REPEATABILITY OF SAMPLING FOR ENDOTOXIN AND DUST WITHIN 3 COTTON TEXTILE MILLS

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Abstract

Endotoxin is one of the contaminants of cotton dust that may be responsible for respiratory illness within textile workers. Epidemiological studies have shown that both dust and endotoxin in the air of cotton mills are closely correlated to the presence of respiratory symptoms. Currently occupational exposure standards in both the UK and USA are set on dust levels rather than endotoxin, primarily as it is considered that endotoxin sampling is unreliable and not reproducible and could give significantly varying results when performed by different technicians.

We analysed the dust and endotoxin concentrations of airborne samples (as determined by endotoxin per m3 of air and per mg of dust in repeated samples obtained in 3 mills visited during the prospective study of textile workers. 3 different technicians performed airborne sampling in 3 mills 1 year apart in each case. Paired samples were obtained both across years and within years. Within year samples were obtained predominantly on the same day but on two different workers with the same job in the same workroom and mill. Across year samples were obtained often from the same worker sampled a year apart or alternatively from a colleague with the same job in the same workroom and mill, one year apart.

All 3 measured parameters varied significantly from year to year. Correlation coefficients comparing logged values between years were only significantly correlated for endotoxin per m3 air ($R=0.76$ $p<0.001$) and were non-significant for dust levels. Within year correlation's were all strongly positive: dust 0.84 endotoxin per m3 0.85, endotoxin per mg 0.82 $p<0.001$). Coefficients of variation for repeated measures, calculated form logged data, show that within year variation on a sample were 38% for dust, 67% for endotoxin per m3 air and 49% for endotoxin per mg dust. The equivalent values for across year samples were 100%, 87% and 107%. From this data it is likely that the variation in results from sampling and assay variability is in the order of 50% and true change in exposure levels over time account for a further 50%.

The repeatability of endotoxin sampling by different technicians using the same protocol is no worse than that for dust measures and this is not a contra-indication to the use of exposure standards in this pilot study, but a larger more standardised study is recommended.

Introduction

Exposure to cotton dust is associated with a number of respiratory problems including acute and chronic byssinosis, chronic bronchitis and other work related respiratory symptoms. Epidemiological work has shown that acute and chronic byssinosis and other work related respiratory symptoms are related to endotoxin exposure within the textile industry (Niven 1993, Haglind 1984), while chronic bronchitis is not (Niven 1997). However sampling and assay methods for endotoxin are poorly standardised (Jacobs 1997). It is thought that their use for monitoring exposure and setting of standards is as yet inappropriate because dose response relationships and methodology are unclear (Jacobs 1997). It is also considered that measured exposure may be different in different centres, using different tools and technicians. There may also be an unacceptable variability in repeated sample measurements.

We planned to determine the degree of variability of repeated sampling for which the variability is caused in part by the assay and in part by the sampling strategy. We reviewed the results of endotoxin samples taken in three cotton mills sampled on two occasions on subsequent years and where 3 different technicians had been responsible for the sampling and assay procedure. The methodology was standard for each technician (Simpson 1996). Intra observer (paired samples taken on individuals with the same occupational code, same year, same technician) and inter observer (same occupational code, year apart, different technician) were compared for both dust levels and measured endotoxin levels as measured as endotoxin per mg dust and endotoxin per m3 of air sampled.

Methodology

Target Population

All endotoxin sampling performed as part of a longitudinal study of textile workers in Lancashire was reviewed to identify mills and occupational codes for which more than one sampling exercise had been performed. All samples where one occupational code had been sampled on more than one occasion by different technicians within the study were included. This identified 3 mills and 3 technicians. Within these mills all occupational codes (same job title, same work room) which had been sampled more than once by the same technician (intra-observer) and by the different technicians on separate sampling programmes (inter observer) were identified as paired samples. 18 paired intra-observer samples and 16 paired inter observer samples were identified. All these paired samples were included in the analysis.

Dust Sampling

Personal dust sampling was used throughout the study. The methodology which is now the basis for occupational

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exposure standards within the UK has been previously described (Niven 1992). Briefly it entails the use of an IOM sampling head, containing a pre-weighed cassette and micro-glass fibre filter. The head is attached on the overalls of the worker, on the left lapel. The head is connected to a 2L/min sampling pump, whose flow rate is checked before and after sampling. The sampling period is the majority of a work shift with a minimum duration of 3 hours. The sampler is switched off for meal and smoking breaks away from the work site. The cassette is removed after sampling and re-weighed. Personal dust exposure, is determined from the weight of dust collected and sampling volume calculated. A correction factor is determined by the use of control filters to allow for any weight change caused by water absorption or loss. Results are expressed as dust concentrations in mg/m3. The current occupational exposure standard in the UK is 2.5mg/m3 of personal dust exposure.

Endotoxin Exposure

The filters are removed from the cassettes when they have been weighed following sampling for dust as described above. They are placed in endotoxin free glass-ware and stored at -20C until assaying. Extraction is performed in 10 mls of Pyrogen Free Water. For the first set of sampling (technician 1 - 1992), extraction was performed by agitation in a water bath for 60 minutes. Following this, the protocol was slightly modified to involve vortex for 30 seconds followed by agitation in a Spiramix for 60 minutes followed by repeat vortex. Extracts were centrifuged and the supernatant collected for assay. Duplicate samples of the extract were assayed using a quantitative turbidometric LAL assay (LAL 5000) and compared to standard curves. Dilution of extracts was performed to obtain the lowest concentration giving a signal within the standard curve, to minimise any enhancement or inhibition of the assay. The control filters were handled and extracted in identical fashion accept that they were not subject to air sampling. They were also analysed for endotoxin, to correct for any contamination of the original filters or during the assay process. Endotoxin in control filters is generally less than 0.5% of the total endotoxin in exposed filters from cotton workers. The amount of endotoxin is expressed as ng/m3 of air sampled and ng/mg of dust.

Analysis

Dust and endotoxin exposure data is not normally distributed. All results were logged to normalise the distribution for further analysis. Two methods of analysis were employed. Firstly the correlation coefficients for intra-observer and inter observer parameters were determined. Secondly the coefficients of variation of paired samples were determined using analysis of variance.

Results

18 paired samples were identified for intra observer comparison and 16 for inter observer comparison. The crude dust values, airborne endotoxin concentrations and endotoxin concentration in dust for each pair are presented in tables 1 and 2.

The correlation coefficients for the paired sample are presented in table 3. Intra-observer dust levels showed significant correlation ($R= 0.84$, $p<0.001$). In addition, there were strong correlation's between paired levels of endotoxin concentration in air ($R=0.85$, $p<0.001$), endotoxin concentration in dust ($R=0.82$ $p<0.001$) and between dust exposure and endotoxin exposure as measured by either parameter.

For inter observer exposure measures (across years), there were fewer significant correlation's, and specifically there was no correlation between dust levels measured across years. However a strong correlation persisted between endotoxin concentration in air as measured between the two years ($R=0.87$ $p<0.001$).

The coefficients of variation for paired samples are presented in table 4. Within years the lowest coefficient of variation is determined by dust monitoring (38%) compare to endotoxin concentration in air (67%) and endotoxin concentration per mg dust (49%). However in paired samples across years and by different observers the trend is reversed although the overall coefficients of variation are much higher. Endotoxin concentration in air sampled has the lowest coefficient of variation (84%) compared to dust levels (100%) and to endotoxin per mg of dust (107%)

Conclusions

The use of endotoxin measurements for standard setting within industries exposed to organic dusts has been the subject of some debate (Rylander 1997). Within specific individual industries the use of endotoxin levels rather than dust is unlikely to confer any advantage and as it is more expensive, time consuming and as yet not fully understood, it has no practical advantages. However if endotoxin levels across different organic dust industries is more predictive of the effects of exposure than dust levels, then a single standard using endotoxin may be preferable. Another concern has been raised over the repeatability of endotoxin measurements made by different individuals and additionally in different centres (Jacobs 1997). The data presented here (extracted from results of a prospective study of textile workers), indicates that while there are high levels of variation of measurements when made across differing time periods by different technicians in terms of sampling, extraction and assay, that these are no different than the variation observed for dust levels made under the same conditions. Indeed it is likely that the majority of the variation of repeated samples is determined by genuine variation in exposure over time.

Paired samples measuring dust and endotoxin levels obtained from different workers doing the same job in the same room at the same time, also show a moderate degree of variation.

This suggests that a significant proportion of the variability in exposure is produced by the individual being sampled. This may relate to a number of factors including:- work practices (tidy worker/dirty worker), ergonomic factors (height, weight etc. determining distance from source of dust) and different local sources depending on the individual machines being supervised by the different workers.

Although different technicians performed the sampling, extraction and assay process across several years and the LAL reagent and endotoxin standard used for standard curve varied, the protocol was similar and the equipment and other materials used were identical. It is not known how much effect, the use of different equipment, manufacture and type of assay would introduce.

The methodology employed within this study, was a simple water extraction method, which is known to extract only a proportion of the endotoxin from the total endotoxin within the sample (Gould 1987). Despite this, the variation in exposure levels were not worse than those of dust measurements. Indeed the finding that across years endotoxin concentrations in air remained correlated while dust levels did not, would be in favour of using endotoxin rather than dust. However, before such a recommendation can be made similar studies need to made in different industrial settings with exposure to dust or aerosols containing endotoxin. In addition a standard methodology should be produced for all endotoxin measuring centres (Wood 1997) and a study comparing results of endotoxin extraction and assay performed in multiple centres performed
.
In conclusion this study has demonstrated that the variation of exposure measures, made by different technicians is no different for endotoxin than it is for dust concentrations. While additional work is required across different scientific centres and further work is performed to elucidate the specific role of endotoxin in the physiological effects of organic dust exposure in both the short and long term, the study supports the possible future use of endotoxin measures for exposure standard setting within organic dust industries.

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Table 1. Dust and endotoxin levels in intra-observer study, same technicians paired samples on occupation.

Pair number	Occupation	Sample A dust	Sample A endotoxin		Sample B dust	Sample B endotoxin	
		mg/m3	ng/m3	ng/mg dust	mg/m3	ng/m3	ng/mg dust
Mill A. 1994							
1.	Winder	0.689	235	341	0.734	139	190
2.	Feeder (opening)	1.070	2543	2377	0.719	973	1352
3.	Labourer (winding)	0.497	144	290	0.967	294	304
Mill B. 1994							
4.	Spped-frame (card)	0.750	1060	1412	0.747	797	1067
5.	Draw-frame (card)	3.317	1887	569	0.711	390	569
Mill C. 1994							
6.	Labourer (card)	2.910	378	131	4.192	691	157
7.	Winder	0.795	415	522	1.445	1058	732
8.	Doubler (doubling)	1.506	1604	1065	1.139	1651	1450
Mill A 1992							
9.	Packer (wind)	0.159	63	398	0.149	27	179
10.	Winder (wind)	0.260	134	515	0.243	112	462
11.	Winder (wind)	0.294	56	192	0.154	32	210
Mill B 1992							
12.	Feeser (open)	2.138	7351	3448	1.995	4955	2483
13.	Spinner (Ring)	0.398	106	282	0.351	387	1102
14.	Card attendant	1.302	1488	1143	0.829	1515	1827
Mill C 1995							
15.	Winder (wind)	2.080	1092	525	1.290	338	262
16.	O/E spinner	2.210	526	238	2.450	511	208
17.	O/E spinner	2.450	511	208	1.310	480	367
18.	O/E spinner	1.310	480	367	2.210	526	238

O/E = open ended.

Table 2. Dust and endotoxin levels in inter-observer study, across years, different technicans.

Pair number	Occupation	Sample A dust	Sample A endotoxin		Sample B dust	Sample B endotoxin	
		mg/m3	ng/m3	ng/mg dust	mg/m3	ng/m3	ng/mg dust
Mill A. 1992 - 1994							
1.	Feeder	1.277	1250	979	5.388	639	119
2.	Draw-frame (card)	0.532	675	1246	2.014	1138	559
3.	Spinner (ring)	0.161	73	451	0.347	66	190
4.	Winder (wind)	0.266	101	390	0.711	187	265
5.	Beamer (beaming)	0.154	32	210	1.124	84	75
6.	Doubler (doubling)	0.387	70	182	1.037	94	91
Mill B. 1992 - 1994							
7.	Feeder (open)	1.995	4955	2484	1.070	2544	2377
8.	Blowman (blow)	2.138	7351	3438	0.719	972	1353
9.	Card attendant	1.066	1502	1485	1.092	1035	945
10.	Spinner (ring)	0.375	196	693	0.599	137	229
11.	Winder (wind)	0.286	51	180	0.732	219	267
Mill C. 1994 - 1995							
12.	Feeder (open)	2.318	6936	2992	12.36	3717	301
13.	Labourer (card)	3.651	535	143	1.460	424	290
14.	O/E spinnner	4.292	398	93	1.990	505	271
15.	Winding (wind)	1.120	736	627	1.685	715	394
16.	Doubler (doubling)	1.323	1627	1207	1.16	620	535

Table 3. Correlation coefficients for exposure parameters in the intra and inter observer study.

	Intra-observer (n=18)		Inter observer (n =16)	
Parameters correlates	R value	Significance	R value	Significance
Dust sample A to dust sample B	0.84	< 0.001	0.55	n.s.
Endotxin per m3 sample A to B	0.85	< 0.001	0.87	< 0.001
Endotoxin per mg dust A to B	0.82	< 0.001	0.57	n.s.

Table 4. Coeeficients of variation for paired samples as determined for the intra and inter observer analysis.

Parameter	Coefficient of variation	
	Intra-observer	Inter-observer
Dust (mg/m3)	38%	100%
Endotoxin (ng/m3 of air)	67%	84%
Endotoxin (ng/m3 of dust)	49%	107%