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Assessment of Exposure to Organic Dust in a Hemp Processing Plant

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The aim of this preliminary study was to assess exposure to various constituents of the organic dust generated during the processing of hemp in a small group of exposed workers. Airborne levels of inhalable dust, endotoxin and soluble protein, and the respirable, thoracic and inhalable fractions of fungal, bacterial and actinomycete contamination were measured in the personal breathing zone of exposed workers. Inhalable dust, endotoxin, fungal and bacterial contamination all exceeded levels found in similar vegetable fibre processing factories, since inhalable dust levels ranged from 10.4 to 79.8 mg/m^3 and inhalable bacterial levels between 4.7 and 190×10^6 cfu/m^3. Soluble protein and endotoxin (r=0.99, P<0.0001), endotoxin and inhalable dust (r=0.94, P<0.005) and inhalable dust and protein (r=0.98, P<0.0001) were significantly correlated, suggesting that there was little variation in the composition of the dust from different sites or activities around the workplace. Andersen sampling gave an indication of background microbe levels, although no attempt was made to identify the specific microorganisms as all plates were significantly overgrown. Airborne assessments demonstrated that exposures were highly task specific. For example, sweeping the floor generated the highest exposure levels of total dust, protein, endotoxin, bacteria and fungi. Therefore, we have shown that a modern-day hemp fibre processing plant produces significant quantities of respirable dust which is highly contaminated with endotoxin and microorganisms. This organic dust has the potential to cause a range of ill health problems.

Keywords: hemp; dust; protein; endotoxin; micro-organism exposure; personal monitoring; occupational lung disease

INTRODUCTION

Organic dust arising from the processing of hemp into fibre in either the paper or textile industries was first recognised as the cause of chest symptoms by Ramazzini (1940). Velvart and Stavroska (1963), reported hemp worker's disease in 34 out of 55 hemp workers and found that the severity of the disease was related to dust exposure in the workplace. Organic dusts are complex mixtures that may contain plant cell debris, insects, mites as well as viable and non-viable microorganisms (spores of fungi, actinomycetes and bacteria, and their components such as endotoxins and mycotoxins). Associations have been found between the concentrations of Gram negative bacteria and the prevalence of byssinosis (Rylander and Lundholm, 1978) and the endotoxin from organic dusts has been found to be associated with lung symptoms in studies of cotton workers (Simpson *et al.*, 1998).

Byssinosis was originally used to describe the respiratory disease found in cotton workers, however, it is now particularly used to describe first working day chest tightness that occurs in individuals exposed to hemp, flax and other dust from organic vegetable fibres (Fishwick and Pickering, 1992). Valic and Zuskin (1972) compared the prevalence of respiratory symptoms in textile workers and found that hemp (44%) and flax (43%) were more associated with a greater prevalence of byssinosis than cotton (27%).

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This supports the finding that hemp dust extract is more potent that cotton dust extract in causing epithelial damage (Bates *et al.*, 1995). There is continuing uncertainty, however, about the aetiology of the disease (Fishwick *et al.*, 1992, 1994), and only by assessing exposure levels associated with current work will further light be shed on this disease. Table 1 shows the levels of exposure to dust and endotoxin measured in cotton mills and hemp processing factories in previous studies.

Endotoxin has been suggested as a putative agent for byssinotic symptoms reported by workers exposed to organic dusts. It is a lipopolysachharide component of the cell wall of Gram negative bacteria. High levels of endotoxin have been found in cotton mills (17 000 EU/m^3; Christiani *et al.*, 1993) and grain mills (88 000 EU/m^3; Olenchock *et al.*, 1990). However guidelines published for a no-effect level for environmental endotoxin based on values for persons with histories of atopy or asthma are 2000 EU/m^3 for toxic pneumonitis, 100 EU/m^3 for airways inflammation and 1000 EU/m^3 for systemic effects (Rylander, 1997). The Dutch Expert Committee on Occupational Standards recommends a limit of 50 EU/m^3 over an 8 h exposure period (Heederik and Douwes, 1997).

Furthermore, previous work investigating hemp processors has identified four different bacteria from throat cultures of hemp workers (Zuskin *et al.*, 1992), although Velvart and Stavroska (1964) suggested that the bacterial and fungi found in hemp dust and sputum were not capable of causing the health effects seen.

The aim of this preliminary study based on a small number of modern day hemp workers was to characterise the components of hemp dust and to establish current exposure levels to these components. Personal and static sampling have been used to assess inhalable dust, protein, endotoxin, bacteria, fungi and actinomycete exposure.

METHODS

The factory and process

The hemp processing factory, a site representative of the UK processing of hemp, was visited on one study day in order to perform both personal and microbiological sampling. Sampling was performed during the morning shift when a mechanical fault limited hemp processing and during the afternoon shift when representative sampling was undertaken during full production. The process was carried out in one large area and consisted of the following stages. Large retting (or rotting) bales of hemp were transported to a guillotine via a conveyor belt. The straw was then dried through an oven tunnel and conveyed to a decorticator where it was passed through a series of rollers. The fibre/shiv/straw mix was then conveyed to a platform where the platform operator then manually forked the mix into one of three carding machines, where fibre was separated from shiv. The factory employed ten workers. On the day of the sampling visit, a machinery breakdown occurred, which was not rectified until the afternoon. Table 2 shows

Table 2. Sampling strategy[a]

Factory status	AM Partially operational	PM Fully operational
Personal dust sampling	✓	×
Personal endotoxin sampling	✓	×
Personal protein sampling	✓	×
Size selective microbial sampling	×	✓
Andersen sampling	✓	✓

[a]✓=sampled; ×=not sampled

Table 1. Levels of dust and endotoxin measured in previous studies

Reference	Factory, area	Mean total dust mg/m^3 (range)	Mean endotoxin EU/m^3 (range)
Christiani *et al.* (1993)	Cotton mill, carding	1.58 (0.74–2.58)	3440 (2510–16 970)
Gokani *et al.* (1987)	Cotton mill, carding	3.74 (2.83–6.85)	17 800 (69–66 000)
Rylander *et al.* (1993)	Cotton mill, carding	0.36	3200
Zuskin *et al.* (1990)	Hemp	22.4 (3.3–68.5)	ND
Zuskin *et al.* (1992)	Hemp	21.4 (4.1–45.6)	ND
Simpson *et al.* (1999)	Cotton mill (open/blow)	Median 1.07 (0.72–5.39) Personal sampling IOM	Median 9730 (710–69 360)
	Wool workers (carding)	Median 13.18 (3.69–61.69) Personal sampling IOM	Median 6940 (910–30 450)
Ogden *et al.* (1993)	Cotton mill	0.27 to 80 (median values) Personal sampling IOM	ND

the sampling strategy used and the operational state of the factory.

Personal sampling and analysis of total dust, protein and endotoxin

Individual breathing zone levels of inhalable dust, endotoxin and soluble protein were measured in seven randomly selected workers. Personal sampling was undertaken when the factory was not fully operational.

The bioaerosol was sampled for between 1 and 5 h, and collected on to a conditioned pre-weighed 0.8 μm polycarbonated filter (Millipore) in an Institute of Occupational Medicine (IOM) sampling head, with Gillian GilAir 5 personal sampler at 2 l./min. After gravimetric analysis, to determine the inhalable dust, the filters were eluted into 5 ml pyrogen-free distilled water and the endotoxin level was determined by the kinetic QCL test (BioWhittaker), a commercial quantitative kinetic assay based on the *Limulus* lysate assay (Rylander and Morley, 1982). Results were derived as Endotoxin Units (EU; 10 EU equates to 1 ng endotoxin). The protein concentration of the filter eluates were estimated by Bichinconnic acid method (Smith *et al.*, 1985) using an automated Cobas Fara using reagents obtained from Sigma Chemicals (UK).

Size selective personal sampling for viable bacteria, fungi and actinomycete

Four randomly selected workers (three production operators and one fork lift truck driver) wore personal bioaerosol samplers during the afternoon when the factory was fully operational, that were size selective for inhalable, thoracic and respirable fractions. This technique has previously been described (Crook *et al.*, 1997). An IOM head containing an 0.8 μm polycarbonated filter with two sterilised polyurethane foams (pufs) (puf 2: pore density of 45–90 pores per inch (ppi) and puf 1:45 ppi) were used with Gillian GilAir 5 personal sampler at 2 l./min. The inhalable fraction was calculated as the sum of the two pufs with the filter, the thoracic fraction being the sum of puf 2 and the filter and the respirable fraction taken as the filter alone. In the laboratory, each puf and filter was removed from the cassette and extracted into 5 ml Peptone Inositol Tween. Ten fold dilution series were used to inoculate agar plates. A range of agar media and incubation temperatures were used to isolate the different microorganisms: bacteria were grown at 25°C on Nutrient agar, fungi were grown on Malt (at 25°C and 45°C) and Dichloran-Glycerol (DG18) (25°C) and thermophilic actinomycetes were grown at 55°C on R8 agar (Amner *et al.*, 1989). The average number of colonies were counted and expressed as colony forming units (CFU)/m^3 air sampled.

Static Andersen sampling

Background viable microbial contamination was assessed by the Andersen six stage sampler, by impacting viable airborne particles in 6 fractions directly onto agar plates. The sampler was operated at 25 l./min and all samples were measured in duplicate and for two different times (chosen from 15, 30 or 60 s). A site close to the 'carding' area of the factory was chosen, and samples were obtained during both partial and full operation of the factory.

Plastic petri dishes containing the same agar as described above were used to quantify bacteria, fungi and actinomycete. Plates were incubated in the laboratory, at the above stated temperatures, on the day of sampling. Colony counts were made 48 h after collection, and again at 7 days. The average number of colonies were counted and amended for positive hole correction (Andersen, 1958). No attempt was made to speciate bacteria, fungi or actinomycete as all plates were significantly overgrown.

Scanning electronic microscopy of hemp dust

A sample of floor dust collected from around the retting bails was analysed by scanning electron microscopy (SEM) to determine the particle size and presence of storage mites.

Statistical methods

The correlation between levels of endotoxin, inhalable dust and protein was tested by Pearson's correlation test.

RESULTS

Personal sampling and analysis of inhalable dust, protein and endotoxin

Seven workers wore personal samplers, and were included in the study. Job titles and tasks performed are shown in Table 3. The levels of inhalable dust, soluble protein and endotoxin measured in the breathing zone of these workers during limited hemp fibre production are shown in Table 4. The mean inhalable

Table 3. Individual workers exposure to total dust, endotoxin and total protein during a work shift with limited hemp fibre production

Job title	No of workers sampled	Tasks
Workshop fitter	1	Machinery repair; general maintenance
Forklift truck driver	1	Moving bales and finished products
Production Operator	4	Cleaning, operating machinery, feeding carding machines, feeding guillotine
Packer	1	Packing final products

Table 4. Levels of dust, endotoxin and protein measured in the breathing zone of the seven workers studied

Task	Sampling time (min)	Inhalable dust (mg/m^3)	Endotoxin (EU/m^3)	Total protein (mg/m^3)
Machinery repair	204	10.4	4734	0.18
Driving forklift truck	286	18.8	19 895	0.53
General machinery operation	90	16.4	11 747	0.35
Packing	251	19.5	8254	0.32
General machinery operation	283	48.1	22 279	0.85
Cleaning	63	79.8	59 801	1.78
Feeding carding machine	271	13.6	10 276	0.25
Mean		29.5	19 569.4	0.61
Median		18.8	11 747	0.35
Geometric Mean		22.86	14 345	0.46
95% confidence interval		6.0–53.0	2177–36 962	0.01–1.13

dust concentration for all tasks was 29.5 mg/m^3 (range 10.4–79.8 mg/m^3), for total protein 0.61 mg/m^3 (range 0.18–1.78 mg/m^3) and for endotoxin 19 569 EUm^3 (range 4734–59 801 EU/m^3). The task associated with the highest exposure of dust, protein and endotoxin was sweeping by the production operator during routine cleaning of the workplace. The lowest exposure was recorded by the workshop fitter during machinery repair. This worker spent some time away form the main processing area during the sampling period (approximately 30 min). Interestingly, there was a highly significant correlation between inhalable dust and total protein (r=0.98, P<0.0001), inhalable dust and endotoxin (r=0.94, P<0.005) and total protein and endotoxin (r=0.99, P<0.0001).

Size selective personal sampling for viable bacteria, fungi and actinomycete

Four workers wore size selective personal samplers with pufs to determine the inhalable, thoracic and respirable fraction of microbial exposure. These measurements were taken when the factory was fully operational (Table 5). The mean respirable levels of bacteria were (0.34×10^6 cfu/m^3), fungi (0.16×10^6 cfu/m^3) and actinomycetes (0.057×10^6 cfu/m^3). The task associated with the highest exposure to microorganisms was sweeping.

Andersen sampling

Andersen sampling was performed at a single site in both the morning (during limited production) and in the afternoon, when production was at full capacity. A comparison of the counts during partial and full production is shown in Table 6(a) and (b). Fungal counts in the first five fractions obtained whilst the factory was fully operational could not be made, as most plates were overloaded during a 15 s sampling time. However, comparison of the sixth fraction between partial and full operation of the factory showed between a two and six fold increase in all fungal counts (except actinomycetes) when production was at full capacity compared to limited hemp production. The total actinomycete counts were unaffected by hemp production, remaining at approximately 1.4×10^5 cfu/m^3 regardless of activity within the factory. Bacterial counts are not presented here as the nutrient agar plates were too overgrown to accurately count individual colonies.

Table 5. Total levels of airborne viable bacteria, fungi and actinomycetes in the breathing zones of four hemp processing workers in the inhalable, thoracic and respirable fractions (cfu/m^3)

Task	Microorganism	Inhalable $\times10^6$	Thoracic $\times10^6$	Respirable $\times10^6$
General machinery operation	Bacteria	19.00	5.90	0.38
	Fungi	2.00	1.00	0.15
	Actinomycetes	0.35	0.21	0.09
General machinery operation	Bacteria	9.80	5.60	0.14
	Fungi	1.30	0.50	0.13
	Actinomycetes	0.24	0.08	0.04
Driving forklift truck	Bacteria	4.70	2.20	0.17
	Fungi	1.20	0.65	0.13
	Actinomycetes	0.16	0.14	0.05
Sweeping factory floor/cleaning	Bacteria	190.00	37.0	0.70
	Fungi	13.00	1.90	0.23
	Actinomycetes	1.40	0.13	0.05

Table 6. Background levels of bacteria, fungi and actinomycetes fractionated using Andersen sampling from a site close to the carding area of the factory during (a) limited hemp fibre production and (b) fully capacity hemp fibre production[a]

Fraction		Malt 25°C fungi	Malt 40°C thermophillic fungi	R8 55°C Actinomycete	DC18 25°C fungi
(a) Carding area: limited production					
>8 μm	1	2.8×10^4	0.066×10^4	2.5×10^4	2.7×10^4
5–10.5 μm	2	2.4×10^4	0.05×10^4	1.9×10^4	3.4×10^4
3–6 μm	3	3.5×10^4	0.062×10^4	2.9×10^4	5.8×10^4
2–3.5 μm	4	2.6×10^4	0.054×10^4	2.3×10^4	5.7×10^4
1–2 μm	5	1.5×10^4	0.02×10^4	2.6×10^4	3.0×10^4
<1 μm	6	1.2×10^4	0.028×10^4	1.4×10^4	2.1×10^4
Total cfu/m^3		1.4×10^5	0.028×10^5	1.4×10^5	2.3×10^5
(b) Carding area: full production					
>8 μm	1	>	>	2.5×10^4	>
5–10.5 μm	2	>	>	1.9×10^4	>
3–6 μm	3	>	>	3.6×10^4	>
2–3.5 μm	4	>	>	2.3×10^4	>
1–2 μm	5	>	0.33×10^4	2.6×10^4	7.8×10^4
<1 μm	6	3.3×10^4	0.17×10^4	2.2×10^4	8.3×10^4
Total cfu/m^3		–	–	1.5×10^5	–

[a]>too overgrown to be counted.

Scanning electron microscopy

No storage mites were detected in the hemp dust, although particles with a diameter of less than 5 μm (Fig. 1) were identified.

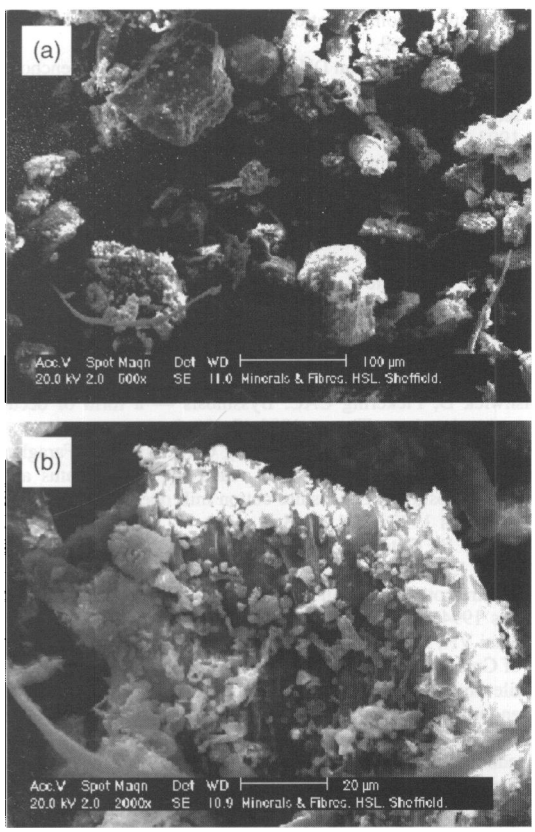

Fig. 1. Scanning electron micrograph of hemp dust sample: (a) magnification ×180; (b) magnification ×900.

DISCUSSION

In this small preliminary study of current hemp workers, we have shown that workers were exposed to extremely high levels of organic dust which was heavily contaminated with protein, endotoxin and micro-organisms. In all workers studied, the levels of dust measured exceeded 10 mg/m^3, albeit for a maximum exposure period of two and a half hours. Furthermore, levels of bacteria and fungi frequently exceeded one million cfu per m^3 of air. Certain of these measurements were taken when the factory was not fully operational, and these should therefore be considered as an underestimate of the exposure which would be encountered during normal operation. Dust levels measured at the hemp processing factory were similar to those previously reported for this process (Zuskin *et al.*, 1990), but were more than ten fold greater than those commonly found in cotton mills (Christiani *et al.*, 1993; Gokani *et al.*, 1987) and animal confinement buildings (Wathes *et al.*, 1997; Donham, 1986). Studies of cotton dust exposure documented mean levels of inhalable dust of 1.58 and 3.74 mg/m^3. The range of dust levels seen in this study was 10.4–79.8 mg/m^3, and the highest value was obtained from a worker sweeping up, presumably due to the large particulate aerosols generated from machines and the floor during this operation. Furthermore, a high proportion of all particles were found to be in the optimally respirable range. Such high exposures have the potential to cause significant health effects, a fact recognised by the company. All workers were supplied with personal respiratory protective equipment.

The levels of endotoxin measured in this workplace were also high, and can be compared to endotoxin levels previously measured in cotton mills by static

sampling where Christiani *et al.* (1993) and Gokani *et al.* (1987) measured levels of 3440 and 17 800 EU/m^3 respectively.

Despite these exposure levels, there are currently no specific exposure limits for endotoxin exposure in the United Kingdom. Furthermore, investigators have reported respiratory symptoms with exposures as low as 90 and 115 EU/m^3 in workers exposed to organic dust (Castellan *et al.*, 1987). The measured personal exposures far exceed those recommended for a no-effect level (Rylander, 1997; Heederik and Douwes, 1997) and demonstrate a source of high endotoxin exposure. Indeed, data from challenge work suggests that relatively low doses of endotoxin (20 μg) may cause significant pulmonary physiological and immunological changes, unaccompanied by systemic or respiratory symptoms (Michel *et al.*, 1992).

Size selective sampling of the microbial portion of the organic dust showed that bacteria formed the bulk of the inhalable, thoracic and respirable fraction.

Microbiological analysis of air quality demonstrated significant bacterial and fungal contamination, so much that individual identification of species was not possible. Actinomycetes and fungi have been reported to be present in the air spora of cotton mills (9.8×10^6 and 1.2×10^6 for actinomycetes and 1.2×10^6 and 4×10^5 spores/m^3 for fungi) (Lacey and Lacey, 1987). In the hemp factory, total contaminating actinomycete and fungi levels were high at 6×10^5 and 7×10^6 cfu/m^3 respectively. Microbial contamination expressed as cfu/m^3 gives an indication of the total viable micro-organisms whereas spores/m^3 gives the total viable plus non-viable.

Andersen samplers were used to impact particles directly onto agar to increase survival of some delicate micro-organisms and to provide size distribution data. However, due to the dustiness of the mill, short sampling times were used, and so the data can only be considered as semi-quantitative. Micro-organisms were distributed on all stages of the sampler suggesting many of the particles were less than 5 μm in diameter.

Clearly, the generalisation of this data to other similar processes must be handled with caution. This study was preliminary with a limited sampling strategy, when certain components of machinery were not in use. Nevertheless, it provides data in an area where there is a genuine shortage of measurements, and provides important information regarding a variety of hemp dust constituents.

The previously reported high prevalence of chronic respiratory symptoms in hemp workers (Valic and Zuskin, 1972) suggests that exposure to hemp dust is a potent risk factor for the development of respiratory disease. As with other workers exposed to organic dusts, such as cotton workers and swine workers, endotoxin and microbiological exposures can be high, and the most high levels particularly relating to cleaning tasks. Endotoxin appears to be a strong candidate for the agent responsible for at least a proportion of the systemic and respiratory ill health seen, although may not entirely responsible for the health effects seen (Christiani *et al.*, 1999).

There was a significant correlation between all constituents of the organic dust suggesting that its composition was consistent, regardless of task within the workplace. This can be contrasted with the change in the nature of the microbial contaminants observed at different production levels within the factory. The nature and origin of the total protein level is not known.

The information from this study was fed back to the management at this site, and a state of the art new facility is planned. It is our intention to revisit and re study airborne levels of dust, microbiological gents and protein after this major intervention.

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