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Advancing the science of bioaerosols' exposure assessment

Research on bioaerosols has experienced, and continues to experience, stellar growth. This is evident in the rocketing number of scientific articles published during recent years (twice more papers in 2013 than in 2003 —based on keywords on Web of Science, and about 10% of the articles in the last volume of AOH). This outbreak of research is due, among others, to (i) an emerging interest in the role of environmental exposure to biological agents (in public and occupational health), (ii) the development of news biotechnologies used in some industrial sectors and (iii) the access to news molecular tools allowing finer bioaerosols characterization. Bioaerosols (synonymous of organic dust) are defined as airborne particle (such as a fungal spore and hyphae, bacteria, endotoxin, β(1→3)-glucans, mycotoxin or high molecular weight allergens) that is composed of or derived from biological matter. They are found everywhere on earth. Indeed, environmental bacteria, viruses and fungi are a part of our natural environment, having coevolved with all the other living organisms, including humans. However, due to the presence of great amounts of organic matter, the release of bioaerosols can be very high in certain industrial sectors (for instance: agirculture, waste management, textile, wood…). Besides infectious diseases, they can cause also allergenic, toxic or irritant reactions. The presence of high levels of bioaerosols is most of the time the result of a natural colonisation of the organic substrate present in the workplace, but sometimes certain specific microorganisms are essential for the processes at work and are deliberately added (for instance: breweries, wineries, biotechnology, chemical or pharmaceutical production, biologically processed foods). Each bioaerosol sample is unique as its composition varies in time and space (abundance and diversity of species, quantity of pro-inflammatory components such as endotoxins and β-D-glucans). This often leads to high variations between samples from the same workplace, which can be due to external factors, but also to the dynamic evolution of the colonised substrate and the fast multiplication rate of microorganisms. These variations in airborne concentrations are often considerably large compared to chemical pollutants. Bioaerosols from some typical work sectors are associated with well known diseases. For instance, the baggasosis in sugarcane industry, the farmers lung in agriculture, the byssinosis in cotton industry).

In this issue, four articles focus on the bioaerosol problems in four very different workplaces. In two studies (Simon *et al*.; Madsen *et al*.), bioaerosols were measured in occupational situations where microorganisms are used deliberately: as agents of cheese maturation in a French cheese factory, and as biopesticides in a Danish potted plant production site, respectively. Another study from Denmark (Basinas *et al.)* explored the influence of farm characteristics and

the specific work tasks performed by dairy farmers on their personal exposure to organic dust and endotoxins. The fourth study (van Kampen et al.) estimated the airborne microbial load in German composting plants by using different quantification methods, and also explored the influence of the workplace characteristics and work processes associated with the highest levels of exposure. These four articles highlight: (i) the complexity of reliably measuring and characterising the airborne microorganism communities and/or their components, and (ii) the difficulty in determining which factor or which specific work task is associated with the greatest level of exposure. They show that occupational biological risks can be estimated using a variety of different methods and that each situation is unique and requires specific methodology. Culture-dependent methods are by far the most widely used procedures for assessing the microbiological content of bioaerosols. These are also one of the rare methods for which exposure recommendations exist. However, it is now widely accepted that such methods significantly underestimate the total quantity of microorganisms present since the vast majority of them cannot be cultivated (Oppliger *et al.* 2008; Fallschissel *et al.* 2010). Moreover, dead airborne bacteria or fungi retain their allergenic or toxic properties and are therefore also relevant to any occupational health assessment. The indirect measurement of microorganism levels by measuring their components (traditionally, endotoxins for Gram-negative bacteria and β-D-glucans for fungi) is another very frequently used method which allows researchers to take into account the concentration of biological components related to health effects since these two components have inflammatory properties.. However, measuring only endotoxins or β-D-glucans could be limiting because they are specific to certain microorganisms. Thus, in the future, in order to better estimate the risks related to the physiological effects of bioaerosols on humans, it will be necessary to develop new methods, or to better use existing ones, for the measurement of relevant indicators. For example, we need to know whether, other bacterial or fungal components can induce an inflammatory reaction or a cytotoxic effect. Moreover it is also essential to have a better knowledge on the physiological effects of a mixture of microorganisms since synergistic effects can occur. This is precisely what two of the studies presented in this issue have done by using in-vitro cellular tests.

Madsen *et al.* have measured the total inflammatory potential of bioaerosol samples by using a granulocyte-like cell assay which measures markers of oxidative stress produced by cells exposed to those samples. This test had been developed to assess microbial contamination of medicines, and was recently used to assess the total inflammatory potential of bioaerosols (Timm *et al.* 2006; 2009). Van Kampen et al. have measured the pyrogenic activity of bioaerosol samples by using a whole blood assay which measured the cytokines (pro-inflammatory

markers) released by blood cells exposed to those samples. This test was first described in 2005 (Kindinger *et al.)* and was then modified to assess occupational microbial exposure (Liebers et al. 2009). Both of these tests could be useful to investigate the mechanism of action of different mixture of bioaerosols and deserve researcher's attention.

In parallel to the assessment of the inflammatory or cytotoxic potential of bioaerosols, we need to have a better understanding of the exact composition of airborne microbial communities (also called microbiota). Simple molecular techniques such as quantitative PCR are of major interest, allowing larger scale sampling studies and evaluation of exposure to agents for which only short averaging time measurements could be taken in the past. Today, the characterisation and comparison of microbial communities, and the detection of changes in their species composition, can be also carried out using next-generation DNA sequencing technologies. These new analytical methods are cheaper and faster than traditional sequencing. They have been applied to assess the diversity of microorganisms not only in different human or animal microbiota, but also in air samples. They enable us to describe the fine-scale structure of entire microbial communities thus allowing the detection, not only of the most dominant microbial community members, but also of rare taxa. It is therefore now possible to study how environmental factors shape bacterial communities as well as which factors determine the presence, abundance and diversity of species within them. However, one has to be conscious that these technologies have still a lot of analytical constraints and cannot be currently applied "in the field" to assess occupational risks. But, even if it is not immediately useful for occupational hygienists, the development and use of new measurement techniques are of great importance; they help us to gain ever greater insights into exposure to biological agents in occupational settings. The tests measuring the physiological effects of bioaerosols, together with recent developments in molecular technology, may enable substantial advances in the near future. We can fairly say that bioaerosol sciences are currently still in a developmental phase and that a lot of important new discoveries are waiting to be made in the coming years which will be, one day, very useful for applied occupational hygiene.

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