways (Close 1980, Donham 1977, Preller 1995). High-concentration exposure can lead to chemical burns of the skin and eyes and even

death (Hurst 1995). Low-level chronic exposures may lead to wheeze, chest tightness, chronic lung inflammation, chronic cough, chronic bronchitis, and decreased lung function (Donham 1986, 1988, 1989, Latenser 2000, Reynolds 1996).

Concentrations of ammonia inside U.S. dairy barns have ranged from 0.1 to 26 ppm (Kullman 1998). Swierstra et al. measured much higher levels of ammonia concentrated in the exhaust from Swedish dairy farms (up to about 11,000 ppm) but did not report more diluted ambient concentrations (Swierstra 2001). Groot Koerkamp (1998) reported ammonia levels of 0.3 – to 1.3 ppm on site for cattle which is slightly lower than levels emitted from swine and poultry CAFOs. Svensson (1994) studied emissions of ammonia during land applications and reported levels ranging from 0.8 ppm to 53 ppm.

Bioaerosols

Bioaerosols are a major component of the toxicants released into the ambient air around CAFOs. Bioaerosols are particles of biological origin suspended in air. These include bacteria, fungi, fungal and bacterial spores, viruses, mammalian cell debris, products of microorganisms, pollens, and aeroallergens (Douwes 2002, Heederik 2002, Jaakkola 1991). Typical aerosol size of bioaerosols range from 0.01 to 100 μm; however, most bacterial cells fall in the range of 0.5 to 5 μm and fungal spores range between 2 to 10 μm (Thorne 1999, Eduard 1997, Douwes 2002). Endotoxin and mycotoxin are microbial metabolites which play a major role in inflammatory lung reactions (Heederik 1991). Endotoxin and mycotoxin readily attach to larger particles.

CAFO environments provide ample substrate for the growth of bacteria and fungi. Animals, manure, bedding, compost, soil, and feeding materials are all avenues for microorganism growth (Eduard 1997, Seedorf 1998). Microorganisms may become airborne during many activities including feeding, bedding, milking and cleaning (Lange 1997). Exposure to occupational bioaerosols and their components have been linked with numerous adverse health effects in humans including organic dust toxic syndrome, hypersensitivity pneumonitis, restrictive physiology, progressive dyspnea, allergies, bronchitis, asthma, and asthma-like syndrome. Exposure to endotoxin in bioaerosols can cause cough, chest tightness, dyspnea, fever, rigors, myalgia, arthralgia, joint pain, and other "flu-like" symptoms (Merchant 1986, Reynolds 1996, Ross 2000). Currently there are no occupational exposure limits for bioaerosols and the Environmental Protection Agency (EPA) and Agency for Toxic Substances and Disease Registry (ATSDR) have not made recommendations about community exposure limits. A limit of $4.3x10^5$ CFU/m^3 has been recommended to ensure worker health (Donham 1988, 2000b).

Mean concentrations of total bacteria were reported as 10^6 CFU/m^3 for poultry, 10^5 CFU/m^3 for swine, and 10^3 CFU/m^3 in dairies (Seedorf 1998, Duchaine 2000, Karwowska 2005). Mean fungal concentrations were 10^4 CFU/m^3 for poultry, 10^5 CFU/m^3 for swine, and 10^2 CFU/m^3 in dairies (Clark 1983, Cormier 1990, Crook 1991, Seedorf 1998). Kullman et al. (1998) found airborne concentrations of culturable fungi and bacteria ranged from 10^2 to 10^6 CFU/m^3 (Kullman 1999).

Odors

Among the emissions from CAFOs, odors are the most commonly recognized by workers and the communities surrounding these sites. In addition to being an extreme nuisance, sufficient odor exposure can cause adverse health effects (Thu 1997). The biological breakdown of feed while in the animal gut and of the manure after excretion produces odoriferous organic compounds (Thorne 2002). Some of the compounds and associated smells emitted from CAFOs are organic acids including butyric acid (rancid butter odor), valeric acid (putrid fecal smell), sulfur-containing compounds including hydrogen sulfide (rotten egg smell) and dimethyl sulfide (odor of rotting vegetables), and nitrogen containing compounds including ammonia, skatole (fecal odor), and indole (intense fecal smell) (Cheremisinoff 1975, Thorne 2002). In a recent study, 331 volatile organic compounds and fixed gases were characterized (Schiffman 2001). Included in these compounds were many acids, alcohols, aldehydes, amides, amines, aromatics, esters, ethers, fixed gases, halogenated hydrocarbons, hydrocarbons, ketones, nitriles, other nitrogen-containing compounds, phenols, sulfur-containing compounds, steroids, and other compounds.

Since the 1980's there is much indication from occupational health literature that CAFOs create health issues for workers. Recent studies have further validated this claim (Schiffman 2001). Health symptoms have been reported from low-level exposures to odors including eye, nose, and throat irritation, headache, nausea, diarrhea, hoarseness, sore throat, cough, chest tightness, nasal congestion, palpitations, shortness of breath (dyspnea), stress, drowsiness, and alteration in mood. Research shows that people living near CAFOs have reported decreased health, decreased quality of life, mood changes

including increase in worry, tension, depression, anger, less vigor, and confusion (Schiffman 1998, 2001, Thu 1997, Wing 2000).

A report from a recent workshop on potential health effects of odors sponsored by the U.S. Environmental Protection Agency (USEPA) and the National Institute on Deafness and Communication Disorders (NIDCD) concluded that there are three potential ways in which odorous emissions from manures and biosolids may produce health symptoms (Schiffman 2000). In the first paradigm, health symptoms are induced by the irritant properties of the odorants. For a broad range or mixture of molecules, irritancy occurs at a concentration about 3–10 times higher than the odor threshold. While the concentration of each individual compound identified in odorous air from CAFOs seldom exceeds the concentration known to cause irritation, the combined load of the mixture of odorants can exceed the irritation threshold. Thus, the irritation induced by the odorous mixture derives from the addition and sometimes synergism of individual compounds (Schiffman 2000).

In the second paradigm, health symptoms occur at odorant concentrations that are above odor detection thresholds but far below the levels that cause irritation. This typically occurs with odorant classes such as sulfur-containing compounds and organic amines. The physiological basis by which sulfur gases or organic amines induce health symptoms when odor potency far exceeds the irritant potency is not well understood. However, brain imaging studies suggest a genetic factor may play a role since noxious odors stimulate different brain areas than those that process pleasant odors (Schiffman 2000).

In the third paradigm, the odorant is a component of a mixture that contains a co-pollutant that initially causes the reported health symptom. Odorous airborne emissions from CAFOs can contain other components such as endotoxin and organic dust that may induce the symptoms. If a person's initial exposure to odor from a CAFO occurs in the presence of dust or endotoxin, he may associate the odor with the health effects from the co-pollutant. Subsequent exposure to the odor in the absence of the co-pollutant can then produce the symptoms via Pavlovian conditioning (Schiffman 2000).

Measurement

With few exceptions, such as the Jerome 631-X Hydrogen Sulfide Analyzer (Arizona Instruments, Phoenix, AZ), there is little consensus on devices for measurement of occupational and environmental contaminants in the agricultural industry. The literature reports vast ranges of contaminants for everything from particulate to gases and odor. These ranges can be attributed somewhat to the variety of sampling methods and equipment used by individual researchers. Standard methods exist for the most commonly measured agricultural occupational contaminants. However, those methods are somewhat outdated and fail to utilize new technology. The National Institute for Occupational Safety and Health (NIOSH) maintains analytical sampling methods for a variety of chemicals including total and respirable particulate, hydrogen sulfide, ammonia, and carbon dioxide. The most recent update to these methods was in January 1998 for Method 0600 for respirable particulate (NIOSH 2007). More recent studies have used improvements based on older technology and newer real time instruments to measure particulate and certain gases. The new technology does have associated

negatives including cost, ease of use, and resiliency to environmental parameters while in the field.

Interventions

The emissions from concentrated animal feeding operations initiate primarily from buildings, manure storage, and land application of manure (Hardwick 1985). Anaerobic processes may be important in creating high concentrations of sulfides and other gases. Several types of engineering controls have been designed to reduce the amount of dust, odor, and gas released into the environment, but cost-effective interventions are still critically needed. A review by Lorimor et al. summarizes current literature regarding emission control technology, and a study by Goodrich et al. examined implementation of current technology in a livestock facility (Goodrich 2001, Lorimor 2002). In general, outdoor storage of manure reduces indoor contaminant levels for CAFO workers. The reduction of odor and gases inside buildings can be accomplished through several methods. The use of bedded solid manure systems, similar to what is used in natural open-air pastures, is thought to reduce odor levels (Lorimor 2002). These systems use sawdust or cornstalks instead of water to mix with and dilute manure, by creating the potential for greater dust emission.

Several studies have evaluated the effects of sprinkling different types of vegetable oils including soybean, canola, and rapeseed into air inside CAFOs (Goodrich 2001, Jacobson 1998, Kirychuk 1999, Nonnenmann 1999, Zhang 1996). This technology can take advantage of the existing water sprayer systems that many facilities have installed to aide with clean up. Mixtures of oil and water are periodically distributed into

confinement air. The effectiveness of this technology is debatable. One study claims reductions of 40 - 50% for dust, up to 60%, for hydrogen sulfide, and up to 60% for odor. No reduction of ammonia was found (Jacobson 1998). A second study claims reductions of 60 - 80% for dust, 30%for ammonia, 20% for hydrogen sulfide, and 10% for odor (Goodrich 2001). Problems with this type of control include installation of sprinklers for barns lacking water spraying systems, clogging and poor distribution of present systems, and buildup of oil residues on building surfaces (Goodrich 2001, Lorimor 2002).

Ozone air treatment can be useful in the reduction of odor. Ozone is distributed with ventilation air (Bottcher 2000). When added to a building in the winter, researchers found reductions of odor by 25% and hydrogen sulfide by 33% as compared to a control barn. H_2S levels were measured using a Jerome meter and odor was measured in the laboratory using dynamic forced choice olfactometer as described by Nicolai (Nicolai 1997). Dust levels were reduced with ozone treatment, but ammonia levels were not changed. During warmer months, when curtain ventilation systems were operated, reduction was not seen through ozone treatment (Goodrich 2001). The disadvantages of ozone treatment include its lack of function when barns are open due to an unstable nature, and toxicity to animals, humans, and the environment at high levels. Ozone is considered an environmental contaminant; so there are issues revolving around adding one contaminant to the environment to reduce another (Goodrich 2001, Lorimor 2002).

Chemical addition, especially during manure pump out, can help to reduce gas emissions. Hydrogen peroxide addition to pits during agitation and pumping showed reductions in hydrogen sulfide levels by 67% over control barns (Lorimor 2002). In a study by the National Pork Board, 35 products were investigated (Lorimor 2002). Only

one product was shown to reduce both hydrogen sulfide and ammonia, but none were able to significantly reduce odor levels. The disadvantages of chemical addition are that few products are able to reduce all contaminants and the associated expense of use (Lorimor 2002).

Diet manipulation by use of standardized ideal digestibility (SID) formulation, addition of amino acids, and reduction of nitrogen and sulfur has been used to reduce ammonia and hydrogen sulfide levels (Canh 1998, Goodrich 2001). Researchers found that use of SID diets, while having no effect on energy, nitrogen digestibility, and excretion, reduced slurry pH and ammonia and hydrogen sulfide emissions (Goodrich 2001. Disadvantages include lack of odor reduction with SID use, and concern regarding production given reduced nutrient levels (Canh 1998, Goodrich 2001).

A relatively simple method is that of a cover for lagoons to trap odor from being emitted. Covers can be made of many different types of materials. Synthetic impermeable covers are manufactured from wood, concrete, fiberglass, or plastic. Covers have been reported to reduce gas and odor up to 90% (Zahn 2001). Floating biocovers made from material, such as straw, cornstalks, sawdust, wood shavings, rice hulls, other material such as polystyrene foam, plastic mats, clay balls, and geotextile have been used as floating covers. University of Minnesota researchers saw an 85% odor reduction by the use of a 12-inch layer of straw, and up to 62% odor reduction and up to 84% hydrogen sulfide reduction using 8-inch Macrolite® clay balls. Researchers at Iowa State University saw more than 90% odor reduction and up to 95% ammonia reduction by using 1.5-inch Leca® clay balls (LPES 2002a). The disadvantages include the initial cost for synthetic covers and the short life span of biocovers. Biocovers must be

reapplied at more frequent intervals than synthetic covers (6 months or 10 – 15 years). With the proper selection, a cover can provide an effective and economical solution to odor emission from manure storage.

Composting is a method of aerobic treatment applied to solid or semi-solid manure. During composting, microorganisms degrade organic material such as manure, leaves, and food wastes. Composting can reduce material bulk by 50%, and has been known to reduce odor and hydrogen sulfide levels (NRAES 1992, Zhang 1997). Ammonia levels can be reduced by adding dry bulking agents such as straw (LPES 2002b)

Biological treatment of manure has also been useful for odor reduction. Both aerobic and anaerobic treatment can be used to treat manure. Complete aerobic treatment can eliminate odor produced by manure. Generally, aerobic treatment is only suitable for slurry or dilute effluent because solid manure increases the amount of aeration and mixing required (LPES 2002b). Aerobic reactors can be added to slurry tanks or to lagoons. Oxygen is added in several methods including bubbling, whipping, or liquid spraying into the air (Lorimor 2002). One disadvantages of aeration include its high cost and large amounts of energy are required to provide proper amounts of oxygen (Zhang 1997). Aeration in lagoons can create more biosolids than anaerobic systems, odor levels can be increased if too little oxygen is introduced into the system, and ammonia levels can be increased if too much oxygen is introduced into the system (LPES 2002b)

Anaerobic treatment occurs in the absence of oxygen. Anaerobic lagoons are the most common type of anaerobic digestion and storage system. When anaerobic lagoons are properly maintained with a balance of acid-forming and methane-forming bacteria

they produce minimal odor. Odor emission increases when the balance is disturbed by short retention times, lack of dilution water, and over-loading with animal waste. If the CAFO is above animal capacity, the lagoon will not be able to support the amount of wastes produced by the excess animals. Odor emissions can be released when the lagoon is disturbed during startup, windy conditions, agitation and pumping, and spring turnover (Lorimor 2002, LPES 2002b).

Anaerobic digesters are operated under more strictly controlled conditions than required for normal lagoons. Specific reactors include the plug-flow, complete-mix, contact, and upflow anaerobic sludge blanket digesters. Although optimal reaction temperature is over 49 °C, cost effectiveness usually creates operation temperature between 35 and 38 °C. Using adequate retention time and proper temperature, anaerobic digesters can eliminate the majority of odor producing compounds. Disadvantages include high-energy costs and high failure rates. The overall chance of failure is about 50% in the United States. Failure rates for plug-flow and complete mix technologies are 63% and 70%, respectively (LPES 2002b).

Chapter 2

Comparison of SKC Biosampler and Anderson Two-Stage Viable Particle Sampler for Measuring Airborne Bacteria and Fungi at Two Colorado Dairies

Abstract

There is a limited amount of information available on dairy farmers' exposure to airborne bacteria and fungi, and even less information on exposures to CAFO emissions other than inside barns. The objective of this study was to evaluate the effectiveness of an innovative lagoon treatment and compare the Anderson sampler and the SKC Biosampler. We investigated the performance of these two samplers and three selective media for the collection of viable microorganisms on two Colorado dairies. Samples were collected with an Anderson two stage viable particle sampler and a SKC Biosampler using R2A for mesophilic bacteria, EMB for Gram-negative bacteria, MEA for fungi at locations near lagoons. Overall, the Anderson sampler collected geometric mean concentrations of 1282, 667, and 781 CFU/m^3 for culturable mesophilic and Gram-negative bacteria and fungi, respectively. The SKC Biosampler collected geometric mean concentrations of 390, 268, and 256 CFU/m^3 for mesophilic and Gram-negative bacteria and fungi, respectively. The SKC Biosampler was found to enumerate approximately 30% of mesophilic bacteria, 40% of Gram-negative bacteria, and 33% of

fungi relative to the Anderson sampler. Similar results were found for individual seasons with values ranging from 16 to 45%. Mesophilic bacteria, Gram-negative bacteria, and fungi concentrations were all significantly higher for the Anderson sampler compared to the SKC Biosampler in all 4 seasons except for fungi in winter. Concentrations determined by these samplers were not consistently correlated. Respirable microorganism concentrations were consistently higher than non-respirable concentrations in total and over all four seasons except for Gram-negative bacteria in the fall. In general these concentrations are lower than suggested guidelines; however they may contribute to long term health concerns for workers and surrounding communities.

Introduction

The industrialization of livestock production has led to concern over public health impacts from air emissions. Hazardous emissions from CAFOs include gases, vapors, particulates, bioaerosols, and other semivolatile and volatile organic compounds. Research has established that these air emissions contribute negatively to the health and quality of life of CAFO workers, animals, and surrounding communities (Schiffman 1995, 1998, 2000, 2001, 2005, Wing 2000).

Bioaerosols are a major component of the toxicants released into the ambient air around CAFOs. Bioaerosols are particles of biological origin suspended in air. These include bacteria, fungi, fungal and bacterial spores, viruses, mammalian cell debris, products of microorganisms, pollens, and aeroallergens (Heederik 2002, Douwes 2002). Typical aerosol size of bioaerosols range from 0.01 to 100 μm; however most bacterial cells fall in the range of 0.5 to 5 μm and fungal spores range between 2 to 10 μm (Thorne

1999, Eduard 1997). CAFO environments provide ample substrate for the growth of bacteria and fungi. Animals, manure, bedding, compost, soil, and feeding materials are all media for microorganism growth (Eduard 1997, Seedorf 1998). Microorganisms may become airborne during many activities including feeding, bedding, milking and cleaning (Lange 1997). Although few occupational standards exist, exposure to occupational bioaerosols has been linked with numerous adverse health effects in humans including organic dust toxic syndrome, hypersensitivity pneumonitis, allergies, bronchitis, and asthma (Merchant 1986, Kirkhorn 2000, Ross 2000, Seifert 2003, Donham 2007).

Mean airborne concentrations of total bacteria were reported as 10^5 to 10^6 CFU/m^3 for poultry, 10^4 to 10^8 CFU/m^3 for swine, and 10^3 to 10^5 CFU/m^3 in dairies (Reynolds 1994, Kullman 1998, Seedorf 1998, Duchaine 2000, Karwowska 2005, Lee 2006). Mean fungal concentrations ranged from 10^3 to 10^4 CFU/m^3 for poultry, 10^3 to 10^5 CFU/m^3 for swine, and 10^2 to 10^4 CFU/m^3 in dairies (Clark 1983, Cormier 1990, Crook 1991, Seedorf 1998, Kullman 1998, Lee 2006). In comparison, indoor residential and commercial building levels of bacteria and fungi are between 10 and 10^3 CFU/m^3 (DeKoster 1995, Reynolds 2001).

The state of Colorado defines a dairy CAFO as one which contains 700 head or 800,000 pounds live weight (Kress 2007). In 2006, the state of Colorado ranked 16th in the United States in total milk production with 2,547,050,000 pounds of milk an 8.5% increase over 2005. Colorado had 170 licensed dairy herds each with an average of 647 cows for a total of 110,000 cows. In total, the United States' 9,112,000 cows produced 181,798,000,000 pounds of milk in 2006.

Increase in milk production and larger herd sizes are trends that U.S. dairy producers can expect to see for years to come (Cooley 2007).

In this study we compared two viable bioaerosol samplers: the Anderson two stage viable particle sampler (ThermoAnderson, Smyrna, GA.); and the SKC Biosampler (SKC Inc., Eighty Four, PN.). The Anderson two stage aluminum viable particle sampler is used whenever a size distribution is not needed and only respirable and non-respirable segregation or total counts are needed. Ninety-five to one hundred percent of the viable particles above 0.8 μm in an aerosol can be collected on a variety of general purpose solid bacteriological agar (ThermoAnderson, Smyrna, GA.). This sampler separates viable particles into two size ranges with the 50% cut-off diameter of stage 1 at 8.0 μm and stage 2 at 0.95 μm for spherical particles of unit density or their aerodynamic equivalent (Hatch 1955). There has only been one other published study (Fabian 2005) comparing the SKC Biosampler and the Anderson sampler in any environment. Fabian et al. used a one-stage N6 Anderson sampler (Graseby-Anderson Instruments, Smyrna, GA). This stage collects particles with a 50% cut-off aerodynamic of 0.65 μm.

In the SKC Biosampler, the airborne microorganisms are drawn into three nozzles through which they are projected at an angle toward a curved surface where they are collected by the combined forces of impaction and centrifugation. During normal operation, the sampler is used with a liquid that swirls upward on the sampler's inner wall and removes collected particles. The swirling motion of the collection liquid generates very few bubbles thus producing minimal aerosolization of collection particles (SKC 2007, Lin 1999). When 20 milliliters of water is used as the collection fluid, the physical collection efficiency of the Biosampler has been shown to be about 79% for 0.3 μm

particles, 89% for 0.5 μm particles, 96% for 1.0 μm particles, and 100% for 2.0 μm particles (Willeke 1998).

The objective of this study was to compare two viable microbial samplers, the Anderson two stage viable particle sampler and the SKC Biosampler, using three selective media, R2A for mesophilic bacteria, EMB for Gram-negative bacteria, and MEA for fungi.

We characterize several types of microorganisms on two Colorado dairies. One dairy employs the use of a novel lagoon intervention designed to increase the dissolved oxygen level of the lagoon in hopes of reducing emissions. The second dairy served as a control.

Materials and Methods

Sampling Sites

The dairy characteristics are listed in Table II. The study dairy had total land area of approximately 60 acres with a single lagoon of approximately 8 acres. The study dairy milked approximately 1350 cows, with approximately 125 cows kept dry and not milked. The study dairy raised the female calves on site. The waste treatment system was as follows: The milking parlor was rinsed with fresh water, approximately 20,000 gallons per day while recycled water, approximately 300,000 to 400,000 gallons per day, was used to rinse the other areas. Wastes flowed by gravity into a leaky dam separation and settling basin and then into a primary lagoon. Dry lots were scraped as are the settling basins and the solids were composted. The lagoon was treated with a novel algae

intervention. Algae were grown in a greenhouse on site next to the lagoon and pumped through micro diffusers into the lagoon.

Table II. Summary of Dairy Characteristics

	Study Dairy	Control Dairy
Land Area (acres)	60	340
Lagoon Area (acres)	8	6
Milking Cows	1350	3000
Dry Cows	125	350
Calves	yes	no
Manure Treatment	Algae Treatment Leaky Dam Separation Settling Basin Primary Lagoon Gravity Fed Scraped Dry Lots Solids Composted Recycled Liquids Straight Milking Parlor	Leaky Dam Separation Earthen Basin Separation Primary and Secondary Lagoons Gravity Fed Scraped Dry Lots Solids Composted Recycled Liquids Rotary Milking Parlor

The algae increased the percent of dissolved oxygen in the lagoon to above 1mg/L with the purpose of transforming the once anaerobic environment into an aerobic environment with the hope of reducing lagoon emissions.

The control dairy had total land area of approximately 340 acres with two lagoons of approximately 3 acres each. The control dairy milked approximately 3000 cows, with approximately 350 cows kept dry and not milked. The control dairy did not raise the female calves on site. The waste treatment system was as follows: The milking parlor was rinsed with fresh water, approximately 180,000 gallons per day including drinking water while recycled water, approximately 80,000 gallons per day, was used to rinse the other areas. Wastes flow by gravity into a leaky dam separation and an earthen basin

separation then into a primary and secondary lagoon. Dry lots and settling basins were scraped and the solids were composted.

Sampling Schedule

We measured the airborne bacteria and fungal concentration at each dairy at a predominately downwind location from the lagoons. The samplers were juxtaposed at approximately one meter above ground level. This study was designed to sample each dairy 40 days with 10 sample days each season for a total of 80 sample days. Weather conditions prohibited sampling on several days. The study dairy was sampled 25 total days with the Anderson two stage viable particle sampler over each season as follows: 9 fall, 4 winter, 6 spring, and 6 summer. The SKC Biosampler was sampled 27 total days over each season as follows: 6 fall, 4 winter, 9 spring, and 8 summer. The control dairy was sampled 40 days with the Anderson two stage viable particle sampler over each season as follows: 10 fall, 10 winter, 10 spring, and 10 summer. The SKC Biosampler was sampled 36 total days over each season as follows: 10 fall, 6 winter, 10 spring, and 10 summer. Both dairies were scheduled for 40 days each; however, meteorological conditions prevent sampling with both instruments on all 40 days.

Sampling and Analysis

Meteorology

Meteorological data including temperature, relative humidity, and wind speed was measured using a Vantage Pro Weather Station (Davis Instruments, Hayward, CA). The Weather Station was mounted to a pole approximately 2 meters above ground level. The Weather Station consisted of a combination of a wind vane and anemometer and a

temperature and relative humidity sensor. All were mounted at the apex of the pole. The temperature sensor was shielded from direct sunlight. The pole was orientated such that the wind vane was directed south and the temperature relative humidity sensor was directed north, as directed by the instruction manual. Data were logged using Davis WeatherLink for Vantage Pro data collection, analysis, and display software for Windows. Data were collected over 1-minute intervals as mandated by the software, and displayed as average values.

Anderson Two Stage Viable Particle Sampler

Total culturable organisms were quantified using an Anderson two stage viable particle sampler (ThermoAnderson, Smyrna, GA), following NIOSH method 800. Air was sampled directly onto culture plates of selection media at a flow rate of 28.3 L/min for approximately 2, 5, and 10 minutes. Time intervals were varied from 2 to 10 minutes to produce the best possible data given unknown airborne bioaerosols concentrations. Anderson samplers were autoclaved prior to each trip, and wiped with isopropyl alcohol swabs between samples.

Selective culture media were prepared by aseptically pipetting 20 ml of EMB for Gram-negative bacteria, MEA with chloramphenicol for fungi, and R2A with cycloheximide for mesophilic bacteria at 45-55 °C into 100x15mm disposable plastic plates. All media were acquired from Difco (Becton, Dickinson and Company, Sparks, MD). Blank plates were handled in a similar manner to sampling plates without attaching the pump. All media plates were stored at 4 °C during transportation. Duplicate determinations were run during the sampling window and averaged to establish a more accurate count. EMB and R2A media plates were incubated at 37 °C, while MEA

was incubated at 25 °C. All plates were counted for 5 days at 24-hour intervals until growth had ceased or overgrowth occurred. Corrections were made using the positive-hole method (Macher 1989), which accounts for the probability of multiple particles impacting through the same hole. Concentrations were reported as colony forming units per cubic meter of air (CFU/m^3).

SKC Biosampler

Total culturable organisms were quantified using a SKC Biosampler (SKC). Air was sampled directly into collection media at a flow rate of 12.5 L/min for approximately 30 minutes. A thirty minute time period was decided upon after several trial periods. A short time period also reduces the chance of media evaporation. The biosamplers were autoclaved prior to each trip. Collection media were prepared by aseptically pipetting 20 ml of sterile phosphate buffered saline 1x (PBS) (Cellgro Herndon, VA) into sterile tubes. The PBS was poured into the Biosampler at the beginning of the sampling period and back into the tubes after sampling for transportation to the lab. All Media were stored at 4 °C during transportation.

The same selective culture media were prepared as with the Anderson sampler. After sampling, the Biosampler media (PBS) were prepared for plating using 10-fold serial dilutions. One milliliter (ml) from of the bulk media was pipetted into 9 ml of sterile PBS and vortexed to reach a 10^{-1} dilution. From there 1 ml was then pipetted into 9 ml sterile PBS and vortexed to reach a 10^{-2} dilution. This process was repeated until a 10^{-7} dilution was reached. One ml of each dilution was plated once on each type of media plate. Fabian et al. (2005) found that multiple plates from multiple SKC Biosamplers sampled in the same locations indoors and outdoors of flooded houses were

statistically indistinguishable (Fabian 2005). Sterile EMB and R2A media plates were incubated at 37 °C, while MEA was incubated at 25 °C. All plates were counted for 5 days at 24-hour intervals until growth had ceased or overgrowth occurred. Concentrations were reported as colony forming units per cubic meter of air (CFU/m^3).

Statistical Analysis

Excel databases were combined and analyzed using SAS Version 9.1 (SAS Institute, Cary, NC). Descriptive statistics were used to characterize environmental measurements. The normality of the data was tested using the Shapiro-Wilks test. All variables, except meteorological conditions, were log transformed before completing statistical analysis. Geometric means and geometric standard deviations were calculated for environmental data that could be described as lognormal. Comparisons between samplers, dairies, and seasons were made using linear analysis of variance with two-way interactions. Included variables were as follows: dependent (mesophilic bacteria CFU/m^3, gram-negative bacteria CFU/m^3, and fungi CFU/m^3); class (dairy, season, and sampler); quantitative (temperature, relative humidity, wind speed, wind direction). Means were compared using Tukey's test procedure. Pearson correlation coefficients were calculated to evaluate associations among environmental parameters and microorganism concentrations.

Results

Seasons were defined as follows: December, January, and February were considered winter. March, April and May were considered spring. June, July, August were considered summer. September, October, and November were considered fall.

Meteorological data are summarized by season in Table III. Overall, atmospheric air temperatures ranged from -12.5°C to +41.1°C with a mean of +13.3°C. Winter, spring,

Table III. Dairy Meteorological Conditions by Season.

	Season	Study Dairy					Control Dairy				
		n	M	(SD)	Min	Max	n	M	(SD)	Min	Max
Temp. (°C)	winter	10	7.7	(5.1)	0.0	15.7	10	7.8	(4.5)	-1.3	15.9
	spring	10	9.5	(4.2)	3.2	16.9	10	8.1	(5.1)	-1.0	17.0
	summer	10	30.1	(4.0)	25.7	40.1	10	26.9	(6.4)	17.9	41.1
	fall	10	13.9	(13.6)	-9.9	28.9	10	2.1	(6.2)	-12.5	12.2
RH (%)	winter	10	31.5	(19.3)	14.4	72.3	10	34.4	(12.0)	7.9	46.0
	spring	10	37.9	(15.1)	12.4	63.0	10	42.2	(10.3)	25.9	62.1
	summer	10	29.1	(14.6)	0.6	48.3	10	45.1	(8.2)	39.7	63.4
	fall	10	48.7	(20.6)	23.3	90.3	10	57.2	(14.5)	38.8	92.3
WS (m/s)	winter	10	1.8	(1.7)	0.5	6.0	10	2.2	(1.0)	0.8	4.1
	spring	10	3.0	(2.5)	0.9	8.7	10	2.8	(0.93)	1.6	4.7
	summer	10	1.6	(0.55)	1.0	2.8	10	2.5	(0.59)	1.8	3.8
	fall	10	1.5	(0.77)	0.6	3.0	10	2.5	(0.63)	1.4	3.6

and fall temperatures were all very similar with a mean around 8.0 °C. Relative humidity ranged from 0.6% to 92.3% with a mean of 40.7%. Winter produced the lowest relative humidity of 33% and fall the most with 53%. Wind speeds ranged from 0.48 m/s to 8.66 m/s with a mean of 2.2 m/s. All four seasons wind speeds averaged between 2.0 and 2.9 m/s.

Summary statistics for culturable mesophilic bacteria, Gram-negative bacteria, and fungi are shown by sampler and season in Table IV. Total concentrations of all three microorganisms were significantly different for the two samplers ($p<0.001$). The Mesophilic bacteria concentrations collected by the Anderson sampler ranged from 173 CFU/m^3 in fall to 9064 CFU/m^3 in summer. While those collected by the SKC Biosampler ranged from 36 CFU/m^3 in summer to 4800 CFU/m^3 in spring. Mesophilic

bacteria concentrations were significantly different for the two samplers in all four seasons (summer $p<0.001$, winter, spring, and fall ($p<0.01$). Anderson sampler concentrations were consistently higher than the SKC Biosampler. Overall, the SKC

Table IV. Comparison of Anderson Sampler Concentrations and SKC Biosampler by Season and Microorganism

Season, Microorganism	Sampler						
	Anderson (CFU/m^3)			Biosampler (CFU/m^3)			Biosampler/Anderson (%)
	GM	(GSD)	n	GM	(GSD)	n	
Total			65			65	
Mesophilic Bacteria ***	1282	(2.6)		383	(3.2)		29.9
Gram-negative Bacteria ***	667	(2.5)		265	(4.5)		39.7
Fungi ***	781	(2.5)		252	(4.0)		32.3
Winter			14			11	
Mesophilic Bacteria **	1034	(2.0)		331	(3.3)		32.0
Gram-negative Bacteria *	422	(2.9)		188	(5.8)		44.5
Fungi	479	(3.3)		191	(9.0)		39.9
Spring			16			19	
Mesophilic Bacteria **	1083	(1.9)		414	(3.2)		38.2
Gram-negative Bacteria **	785	(1.7)		256	(4.2)		32.6
Fungi **	816	(2.9)		223	(3.4)		27.3
Summer			16			18	
Mesophilic Bacteria ***	2171	(2.7)		353	(3.0)		16.3
Gram-negative Bacteria **	854	(2.7)		241	(4.8)		28.2
Fungi ***	959	(2.1)		170	(3.8)		17.7
Fall			19			17	
Mesophilic Bacteria **	1112	(2.8)		451	(3.2)		40.6
Gram-negative Bacteria *	663	(2.3)		298	(5.1)		44.9
Fungi **	909	(1.8)		257	(6.5)		28.3

Note: * = < 0.05 ** = < 0.01 *** = < 0.001 that mean concentrations in that row are not equal by ANOVA

Biosampler collected only 29.9% of the mesophilic bacteria that was collected by the Anderson sampler. By season it ranged from 16.3% in summer to 40.6% in fall. Gram-

negative bacteria concentrations collected by the Anderson sampler ranged from 39 CFU/m^3 in winter to 5071 CFU/m^3 in fall. While those collected by the SKC Biosampler ranged from 4 CFU/m^3 in summer to 2933 CFU/m^3 in summer. Gram-negative bacteria concentrations were significantly different for the two samplers in all four seasons (spring and summer $p<0.01$, winter and fall $p<0.05$). Anderson sampler concentrations were consistently higher than the SKC Biosampler. Overall the SKC Biosampler only collected 39.7% of the Gram-negative bacteria that was collected by the Anderson sampler. By season it ranged from 28.2% in summer to 44.9% in fall.

Fungi concentrations collected by the Anderson sampler ranged from 32 CFU/m^3 in winter to 7724 in spring CFU/m^3 in summer. While those collected by the SKC Biosampler ranged from 4 CFU/m^3 in winter to 2933 CFU/m^3 in fall. Fungi concentrations were also significantly different for the two samplers in three seasons (summer $p<0.001$, spring and fall $p<0.01$). Again, Anderson sampler concentrations were consistently higher than the SKC Biosampler. Overall the SKC Biosampler only collected 32.3% of the fungi that was collected by the Anderson sampler. By season it ranged from 17.7% in summer to 39.9% in winter.

Mesophilic bacteria, gram-negative bacteria, and fungi are summarized by sampler in a box-whisker plot in Figure I. The whiskers delineate the 10^{th} and 90^{th} percentiles and the box lines represent median, lower, and upper quartiles of the data. Respirable and non-respirable culturable mesophilic bacteria, Gram-negative bacteria, and fungi collected by the Anderson sampler are shown by season in Table V. Respirable mesophilic bacteria ranged from 92 to 5442 CFU/m^3 and non-respirable mesophilic bacteria ranged from 49 to 3975 CFU/m^3. Respirable Gram-negative bacteria ranged

from 14 to 6095 CFU/m^3 and non-respirable gram-negative bacteria ranged from 14 to 2915 CFU/m^3. Respirable fungi ranged from 18 to 5336 CFU/m^3 and non-respirable fungi ranged from 14 to 4682 CFU/m^3.

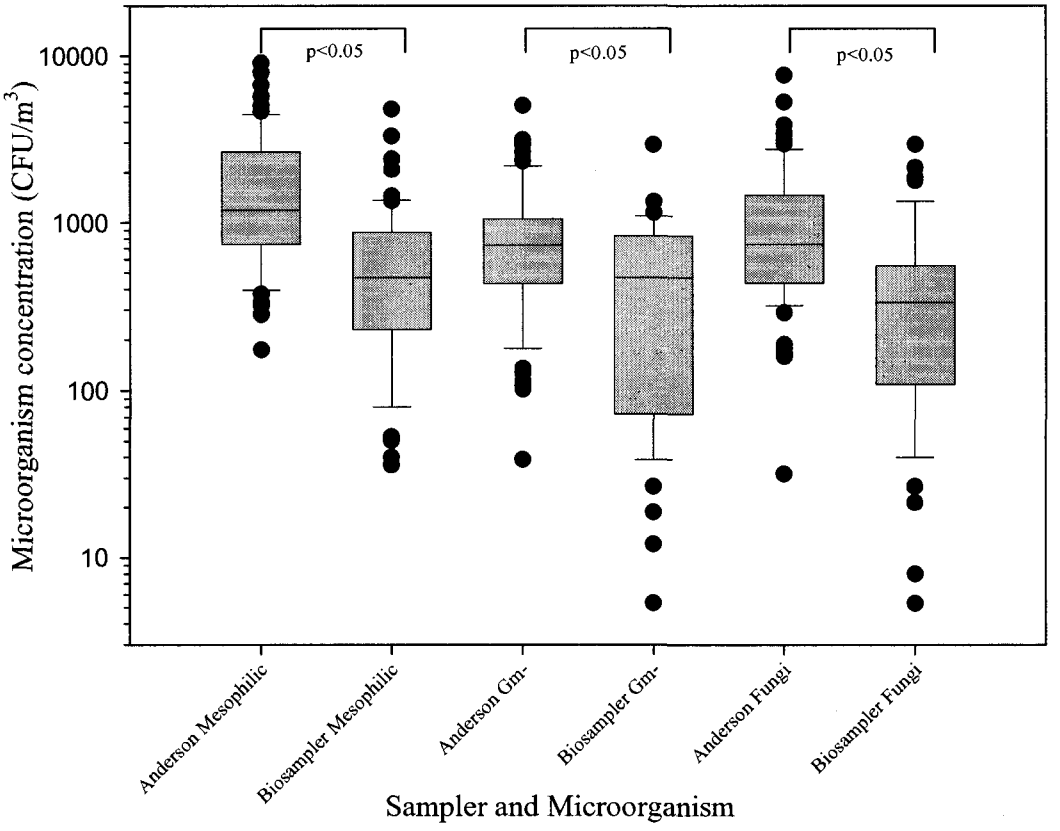

Figure I. Box-Whisker Plot of Bioaerosols Concentrations by Sampler at Dairy Lagoons
(Whiskers delineate 10th, 90th percentile and box lines represent median, lower, and upper quartiles of the data)

Respirable microorganism concentrations were consistently higher than non-respirable concentrations in total and over all four seasons except for Gram-negative bacteria in the fall.

The geometric mean for Gram-negative bacteria was approximately 52% of the geometric mean for mesophilic bacteria (667 and 1282 CFU/m^3, respectively) for the Anderson sampler.

Table V. Comparison of Anderson Sampler Respirable and Non-Respirable Concentrations by Microorganism

Season, Microorganism	Size					
	Respirable (CFU/m^3)			Non-Respirable (CFU/m^3)		
	GM	(GSD)	n	GM	(GSD)	n
Total			65			65
Mesophilic Bacteria	652	(2.7)		242	(2.6)	
Gram-negative Bacteria	479	(2.6)		380	(2.6)	
Fungi	350	(2.4)		278	(2.5)	
Winter			14			14
Mesophilic Bacteria	478	(2.1)		178	(2.9)	
Gram-negative Bacteria	466	(1.9)		255	(3.4)	
Fungi	200	(2.0)		196	(2.9)	
Spring			16			16
Mesophilic Bacteria	513	(1.6)		280	(2.0)	
Gram-negative Bacteria	458	(2.2)		350	(2.8)	
Fungi	408	(1.7)		305	(2.8)	
Summer			16			16
Mesophilic Bacteria	1300	(2.6)		320	(2.9)	
Gram-negative Bacteria	669	(2.9)		540	(3.3)	
Fungi	491	(3.3)		334	(2.8)	
Fall			19			19
Mesophilic Bacteria	528	(2.8)		215	(3.1)	
Gram-negative Bacteria	386	(2.6)		430	(2.0)	
Fungi	343	(2.2)		319	(2.2)	

The geometric mean for Gram-negative bacteria was approximately 69% of the geometric mean for mesophilic bacteria (268 and 390 CFU/m^3, respectively) for the SKC Biosampler. For the Anderson sampler, culturable mesophilic bacteria was comprised of 41%, 72%, 39%, and 60 % Gram-negative bacteria for winter, spring, summer, and fall,

respectively. For the SKC Biosampler culturable mesophilic bacteria was comprised of 36%, 62%, 68%, and 66 % gram-negative bacteria for winter, spring, summer, and fall, respectively.

Pearson correlation coefficients were calculated to evaluate relationships between each of the microorganisms by sampler and meteorological conditions. Generally, correlation was poor between analytes. There was moderate to high correlation between total microorganisms and respirable and non-respirable fractions as collected by the Anderson sampler (Table VI). The highest correlations ($r = 0.86$ to 0.94) were within microorganism type. Though not seen in Table VI, there was high correlation between respirable and non-respirable fractions within microorganism type ($r = 0.80, 0.77, 0.73$) for mesophilic bacteria, Gram-negative bacteria, and fungi, respectively. Correlation results were similar when computed for each season. There was no correlation between samplers for measuring culturable bacteria and fungi. This lack of a consistent quantitative relationship between samplers was evident regardless of season or dairy. In general, the SKC Biosampler collected 17.7% to 44.9% of the Anderson sampler.

Discussion

This study was designed to test the null hypothesis that there is no difference between the Anderson two stage viable particle sampler and the SKC Biosampler for measuring airborne culturable bacteria and fungi. Evaluated with a sample size of 65 Anderson and 65 SKC Biosampler samples, there was a statistically significant difference between the Anderson and SKC Biosampler in all seasons and all media except for fungi in winter (Table IV).

Table VI. Significant Pearson Correlation Coefficients for Anderson Sampler

	Respirable Mesophilic Bacteria	Non-Respirable Mesophilic Bacteria	Respirable Gram-negative Bacteria	Non-Respirable Gram-negative Bacteria	Respirable Fungi	Non-Respirable Fungi
Total Mesophilic Bacteria	0.89 ***	0.89 ***	0.41 ***	0.26 *	0.43 ***	0.26 *
Total Gram-neagtive Bacteria	0.26 *	0.34 **	0.94 ***	0.89 ***	0.33 **	0.46 ***
Total Fungi	0.31 *	0.36 **	0.50 ***	0.38 **	0.88 ***	0.86 ***

Note: * = $p < 0.05$ ** = $p < 0.01$ *** = $p < 0.001$ Respirable and Non-respirable microorganisms obtained from bottom and top stages of Anderson sampler, respectively.

Only one other study has compared the SKC Biosampler and the Anderson sampler in any environment (Fabian 2005). Fabian et al. compared the SKC Biosampler and a one-stage N6 Anderson sampler (Graseby-Anderson Instruments, Smyrna, GA) indoors and outdoors of flood damaged homes following a major regional flood in Colorado. The N6 Anderson sampler has a single stage that collects particles with a 50% cut-off aerodynamic of 0.65 μm. Fabian et al. found that bacterial concentrations recovered on tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI) plates in Anderson samplers agreed with general trends observed from culturing microorganisms retained in SKC Biosamplers. However, bacteria concentrations collected with the SKC Biosampler were significantly higher than those collected with the Anderson sampler in eight of nine houses. Bacteria concentrations recovered with the SKC Biosampler were between 10^2 to 10^4 times higher than those collected by the Anderson sampler. Fungi concentrations recovered on MEA plates from Anderson samplers agreed with general trends observed from culturing fungi from samples retained in the SKC Biosampler. Though not significant, fungi concentrations recovered from the SKC Biosampler were between 10^2 and 10^3 times higher than the Anderson sampler. Fabian et al. concluded that several reasons may have led to the differences between samplers including: 1) sample time; 2) retention differences intrinsic to the equipment; (3) particle stress; (4) differences in particle-size collection (Fabian 2005).

Other dairy studies collected mesophilic bacteria and fungi data in dairy barns with other samplers including a MAS-100 (Merck), which is based on the principle of a six stage Anderson sampler, and an all glass impinger (AGI-30, ACE Glassworks, Vineland, NJ). Mesophilic bacteria concentrations ranged from 160 and 1662 CFU/m^3 at

two milking points, and from $1.7x10^3$ to 10^6 (Karwowska 2005, Lange 1997, Dutkiewiez 1994, Batel 1979). Fungi concentrations ranged from 167 and 311 CFU/m^3 at two milking points, and from $1.7x10^2$ to 10^7 (Karwowska 2005, Dutkiewiez 1994, Lappalainen 1996, Hanhela 1995, Lange 1997, Batel 1979). Our data were considerably less than all but those collected by Karwowska at Polish dairy farms using a MAS-100 (Karwowska 2005). However, our data were collected downwind from lagoons several hundred meters from the nearest barn rather than inside dairy barns and inside milking parlors.

An et al. used the SKC Biosampler with 0.9% saline solution as a reference sampler in a study measuring airborne bacteria and fungi in a residential living room and outside a midsized building. At the indoor sampling site their mean concentration of airborne culturable bacteria and fungi were both 300 CFU/m^3. At the outdoor sampling site they observed a maximum concentration of 3000 CFU/m^3 for culturable bacteria and 200 CFU/m^3 for airborne culturable fungi (An 2004). Our outdoor culturable bacteria values were similar to An's reported residential living room value of 300 CFU/m^3, and our SKC Biosampler collected a geometric mean value of 252 CFU/m^3 for culturable fungi at outdoor locations.

Our Anderson Gram-negative bacteria data fell between data from studies conducted at swine and poultry facilities with Anderson samplers. Gram-negative bacteria data from swine facilities including farrowing and fattening operations were 80, 140, $7.7x10^3$, and $8.8x10^4$ CFU/m^3 (Clark 1983, Cormier 1990, Heederick 1991). Gram-negative bacteria data from a poultry facility were $4.1x10^4$ CFU/m^3 (Clark 1983). Our Anderson fungi data fell between data from studies conducted at swine and poultry

facilities with Anderson samplers. Fungi data from swine facilities including farrowing and fattening operations were 150, 190, 300, and from $2.0x10^3$ to 10^5 CFU/m^3 (Cormier 1990, Clark 1983, Crook 1991). Fungi data from a poultry facility were 500 CFU/m^3 (Clark 1983).

Our data do not agree with a study characterizing dust from swine confinements by Donham et al. where Gram-positive organisms were tenfold higher than both Gram-negative and fungi (Donham 1986). Although the Donham study characterized bacteria from dust samples, this study sampled viable organisms directly from ambient aerosols. The difference in sampling methodology could explain the variation in the numbers.

Culturable mesophilic bacteria collected in the summer were 2171 and 353 CFU/m^3 for the Anderson sampler and SKC Biosampler, respectively. In winter culturable mesophilic bacteria collected were 1034 and 331 CFU/m^3 for the Anderson sampler and SKC Biosampler, respectively. The SKC Biosampler collected only 16% and 32% of the mesophilic bacteria that was collected by the Anderson Sampler for summer and winter, respectively. Jo and Kang (Jo 2005) performed a similar study in Korean swine and poultry sheds using a single stage Anderson sampler in summer and winter. In swine sheds they found total viable bacteria to be $1.34x10^5$ and $3.3x10^4$ CFU/m^3 for winter and summer, respectively. In poultry sheds they found total viable bacteria to be $2.8x10^5$ in summer. These values are more than 15 times our Anderson sampler values for summer and more than 100 time our winter data. Again, these values were from inside buildings.

Culturable fungi collected in the summer were 959 and 170 CFU/m^3 for the Anderson sampler and SKC Biosampler, respectively. In winter, fungi concentrations

were 479 and 191 CFU/m^3 for the Anderson sampler and SKC Biosampler, respectively. The SKC Biosampler collected only 18% and 40% of the culturable fungi that was collected by the Anderson sampler for summer and winter, respectively. Jo and Kang (Jo 2005) found total viable fungi to be 454 and $7.1x10^3$ CFU/m^3 for winter and summer, respectively. In poultry sheds they found total viable bacteria to be $7.4x10^3$ CFU/m^3 in summer. Our fungi values were nearly the same for their winter swine collection. However, our summer values were 7 times less for both swine and poultry values. We also collected data in spring and fall and found similar results to our winter and summer data. Jo and Kang sampled an indoor environment, whereas our samples were collected outside downwind of the lagoons.

Our Anderson sampler concentrations varied more in summer than any other season. Values in summer approximately double those from winter. Values for summer Gram-negative bacteria and fungi were very similar to spring and fall values. SKC Biosampler values varied less than the Anderson sampler values. Highest values for mesophilic bacteria were found in spring and fall. Gram-negative bacteria values were similar over all seasons but slightly lower in winter. Fungi values were slightly higher in spring and fall. The mean temperatures for winter, spring, and fall were all within 1.0 °C, while summer temperatures were 20 °C higher.

Culturable fungi levels were 781 and 252 CFU/m^3 for the Anderson sampler and SKC Biosampler, respectively. The SKC Biosampler only collected 32% of the culturable fungi that was collected by the Anderson sampler.

Our data were similar to the lower side of other data collected in dairy barns with Anderson samplers ranging from 153 to 2225 CFU/m^3, 10^3 to 10^5, and 10^6 CFU/m^3 (Adhikari 2004, Pasanen 1989, Duchaine 1999).

Culturable microorganisms collected using the Anderson sampler were separated into respirable and non-respirable groups (Table V). Respirable mesophilic bacteria, Gram-negative bacteria, and fungi (geometric means 648, 350, and 391 CFU/m^3, respectively) were all higher than corresponding non-respirable values (geometric means 482, 244, and 288 CFU/m^3, respectively). Winter and spring values for respirable and non-respirable mesophilic bacteria were nearly equal while in summer and fall non-respirable values were approximately 51% and 73% that of respirable values. Winter and summer values for respirable and non-respirable gram-negative bacteria were nearly equal while in spring and fall non-respirable values were approximately 69% and 63% that of respirable values. Values for non-respirable fungi were approximately 77%, 87%, 62%, and 74% that of respirable values for winter, spring, summer, and fall, respectively. Although overall microorganism concentrations are relatively low—most values are comparable to those found at indoor locations—respirable concentrations are higher than non-respirable concentrations. Respirable microorganisms in the agricultural environment are of great concern given that they have the ability to travel deep into the lung and cause multiple respiratory diseases.

Listed in Table IV, in total the SKC Biosampler only collected between 30 and 40% of the culturable microorganisms that were collected by the Anderson sampler. Broken up by season, the values ranged from 16 to 45% but most values fell between 27

and 41%. Several factors may have contributed to the lower concentration collected by the Biosampler. The Anderson collects airborne microorganisms at a flow rate more than double that of the Biosampler. While the reported concentrations were standardized to one cubic meter of air, it is possible that in the outdoor environment, the Anderson sampler was less affected by environmental factors such as wind. The higher flow rate used for the Anderson might have been able to overcome higher wind speeds as compared to the SKC Biosampler and was able to collect a larger number of microorganisms. The Anderson and Biosampler inlets are different in that the Anderson inlet is parallel to the ground while the Biosampler inlet is perpendicular. It is possible that some particles may have settled onto the Anderson through gravity. When particles are collected by impingement, as in the Biosampler, air flow velocity through collector's nozzles often reaches sonic velocity, which is known to break-up clumps of culturable microorganisms into individual cells. This way a particle initially containing several cells will be broken into separate cells to be counted as multiple microorganisms. In an impactor, like the Anderson sampler, a particle impacting on the agar surface will be counted as one CFU even if it contains several culturable microorganisms. Therefore, the SKC Biosampler might be expected to yield larger values than the Anderson through particle deagglomeration during the impingement process. This high velocity may also affect viability of microorganisms collected therefore decreasing counts.

Sample timing may explain some of the difference in samplers. The Anderson sampler was run for 2, 5, and 10 minutes with 1-2 minutes between samples to clean the sampler while the SKC Biosampler was running for 30 minutes. It is possible that the Anderson was able to collect higher concentrations from sporadic bursts of bioaerosols in

the 2, 5 or 10 minute periods. These same bursts would have also been collected by the SKC Biosampler, but the concentrations would look smaller when averaged over the 30 minute sampling period. Variability in bioaerosols during these discordant sampling intervals may also help explain or contribute to the lack of correlation between the two samplers.

Difference in particle-size collection may have led to differences between samplers. The two stage Anderson sampler separates viable particles into two size ranges with the 50% cut-off diameter of stage 1 at 8.0 μm and stage 2 at 0.95 μm for spherical particles of unit density or their aerodynamic equivalent (Hatch 1955). The physical collection efficiency of the SKC Biosampler has been shown to be about 79% for 0.3 μm particles, 89% for 0.5 μm particles, 96% for 1.0 μm particles, 100% for 2.0 μm particles (Willeke 1998).

While many studies have used the Anderson sampler—in multiple forms—and the SKC Biosampler to evaluate viable microorganism concentrations in agricultural and other environments, only one other study has directly compared the two (Fabian 2005). The SKC Biosampler has been compared and found to have several advantages over the AGI-30 (Ace Glass Inc., Vineland, N.J.) the main advantage being that it can be used with a longer lasting non-volatile liquid to allow sampling of up to 8 hours (Lin 1997, 1998, 2000). Both samplers are relatively user friendly; however, the SKC Biosampler requires an additional step to dilute and plate collection media. The SKC Biosampler was also more sensitive in extremely hot or cold temperatures to evaporation or freezing than the Anderson sampler.

Conclusions

This study showed that culturable microorganism levels collected at outdoor points away from animal barns were generally lower than, but within some ranges typically reported, those inside livestock barns. Seasonal variations had little practical impact on the measurable concentrations of culturable bacteria and fungi, but seasons were still significantly different. Our data show that the Anderson sampler was able to collect significantly more culturable bioaerosols than the SKC Biosampler for mesophilic and Gram-negative bacteria and fungi in all four seasons except fungi in winter. Respirable microorganism concentrations were consistently higher than non-respirable concentrations in total and over all four seasons except for Gram-negative bacteria in the fall.

Chapter 3

Comparison of Environmental Emissions from Colorado Dairies

Abstract

There is a limited amount of information available on dairy farmers' exposure to hydrogen sulfide, ammonia, odor, particulate and endotoxin. There is even less information on exposures to these CAFO emissions at areas other than inside barns. Communities surrounding CAFOs are continually exposed to odor emissions reducing quality of life and causing symptoms including headaches, runny nose, sore throat, excessive coughing, diarrhea, and burning eyes. Often times CAFOs are built in areas of lower socioeconomic status. The objective of this study was to evaluate the effectiveness of an innovative lagoon treatment to determine if that treatment can reduce the emissions of gases and odors from a dairy lagoon. We investigated the lagoon emissions from two Colorado dairies over four seasons. Peak ammonia levels ranged from 2.0 to 142 ppm at the study dairy and 2.0 to 23 ppm at the control dairy. In total, the study dairy (GM 10.0 ppm) had significantly ($p<0.05$) higher peak ammonia values than the control dairy (GM 6.4 ppm). Mean hydrogen sulfide values ranged from 4.0 to 394 ppb at the study dairy and 4.0 to 890 ppb at the control dairy. Maximum values ranged from 37 to 17,000 ppb at the study dairy and from 210 to 5,200 ppb at the control dairy. In total, there was no difference between the two dairies for average H_2S. However, the control dairy had

significantly ($p<0.05$) higher peak (GM 1067 ppb) hydrogen sulfide than the study dairy (GM 351 ppb). Odor values ranged from 0 to 15 dilutions to threshold (D/T) at both dairies, with 2 D/T occurring most often at both dairies. Inhalable particulate ranged from below the limit of detection (LOD) to 2.3 mg/m^3 at the study dairy and from below the LOD to 1.5 mg/m^3 at the control dairy. Inhalable endotoxin ranged from 2.1 to 270.7 EU/m^3 at the study dairy and from 2.3 to 487.2 EU/m^3 at the control dairy. Inhalable endotoxin per mg of dust ranged from 6.7 to 1237 EU/mg at the study dairy and from 6.6 to 2270 EU/mg at the control dairy. Total particulate ranged from below the LOD to 2.4 mg/m^3 at the study dairy and from below the LOD to 0.21 mg/m^3 at the control dairy. Total endotoxin ranged from 2.5 to 6587 EU/m^3 at the study dairy and from 2.0 to 2986 EU/m^3 at the control dairy. Total endotoxin per mg of dust ranged from 17.9 to 100,413 EU/mg at the study dairy and from 12.2 to 39817 EU/mg at the control dairy. In general, these concentrations are lower than suggested guidelines. However, they may contribute to long term health concerns for workers and surrounding communities. Overall, our data do not produce a definitive answer regarding the effectiveness of the algae lagoon treatment.

Introduction

The industrialization of livestock production has led to concern over public health impacts from air emissions. Hazardous emissions from CAFOs include gases, vapors, particulates, bioaerosols, odors, and other semivolatile and volatile organic compounds. Research has established that these air emissions contribute negatively to the health and quality of life of CAFO workers, animals, and surrounding communities.

The state of Colorado defines a dairy CAFO as one which contains 700 head or 800,000 pounds live weight (Kress 2007). In 2006, the state of Colorado ranked 16th in the United States in total milk production with 2,547,050,000 pounds of milk for an 8.5% increase over 2005. Colorado had 170 licensed dairy herds each with an average of 647 cows for a total of 110,000 cows. In total, the United States' 9,112,000 cows produced 181,798,000,000 pounds of milk in 2006. Increase in milk production and larger herd sizes are trends that U.S. dairy producers can expect to see for years to come (Cooley 2007).

Air emissions from CAFOs contain numerous toxic and odoriferous compounds. These include gases and vapors such as ammonia (NH_3), hydrogen sulfide (H_2S), and dimethyl sulfide (Merkel 1969, Donham 1985a); particulates (Donham 1986a,b); bioaerosols including bacteria, fungi, and endotoxin (Heederik 2002, Douwes 2002); and volatile organic compounds including acetaldehyde, acetone, benzene, chloroform, hexane, methanol, phenol, toluene, and xylene (Merkel 1969, Cheremisinoff 1975, Schiffman 2001).

The limited amount of environmental data and the heterogeneous characteristics of the emissions from CAFOs complicate the heavily debated topic over regulation of those emissions. The most often regulated emissions include H_2S, NH_3, and odor. H_2S is one of the most significant gases emitted from CAFOs. Arising from the storage, handling, and anaerobic digestion and decomposition of animal wastes, H_2S is a pulmonary irritant and an asphyxiant (Donham 1985b, Partti-Pellinen 1996). NH_3 is a major constituent of animal waste and is released from CAFOs, manure storage vessels,

and land application of manure. NH_3 is highly water-soluble and is rapidly absorbed in the upper airways, resulting in damage to the airway epithelia (Donham 1985b, Close 1980, Leduc 1992).

Endotoxin is a lipopolysaccharide protein complex component of the outer wall of Gram-negative bacteria. Endotoxin is a potent inflammatory agent that produces systemic effects and lung obstruction, even at low levels of exposure (Thorne 2000). Animal feces and plant materials contaminated with bacteria are major contributors of endotoxin to organic dust. Exposure to such dust is prevalent in livestock farming (Thorne 1997).

Among the emissions from CAFOs, odors are the most commonly recognized by the communities surrounding these sites. In addition to being an extreme nuisance, sufficient odor exposure can cause adverse health effects (Miner 1980, Overcash 1983, Schiffman 1995). The biological breakdown of feed while in the animal gut and of the manure after excretion produces odoriferous organic compounds.

Confinement dust is primarily composed of organic compounds. The majority of the dust arises from feed and fecal particles. NH_3, molds, bacteria, and endotoxin can attach to dust particles and become deposited in the lung (Donham 1986a, Tripp 1999). Dust particles can settle at different levels of the respiratory system depending on size. Particles greater than 10 microns are generally deposited in the upper respiratory tract. Particles from 3 to 10 microns are most often deposited in the major airways of the lower respiratory tract and particles smaller than 3 microns are respirable and can reach deep into the lung parenchyma (Tripp 1999). Adverse health effects associated with confinement workers include cough, wheeze, shortness of breath, chronic bronchitis, decrease in lung function, asthma-like syndrome, and organic dust toxic syndrome (Clark

1983, Crook 1991, Donham 1989, Thorne 1999). Donham et al. have found that high proportion of disease occurs in workers at dust levels above 2.5 and 0.23-mg/m^3 for total and respirable, respectively, and have recommended that these levels serve as occupational limits for CAFO workers (Donham 1988, 1995, 2000a, 2000b). Kullman et al. found personal breathing zone dust levels averaged: 1.78 mg/m^3 for inhalable fractions and 0.07 mg/m^3 for respirable fractions on 85 Wisconsin dairy farms (Kullman 1998). Firth et al. measured personal inhalable dust levels at New Zealand dairy, sheep, arable, and mixed farms. They found median inhalable levels of 0.60, 0.70, 1.71, and 0.54 mg/m^3, respectively. Interquartile ranges were from 0.22 to 2.45 mg/m^3, overall (Firth 2006).

Several types of engineering controls have been designed to reduce the amount of dust, odor, and gas released into the environment but cost-effective interventions are still critically needed. A review by Lorimor et al. (2002) summarizes current literature regarding emission control technology, and a study by Goodrich et al. examined implementation of current technology in a livestock facility. In general, outdoor storage of manure reduces indoor contaminant levels for CAFO workers. The reduction of odor and gases inside buildings can be accomplished through several methods. The use of bedded solid manure systems, similar to what is used in a natural open-air pastures, is thought to reduce odor levels (Lorimor 2002). Several studies have evaluated the effects of sprinkling different types of vegetable oils including soybean, canola, and rapeseed to reduce particulate and gas levels (Goodrich 2001, Jacobson 1998, Kirychuk 1999, Nonnenmann 1999, Zhang 1996).

Ozone air treatment can be useful in the reduction of odor. Ozone is distributed with ventilation air (Bottcher 2000). Chemical addition, especially during manure pump out, can help to reduce gas emissions. Hydrogen peroxide addition to pits during agitation and pumping showed reductions in hydrogen sulfide levels by 67% over control barns (Lorimor 2002). Diet manipulation by use of standardized ideal digestibility (SID) formulation, addition of amino acids, and reduction of nitrogen and sulfur has been used to reduce ammonia and hydrogen sulfide levels (Canh 1998, Goodrich 2001). A relatively simple method is that of a cover for lagoons to trap odor from being emitted. Covers can be made of many different types of materials. Synthetic impermeable covers are manufactured from wood, concrete, fiberglass, or plastic. Covers have been reported to reduce gas and odor up to 90% (Zahn 2001). Composting is a method of aerobic treatment applied to solid or semi-solid manure. During composting, microorganisms act upon and degrade organic material such as manure, leaves, and food wastes. Composting can reduce material bulk by 50% and has been known to reduce odor and hydrogen sulfide levels (NRAES 1992, Zhang 1997). Ammonia levels can be reduced by adding dry bulking agents such as straw (LPES 2002)

Biological treatment of manure has also been useful for odor reduction. Both aerobic and anaerobic treatment can be used to treat manure. Complete aerobic treatment can eliminate odor produced by manure. Generally, aerobic treatment is only suitable for slurry or dilute effluent because solid manure increases the amount of aeration and mixing required (LPES 2002). Aerobic reactors can be added to slurry tanks or to lagoons. Oxygen is added in several methods including bubbling, whipping, or spraying liquid into the air (Lorimor 2002).

Anaerobic treatment occurs in the absence of oxygen. Anaerobic lagoons are the most common type of anaerobic digestion and storage system. When properly maintained with a balance of acid-forming and methane-forming bacteria an anaerobic lagoon can produce minimal amounts of odor (Lorimor 2002, LPES 2002).

This study was designed to test the null hypothesis that there is no difference between the environmental emissions from a dairy with a typical anaerobic lagoon system and a dairy with a typical anaerobic lagoon system with the addition of a novel algae intervention designed to create an aerobic lagoon. We measured total and inhalable particulate and endotoxin, gases and vapors including H_2S, NH_3, carbon dioxide, and odor.

Materials and Methods

Sampling Sites

The dairy characteristics are listed in Table II. The study dairy had total land area of approximately 60 acres with a single lagoon of approximately 8 acres. The study dairy milked approximately 1350 cows, with approximately 125 cows kept dry and not milked. The study dairy raised the female calves on site. The waste treatment system was as follows: The milking parlor was rinsed with fresh water and recycled water was used to rinse the other areas. Wastes flowed by gravity into a leaky dam separation and settling basin and then into a primary lagoon. Dry lots were scraped as were the settling basins and the solids were composted. The lagoon was treated with a novel algae intervention

(Agsmart 2007). Algae was grown in a greenhouse on site next to the lagoon and pumped through micro diffusers into the lagoon. The algae were intended to increase the dissolved oxygen in the lagoon to above 1 mg/ml with the purpose of transforming the once anaerobic environment into an aerobic environment with the goal of reducing lagoon odor emissions and solids (Agsmart 2007).

The control dairy had total land area of approximately 340 acres with two lagoons of approximately 3 acres each. The control dairy milked approximately 3000 cows, with approximately 350 cows kept dry and not milked. The control dairy did not raise the female calves on site. The waste treatment system was as follows: The milking parlor was rinsed with fresh water and recycled water used to rinse the other areas. Wastes flowed by gravity into a leaky dam separation and an earthen basin separation then into a primary and secondary lagoon. Dry lots and settling basins were scraped and the solids were composted.

Sampling Schedule

We measured total and inhalable particulate and endotoxin, gases and vapors, including H_2S, NH_3, carbon dioxide, and odiferous compounds, at each dairy at a predominately downwind location from the lagoons. The samplers were juxtaposed at approximately one meter above ground level except for the Jerome sampler, which was approximately 0.5 meters above the ground. The study and control dairies were each sampled 40 total days over each season as follows: 10 fall, 10 winter, 10 spring, and 10 summer. Some hydrogen sulfide, ammonia, and particulate samples are missing due to equipment malfunction.

Sampling and Analysis

Meteorology

Meteorological data including temperature, relative humidity, and wind speed was measured using a Vantage Pro Weather Station (Davis Instruments, Hayward, CA). The Weather Station was mounted to a pole approximately 2 meters above ground level. The Weather Station consisted of a combination of a wind vane and anemometer and a temperature and relative humidity sensor. All were mounted at the apex of the pole. The temperature sensor was shielded from direct sunlight. The pole was orientated such that the wind vane was directed south and the temperature relative humidity sensor was directed north, as directed by the instruction manual. Data was logged using Davis WeatherLink for Vantage Pro data collection, analysis, and display software for Windows. Data was collected over 1-minute intervals as mandated by the software, and displayed as average values.

Total Particulate and Endotoxin

Total particulate and endotoxin samples were collected according to NIOSH method 0500 (NIOSH 2007a). Particulate was collected on a 37-mm closed-faced cassette on polyvinyl chloride filters with a 5 μm pore diameter (SKC, Inc. Eighty Four, PA). Sampling pumps were calibrated at 2.0 liters per minute (L/min). Filters were pre- and post-weighed using a Mettler MT5 microbalance (Mettler-Toledo, Inc.) and were blank-corrected to account for humidity differences and measurement drift. Samples were collected for 8 hours at a flow rate of 2 L/min. After sample collection the filters were stored at 4°C under desiccation and analyzed for endotoxin using a new Recombinant Factor C Endotoxin Assay (Biowhittaker, Walkersville, MD). Sample

concentrations were reported as mg/m^3 for total dust and EU/m^3 for total endotoxin. The limit of detection (LOD) for this total dust method was 0.03 mg. This value was determined from the mean of blanks plus 3 standard deviations.

Inhalable Particulate and Endotoxin

Inhalable particulate and endotoxin were collected using Institute of Occupational Medicine (IOM) samplers and SKC Button samplers (SKC Inc. Eighty Four, PA.) with 25mm filters with 5 μm pore size (SKC, Inc. Eighty Four, PA). For IOM samples, the entire filter cassettes were pre- and post-weighed on a Mettler MT5 microbalance (Mettler-Toledo, Inc.) and were blank-corrected to account for humidity differences and measurement drift. Samples were collected for 8 hours at a flow rate of 2 L/min. After sample collection the filters were stored at 4°C under desiccation and analyzed for endotoxin using a new Recombinant Factor C Endotoxin Assay (Biowhittaker, Walkersville, MD). Sample concentrations were reported as mg/m^3 for inhalable dust and EU/m^3 for inhalable endotoxin. The limit of detection (LOD) for this inhalable method was 0.05 mg. This value was determined from the mean of blanks plus 3 standard deviations.

Hydrogen Sulfide

H_2S concentrations were measured using a Jerome 631-X Hydrogen Sulfide Analyzer (Arizona Instruments, Phoenix, AZ). A Jerome meter is a real-time instrument with a measuring range of 3 parts per billion (ppb) to 50 parts per million (ppm). The limit of detection is 3 ppb. The instrument is accurate to 3 ppb within a temperature range of 0–40 °C. Briefly, an internal pump draws a known volume sample of the ambient air over a thin gold film sensor for a precise period of time. The gold film

undergoes a change in electrical resistance proportionate to the concentration of hydrogen sulfide in the sample. The Jerome was factory calibrated once per year. Before and after each sampling period the internal sensor was heat-cycle-regenerated to remove saturated hydrogen sulfide. After each regeneration cycle, the display is manually zeroed and confirmed using a zero-air filter. A microprocessor automatically re-zeros the digital meter at the start of each sample cycle and freezes the meter reading until the next sample cycle is activated, thus eliminating drift between samples. Internal filters scrub out possible contaminants to increase specificity for hydrogen sulfide. An external ammonia filter was added inline. The Jerome measured and logged data at one-minute intervals over the sampling period. H_2S concentrations were reported in ppb. Peak concentrations were also recorded.

Ammonia

NH_3 measurements were performed using a Pac III, a direct reading and data logging device from Draeger (Draeger Safety Inc., Pittsburg, PA.). The Pac III has a measurement range from 1 to 300 ppm with a resolution of 1 ppm. The instrument is accurate to 3% of the measured value within a temperature range of -20 to 55 °C. The Pac III was factory calibrated once each year and after sensor replacement. The Pac III measured and logged data at one-minute intervals over the sampling period. Mean peak NH_3 concentrations were reported in ppm.

Carbon Dioxide

Carbon dioxide (CO_2) gas was measured using a Q-Trak (TSI Inc., Shoreview, MN). The Q-Trak is a direct reading instrument with data logging capabilities. The Q-Trak has a measurement range from 0 to 5000 ppm with a resolution of 1 ppm. The

instrument is accurate to 3% of the measured value plus 50 ppm within a temperature range of 0 to 50 °C. CO_2 is measured using a non-dispersive infrared detector. The device was set to log data at one minute intervals. The Q-trak was factory calibrated using standard gases yearly. Mean concentrations were reported as ppm.

Odor

Odor was measured using a model 1959-A/SCC scentometer (Barnebey & Sutcliffe Corporation, Columbus, OH). Although not as precise as olfactometry, the scentometer provides a basic gauge of odor level in the field and is inexpensive. Also, this is one device currently used in enforcement of Colorado air quality standards for swine confinements. Briefly, the scentometer is a rectangular clear plastic box containing two chambers filled with activated charcoal, two nasal sampling ports, two 1/2" diameter ports (one for each charcoal chamber), and six ambient air inlets (1/32", 1/16", 1/8", 3/16", 1/4", and 1/2" in diameter). Air is drawn through the two charcoal beds to remove any odor and then is mixed with contaminated air. The size of the contaminated air inlet hole is used to indicate the field odor concentration. The concentration is expressed as the number of times the odor is as strong as the threshold and is written as dilution to threshold (D/T) (Barnebey 1962). Scentometer readings proceed stepwise in terms of odor strength from 0 (no noticeable odor), 2 (a noticeable odor), 7 (an odor most people would find objectionable), 15 (most would declare it a nuisance), 31 (extremely nauseating), 170, and 350. Calibration is neither required nor needed. Barnebey and Sutcliffe recommend that the charcoal be changed every 6 months with moderate use. Measurements were taken 3-4 times over the 8-hour sampling period and were reported as maximum and modes.

Endotoxin Measurement - Recombinant Factor C Endotoxin Assay

The concentration of endotoxin was determined using a novel Recombinant Factor C Endotoxin (rFC) Assay (Biowhittaker, Walkersville, MD). The activation of rFC is determined by the fluorescence generated by the enzymatic cleavage of a peptide-coumarin substrate. Fluorescence is measured after one-hour incubation with endotoxin standards at 37°C. The log fluorescence is proportional to the log endotoxin concentration and is linear in the 0.01-10EU/ml range. The minimum detection of endotoxin is ~0.01EU/ml. The rFC assay has been found to detect no (1,3)-glucan activity; an improvement in specificity compared to the most commonly used assays.

Samples were extracted in sterile, pyrogen-free (pf) water containing 0.05% Tween-20 for 1 hr at 22°C with continuous shaking. Extracts were centrifuged and supernatants were transferred into pf cryotubes. They were then analyzed using the Recombinant Factor C Endotoxin Assay. Two-fold serial dilutions of endotoxin standards and sample extracts were prepared using sterile, pf water with Tween-20 in borosilicate glass tubes that had been heated for 4 hr at 200°C to remove endotoxin activity. The samples were added to a 96-well plate followed by 100 microliters of a mixture of enzyme, buffer and fluorogenic substrate. The plates were incubated at 37°C for one hour and read in a fluorescence microtiter plate reader (Biotek Instruments FLX800TBIE) at Excitation/Emission 380/440 nm. Background (1 EU/ml) fluorescence was subtracted and log delta fluorescence plotted against log endotoxin concentration. Endotoxin concentrations of samples were calculated according to the standard curve. Four assay reagent blank wells served as reference and control for the pf status of the reagent water, centrifuge tubes, pipette tips and microplates.

Quality assurance spiking assays were performed to assess matrix interference or enhancement.

Statistical Analysis

Excel databases were combined and analyzed using SAS Version 9.1 (SAS Institute, Cary, NC). Descriptive statistics were used to characterize environmental measurements. The normality of the data was tested using the Shapiro-Wilks test. All variables, except meteorological conditions, were log transformed before completing statistical analysis. Geometric means and geometric standard deviations were calculated for environmental data that could be described as lognormal. Comparisons between dairies and seasons were made using linear analysis of variance with two-way interactions. Included variables were as follows: dependent (mean CO_2, mean H_2S, mean peak H_2S, mean peak NH_3, odor mode, peak odor, total and inhalable particulate (mg/m^3) and endotoxin (EU/m^3)); class (dairy and season); quantitative (temperature, relative humidity, wind speed, wind direction). Means were compared using Tukey's test procedure. Pearson correlation coefficients were calculated to evaluate associations among environmental parameters and gas, odor, particulate, and endotoxin concentrations. Values that fell below the LOD, occurred for particulate sampling methods only, were replaced with the LOD divided by the square root of 2 for statistical analysis.

Results

Meteorology

Seasons were defined as follows: December, January, and February were considered winter. March, April and May were considered spring. June, July, August were considered summer. September, October, and November were considered fall. Meteorological data is summarized by season in Table III. For the study dairy atmospheric air temperatures ranged from -9.9°C to +40.1°C with a mean of +15.3°C. Relative humidity ranged from 0.6% to 90.3% with a mean of 36.8%. Wind speeds ranged from 0.5 m/s to 8.7 m/s with a mean of 2.0 m/s. For the control dairy atmospheric air temperatures ranged from -12.5°C to +41.1°C with a mean of +11.2°C. Relative humidity ranged from 7.9% to 92.3% with a mean of 44.7%. Wind speeds ranged from 0.8 m/s to 4.7 m/s with a mean of 2.5 m/s.

Ammonia

Table VII lists the geometric means (GM) and geometric standard deviations (GSD) for the peak values of NH_3 in total and for each season for the study and control dairies. Peak values are displayed because the daily averages of 480 or more samples were all below the 1.0 ppm LOD of the Draeger Pac III (Draeger Safety Inc., Pittsburg, PA.). Peak values ranged from 2.0 to 142 ppm at the study dairy and 2.0 to 23 ppm at the control dairy. In total, the study dairy (GM 10.0 ppm) had significantly ($p<0.05$) higher peak ammonia values than the control dairy (GM 6.4 ppm). In summer, the study dairy (GM 26.0 ppm) had significantly ($p<0.0001$) higher peak ammonia than the control dairy (GM 7.0 ppm). All other seasons were not significantly different. Peak NH_3 levels are summarized by dairy in a box-whisker plot in Figure II. The whiskers delineate the 10^{th}

and 90th percentiles and the box line represent median, lower, and upper quartiles of the data.

Hydrogen Sulfide

Two values were determined for H_2S. The mean concentration in ppb was determined for the approximately 8-hour sampling period, and the maximum concentration in ppb was also determined by sampling period. Table VII lists values for the study and control dairies. Average values ranged from 4.0 to 394 ppb at the study dairy and 4.0 to 890 ppb at the control dairy. Maximum values ranged from 37 to 17,000 ppb at the study dairy and from 210 to 5,200 ppb at the control dairy. In total, there was no difference between the two dairies for average H_2S; however, the control dairy had significantly ($p<0.05$) higher maximum (GM 1067 ppb) hydrogen sulfide than the study dairy (GM 351 ppb). In summer, the study dairy had a significantly ($p<0.01$) higher average H_2S value (GM 83 ppb) than the control dairy (GM 32 ppb). In winter, spring, and fall the control dairy had significantly ($p<0.001$, $p<0.01$, and $p<0.001$, respectively) higher maximum H_2S values (GM 853, 973, and 1111 ppb, respectively) than the study dairy (GM 100, 355, 300 ppb, respectively). Mean and mean peak H_2S levels are summarized by dairy in a box-whisker plot in Figure III. The whiskers delineate the 10th and 90th percentiles and the box line represent median, lower, and upper quartiles of the data.

Table VII. Comparison of Gases and Odor Concentrations by Season and Dairy

Season, Toxicant	Location					
	Study Dairy			Control Dairy		
	GM	(GSD)	n	GM	(GSD)	n
Total						
Carbon Dioxide (ppm)	443	(1.3)	40	460	(1.2)	40
Hydrogen Sulfide (ppb)	28	(2.9)	36	30	(3.3)	34
Hydrogen Sulfide Maximum (ppb) [a] *	351	(4.3)	36	1067	(2.4)	34
Ammonia Maximum (ppm) [b] *	10.0	(3.4)	24	6.4	(1.9)	31
Odor (D/T) [c]	2		40	2		40
Odor Maximum (D/T) [d]	15		40	15		40
Winter						
Carbon Dioxide (ppm)	508	(1.1)	10	491	(1.1)	10
Hydrogen Sulfide (ppb)	13	(2.2)	8	24	(2.1)	10
Hydrogen Sulfide Maximum (ppb) [a] ***	100	(1.6)	8	853	(2.6)	8
Ammonia Maximum (ppm) [b]	3.0	(1.4)	3	5.0	(2.2)	8
Odor (D/T) [c]	2		10	2		10
Odor Maximum (D/T) [d]	15		10	7		10
Spring						
Carbon Dioxide (ppm)	473	(1.1)	10	504	(1.1)	10
Hydrogen Sulfide (ppb)	26	(1.8)	9	33	(5.2)	7
Hydrogen Sulfide Maximum (ppb) [a] **	355	(3.0)	9	973	(2.5)	7
Ammonia Maximum (ppm) [b]	5.3	(2.3)	7	8.5	(1.8)	9
Odor (D/T) [c]	2		10	2		10
Odor Maximum (D/T) [d]	7		10	15		10
Summer						
Carbon Dioxide (ppm)	321	(1.1)	10	371	(1.1)	10
Hydrogen Sulfide (ppb)**	83	(3.1)	10	32	(2.9)	9
Hydrogen Sulfide Maximum (ppb) [a]	1091	(5.8)	10	1340	(2.1)	9
Ammonia Maximum (ppm) [b] ***	26.0	(3.2)	10	7.0	(2.2)	6
Odor (D/T) [c]	2		10	2		10
Odor Maximum (D/T) [d]	7		10	7		10
Fall						
Carbon Dioxide (ppm)	498	(1.4)	10	488	(1.1)	10
Hydrogen Sulfide (ppb)	19	(2.0)	9	31	(4.0)	10
Hydrogen Sulfide Maximum (ppb) [a] ***	300	(3.0)	9	1111	(2.7)	10
Ammonia Maximum (ppm) [b]	7.7	(1.7)	4	5.9	(1.7)	8
Odor (D/T) [c]	2		10	2		10
Odor Maximum (D/T) [d]	7		10	7		10

Note: D/T = dilution to threshold a = GM of maximum H_2S values; b = GM of maximum NH_3 values; c = mode of odor values; d = maximum occurring odor value; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ that mean concentrations in that row not equal by ANOVA

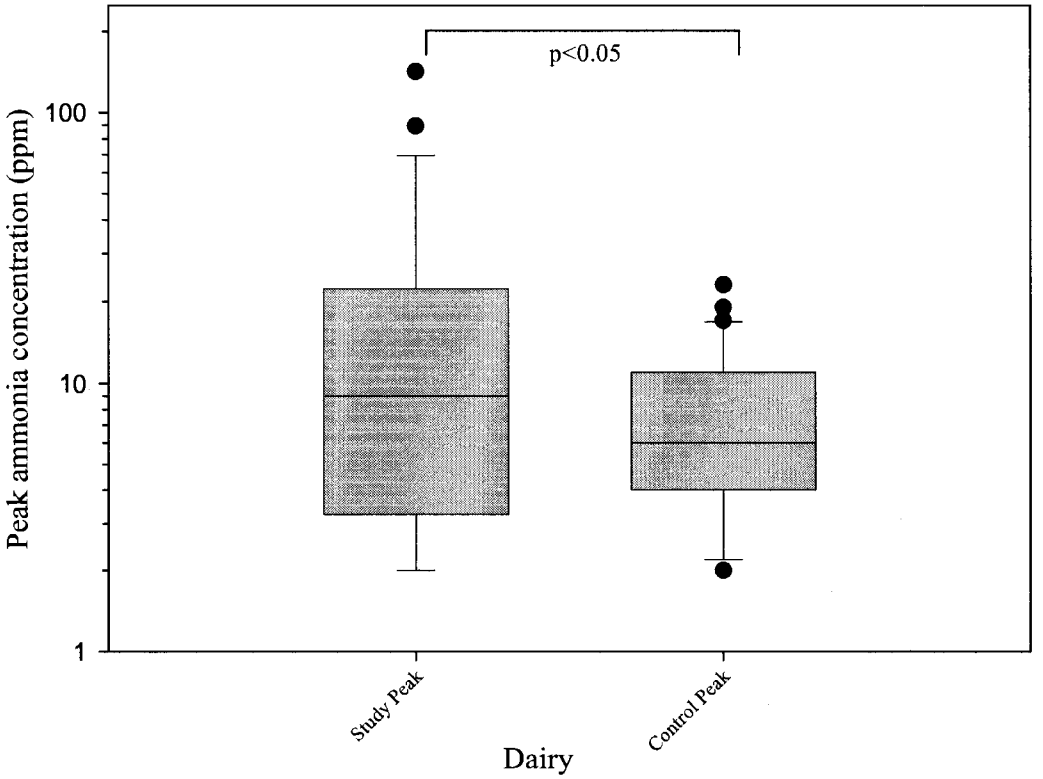

Figure II. Box-Whisker Plot of Peak Ammonia Concentrations at Study and Control Dairy Lagoons

(Whiskers delineate 10th, 90th percentile and box lines represent median, lower, and upper quartiles of the data)

Odor

Odor measures ranged from 0 (no noticeable odor) to 15 D/T (most would declare a nuisance) at both dairies. A value of 0 corresponded to no odor, 1 corresponded to noticeable without dilution, while 2 was the lowest value detectable using the scentometer. Odor values are listed in Table VII. Values for odor are modes while the maximum odor value is the highest occurring value at any time during the sampling period. In total, the mode value and the maximum value were equal for the study and control dairies.

Though not significantly different, the study dairy had higher odor than the control dairy while in spring the control dairy had higher odor. Summer and fall were equal between the study and control dairies.

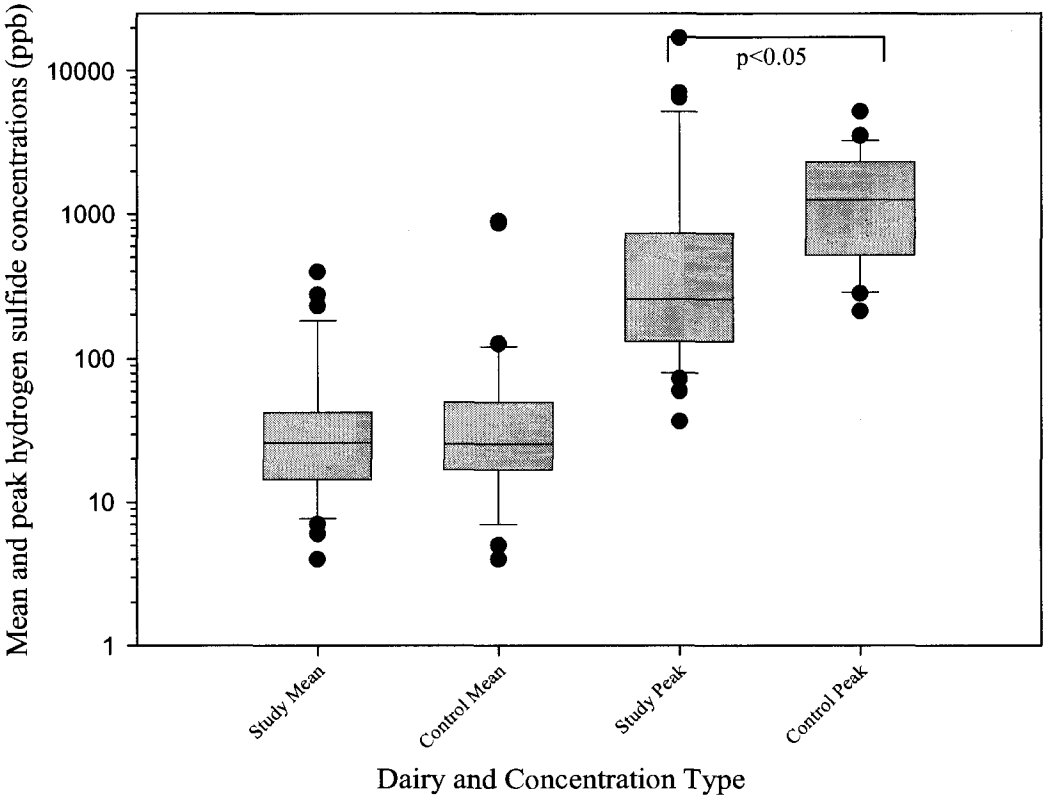

Figure III. Box-Whisker Plot of Mean and Peak Hydrogen Sulfide Concentrations at Study and Control Dairy Lagoons
(Whiskers delineate 10th, 90th percentile and box lines represent median, lower, and upper quartiles of the data)

Endotoxin and Particulate

Inhalable particulate and endotoxin concentrations are listed in Table VIII. Inhalable particulate ranged from below the LOD to 2.3 mg/m^3 at the study dairy and from below the LOD to 1.5 mg/m^3 at the control dairy. Inhalable endotoxin ranged from 2.1 to 270.7 EU/m^3 at the study dairy and from 2.3 to 487.2 EU/m^3 at the control dairy.

Inhalable endotoxin per mg of particulate ranged from 6.7 to 1237 EU/mg at the study dairy and from 6.6 to 2270 EU/mg at the control dairy. In fall, the study dairy had significantly higher ($p<0.05$) inhalable endotoxin (GM 10.1 EU/m^3) than the control dairy (GM 9.7 EU/m^3). Total and inhalable particulate levels are summarized by size fraction and dairy in a box-whisker plot in Figure IV.

Table VIII. Comparison of Inhalable Particulate and Endotoxin Concentrations by Season and Dairy

Season, Toxicant	Location					
	Study Dairy			Control Dairy		
	GM	(GSD)	n	GM	(GSD)	n
Total						
Inhalable Particulate (mg/m^3)	0.096	(3.0)	41	0.104	(3.5)	61
Inhalable Endotoxin (EU/m^3)	17.3	(3.2)	31	20.1	(3.4)	54
Inhalable Endotoxin/Particulate (EU/mg)	192	(5.2)	31	186.5	(4.2)	54
Winter						
Inhalable Particulate (mg/m^3)	0.105	(4.1)	10	0.106	(2.7)	12
Inhalable Endotoxin (EU/m^3)	48.5	(6.7)	4	22.7	(3.7)	11
Inhalable Endotoxin/Particulate (EU/mg)	817.4	(5.2)	4	201.7	(3.6)	11
Spring						
Inhalable Particulate (mg/m^3)	0.091	(2.5)	10	0.09	(3.1)	12
Inhalable Endotoxin (EU/m^3)	18.1	(2.4)	9	45.8	(2.0)	12
Inhalable Endotoxin/Particulate (EU/mg)	179.5	(3.9)	9	509.5	(2.7)	12
Summer						
Inhalable Particulate (mg/m^3)	0.071	(3.5)	10	0.234	(4.2)	20
Inhalable Endotoxin (EU/m^3)	17.7	(2.4)	9	18.9	(3.4)	16
Inhalable Endotoxin/Particulate (EU/mg)	264.3	(6.3)	9	68.7	(5.2)	16
Fall						
Inhalable Particulate (mg/m^3)	0.135	(2.6)	13	0.044	(1.7)	18
Inhalable Endotoxin (EU/m^3) *	10.1	(3.3)	9	9.7	(3.3)	16
Inhalable Endotoxin/Particulate (EU/mg)	78.4	(3.8)	9	219.7	(2.4)	16

*Note: * = $p < 0.05$ that means in that row are not equal by ANOVA*

Total and inhalable endotoxin concentrations are summarized by size fraction and dairy in a box-whisker plot in Figure V. The whiskers delineate the 10^{th} and 90^{th} percentiles

and the box lines represent the median, lower, and upper quartiles of the data.

Total particulate and endotoxin concentrations are listed in Table IX. Total particulate ranged from below the LOD to 2.4 mg/m^3 at the study dairy and from below the LOD to 0.21 mg/m^3 at the control dairy. Total endotoxin ranged from 2.5 to 6587 EU/m^3 at the study dairy and from 2.0 to 2986 EU/m^3 at the control dairy. Total endotoxin per mg of particulate ranged from 17.9 to 100,413 EU/mg at the study dairy and from 12.2 to 39817 EU/mg at the control dairy. In fall, the study dairy had significantly higher ($p<0.05$) total particulate (GM 0.037 mg/m^3) than the control dairy (GM 0.028 mg/m^3).

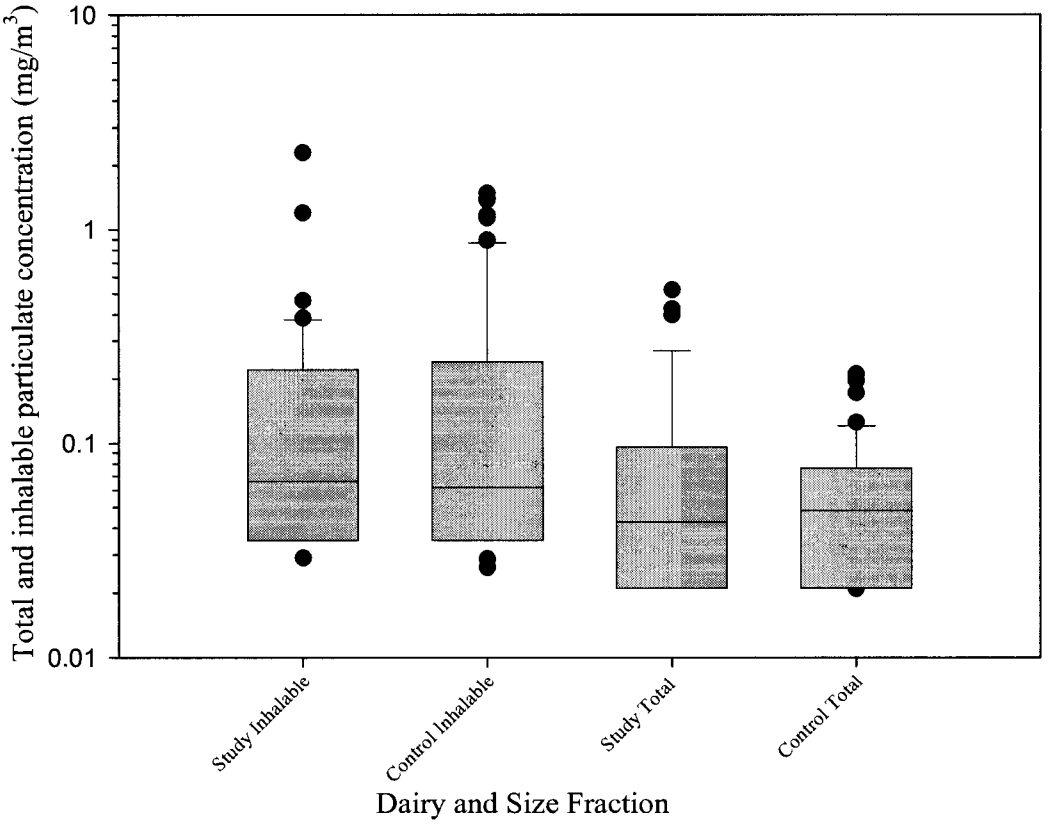

Figure IV. Box-Whisker Plot of Total and Inhalable Particulate Concentrations at Study and Control Dairy Lagoons
(Whiskers delineate 10th, 90th percentile and box lines represent median, lower, and upper quartiles of the data)

In winter , the study dairy had significantly higher ($p<0.05$) total endotoxin (GM 252.7 EU/m^3) than the control dairy (GM 26.3 EU/m^3). In summer, the control dairy had significantly higher ($p<0.05$) total endotoxin per mg particulate (GM 322.4 EU/mg) than the study dairy (GM 131.3 EU/mg).

Correlations

Table X shows the significant Pearson correlation coefficients of interest for environmental parameters.

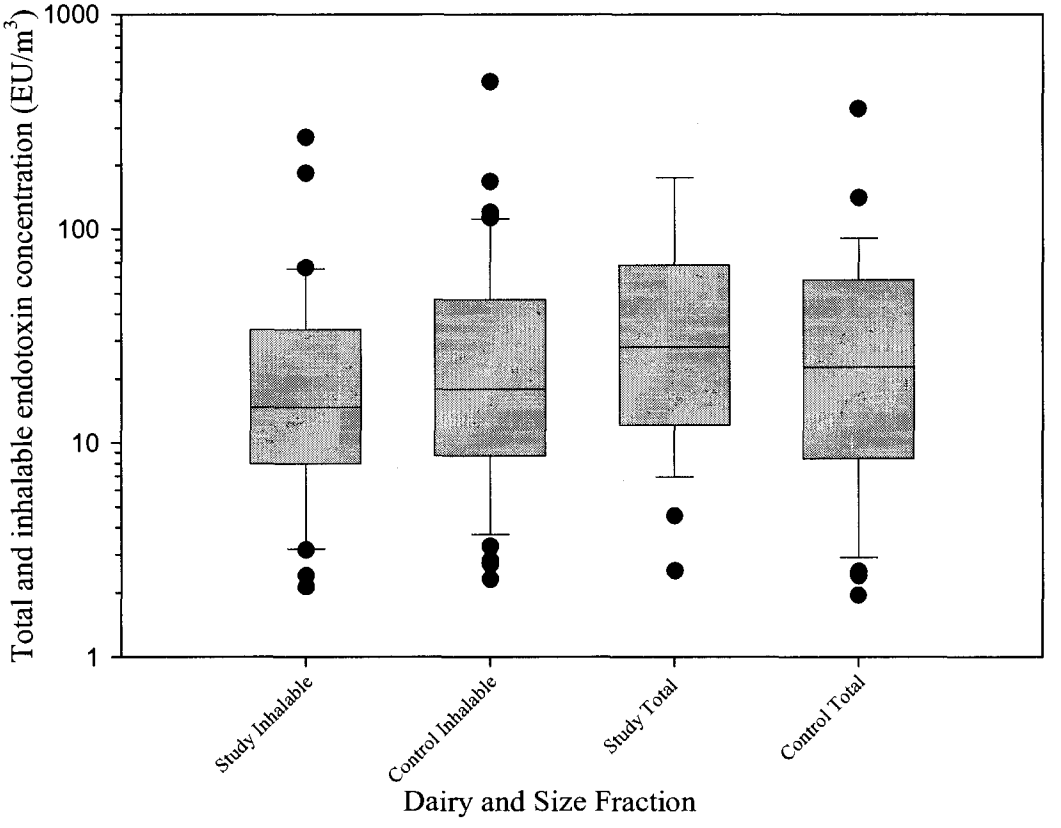

Figure V. Box-Whisker Plot of Total and Inhalable Endotoxin Concentrations at Study and Control Dairy Lagoons
(Whiskers delineate 10th, 90th percentile and box lines represent median, lower, and upper quartiles of the data)

Carbon dioxide was negatively correlated with H_2S, H_2S maximum, NH_3 maximum, and temperature. H_2S was positively correlated with H_2S maximum, NH_3 maximum, and temperature. NH_3 maximum was positively correlated with H_2S maximum and temperature. Odor mode and maximum, particulate, and endotoxin did not significantly correlate with H_2S or NH_3.

Table IX. Comparison of Total Particulate and Endotoxin Concentrations by Season and Dairy

Season, Toxicant	Location					
	Study Dairy			Control Dairy		
	GM	(GSD)	n	GM	(GSD)	n
Total						
Total Particulate (mg/m^3)	0.053	(2.6)	34	0.047	(2.0)	41
Total Endotoxin (EU/m^3)	35.6	(5.2)	29	21.5	(4.4)	37
Total Endotoxin/Particulate (EU/mg)	605.1	(6.9)	29	451.1	(5.1)	37
Winter						
Total Particulate (mg/m^3)	0.043	(4.7)	5	0.051	(2.0)	10
Total Endotoxin (EU/m^3) *	252.7	(12.1)	2	26.3	(11.5)	8
Total Endotoxin/Particulate (EU/mg)	2415.8	(1.2)	2	462.8	(9.5)	8
Spring						
Total Particulate (mg/m^3)	0.05	(1.9)	7	0.057	(2.2)	12
Total Endotoxin (EU/m^3)	25.9	(2.1)	6	20.7	(2.9)	11
Total Endotoxin/Particulate (EU/mg)	499	(2.7)	6	364.1	(5.0)	11
Summer						
Total Particulate (mg/m^3)	0.083	(2.4)	11	0.06	(1.5)	9
Total Endotoxin (EU/m^3)	11.9	(2.5)	10	20.1	(3.7)	8
Total Endotoxin/Particulate (EU/mg) *	131.3	(5.1)	10	322.4	(4.1)	8
Fall						
Total Particulate (mg/m^3) *	0.037	(1.9)	11	0.028	(1.5)	10
Total Endotoxin (EU/m^3)	80.1	(5.9)	11	20.2	(3.5)	10
Total Endotoxin/Particulate (EU/mg)	2176.3	(5.7)	11	731.5	(4.0)	10

Note: * = $p < 0.05$ that means in that row are not equal by ANOVA

Table X. Significant Pearson Correlation Coefficients for Environmental Parameters

	H_2S	H_2S Maximum	NH_3 Maximum	Temperature
CO_2	-0.41 ***	-0.27 *	-0.49 ***	-0.81 ***
H_2S		0.56 ***	0.57 ***	0.36 **
NH_3 Maximum	0.57 ***	0.51 ***		0.49 ***

Note: * = $p < 0.05$ ** = $p < 0.01$ *** = $p < 0.001$

Discussion

This study was designed to test the null hypothesis that there is no difference between the environmental emissions from a dairy with a typical anaerobic lagoon system and a dairy with a typical anaerobic lagoon system plus the addition of a novel algae intervention designed to create an aerobic lagoon. The control dairy had significantly ($p<0.05$) higher total maximum H_2S, but significantly ($p<0.05$) lower total maximum NH_3. Overall, total and inhalable particulate and endotoxin were not significantly different between the study and control dairies. Odor values were also similar between the two dairies.

Carbon dioxide was used as an indicator of air movement and mixing around the dairy lagoons (Table VII). The largest difference between the dairies (13%) was in summer. However, overall and for the three other seasons the difference in CO_2 values ranged between 3-6%. This indicates that while the control dairy had consistently higher wind speeds (Table III), both dairies had similar air movement and mixing around the lagoons.

The negative correlations (Table X) between CO_2 and maximum H_2S and NH_3 indicated that, as the air movement and mixing around the lagoons increased, H_2S and NH_3 decreased. Higher levels of H_2S and NH_3 were most likely dispersed by wind gusts.

Mean H_2S values (Table VII) were consistently higher, though not significantly, at the control dairy in total, winter, spring, and fall. In summer the study had significantly higher ($p < 0.05$) H_2S. Maximum H_2S values were significantly ($p < 0.001$ to 0.05) higher at the control dairy in total and for all seasons except summer. The control dairy had a higher summer maximum H_2S mean value but it was not significantly different from the study dairy. H_2S is an easily recognized component of odor and reductions in H_2S can greatly increase the publics' acceptance of agricultural facilities. The highest recorded value for the study dairy (17,000 ppb or 17 ppm) was higher than the current recommended short-term standards of 10 and 15 ppm for occupational exposures (ACGIH 2007, NIOSH 2007b). This high value was recorded as a one minute average not over 10 minutes as recommended by the standards. NIOSH recommends an immediate danger to life and health (IDLH) limit of 100 ppm (NIOSH 2007b). This value should never be exceeded. The highest recorded value at the control dairy was 5200 ppb or 5.2 ppm. Our average H_2S values at both the study and control dairies were similar to those measured at swine facilities in exhaust air and before, during, and after manure slurry removal. Swine confinement values have been reported to range from 1 to 35,825 ppb (Donham 2006, Heber 2006, Hoff 2006, Ni 2000, Zhu 2000). Values reported inside dairy barns were all below quantifiable levels of 1 ppm (Kullman 1998).

Total maximum NH_3 values (Table VII) were significantly higher ($p<0.05$) at the study dairy. Maximum NH_3 values varied over all four seasons at both dairies. Values

were higher at the control dairy in winter and spring, but lower in summer and fall. The study dairy had a significantly ($p<0.0001$) higher peak ammonia value during summer. The other seasons were not significantly different. The highest recorded value for the study dairy (142 ppm) was higher than the current recommended short term standards of 35 ppm for occupational exposures (ACGIH 2007, NIOSH 2007b). This high value was recorded as a one minute average not over 10 minutes as recommended by the standards. NIOSH recommends an IDLH of 300 ppm (NIOSH 2007b). This value should never be exceeded. Our peak value from the study dairy was approximately 50% of the IDLH. The highest recorded value at the control dairy was 23 ppm. Our maximum NH_3 values at both the study and control dairies were similar to those measured at swine facilities in exhaust air, inside poultry houses, and inside dairy barns. Swine and poultry values ranged from 1 ppb to 73 ppm (Donham 2006, Groot Koerkamp 1998, Heber 2006, Omland 2002, Zhu 2000). Values measured inside dairy barns ranged from 0.1 to 26.1 ppm with a geometric mean of 6.4 ppm (Kullman 1998). Different lagoon practices may be responsible for the higher ammonia levels measured at the study dairy. The control dairy was able to remove liquids from their lagoon. These liquids were applied to nearby fields. This practice reduces the nitrogen levels in the lagoon and can reduce ammonia emissions.

Interestingly, the peak H_2S (17,000 ppb for study dairy and 5200 ppb for control dairy) and NH_3 (142 ppm for study dairy and 23 ppm for the control dairy) values occurred on the same days at the respective dairies. The peak values occurred in summer at the study dairy and in spring at the control dairy.

This trend was fairly consistent for other high values of H_2S and NH_3 at both dairies, as indicated by the correlation between the values (Table X).

Odor values were similar between the dairies (Table VII). Dilution to threshold (D/T) values ranged from 0 to 15. However, 2 was the most commonly observed number over all seasons at both dairies. The study dairy had a higher maximum value of 15 in the winter, while the control dairy had a higher maximum value of 15 in the spring. To reduce operator bias, odor measurements were recorded every two hours at specific time points at each dairy for a total of 4-5 recorded samples per day. Therefore, it is possible that odor measurements may have been higher or lower overall depending on the time of the individual samples. Most odor regulations, including Colorado's odor regulations, require multiple (two or more) samples made within a one hour period but separated by at least 15 minutes in order to provide proof of violation (Colorado 2001). Colorado's regulation is somewhat confusing in that it states that it is a violation for a swine facility to produce odor of 7 D/T at the property boundary and 2 D/T at any receptor, but any other odor producer must remain below 15 D/T if it is used primarily for residential or commercial and below 7 D/T for all other land use areas (Colorado 2001). However, when the source is a manufacturing process or agricultural operation, no violation shall be cited if that operation is employing the best practical treatment, maintenance, and control currently available to maintain the lowest possible emission of odorous gases. Furthermore, it is a violation for all areas when odors are detected at 127 D/T (Colorado 2001). A value of 127 D/T is about 4 times more odor than a value of 31 which most people find to be extremely nauseating. Our H_2S, NH_3, and odor values measured near the lagoons at two dairies are high enough to create a nuisance to anyone living, working,

or playing near these two dairies. Wing et al. have reported that residents living near hog confinements in North Carolina suffer from decreased health and quality of life. Residents have reported increased occurrence of symptoms including headache, runny nose, sore throat, excessive coughing, diarrhea, and burning eyes (Wing 2000). Schiffman et al. (2005) exposed healthy volunteers to diluted swine confinement air containing 24 ppb H_2S, 817 ppb, NH_3, 0.0241 mg/m3 total particulate, 7.4 EU/m^3 endotoxin, and 57 D/T odor. Subjects were 4.1 times more likely to report headaches, 6.1 times more likely to report eye irritation, and 7.8 times more likely to report eye irritation (Schiffman 2005).

Total and inhalable particulate and endotoxin varied little between the dairies (Table VIII and Table IX). In fall, the study dairy had significantly higher ($p<0.05$) inhalable endotoxin (GM 10.1 EU/m^3) than the control dairy (GM 9.7 EU/m^3). In fall, the study dairy had significantly higher ($p<0.05$) total particulate (GM 0.037 mg/m^3) than the control dairy (GM 0.028 mg/m^3). In winter, the study dairy had significantly higher ($p<0.05$) total endotoxin (GM 252.7 EU/m^3) than the control dairy (GM 26.3 EU/m^3). In summer, the control dairy had significantly higher ($p<0.05$) total endotoxin per mg particulate (GM 322.4 EU/mg) than the study dairy (GM 131.3 EU/mg).

Inhalable particulate ranged from below the LOD to 2.3 mg/m^3 at the study dairy and from below the LOD to 1.5 mg/m^3 at the control dairy. Inhalable endotoxin ranged from 2.1 to 270.7 EU/m^3 at the study dairy and from 2.3 to 487.2 EU/m^3 at the control dairy. Our data were similar to those collected by others in dairy and cattle environments both inside and outside barns. Geometric mean inhalable particulate values ranged from 0.22 to 2.67 mg/m^3 for various dairy and cattle tasks and locations (Firth 2006, Kullman

1998, Nieuwenhuijsen 1999, Takai 1998). Inhalable endotoxin levels were similar to those found inside dairy barns and for other dairy tasks. Geometric mean values ranged from 23 to 647 EU/m3 (Kullman 1998, Nieuwenhuijsen 1999, Seedorf 1998). In comparison, inhalable dust and endotoxin values are much higher in swine and poultry facilities, with poultry producing the highest levels. Geometric mean inhalable particulate values ranged from 1.8 to 6.7 mg/m^3 and geometric mean inhalable endotoxin values ranged from 40 to 55,660 EU/m^3 (Nieuwenhuijsen 1999, Seedorf 1998, Takai 1998).

Total particulate ranged from below the LOD to 2.4 mg/m^3 at the study dairy and from below the LOD to 0.21 mg/m^3 at the control dairy. Total endotoxin ranged from 2.5 to 6587 EU/m^3 at the study dairy and from 2.0 to 2986 EU/m^3 at the control dairy. Our total particulate data were similar to other dairy studies ranging from 0.007 to 7.3 mg/m^3 with geometric means ranging from 0.22 to 3.87 mg/m^3 for ambient samples (Cathomsa 2002, Kullman 1998, Omland 2002). Total particulate and endotoxin values at swine and poultry facilities were higher than our data with poultry facilities producing the highest values. These total particulate and endotoxin values ranged from 0.47 to 76.7 mg/m^3 and from 0.1 to 41,310 EU/m^3 (Omland 2002, Radon 2002, Reynolds 1994).

Donham and Reynolds have found that a high proportion of disease occurs in workers at dust levels above 2.5 mg/m^3 total and 0.23 mg/m^3 respirable for swine confinement operations and poultry house operations. Both have recommended that these levels serve as occupational limits for CAFO workers (Donham 1988, 1995, 2000a, 2000b, Reynolds 1996). The U.S. EPA recommends National Ambient Air Quality Standards (NAAQS) for several pollutants and they recommend a level of 0.150 mg/m^3

for PM_{10} for a 24-hour period not to be exceed more than once per year on average in a 3 year period (NAAQS 2007). Donham et al. have recommended 100 EU/m^3 as an occupational limit (Donham 1988). In the Netherlands, the Dutch Expert Committee on Occupational Standards has proposed an occupational exposure limit of 50 EU/m^3 (4.5 ng/m^3) over an 8-hour exposure period (DECOS 1996, Duchaine 2001).

Our highest total and inhalable particulate values are below these recommended occupational limits but they do approach these occupational limits. Some of our higher values exceed the NAAQS of 0.150 mg/m^3. Our mean total endotoxin levels for the study dairy in winter and fall (253 and 80 EU/m^3, respectively) far surpass the recommended levels. Other seasons approach these values. Keep in mind that our data were collected outside close to lagoons, and workers are not assigned to these areas except for brief tasks.

We were unable to perform a pre- and post-intervention study at the same dairy. Ideally, we would collect data for a specific period before the intervention was implemented and then again after the intervention had time to become established in the lagoon. We were unable to collect samples at each dairy on the same day for the same time period. We did not have the personnel or equipment to collect such samples. The study and control dairies were not exactly the same.

The two dairies were fairly different in all aspects except for the lagoon system design (Table II). The control dairy had 2 times the number of cows on 5 times the amount of land. The lagoon systems were almost identical, but the control dairy composted much more waste and flushed much less waste as a function of the pens which housed the cows. The control dairy had large dirt pens with concrete alleys on either side

for feeding and watering. The lots were periodically scraped and wastes composted and only the feeding alleys were flushed to the lagoons. The pens at the study dairy were much smaller with a similar alley for feeding and a small sand area for bedding. The study dairy pen alleys were flushed to the lagoons three times per day and the sand was periodically replaced and recycled.

The control dairy had primary and secondary lagoons. Each lagoon was approximately 3 acres. The study dairy had one lagoon of approximately 8 acres. The control dairy maintained less lagoon capacity than the study dairy with double the amount of cows. A primary and secondary anaerobic lagoon system is thought to emit less gases and odor than a single lagoon system. The lagoons at the control dairy appeared to contain less solid material as compared to the lagoon at the study dairy.

Sampling locations were similar at both dairies. Equipment was placed in a predominantly downwind location approximately 15-20 meters from the lagoon edge near an 110v power outlet. At the study dairy this location was near the building which housed the algae tanks and near an open air recirculation pit which contained a submersible pump used to supply the flushing tanks. The compost fields sat to the Northeast of this location. The closest animal building was over 100 meters away. At the control dairy this location was between the primary and secondary lagoons but downwind from the primary lagoon. The compost fields sat to the North of this location. The closest animal pen was over 100 meters away. At random times the pump inside the recirculation pit at the study dairy would engage to fill the pit with lagoon liquid. At these times hydrogen sulfide, ammonia, and odor would increase.

Conclusions

Overall, our data do not produce a definitive answer as to the effectiveness of the algae lagoon treatment (Agsmart 2007). While total maximum hydrogen sulfide levels were significantly lower at the study dairy, total maximum ammonia levels were significantly lower at the control dairy. The highest overall measures of H_2S and NH_3 were at the study dairy. Odor measurements were not significantly different. Lower H_2S levels can have a large impact on overall odor reduction because it is easily recognized as a rotten odor. Some of the differences in NH_3 levels can be explained by the differences in lagoon practices between the dairies. Overall, total and inhalable particulate and endotoxin were not significantly different except for total endotoxin in winter and summer was significantly lower at the control dairy. However, the algae lagoon treatment makes no claims about reducing particulate and endotoxin. Further data collection will be necessary to substantiate any claims of gas and odor reduction. A similar study must be conducted pre- and post-intervention. If this is not possible, then more similar dairies must be recruited. Also, an ammonia measurement device with a lower resolution would help to elucidate the overall ammonia concentrations.

Chapter 4

Characterization of Occupational Exposures for Various Tasks at Two Colorado Dairies

Abstract

Total and inhalable particulate and endotoxin were measured for six tasks at two Colorado dairies. The majority of the workers were male Hispanics ranging in age from 19 to 57. Most participants had at least some high school education. Length of employment varied greatly from 1 to 236 months. The average length of employment was about 44 months. However, frequent worker turnover was quite common. Most participants were either former smokers or had never smoked. The majority of participants (56% Dairy 1 and 100% Dairy 2) had experienced flu-like symptoms at some point in their employment. Inhalable particulate (8-hour time weighted average (TWA)) ranged from 0.07 to 8.0 mg/m^3 at Dairy 1 and from 0.07 to 5.1 mg/m^3 at Dairy 2. The highest inhalable particulate values at both dairies occurred for loading feed. Overall, Dairy 1 had significantly higher ($p<0.05$) inhalable particulate for milking, loading feed, and distributing feed. Total particulate ranged from 0.03 to 5.3 mg/m^3 at Dairy 1 and from 0.05 to 6.9 mg/m^3 at Dairy 2. The highest total particulate values at both dairies occurred for milking. Overall, Dairy 1 had significantly higher ($p<0.05$) total particulate than Dairy 2 for milking. Inhalable endotoxin ranged from 8.4 to 11096 EU/m^3 at Dairy

1 and from 2.0 to 5286 EU/m^3 at Dairy 2. The highest inhalable endotoxin levels were for calves at Dairy 1 and for milking at Dairy 2. Overall, Dairy 1 had significantly higher ($p<0.05$) inhalable endotoxin than Dairy 2 for loading feed and distributing feed. Total endotoxin ranged from 5.9 to 6758 EU/m^3 at Dairy 1 and from 7.3 to 4649 EU/m^3 at Dairy 2. The highest values at both dairies occurred for milking. Total endotoxin levels were not significantly different between the two dairies for any task or any season. Overall, the only significant differences between the different tasks at the individual dairies occurred at Dairy 1. For IOM samplers, inhalable endotoxin concentrations for working calves was significantly higher ($p<0.05$) than working sick cows and distributing feed. For Button samplers, inhalable endotoxin concentrations for milking were significantly higher ($p<0.05$) than working calves. For total samplers, total endotoxin concentrations for milking were significantly higher ($p<0.05$) than working calves. There were no significant differences between tasks at Dairy 2 and there were no significant differences between tasks as a whole. Our levels of inhalable particulate are similar to other milking studies but 2-3 times higher than other feeding studies and our levels of inhalable endotoxin are more than 25 times higher than other milking studies and up to 2-3 times higher than other feeding studies. Overall, our particulate levels do not exceed current U.S. occupational exposure limits. Particulate and endotoxin levels may be reduced for milking tasks by increasing ventilation rates inside the milking parlors and for feeding tasks by installing or properly maintaining tractor cabin filtration systems.

Introduction

Animal confinement workers spend an increased amount of time indoors and around animals, experiencing greater exposures than in the past, and suffering increased rates of respiratory disease – up to 30% are affected by chronic obstructive pulmonary disease (COPD) (Cathomas 2002, Donham 1995, Kullman 1998).

Confinement dust is primarily composed of organic compounds. The majority of the dust arises from feed and fecal particles. Ammonia, molds, bacteria, and endotoxin can attach to dust particles and become deposited in the lung (Donham 1986, Tripp 1999). Dust particles can settle at different levels of the respiratory system depending on size. Particles greater than 10 microns are generally deposited in the upper respiratory tract. Particles from 3 to 10 microns are most often deposited in the major airways of the lower respiratory tract, and particles smaller than 3 microns are respirable and can reach deep into the lung parenchyma (Tripp 1999).

Endotoxin is a lipopolysaccharide protein complex component of the outer wall of gram-negative bacteria. Endotoxin is a potent inflammatory agent that produces systemic effects and lung obstruction, even at low levels of exposure (Thorne 2000). Animal feces and plant materials contaminated with bacteria are major contributors of endotoxin to organic dust. Exposure to such dust is prevalent in livestock farming (Thorne 1997).

Adverse health effects associated with confinement dust and endotoxin include cough, wheeze, shortness of breath, chronic bronchitis, decrease in lung function, asthma-like syndrome, and organic dust toxic syndrome cough, chest tightness, mucous membrane irritation, decrease in lung function, chronic airways obstruction, byssinosis, bronchial hyperreactivity, dyspnea, fever, rigors, myalgia, arthralgia, and other influenza-

like symptoms (Donham 1989, Merchant 1986, Reynolds 1996, Ross 2000, Thorne 1999). Workers in cotton and flax mills, wool carpet workers, swine confinement workers, and animal feed workers have reported symptoms linked to endotoxin exposure (Castellan 1995, Ozesmi 1987, Thorne 1999).

Donham et al. have found that high proportion of disease occurs in workers at dust levels above 2.5 and 0.23-mg/m^3 for total and respirable, respectively, and have recommended that these levels serve as occupational limits for CAFO workers (Donham 1988, 1995, 2000a, 2000b, Reynolds 1996). Donham et al have recommended 100 EU/m^3 as an occupational limit (Donham 1988). In the Netherlands, the Dutch Expert Committee on Occupational Standards has proposed an occupational exposure limit of 50 EU/m^3 (4.5 ng/m^3) over an 8-hour exposure period (DECOS 1996, Duchaine 2001).

Multiple agencies have recommended exposure limits for several of the contaminants found at dairy farms (Table I). The Occupational Safety and Health Administration (OSHA) promulgate 8-hour time weighted average (TWA) enforceable occupational standards called Permissible Exposure Limits (PELs). The American Conference of Governmental Industrial Hygienists (ACGIH) and The National Institute for Occupational Safety and Health (NIOSH) also develop 8-hour TWAs and short term exposure limit (STEL) occupational standards called Threshold Limit Values (TLVs) and Recommended Exposure Limits (RELs). These agencies maintain standards for both total and respirable particulate not otherwise classified (PNOC) and grain dust. Total, inhalable, and respirable PNOC and grain dust exposure limits range from 3 to 15 mg/m^3 (ACGIH 2007, NIOSH 2007a, OSHA 2007).

Kullman et al. found personal breathing zone dust levels averaged 1.78 mg/m3 for inhalable fractions and 0.07 mg/m3 for respirable fractions on 85 Wisconsin dairy farms (Kullman 1998). Firth et al. measured personal inhalable dust levels at New Zealand dairy, sheep, arable, and mixed farms. They found median inhalable levels of 0.60, 0.70, 1.71, and 0.54 mg/m^3, respectively. Interquartile ranges were from 0.22 to 2.45 mg/m^3, overall (Firth 2006).

The state of Colorado defines a dairy CAFO as one which contains 700 head or 800,000 pounds live weight (Kress 2007). In 2006, the state of Colorado ranked 16^{th} in the United States in total milk production with 2,547,050,000 pounds of milk for an 8.5% increase over 2005. Colorado had 170 licensed dairy herds each with an average of 647 cows for a total of 110,000 cows. In total, the United States' 9,112,000 cows produced 181,798,000,000 pounds of milk in 2006. Increase in milk production and larger herd sizes are trends that U.S. dairy producers can expect to see for years to come. Therefore, the numbers of dairies and dairy workers are rapidly increasing (Cooley 2007).

The purpose of this study was to measure total and inhalable particulate and endotoxin, characterize worker exposure, and evaluate differences among tasks at two Colorado dairies with different milking parlor systems.

Materials and Methods

Sampling Sites

Two Colorado dairies agreed to participate in this study. The dairy characteristics are listed in Table II. Dairy 1 had total land area of approximately 60 acres with a single

lagoon of approximately 8 acres. Dairy 1 milked approximately 1350 cows with approximately 125 cows kept dry and not milked. Dairy 1 raised the female calves on site. This dairy has a straight milking parlor capable of milking 40 cows at one time. The waste treatment system was as follows: The milking parlor was rinsed with fresh water and recycled water used to rinse the other areas. Wastes flowed by gravity into a leaky dam separation and settling basin and then into a primary lagoon. Dry lots were scraped as are the settling basins and the solids were composted. The lagoon was treated with a novel algae intervention (Agsmart 2007). Algae was grown in a greenhouse on site next to the lagoon and pumped through micro diffusers into the lagoon. The algae were intended to increase the dissolved oxygen in the lagoon to above 1 mg/ml with the purpose of transforming the once anaerobic environment into an aerobic environment with the goal of reducing lagoon odor emissions and solids (Agsmart 2007).

Dairy 2 had total land area of approximately 340 acres with two lagoons of approximately 3 acres each. Dairy 2 milked approximately 3000 cows and had approximately 350 cows kept dry and not milked. Dairy 2 did not raise the female calves on site. The milking parlor was a rotary system capable of milking 80 cows at one time. The waste treatment system was as follows: The milking parlor was rinsed with fresh water and recycled water used to rinse the other areas. Wastse flowed by gravity into a leaky dam separation and an earthen basin separation then into a primary and secondary lagoon. Dry lots were scraped as are the settling basins and the solids were composted.

Sampling Schedule

We measured total particulate and endotoxin at each dairy for milking and

maintenance tasks, and inhalable particulate and endotoxin at each dairy for milking, working calves, working sick cows, maintenance, driving feed truck, and loading feed truck. All samples were personal samples attached to the specific worker near their breathing zone except the feed truck driver and feed truck loaders which were located in the specific truck or loader near the breathing zone of the worker.

Each dairy was sampled 40 total days over each season as follows: 10 fall, 10 winter, 10 spring, and 10 summer. When possible the same workers were sampled; however, this was not always possible with days off and high job turn over.

Sampling and Analysis

Meteorology

Meteorological data, including temperature, relative humidity, and wind speed, were measured using a Vantage Pro Weather Station (Davis Instruments, Hayward, CA). The Weather Station was mounted to a pole approximately 2 meters above ground level. The Weather Station consisted of a combination of a wind vane and anemometer and a temperature and relative humidity sensor. All were mounted at the apex of the pole. The temperature sensor was shielded from direct sunlight. The pole was orientated such that the wind vane was directed south and the temperature relative humidity sensor was directed north, as directed by the instruction manual. Data were logged using Davis WeatherLink for Vantage Pro data collection, analysis, and display software for Windows. Data were collected over 1-minute intervals as mandated by the software, and displayed as average values.

Total Particulate and Endotoxin

Total particulate and endotoxin samples were collected according to NIOSH method 0500 (NIOSH 2007b). Particulate was collected on a 37-mm closed-faced cassette on poly vinyl chloride filters with a 5 μm pore diameter (SKC, Inc. Eighty Four, PA). Filters were pre- and post-weighed using a Mettler MT5 microbalance (Mettler-Toledo, Inc.) and were blank-corrected to account for humidity differences and measurement drift. Samples were collected for 8 hours at a flow rate of 2 L/min. After sample collection the filters were stored at 4°C under desiccation and analyzed for endotoxin using a new Recombinant Factor C Endotoxin Assay (Biowhittaker, Walkersville, MD). All results were reported as 8-hour TWAs. Sample concentrations were reported as mg/m^3 for total dust and EU/m^3 for total endotoxin. The limit of detection (LOD) for this method was 0.03 mg. This value was determined from the mean of blanks plus 3 standard deviations.

Inhalable Particulate and Endotoxin

Inhalable particulate and endotoxin were collected using Institute of Occupational Medicine (IOM) samplers and SKC Button samplers (SKC Inc. Eighty Four, PA.) with 25mm filters with 5 μm pore size (SKC, Inc. Eighty Four, PA). Button samplers were used to measure inhalable particulate and endotoxin for milking tasks. It was our experience that the Button samplers provided better filter protection from splashed waster and manure as compared to the IOM sampler. Personal sampling pumps were calibrated to 2.0 L/min for IOMs and 4.0L/min for button samplers. Samples were collected for 8 hours. For IOM samples the entire filter cassettes were pre- and post-weighed on a Mettler MT5 microbalance (Mettler-Toledo, Inc.). All results were reported as 8-hour

TWAs. Sample concentrations were reported as mg/m^3 for inhalable dust and EU/m^3 for inhalable endotoxin. The LOD for this method was 0.05 mg. This value was determined from the mean of blanks plus 3 standard deviations.

For the SKC Button only the filters were pre- and post-weighed. All filters were blank-corrected to account for humidity differences and measurement drift. After sample collection the filters were stored at 4°C under desiccation and analyzed for endotoxin using a new Recombinant Factor C Endotoxin Assay (Biowhittaker, Walkersville, MD). All results were reported as 8-hour TWAs. Sample concentrations were reported as mg/m^3 for inhalable dust and EU/m^3 for endotoxin. The LOD for this method was 0.04 mg. This value was determined from the mean of blanks plus 3 standard deviations.

Endotoxin Measurement - Recombinant Factor C Endotoxin Assay

The concentration of endotoxin was determined using a novel Recombinant Factor C Endotoxin (rFC) Assay (Biowhittaker, Walkersville, MD). The activation of rFC is determined by the fluorescence generated by the enzymatic cleavage of a peptide-coumarin substrate. Fluorescence is measured after one-hour incubation with endotoxin standards at 37°C. The log fluorescence is proportional to the log endotoxin concentration and is linear in the 0.01-10EU/ml range. The minimum detection of endotoxin is ~0.01EU/ml. The rFC assay has been found to detect no (1,3)-glucan activity; an improvement in specificity compared to the most commonly used assays.

Samples were extracted in sterile, pyrogen-free (pf) water containing 0.05% Tween-20 for 1 hr at 22°C with continuous shaking. Extracts were centrifuged and supernatants were transferred into pf cryotubes. They were then analyzed using the Recombinant Factor C Endotoxin Assay. Two-fold serial dilutions of endotoxin standards

and sample extracts were prepared using sterile, pf water with Tween-20 in borosilicate glass tubes. The samples were added to a 96-well plate followed by 100 microliters of a mixture of enzyme, buffer and fluorogenic substrate. The plates were incubated at 37°C for one hour and read in a fluorescence microtiter plate reader (Biotek Instruments FLX800TBIE) at Excitation/Emission 380/440 nm. Background (1 EU/ml) fluorescence was subtracted and log delta fluorescence plotted against log endotoxin concentration. Endotoxin concentrations of samples were calculated according to the standard curve. Four assay reagent blank wells served as reference and control for the pf status of the reagent water, centrifuge tubes, pipette tips and microplates. Quality assurance spiking assays were performed to assess matrix interference or enhancement.

Task Descriptions

Six tasks at two Colorado dairies were characterized. The tasks included milking, loading feed, distributing feed, maintenance, working calves, and working sick cows. Dairy 1 included all six tasks. Dairy 2 included milking, loading feed, and distributing feed. Milking tasks included moving cows into position to be milked, cleaning cows prior to milking, attaching milking device, applying anti-mastitis medicine post milking, and moving cows out of position. Loading feed tasks included driving a front end loader to load different types of feed by weight into a truck or trailer hopper. Normally the windows of the loader would be closed in cold weather and open in warmer weather. The cabs of the loaders were very dirty and dusty even with closed windows. Feed distribution tasks included driving either a truck with mixing hopper or tractor pulling a trailer mixing hopper to deliver the feed to the cows. As with the loaders, normally the windows of the truck or tractor would be closed in cold weather and open in warmer

weather. The cabs of both the truck and tractor were very dirty and dusty even with closed windows.

Maintenance tasks varied the most but often included scraping and moving manure, working compost, moving cows, and artificial insemination. Working calves included moving calves, feeding (opening feed bags and distributing) and watering calves, checking health, and administering medication. Working sick cows included checking sick cows, administering medication, milking sick cows, checking pregnant cows, moving sick and pregnant cows, delivering calves, and moving calves.

Workers performed the same tasks throughout the day (8-12 hours). Milking was performed by 3-6 workers, feeding was performed by 1 worker, maintenance was performed by 1-3 workers, sick cows was performed by 1-2 workers, and feeding calves was performed by 1-2 workers.

Questionnaire

Participants were asked to complete a simple questionnaire designed to collect general information about demographics and health status. The questionnaire was provided in the language of the participants' choice. An English copy of the questionnaire can be seen in Appendix I. This study was approved by Colorado State University's Institutional Review Board for research.

Statistical Analysis

Excel databases were combined and analyzed using SAS Version 9.1 (SAS Institute, Cary, NC). Descriptive statistics were used to characterize environmental measurements. The normality of the data was tested using the Shapiro-Wilks test. All variables, except meteorological conditions, were log transformed before completing

statistical analysis. Geometric means and geometric standard deviations were calculated for environmental data that could be described as lognormal. Comparisons between samplers, dairies, and seasons were made using linear analysis of variance with two-way interactions. Included variables were as follows: dependent (total particulate mg/m^3, inhalable particulate mg/m^3, total endotoxin EU/m^3, and inhalable endotoxin EU/m^3); class (dairy, season, and task). Means were compared using Tukey's test procedure. Pearson correlation coefficients were calculated to evaluate associations among environmental parameters and particulate and endotoxin concentrations. Values that fell below the LOD, occurred for particulate sampling methods only, were replaced with the LOD divided by the square root of 2 for statistical analysis.

Results

Meteorology

Seasons were defined as follows: December, January, and February were considered winter. March, April and May were considered spring. June, July, August were considered summer. September, October, and November were considered fall. Meteorological data is summarized by season in Table III. For the Dairy 1 atmospheric air temperatures ranged from -9.9°C to +40.1°C with a mean of +15.3°C. Relative humidity ranged from 0.6% to 90.3% with a mean of 36.8%. Wind speeds ranged from 0.5 m/s to 8.7 m/s with a mean of 2.0 m/s. For the Dairy 2 atmospheric air temperatures ranged from -12.5°C to +41.1°C with a mean of +11.2°C. Relative humidity ranged from 7.9% to 92.3% with a mean of 44.7%. Wind speeds ranged from 0.8 m/s to 4.7 m/s with a mean of 2.5 m/s.

Demographics

Demographics and questionnaire answers are summarized in Table XI. The majority of the workers were male Hispanics ranging in age from 19 to 57. Most participants had at least some high school education. Length of employment varied greatly from 1 to 236 months. The average length of employment was about 44 months; however, frequent worker turnover was quite common. Most participants were either former smokers or had never smoked, which is lower than the U.S. average. The majority of participants had experienced flu-like symptoms at some point in their employment. Many participants claimed to have experienced the following symptoms in

Table XI. Demographics and Questionnaire Results

	Location					
Category	Dairy 1			Dairy 2		
	Mean or %	Range	n	Mean or %	Range	n
Race (Hispanic)	94%		16	100%		6
Gender (male)	88%		16	100%		6
Age	35	19 - 57	14	28	23 - 35	6
Highest Education Completed	12% Grade School 25% Some High School 38% High School Grad 25% Post High School		16	83% High School Grad 17% Post High School		6
Length of Employment (months)	46	1 - 236	16	38	6 - 72	6
Smoker	50% Former 38% No 12% Yes		16	83% No 17% Yes		6
Flu-like Symptoms	56% Yes		16	100% Yes		6
Symptoms last 3 months	44% Yes		16	67% Yes		6
Wear a Bandana?	81% No		16	33% Yes		6
Wear a Dust Mask?	94% No		16	100% No		6
Wear a Respirator?	100% No		16	100% No		6

the past 3 months: mild to severe eye irritation, blurred vision, nose irritation, mucous or phlegm, tingling in fingers, shortness of breath, chest discomfort, chest wheeze, throat irritation, and cough. Most participants did not wear any type of personal protective equipment such as a dust mask, although 3 workers at Dairy 1 and 2 workers at Dairy 2 often used bandanas.

Particulate

Inhalable particulate levels for tasks are summarized in Table XII. Inhalable particulate ranged from 0.07 to 8.0 mg/m^3 at Dairy 1 and from 0.07 to 5.1 mg/m^3 at Dairy 2. The highest inhalable particulate values at both dairies occurred for loading feed. Overall, Dairy 1 had significantly higher ($p<0.05$) inhalable particulate for milking, loading feed, and distributing feed. In summer, Dairy 1 had significantly higher ($p<0.0001$) inhalable particulate than Dairy 2 for milking. In fall, Dairy 1 had significantly higher ($p<0.0001$ loading and $p<0.01$ distributing) inhalable particulate than Dairy 2 for loading and distributing feed. Inhalable particulate levels for milking, loading feed, and distributing feed tasks at both dairies are summarized in a box-whisker plot in Figure VI. Inhalable particulate levels for maintenance, working sick cows, and working calves tasks at the study dairy are summarized in Figure VII. The whiskers delineate the 10th and 90th percentiles and the box lines represent median, lower, and upper quartiles of the data.

Total particulate levels for milking and maintenance tasks at both dairies are summarized in Figure VIII. The whiskers delineate the 10th and 90th percentiles and the box lines represent median, lower, and upper quartiles of the data. Total particulate levels for tasks are seen in Table XIII.

Table XII. Comparison of Inhalable Particulate Concentrations for Tasks by Season and Dairy

Season, Task	Location							
	Dairy 1				Dairy 2			
	GM	(GSD)	n	range	GM	(GSD)	n	range
Total								
Milking *	0.58	(2.6)	38	0.06 - 3.8	0.30	(1.8)	99	0.07 - 1.9
Loading Feed *	1.5	(2.3)	35	0.26 - 8.0	0.57	(3.1)	38	0.06 - 5.1
Distributing Feed *	0.85	(2.2)	35	0.19 - 3.8	0.47	(2.9)	39	0.07 - 2.9
Maintenance	1.3	(1.7)	14	0.07 - 4.4	NA	NA	NA	NA
Sick Cows	1.1	(1.7)	15	0.4 - 2.6	NA	NA	NA	NA
Calves	1.2	(2.6)	46	0.074 - 6.3	NA	NA	NA	NA
Winter								
Milking	0.57	(1.8)	9	0.20 - 1.4	0.29	(1.8)	22	0.09 - 1.3
Loading Feed	0.74	(1.6)	7	0.34 - 1.3	0.71	(2.7)	10	0.11 - 2.4
Distributing Feed	0.72	(2.4)	7	0.20 - 2.4	0.96	(3.0)	10	0.16 - 5.0
Maintenance	1.7	(1.9)	4	0.82 - 2.7	NA	NA	NA	NA
Sick Cows	0.86	(1.3)	3	0.70 - 1.2	NA	NA	NA	NA
Calves	1.3	(1.9)	14	0.28 - 3.5	NA	NA	NA	NA
Spring								
Milking	0.34	(2.6)	10	0.06 - 1.5	0.29	(2.0)	22	0.07 - 0.80
Loading Feed	1	(2.0)	10	0.26 - 3.1	0.53	(2.3)	11	0.10 - 2.9
Distributing Feed	0.82	(2.5)	10	0.20 - 3.8	0.73	(1.7)	10	0.40 - 1.6
Maintenance	1.6	(1.8)	2	1.0 - 1.3	NA	NA	NA	NA
Sick Cows	1.2	(2.1)	4	0.42 - 2.6	NA	NA	NA	NA
Calves	0.76	(3.4)	10	0.074 - 3.4	NA	NA	NA	NA
Summer								
Milking ***	0.72	(3.2)	14	0.06 - 3.8	0.26	(1.6)	30	0.14 - 0.54
Loading Feed	2.4	(2.0)	8	0.90 - 8.0	1.1	(1.3)	8	0.70 - 1.3
Distributing Feed	1.2	(1.9)	9	0.30 - 2.5	1.1	(2.5)	8	0.21 - 3.5
Maintenance	1.6	(1.8)	5	1.0 - 4.4	NA	NA	NA	NA
Sick Cows	0.87	(1.7)	4	0.50 - 1.6	NA	NA	NA	NA
Calves	0.89	(3.4)	10	0.15 - 6.3	NA	NA	NA	NA
Fall								
Milking	1.0	(1.5)	5	0.64 - 1.7	0.38	(1.7)	25	0.15 - 1.9
Loading Feed ***	2.3	(2.2)	10	0.45 - 5.6	0.13	(2.0)	10	0.07 - 0.42
Distributing Feed **	0.76	(2.3)	9	0.25 - 1.9	0.15	(1.8)	10	0.06 - 0.34
Maintenance	0.89	(1.3)	3	0.70 - 1.2	NA	NA	NA	NA
Sick Cows	1.4	(1.5)	4	0.80 - 2.1	NA	NA	NA	NA
Calves	1.9	(1.6)	12	0.86 - 3.9	NA	NA	NA	NA

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ that mean concentrations in that row not equal by ANOVA

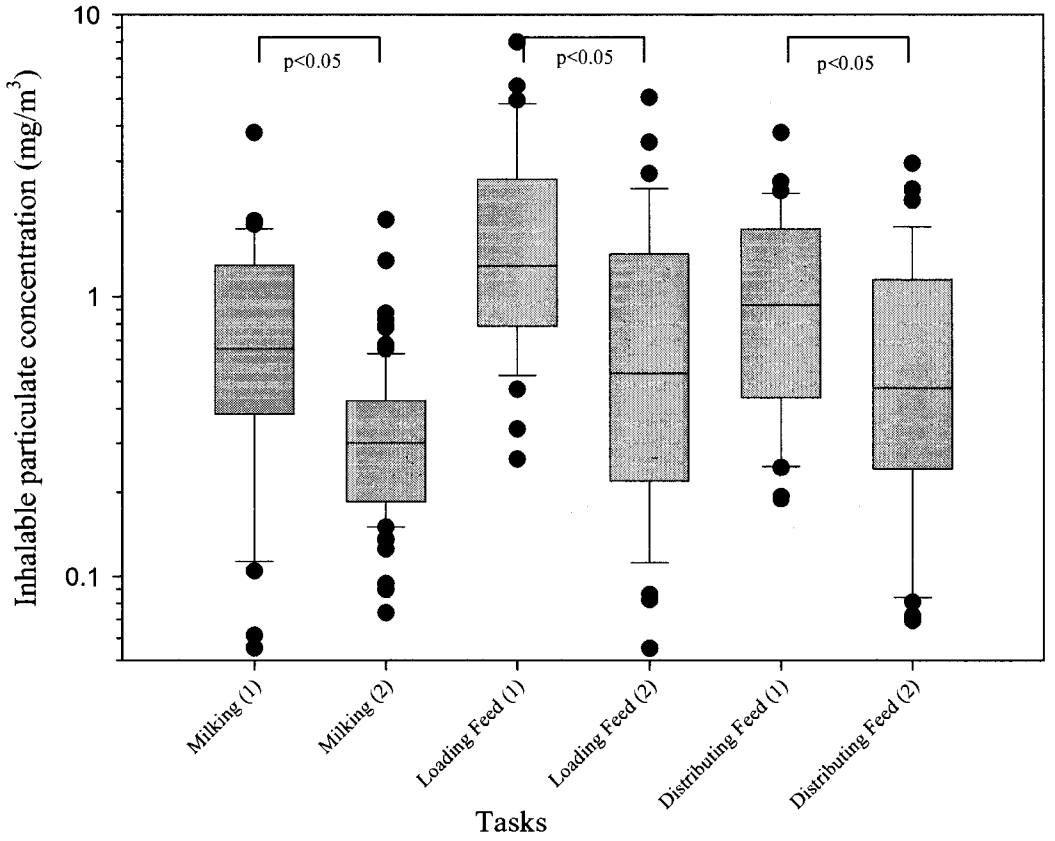

Figure VI. Box-Whisker Plot of Inhalable Particulate Concentrations for Milking, Loading Feed, and Distributing Feed Tasks at Dairy (1) and Dairy (2)
(Whiskers delineate 10th, 90th percentile and box lines represent median, lower, and upper quartiles of the data)

Total particulate ranged from 0.03 to 5.3 mg/m^3 at the Dairy 1 and from 0.03 to 6.9 mg/m^3 at Dairy 2. The highest total particulate values at both dairies occurred for milking. Overall, Dairy 1 had significantly higher ($p<0.05$) total particulate than Dairy 2 for milking.

Endotoxin

Inhalable endotoxin concentrations for tasks are listed in Table XIV. Inhalable endotoxin levels for milking, loading feed, and distributing feed tasks at both dairies are summarized in a box-whisker plot in Figure IX. Inhalable endotoxin levels for

maintenance, working sick cows, and working calves tasks at the study dairy are summarized in Figure X. The whiskers delineate the 10^{th} and 90^{th} percentiles and the box lines represent median, lower, and upper quartiles of the data.

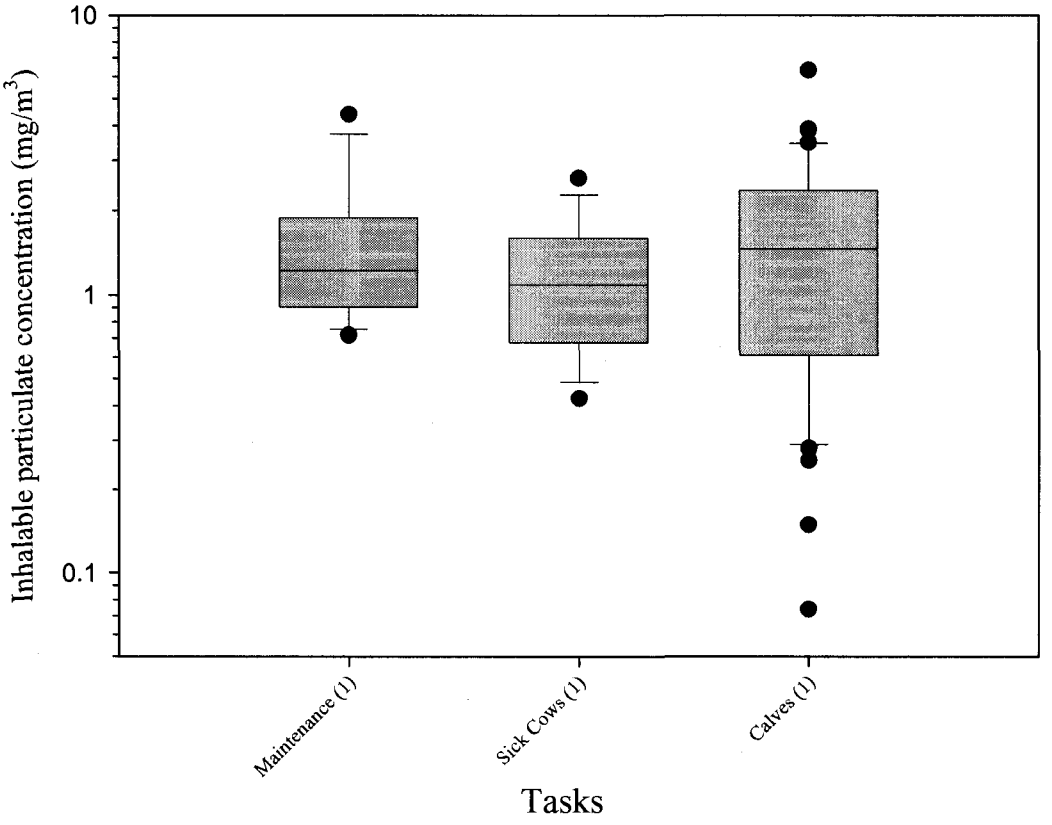

Figure VII. Box-Whisker Plot of Inhalable Particulate Concentrations for Maintenance, Working Sick Cows, and Working Calves at Dairy (1)
(Whiskers delineate 10th, 90th percentile and box lines represent median, lower, and upper quartiles of the data)

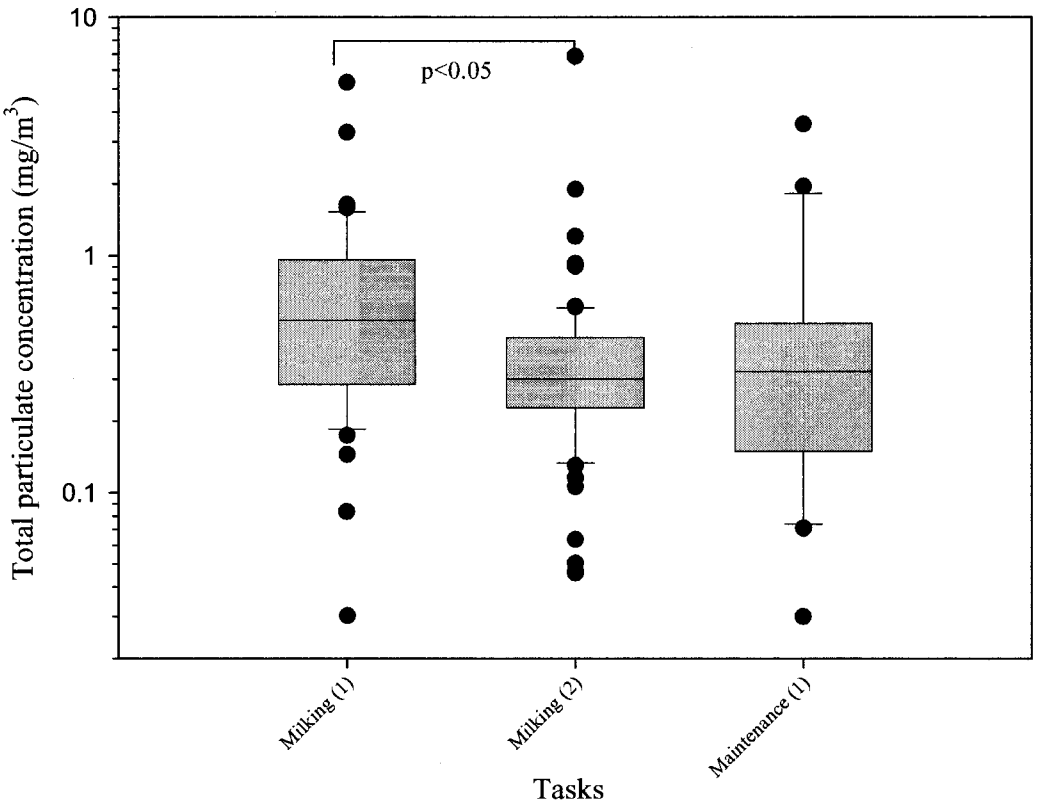

Figure VIII. Box-Whisker Plot of Total Particulate Concentrations
for Milking and Maintenance Tasks at Dairy (1) and Dairy (2)
(Whiskers delineate 10th, 90th percentile and box lines represent median, lower, and upper quartiles of the data)

Inhalable endotoxin ranged from 8.0 to 11096 EU/m^3 at Dairy 1 and from 2.0 to 5286 EU/m^3 at Dairy 2. The highest inhalable endotoxin levels were seen for working calves at Dairy 1 and for milking at Dairy 2. Overall, Dairy 1 had significantly higher ($p<0.05$) inhalable endotoxin than Dairy 2 for loading feed and distributing feed. In spring, Dairy 1 had significantly higher ($p<0.05$) inhalable endotoxin than Dairy 2 for milking.
In summer, Dairy 1 had significantly higher ($p<0.05$) inhalable endotoxin than Dairy 2 for loading feed. In fall, Dairy 1 had significantly higher ($p<0.0001$ loading and $p<0.001$ distributing) inhalable endotoxin than Dairy 2 for loading and distributing feed.

Table XIII. Comparison of Total Particulate Concentrations for Tasks by Season and Dairy

Season, Task	Location							
	Dairy 1				Dairy 2			
	GM	(GSD)	n	range	GM	(GSD)	n	range
Total								
Milking *	0.53	(2.5)	47	0.03 - 5.3	0.30	(2.1)	81	0.05 - 6.9
Maintenance	0.32	(3.1)	26	0.03 - 3.6	NA			
Winter								
Milking	0.40	(5.2)	9	0.03 - 5.3	0.30	(2.1)	21	0.05 - 1.2
Maintenance	0.21	(6.2)	4	0.03 - 2.0	NA			
Spring								
Milking	0.50	(1.8)	11	0.2 - 1.5	0.25	(1.9)	19	0.05 - 0.61
Maintenance	0.22	(2.0)	10	0.078 - 0.5	NA			
Summer								
Milking	0.62	(2.0)	11	0.17 - 1.6	0.29	(2.3)	21	0.13 - 6.9
Maintenance	0.44	(2.5)	5	.21 - 1.6	NA			
Fall								
Milking	0.58	(2.2)	16	0.14 - 3.3	0.38	(2.0)	20	0.05 - 1.9
Maintenance	0.50	(3.5)	7	0.07 - 3.6	NA			

* = $p < 0.05$ that mean concentrations in that row not equal by ANOVA

Inhalable endotoxin per mg of particulate (Table XV) ranged from 7.5 to 144,886 EU/mg at Dairy 1 and from 1.5 to 21,114 EU/mg at Dairy 2. The highest value at Dairy 1 occurred for distributing feed and at Dairy 2 for milking. Overall, Dairy 2 had significantly higher ($p<0.05$) inhalable endotoxin per mg particulate than Dairy 1 for milking, but Dairy 1 had significantly higher ($p<0.05$) inhalable endotoxin per mg particulate than Dairy 2 for loading feed. In winter, Dairy 1 had significantly higher ($p<0.05$) inhalable endotoxin per mg particulate than Dairy 2 for loading feed. In summer, Dairy 2 had significantly higher ($p<0.0001$) inhalable endotoxin per mg particulate than Dairy 1 for milking.

Table XIV. Comparison of Inhalable Endotoxin Concentrations for Tasks by Season and Dairy

Season, Task	Location							
	Dairy 1				Dairy 2			
	GM	(GSD)	n	range	GM	(GSD)	n	range
Total								
Milking	571.3	(2.9)	38	22 - 2540	657.3	(3.4)	99	4 - 5286
Loading Feed *	386.1	(3.0)	35	32 - 2862	68.7	(5.0)	38	3 - 1611
Distributing Feed *	252.8	(4.3)	35	8 - 1671	67.3	(5.1)	39	2 - 1701
Maintenance	416	(2.1)	14	89 - 2809	NA	NA	NA	NA
Sick Cows	255.7	(2.6)	15	29 - 845	NA	NA	NA	NA
Calves	666.9	(3.3)	46	56 - 11096	NA	NA	NA	NA
Winter								
Milking	844.2	(2.0)	9	384 - 1557	431.9	(3.8)	22	3.8 - 1676
Loading Feed	299.2	(3.0)	7	61 - 698	143.8	(8.3)	10	2.5 - 1701
Distributing Feed	274.6	(4.3)	7	46 - 1349	99.4	(9.9	10	1.9 - 1611
Maintenance	500.2	(11.5)	4	89 - 2809	NA	NA	NA	NA
Sick Cows	63.3	(3.1)	3	29 - 140	NA	NA	NA	NA
Calves	465.6	(1.7)	14	278.7 - 1009	NA	NA	NA	NA
Spring								
Milking	264.4	(2.5)	10	71 - 1503	533.6	(3.9)	22	19 - 5286
Loading Feed	228.7	(3.8)	10	32 - 1859	104.9	(2.4)	11	43 - 1033
Distributing Feed *	299.5	(3.7)	10	32 - 1671	125.8	(2.0)	10	41 - 296
Maintenance	263.3	(2.0)	2	159 - 436	NA	NA	NA	NA
Sick Cows	202	(2.0)	4	92 - 300	NA	NA	NA	NA
Calves	309.7	(3.2)	10	56 - 1561	NA	NA	NA	NA
Summer								
Milking	672.8	(3.4)	14	22 - 2540	521.9	(2.6)	30	87.4 - 3085
Loading Feed *	637.8	(2.2)	8	249 - 2524	109.0	(1.8)	8	41 - 232
Distributing Feed	346.2	(4.5)	9	7.7 - 1223	114.7	(1.8)	8	40.5 - 1519
Maintenance	715.1	(2.3)	5	317 - 2259	NA	NA	NA	NA
Sick Cows	459.3	(2.0)	4	219 - 845	NA	NA	NA	NA
Calves	459.3	(3.3)	10	150 - 5613	NA	NA	NA	NA
Fall								
Milking	980.9	(1.4)	5	650- 1370	1382.8	(2.9)	25	58 - 5065
Loading Feed **	483.3	(2.6)	10	100 - 2862	10.2	(2.6)	10	2.7 - 46
Distributing Feed **	147.3	(5.4)	9	8.4 - 730	11.3	(2.6)	10	2.7 - 91
Maintenance	202.3	(1.4)	3	156 - 292	NA	NA	NA	NA
Sick Cows	394.6	(1.8)	4	183 - 806	NA	NA	NA	NA
Calves	991.8	(3.3)	12	92 - 11096	NA	NA	NA	NA

* = $p < 0.05$, ** = $p < 0.001$ that mean concentrations in that row not equal by ANOVA

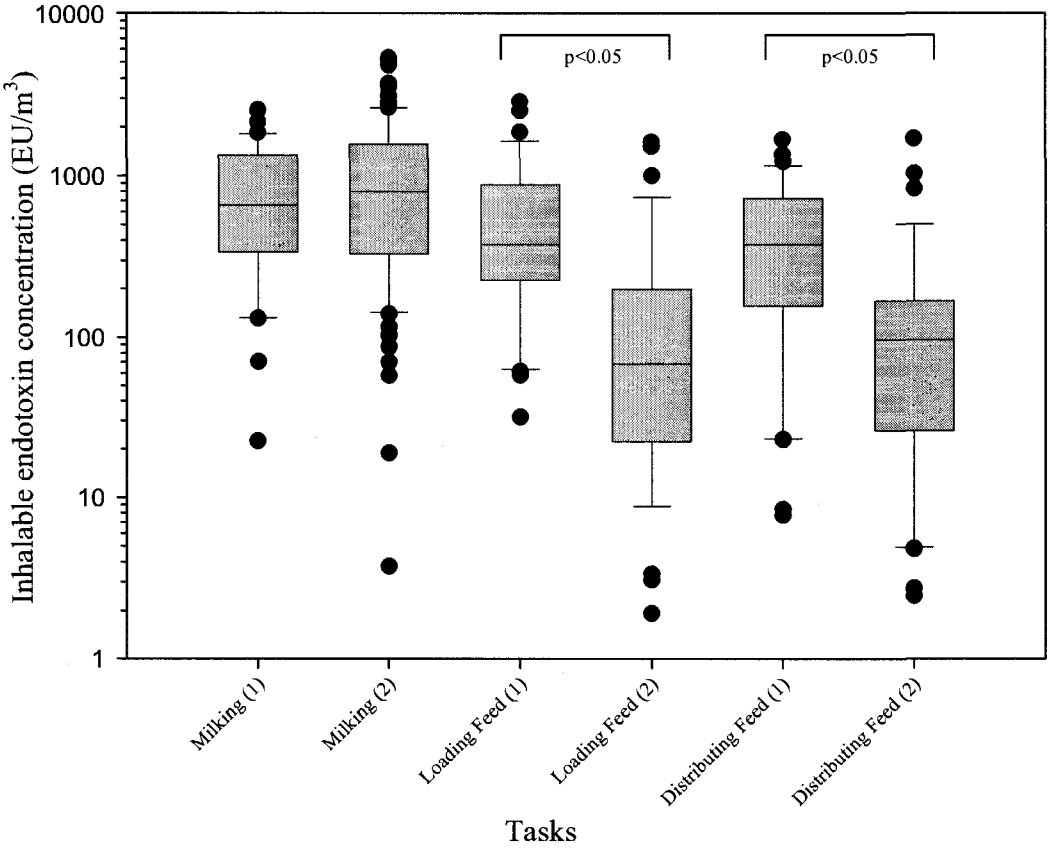

Figure IX. Box-Whisker Plot of Inhalable Endotoxin Concentrations for Milking, Loading Feed, and Distributing Feed Tasks at Dairy (1) and Dairy (2)
(Whiskers delineate 10th, 90th percentile and box lines represent median, lower, and upper quartiles of the data)

In fall, Dairy 2 had significantly higher ($p<0.05$) inhalable endotoxin per mg particulate than Dairy 1 for milking.

Total endotoxin concentrations are listed in Table XVI. Total endotoxin levels for milking and maintenance tasks at both dairies are summarized in Figure XI. The whiskers delineate the 10^{th} and 90^{th} percentiles and the box lines represent median, lower, and upper quartiles of the data.

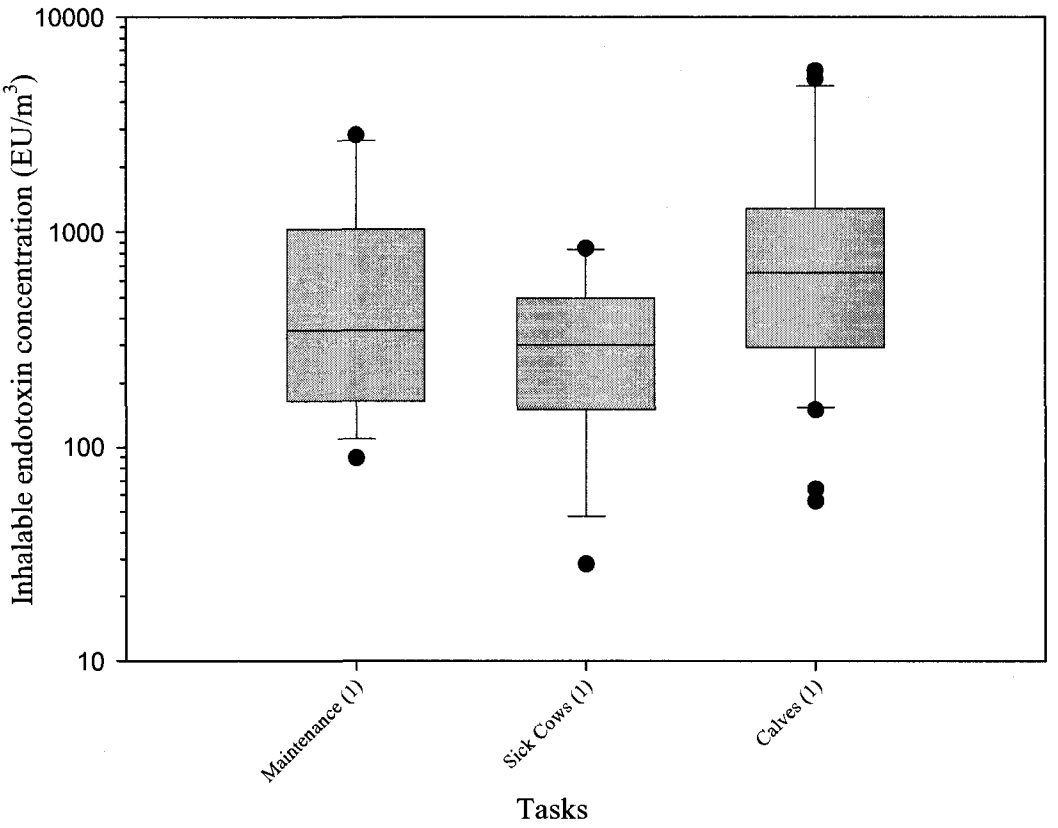

Figure X. Box-Whisker Plot of Inhalable Endotoxin Concentrations for Maintenance, Working Sick Cows, and Working Calves at Dairy (1)
(Whiskers delineate 10th, 90th percentile and box lines represent median, lower, and upper quartiles of the data)

Total endotoxin ranged from 5.9 to 6758 EU/m^3 at Dairy 1 and from 9.9 to 4649 EU/m^3 at Dairy 2. The highest values at both dairies occurred for milking. Total endotoxin levels were not significantly different between the two dairies for any task or any season. Total endotoxin per mg of particulate (Table XVII) ranged from 15 to 96,506 EU/mg at Dairy 1 and 46 to 19,778 EU/mg at Dairy 2.

Table XV. Comparison of Inhalable Endotoxin/Particulate Concentrations for Tasks by Season and Dairy

Season, Task	Location							
	Dairy 1				Dairy 2			
	GM	(GSD)	n	range	GM	(GSD)	n	range
Total								
Milking *	968.4	(2.1)	38	304 - 4378	2218	(2.8)	99	13 - 21114
Loading Feed *	253.3	(2.7)	35	29 - 2791	124.7	(3.0)	38	1.5 - 435
Distributing Feed	399.2	(4.0)	35	7.5 - 144886	143.3	(3.5)	39	1.5 - 5586
Maintenance	327.9	(2.1)	14	109 - 1356	NA	NA	NA	NA
Sick Cows	244.1	(2.3)	15	35 - 986	NA	NA	NA	NA
Calves	582.9	(2.8)	46	107.8 - 7426	NA	NA	NA	NA
Winter								
Milking	1731.1	(2.2)	9	704 - 2955	1657.7	(3.8)	22	13 - 5547
Loading Feed *	471	(3.9)	7	78 - 2071	104.9	(1.8)	10	1.5 - 5586
Distributing Feed	474.5	(2.1)	7	244 - 1096	203.0	(4.2)	10	1.5 - 367
Maintenance	384.9	(5.9)	4	109 - 1356	NA	NA	NA	NA
Sick Cows	85	(3.5)	3	35 - 207	NA	NA	NA	NA
Calves	340.4	(2.7)	14	107.8 - 1407	NA	NA	NA	NA
Spring								
Milking	903.5	(2.6)	10	304 - 2994	1811.7	(2.7)	22	150 - 6686
Loading Feed	221.9	(3.2)	10	29 - 1221	198.2	(1.5)	11	124 - 431
Distributing Feed	564.5	(7.5)	10	7.5 - 144886	171.7	(1.4)	10	116 - 262
Maintenance	235.5	(2.4)	2	127 - 436	NA	NA	NA	NA
Sick Cows	170.9	(1.4)	4	116 - 218	NA	NA	NA	NA
Calves	409.8	(2.4)	10	129.7 - 2136	NA	NA	NA	NA
Summer								
Milking **	929.6	(2.1)	14	404 - 4378	2022.4	(2.1)	30	538 - 8750
Loading Feed	270.3	(2.7)	8	117 - 2791	105.5	(1.8)	8	32 - 182
Distributing Feed	536	(2.2)	9	233 - 2188	101.7	(3.5)	8	116 - 435
Maintenance	438.3	(1.7)	5	226 - 941	NA	NA	NA	NA
Sick Cows	554.8	(1.7)	4	343 - 986	NA	NA	NA	NA
Calves	1150.8	(2.8)	10	316 - 7426	NA	NA	NA	NA
Fall								
Milking *	956.3	(1.3)	5	765 - 1364	3605.7	(2.5)	25	203 - 21115
Loading Feed	214.1	(1.7)	10	94 - 638	83.0	(2.1)	10	30 - 246
Distributing Feed	193.7	(2.8)	9	34 - 818	122.2	(2.3)	10	21 - 269
Maintenance	226.5	(1.2)	3	193 - 252	NA	NA	NA	NA
Sick Cows	290.6	(1.3)	4	234 - 392	NA	NA	NA	NA
Calves	535.3	(2.6)	12	159.9 - 2861	NA	NA	NA	NA

* = $p < 0.05$, ** = $p < 0.001$ that mean concentrations in that row not equal by ANOVA

Table XVI. Comparison of Total Endotoxin Concentrations for Tasks by Season and Dairy

Season, Task	Location							
	Dairy 1				Dairy 2			
	GM	(GSD)	n	range	GM	(GSD)	n	range
Total								
Milking	815.6	(2.3)	47	139 - 6758	779.5	(3.5)	81	9.9 - 4649
Maintenance	223.4	(3.8)	26	5.9 - 2435	NA			
Winter								
Milking	549	(3.0)	9	139 - 2923	563.6	(6.2)	21	9.9 - 3470
Maintenance	723.0	(2.6)	4	204.9 - 773	NA			
Spring								
Milking	673	(1.8)	11	253 - 1548	618.2	(2.6)	19	49 - 1745
Maintenance	125.7	(6.0)	10	5.9 - 1273	NA			
Summer								
Milking	893	(2.5)	11	207 - 5415	477.1	(3.0)	21	15.8 - 1481
Maintenance	294.4	(2.4)	5	143 - 1033	NA			
Fall								
Milking	1016	(2.3)	16	387 - 6758	1981	(1.7)	20	629 - 4649
Maintenance	323.2	(3.8)	7	37 - 2435	NA			

The highest values at both dairies occurred for milking. In fall, Dairy 2 had significantly higher ($p<0.05$) total endotoxin per mg particulate than Dairy 1 for milking.

Overall, the only significant differences between the different tasks at the individual dairies occurred at Dairy 1. For IOM samplers, inhalable endotoxin associated with working calves (GM 422 EU/m^3) was significantly higher ($p<0.05$) than that with working sick cows (GM 256 EU/m^3) and distributing feed (GM 253 EU/m^3). For button samplers, inhalable endotoxin for milking (571 EU/m^3) was significantly higher ($p<0.01$) than for working calves (297 EU/m^3). For total samplers, total endotoxin for milking (816 EU/m^3) was significantly higher ($p<0.001$) than working calves (223 EU/m^3). There

were no significant differences among tasks at Dairy 2, and there were no significant differences between tasks as a whole.

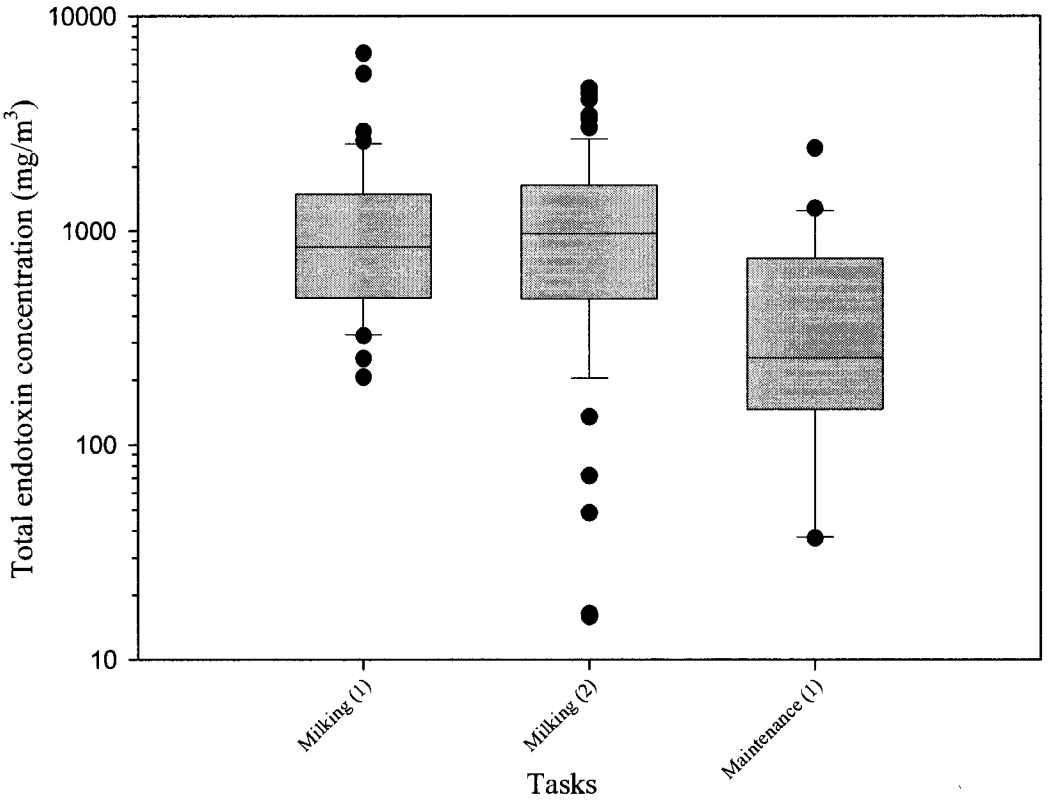

Figure XI. Box-Whisker Plot of Total Endotoxin Concentrations for Milking and Maintenance Tasks at Dairy (1) and Dairy (2)
(Whiskers delineate 10th, 90th percentile and box linesrepresent median, lower, and upper quartiles of the data)

Discussion

This study was designed to test the null hypothesis that there is no difference between the individual tasks at different dairies and no difference among the tasks as a whole. We measured total and inhalable particulate and endotoxin for various tasks at two Colorado dairies.

Table XVII. Comparison of Total Endotoxin/Particulate Concentrations for Tasks by Season and Dairy

Season, Task	Location							
	Dairy 1				Dairy 2			
	GM	(GSD)	n	range	GM	(GSD)	n	range
Total								
Milking	1617	(3.0)	47	140 - 96506	2459	(3.1)	81	46 - 19778
Maintenance	905	(2.8)	26	15 - 8266	NA			
Winter								
Milking	2702	(8.4)	9	412 - 96506	2037	(5.0)	21	71 - 19778
Maintenance	1447	(6.7)	4	189 - 8266	NA			
Spring								
Milking	1344	(1.7)	11	570 - 4276	2208	(2.0)	19	458 - 6278
Maintenance	654.7	(7.4)	10	15 - 4431	NA			
Summer								
Milking	1431	(2.4)	11	258 - 4619	1649	(3.7)	21	46 - 4999
Maintenance	670	(3.6)	5	15 - 3772	NA			
Fall								
Milking *	1684	(3.0)	16	140 - 8293	4616	(1.8)	20	1391 - 10525
Maintenance	646.6	(1.4)	7	453 - 992	NA			

* = $p < 0.05$ that mean concentrations in that row not equal by ANOVA

The two dairies were vastly different in all aspects except for lagoon systems and feeding cows. Dairy 2 had twice as many cows and 5 times the amount of land. The lagoon systems were similar, but Dairy 2 composted much more waste as a function of the pens which housed the cows. The pens at Dairy 2 were scraped and composted, and only the feeding areas were flushed to the lagoons. The pens at Dairy 1 were much smaller and were only flushed to the lagoons. The milking parlors were very different. Dairy 1 parlor was capable of milking 40 stationary cows at one time with 20 on each side of a long walkway. The workers moved along the walkway to perform milking tasks. Dairy

2 parlor was capable of milking 80 cows at one time on a large rotating circular platform. Cows walk onto the platform, rotate around, and then walk off after the milking is complete. Workers were mostly stationary.

Workers at Dairy 1 performed 6 total tasks including milking, loading feed, distributing feed, maintenance, working sick cows, and working calves. Workers at Dairy 2 had 3 total tasks including milking, loading feed, and distributing feed. Dairy 2 did not raise calves on site. Dairy 2 outsourced the majority of their veterinary needs so there were no sick cow tasks. The maintenance workers at Dairy 2 refused to participate in our study.

The highest inhalable particulate levels at both dairies occurred for loading feed. However, the highest particulate level for working calves at Dairy 1 (6.3 mg/m^3) was higher than the highest level for loading feed at Dairy 2. The geometric mean levels for particulate for all tasks at Dairy 1 were higher than those at Dairy 2. Milking tasks had the lowest levels of inhalable particulate as expected given that these environments appeared to be the cleanest and wettest of all tasks. Although, milking at Dairy 1 and loading feed at Dairy 2 had almost identical inhalable particulate levels (GM 0.58 and 0.57 mg/m^3 respectively). Inhalable particulate levels for maintenance, working sick cows, and working calves at Dairy 1 were all similar. Inhalable particulate levels were highest for loading and distributing feed during summer as expected given that the windows of the loaders, tractors, and trucks were almost always open.

The highest total particulate levels at both dairies occurred for milking; however, they were much lower than inhalable particulate levels. Unlike the levels for inhalable particulate, milking tasks at Dairy 1 produced the highest levels of total particulate. The

highest GM inhalable endotoxin levels were seen for working calves at Dairy 1 and for milking at Dairy 2. The lowest GM inhalable endotoxin levels at both dairies were for distributing feed.

The highest GM inhalable endotoxin per mg particulate values at both dairies occurred for milking. These levels are not surprising given that milking tasks at both dairies had the lowest inhalable particulate levels but the highest inhalable endotoxin levels. Again the milking parlor was thought to be the cleanest environment at each dairy. In summer, working calves produced higher GM inhalable endotoxin per mg particulate than did milking at Dairy 1.

The highest GM total endotoxin values at both dairies occurred for milking. Total endotoxin levels were not significantly different between the two dairies for any task or any season. The highest total endotoxin per mg of particulate values at both dairies occurred for milking. Again, these levels are not surprising given that milking tasks at both dairies highest inhalable endotoxin levels. Again the milking parlor was thought to be the cleanest environment at each dairy. In spring, maintenance produced higher inhalable endotoxin per mg particulate than did milking at Dairy 1.

Our data was similar to those collected by others in dairy and cattle environments both inside and outside barns. Geometric mean inhalable particulate values ranged from 0.22 to 2.67 mg/m^3 for various dairy and cattle tasks and locations (Firth 2006, Kullman 1998, Nieuwenhuijsen 1999, Takai 1998). Inhalable endotoxin levels were similar to those found inside dairy barns and for other dairy tasks. Geometric mean values ranged from 23 to 647 EU/m3 (Kullman 1998, Nieuwenhuijsen 1999, Seedorf 1998). Niewenhuijsen et al. measured inhalable particulate and endotoxin in several agricultural

tasks in California. They found geometric mean levels of 0.61 mg/m^3 and 24 EU/m^3 for milking and 0.47 mg/m^3 and 120 EU/m^3 for feeding cows (Nieuwenhuijsen 1999). Our levels of inhalable particulate are similar for milking but 2-3 times higher for feeding and our levels of inhalable endotoxin are more than 25 times higher for milking and 2-3 times higher at Dairy 1 but 2 times lower at Dairy 2 for feeding. The California feeding methods are not described.

In comparison, inhalable dust and endotoxin values are much higher in swine and poultry facilities, with poultry producing the highest levels. Geometric mean inhalable particulate values ranged from 1.8 to 6.7 mg/m^3 and geometric mean inhalable endotoxin values ranged from 40 to 55,660 EU/m^3 (Nieuwenhuijsen 1999, Seedorf 1998, Takai 1998

Our total particulate data were similar to other dairy studies ranging from 0.07 to 7.3 mg/m^3 with geometric means ranging from 0.22 to 3.87 mg/m^3 for ambient samples (Cathomas 2002, Kullman 1998, Omland 2002). Total particulate and endotoxin values at swine and poultry facilities were higher than our data with poultry facilities producing the highest values. These total particulate and endotoxin values ranged from 0.47 to 76.7 mg/m^3 and from 0.1 to 41,310 EU/m^3 (Omland 2002, Radon 2002, Reynolds 1994).

OSHA, NIOSH, and ACGIH maintains 8-hour time weighted average (TWA) occupational standards for total, inhalable, and respirable particulate not otherwise classified (PNOC) and grain dust ranging from 3 to 15 mg/m^3 (ACGIH 2007, NIOSH 2007a, OSHA 2007). However, agricultural dusts other than specific grain dusts, such as wheat, oat, and barley, are not covered by these standards.

Donham et al. and Reynolds et al. have found that a high proportion of disease occurs in agricultural workers at dust levels above 2.5 mg/m^3 total and 0.23 mg/m^3 respirable for swine confinement operations and poultry house operations. Both have recommended that these levels serve as occupational limits for CAFO workers (Donham 1988, 1995, 2000a, 2000b, Reynolds 1996). Donham et al. have recommended 100 EU/m^3 as an occupational limit (Donham 1988). In the Netherlands, the Dutch Expert Committee on Occupational Standards has proposed an occupational exposure limit of 50 EU/m^3 (4.5 ng/m^3) over an 8-hour exposure period (DECOS 1996, Duchaine 2001).

Our highest total and inhalable particulate values were 2-3 times the recommended limits by Donham et al. and Reynolds et al. Mean particulate levels for all tasks were below the Donham et al. and Reynolds et al. recommended level of 2.5 mg/m^3. However, some tasks at both dairies (22% of working calves samples and 30% of loading feed samples) produced at least one particulate sample above the recommended level of 2.5 mg/m^3. Our mean total and inhalable endotoxin levels for every task at both dairies (84 - 100% of all samples) surpass the recommended levels of 50 - 100 EU/m^3. Most tasks are 4-10 times higher than these recommended levels.

New workers at both dairies normally begin in the milking parlors or feeding calves (Dairy 1). The most experienced workers feed cows or work with sick cows. The new workers are exposed to high levels of endotoxin during milking and calf tasks, and, while feeding cows may be considered the easiest task, it produces some of the highest particulate and endotoxin levels. Reducing these levels would prove difficult for some tasks. Dairy 2 had much better ventilation in the milking parlor than Dairy 1. This may have led to their almost 50% lower particulate levels. The milking parlors produced

similar endotoxin levels. Exposures for feeding tasks could be reduced by installing cabin filtration systems (or maintaining the current systems) on the feed loaders and distributors. Because of the nature of the other tasks (maintenance, sick cows, and calves), providing personal protective equipment, such as dust masks, might be the best option. However, the workers must be trained how to properly use the equipment.

Conclusions

Dairy 1 had significantly higher inhalable particulate for milking and loading and distributing feed, significantly higher inhalable endotoxin for loading and distributing feed, and significantly higher total particulate for milking. Overall, the only significant differences between the different tasks at the individual dairies occurred at Dairy 1. For IOM samplers, inhalable endotoxin for the task of working calves was significantly higher than that for working sick cows and distributing feed. For button samplers, inhalable endotoxin for milking was significantly higher than working calves. For total samplers, total endotoxin for milking was significantly higher than for working calves. There were no significant differences between tasks at Dairy 2, and there were no significant differences between tasks as a whole. Overall, the greatest risk of exposure comes from endotoxin. Every task was above the recommended levels for good worker health. Particulate levels for all tasks were generally below levels of concern.

Chapter 5

Recommendations for Improvements and Future Research

This study was designed and conducted at two Colorado dairies to determine whether a novel algae lagoon intervention treatment is able to reduce environmental gas and odor emissions. We also measured occupational exposures for various tasks at the two dairies, and compared two viable microbial samplers to determine the differences between the two devices. This study collected data for three unique aspects of emissions and exposures from dairies. We collected a variety of data at lagoons and for various tasks at two dairies.

Overall, our data do not provide a definitive answer as to the effectiveness of the algae lagoon intervention. However, mean peak H_2S levels were significantly lower at the study lagoon compared to the control lagoon. H_2S contributes to the amount of odor emitted from a dairy lagoon. H_2S is easily identified by its rotten odor. NH_3 levels were significantly higher at the study lagoon compared to the study lagoon. However, the values were similar in three seasons except summer. The summer levels were almost 4 times higher at the study dairy. The control dairy's location allowed them to use field application of liquids to keep nitrogen levels in their lagoon down. They study dairy did not use this process.

The highest overall peak measures of H_2S and NH_3 were at the study dairy. Odor measurements were not significantly different. Overall, total and inhalable particulate and endotoxin were not significantly different except for total endotoxin in winter and summer was significantly lower at the control dairy. However, the algae lagoon treatment makes no claims about reducing particulate and endotoxin.

Culturable microorganism levels collected at outdoor points away from animal barns were generally lower than—but within some ranges—typically reported inside livestock barns. Microorganism levels for each sampler were significantly different in all seasons. Analysis of our study data showed that the Anderson two stage viable particle sampler was able to collect significantly more culturable bioaerosols than the SKC Biosampler for mesophilic and Gram-negative bacteria and fungi in all four seasons except for fungi in winter. Respirable microorganism concentrations were consistently higher than non-respirable concentrations in total and over all four seasons except for Gram-negative bacteria in the fall.

The study dairy had: significantly higher inhalable particulate for milking and loading and distributing feed; significantly higher inhalable endotoxin for loading and distributing feed; and significantly higher total particulate for milking. Overall, the only significant differences among the different tasks at the individual dairies occurred at the study dairy. For IOM samplers, inhalable endotoxin for the task of working calves was significantly higher than for working sick cows and distributing feed. For button samplers, inhalable endotoxin for milking was significantly higher than for working calves.

For total samplers, total endotoxin for milking was significantly higher than for working calves. There were no significant differences among tasks at the control dairy, and there were no significant differences between tasks as a whole.

The two dairies were fairly different in most aspects. The control dairy or Dairy 2 had twice the number of cows and 5 times the amount of land. The lagoon systems were similar, but the control dairy composted much more waste and flushed much less waste as a function of the pens which housed the cows. The control dairy had large dirt pens with concrete alleys on either side for feeding and watering. The lots were periodically scraped and wastes composted and only the feeding alleys were flushed to the lagoons. The pens at the study dairy were much smaller with a similar alley for feeding and a small sand area for bedding. The study dairy pen alleys were flushed to the lagoons three times per day and the sand was periodically replaced and recycled.

The control dairy had primary and secondary lagoons. Each lagoon was approximately 3 acres. The study dairy had one lagoon of approximately 8 acres. The control dairy maintained less lagoon capacity than the study dairy with double the amount of cows. A primary and secondary anaerobic lagoon system is thought to emit less gases and odor than a single lagoon system. The lagoons at the control dairy appeared to contain less solid material as compared to the lagoon at the study dairy. The control dairy reduced the level of liquid in its lagoons by field application. This practice reduces the level of nitrogen and NH_3 emissions.

Sampling locations were similar at both dairies. Equipment was placed in a predominantly downwind location approximately 15-20 meters from the lagoon edge

near an 110v power outlet. At the study dairy this location was near the building which housed the algae tanks and near an open air recirculation pit which contained a submersible pump used to supply the flushing tanks. The compost fields sat to the Northeast of this location. The closest animal building was over 100 meters away. At the control dairy this sampling location was between the primary and secondary lagoons but downwind from the primary lagoon. The compost fields sat to the North of this location. The closest animal pen was over 100 meters away. At random times the pump inside the recirculation pit at the study dairy would engage to fill the pit with lagoon liquid. At these times hydrogen sulfide, ammonia, and odor would increase.

Several occupational tasks were different between the two dairies. The study dairy workers performed 6 total tasks while the control dairy had only 3 total tasks. The control dairy did not raise calves on site. The control dairy outsourced the majority of their veterinary needs, and their maintenance workers refused to participate in our study.

The milking parlors were very different. The study dairy parlor was capable of milking 40 stationary cows at one time with 20 on each side of a long walkway. The workers moved along the walkway to perform milking tasks. The control dairy parlor was capable of milking 80 cows at one time on a large rotating circular platform. Cows walked onto the platform, rotated around, and then walked off after the milking was complete. Workers were mostly stationary.

Feeding cows was fairly similar between the two dairies. Both dairies had feed areas where bulk feed was stored. Feed included alfalfa, cracked corn, wood chips, cotton seed, corn gluten, and brewer's grain. Workers loaded preset amounts of feed into a mixing hopper using a front end loader. The loaders used by each dairy varied on a day

to day basis but they were similar. Workers would normally close the windows in colder weather and open them in warmer weather. The mixing hoppers were similar. The study dairy used a tractor to pull a mixing hopper trailer while the control dairy used a mixing hopper truck. The truck or tractor was driven to the cows to distribute the feed via a conveyer belt. Approximately 75% of the workers' time was spent inside the loader.

Slight variations in these tasks may explain some of the variation in occupational exposures. The milking parlor at the control dairy had large doors on two sides that were opened in warmer weather for ventilation. This ventilation may have reduced the particulate and endotoxin levels at the control dairy.

Participants were asked to fill out a questionnaire to collect demographic data and data about different symptoms that the workers might have experienced. Worker demographics were very similar. Most were Hispanic males between the ages of 20-35 with similar education. Most workers were non-smokers. Most workers had experienced flu-like symptoms at some point during their employment and other symptoms during the past 3 months before the questionnaire. More workers at the control dairy experienced these symptoms. Flu-like symptoms include: fever, chills, cough, tiredness, weakness, muscle, and joint pain. Other symptoms include: eye irritation, blurred vision, nose irritation, mucous/phlegm, tingling finger, shortness of breath, chest discomfort, chest wheezing, throat irritation, and cough.

We were unable to perform a pre- and post-intervention study at the same dairy. Ideally, we would collect data for a specific period before the intervention was implemented and then again after the intervention had time to become established in the lagoon. This would give the most accurate estimate of the ability of the algae

interventions ability to reduce gases and odor. If possible, finding several more similar dairies to participate would be the second option. Dairies with similar land, animal, and lagoon sizes would provide a more accurate estimate. Originally this study was designed with 4 dairies in total and 2 with the algae intervention. However, 3 of 4 withdrew from participation for various reasons including cost, change in ownership, and bankruptcy. We were forced to use a less than ideal candidate as the control dairy. Another option would be to use identical research lagoons.

We were unable to collect samples at each dairy on the same day for the same time period. We did not have the personnel or equipment to collect such samples. With our limited budget we were only able to purchase one each of the Jerome hydrogen sulfide analyzer and Pac III ammonia analyzer. Our access to personal sampling pumps was also limited. For this reason sampling at two dairies on the same day would have been impossible. It also prohibited us from collecting occupational hydrogen sulfide and ammonia data. Our budget also dictated that we purchase the PAC III ammonia analyzer which has a resolution of 1 ppm. Other more sensitive devices are available at a higher cost.

A large portion of this study could not be completed because of lack of data. We had planned to collect odor data using a Cyranose 320 electronic nose. This device uses multiple sensors to create a digital smell print. The Cyranose 320 is trained to identify compounds in the laboratory and then is used to identify those same compounds in the field. A comprehensive literature search was performed to select several compounds to compare between the Cyranose 320 and GC. Included in these compounds were many acids, alcohols, aldehydes, amides, amines, aromatics, esters, ethers, fixed gases,

halogenated hydrocarbons, hydrocarbons, ketones, nitriles, other nitrogen-containing compounds, phenols, sulfur-containing compounds, steroids, and other compounds. These data were to be compared with hydrogen sulfide, ammonia, scentometer odor data, and gas chromatography results. We collected air samples by the lagoons using charcoal tubes. These tubes were analyzed by gas chromatography (GC) for several compounds that make up odor including acetone, n-butanol, and n-propanol. These compounds were chosen based on the response from the Cyranose 320.

While useful in the laboratory, the Cyranose 320 was impractical in the field. The device failed to identify any of the trained compounds and even failed to identify manure retrieved from the same lagoons. The device was extremely sensitive to heat and cold which restricted the days and times that it could be used. The GC field samples failed to produce any result. There are several reasons that might explain these results. Samples were stored at temperatures according to the particular standards but many for longer than recommended times. It is possible that compounds off-gassed prior to analysis. When recently collected (within 1-3 days) samples were analyzed, they produced the same negative results. However, the samples that were quickly analyzed were only a small part of the total amount of samples. Operator error may have led to the negative results. The operator's use of the GC was infrequent and his technique unpracticed. The samples were collected at a very low flow rate (100 – 200 ml/minute) according to the applicable standards to reduce break through. In total these samples were less than 100 liters of air. It is possible that with a much larger volume of air certain compounds may have been identified. Further experiments would need to be conducted to determine what flow rates and volumes of air produce the best results.

Laboratory spike samples, at levels many times what would be found in the ambient air near the lagoons, produced identifiable GC peaks when analyzed. These samples produced similar results even after storage up to 7 days.

Respirable data on particulate were not collected for the various tasks at the two dairies because the preliminary samples returned results that were consistently below the limit of detection. Respirable data would have been a very important component of the occupational aspect of this study given the implication for worker health.

Overall, the levels of environmental contaminants and bioaerosols measured at the lagoons of both dairies are below recommended environmental levels and levels to ensure good occupational health. Some researchers claim that long term exposure to levels similar to our values of environmental contaminants can reduce health and quality of life. Residents have reported increased occurrence of symptoms including headache, runny nose, sore throat, excessive coughing, diarrhea, and burning eyes (Wing 2000). Schiffman et al. exposed healthy volunteers to diluted swine confinement air containing 24 ppb H_2S, 817 ppb, NH_3, 0.0241 mg/m3 total particulate, 7.4 EU/m^3 endotoxin, and 57 D/T odor. Subjects were 4.1 times more likely to report headaches, 6.1 times more likely to report eye irritation, and 7.8 times more likely to report eye irritation (Schiffman 2005).

Endotoxin was the only occupational level of concern. The endotoxin levels for all tasks exceeded recommended levels to ensure good worker health. Particulate and endotoxin levels can be reduced for occupational task through engineering controls and personal protective equipment. Exposures in the milking parlors can be reduced by increasing the ventilation. Dairy 2 had lower particulate and endotoxin than Dairy 1.

Dairy 2 had large garage doors on both sides of the milking parlor that allowed the workers to increase ventilation created by cross winds. Exposures for feeding tasks can be greatly reduced by installing and maintaining cabin filtration systems in the tractors and trucks. Air conditioning must also be installed and the drivers would have to change their personal practices of opening windows. Exposures for other tasks can be reduced through the use of personal protective equipment such as dusk masks and respirators that reduce the amount particulate and endotoxin that can reach the workers breathing zone.

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Appendix

Questionnaire

Date_____ Worker ID #_____ Farm ID #_____

1. Race / Nationality:

   _____American Indian or Alaskan Native
   _____Asian
   _____Black or African American
   _____Hispanic or Latino
   _____Native Hawaiian or Other Pacific Islander
   _____Caucasian
   _____Other

2. Gender: _____Male _____Female

3. Age? _____

4. What is the highest level of education you have completed?

   _____Eighth grade or less
   _____Technical school graduate
   _____Some high school
   _____Some college
   _____High school graduate or GED certificate
   _____College graduate
   _____Some technical school
   _____Post graduate or professional degree
   _____Don't know

5. How long have you been working at this dairy? _____

6. What is your primary job at this dairy? (Please check more than one if you have more than one primary job.)

   _____General Maintenance (milking parlors)
   _____Milking
   _____General Maintenance (lagoon/waste maintenance)
   _____Breeding
   _____Corral Maintenance (cleaning and flushing barns)
   _____Feeding
   _____Corral Maintenance (repairing pens and gates)
   _____Calf/Heifer Rearing
   _____Veterinarian (hospital treatments)
   _____Moving Animals

7. Do you smoke?

   _____Yes, current smoker _____No, never smoked _____No, former smoker

8. Have you **EVER** had flu-like symptoms during or after working at a dairy?
   (Flu-like symptoms include: fever, chills, cough, tiredness, weakness, muscle and joint pains)
   _____Yes _____No _____Don't Know

   If yes, when and where?_____

   _____

9. Have you experienced any of the following (see box below) respiratory or other symptoms from work during the past **THREE (3)** months? _____Yes _____No

If yes, please rate the severity of your symptoms by placing an (X) in the box next to the symptom and below the box that best rates the severity of that symptom.

		None (1)	Mild (2)	Moderate (3)	Severe (4)	Don't Know
1	Eye Irritation					
2	Blurred Vision					
3	Nose Irritation					
4	Mucous or Phlegm					
5	Tingling Fingers					
6	Shortness of Breath					
7	Chest Discomfort					
8	Chest Wheezing or Whistling					
9	Throat Irritation					
10	Cough					
11	Other (specify)					

10. Do you wear a bandana while at work?

_____Yes, always _____Yes, sometimes _____No

_____Don't know

11. Do you wear a dust mask while at work?

_____Yes, always _____Yes, sometimes _____No

_____Don't know

12. Do you wear a respirator with a cartridge while at work?

_____Yes, always _____Yes, sometimes _____No

_____Don't know

Thank you for your participation in our study.

Glossary

Aerobic -	presence of oxygen
Anaerobic -	absence of oxygen
Biological Oxygen - Demand	a chemical term procedure for determining how fast biological organisms use up oxygen in a body of water
Cloramphenicol -	broad-spectrum antibiotic $C_{11}H_{12}Cl_2N_2O_5$ isolated from cultures of a soil actinomycete (*Streptomyces venezuelae*) or prepared synthetically
Confinement Air/Dust -	made up of a complex mixture of ammonia, carbon monoxide, carbon dioxide, hydrogen sulfide, feed particles, insect parts, pollen, grains, mineral ash, animal dander, dried feces and urine, bacteria, viruses, fungi, endotoxin, proteins, and proteolytic enzymes
Cyclohexamide -	an agricultural fungicide $C_{15}H_{23}NO_4$ that inhibits protein synthesis and is obtained from a soil bacterium (*Streptomyces griseus*)
Dissolved Oxygen -	refers to oxygen gas that is dissolved in water often measured in mg/L
Endotoxin -	a lipopolysaccharide protein complex component of the outer wall of Gram-negative bacteria, endotoxin is a potent inflammatory agent that produces systemic effects and lung obstruction
Federal Region VIII -	States of Colorado, Montana, North Dakota, South Dakota, Utah, and Wyoming
Manure Slurry -	Mixture of feces and urine
Mesophilic -	an organism that grows best in moderate temperature, typically between 25 and 40 °C (77 and 104 °F), mainly applied to microorganisms

Olfactometer - an instrument for measuring the sensitivity of the sense of smell in regard to intensity, concentration, or quality of an odor

List of Abbreviations

°C	Degrees Celsius
ACGIH	American Conference of Governmental Industrial Hygienists
AGI	All Glass Impinger
ATSDR	Agency for Toxic Substances and Disease Registry
BOD	Biological Oxygen Demand
CAFO	Concentrated Animal Feeding Operation
CFU/m^3	Colony Forming Units per cubic meter air
CO_2	Carbon Dioxide
COPD	Chronic Obstructive Pulmonary Disease
DECOS	Dutch Expert Committee on Occupational Standards
D/T	Dilution to Threshold
EHSRC	Environmental Health Sciences Research Center
EMB	Eosin Methylene Blue
EPA	Environmental Protection Agency
EU/ml	Endotoxin Units per milliliter
EU/m^3	Endotoxin Units per cubic meter air
EU/mg	Endotoxin Units per milligram
GC	Gas Chromatography
GM	Geometric Mean
Gm-	Gram-Negative
GSD	Geometric Standard Deviation
HICAHS	High Plains Intermountain Center for Agricultural Health and Safety
H_2S	Hydrogen Sulfide
IDLH	Immediate Danger to Life and Health
IOM	Institute of Occupational Medicine
L/min	Liters per minute
LOD	Limit of Detection
MEA	Malt Extract Agar
mg	milligrams
mg/L	milligrams per liter
mg/m^3	milligrams per cubic meter air
mg/ml	milligrams per milliliter

m/s	meters per second
μm	micrometers
NAAQS	National Ambient Air Quality Standards
ng/m^3	nanograms per cubic meter air
NH_3	Ammonia
NIDCD	National Institute on Deafness and Other Community Disorders
NIOSH	National Institute for Occupational Safety and Health
nm	nanometers
OSHA	Occupational Safety and Health Administration
PBS	Phosphate Buffered Saline
PEL	Permissible Exposure Limit
Pf	Pyrogen free
PM_{10}	Particulate matter less than 10 microns
PNOC	Particulate Not Otherwise Classified
ppb	parts per billion
ppm	parts per million
REL	Recommended Exposure Limit
rFC	Recombinant Factor C
SID	Standardized Ideal Digestibility
STEL	Short-Term Exposure Limit
TLV	Threshold Limit Value
TSA	Tryptic Soy Agar
TWA	Time-Weighted Average
USEPA	United States Environmental Protection Agency
WHO	World Health Organization