

## O1

### MONITORING AND EVALUATION OF BIOAEROSOL EXPOSURE

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Airborne and settled particulate material of microbial, plant or animal origin is often referred to collectively as bio-aerosols or organic dust. It may comprise pathogenic and non-pathogenic microorganisms, toxins and allergens. The effects of exposure to bio-aerosols can include infectious diseases, acute toxic effects, allergies, and cancer.

Assessment of exposure to bio-aerosols offers challenges distinct from those for inorganic aerosols and chemical agents. Measurement of microorganisms relies upon collection of a 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 components are not detected whereas they may have potential toxic or allergic properties. 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 quantitative identification and low counting accuracy. Advanced methods such as PCR based technologies, FISH, and immunoassays have opened new avenues for detection and speciation regardless of whether organisms are culturable. Finally, specific bio-aerosol associated agents can be measured using specific immuno-assays, other bio-assays or GC-MS. These agents may either be directly pathogenic (e.g. allergens, bacterial endotoxin, fungal mycotoxins and  $\beta(1,3)$ -glucans) or may be general markers of exposure (e.g. ergosterol, fungal extracellular polysaccharides). However, for many of these agents no commercially available methods are currently available.

Storage and extraction procedures are critical in bio-aerosol measurements, particularly for agents such as viruses, proteins, endotoxin etc. Variability in bio-aerosol exposure (both in space and time) is often large thus requiring large measurement series to detect dose response relationships and/or differences between contaminated and background areas. In addition, bio-aerosol exposures are ubiquitous and thus everyone is exposed throughout life which complicates risk assessment. Therefore, with the exception of a few allergens and toxins no legal exposure limits exist for bio-aerosol exposures in most countries.

In conclusion, 1) selection of the most appropriate exposure assessment method(s) for bio-aerosols is highly dependent on the specific goals of the study; 2) interpretation of bio-aerosol measurement results is impossible without detailed information about the sampling and analytical procedures; 3) due to large uncertainties in exposure assessment methods risk assessment is complicated, hampering legal exposure limits to be developed.

## O2

### **EXPOSURE TO INHALABLE FLOUR DUST, WHEAT-ALLERGENS AND FUNGAL ALPHA-AMYLASE IN FLOUR AND ENZYME PROCESSING INDUSTRIES AND BAKERIES**

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Exposure to flour dust and especially to (flour) allergens is associated with an increased asthma risk in bakers. However, hardly any information on exposure levels is available in other companies than bakeries. The objectives of the present study were to measure full-shift exposure to inhalable dust, wheat-allergens and alpha-amylase in different industries with flour and enzyme exposure, and to investigate relevant exposure determinants which indicate directions for control strategies.

Personal full-shift inhalable dust exposure was determined for about 650 workers in four sectors, i.e. small traditional bakeries, large industrialised bakeries, flour mills, and suppliers of bakery ingredients. Wheat-allergens and fungal alpha-amylase concentrations were analysed using earlier described immuno-assays. 87 workers were measured on two or three days. For each worker, the following information was recorded: job title, tasks performed during the day, work practices, type of products manufactured and used, and the presence of potentially effective control measures.

On average, highest dust concentrations were found in flour mills (GM 3.2 mg/m<sup>3</sup>, GSD 4.7, N=156) and suppliers of bakery ingredients (GM 2.0 mg/m<sup>3</sup>, GSD 5.9, N=128). Wheat-allergen exposure was highest in flour mills (GM 11.7 µg/m<sup>3</sup>, GSD 10.1) and small bakeries (GM 5.5 µg/m<sup>3</sup>, GSD 9.3, N=134). Highest exposure levels for fungal alpha-amylase were found for suppliers of bakery ingredients (GM 32.4 ng/m<sup>3</sup>, GSD 22.2) and flour mills (GM 8.0 ng/m<sup>3</sup>, GSD 9.8) where workers regularly handled the enzymes in a relatively pure formulation. The Dutch Expert Committee of the Health Council has recommended a health-based occupational exposure limit of 0.5 mg/m<sup>3</sup> for flour dust. In total, 81% of the persons were exposed to higher concentrations.

Relatively high exposed functions were dough makers in large bakeries; milling operators, cleaners, and operators involved in bagging in flour mills; and weighers and operators involved in dumping or bagging in bakery ingredients supplying companies.

Multiple regression analysis was used to identify significant predictors of exposure. For all sectors, job title explained most of the variability in alpha-amylase exposure (30-48%). Especially for inhalable dust and wheat allergens exposure in industrialised bakeries and flour mills more variability was explained by the tasks performed (16-36%) than with job title. Regression coefficients were highest for tasks such as weighing, dusting, mixing, dumping, cleaning and filling/bagging with differences for type of exposure, indicating that control measures should preferably be focussed on these tasks. The low explained variability suggests that differences in task performance should be investigated more thoroughly to recognize good work practices.

### **O3**

#### **OCCUPATIONAL ISSUES REGARDING NAIL DUST IN PODIATRIC PRACTICE**

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Harmful nail dust is produced upon reduction of onychauxic (thickened) toenails via the use of a rotating burr attached to a nail drill. The issue of nail dust as a possible occupational hazard has been debated for some time, with regard to the possible respiratory risks to podiatric practitioners. There is no doubt that such a risk exists. However, other occupational risks from the hazard have not been considered. This study was unique in examining the potential hazards to the ocular welfare and safety of the practitioner, as no baselines existed for many of the areas under question. A number of hypotheses were therefore constructed and the thesis subsequently comprises a series of unique smaller studies, culminating in addressing the research question. The study is essentially multidisciplinary, combining expertise in podiatry, microbiology, ophthalmology and Health and Safety.

A postal questionnaire was completed by members of the profession identifying their experiences of ocular injury, use of nail drills and eye protection. The microbial content of dust bags (attached to the nail drills) was also examined. The possible route of transference of these microbes to the ocular mucosa was also addressed. This included direct contact (through the contamination of the practitioners' hands) and airborne transmission routes. Significantly increased numbers of viable microbial colonies were isolated from both routes following the drilling procedure.

The study also uniquely identified the movement of nail dust particles upon leaving the surface of the drill burr. Employing the Tyndall lamp technique and additionally the use of high speed videography, this visual component stunningly illustrates the dust particle clouds produced by the drilling procedure enveloping not only the breathing and ocular zones of the practitioner, but travelling beyond head height.

The results of the study indicate a marked increase in the prevalence rates of asthma and allergic-type complaints among podiatrists in comparison to the general population, alongside the existence to irrefutably support the potential for ocular injury/infection and cross-infection in podiatric practice.

The research evidence disseminated through this study has enhanced awareness, not only by practitioners, but also Occupational Health and Health and Safety practitioners nationally.

## O4

### GROWTH OF MOULDS, FUSARIUM DNA AND MYCOTOXINS FROM SETTLED DUST

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#### Background

Mothers engaged in grain handling have a twofold risk of preterm births or late abortions compared to the general population in the same area. The association is strongest in wet seasons, known to favour growth of fungi that may produce mycotoxins with immunotoxic and hormone like effects. *Fusarium* spp. are the predominant toxigenic field fungi while *Penicillium* spp. and *Aspergillus* spp. are the most important storage fungi in the northern temperate regions. The present study assesses growth of moulds, detection of *Fusarium*-DNA and mycotoxins from dust associated with grain handling.

#### Methods

Samples of settled grain dust were collected from 92 Norwegian farms during handling of grain from the 1999 and 2000-year production. Samples were both prepared for culturing of *Aspergillus*, *Penicillium* and several *Fusarium* spp. and analysed by PCR for detection of species-specific *Fusarium*-DNA sequences and a gene for trichothecene-production. Zearalenone (ZEN) and ochratoxin A (OTA) were extracted and quantified by competitive direct ELISA (detection limit (DL) 1 ppb for OTA, 50 ppb for ZEN) originally developed for feed, corn and grains. In 20 of these samples, ZEN and OTA were also quantified by the more established HPLC method. Trichothecenes will be analysed by GC-MS.

#### Preliminary results

Thirty-three of the 49 samples of grain dust cultured so far were positive for *Fusarium* growth, while 30 were positive for *Penicillium* and 8 were positive for *Aspergillus*. PCR analyses for general detection of *Fusarium* were positive in all 109 samples. *F. avenaceum* predominated among field fungi, whereas *Penicillium* spp. predominated among storage fungi, as expected. Cultured *Fusarium* spp. from grain dust correlated weakly with the corresponding *Fusarium* spp. detected by PCR ( $r=0.11 - 0.36$ ). The 109 dust samples analysed by ELISA had a mean of 37 ppb ZEN, (range 13-122 ppb,  $n=109$ , 15 samples > DL), whereas all 109 samples were over the DL for OTA (mean 8.3 ppb, range 1.7-54 ppb). The 20 samples analysed by HPLC had a mean of 10.6 ppb ZEN (range 0-62,  $n=20$ , 5 samples > DL) and 4.0 ppb OTA (range 0-68,  $n=21$ , 12 samples > DL). Irrespective of the method used to measure ZEN and OTA, neither of these was correlated to ZEN-or OTA-producers found by grain dust cultivation or by PCR. The correlations between ELISA-analyses and HPLC-analyses was weak to moderate, showing  $r=0.17$  for ZEN and  $r=0.51$  for OTA.

#### Discussion

PCR analysis of *Fusarium* spp. detects dead as well as viable fungi, and is expected to detect more fungi than results from culturing. This was confirmed by the poor correlation between culture and PCR result. The lack of correlation of mycotoxin-producing fungi and the measured mycotoxins, may be due to degradation of the fungi after the production of toxin, and to competitive growth of non-toxin-producing species. It is well known that the mycotoxins are much more stable than the fungi that produced it. The poor agreement between ELISA- and HPLC-results, may partly be due to large relative errors from low mycotoxin levels in most of the samples, and because the two methods detect partly different metabolites. Whereas HPLC only detected ZEN and OTA, the ELISA-measurements also included some of the related toxic substances. Due to the latter, HPLC cannot be regarded as a

fully validation of the ELISA results. The results from the trichothecene analysis and associations with cultivation of moulds and PCR-analysis will be presented.