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Inflammatory potential of dust from schools and building related symptoms

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Aims: To test whether the inflammatory potential of dust samples might be used to differentiate schools with high and low prevalence of building related symptoms (BRS) among the occupants.

Methods: Ten schools with high prevalence of BRS and 10 schools with low prevalence were selected. Dust collected from floors, horizontal surfaces, and exhaust outlets was tested at five concentrations on the lung epithelial cell line A549. The potency of the dust (PF) to stimulate IL-8 secretion was calculated from the initial linear part of the dose-response curves. The organic fraction of the dust samples was determined by incineration.

Results: The schools with low prevalence of symptoms had a BRS% of 4.4–11.0 and the schools with high prevalence a BRS% of 19.6–31.9. The PF of floor dust and surface dust correlated, and the PF was associated with the organic content of the dust. The schools with low prevalence of symptoms had a significantly lower PF than the schools with high prevalence. Using the cut point value of 4.5 ng IL-8/mg floor dust, significantly more high prevalence schools were found above the cut point than below.

Conclusion: The PF of the floor dust samples correlated significantly with the prevalence of symptoms in the schools. The content of endotoxin and microorganisms did not seem to explain the inflammatory potential of the dust or BRS, and the substances in the dust causing the inflammatory potential are presently unknown.

Non-specific symptoms such as mucous membrane irritations in the eyes, nose, and the upper respiratory tract, cough, dryness of the skin, and symptoms such as headache, dizziness, fatigue and lethargy, and lack of concentration, have all been related to the quality of the indoor air or the indoor environment in non-industrial buildings like offices, schools etc.^{1,2} Both the type and the severity of symptoms may vary from person to person, even within the same building. The building related symptoms (BRS) begin gradually, within days or months of occupation of a building with problems or after changes are made in a building.³ The symptoms usually disappear during absence from work.⁴

BRS are multifactorial in origin,⁵ and both chemical,³ biological,⁶ physical,⁷ and psychosocial⁸ factors may be relevant. It is therefore difficult to point out one or just a few chemical factors or components of microbial origin that may cause or add to the multitude of symptoms related to the indoor environment.

Inflammation is a common pathogenic mechanism behind many of the symptoms and illnesses related to exposure to organic dust.⁸ Inflammation may therefore be considered an integrated effect of the total biological and chemical exposure load from an indoor environment. Hence, measurements of the inflammatory potential of dust samples from the indoor environment might be an attractive supplement or alternative to many chemical and biological single factor analyses presently used to evaluate the indoor climate.

The epithelial cells of the airways not only constitute a physical barrier, they are also involved in the first line of defence against intruding agents or microorganisms.^{9–11} They contribute to the protection by production of proinflammatory cytokines, inflammatory eicosanoids, and specific cell adhesion molecules.¹¹ When an inflammatory agent such as endotoxin is inhaled, a cascade of proinflammatory cytokines are secreted from, for example, the macrophages and epithelial cells of the lungs.¹² These cytokines include tumour necrosis factor (TNF), and the interleukins (IL) IL-1, IL-6, and IL-8. IL-8 has neutrophilic attracting and activating properties as

well as acting as attractant for B lymphocytes and basophils,¹² and is thus a good indicator of inflammation.

Previously, we published results from a school study showing no or only weak correlation between the inflammatory potential of dust samples from schools and the content of bacteria, fungi, and endotoxins in the dust.¹³ For the same schools results of a questionnaire study were recently published.^{14,15}

The aim of this study was to test whether the inflammatory potential of surface dust samples from these schools, measured as IL-8 secretion from lung epithelial cells (A549 bioassay), might be used to differentiate schools with a high and a low prevalence of BRS among the occupants.

METHODS

Questionnaires and technical investigation

"The Copenhagen School Study" was performed as a cross sectional study including employees and pupils from the 8th grade and up (> 13 years) from 75 schools in Copenhagen. The questionnaire included questions about symptoms of the eyes, nose, throat, and skin, as well as general symptoms such as headache, fatigue, and difficulties in concentration. The answering categories were: "never/seldom", "now and then", "several times a week", or "daily". Questions regarding hay fever, asthma, and other illnesses diagnosed by a doctor were also included. A total of 7884 questionnaires were returned, giving a response rate of 66%. A BRS value was calculated for each school, using the mean prevalence of eight symptoms: eye irritation, nose irritation, nose congestion, irritation of the

Abbreviations: BRS, building related symptoms; CFU, colony forming units; CP, cut point; ELISA, enzyme linked immunosorbent assay; EU, endotoxin units; FBS, fetal bovine serum; IL, interleukin; LPS, lipopolysaccharide; PF, potency factor, the inflammatory potential; RMSE, relative mean square error; SE, standard error; TNF, tumour necrosis factor; TVOC, total volatile organic compounds

throat, itching/flushing facial skin, headache, fatigue, and difficulties in concentration.

From the calculated BRS values 20 schools were selected: 10 schools with the lowest prevalence of BRS and 10 schools with the highest prevalence of BRS. The prevalence status of the schools was blinded to the investigator during sampling and analysis of dust samples. Investigations of the schools were combined with information on the building provided by each of the schools and from a visual registration of building characteristics.¹⁵ A minimum of four rooms in each school were selected; information about number of persons occupying the room, heating and ventilation system, cleaning status, visible damp stains, and visible mould growth was registered. A thorough examination of the loft (regarding ventilation, smell, damp, and mould) and the air handling unit (operation and appearance) was performed by an experienced civil engineer. Measurements of temperature, CO₂, and total volatile organic compounds (TVOCs) were performed by the Danish Building and Urban Research Institute, Hoersholm, Denmark.

Dust

Dust was collected from the chosen rooms of the 20 schools. Floor dust was sampled with the HVS-3 sampler (ASTM Designation: D 5438-94) connected to a vacuum cleaner ($n = 96$).¹⁶ Dust from horizontal or near horizontal surfaces (surface dust) ($n = 21$) and dust from exhaust outlets ($n = 41$) was sampled by a portable vacuum cleaner with the VacuuMark disposable nozzle, containing a filter with a pore size of 5–6 microns (retain particles down to 0.3 μm) in a filter box (Petersen Bach, Denmark). The surface dust samples from the rooms of each school were pooled in order to gain enough dust for all the analysis. The fibre part of the dust samples was torn in a knife mill (Type A10, Funkenstört, KB 5/10, Janke & Kunkel GmbH & Co.KG) for one minute. The total dust sample was then sieved on a 300 μm filter in a sieving machine (Retsch, Type VE 1000, F. Kurt Retsch GmbH & Co. KG, Haan, Germany) for 15 minutes at an amplitude of 1.5 mm, and stored at -20°C .

Each dust sample (particles $<300 \mu\text{m}$) was divided into subsamples for different analyses: (1) test of the inflammatory potential in the A549 bioassay; (2) microbiological analysis¹³; and (3) determination of organic/inorganic content. The percentage of fibre (hair, fibres from clothes, etc) was maintained in the subsamples.

The dust samples for test in the A549 bioassay were sterilised by γ radiation at 35 kGy (Risø, Roskilde, Denmark) and stored at -20°C . The results of these samples are referred to as total dust.

A549 bioassay

The A549 bioassay was performed as described earlier.^{13 17 18} In short, 1×10^5 cells of the human lung epithelial cell line A549 (ATCC no. CCL-185) were grown in 24 well multi-dishes (GIBCO BRL), and incubated for 48 hours at 36.5°C , 5% CO₂, and 100% relative humidity with a change of medium after 24 hours. The dust was suspended in cell culture media and sonicated in a water bath (Branson 2200 E3, 47 kHz $\pm$ 6%) for three times one minute before addition to the cells. Six concentrations (0, 0.1, 0.5, 1, 3, and 5 mg dust/ml) of each dust sample in triplets were tested together with two positive control samples (100 μg LPS/ml (*E coli* O55:B5 (Sigma, St Louis, MO, USA; catalogue no: L 2637) and 10 ng TNF α /ml (Genzyme, Cambridge, MA, USA; catalogue no: GENTNF-H)). The positive control samples from all the experiments performed were included in an X-R control chart to ensure that the method stayed in control.¹⁹ IL-8 secretion was measured in the media after 24 hours incubation by ELISA techniques (Genzyme and R&D systems Inc., Minneapolis, MN, USA). Before the media was harvested the morphology of the cells was assessed under a microscope, for any visual cytologic

effect on the cells (rounded, detached, or lysed cells). When nothing else is stated the cells appeared as unstimulated cells.

A pilot study on the interference of dust particles with the measured IL-8 concentration was performed by adding in duplicate increasing amounts of dust to samples with a known concentration of IL-8 (1000 pg IL-8/ml (standard from the IL-8 ELISA kit from Genzyme)). The experiments were performed under the same conditions as the A549 bioassay, but with no cells present. IL-8 was measured after 24 hours incubation by the ELISA kit from R&D Systems. At a concentration of 1 mg/ml of dust, a 38% reduction in the measured IL-8 concentration was observed. The reduction increased to 80–90% at concentrations of 3–5 mg/ml of dust. No effects were obtained by addition of salt (0.5 and 1 M final conc. NaCl (Bie Berntsen, Denmark)) immediately before harvesting the media. When increasing the pH (7.6 $\rightarrow$ 9.2) with NaOH, practically no IL-8 was measured in any of the wells.

To overcome problems with dust interference, the inflammatory potential of the dust sample, termed the potency factor (PF), was expressed as the slope of the initial linear part of the dose-response curve. Day to day variation in the bioassay was taken into account by standardising the PF against the positive control, relative to the mean of all the positive controls in the study: $\text{PF}_{\text{corr}} = \alpha / (\bar{X}/T)$, where α is initial linear slope of the dose-response curve, $\bar{X}$ is the mean of the control sample in triplicate measured on the same day, and T is the mean of multiple control measurements on different days.¹⁸

Organic content

The content of organic matter in the dust samples was determined by incineration. The dust samples was placed on Whatman CF/C glasfibre filters of 25 mm (Frisenette Aps, Denmark) and placed in a muffle furnace (Carbolite GM3) preheated to 480°C for four hours. After acclimatisation the samples were weighed and the content of organic material was calculated (expressed as weight loss). Recalculation of the potency factor (PF), including only the organic fraction is referred to as PF of organic fraction.

Statistics

Correlation tests of data not following a normal distribution were performed by the non-parametric Hotelling and Pabst test for rank order correlation; Pearson's r based on the ranks, rather than the original observations, reduces to Spearman's rank difference correlation coefficient r_s .²⁰ The Mann-Whitney U rank sum test was used to test for differences in continuous variables between groups (for example, schools with low and high prevalence of symptoms). A significance level of $\alpha = 0.05$ was used, when nothing else is stated.

RESULTS

Dust tested in the A549 bioassay

Dust samples tested in the A549 bioassay most frequently showed dose-response curves with increase of the IL-8 secretion until stimulation with 0.5 mg or 1 mg of dust, and then a decline towards zero with stimulation at higher dust concentrations (fig 1). For surface dust the maximum IL-8 secretion ranged from 2.8 ng to 19.0 ng IL-8/ml with background values of 0.11 ng to 0.8 ng IL-8/ml. For floor dust samples the maximum IL-8 secretion ranged from 0.97 ng to 5.9 ng IL-8/ml with background values between 0.11 and 1.8 ng IL-8/ml, and for exhaust outlet dust the maximum IL-8 secretion ranged from 0.34 ng to 13.9 ng IL-8/ml, with background values between 0.16 ng and 0.37 ng IL-8/ml. In some wells stimulated with 1 mg of dust many of the cells were rounded up and detached from the bottom of the well. Cells stimulated with floor dust from three of the 20 schools were rounded up in the wells stimulated with 3 and 5 mg of dust. Lysed and clearly dead cells were seen in the wells stimulated with 5 mg of floor dust from three other schools. Table 1 summarises the potency factors.

Table 1 Potency factor (PF) of the different dust samples tested in the A549 bioassay*

Dust samples from:	IL-8 induction ng IL-8/mg dust median (min–max)	TNF corrected IL-8 induction ng IL-8/mg dust median (min–max)
Surface	8.3 (0.78–38.5)	11.0 (1.07–28.6)
Floor	2.4 (0.12–7.93)	2.8 (0.12–11.8)
Exhaust outlets	8.6 (0.16–28.5)	7.4 (0.15–30.5)

*Because of large variations in the LPS control, corrected values are only shown for the TNF control.

Figure 1 Examples of dose-response curves of the A549 lung epithelial cells stimulated with dust from surfaces, the floor, and the exhaust outlet from a school with high prevalence of symptoms. The mean of a triplet is shown together with the standard deviation.

Positive controls

The LPS control had a mean value of 3.8 (SE 2.1) ng IL-8/ml (209 experiments) with a relative mean square error (RMSE = SE/ $\bar{X}$) = 0.55. The TNF control had a mean of 12.4 (SE) 3.2 ng IL-8/ml (207 experiments) and an RMSE = 0.26.

Correlations between potency factors, building status, and organic content in the dust

A significant positive correlation between the mean PF of the floor samples and the PF from the surface dust samples ($r_s = 0.43$) was found. No correlation was found between the mean PF of the samples from exhaust outlets and the mean PF of the two other types of dust. No correlation was found between PF of floor dust and temperature, the concentration of CO₂, total VOCs, or the age of the building. However, the PF of dust from mechanical ventilation outlets was higher than the PF of dust from natural ventilation outlets ($p < 0.0001$,

Table 2 Mean content of organic dust from the three different types of dust samples

Dust samples from:	Organic content (min–max)
Surface	52.3% (20.1–76.8%)
Floor	35.5% (7.9–73.1%)
Exhaust outlets	33.7% (2.3–81.5%)
Mechanical ventilation	56.2% (18.3–81.5%)
Natural ventilation	17.1% (2.3–64.7%)

Mann-Whitney test). No difference of the PF calculated from the fraction of organic dust was found between natural and mechanical ventilation outlets.

Table 2 summarises the mean organic content in the dust samples. A significant positive correlation was found between the TNF corrected PF of all the dust samples and the content of organic dust, with a Spearman rank correlation coefficient (r_s) of 0.66. For the surface dust the coefficient was 0.9, for the floor dust 0.53, and for the dust samples from exhaust outlets 0.74. When correlating the PF calculated from the total dust sample versus PF calculated from the fraction of organic dust, a significant positive correlation was also found ($r_s = 0.59$).

Grouping of schools regarding prevalence of symptoms

The eight BRS were dichotomised, so that answers “never/seldom” and “now and then” were coded “seldom”, and answers “several times a week” and “daily” were coded “often”. For each school an average prevalence for having the symptoms often among the occupants was calculated. The 10 schools with low prevalence of symptoms had a BRS% of 4.4–11.0, whereas the schools with high prevalence of symptoms had a BRS% of 19.6–31.9.

Table 3 presents the inflammatory potential (PF) of sampled dust from selected rooms of the 10 schools with low prevalence of BRS and the 10 schools with high prevalence. The schools with the lowest symptom prevalence had a significantly lower median PF of dust from floors ($p < 0.0001$). Figure 2 shows that choosing a cut point of 2 ng IL-8/mg floor dust, 78% of the dust samples were from schools with low prevalence of symptoms, and 91% of the dust samples above the second cut point of 4.5 ng IL-8/mg floor dust (CP2), were from schools with high prevalence of symptoms. No statistically significant difference in PF between the schools with low and high prevalence of BRS was found for the surface dust or for dust from ventilation outlets (table 3).

The cut point of 4.5 ng IL-8/mg floor dust (CP2) was used to see whether any difference appeared in building characteristics from schools with high prevalence of BRS above and below the CP2. The low prevalence schools were predominantly below CP2 and the high prevalence schools predominantly above the CP2 ($p = 0.0001$). A smaller volume per person was found for high prevalence schools above the CP2, and for all the schools above the CP2 than below the CP2. The area

Table 3 Inflammatory potential corrected by the TNF control of sampled dust from selected rooms of the 10 schools with low prevalence of symptoms and the 10 schools with high prevalence of symptoms, defined according to average prevalence of BRS

Dust samples from:	Inflammatory potential (ng IL-8/mg dust), median (min–max)	
	10 low prevalence schools	10 high prevalence schools
Surface ($p=0.8$) (pooled samples)	9.8 (3.6–14.9) (n=10)	11.7 (1.1–28.6) (n=11)
Floor ($p<0.0001$)	1.8 (0.1–6.1) (n=46)	3.5 (0.8–11.8) (n=49)
Exhaust outlets ($p=0.19$)	3.8 (0.2–30.5) (n=23)	10.2 (0.7–20.6) (n=19)

Statistics: Mann Whitney U rank sum test.

Figure 2 Grouping the PF values (ng IL-8/mg dust) regarding the origin of the dust sample from schools with low and high prevalence (LP and HP) of BRS. The cut point values (CP1 and CP2) were chosen arbitrarily. About the same number of samples are from schools with low and high prevalence of symptoms. Of the samples below the CP1, 78% are from schools with low prevalence of symptoms and 22% from schools with high prevalence. Above CP2, 9% of the samples are from schools with low prevalence of symptoms and 91% from schools with high prevalence. In the between area (>CP1 and <CP2) the distribution of schools with low and high prevalence of symptoms are about half of each.

Table 4 Individual symptoms of the BRS index tested against the PF

Symptom	p value
At least 2 symptoms of the mucous membrane and skin	0.033
Eye irritation	
Running eyes	0.747
Itching eyes	0.046
Nose irritation	
Itching nose	0.994
Nose congestion	0.026
Irritation of the throat	
Hoarseness	0.657
Dry throat	0.269
Itching/flushing facial skin	
Dry skin	0.421
Prickly sensation of the skin	0.077
At least one symptom from the central nervous system	0.004
Headache	0.068
Fatigue	0.031
Difficulties to concentrate	0.524

Statistics: 2×2 χ^2 test.

covered with mould was found to be larger below the CP2 than above. Rooms with no recirculation of air were associated with PFs above the CP2. Rooms where the ventilation system was stopped during the night were found above the CP2.

Correlations between potency factors and symptoms

A 2×2 χ^2 test was used to calculate the correlation between PF and the individual symptoms of BRS (table 4). Itching eyes, nose congestion, and fatigue were positively correlated with the PF ($p < 0.05$). Prickly sensation of the skin, and headache were borderline correlated with the PF ($p < 0.10$). A positive index of at least two of five symptoms of the mucous membrane and the skin, and a positive index of at least one symptom from the central nervous system were positively correlated to the PF ($p < 0.05$).

DISCUSSION

The present study shows that the inflammatory potential of floor dust can differentiate between schools with low versus high prevalence of BRS—that is, dust from the schools with high prevalence of symptoms also have a higher PF. This indicates that measurements of the PF of dust may be a useful screening tool for evaluation of heavily populated buildings as schools. A high PF of the dust may indicate that the BRS reported in a problem building are associated with an exposure, which is reflected in the dust. No correlation between the PF of surface dust and BRS was found, possibly due to the low number of samples ($n = 21$); however, a positive correlation between PF of floor dust and surface dust was

found ($r_s = 0.43$), indicating that these two types of dust reflect each other.

The employed method may have some limitations. It may be argued that floor dust is not a particularly good proximeasure of the actual risk of dust exposure, since a fraction of the floor dust is composed of large and heavy particles which never become airborne. It should, however, be emphasised that a high PF of dust does not necessarily mean that the BRS are caused by inhalation of dust particles as such. It may well be that the causative agents are merely reflected in the dust, and it could be speculated that absorption of, for example, reactive VOCs can make the dust more potent. Therefore dust in this study is used as a characterisation of the source, reflecting the chemical and biological exposures of the room. The A549 bioassay also has the disadvantage of missing the interaction between different cell types as found in the complete human immune system, hence the A549 results may not be easy to interpret in terms of the actual mechanisms causing the symptoms.

Stimulation of the lung epithelial cells with dust gave a biphasic course of the dose-response curves (fig 1). This could be explained by the cytotoxic effect on the cells as observed for some samples at the higher concentrations of dust or that high dust concentrations interfere with the IL-8 measurements. Since the potency factor (PF) was calculated from the initial linear part of the dose-response curve, this interference had limited impact on the calculation of the PF, at the most a small underestimation of the PF. Hence, no attempts were made to reduce the interference.

Treatment of dry dust with γ radiation will to some extent lead to the formation of free radicals. However, these will quite rapidly react with other compounds and become stable again. When using γ radiation to kill microorganisms, one may speculate that intracellular mediators and toxins may leak from the organism, for example, through disruption of the cell membrane. This could add to the reactivity of the dust.

The active components responsible for the difference in PF of dust from schools with low and high prevalence of symptoms are still unknown. The organic fraction of the dust sample correlated with the PF of the dust, indicating that the inflammatory agents are to be found mainly in the organic fraction of the dust. Dust from mechanical ventilation outlets contained a higher content of organic dust than dust from the natural ventilated outlets. This was also reflected in the A549 bioassay. Furthermore, schools with a high prevalence of symptoms had significantly more rooms with mechanical ventilation.¹⁵

Previous studies have indicated that endotoxin and microorganisms in house dust may contribute to sick building syndrome and aggravation of allergic asthma.²¹ In a previous study the mean endotoxin concentration was found to be 76.4 EU/mg, ranging from 1.36 to 204 EU/mg dust in these school dust samples (table 5).¹³ A significant, positive correlation was previously shown for the PF of the surface dust samples and endotoxin (table 5).¹³ This correlation was also seen when correction was made for the content of organic matter (unpublished results). However, the measured endotoxin concentrations in the dust samples were below the detection limit of the bioassay (17 $\mu\text{g}/\text{ml}$).¹⁷ Endotoxin, therefore, may act as a proximeasure of some other unknown biological active microbial components in the dust samples. Alternatively, it is possible that the potency of endotoxin in house dust is enhanced by the co-presence of other biological active components. The endotoxin concentration in the different dust samples (surface, floor, and exhaust outlet) of this study was not significantly different in schools with high and low prevalence of symptoms.¹⁵ Hence, endotoxin is not a likely explanation of the inflammatory potential of dust from schools. Measurements of (1 $\rightarrow$ 3)- β -D-glucan, from moulds, were not made. Glucans however have the capacity in vitro to induce secretion of cytokines such as TNF α , and a correlation between levels of

Table 5 Endotoxin and viable counts, shown as median (range)

	Surface Median (min-max)	Floor Median (min-max)	Ventilation outlets Median (min-max)
Endotoxin (EU/mg dust)	59 (9.3–204) $r_s=0.31$	72 (10–195) $r_s=0.07$	12 (1.4–64) No correlation
Actinomycetes (cfu/mg dust)	8.1 (0.1–357) No correlation	28 (0.6–2402) No correlation	0.6 (0.6–21) $r_s=0.14$
Bacteria (cfu/mg dust)	1185 (335–4790) No correlation	2072 (10–21840) $r_s=0.14$	65 (0.6–33348) $r_s=0.01$
Mould (cfu/mg dust)	69 (16–411) No correlation	31 (1.1–400) No correlation	12 (0.6–1310) $r_s=0.46$

Spearman's rank correlation coefficient (r_s) is given where significant positive correlation is found between viable counts and PF in the rank correlation test. Modified from Allermann and Poulsen.¹³

(1→3)- β -D-glucan and reported symptoms as well as lung function changes have been found.^{22, 23}

The content of both bacteria and fungi has been shown to correlate with BRS.^{2, 23} In the school dust samples the highest number of viable bacteria (including actinomycetes) was previously found in the floor dust, and the highest number of viable fungi was found in the surface dust (table 5).¹³ No correlation was observed between the viable counts of microorganisms and the PF from the surface dust samples (table 5).¹³ The number of bacteria in the dust samples was generally higher than the number of actinomycetes and fungi. The concentration of bacteria (median value) in the floor dust was significantly higher ($p = 0.009$) in samples from the schools with low prevalence of symptoms.¹⁵ Thus, the concentration of viable microorganisms does not seem to contribute significantly to the inflammatory potential of dust in these schools.

Conclusion

The obtained potency factors (PF) of the floor dust samples tested in the A549 bioassay showed a highly significant correlation with the prevalence of BRS, with the low PFs originating from the schools with low prevalence of symptoms and vice versa. The organic fraction of the dust samples correlated with the PF of the dust, indicating that the inflammatory agent is likely to be found in the organic fraction of the dust.

No single factors could explain the observed differences between the PF of dust samples; the inflammatory potential of the dust may have multifactorial causes with no known single parameter being of major importance.

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