Abstract

Background

Biologically derived airborne contaminants include bioaerosols and volatile organic compounds originating in an organism. Bioaerosols are defined as suspended particles consisting of or derived from living organisms. Viruses, pathogenic and non-pathogenic microorganisms, allergens, endotoxins, mycotoxins, peptidoglycans and others also fall under this definition. Biological hazards are associated with a variety of adverse health effects affecting workers as well as the public. Chronic respiratory exposure to endotoxins, which are integral components of gram-negative bacteria cell walls, is associated with adverse health outcomes such as fever, headache, sensitization of the nasal cavities and throat, cough, dyspnea, heavy breathing, acute constriction of the respiratory tracts and inflammation.

The two long known industries selected for this study were animal feedstock production and metal machining. The former produces food mixtures for poultry, beef, sheep, horses etc., while the latter is characterized by the use of metalworking fluids as coolants, lubricants and rust protective agents in mechanical metal processing (milling, lathing, cutting, drilling). The emulsion of organic oil and water provides a nutritional broth sufficient for the proliferation of bacteria and fungi. Studies of the adverse health effects of exposure to organic dust in animal feedstock production facilities have reported increased prevalence of respiratory diseases including asthma, symptoms of hypersensitivity pneumonitis and organic dust toxic syndrome (ODTS) among employees.

The goal of the research was to identify exposure sources to bioaerosols and endotoxins in animal feedstock facilities and metal machining workshops using metalworking fluids, and investigate associations between airborne levels of solid and liquid aerosols and levels of bioaerosols and endotoxins contained therein.

Research methods

This study was cross sectional. Two large, two medium and one small metal machining workshops and seven animal feedstock production facilities comprised the sample population. Data were collected in eight processes characterizing animal feedstock production and three processes characterizing metal machining. These processes were investigated due to their prevalence at the selected sites and their inherited potential of particles and bioaerosols dispersion.

The research methodology included the following stages: a pilot examination of the suitability of the methods to be employed for measuring bioaerosols within the expected range of concentrations; an industrial hygiene survey at each work place to recognize processes and tasks, processes flow, technological characteristics of the processess , work procedures and activities, work practices, existing control measures; identification of potential hazards, routes of exposure, , interaction between worker and exposure source, exposure frequency and duration, factors contributing to exposure; environmental monitoring to assess exposure to dust and bioaerosols; construction of a data base for analysis; data analysis and conclusions.

The pilot study evaluated suitabilility of methods for measuring bioaerosols in the expected range of concentrations, as well as the suitability of parameters for assessment of bioaerosols and endotoxins in the work environment. Based on this pilot, leading and backup methods were selected. Additional variables examined so as to attain optimal data collection included: selection of culture substrate,

effective sampling time, incubation duration, and the preferred medium dilution for the analysis of microorganisms on gelatin filters.

Airborne levels of the following variables were measured to characterize the work environment: 1. Inhalable fraction of suspended particles (organic grain dust and metalworking fluid aerosol), 2. Endotoxins, 3. Total bacteria and fungi, 4. Gram-negative bacteria.

Personal and area samples were taken to characterize the potential exposure and study associations between variables, taking into account exposure frequency and duration of processes and tasks. Inhalable fractions of dust were determined gravimetrically; endotoxins were sampled on fiberglass filters and analyzed by the turbidimetric Limulus Amebocytes Lysate (LAL) method. Viable bacteria and fungi were sampled and examined by two methods, one based on sampling with a Button^{TM} aerosol sampler equipped with a sterile gelatin filter, and the other based on use of an Anderson multipore single stage sampler (N-6). Colonies of bacteria and fungi on selective agar substrates were counted after incubation. Bacteria genus and species were identified by rRNA gene PRC and sequencing, after isolation of colonies of the most common microorganisms.

Descriptive statistics was performed after examining the distributions of data. The differences between averages of continuous parameters, including exposure levels, were tested for significance by t tests, with $p \leq 0.05$ being defined as significant. Associations between the variables were analyzed by Pearson correlations and linear regression.

Results

Work processes among the various animal feedstock production facilities were similar. The same applies to work processes utilizing metalworking fluids in metal machining workshops. The source of microorganisms and endotoxins in animal feedstock facilities is the organic grains which are supplied as raw materials. The pattern of the potential exposure to dust and bioaerosols in feedstock production is not homogenous and is affected very much by process technology, nature of tasks and activities and their extent, facility size, presence vs. lack of engineering control, maintenance level of machines and facility, work practice, use of PPEs and extent of employee compliance with safety and hygiene instructions.

The most common metalworking fluid used in metal machining is a blend of soluble oils and additives emulsified in water. Semi-automatic CNC machines are common and workers remain by the machines along most of the shift. There is much more similarity in the patterns of the potential exposure to bioaerosols among metal machining processes than among processes in animal feedstock production facilities.

The geometric mean of grain dust in animal feedstock production facilities approached 75% of the threshold limit value (TLV) set by the ACGIH for grain dust, while 48% of the dust results exceeded the TLV and 71% were above half of the TLV (action level). Airborne endotoxin levels were scattered over a very wide range and the average concentration (179.4 EU/M^3) was higher than the Dutch OEL (EU/M^3) which is the only OEL established within western countries. This OEL was exceeded among 62% of the endotoxin results including the levels found in personal samples. Concentrations of bacteria and fungi in the order of magnitude of 10^3 – 10^5 CFU/M^3 were common in feedstock production and the proportion exceeding maximal recommended exposure values was 35% and 50% for bacteria and fungi, respectively. The mean and median of viable gram-negative bacteria and total viable bacteria count and fungi count reached 159 and 189, 8,856 and 7,099 and 1,858 and 915 CFU/M^3, respectively. The difference between the mean concentration in processes and that of the background was statistically significant for all variables measured. Satisfactory resemblance was found between airborne levels of gram-negative bacteria and fungi recovered from collection on gelatin filters and the corresponding levels recovered from direct collection on petri dishes. However, such resemblance was not obtained between corresponding total bacteria counts, which were higher after collection on petri dishes.

The highest levels of airborne particles, endotoxins and bacteria and fungi in animal feedstock production were found in the task of discharging grains from trucks into a receiving pit.

The levels of inhalable particles, endotoxins, bacteria and fungi in metal machining workshops were much lower than the parallel levels found in animal feedstock production facilities rendering statistically significant difference between averages calculated for each variable in both industries. The geometric means of the airborne levels of inhalable particles, endotoxins, viable gram-negative bacteria, total viable bacteria count and fungi count in metal machining workshops using metalworking fluids were, respectively, 0.32 mg/M^3, 6.1 EU/M^3, 18.1, 132.6, and 44.5 CFU/M^3. The difference between the mean concentration in processes and that of the background was not statistically significant for inhalable particles, total viable bacteria and total spores forming fungi. In general, the exposure to bioaeroaols in metal machining workshops did not exceed recommended maximal values. In these workshops the airborne levels of gram-negative bacteria and fungi recovered from collection on gelatin filters resembled corresponding levels recovered from direct collection on petri dishes but the recovered total viable bacteria count was higher when collected on petri dishes.

The coefficients of Pearson were positive strong and statistically significant for the correlation between inhalable grain dust and airborne endotoxins (0.83) as well as between gram-negative bacteria and endotoxins (0.69) in animal feedstock production. The coefficient of the correlation between inhalable aerosol and endotoxins in metal machining workshops was medium (0.496) but significant, and that found between gram-negative bacteria and endotoxins was weak (0.28) and not statistically significant. Satisfactory linear regression coefficients were obtained for associations between variables measured in animal feedstock production but only between inhalable particles and endotoxins in the milling process at workshops using metalworking fluids.

<u>Discussion</u>

The potential for respiratory exposure of workers to bioaerosols in animal feedstock production facilities and metal machining workshops using metalworking fluids exists as does that for potential dermal and ingestion contact with bioaerosol-bearing inhalable particles. Organic dust is dispersed during all processes handled in animal feedstock production but to different extents. The examination of the exposure potential in the production facilities based on airborne levels only may be misleading because the duration of exposure in the different tasks may play an important role in the overall assessment. The presence of a worker in certain work stations may be infrequent and the total time spent by a worker in these stations may be short leading to relatively low doses of exposure despite high environmental levels of bioaerosols resulting in the process. This is in contrast to metal machining processes where the total time spent by a worker near a processing machine during the work shift is long and significant. In general, workers at animal feedstock production may not be present in areas where peak concentrations of bioaerosols, especially endotoxins, may induce organic toxic dust syndrome. Nevertheless, endotoxins levels of exposure in some of the personal and area samples taken exceeded the suggested threshold levels that may induce inflammatory reaction in the respiratory tracts.

The exposure pattern in metal machining workshops is different from that in animal feedstock production facilities. In the former, the liquid medium and limited organic nutritional substrate for bacterial growth as well as the use of metalworking fluids in closed machines reduces the potential for environmental dispersion and exposure despite the very strong collision between the rotating parts and the metalworking fluid inside the machine which encourages formation of droplet aerosols. This collision may also contribute to destruction of bacteria and fungi cells decreasing the concentrations of viable cells in the air but increasing cell fragment formation, thus explaining the reasonable regression coefficient found between levels of inhalable particles and airborne endotoxins.

Dust levels, as well as gram-negative bacteria and total bacteria levels in the air may predict the level of airborne endotoxins in animal feedstock production processes. Conversely, the absence of good correlations between exposure predictors in metal machining processes using metalworking fluids suggests more than one source for the presence of aerosols and bioaerosols while the contribution of the metalworking fluid alone to the overall presence remains unquantified.

Gelatin filters are preferable over petri dishes mounted on an Anderson multipore single stage sampler for routine sampling of bioaerosols, due to their applicability to personal samples and time-weighted average sampling. Gelatin filters also enable the simultaneous sampling of different types of bioaerosols without a need for different selective growth substrates as required when using petri dishes.

Applicability of the study for exposure and risk assessment in animal feedstock production and metal machining is discussed and recommendations as to reduction of exposure and future studies are given.