Monitoring and Evaluation of Bioaerosol Exposure

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Introduction

Bioaerosols are defined as aerosols or particulate matter of microbial, plant or animal origin. Bioaerosols may consist of pathogenic or non-pathogenic live or dead bacteria and fungi, viruses, allergens, bacterial endotoxins, mycotoxins, peptidoglycans, ß(1-3)-glucans, pollens, plant fibres, etc. Exposures to bioaerosols in the occupational environment are associated with a wide range of health effects, including infectious diseases, toxic effects, allergies, and cancer [Douwes et al., 2003]. Workers from a large number of industries are potentially at risk including workers in agriculture, meat production, food and animal feed industry, waste recycling and composting industry, detergent industry, wood and paper industry, metal machining industries, biotechnology industries, the medical and public health sector, as well as, veterinarians, pet shop keepers, laboratory animal workers, etc.

One recent example of a high profile bioaerosol exposure with major health impact was the outbreak of Legionnaires disease at a flower show in the Netherlands in 1999. Visitors and workers were exposed to bioaerosols containing *Legionella pneumophila* originating from contaminated spas and sprinklers [Den Boer et al., 2002]. A total of 188 confirmed (155) or probable (33) cases were identified of whom 29 died. Other examples include exposures of enzymes such as α-amylase in the bread baking and flour producing industry [Houba et al., 1998], and subtilisins in the detergent industry [Sandiford et al., 1994; Schweigert et al., 2000] causing allergic rhinitis and asthma in workers handling the enzymes and/or intermediate products that contain enzymes [Cullinan et al., 2000, 2001]. Another example is exposure to high levels of microorganims and endotoxin such as occur in waste recycling workers (e.g. waste sorting, organic waste collection and composting; [van Tongeren et al., 1997; Douwes et al., 2000; Wouters et al., 2002]) causing airway inflammation and respiratory conditions such as "organic dust toxic syndrome", asthma, and "extrinsic allergic alveolitis" [Poulsen et al., 1995; Thorn and Rylander, 1998; Douwes et al., 2000; Wouters et al., 2002].

Despite the recognition of the importance of bioaerosol exposure on human health, the precise role of biological agents in the development and aggravation of symptoms and diseases is still only poorly understood. This is primarily due to the lack of valid *quantitative* exposure assessment methods for bioaerosols. In this paper we will briefly discuss exposure assessment methods and evaluation of biological agents in the work place.

Exposure assessment

Exposure assessment plays a central role in occupational health studies seeking to characterize population risks, in screening studies aimed at identifying individuals at risk, and in interventions designed to reduce risk. However, since exposure limits are available for only few biological agents (see below) exposure assessment is often only used in basic

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hazard evaluations (are the agents present?) or in specialized epidemiological studies to characterise population risks (is there an exposure response relationship?).

Assessment of exposure to bioaerosols offers challenges distinct from those for inorganic aerosols and chemical agents [Heederik et al., 2003]. Measurements of micro-organisms relies upon collection of an airborne sample into or onto solid, liquid, or agar media with subsequent microscopic, microbiologic, biochemical, immunochemical or molecular biological analysis. Two approaches are being distinguished for evaluation of microbial exposure: "culture-based methods", and "non-culture methods". Counting culturable microorganisms is a very sensitive method, which also permits identification of species. However, viable sampling is limited to short sampling times (to reduce viability loss) which may introduce considerable measurement error. In addition, dead or non-culturable microorganisms and specific microbial agents are not detected whereas they may have potential toxic or allergic properties. Moreover, in epidemiology focussing on non-infectious health outcomes, culture methods have proven to be of limited use for exposure assessment. Non-culture methods attempt to enumerate organisms without regard to viability using microscopy for counting spores or cells. They allow full shift measurements but have specific problems such as limited potential for qualitative identification and low counting accuracy. Advanced methods such as PCR-based technologies and immunoassays have opened new avenues for detection and speciation regardless of whether organisms are culturable. Finally, specific bioaerosol associated agents can be measured using specific immuno-assays, other bio-assays or mass spectrometry technique. These agents may either be directly toxic (e.g. allergens, bacterial endotoxin, fungal mycotoxins and ß(1,3)-glucans) or may be general markers of exposure (e.g. fungal ergosterol or fungal extracellular polysaccharides). Non-culture methods appear more promising for exposure assessment but the experience is still limited and for many of the relevant agents no commercially available methods are currently available.

Evaluation

Risk assessment for bioaerosol exposure is complicated due to 1) the great variability in bio-aerosol exposure (both in space and time) requiring large measurement series to detect dose-response relationships and/or differences between contaminated and background areas; 2) the lack of valid and sensitive quantitative exposure assessment methods; and 3) the fact that bio-aerosol exposures also commonly occur outside the work place. Therefore, with the exception of a few allergens and toxins (see below) no legal exposure limits exist for comparison with bio-aerosol exposure data. Some exposure standards are therefore based on hazard evaluation (presence of the agent) and not on health based risk assessment procedures as known for many chemical substances. Examples are the "standards" for number of colony forming units in the air (for bacteria and fungi), which are merely rules of thumb, based on a comparison with background levels. A recent version of such a standard is the one for *Legionella pneumophila* in drinking water.

Wood dust standards have been adopted in several countries (e.g. 5 mg/m^3 in the US and 2 mg/m^3 in The Netherlands (the proposed Health Based Recommended Occupational Exposure Limit (HBROEL) is lower); based on 8 hour time weighted averages (8-TWA) of inhalable dust). In a recent literature review by Demers et al ([1997], a standard of 1 mg/m^3 for softwoods was suggested to protect workers from non-malignant effects. An 8-TWA "threshold limit value" (TLV) of 4 mg/m^3 has been established for total grain dust (wheat, oats, barley) by the American Conference of Governmental Industrial Hygienists (ACGIH) since 1980 [ACGIH 1980].

Standards for some allergens have been adopted or suggested including the TLV for workplace airborne exposure of subtilisins (60 ng/m^3 ceiling concentration; [ACGIH 1996]). Subtilisins are bacterial enzymes with strong sensitising properties used in detergents. The TLV has been criticized mainly because this standard is not based on health considerations and there is serious doubt whether this TLV actually protects against sensitisation. Some evidence suggests that sensitization may occur at levels below the TLV [Brisman, 1994; Heederik et al., 2002]. In fact, the Health and Safety Executive (HSE) in the UK is proposing to withdraw the British limit (OES: 60 ng/m^3, TWA) because no safe exposure limit for subtilisins could be identified. The ACGIH has also adopted an exposure standard of 0.5 mg/m^3 for inhalable flour dust (8-TWA) [ACGIH 1990].

For endotoxin a health-based exposure limit has been proposed in The Netherlands by the Dutch Health Council of 50 EU/m^3 (8-TWA) [DECOS 1998]. After advice from unions and employers a higher value of 200 Endotoxin Units/m^3 was proposed and the Minister of Social Affairs initially decided to implement this value as from 1 January 2003. However, several industries commented that they were not able to comply even with this higher value. The Socio-Economic Council (SER) therefore again advised the Minister to introduce the standard of 200 EU/m^3 at a later stage, in combination with the development of a research programme that should lead to the development of exposure control measures for different industries. The decision of the Minister of Social Affairs is not yet known. Since differences in storage, extraction and analysis of endotoxin samples may result in large differences in exposure estimates [Hollander et al., 1993; Douwes et al., 1995; Thorne et al., 1997, 2003; Chun et al., 2000; Duchaine et al., 2001; Reynolds et al., 2002] it has been decided to adopt the CEN draft protocol for measurement of endotoxin [CEN, 2001]. However, the CEN protocol does not describe extraction and measurement procedures very specifically thus potentially resulting in significant variations in exposure assessment between laboratories. For standard setting purposes further validation and

standardization of sampling, extraction and analytical procedures are urgently needed. Therefore, careful introduction of the CEN protocol is also advised by the SER.

In conclusion

Methods to assess bioaerosol exposures quantitatively have been developed but are currently only poorly validated and they are not widely available. This combined with the relatively large spatial and temporal variation in exposure complicates exposure and risk assessment, hampering legal exposure limits to be developed (with the exception of a few specific components such as specific allergens and endotoxin). Therefore, more research is needed to establish better exposure assessment tools and to validate newly developed methods. Validation of methods is particularly needed for those agents where occupational exposure limits have been established or proposed (e.g. allergens and endotoxin) resulting in internationally accepted protocols that should include concise and uniform guidelines on sampling, storage, extraction and analytical procedures.

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