Discussion paper

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Quantitative exposure assessment of organic dust

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Organic dust consists of particles with a biological origin. Airborne levels of bacteria, fungi, endotoxins, and glucans have been investigated in epidemiological studies of mucous membrane irritation, inflammation, and airway obstruction. Organic dust can be measured by gravimetry of filter samples. Microorganisms can be quantified by culture, microscopic, and DNA-based methods. Culture methods underestimate microbial exposure and have poor precision. However, high sensitivity and identification of species that indicate fungal contamination have advantages in indoor air studies. Microscopic and DNA-based methods quantify microorganisms independent of cultivability. Specific organisms can even be quantified with molecular techniques. Quantification of specific organic dust components is preferred to dust levels. However, no occupational exposure limits exist for specific agents, although criteria for endotoxin and fungal spores have been proposed. In exposure assessments of microbial agents, larger numbers of samples may be needed compared to chemical agents because of higher exposure variability.

Key terms endotoxin; fungal spore; measurement method; measurement strategy; microorganism; work environment.

The term "organic dust" is often used synonymously with "bioaerosol" in the occupational health context and can be defined as "particles with biological origin suspended in air". Particles are often fragments of larger organisms or products thereof, such as wood dust, flour, textile fibers, paper fibers, paper dust, skin scales, hair, body fragments, evaporated urine droplets, and faeces. Microorganisms, such as fungi, bacteria, and viruses, are small enough to remain airborne once they have been dispersed into the air, and can be found as single organisms and aggregates of cells or spores. Microorganisms, especially bacteria, can even be attached to the substrate they colonize.

As microorganisms are subjected to strain from desiccation when dispersed into the air, a large proportion of airborne microbial cells are dead and cell debris is probably also common. Bioaerosols can be very complex; for example, grain dust may contain fragments from grain, husk and straw, soil particles, pollen, bacterial spores and cells, fungal spores and hyphae, fragments and faeces of storage mites and insects, and microbial components such as endotoxins, glucans, peptidoglycans, mycotoxins, antigens, and allergens. Figure 1 shows examples of particles that have been found in bioaerosols.

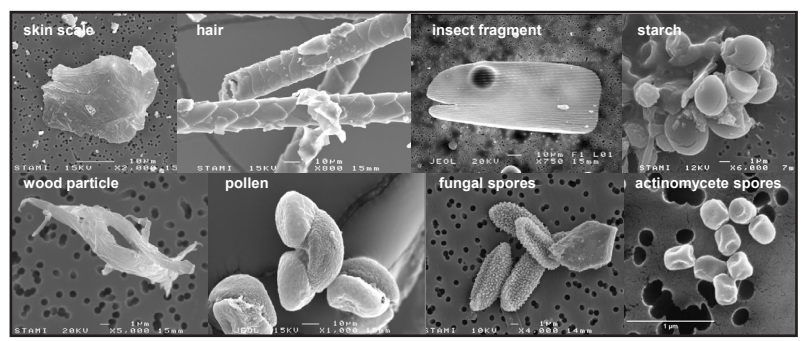

Figure 1. Examples of organic dust particles viewed through a scanning electron microscope. Micrographs by Asbjørn Skogstad and Lene Madsø, National Institute of Occupational Health, Oslo, Norway.

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Organic dust has been studied in many epidemiological studies because exposure may cause mucous membrane irritation in the eyes and upper and lower airways, inflammation by allergic and non-allergic mechanisms, and airway obstruction (1). Table 1 gives an overview of dust types that have been associated with respiratory disease.

Fungi need an organic substrate, oxygen, and water for growth. Bacteria can also grow on inorganic compounds and may use sulfate and nitrate as an oxygen source, which are transformed into hydrogen sulphide and ammonia, as well as water. As the first two requirements usually are met, access to water is the main limiting factor for microbial growth. The proliferation of microorganisms can be extremely rapid. Some bacterial species can multiply by cell division every 20 minutes; under ideal conditions, 17 million cells would result from a single viable cell after eight hours in culture. Some fungi can complete a lifecycle from one spore to a sporulating colony, producing millions of spores within 24 hours. Thus, under favorable conditions, microbial contamination can develop extremely fast. This property is distinct from other biological and chemical agents.

It is therefore not surprising that microorganisms are common contaminants of plant and animal products, and are likely to be found in workplaces where such materials are grown, processed, and handled. Bacteria, fungi, and their components endotoxin and glucans, have been the focus of many occupational studies. In contrast, few studies have been published on viruses, amoebae, and mites. The bacteria include two major groups: gram-positive (G+) and gram-negative (G-) bacteria. Their names reflect that the two groups are stained differently by a technique developed by the Danish bacteriologist Hans Christian Gram. It was later found that the cell wall of the G- bacteria contains endotoxins that are potent inflammatory agents not present in the cell walls of G+ bacteria. The cell walls of both groups contain peptidoglycans, which also have inflammatory properties. As the cell walls of G+ bacteria are thicker, these bacteria contain more peptidoglycans than the G- bacteria. The actinomycetes resemble the fungi in morphology and they also can produce numerous spores, but they belong to the G+ bacteria and reproduce only by asexual spores and hyphal growth. Some fungi may excrete mycotoxins in the substrates that they colonize. These toxins can be extremely toxic, for example aflatoxin B1 is the most toxic compound known at present (2). Mycotoxins may also be found in the spores, for example, citrinin in spores from *Penicillium verrucosum* (3).

Table 1. Non-infectious respiratory diseases associated with organic dust. (ODTS = organic dust toxic syndrome = inhalation fever)

Agent	Respiratory diseases
Cotton dust	Byssinosis, airway obstruction
Grain dust	ODTS, airway obstruction
Wood dust	Asthma, respiratory cancer
Flour dust	Rhinitis, allergy, asthma
Laboratory animal dust	Rhinitis, allergy, asthma
Fungal spores	Chronic bronchitis, rhinitis, airway obstruction, ODTS, allergic alveolitis
Endotoxins	Chronic bronchitis, ODTS, asthma
Pollen	Hayfever
Allergens (eg, house dust mites, α-amylase, rat urine and shellfish)	Rhinitis, allergy, asthma

Measurement methods

Aerosols originating from plant material are often measured by gravimetry of filter samples. The composition of the aerosol can be evaluated qualitatively by considering the processes that generate the aerosol. Additional information can be obtained by viewing particles with a microscope and ashing filter samples assuming that the weight loss is due to organic material. The latter is not a perfect measure, however, because inorganic substances may lose water and/or may gain weight by oxidation. Pollen are preferentially counted by light microscopy but have been quantified in only few occupational studies. The exposure to pollen in indoor environments is expected to be low because pollen enter by outdoor air and are usually removed by filtration in ventilation systems or cracks and crevices in the building envelope. Pollen also settle relatively quickly due to their relative large size, typically 10–100 µm in diameter. Light microscopy can also be used for identification of mites, but this is only performed in fine or floor dust samples because mites are fairly large, typically 300 µm in size and they settle very quickly if dispersed into the air. However, mite fragments and faeces can be found in air samples, but the latter are morphologically difficult to recognize. Instead, mite allergens can be detected by immuno-chemical methods as these allergens are particularly concentrated in faecal pellets.

Microorganisms can be quantified by culture, microscopic, and DNA-based methods. Some culture methods collect particles directly by impaction on semi-solid nutrient plates, which are cultivated under standardized conditions. The colonies that grow on the plates are counted and results are given as colony-forming units. Samples can also be collected with impingers into a liquid or on filters. Sampling of microorganisms by impaction, impingement (impaction into a liquid), and filtration subject microorganisms to strain that may lead to a loss

of culturability, especially the vegetative cells of bacteria, while spores from moulds and bacteria are more robust. Filter samples can also be dispersed in a liquid prior to cultivation. The resulting suspension can be diluted before culture and subsamples can be cultivated under different conditions, allowing different organisms to be detected. It should be noted, however, that aggregates may disperse when collected or resuspended in a liquid leading to higher colony counts than methods with direct cultivation of the collection plates.

The most important advantages of the culture methods are high sensitivity and their potential to identify cultivable organisms. These features are important in studies of fungal contamination of indoor environments where fungal concentrations are low and the presence of species like *Aspergillus versicolor* indicates indoor fungal growth. Viability is crucial in the study of infectious organisms. The main disadvantages of culture methods are poor precision and a highly variable underestimation of exposure. The underestimation depends on sampling strain, microbial robustness, and the size of aggregates that may grow only into one colony. Furthermore, aggregates may break up when dispersed in a liquid before samples are cultivated, and number of colony-forming units depends on culture conditions, nutrient medium, and the presence of other species. Results based on cultivation are, therefore, at best semi-quantitative (4).

Inhalation of non-cultivable or dead microorganisms may cause similar health effects as cultivable microorganisms, although viable invasive microorganisms probably represent higher health risks than non-viable organisms. Culture methods highly underestimate microbial exposure because a large proportion of the microorganisms cannot grow in culture. Therefore, microscopic and molecular biological methods have been developed that can quantify microorganisms independent of cultivability (4, 5).

Fungal spores can be recognized by the morphology of spores and spore aggregates, and counted with light and scanning electron microscopes, but the ability of these methods to discriminate between species is limited – although some species and families can be recognized. Even spores of the actinomyces that are smaller than fungal spores can be quantified with scanning electron microscopes. Bacterial cells show fewer morphological features, but can be counted with a fluorescence microscope after staining with a fluorochrome.

Molecular techniques such as polymerase chain reaction (PCR) and DNA hybridization have provided significant advances in rapid detection and identification of microorganisms, irrespective of their viability or cultivability. By the multiple synthesis of specific DNA fragments in vitro, microbial DNA can be quantified and identified to genus, group, or species level, depending on the reagents used (5). Although these methods have, so far, not been frequently applied in occupational exposure studies, specific reagents for the detection of a great number of occupationally relevant microorganisms are available.

An important difference is that both molecular biology and culture methods quantify spores and hyphae jointly, whereas microscopic methods can differentiate between spores and hyphae, although only few studies have reported hyphal counts (6). An overview of recommended measurement methods for microorganisms is given in table 2.

Microbial metabolites have been quantified because of their toxic potential. Endotoxin is the organic dust agent that has been most often measured in epidemiological studies. It is commonly measured by a kinetic chromogenic limulus amoebocyte lysate (LAL) assay that utilizes enzymes extracted from amoebocytes from the horseshoe crab (7). As dust samples are extracted before analysis, only the water-soluble endotoxins are detected. There are also substantial differences between analytical results of replicate samples analyzed by different laboratories even when they use the same methods and reagents (8, 9). However, the LAL method has high sensitivity; exposure–response associations have been found with endotoxin detected by this method in numerous studies. The (1→3)-β-D-glucans are regarded as markers of fungi, although such glucans can also be found in some bacteria and plants, and have inflammatory and immune-modulating properties. Glucans can be detected by a variant of the LAL assay and an enzyme immunoassay (1). Other metabolites are the mycotoxins and chemical markers. Mycotoxins are often extremely toxic compounds, but the exposure can be difficult to quantify in personal samples because their concentrations in air are low. Very few measurements of airborne mycotoxins have been reported thus far (5). Some chemical markers (eg, ergosterol for fungi,

Table 2. Recommended methods for quantification of airborne microorganisms. (SEM = scanning electron microscopy; LM = light microscopy; FM = fluorescence microscopy; PCR = polymerase chain reaction)

Agent	Optimal methods
Microorganisms (cultivable and non-cultivable)	
Fungal spores	SEM, LM, FM
Fungal hyphae	SEM, LM
Fungal spores and hyphae	PCR
Actinomycetes	SEM, PCR
Bacteria	FM, PCR
Specific organisms at low concentrations	
Indoor air, infectious organisms	PCR, culture

3-hydroxy fatty acids for endotoxin and G- bacteria, and muramic acid for G+ bacteria) are not particularly toxic, but can be used as surrogate measures for microbial groups.

Allergens and antigens can originate from plant and animal sources and, to some extent, from microorganisms. These components of organic dust can be detected by enzyme immunoassays after they have been extracted from the filter. An overview of measurement methods for microbial metabolites and allergens is shown in table 3. These methods have not yet been standardized. Rat and mouse allergen levels, analyzed in parallel samples by different immunochemical methods, differed by up to 3000-fold. However, results obtained by different laboratories using the same method were more similar and differed only by a factor of 2 (10).

Measurement strategies

At first, the agent(s) of interest must be selected. Measurement of the aerosol mass has been used in many studies and can provide useful results if the dust composition is similar throughout a workplace or an industry. For example, in the furniture industry, it would be a reasonable assumption that most of the dust involved in many jobs consists of wood particles. However, it cannot be generally assumed that the composition of the dust does not vary within an industry. For example, the toxicity of the dust in a cotton textile factory processing raw cotton depends on the bacterial contamination of the raw material, while the dust is much more toxic at the start of the production line than at the end. This is because the likely causal factor of lung function loss in cotton workers is endotoxin, which is gradually removed during the process from cleaning and carding to spinning of cotton yarn.

Table 3. Methods for quantification of microbial metabolites. (LAL = limulus amoebocyte lysate assay; EIA = enzyme immunoassay; GC–MS = gas chromatography–mass spectrometry; LC–MS = liquid chromatography–mass spectrometry; HPLC = high performance liquid chromatography).

Metabolite	Origin	Method
Toxins		
Endotoxin	Gram-negative bacteria	LAL
Glucans	Fungi (also in bacteria and plants)	LAL, EIA
Mycotoxins	Fungi	GC–MS, LC–MS, EIA, HPLC
Allergens		
Amylase	Aspergillus sp	EIA
Chemical markers		
Muramic acid	Gram-positive bacteria (some in gram-negative bacteria)	GC–MS
Ergosterol	Fungi	GC–MS
3-hydroxy fatty acids	Gram-negative bacteria	GC–MS

The measurement of specific agents in organic dust is expected to provide a better assessment of relevant exposure. Prior knowledge of likely causal agents and their risk levels is needed. Until now, occupational exposure criteria have only been evaluated for endotoxin and fungal spores. For endotoxin, occupational exposure limits of 50–100 endotoxin units/m^3 have been proposed based on the lowest observed effect levels for decline in the forced expiratory volume in one second (FEV_1) (11, 12). For fungal spores, the lowest observed effect levels for respiratory symptoms, FEV_1 changes, and airway inflammation were approximately 10^5 spores/m^3 for most fungi except mycotoxin-producing and invasive species that were more toxic in both in vitro and in vivo studies (13). For allergens from flour in bakeries, laboratory animals, and enzymes in the food industry, exposure–response associations have been found in toxicological and epidemiological studies. However, the use of different methods or reagents in exposure assessment, complicates comparisons between studies and the interpretation of the exposure levels. Nevertheless, assessments of allergen exposure may be feasible if the same measurement methods and reagents are applied that have been used in the studies performed for comparison purposes. Tables 4 and 5 give suggestions for agents that may be relevant for inclusion in exposure assessments in the food industry and other industries, respectively.

A basic problem in quantitative exposure assessment is the variability of exposure. As an example, stationary measurements of fungal spores with a sampling time of eight hours, at a fixed location in a sorting plant for timber, showed day-to-day variations from $<0.1 \times 10^6$ to 20×10^6 spores/m^3 (figure 2). However, the exposure variability of microbial agents can be even greater because microorganisms may proliferate rapidly during favorable conditions.

Day-to-day variability represents less problems in epidemiological studies of short-term effects where the mean exposure during the entire study period that is related to the observed effects can be measured. However, exposure measurement of all exposed time – in occupational epidemiological and hygiene studies of longer duration – is not feasible, and exposure has to be estimated from a limited number of measurements. A group-based strategy is usually chosen in order to limit costs. This strategy assumes that workers performing similar jobs also have similar exposure levels and that measurements performed on a subset of the workers are valid for the other workers within the group. Then

Table 4. Specific agents that can be present in organic dust from the food processing industry.

Production/industry	Fungal spores	Bacteria	Actionomycetes	Endotoxin	Allergens	Enzymes
Agriculture	✔	✔	✔	✔	✔	
Grain silo	✔	✔	✔	✔	✔	
Bakeries	✔				✔	✔
Breweries	✔					
Cheese	✔					
Mushroom	✔		✔			
Fruit/vegetable sorting	✔					
Potato flour		✔		✔		
Sugar			✔			
Seafood		✔		✔	✔	✔

Table 5. Specific agents that can be present in organic dust from industries other than food processing.

Production/industry	Fungal spores	Bacteria	Actionomycetes	Endotoxins	Allergens	Enzymes	Other
Sawmill	✔	✔	✔	✔	✔		Wood dust
Furniture					✔		Wood dust
Biotechnology	✔	✔			✔	✔	
Detergents					✔	✔	
Waste water treatment		✔		✔			
Renovation	✔	✔		✔			
Composting organic waste	✔	✔	✔	✔			
Laboratory animals		✔		✔	✔		
Humidification (eg, printing office, textile factory)	✔	✔	✔	✔			Amoebae
Library (water damaged books)	✔	✔		✔	✔		
Mechanical industry (cutting fluids)		✔		✔			

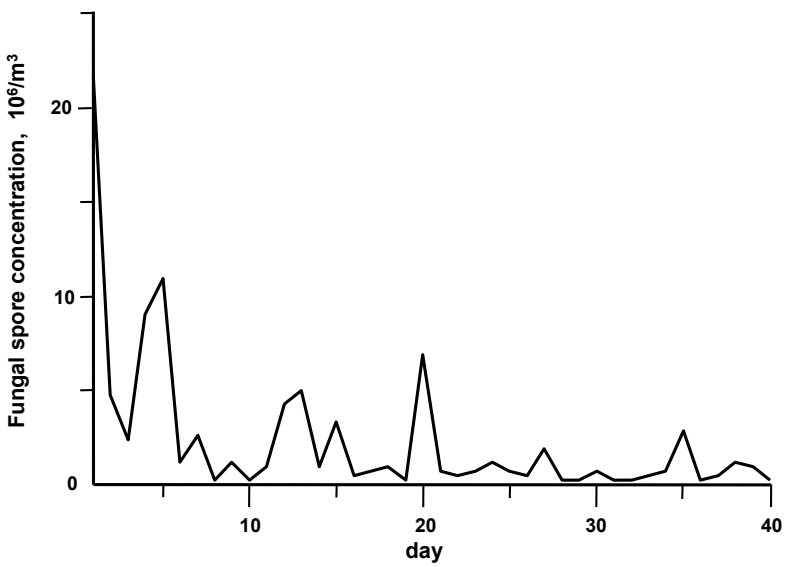

Figure 2. Variability of fungal spore concentrations on consecutive days in a sorting and trimming department of a Norwegian sawmill. Shift mean concentrations measured by stationary sampling are shown.

the measurements of all workers can be combined, and the mean exposure of the whole group is determined with better precision than the mean exposure of the individual workers because the group mean is based on a larger number of measurements. The efficiency of this strategy depends on the similarity of exposure levels between workers as combining workers with diverse exposure levels in groups may lead to poor precision of exposure–response associations in the epidemiological analysis (14). It should be noted that exposure variability of microbial agents can be substantially greater than commonly found for chemical agents. The number of samples must then be increased in order to obtain exposure estimates with sufficient precision.

Concluding remarks

Organic dust may contain many different agents. Among these agents, only endotoxins and fungal spores can be compared with the proposed observed effect levels. However, dust levels, within a branch/industry, can be compared to published effect levels found in epidemiological studies (eg, the wood industry). Exposure variability of biological agents can be high which may increase the number of samples that have to be collected.

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