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Characterization of Dusts Collected from Swine Confinement Buildings

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As part of a project to evaluate health hazards for workers in swine confinement buildings, the air in 21 different buildings was sampled with 37mm cassette filters with and without cyclone preselectors and with cascade impactors. Filter results yielded a mean total aerosol of 6.3 mg/m³, a mean respirable aerosol of 0.5 mg/m³; the geometric mean diameter was 2.9 microns. Cascade impactor measurements revealed a mean total aerosol of 7.6 mg/m³, a respirable aerosol of 2.5 mg/m³ and a mass median diameter of 9.6 microns. The two major constituents in these aerosols were grain particles and dried fecal matter. The grain particles were larger than fecal particles and proportionately more abundant in finishing buildings where 50 kg-100 kg animals are housed. Therefore the respirable fraction was less in finishing buildings than in farrowing and nursery buildings. Culturing of settled dusts yielded six different mold species, with the highest counts for *Verticillium* sp. $(5\times10^2 \text{ cfu/mg dry dust})$ grown at 37° C. Thermophilic *Actinomycetes* and both gram negative and gram positive bacteria were isolated. Azocasein proteinase activity was found in most dust samples analyzed. This dust had a protein content of about 23% and a mean adsorbed ammonia content of 0.4%.

Introduction

A variety of respiratory conditions have been recognized in people who work in swine confinement buildings. (1) These conditions include bronchitis, reversible airways obstruction and symptoms similar to either hypersensitivity pneumonitis or organic dust toxic syndrome. (2-6) The major environmental contaminants in this work place include toxic and irritating gases and aerosolized particles. (1,2,7-12) We feel the observed human health effects result from the combined effects of inhaled dusts and gases. (1,2) The gas problem has been evaluated by several researchers qualitatively and quantitatively to the extent that exposures to ammonia, hydrogen sulfide, carbon monoxide, carbon dioxide and methane are defined reasonably well. (1-3,7,8) Little research has been done, however, to characterize the dust in these buildings from a human health standpoint.

One researcher found total dust aerosols in the air of swine confinement buildings on four farms ranged from 1.2 to 6.7 mg/m³ (mean = 3.4) with a respirable fraction of 15%. ⁽²⁾ In another study the respirable fraction was measured at 50%. ⁽¹⁾ Although these levels did not exceed standards for nuisance dust, there is substantial evidence that this dust contained biologically active components such as large quantities of microbial agents, ⁽¹²⁾ endotoxins, ⁽¹³⁾ and protein, ⁽⁷⁾ suggesting the need for lower exposure limits than the nuisance dust standard. ⁽⁷⁻¹⁰⁾ It is quite difficult to make generalizations from the literature relating dust levels and livestock health problems. The published reports are few and scattered; there

was no standardization of sampling procedures, sampling locations or sampling volumes.

This study was an attempt to characterize more systematically the physical and chemical properties of the dust in swine confinement atmospheres.

Methods

Measurements of Mass

Area dust samples were collected in 21 randomly selected swine confinement operations from a 27-county area in eastern Iowa. This group of buildings was a subset of a larger probability sample of Iowa swine operations (n=2100) which was stratified by county, size of operation and the degree of respiratory symptoms reported by workers on a screening questionnaire. (4) The environmental studies were carried out during the months of December, January and February. Dust samples were collected on preweighed 37mm polyvinyl chloride membrane filters, using low volume personal sampling pumps (Model G Pump, Mine Safety Appliance, Pittsburgh, Pa.) precalibrated to 1.7 L/min. Total particles were collected in close-faced cassettes; respirable fractions were collected through 10mm cyclone preselectors. A total of six pairs of total mass and respirable fractions were collected side-by-side in a grid fashion to cover the entire working area of each building.

Aerodynamic size characteristics of particles in swine buildings were measured by either a Gelman 7-stage cascade impactor (Gelman 1 ACFM Particle Fractioning Sampler, Gelman Scientific, Ann Arbor, Mich.) or Andersen 8-stage cascade impactor (Andersen 1 ACFM Ambient Particle Sizing Sampler, Andersen Samplers, Inc., Atlanta, Ga.). These samplers were located at breathing zone level approx-

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TABLE I
Mass of Aerosolized Particles in Swine Confinement Buildings

Personal Sampler	Farrow(n=9)	Nursery/Grower(n=8)	Finish(n=4)	All Buildings(n=21)
Mean Total Mass (mg/m³)	3.15	5.2	15.3	6.25
Geometric Standard Deviation	1.6	1.4	1.4	1.6
Mean Respirable Mass (mg/m³)	0.34	0.37	0.92	0.53
Geometric Standard Deviation	1.35	1.2	3.4	2.0
Respirable Fraction (percent) ^A	10.7	7.1	6.0	8.4
Cascade Impactor				
Mean Total Mass (mg/m³)	4.1	10.8	9.0	7.6
Mean Respirable Mass (mg/m³)	2.7	2.0	3.2	2.5
Geometric Standard Deviation	3.45	1.75	3.9	2.7
Respirable Fraction (percent) ^B	20.1	13.4	12.4	10.2
Mass Median Diameter (microns)	8.95	9.8	10.7	9.6

^ARation of simultaneous side-by-side dust collections, with and without cyclone preseparator.

imately in the center of the work area. Mass median diameters were calculated from the distribution of weights of the samples in the various stages of the impactor.

The mass of respirable fractions also was calculated by summing the products of the percent lung deposition [in accordance with the ACGIH curve⁽¹⁵⁾] at the stated aerodynamic cut-off diameter of each impactor stage (as specified by the manufacturer), times the mass of particles on each stage, as expressed in the following equation:

$$R_{\text{mass}} = \sum_{i=1}^{8} \frac{f_i \ m_i}{m_i}$$

Where: R_{mass} = mass of respirable fractions;

f_i = percent deposition of particles (according to the ACGIH curve) at the stated aerodynamic 50% cut-off diameter (according to the manufacturer);

m_i = mass of particles collected at each stage (subscripted i).

Evaluation by Light Microscopy

Dust samples from four confinement buildings were collected in closed-faced cassettes with PVC membrane filters and in the cascade impactor on Whatman No. 41 filter pads. Sampling pumps were run a variable period of time (from 2-8 min) just until a brown stain developed on the filter membrane below the inlet port on the 37 mm cassettes.

Dust samples were mounted and examined by light microscopy, in which a slight modification of a previously described standard method was used. (16) Clear cellophane adhesive tape was pressed firmly on the surface of either the 37mm PVC or Whatman 41 filters used to sample air in the buildings. We attempted to sample a representative area of the filter, including the central brown-stained area of

cassette filters where dust was concentrated plus about a 10 mm area on either side of the center. Then the tape was mounted on glass slides, and the particles were examined with a light microscope (Model Starphase Light Microscope, American Optical, Buffalo, N.Y.) using an immersion oil (Cargille immersion oil) with the same refractive index as the respective filter material (the refractive indices were paper-1.54, glass-1.52, and membrane-1.51). Dust particles from the total sample and from each stage of the cascade impactor were sized by light microscopy, measuring their projected area with a porton reticle (BGI Inc., Waltham, Mass.) in the ocular of the microscope. The reticle was calibrated (separately with each objective used) with a stage micrometer (BGI Inc., Waltham, Mass.). The particles also were analyzed using polarized, phase contrast (Model Phasestar Light Microscope, American Optical, Buffalo, N.Y.) and fluorescent light microscopy. The various components were identified by comparing them to known standards, which were prepared in the laboratory, and to published photographs of known particles. (16) Iodine and Nile blue sulfate stains were used as adjuncts in identifying starch granules and fecal material, respectively.

Analysis for Chemical and Microbial Components of Dust

Settled dust samples taken from swine confinement operations were analyzed for adsorbed ammonia, urea and total protein nitrogen using standard microdiffusion methods. Quality control was maintained by running duplicate samples with standard protein solutions.

Proteinase activity on dust samples from six different swine finishing buildings was determined using the azocasein assay. (18) An azocaseinolytic unit is that activity which hydrolyzes 1 mg of azocasein per hour under the given assay conditions.

Fungi, actinomycetes and bacteria were isolated from a pooled sample of settled dust from three finishing buildings

^BRatio of the sum of the respirable fraction of the mass collected at each stage, to the total mass.

as well as on two additional samples from individual buildings. Quantitative estimates of fungi were obtained by using the pour plate technique⁽¹⁹⁾ on agar malt, yeast extract agar (malt extract, 3 g; yeast extract, 3 g; peptone, 5 g; glucose, 10 g; agar, 20 g; 1000 mL distilled water, pH 6.0) containing 50 μ g/mL chloramphenicol to suppress bacterial growth. After incubation at 25° C, 37° C and 50° C, colonies were counted and identified by standard morphological criteria. Quantitative estimates of actinomycetes and bacteria were obtained by using the spread plate technique⁽¹⁹⁾ on nutrient agar (Oxoid USA, Inc., Columbia, Md.) plates containing 50 μ g of cycloheximide per mL to suppress fungal growth. After incubation at 37° C and 55° C, colonies were counted. The identity of actinomycetes was determined morphologically and bacteria were differentiated by gram staining.

Results

Measurements of Mass and Size

Compared to a desirable work environment, the aerosol concentrations were relatively high in the buildings (Table I). For measurements taken with 37-mm cassettes, the mean total aerosol concentration for all buildings sampled was 6.25 mg/m³; however, the total aerosols varied according to the use (type) of building. The farrowing buildings had the lowest mean amounts at 3.2 mg/m³, followed by growernursery units at 5.2 mg/m³ and finishing units at 15.3 mg/m³ (t-test comparisons among farrowing vs. finishing, p \leq .01; grower vs. finishing, p \leq .05). Based on cassette filters with and without cyclone preseparators, the mean respirable fractions consistently decreased from farrowing (10.7%) to nursery (7.1%) to finishing building (6.0%) (t-test, .05 < p < .10). Thus, a larger proportion of the particles were of respirable size in buildings that housed the younger animals.

For measurements taken with the cascade impactor, we again found a larger percent respirable fraction in buildings housing younger animals; farrowing buildings had 20.1% respirable fraction, followed by nursery-grower buildings (13.4%) and finishing buildings (12.4%) (Table I) (t-test, farrowing vs. growing and farrowing vs. finishing, $p \le .05$). These values, however, were approximately twice the values of respirable particles as measured by the cassette sampler with and without cyclone preselector (paired t-test, p < .025). All of the reasons for this difference are not clear, but one reason could be particle bounce and reentrainment in the cascade impactors.

The mean mass median diameter of particles for all buildings as measured by the cascade impactor was 9.6 microns (Table I). There was a difference in mass median diameter between building types similar to that indicated by cyclone separation (paired t-test, farrowing vs. finishing, $p \leq .05$). Sizing by light microscopy (Figure 1) showed the particles collected from the air of a swine confinement nursery to be a log-normal polydisperse aerosol with a geometric mean of 2.9 microns and geometric deviation of 3.4. The corresponding calculated mass median diameter is approximately 50 microns. The results of size analyses for each stage of the cascade impactor filters from four finishing confinement buildings are given in Table II. The apparent variation in

particle density with diameter is insufficient to account for the different mass median diameters resulting from the two methods.

Evaluation By Light Microscopy

Qualitative microscopic analysis of swine confinement aerosols revealed them to be heterogeneous in nature with a great diversity of shape and composition. Some of the components identified included the following:

- 1. feed: starch granules, grain meal, trichomes and corn silk
- 2. swine fecal material (included bacteria, gut epithelial cells and undigested feed)
- 3. swine dander
- 4. mold: hyphae, spores and sporangia
- 5. pollen and grains
- 6. insect parts
- 7. mineral ash

Feed components and fecal material composed the bulk of the collected particles. The size ranges of the feed dust and swine dander were such that they were largely collected by the Preselector and Stage 1 of the cascade sampler. These

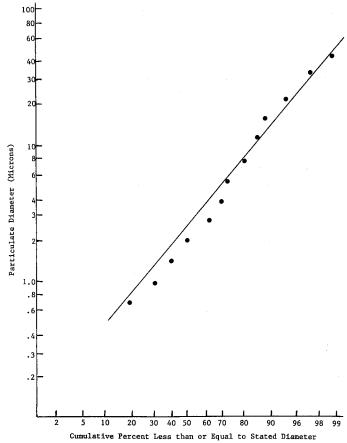


Figure 1 — Size distribution of 600 aerosolized particles $^{\rm A}$ measured by light microscopy, $^{\rm B}$ in a swine confinement nursery.

^AParticles were collected on a pvc membrane filter in a closed-face 37 mm cassette using a low-volume air pump.

^BAmerican Optical Model Trans Star Microscope affixed with a porton reticle.

TABLE II
Size Measurement by Light Microscopy of Aerosolized Particles
Collected by a Cascade Impactor in Four
Swine Confinement Buildings

Andersen Sampler Stage	PS ^A	1	2	3	4	5	6	7
Diameter, microns								
Observed Geometric Mean ^B	9.7	5.9	3.6	2.0	1.6	0.9	0.5	0.4
Expected Mean ^c	9.8	5.8	4.7	3.2	2.0	1.05	0.7	0.35
Observed Geometric Standard Deviation	2.3	2.0	2.0	1.8	1.7	1.7	1.6	1.4
Apparent Density ^D	1.01	0.99	1.14	1.26	1.12	1.08	1.18	0.94

^APreSeparator.

stages correspond with deposition in the nose and pharynx. Particles of fecal origin were found on all stages from the Preselector to Stage 6 (the latter corresponds with sedimentation in the respiratory alveoli), but fecal material composed the major component of Stages 3, 4 and 5. Fecal material took Nile blue sulfate (a fatty acid stain) readily and fluoresced distinctively when examined under ultraviolet microscopy.

Analysis for Chemical and Microbial Components of Dust

The predominant organisms isolated were mesophilic gram positive bacteria $(1.3 \times 10^3 \text{ cfu/mg})$ with gram negative bacteria and mesophilic fungi present in tenfold lower amounts. The major fungal species isolated were *Penicillium*, Fusarium and Verticillium. Although the thermophilic actinomycete species Thermoactinomycetes candidus, Micropolyspora faeni and Saccharomonospora viridis were isolated, they were present in very low amounts. Results from the two additional dust samples from other buildings were similar to those from these pooled samples in that the predominant organisms were mesophilic gram positive bacteria (1.9×10^3) and 4.8×10^3 cfu/mg dust) with gram negative bacteria and fungi present in tenfold lower amounts. The major fungal species were Alternaria, Fusarium, Cladiosporium, Aspergillus flavus and Scopulariopsis. Although low levels of Streptomyces sp. were isolated from two of the samples, no thermophilic actinomycetes were isolated. Proteolytic activity was detected in five of the six dust samples examined and ranged from 0.000 to 0.164 units/mg dust with a mean of 0.046 units/mg.

Microdiffusion analysis (16) of dust samples, in which an ammonia specific electrode was used, detected levels of ammonia gas adsorbed to the particulates in a range of 2.5-4.0 mg N/gm dust (mean = $3.9 \pm 0.25 \text{ mg N/gm dust}$). A

modified micro-Kjeldahl technique⁽²⁰⁾ then was used to determine protein nitrogen values on the same samples. Protein nitrogen values ranged from 28.1 - 42.0 mg N/gm Dust (mean = $36.1 \pm 3.8 \text{ mg N/gm}$ Dust). The average crude protein value was calculated to be 22.6%.

Discussion

The total aerosols measured exceeded the TLV for total nuisance dust but not for respirable nuisance dust in half of the finishing buildings evaluated. Farrowing and nursery-grower buildings rarely exceeded the TLV for nuisance dust. Aerosol limits for nuisance dust, however, probably are not adequate here. This dust contains foreign protein, grain dust, insects and insect parts, fecal dust, bacteria, mold spores, proteinase and possibly other biologically active substances. The presence of adsorbed ammonia complicates assessment of the potential health hazards of this dust. Irritation was reported at dust levels of 2 mg/m³. (3) Research needs to be done to determine the lower limits of aerosols and gases necessary to prevent an adverse response.

It is interesting to note that the results of both the 37mm cassettes and cascade impactors indicated that both the total aerosol concentration and the aerodynamic particle size increased from farrowing to nursery-grower to finishing buildings. Gross examination of the aerosolized dust collected in farrowing and nursery grower buildings revealed a fine, dense and blackish-appearing material, while dust in the finishing units was a more fluffy, coarse, tannish-appearing material. We speculate that these differences are a result of the relatively greater ratio of feed to fecal components of dust in the finishing vs. farrowing and nursery buildings. The finishing units use more feed than do the farrowing and nursery buildings; the ground feed often may be dropped several feet from an auger to the feeder resulting

^BProjected area diameters measured by light microscopy.

^CEffective cut off diameter, as published by the manufacturer of the cascade impactor; *Operating Manual for Andersen Samplers Inc.*, revised January 1979, Andersen Samplers Inc., 4215-C Wendell Drive, Atlanta, GA 30336.

^DApparent density = (expected mean size)²/(observed geometric mean)², based on equivalent aerodynamic and stokes diameters.

in significant dust generation; and feed is more likely to be spilled from the feeders onto the floor. The proportionately higher intrinsic concentrations of infectious organisms and endotoxins expected within fecal material vs. feed suggests that the same threshold aerosol concentration to avoid adverse lung response may not be applicable to all production stages. This theory needs further investigation.

Statistical analysis of size data obtained by light microscopy showed that 66% of the total particulates were less than 4.6 microns in diameter and within the respirable range. This finding correlates well with other reports that 30% to 90% of particles in confinement aerosols are less than 5 microns in diameter. (8,10) Clearance of these respirable particles generally is by alveolar macrophage ingestion and lymphatic drainage, (21,22) which may involve hypersensitivity pneumonitis and/or organic dust toxic syndrome as a tissue and systemic response.

Closed-faced cassettes were employed to protect the sample from flies and other foreign debris that may become temporarily airborne as a result of worker or animal activity. Therefore, a possible criticism of our methods is that the light microscopy particle sizing may have been conducted on non-representative size particles. Particles — particularly those with greater mass — tend to pile up and agglomerate directly under the inlet port. We do not feel this was a significant problem, however, because the sampling pump was run only long enough to have a sample that just was visible, not a large pellet. Thus, the problems of particle overlap and agglomeration were kept to a minimum. In addition we examined and counted the peripheral areas of the filter as well as the center portion. We observed no apparent bias of particle sizes across the filter.

The presence of 3.9 mg adsorbed ammonia per gm of dust carried by these particles into the respiratory system could be an important consideration in evaluating their potential health effects. Because ammonia is water soluble and usually absorbed by mucous in the upper respiratory tract, the lungs may be protected from the effects of exposure to moderate levels of ammonia in the vapor phase. (23) As particles are inhaled, however, adsorbed ammonia would be delivered to the deeper, more sensitive portions of the pulmonary system. The irritant effects of ammonia also may increase the inflammatory response from the inhaled aerosol.

Qualitative analysis of the dust collected from four buildings on the cascade impactor stages 3-7 showed it to comprise largely fecal particles (ranging from 2 to $0.4~\mu m$ diameters). It also is interesting to note in Table II the apparent density of these smaller particles as compared to larger particles. Since particles of 2.0 - 0.4 microns have maximal deposition in the pulmonary alveoli, it appears that swine fecal materials (which include enteric bacteria and epithelial gut cells) constitute the major dust burden to the alveoli. It has been reported previously that workers exposed to laboratory animals may experience adverse health effects, including allergic reactions and direct irritation in response to inhaled animal proteins. (23) It is felt that confinement opera-

tors may have similar symptoms after inhaling swine proteins suspended in the air of confinement buildings.

Further study of the cascade impactor series showed the feed grain dust in the buildings to be filtered largely onto the preselector and first stage. Impaction at these levels corresponds with deposition on the skin, in the nose and in the pharynx. Most of the feed dust is coarse, settles quickly and makes up the bulk of the nonrespirable fraction. It has been observed that the dust concentration in confinement buildings with wet feeding systems does not differ appreciably from that of barns in which dry feeding systems are used. Furthermore, a study by Bundy indicated that dust levels are related to animal activity rather than feeding methods. Therefore, it appears that the bulk of the respirable matter in confinement aerosols consists of fecal particles from the animals and is generated by movement of the animals.

The protein content of this dust was 23%, which is consistent to that reported in a previous study. (6) Certainly this substantiates the suspected allergenic potential of this dust. The swine house dusts examined here contained multiple components of potential biological relevance: bacteria, fungi and actinomycetes as well as proteolytic enzymes. Whether these components are present in significant amounts and whether workers are exposed to significant levels to account for the observed respiratory problems remains to be determined. The microbial content of these swine house dusts is 3-4 orders-of-magnitude lower than that reported for moldy silage dust associated with pulmonary mycotoxicosis and farmer's lung disease. (25) The minimum levels relevant for sensitization or complement activation within the lung and its airways, however, have not yet been established. Proteinase levels in these swine house dusts were only ten times lower than those reported for moldy silage dusts; (26) however, biologically relevant amounts necessary for release of inflammatory mediators within the lung is not yet known.

Both gram positive and negative bacteria previously have been found in association with swine confinement dust. (12) This is not surprising since fecal material appears to be a major constituent of this dust and bacteria may make up as much as 50% of fecal solids. (27) Although we did not analyze for endotoxins, gram negative bacteria are a major endotoxin source, and endotoxins have been reported before in swine house dust. (13) It has been hypothesized that these endotoxins may be responsible for some of the adverse health effects seen in exposed workers. (28)

We note that analyses for protein, adsorbed ammonia and microbial content were conducted on settled dust. Other recent investigations in Sweden⁽²⁹⁾ found that airborne dusts were qualitatively similar to these settled dusts in terms of the relative microbial distributions (Table II); however, the actual concentrations of each organism were higher than reported herein. Therefore, these measurements on settled dust should be considered as a screening procedure, not a replacement for evaluations on aerosolized samples. In fact adsorbed ammonia and amount of gram negative bacteria (and endotoxin) may be greater in aerosolized dust because of its large component of fecal particles.

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TABLE III

Fungae and Bacterial Isolations from Pooled Samples of Settled Dust from Three Swine Confinement Buildings

Organism	Colony Forming Units/Per mg Dry Dust	(Percent Within Group)	Percent Overall
Fungi 25° C - total	3.94 × 10 ²		16%
Penicillium sp.	2.46×10^{2}	(63%)	
Fusarium sp.	1.16×10^2	(30%)	
Aspergillus flavus	0.16×10^{2}	(4%)	
Scopulariopsis sp.	0.16×10^2	(4%)	
Fungi 37° C - total	6.10×10^2		25%
Verticillium sp.	5.00×10^{2}	(82%)	
Phycomycete sp.	1.10×10^2	(18%)	
Fungi 50° C - total	1.5		<0.1%
Penicillium sp.	0.75	(50%)	
Paecilomyces sp.	0.75	(50%)	
Bacteria 37° C - total	1.4×10^{3}		58%
Gram positive	1.3×10^{3}	(96%)	
Gram negative	0.1×10^3	(4%)	
Actinomycetes 37° C None isolated			
Bacteria 55° C - total	4.0		<0.1%
Actinomycetes 55° C - total	11.0		<0.1%
Thermoactinomyces candidus	5.0	(45%)	
Micropolyspora faeni	4.0	(36%)	
Saccharomonospora viridis	2.0	(18%)	

Finally, we note that the complexity of these aerosols makes it difficult to anticipate their long range health effects on workers. The combination of toxic gases, mold spores and the other heterogeneous components of these aerosols may have the potential for causing a synergistic effect or additive effect, as compared to the effects of the individual elements. Further studies are indicated to assess the possible health effects on a prospective basis.

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