COMPARATIVE EXPOSURES TO ENDOTOXIN IN WORKERS EXPOSED TO ORGANIC DUSTS

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Abstract

Increasingly it is realised that exposures to organic dusts are harmful to respiratory health. Endotoxins are a common contaminant of such dusts and are recognised as possible aetiological agents in respiratory disease in exposed individuals with such occupations. Exposures in different working environments and occupations have been measured by different investigators. Comparisons, however, are invalid due to the use of differing sampling, extraction and assay techniques. This paper presents data drawn from a study of 9 different industries comparing endotoxin exposures. Endotoxin exposures are presented as both environmental levels (per unit sampled air) and by contamination within the dust sampled (per mg of dust collected).

Introduction

The inhalation of organic dusts is implicated in the aetiology of respiratory diseases including byssinosis, extrinsic allergic alveolitis, asthma, chronic bronchitis and the organic dust toxic syndrome. Organic dusts are complex mixtures derived from animal and vegetable material. When considering occupational exposures variations are found not only between but within industries. Within organic dusts the finding of endotoxins and their identification as a potential aetiological agent has been explored. The term endotoxin is used to describe a heterogeneous group of high molecular weight lipopolysaccharide compounds, derived from the cell wall of gram negative bacteria. They have been implicated in the causation of byssinosis, humidifier fever, the sick building syndrome, chronic bronchitis and more recently in the severity of asthma (Michel 1991).

Investigators have isolated and measured both the endotoxin contamination of settled and airborne organic dusts. With these results attempts have been made to identify levels of exposure above which harmful effects become apparent (Castellan 1987 Milton 1994). In addition attempts have been made to compare exposures across different industries. The utilisation of these results for comparative purposes is invalid because of differences in methodology used by different investigators. Firstly to consider the collection of the dust, settled and airborne dust sampling techniques have been used. Within the airborne dusts different exposures have been measured using static and personal sampling techniques, and in some instances respirable fractions. Secondly with regard to the analysis of samples for their endotoxin content different methods for storage, extraction and analysis (quantitative and semi quantitative) have been used.

The aim of this study was to provide valid comparative personal exposures for both organic dusts and endotoxin across a range of industries. With this information comparative data for the levels of contamination of dusts within industries is presented. In addition amongst the industries covered are mushroom cultivation and weaving, which to the authors knowledge have not previously been surveyed.

Methods

A number of industrial settings were selected for study. Approaches were then made to senior management, safety officers, workers representatives and occupational health and hygiene departments where applicable. The nature of the survey explained and co-operation of participants elicited.

A representative sample of the work force at each site was selected to record personal total inspirable dust exposures during a typical work shift. The IOM (Institute of Occupational Medicine) sampling head was used to collect the dust onto Whatman 25 mm glass fibre filter papers. The head was worn by operatives at the level of the left clavicle. The filter papers were weighed pre and post collection using a Sartorius micro balance to 3 decimal places. Prior to down weighing "fly" or large particles (such as feathers) were removed. Control filters were also taken to each site. This was necessary to correct for changes in weight of the filters due to changes in water content, and as will be appreciated later to look for cross contamination with endotoxin.

Calibrated battery operated rechargeable Cassella personal sampling pumps were used to draw air across the filter. The pump flow rate was set at 2 l/min at the commencement of sampling and the flow rate checked at the end of sampling. Using this flow rate and the time of the sampling period, the volume of air sampled could be calculated. The results are expressed as per m^3 of sampled air.

Following gravimetric analysis the individual filters were transferred using flamed forceps to endotoxin free glass ware marked with a unique identity number. The samples were then stored at < - 20 C until analysis. Through out the analysis endotoxin free products were used including

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glassware and water. When handling volumes of 1 ml or less adjustable volumetric pipettes (Gilson Pipetman P) and polypropylene pipette tips were used . Random samples of 10 polypropylene tips were taken and soaked for 24 hours in endotoxin free water then analysed for contamination this was found to be zero.

Collected samples were thawed at room temperature for an hour prior to a simple water extraction technique. 10 mls of water were added to the 10 oz universal containers used to store the filters in. Samples were vortexed for 60 seconds, prior to agitation on roller bars for 60 minutes and were finally vortexed for a further 60 seconds. This resulted in the disintegration of the filter paper. A 4 ml aliquot of this solution was then centrifugedat 3000 g for 15 minutes. Serial dilutions were then made with 0.5 mls of the supernatant ranging from 1:10 to 1:10,000.

The samples were then analysed using a quantitative kinetic turbidimetric method (LAL 5000e series 2 machine Associates Cape Cod). The lowest concentration of the serial dilution within the range of the standard curves was used to evaluate the measured concentrations. The pH of the final solutions assayed were checked using an electronic monitor. Water negative controls were also included with each analysis. Two different batches of LAL reagent were used through out lot 12-16-608-t and lot 42-133-575-t (Associates Cape Cod). Standard curves were generated with control standard endotoxin E Coli 0113 lot # 60 (Associates Cape Cod) and these two LAL lots. Both curves generated had correlation coefficients of greater than 0.990.

The control filters from each site were analysed for their endotoxin content, the results from these were then used to correct the measured collected values. The rational for this was both to account for contamination of clean filters and also possible cross contamination from the filter holding cassette in the IOM head. Complete depyrogenation by physical methods was impossible because of the nature of the component parts.

Using the previously calculated volume of air sampled the results are expressed as ng/m^3. With the control standard endotoxin and LAL lots used a conversion factor of 10 can be used to convert the results to endotoxin units. In addition the contamination of the dusts collected with endotoxin is also presented and expressed as mcg of endotoxin per mg of dust.

Results

The industries surveyed were textiles (cotton spinning, wool scouring/ combing and weaving), agriculture (grain handling, wood, mushroom cultivation and animal feed manufacture) and animal handling (swine and poultry confinement). These were spread over 36 different sites. A total of 267 filters were collected 8 (3%) were unfit for analysis because of damage to the filter paper or loading. This resulted in 259 samples for which both collected dust and endotoxin could be measured. This represented on overall sampling rate of 25% of the studied population. This figure varied according to the number of sites visited and the diversity of jobs. Consequently for swine confinement where 11 sites (each employing from 1 to 8 workers) were visited and jobs varied considerably 62.8% of the workforce were sampled. Conversely the lowest sampling rate (13.7%) was found in the cotton spinning industry where 2 sites were visited and large numbers of individuals were employed performing the same tasks. Table 1 presents the sampling rates for the individual industries and the correlation's found between the collected dusts and the measured endotoxin.

Comparative dust exposures expressed as a median and range in mg/m^3 are displayed in figure 1. The highest exposures occurred in the wool and grain industries. In both these industries the extreme values (up to 72.5 mg/m^3) were collected during cleaning activities. The highest median values were found in the industries involved with animal handling (poultry 12.8 mg/m^3 and swine 6.7 mg/m^3).

Comparative endotoxin exposures again expressed as a median and range in mcg/m^3 are displayed in figure 2. The highest exposures for both the range and median exposures are found in the poultry(median 12 mcg/m^3 highest 72 mcg/m^3), swine confinement (median 0.6 mcg/m^3 highest 14.9 mcg/m^3) and cotton spinning (median 0.4 mcg/m^3 highest 6.9 mcg/m^3) industries.

With the collected dust and the measured endotoxin it was then possible to calculate comparative contamination of dusts, these values again expressed as median and ranges in figure 3. Not suprisingly when considering the previous data the most highly contaminated dust occur in the poultry (median 1 mcg/mg highest 3.6 mcg/mg), cotton (median 0.5 mcg/mg highest 3 mcg/mg) and swine (median 0.2 mcg/mg highest 1.6 mcg/mg) industries.

Discussion

The validity of the hygiene results should be considered in detail before the acceptance of the results. The methodology employed in the collection of the dust has been used for a number of years within the department and shown to give reproducible results (unpublished data). The equipment itself is regularly serviced and calibrated, as appropriate, prior to use on each occasion. The method for endotoxin extraction (Gould 1987) and analysis (Milton 1987), is a standard accepted technique. It is accepted that the extraction procedure will not be exhaustive but is simple and easily reproducible.

The calculation of exposures as concentrations is limited in its accuracy by the truthfulness of the individuals wearing the samplers with regard to how long they have worn them

for switched on. The other operative dependent problem is one of dust loading, or spoiling of the filters. This can be readily detected, however, when inspecting the filters after collection of dusts during down-weighing. Out of the total of 267 filters only 8 were spoiled. The removal of fly during the down weighing could also be viewed as a source of error. With the exception of the cotton industry the particles removed were large and obviously non-respirable, examples being feathers and bits of straw. In the cotton industry the fly forms a fine web like lattice on the surface of the filter paper which is easily removable with sterile forceps. Results from the department have previously shown that the correlation between the measured dust without fly compared to the dust with fly is superior with regard to the prediction of respiratory symptoms (Niven 1993). The results presented are therefore the concentration of dust and endotoxin measured after the removal of fly.

The final area to consider with respect to the validity of the collected sample is whether the collected samples are representative for the industries. The main consideration with regard to grain and animal handling and areas within saw mills is the effect of different climatic conditions on both the level and possibly the composition of the dusts. The sampling of a number different sites over the year during which the dust samples were collected should help to overcome this problem. For the other industries, climatic conditions will have little if any effects. For the textile industries differing fibre types processed at different times may produce different dusts. Also to be considered, is whether the time at which the sample was collected was representative for the occupation within that room. Where possible more than one sample has been collected and an average taken.

No records for exposures in the mushroom or weaving industry have previously been published. In comparison with other industries reported in this paper both have relatively low exposures. The highest exposures are, not surprisingly, found in the industries where workers are exposed to waste material derived from animals. A wide range of exposures are seen within industries reflecting the diversity of jobs within the industries and also reinforcing the fact that the dust levels and compositions will vary. Within the textile industry when looking at the contamination of dusts with endotoxin the effect of processing on reducing the exposure levels to endotoxin has previously been demonstrated. This is particularly well shown in the wool cleaning industry where washing in hot water with alkali followed by drying with hot air greatly reduces but does not eradicate all the endotoxin contamination (Simpson 1995). The level of contamination of dusts is important as it gives a guide as to the potential for endotoxin exposure if the dust becomes airborne.

In the United Kingdom occupational exposure limits exist for exposures to dust. There are two different categories a maximum exposure limit (MEL) and an occupational exposure standard (OES). In essence an OES reflects an exposure value at which it is felt no health risk is present and an MEL is one at which a risk still remains but the level is set taking into account socio economic factors. For an MEL attempts have to made to reduce exposures as far below the MEL as is reasonably practible. These limits are set following recommendations to the Health and Safety Commission (HSC) by advisory committees. The OESs are found in an HSC approved list published by the Health and Safety Executive (HSE) in a document entitled EH 40/94 (HSE 1994). The MELs are approved by the Secretary of State and listed under schedule 1 of the Control Of SubstancesHazardous to Health regulations. The MELs are also published in the HSE document EH 40/94. With the exception of cotton spinning OELs are based on total personal dust exposures. There are plans to change the exposure limits in the cotton spinning industry from the existing static sampling levels to personal dust exposures. For dusts not covered by a specific OEL, a nuisance level of 10 mg/m^3 is set. Notably for grain an MEL of 10 mg/m^3 and for hard wood dust exposures an MEL of 5 mg/m^3 exists. When considering this information the measured dust exposures are found to exceed these limits.

For endotoxin no specific control limits exist. However investigators have previously reported deleterious effects with exposures as low as 9 and 15 ng/m^3 in workers occupationally exposed to organic dusts (Castellan 1987, Milton 1994). Single exposures to isolated endotoxin have been found to have an effect in asthmatic individuals at a level of 20 mcg (Michel 1989), chronic bronchitics at 40 mcg (Cavagna 1969) and healthy individuals at 200 mcg (Rylander 1989). The measured values obviously far exceed the values quoted for individuals occupationaly exposed and some values approach levels found to have an effect in single isolated challenges.

Finally to consider the correlations found between the collected dust and endotoxin and their validity. In industries where the nature of the dust changes due to processing or different sites, the correlation for the whole industry specific cohort would be expected to be poor. The unequal distribution of samples across rooms and sites within industry cohorts can lead to a distortion of true correlation between dust and endotoxin. This is best demonstrated in the wool industry where a coefficient of 0.82 is obtained. The explanation for this finding, is that due to the diversity of the jobs in the carding room the majority of the samples were collected from this area. The contamination of dust in this room remains relatively constant although dust exposures vary. Considering the cohort of industries as a whole a strong correlation is found. The reason for this can be appreciated from the presented data, in general the industries where consistently high dust exposures are found also have highly contaminated dusts and conversely industries where the lowest dust exposures were found also had little contamination. This means that correlation coefficients

presented for dust and endotoxin in this and other work should be interpreted with caution and in conjunction with the documented variation in the levels of dust contamination between rooms.

Conclusion

Valid comparative data for dust and endotoxin exposures has been presented. This work highlights that workers with in the animal handling industries are exposed to high levels of dust and endotoxin. Workers during cleaning operations are exposed to particularly high exposures. In addition dust exposures where standards exist are being exceeded and endotoxin exposures are found to be above levels at which they have been implicated in the aetiology of respiratory morbidity. The effects of these exposures needs to be ascertained, 'safe' exposure limits determined and effective controls implemented.

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Table 1. Distribution of collected samples across industries and the relationship between the collected dust and measured endotoxin.

Industry	Nos of samples	% Sampled	Correlation (r)	Significance (p)
Mushroom	30.00	24.60	-0.03	none
Swine	27.00	62.80	0.27	none
Grain	31.00	44.90	0.71	p<0.001
Poultry	33.00	39.30	0.62	p<0.001
Saw mills	37.00	36.60	0.12	none
Cotton spinning	31.00	13.70	0.49	p<0.01
Wool mill	28.00	20.10	0.82	p<0.001
Animal feed	6.00	17.60	0.77	none
Weaving	36.00	16.80	0.77	p<0.001
Total	**259.00**	**25.10**	**0.70**	**p<0.001**

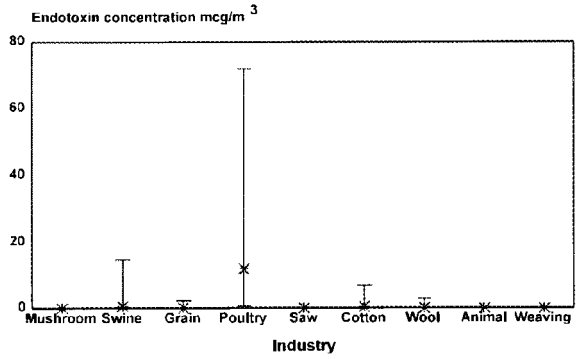

Figure 1. Comparison of personal dust exposures by industry (median and range)

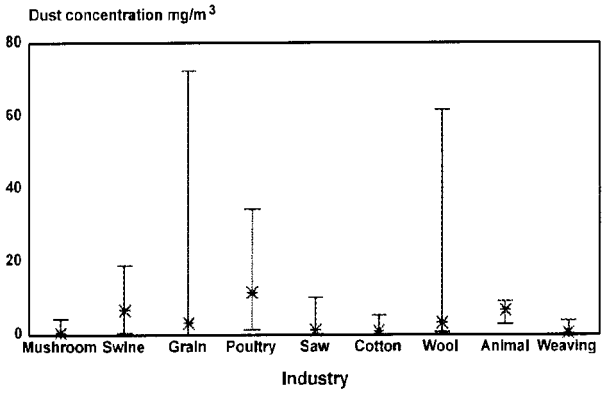

Figure 2. Comparison of personal endotoxin exposures by industry (median and range).

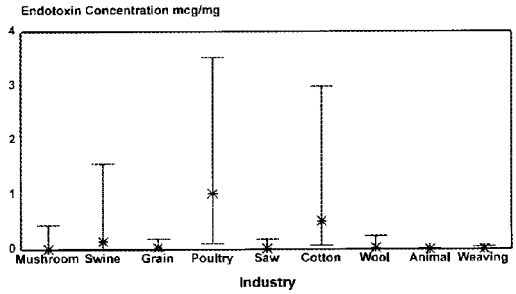

Figure 3 Comparison of contamination of dust with endotoxin by industry (median and range).