ORIGINAL ARTICLE

Agricultural seed dust as a potential cause of organic dust toxic syndrome

L A M Smit, I M Wouters, M M Hobo, W Eduard, G Doekes, D Heederik

Occup Environ Med 2006;**63**:59–67. doi: 10.1136/oem.2005.021527

See end of article for authors' affiliations

Correspondence to: Ms L Smit, Institute for Risk Assessment Sciences, Division of Environmental and Occupational Health, PO Box 80176, 3508 TD Utrecht, the Netherlands; L.Smit@iras.uu.nl

Accepted 7 September 2005

Aims: Episodes of serious work related health problems resembling organic dust toxic syndrome (ODTS) in workers of a grass seed quality inspection laboratory prompted the authors to study personal endotoxin exposure levels in this facility and in the agricultural seed processing industry. In addition, microbial and inflammatory characteristics of agricultural seeds were studied.

Methods: The authors assessed inhalable dust and endotoxin levels in 101 samples from 57 workers in grass, cereal, and vegetable seed plants who were handling mainly grass seeds as bulk product, and horticulture seeds in smaller quantities. Additionally, real-time dust exposure was measured using a DataRAM monitor in 12 grass seed workers to obtain more information on exposure patterns during specific tasks. Endotoxin concentrations in seed extracts were determined by LAL assay and seed samples were analysed by scanning electron microscopy. Release of inflammatory cytokines was measured in supernatants of whole blood samples stimulated with lipopolysaccharide (LPS) or agricultural seed extracts in a human whole blood assay (WBA).

Results: Endotoxin concentrations in personal samples were high (geometric mean 1800 EU/m^3), particularly in the grass seed quality inspection lab where endotoxin levels up to 274 000 EU/m^3 were measured. The recommended health based endotoxin exposure limit of 50 EU/m^3 was amply exceeded in almost all personal samples. Job tasks dumping and mixing were associated with highest dust and endotoxin exposures, which was confirmed by real-time measurements. Microbial infestation was found in almost all seed samples. WBA results showed that most seed extracts were capable of inducing a pronounced dose dependent cytokine release.

Conclusions: Workers handling grass, cereal, or vegetable seeds are at risk of exposure to high levels of endotoxin containing seed dust. Occupational exposure to inhalable agricultural seed dust can induce inflammatory responses, and is a potential cause of ODTS.

Occupational and environmental exposure to endotoxin, a cell wall component of Gram negative bacteria, is associated with a dose dependent risk of inflammatory health effects.[1] Epidemiological studies in a variety of occupational environments such as pig farming, animal feed, and grain processing, and the waste and compost industry have shown exposure-response relations between endotoxin exposure and acute lung function changes, accelerated chronic lung function decline, work related and chronic respiratory symptoms, and airway hyperresponsiveness.[2–5] Even at relatively low levels, above 50–500 EU/m^3 over eight hours, endotoxin exposure may cause across shift decline in lung function,[6] mucosal membrane irritation, and dry cough.[1] Earlier studies have shown that workers heavily exposed to endotoxin containing organic dust such as hay, silage, and grain dust may develop organic dust toxic syndrome (ODTS), an acute non-allergic flu-like illness characterised by fever, chills, chest tightness, shortness of breath, dry cough, myalgias, and/or fatigue.[7 8] Incidents of ODTS appear to be common among farmers, particularly after work tasks such as cleaning grain bins or removing mouldy feed.[8 9] A number of studies have shown that endotoxin is primarily responsible for inflammatory responses following grain dust exposure, although other components in grain dust such as tannins, β(1→3)-glucans, or mycotoxins may contribute to in vivo and in vitro inflammation as well.[10–12]

In contrast to grain dust exposure, which is a relatively well described occupational health risk, grass seed dust has thus far not been recognised as a potential source of high endotoxin exposure. In a preliminary paper, we earlier reported the occurrence of episodes of serious work related health problems resembling ODTS in workers of a grass seed quality inspection laboratory.[13] An initial airborne dust sample showed high endotoxin levels which prompted us to study personal exposure levels in this facility. In addition, we investigated whether high endotoxin levels are a general phenomenon in the agricultural seed processing industry or an exclusive finding in this particular grass seed quality control laboratory. Therefore we assessed personal inhalable dust and endotoxin exposure in grass, cereal, and vegetable seed processing plants. Real-time dust exposure was measured in a sample of grass seed processing workers to obtain more information on exposure patterns during specific tasks.

Endotoxin activity was determined with the limulus amebocyte lysate (LAL) assay, which may be influenced by components in seed, either by directly activating or by strongly potentiating, resulting in false positive results.[14] If endotoxin or other components in airborne grass seed dust were indeed the cause of observed ODTS cases, grass seed should not only activate the LAL assay but should be capable of activating human inflammatory cells as well. Therefore we studied the capacity of seed extracts to induce cytokine production in a human whole blood assay (WBA).

Abbreviations: LAL, limulus amebocyte lysate; LOD, limit of detection; LPS, lipopolysaccharide; ODTS, organic dust toxic syndrome; SEM, scanning electron microscopy; WBA, whole blood assay

METHODS

Case study

The study was initiated in the late 1990s by a case of chronic and/or recurrent health problems in a small workforce of a seed quality control institute, as reported previously in concise form.[13] At that institute, relatively small seed samples (batches of 0.1–1 kg) from producers are routinely checked for the presence of mould growth and other plant pathogens, examined microscopically to determine species and variety purity, and by in vitro culture to assess germination capacity under standard conditions. After a first visual inspection the samples were cleaned with mechanically shaken sieves to which the samples had been applied manually. The sieving machines were concentrated in a specific cleaning room, with central ventilation, and above each of the sieving machines was a hood to remove dust. Each cleaning ''cycle'' took one to a few hours, during which workers spent most of the time in the room, and often worked close to the sieves—to remove small stones or other objects, to add new seed portions, to clean and replace sieves after the runs, and so on.

Among the fewer than 15 workers who frequently worked in the sieving room, a high frequency of health problems had been noted, especially related to cleaning of grass seeds. In the last few years, seven workers had often suffered during or after grass seed cleaning from upper respiratory tract (stuffy nose, frequent nose bleeding: two cases), lower respiratory tract (chest tightness, dyspnea: five cases), gastrointestinal (diarrhoea, pain, nausea: two cases) and/or flu-like symptoms (fever and shivering: four cases), joint pain (three cases), chronic or recurrent severe fatigue (five cases), and/or severe headache or migraine (three cases).

It appeared that in the past similar syndromes or symptoms had occurred in other workers. In some of them, with positive skin prick reactions to grass pollen extracts, grass (pollen) allergy had been diagnosed as the most likely cause, and they had consequently left the workplace. Most of the more recent symptoms were however not associated with grass pollen or any other demonstrable common atopic sensitisation. Furthermore, although seed dust from the laboratory had been shown to be moderately rich in common mould spores, tests for IgE or IgG mediated mould allergy in cases had been negative.

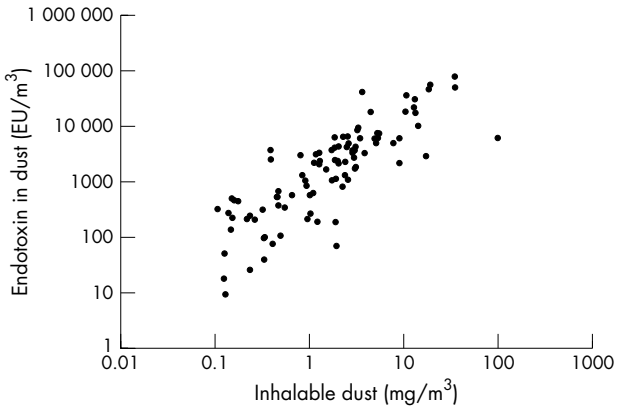

Figure 1 Endotoxin concentrations versus inhalable dust concentrations in 96 personal samples. Pearson's $r=0.82$ ($p<0.0001$; calculated with log transformed data).

As symptoms had increased in frequency and severity after the move of the institute to a new building on a different location, the ventilation system had been checked and renewed, but this also had not eliminated or lessened the health problems. The possibility of high endotoxin exposure had sometimes been suggested but was not considered a likely explanation. The first endotoxin measurements were actually taken mainly with the thought to exclude this possibility.

Personal exposure assessment

In November 1999, personal endotoxin exposure was determined in the seed quality control institute. Two workers in the cleaning department were investigated and four measurements were taken during the sieving of grass seed.

The study was extended to a series of personal exposure measurements in the whole seed processing industry. A representative sample consisting of eight seed processing plants (two grass seed plants, two grass and maize seed plants, one cereal seed plant, and three vegetable seed plants) in different parts of the Netherlands was selected after consultation with the agricultural seed industry association. Seeds are delivered as bulk product at these plants after

Table 1 Inhalable dust and endotoxin exposure levels in the agricultural seed processing industry

		Inhalable dust (mg/m³)		Endotoxin (10^3 EU/m³)	
Plant	n	GM (GSD)	Range	GM (GSD)	Range
November 1999					
Grass seed, quality inspection lab	4	–*	–	88 (2.5)	33–274
November 2000					
Grass and maize seed-1	5	0.9 (2.9)	0.2–1.9	0.6 (2.4)	0.2–1.7
Grass seed-1	6	5.4 (1.5)	3.1–9.0	3.7 (1.7)	1.8–6.1
April 2001					
Grass and maize seed-1	12	1.0 (9.1)	0.1–98.0	0.6 (11.9)	0.01–51
Grass and maize seed-2	11	2.6 (2.0)	1.0–13.3	3.4 (2.3)	0.6–18
Grass seed-2	15	1.5 (3.4)	0.1–17.2	2.9 (3.0)	0.2–10
Cereal seed-1	2	4.0 (1.5)	3.0–5.5	3.6 (2.9)	1.7–7.5
Vegetable seed-1	10	1.0 (3.6)	0.2–14.1	0.6 (6.0)‡	0.04–10
Vegetable seed-2	14	0.9 (3.6)	0.2–12.7	0.7 (5.0)	0.1–22
Vegetable seed-3	16	1.0 (3.4)	0.1–5.3	1.0 (8.5)	0.03–42
January 2002					
Grass seed, research institute	6	16.1 (1.6)	10.4–34.7	41 (1.7)	19–80
All	101	1.6 (4.3)†	0.1–98.0	1.8 (7.7)§	0.01–274

n, number of measurements; GM, geometric mean; GSD, geometric standard deviation.
*Inhalable dust levels not available.
†n = 97.
‡n = 9.
§n = 100.

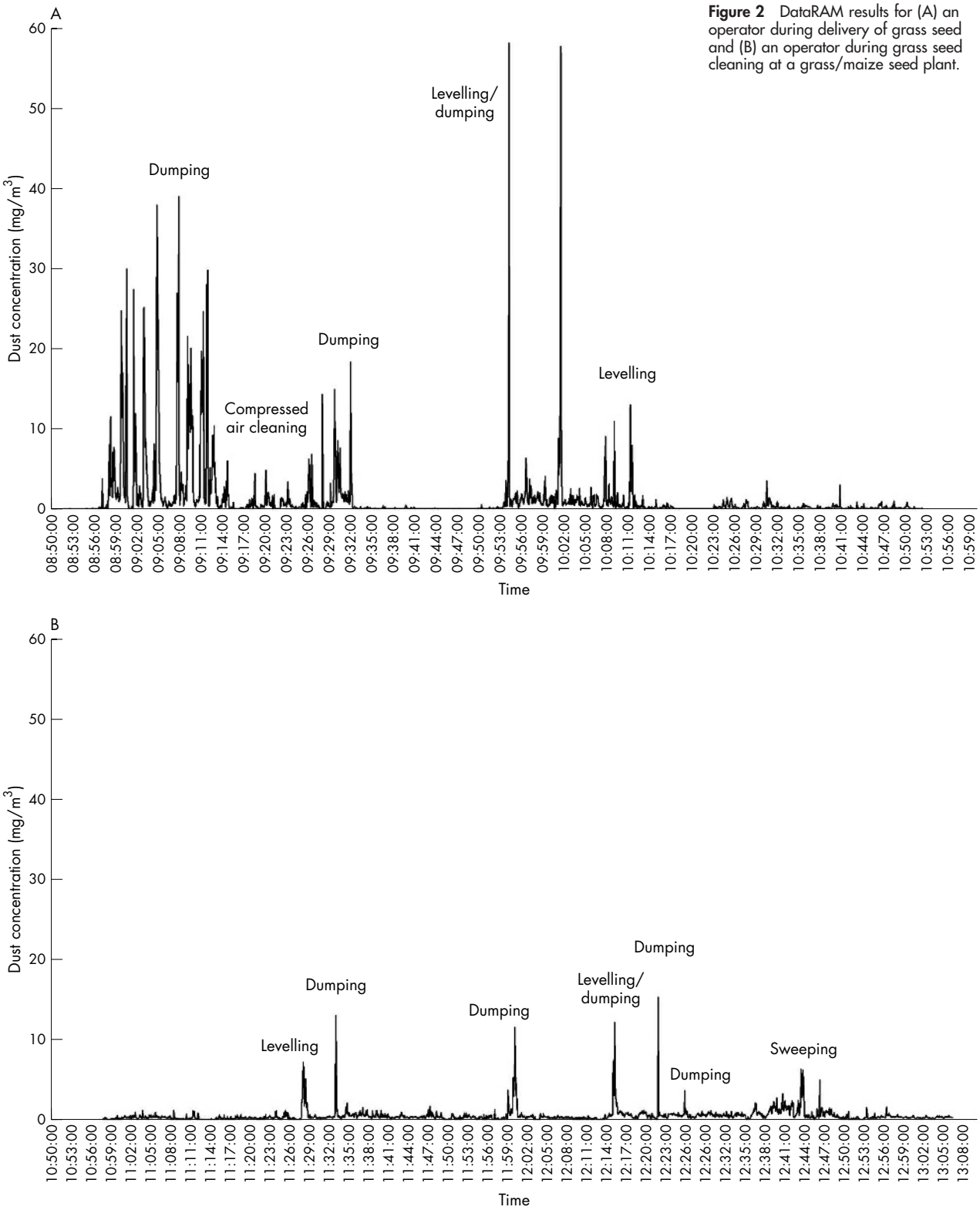

Figure 2 DataRAM results for (A) an operator during delivery of grass seed and (B) an operator during grass seed cleaning at a grass/maize seed plant.

harvesting and threshing by the producers. First, seeds are often dried to reduce the moisture content, then seed is cleaned mechanically by sieving to remove weed seeds, inert matter, and poor quality seeds. Cleaned seed from different boxes is mixed mechanically to form a homogeneous lot, and sometimes disinfection or coating with fungicide is applied before seeds are packaged. Each seed lot is sampled at different stages of processing and tested by an on-site quality control laboratory.

Six main tasks were distinguished in the plants that were visited:

- lift truck driving: receiving deliveries, moving bulk or packaged products

- mixing or dumping: unloading boxes, big bags or containers, operating mechanical mixers
- seed cleaning: operating sieving machines
- disinfecting: operating disinfection machines
- packaging: operating packaging machines that pack processed seeds into sacks or boxes
- on-site laboratory work: visual inspection of seed samples.

Most workers performed one single main task on measurement days, often in combination with tasks such as cleaning (compressed air blowing, sweeping, or vacuum cleaning) or office work.

All plants used a similar production process, but they differed in size, dust control measures (mainly local ventilation), and level of technology. The process is also similar in vegetable seed plants where horticulture seeds such as onion, lettuce, beetroot, and cauliflower seeds are handled.

Personal inhalable dust and endotoxin exposure measurements took place in November 2000 (delivery) and April 2001 (cleaning, packaging). Each plant was visited on two consecutive days and one grass/maize seed plant was visited in both seasons. All production workers and a limited number of lab workers who were present on the sampling days were asked to participate. All 53 invited workers agreed to participate resulting in 91 personal exposure measurements.

In January 2002, additional personal samples were collected in an agricultural research institute where workers suffered from work related respiratory symptoms. Six samples were collected from two participating workers during the threshing of grass.

Every participant was interviewed to obtain data on job title, tasks, and work history. In addition, after each sampling day a short questionnaire on tasks and products that were handled during the measurements was administered. Samples were collected using Gilian GilAir portable pumps (Gilian, West Caldwell, NJ, USA) at a flow rate of 2.0 l/min in combination with PAS6 sampling heads and 25 mm glass fibre filters (Whatman GF/A, Maidstone, UK). Average sampling time was 324 minutes (SD 99 minutes).

In August and September 2002, one of the grass and maize seed plants was visited again to determine real-time dust exposures in 12 workers at three different worksites (delivery, cleaning, and packaging). Dust concentrations were measured for two hours using a personal real-time photometric monitor (DataRAM; MIE, Bedford, MA, USA). During measurements a fieldworker registered tasks and task duration. Dust levels were registered every three seconds, stored in a data logger, and then downloaded to a PC. Simultaneously, inhalable dust samples were collected on 37 mm glass fibre filters (Whatman GF/A) in GSP sampling heads using Gilian GilAir portable pumps at a flow rate of 3.5 l/min. DataRAM dust concentrations were multiplied by a factor 3 to 9 to calibrate against inhalable dust measurements. Number and duration of peaks exceeding arbitrary reference levels of 0.5 and 5 mg/m^3 were determined afterwards by means of software developed in our institute.

Inhalable dust levels were measured gravimetrically, and filters were extracted in pyrogen free water with 0.05% Tween 20 as described previously.[15] Supernatants were stored at −20°C until endotoxin analysis by the LAL assay (Kinetic-QCL no. 50–650 U; BioWhittaker, Walkersville, MD, USA).[15] All personal samples in the seed processing plants were tested in duplicate (average CV 28%). Endotoxin concentrations in the extracts were above the limit of detection for all samples. Results were expressed as endotoxin units (EU)/m^3.

Characterisation of seed dust

Endotoxin in seed extracts

Thirty seven seed samples were selected by the industry and sent in for analysis. Grass seed (n = 15), cereal seed (n = 6), maize seed (n = 6), and vegetable seed samples (n = 10) were selected on the basis of dustiness (as perceived by the industry) and production volume. As harvest methods, weather conditions during harvest, and seed drying and cleaning may influence microbial growth on the seed, the selection included differently harvested seeds—either combined from standing crop, or swathed: cut stalks are left in a ''swath'' or windrow—seeds from different seasons, dried, undried, cleaned, and uncleaned seeds. Approximately 1 g of seed was extracted in pyrogen free water (10% w/v) with 0.05% Tween 20. Samples were rocked for one hour at room temperature and subsequently centrifuged at 1000 g for 15 minutes. Supernatants were stored at −20°C until endotoxin analysis by LAL assay or use as a stimulatory agent in the WBA.

Endotoxin removal using polymyxin-B

To verify that the measured activity in the LAL assay was caused by endotoxin, 15 seed extracts and four extracts of personal samples were incubated with polymyxin-B coated agarose beads which specifically bind endotoxin (P1411; Sigma-Aldrich, St Louis, MO, USA). Undiluted and 1/400 diluted extracts (diluted in pyrogen free water with 0.05% Tween 20) were incubated with polymyxin-B for 30 minutes

Table 2 DataRAM measurements, inhalable dust and endotoxin exposure for 12 workers during grass seed processing

				Peaks above 5.0 mg/m^3			Peaks above 0.5 mg/m^3		
Workplace and job title	DataRAM (mg/m^3)	Inhalable dust (mg/m^3)	Endotoxin (10^3 EU/m^3)	Peaks/hour	Duration (min)	Time between peaks (min)	Peaks/ hour	Duration (min)	Time between peaks (min)
Delivery									
Operator	1.34	12.1	78	60	0.3	0.7	7	8.4	0.3
Lift truck driver	0.86	4.0	44	37	0.2	1.2	5	11.8	0.1
Lift truck driver	0.66	2.1	29	31	0.1	1.7	11	5.4	0.3
Lift truck driver	0.45	3.6	72	39	0.2	1.2	1	46.9	6.9
Cleaning									
Operator	0.57	2.0	11	17	0.2	3	32	1.7	0.2
Operator	0.45	1.7	3.7	11	0.3	5.2	49	1.1	0.1
Lift truck driver	0.20	0.8	1.3	1	0.1	.	109	0.4	0.2
Lift truck driver	0.16	0.7	1.8	4	0.1	13	129	0.2	0.2
Packaging									
Operator	0.15	0.8	1.2	3	0.2	16.4	99	0.2	0.5
Operator	0.07	0.5	0.8	3	0.1	20.2	122	0.1	0.4
Lift truck driver	0.21	0.7	0.5	6	0.2	8.8	56	0.2	0.9
Lift truck driver	0.10	0.5	0.1	2	0.1	30.4	118	0.1	0.4

Peak profile (peaks per hour, average duration, and average time between peaks) for peaks above reference levels of 5 and 0.5 mg inhalable dust/m^3.

at room temperature. After incubation the extracts were centrifuged at 1000 g for 15 minutes and endotoxin activity was determined in the supernatants by LAL assay. Extraction medium and extraction medium with polymyxin-B were spiked with an endotoxin standard (1 EU/ml) to test possible interference from polymyxin-B addition with the LAL assay. Before spiking, media were centrifuged to remove polymyxin-B.

Whole blood assay

Seventeen agricultural seed extracts were tested in a WBA with blood from eight volunteers from our own department. Informed consent was obtained from all participants. Three to five seed extracts were tested per donor and each extract was tested with blood from two different donors to diminish the effect of donor related variation in responsiveness. The WBA was performed as described previously.[16] Briefly, 900 µl aliquots of EDTA anticoagulated peripheral blood were transferred into sterile tissue culture tubes and stimulated with 100 µl of stimulatory agents or control solution. The stimulatory agents—seed extracts and a positive control (lipopolysaccharide (LPS), 1 µg/ml≈10 000 EU/ml)—were serially diluted in RPMI 1640 and tested at a 1:3, 1:9, 1:27, 1:81, and 1:243 dilution, which resulted in final concentrations in the tubes of 333, 111, 37, 12, and 4 EU/ml for LPS. LPS concentrations of seed extracts were determined by LAL assay. RPMI and extraction medium served as control for background cytokine production. Blood samples were incubated for 18 hours at 37℃, 5% CO_2, and 96% relative humidity, after which tubes were centrifuged for 10 minutes at 1000 g. Supernatants were stored at −20℃ until analysis of interleukin-1β (IL1β), IL8, IL6, and tumour necrosis factor alpha (TNFα) by Enzyme Immuno Assays (EIAs; Biosource, Biosource Europe SA, Fleurus, Belgium). Supernatants with cytokine levels below the limit of detection (LOD) were assigned a value of 2/3 of the LOD.

Scanning electron microscopy

Nine seed samples (grass, cereal, and vegetable seed) were qualitatively analysed by scanning electron microscopy (SEM) with magnifications ranging from 100 to 10 000× to obtain visible information of microbial growth.[17] Seeds with a different degree of staining were selected, which was assumed to be related to microbial growth. The presence of fungi was judged by the presence of spores with size >1.5 µm as well as mycelium on the seed surface, actinomycetes by spores with size <1.5 µm and mycelium, and bacteria by accumulation of cells with similar shape and cross section typically <1 µm.

Statistical analysis

Data were analysed using SAS statistical software (SAS System for Windows, version 8.02; SAS Institute, Cary, NC, USA). As inhalable dust, endotoxin concentrations, and DataRam measurements were log normally distributed, data analysis was performed on log transformed concentrations. Repeated measurements for the same workers may be correlated. Therefore, mixed effects models that account for this dependence between repeated measurements were applied, using PROC MIXED, to study effects of tasks on exposure levels among workers in seed processing plants.[18 19] Within worker (day to day) and between worker variance components of one way random effects models (only worker effects) and mixed effects models (including task as fixed effect) were estimated using the restricted maximum likelihood method and a compound symmetric covariance structure. Mixed effects models were applied separately for data collected in grass, grass/maize, and cereal seed plants, and data collected in vegetable seed plants because a strong interaction was found between task and type of plant. Mixed effects models differed significantly from the random effects only models (likelihood ratio test; $p<0.001$). Elevation of geometric mean endotoxin exposure levels for tasks relative to reference tasks were calculated (factor = e^{β}, β = regression coefficient).

Table 3 Microbial growth on seed samples observed by scanning electron microscopy

	Observed microorganisms		
	Fungi	Actinomycetes	Bacteria
Grass seed			
Creeping red fescue (*Festuca rubra*), uncleaned, straight combined, undried	+	+	+
Perennial ryegrass (*Lolium perenne*), uncleaned, swathed, undried	(+)	−	−
Perennial ryegrass, uncleaned, straight combined, undried	+	−	−
Cereal seed			
Summer barley	++	++	++
Summer wheat	++	(+)	(+)
Winter wheat	++	−	?
Vegetable seed			
Cauliflower, uncleaned	−	−	+
Beetroot, uncleaned	−	−	−
Lamb's lettuce, uncleaned	(+)	−	−

++, Clear growth on all inspected seeds; +, clear growth on some spots; (+), sparsely observed; −, not observed; ?, uncertain if observed particles are microorganisms, but not unlikely.

RESULTS

Personal inhalable dust and endotoxin exposure

First samples taken by the occupational health service during grass seed cleaning at the quality control laboratory showed very high endotoxin values of approximately 10 000 EU/m^3, and values were even higher in a next series of four personal samples taken during cleaning of two different species of grass seed—up to 274 000 EU/m^3 (table 1). During a subsequent visit to the institute, when cleaning work activities had been suspended, samples of settled dust were taken from several surfaces in the cleaning rooms (upper side of cupboards, armatures, etc) and dust was collected from the ventilation system. LAL tests with extracts of these dust samples confirmed its high endotoxin content, with values from 5000 to 11 000 EU per mg dust.[13] As a consequence of the findings, the sieving room was closed and thoroughly cleaned and work was only resumed after implementation of severe dust reduction measures, which indeed strongly reduced airborne endotoxin levels (not shown). However it remained very difficult to achieve consistently low values to comply with proposed threshold levels of 50–200 EU/m^3.[20]

Although values in the quality control institute were by far the highest, high airborne endotoxin was a normal finding in many of the investigated seed processing plants (table 1). Dust levels were moderate, although some workers were exposed to levels above the Dutch occupational exposure limit for nuisance dust (10 mg/m^3). Inhalable dust and endotoxin concentrations showed good correlation (fig 1) with dust levels explaining 67% (r^2) of the variability in endotoxin concentrations. Endotoxin levels as high as 500 EU/m^3 were already measured at very low dust exposures (0.2 mg/m^3). The mean concentration of endotoxin per mg dust was independent of dust concentration and type of plant and high at 980 EU/mg (geometric mean (GM)).

Task was strongly associated with endotoxin levels among workers in grass, grass/maize, and cereal seed plants (the between worker variance component (s^2_{bw}) was reduced by

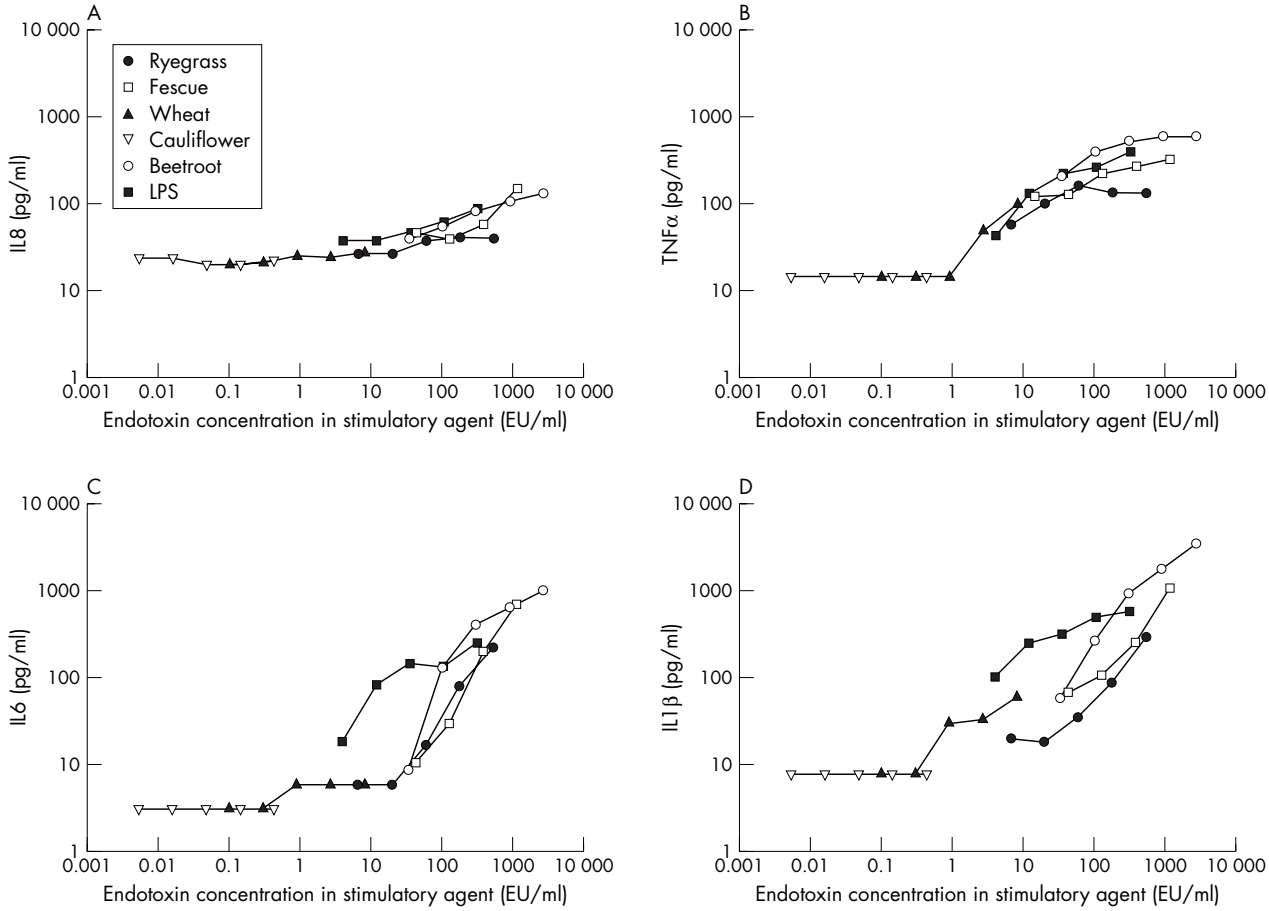

Figure 3 Seed extract and LPS induced IL8 (A), TNFα (B), IL6 (C), and IL1β (D) production in human whole blood. All seed extracts were tested at a 1:3, 1:9, 1:27, 1:81, and 1:243 dilution, and corresponding endotoxin concentrations as determined with LAL assay are plotted on the x axis.

81% when adding task as fixed effect). For vegetable seed plants, task explained 50% of the variability in endotoxin levels between workers. Task did not influence day to day variability (s^2_{ww}) in endotoxin levels, as most workers had the same main task on both sampling days. For the grass, grass/maize, and cereal seed processing plants, lab work was associated with lowest endotoxin levels (GM = 85 EU/m^3; mixed effects model). Endotoxin exposure levels corresponding to other tasks were increased relative to lab work by a factor of 23 for seed cleaning, 25 for disinfecting, 26 for lift truck driving, 34 for packaging, and 74 for mixing or dumping. For the vegetable seed plants, lift truck driving was the reference task (GM = 217 EU/m^3). For other tasks, the GM exposure was increased by a factor of 1.3 for lab work, 1.6 for packaging, 4 for disinfecting, 18 for seed cleaning, and 32 for mixing or dumping. A similar pattern was seen for inhalable dust exposure.

Figure 2 shows DataRAM investigations for two operators (delivery and cleaning) in a grass/maize seed plant. Distinct peak exposures were observed during tasks such as dumping grass seed into boxes, levelling seed in a box, cleaning with compressed air, and sweeping. A similar picture was seen for the other workers, although the number and duration of peaks differed. Dust and endotoxin levels that were measured during the real time exposure measurements are presented in table 2. Highest levels of dust and endotoxin (up to 78 000 EU/m^3) were measured during delivery. Concentrations during seed cleaning were lower, and packaging workers were exposed to relatively lowest levels. Operators were exposed to higher levels than lift truck drivers. High correlations were found between inhalable dust and DataRAM dust concentrations ($r = 0.93$) and between inhalable dust and endotoxin concentrations ($r = 0.92$) (Pearson's correlation coefficient, log transformed concentrations). The number of inhalable dust peaks above 5 mg/m^3 was highest for workers in the delivery hall (31–60 peaks/hour) and lowest for packers (2–6 peaks/hour). Average maximum peak concentration and average peak duration were similar for the three work sites. A different profile was seen for peaks above 0.5 mg/m^3: packers and seed cleaners were exposed to many short lived peaks whereas delivery workers were exposed to a few peaks that lasted for almost the entire measurement (in fact a high background exposure).

Characterisation of seed samples

Endotoxin levels were highest in grass seed extracts (range 59–361×10^3 EU/g) and lower in cereal seed (range 2.5–86×10^3 EU/g) and maize seed (range 0.5–7.1×10^3 EU/g). Endotoxin levels in vegetable seed extracts showed a much larger variation: from 40 EU/g in lettuce seed to above 1.0×10^6 EU/g in lamb's lettuce seed.

Incubation with polymyxin-B resulted in elimination of almost all endotoxin activity (>98%) in 1/400 diluted seed extracts. Polymyxin-B also removed endotoxin (>95%) from four extracts of personal dust samples that were collected at the research institute. In most undiluted extracts, not all endotoxin activity was removed, most likely due to saturation of the binding capacity. Polymyxin-B did not interfere with the LAL assay, as endotoxin activity in spiked extraction

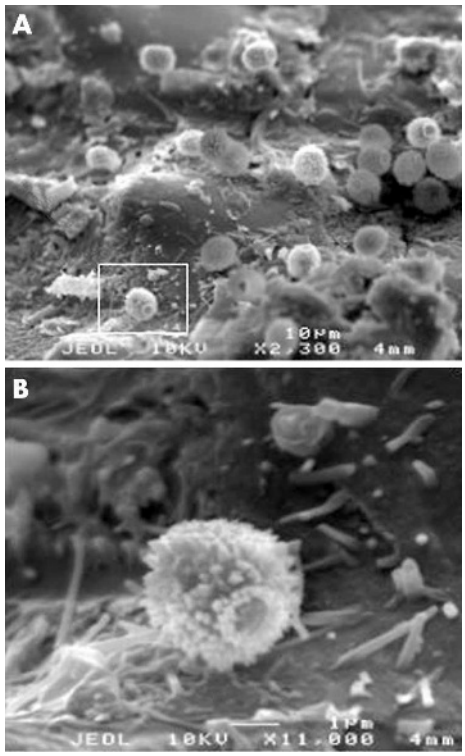

Figure 4 (A) Scanning electron micrographs of summer barley seed with clearly visible fungal spores. (B) The 5× magnified section shows rod/shaped particles with a morphology consistent with bacteria.

medium and extraction medium with polymyxin-B was completely recovered.

WBA results showed that seed extracts were capable of inducing a pronounced dose dependent release of all four tested cytokines. Cytokine release was associated with LAL test reactivity in different seed extracts. However, considerable quantitative and qualitative interindividual variation in dose response curves was observed. Figure 3 shows a typical example of the relation between endotoxin activity in stimulatory agents and induced cytokine production for one of the donors. Dose response curves for seed extracts and LPS appeared similar.

Microbial growth, including bacterial growth, was observed by SEM on most samples (table 3 and fig 4) and was present on the surface of both stained and several not stained seeds. The presence of fungi dominated, whereas bacteria were observed to a lesser extent. Microbial growth was more prevalent on cereal seeds than on grass or vegetable seeds.

DISCUSSION

This study showed that workers in the agricultural seed processing industry are at risk of exposure to high levels of endotoxin containing seed dust and thus of developing work related health effects. Exposure to high levels of endotoxin was most likely associated with episodes of ODTS resembling symptoms in a grass seed quality inspection lab, and with respiratory symptoms in an agricultural research institute. The overall geometric mean endotoxin concentration (1800 EU/m^3) in personal samples that were taken in a representative sample of the agricultural seed processing industry was two times higher than the GM for pig farmers (920 EU/m^3), a population known to be highly at risk for developing various work related respiratory diseases.[21 22] The health based endotoxin exposure limit of 50 EU/m^3 that was recommended by the Dutch Expert Committee on Occupational Standards (DECOS) of the National Health Council[20] was amply exceeded in almost all personal samples. Seed dust was heavily contaminated with endotoxin: endotoxin levels per mg dust were around 50 times higher than levels in house dust,[23 24] and four times higher than levels in dust collected in the potato industry.[25]

Most tested seed extracts were capable of inducing proinflammatory cytokines IL1β, IL6, IL8, and TNFα in WBA experiments. Stimulation with seed extracts resulted in a similar cytokine profile as for LPS stimulation, and responses to seed extracts appeared strongly related to the endotoxin content of extracts as measured by the LAL assay. Thus, the observed high potency of (grass) seed extracts as in vitro inducer of cytokine responses suggests that LAL test activity of seed extracts is not merely a laboratory artefact, and argues for the hypothesis that grass seed dust can potentially cause health effects such as ODTS. Nevertheless, conclusions on the potency of seed extracts in relation to LPS should be made with caution because of the limited number of WBA tests and marked known interindividual differences in cytokine responsiveness.[16] Using a WBA as a model for in vivo effects of endotoxin exposure is debatable because inhaled active agents in seed dust would at first interact with airway mucosal cells and not with peripheral blood cells. However, systemic responses following local inflammatory responses after acute inhalation of LPS have been reported, which supports the use of peripheral blood cells in a stimulation assay.[26 27] Earlier studies have used in vitro cytokine secretion from pulmonary epithelial cell lines and/or alveolar macrophages to assess the inflammatory potential of swine dust,[28 29] and organic dust from waste handling facilities.[30] In other studies peripheral cells were used to assess organic dust induced cytokine release from monocytic cell lines,[31] and inflammatory capacity of fish processing agents in a WBA.[32] Furthermore, Kline *et al* have shown that LPS induced release of IL6 and IL8 by both alveolar macrophages and peripheral blood monocytes correlated with airflow obstruction in response to experimental LPS inhalation, suggesting that peripheral cells can be used as a model for in vivo effects.[33]

In contrast to livestock farming environments where abundantly present Gram negative bacteria in animal faeces are a likely source of endotoxin, observed high exposures to airborne endotoxin in the agricultural seed industry are not easily understood. However, a wide range of epiphytic Gram negative bacteria occurring on plants and plant products during growth or storage may lead to seeds contaminated with Gram negative bacteria and their endotoxins, similarly as previously described for cotton and grain.[34–36] Endotoxin levels varied widely for different seed extracts, which may partly be explained by seed morphology. Large and smooth seeds such as maize seed may contain less bacteria or endotoxin per gram seed than smaller seeds with rugged or hairy surfaces. External factors like harvesting methods and conditions may also play a role to some extent.[37]

For possible future prevention measures, it would be important to know if it was really bacterial contamination that caused high LAL test activity and health complaints during grass seed processing. Microbial growth on a selection of seed samples was observed by scanning electron microscopy. Only the surface of seeds was studied, while microorganisms may grow more abundantly below the upper hull where water activity may be high for longer periods than on the outer surface. Mainly fungal spores were detected, but bacteria were present as well although detected with less certainty. Recognition of bacteria (except Gram positive actinomycetes) is less definite than of spores of actinomycetes and fungi, as morphological features of bacteria are less

Main messages

- Workers in the agricultural seed processing industry are at risk of exposure to high levels of endotoxin containing seed dust.
- Episodes of ODTS resembling symptoms in grass seed exposed workers were most likely related to high levels of endotoxin exposure.
- The job tasks of dumping and mixing were associated with highest dust and endotoxin exposures.
- Seed extracts induced dose dependent release of proinflammatory cytokines in a human whole blood stimulation assay.

Policy implications

- Dust control strategies should focus on tasks that cause peak exposures such as dumping, mixing, sweeping, and compressed air blowing.
- Symptom prevalence and other health outcomes in agricultural seed workers should be investigated.

distinct.[17] Moreover, only the presence of bacteria might be studied, which may be either Gram negative or Gram positive. Nevertheless, the presence of bacteria on seed samples and experiments with polymyxin-B incubation further supported the hypothesis that endotoxin is the main cause of LAL activity in seed extracts, although the question remained whether the inflammatory potency of seed extracts was entirely caused by LPS, or also by other agents, for instance β(1→3)-glucans or endogenous components of seed.

Analysis of the exposure data demonstrated that dumping and mixing were associated with highest dust and endotoxin exposures. This finding was confirmed by real-time DataRAM measurements which showed that most peak exposures were caused by dumping seed into boxes and levelling seed by using a rake. These tasks caused momentary clouds of dust that were responsible for a large amount of total exposure. Packers were exposed to lower dust and endotoxin levels which may partly be explained by the reduction of dustiness and endotoxin contamination after seed cleaning. On-site quality control laboratory workers were generally exposed to low levels of dust and endotoxin, in contrast to workers in the grass seed quality inspection lab. However, it should be noted that the nature of tasks in the grass seed inspection lab was different from the on-site labs. High levels in the cleaning department of the grass seed quality inspection lab resulted most likely from continuously dumping uncleaned seed in sieving machines, whereas on-site lab workers spent most time on visual inspection of seed samples. A control strategy focused on elimination or reduction of tasks that cause peak exposures would lead to considerable reduction of exposure levels. However, background exposure levels at a worksite such as the delivery hall where uncleaned seed is delivered and dumped into drying boxes are such that exposures may remain high even after elimination of individual tasks.

In conclusion, occupational exposure to inhalable grass seed dust, and possibly to cereal and vegetable seed dust may induce inflammatory responses, and is a potential cause of ODTS. Endotoxin appears to be the causative agent in seed dust, although other components in agricultural seed may play a role as well.

ACKNOWLEDGEMENTS

We are grateful to Lene Madsø for SEM analyses, Isabella Oosting, Jack Spithoven, and Griet Terpstra for laboratory analyses, Anoek Besselink for performing the DataRAM measurements, and Wim Braun for DataRAM data management. We wish to thank Plantum NL for coordination, collaboration, and funding.

Authors' affiliations
L A M Smit, I M Wouters, M M Hobo, G Doekes, D Heederik, Institute for Risk Assessment Sciences (IRAS), Division of Environmental and Occupational Health, Utrecht University, the Netherlands
W Eduard, National Institute of Occupational Health, Oslo, Norway

Competing interests: none.

REFERENCES

1 In: **Rylander R**, Christiani DC, Peterson Y, eds. *Report of the Committee on Organic Dusts of the International Commission on Occupational Health*. Gothenburg, Sweden: Institutionen for Hygien Goteborgs Universitet, 1989.
2 **Smid T**, Heederik D, Houba R, *et al*. Dust- and endotoxin-related acute lung function changes and work-related symptoms in workers in the animal feed industry. *Am J Ind Med* 1994;**6**:877–88.
3 **Vogelzang PF**, van der Gulden JW, Folgering H, *et al*. Endotoxin exposure as a major determinant of lung function decline in pig farmers. *Am J Respir Crit Care Med* 1998;**157**:15–18.
4 **Schwartz DA**, Thorne PS, Yagla SJ, *et al*. The role of endotoxin in grain dust-induced lung disease. *Am J Respir Crit Care Med* 1995;**152**:603–8.
5 **Douwes J**, Thorne P, Pearce N, *et al*. Bioaerosol health effects and exposure assessment: progress and prospects. *Ann Occup Hyg* 2003;**47**:187–200.
6 **Zock JP**, Hollander A, Heederik D, *et al*. Acute lung function changes and low endotoxin exposures in the potato processing industry. *Am J Ind Med* 1998;**33**:384–91.
7 **May JJ**, Stallones L, Darrow D, *et al*. Organic dust toxicity (pulmonary mycotoxicosis) associated with silo unloading. *Thorax* 1986;**41**:919–23.
8 **Rask-Andersen A**. Organic dust toxic syndrome among farmers. *Br J Ind Med* 1989;**46**:233–8.
9 **Von Essen S**, Fryzek J, Nowakowski B, *et al*. Respiratory symptoms and farming practices in farmers associated with an acute febrile illness after organic dust exposure. *Chest* 1999;**116**:1452–8.
10 **Jagielo PJ**, Thorne PS, Watt JL, *et al*. Grain dust and endotoxin inhalation challenges produce similar inflammatory responses in normal subjects. *Chest* 1996;**110**:263–70.
11 **Jagielo PJ**, Thorne PS, Kern JA, *et al*. Role of endotoxin in grain dust-induced lung inflammation in mice. *Am J Physiol* 1996;**270**:L1052–9.
12 **Von Essen SG**, O'Neill DP, Olenchok SA, *et al*. Grain dusts and grain plant components vary in their ability to recruit neutrophils. *J Toxicol Environ Health* 1995;**46**:425–41.
13 **Doekes G**, Wouters IM, van Schothorst I, *et al*. Endotoxin in grass seed as a cause of organic dust toxic syndrome [abstract]. *Eur Respir J* 2000;**16**(suppl 31):157s.
14 **Hollander A**, Heederik D, Versloot P, *et al*. Inhibition and enhancement in the analysis of airborne endotoxin levels in various occupational environments. *Am Ind Hyg Assoc J* 1993;**54**:647–53.
15 **Douwes J**, Versloot P, Hollander A, *et al*. Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Appl Environ Microbiol* 1995;**61**:1763–9.
16 **Wouters IM**, Douwes J, Thorne PS, *et al*. Inter- and intraindividual variation of endotoxin- and beta(1 --> 3)-glucan-induced cytokine responses in a whole blood assay. *Toxicol Ind Health* 2002;**18**:15–27.
17 **Eduard W**, Heederik D. Methods for quantitative assessment of airborne levels of noninfectious microorganisms in highly contaminated work environments. *Am Ind Hyg Assoc J* 1998;**59**:113–27.
18 **Peretz C**, Goren A, Smid T, *et al*. Application of mixed-effects models for exposure assessment. *Ann Occup Hyg* 2002;**46**:69–77.
19 **Rappaport SM**, Lyles RH, Kupper LL. An exposure-assessments strategy accounting for within- and between-worker sources of variability. *Ann Occup Hyg* 1995;**39**:469–95.
20 **Heederik D**, Douwes J. Towards an occupational exposure limit for endotoxins? *Ann Agric Environ Med* 1997;**4**:17–19.
21 **Preller L**, Heederik D, Kromhout H, *et al*. Determinants of dust and endotoxin exposure of pig farmers: development of a control strategy using empirical modelling. *Ann Occup Hyg* 1995;**39**:545–57.
22 **Omland O**. Exposure and respiratory health in farming in temperate zones—a review of the literature. *Ann Agric Environ Med* 2002;**9**:119–36.
23 **Gehring U**, Douwes J, Doekes G, *et al*. Beta(1-->3)-glucan in house dust of German homes: housing characteristics, occupant behavior, and relations with endotoxins, allergens, and molds. *Environ Health Perspect* 2001;**109**:139–44.

24 **Douwes J**, Doekes G, Heinrich J, *et al*. Endotoxin and beta(1-->3)-glucan in house dust and the relation with home characteristics: a pilot study in 25 German houses. *Indoor Air* 1998;**8**:255–63.
25 **Zock JP**, Heederik D, Kromhout H. Exposure to dust, endotoxin and micro-organisms in the potato processing industry. *Ann Occup Hyg* 1995;**39**:841–54.
26 **Wang Z**, Manninen A, Malmberg P, *et al*. Inhalation of swine-house dust increases the concentrations of interleukin-1 beta (IL-1 beta) and interleukin-1 receptor antagonist (IL-1ra) in peripheral blood. *Respir Med* 1998;**92**:1022–7.
27 **Thorn J**. The inflammatory response in humans after inhalation of bacterial endotoxin: a review. *Inflamm Res* 2001;**50**:254–61.
28 **Wang Z**, Malmberg P, Ek A, *et al*. Swine dust induces cytokine secretion from human epithelial cells and alveolar macrophages. *Clin Exp Immunol* 1999;**115**:6–12.
29 **Palmberg L**, Larsson BM, Malmberg P, *et al*. Induction of IL-8 production in human alveolar macrophages and human bronchial epithelial cells in vitro by swine dust. *Thorax* 1998;**53**:260–4.
30 **Allermann L**, Poulsen OM. Inflammatory potential of dust from waste handling facilities measured as IL-8 secretion from lung epithelial cells in vitro. *Ann Occup Hyg* 2000;**44**:259–69.
31 **Allermann L**, Poulsen OM. Interleukin-8 secretion from monocytic cell lines for evaluation of the inflammatory potential of organic dust. *Environ Res* 2002;**88**:188–98.
32 **Bonlokke JH**, Thomassen M, Viskum S, *et al*. Respiratory symptoms and ex vivo cytokine release are associated in workers processing herring. *Int Arch Occup Environ Health* 2004;**77**:136–41.
33 **Kline JN**, Cowden JD, Hunninghake GW, *et al*. Variable airway responsiveness to inhaled lipopolysaccharide. *Am J Respir Crit Care Med* 1999;**160**:297–303.
34 **Dutkiewicz J**. Exposure to dust-borne bacteria in agriculture. I. Environmental studies. *Arch Environ Health* 1978;**33**:250–9.
35 **Lacey J**, Dutkiewicz J. Bioaerosols and occupational lung-disease. *J Aerosol Sci* 1994;**25**:1371–404.
36 **Olenchock SA**, Christiani DC, Mull JC, *et al*. Endotoxins in baled cottons and airborne dusts in textile mills in the People's Republic of China. *Appl Environ Microbiol* 1983;**46**:817–20.
37 **Roussel S**, Reboux G, Dalphin J-C, *et al*. Microbiological evolution of hay and relapse in patients with farmer's lung. *Occup Environ Med* 2004;**61**:e3.

ECHO

Screening for colour vision defects is pointless

Please visit the *Occupational and Environmental Medicine* website [www.occenvmed.com] for a link to the full text of this article.

Paediatricians are advocating that childhood screening for congenital colour vision defects (CVD) in the UK be abandoned, based on the results of a retrospective cohort study.

They found little evidence to support screening—used mainly to advise against certain occupations—and say that specialist diagnostic tests, as nationally recommended, are more useful to assess the true risk in specific jobs within occupations.

Their analysis of current and past occupations among the 1958 British birth cohort at age 33 showed that in only a few of the occupations traditionally deemed unsuitable on the basis of safety or product quality were men with CVD significantly underrepresented. These occupations were aircraft and ships' officers, electrical and electronic engineering, and fibre and textile processing. Current occupation in all occupational groups varied significantly, not by colour vision but by sex and social class at birth; nor did colour vision significantly affect choice of career expected from educational attainment and social class. Employment state at 33 was unrelated to CVD. The cohort of 12 534 subjects, screened for colour vision at age 11, comprised 51% men and 49% women; 6.7% and 1.1%, respectively, had CVD.

The study analysed self reported occupation with a taxonomy based on tasks performed in 3500 occupations.

The UK's Health and Safety Executive recognises that jobs within occupations requiring normal colour vision can have different needs. Legally the need for normal colour vision in certain occupations varies internationally, and the value of universal childhood screening, widespread in industrialised countries, is in question.

▲ Cumberland P, *et al*. *Archives of Disease in Childhood* 2005;**90**:906–908.