

INFLAMMATORY POTENTIAL OF DUST FROM SCHOOLS ASSOCIATED WITH BUILDING RELATED SYMPTOMS

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ABSTRACT

The aim was to test whether the inflammatory potential of dust might be used to differentiate schools with high and low prevalence of building related symptoms (BRS). 10 schools with a high prevalence of BRS and 10 with a low prevalence were selected. The potency (PF) of dust samples to stimulate an IL-8 secretion from the A549 cell line was associated with the organic content of the dust. Dust from schools with low prevalence of symptoms had a significantly lower PF than high prevalence schools, and the PF of the floor dust correlated with the prevalence of symptoms (high or low). Using a cut-point value of 4.5 ng IL-8/mg floor dust, significantly more schools with high prevalence of BRS were found above the cut-point than below. The substances in the dust causing the inflammatory potential are at present time unknown.

INDEX TERMS

Building related symptoms, Dust, In vitro, Indoor air, interleukin 8

INTRODUCTION

Non specific symptoms such as mucous membrane irritations in the eyes, nose and the upper respiratory tract, cough, dryness of the skin, and symptoms of the central nervous system (CNS) as headache, dizziness fatigue and lack of concentration, have all been related to the quality of the indoor air or the indoor environment (Redlich et al., 1997). Both the type and the severity of symptoms may vary from person to person, even within the same building. These building related symptoms (BRS) are multifactorial in origin (Lahtinen et al., 1998), and both chemical, biological, physical, and psychosocial (Seltzer, 1995, Jaakkola et al., 1989, Lahtinen et al., 1998) factors have been related to outbreaks of BRS. Chemical and biological factors in the immediate surroundings may be reflected in the dust. Hence, the composition of dust, including organic and inorganic constituents from the building and the building users, varies according to the environment. It is difficult to point out one or just a few chemical factors or components 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 (Nielsen et al., 1995). Inflammation could therefore be considered an integrated effect of the total biological and chemical exposure load from an indoor environment. Airborne dusts will sediment on surfaces, and sampling of dust from surfaces may be used as a crude proximeasure reflecting the total exposure burden of the room.

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The epithelial cells constitute not only a physical barrier, they also contribute to the protection by production of proinflammatory cytokines, inflammatory eicosanoids, and specific cell adhesion molecules (Devalia and Davies, 1993). The proinflammatory cytokine IL-8 has neutrophilic attracting and activating properties as well as acting as attractant for B-lymphocytes and basophils (Wuyts et al., 1998), and is thus a good indicator of inflammation.

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

METHODS

Questionnaires and technical investigation

“The Copenhagen School Study” was performed as a cross sectional study including employees and pupils (>13 years) from 75 schools in Copenhagen. Questionnaires included questions about symptoms of the eyes, nose, throat, and skin, as well as general symptoms as headache, fatigue, and difficulties to concentrate. 7884 questionnaires were returned giving a response rate of 66%. A BRS index for each school was calculated using the mean prevalence of 8 symptoms: eye irritation, nose irritation, nose congestion, irritation of the throat, itching/flushing facial skin, headache, fatigue and difficulties to concentrate.

From the BRS index 20 schools were selected: 10 schools with the lowest prevalence of BRS and 10 schools with the highest. The prevalence of symptoms (low or high) of the schools was blinded to the investigator during sampling and analysis of dust samples.

Dust

Floor dust was sampled with the HVS-3 sampler (ASTM Designation: D 5438-94) connected to a vacuum cleaner (n=96). Dust from horizontal or near horizontal surfaces (Surface dust) (n=21) and dust from exhaust ducts (n=41) was sampled on a filter by a portable vacuum cleaner with the VacuuMark disposable nozzle (Petersen bach, Bjerringbro, 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 cut into pieces. The total dust sample was then sieved on a 300 µm filter and stored at -20 °C. Each dust sample (particles < 300 µm) was divided into sub-samples for different analysis as 1) test of the inflammatory potential in the A549 bioassay (sterilised by γ -radiation at 35 kGy (Risø, Roskilde, Denmark) and stored at -20°C), 2) microbiological analysis (Reported in (Allermann and Poulsen, 1999)) 3) determination of organic/inorganic content. Dust was randomly picked out from the sample, and the percentage of fibre was maintained in the sub-samples.

A549 bioassay

The A549 bioassay was performed as described earlier (Allermann and Poulsen, 1999). In short, the human lung epithelial cell line A549 (ATCC no. CCL-185) was grown in Ham's F12 media supplemented with 100 IU/ml penicillin, 100 µg/ml streptomycin, 2 mM L-Glutamine and 10% heat inactivated Foetal Bovine Serum (FBS) (all reagents from GIBCO BRL, Paisley, Scotland). 1×10^5 cells per well were grown in 24 well multi-dishes (GIBCO BRL), at 36 °C, 5% CO₂ and 100% relative humidity. The dust was suspended in cell culture media and sonicated in a water bath for three times one minute just 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 a positive control sample containing 10 ng Tumor Necrosis Factor- α (TNF- α)/ml (Genzyme, Cambridge, Ma, USA. Catalog no: GENTNF-H)]. The volume was

one ml per well. The IL-8 secretion was measured in the media after 24 hours incubation by ELISA techniques (Genzyme and R&D systems Inc., Minneapolis, MN, USA). 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, i.e. the released IL-8 versus the concentration of dust. Day to day variation in the bioassay was taken into account by standardising the PF against the value of a positive control, relative to the mean of all the positive controls in the study:

$$PF_{\text{corr}} = \alpha / (\bar{X}/T) \quad (1)$$

where α is initial linear slope of the dose response curve, \bar{X} is the mean of the control samples in triplicate measured on the same day, and T is the mean of multiple control measurements on different days (Allermann and Poulsen, 1999).

Organic content

The contents of organic matter in the dust samples were determined by incineration.

Statistics

Correlation test of data not following a normal distribution was performed by the non-parametric Hotelling-Pabs rank correlation test. The Mann-Whitney U rank sum test was used to test for differences in continuous variables between groups (e.g. low and high prevalence of BRS). A significance level of $\alpha = 0.05$ is used, when nothing else is stated.

RESULTS

Dust tested in the A549 bioassay

Surface dust and dust from floors showed typical bell shaped 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 concentration. The inflammatory potential of the different dust samples to elicit an IL-8 secretion from the A549 lung epithelial cells was calculated as the potency factor and summarised in table 1. The maximum IL-8 secretion for surface dust 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. The maximum IL-8 secretion for floor dust ranged from 0.97 ng to 5.9 ng IL-8/ml with a background value between 0.11 and 1.8 ng IL-8/ml.

Some dust samples from exhaust ducts produced similar bell shaped dose response curves as mentioned above. In others a linear increase over the whole dose spectrum was seen. 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.

Table 1. The 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 [n=21]	8.3 (0.78 – 38.5)	11.0 (1.07 – 28.6)
Floor [n=96]	2.4 (0.12 – 7.93)	2.8 (0.12 – 11.8)
Exhaust ducts [n=41]	8.6 (0.16 – 28.5)	7.4 (0.15 – 30.5)

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 ducts and the mean PF of the floor dust samples, or the PF from the surface dust samples. The PF of dust from mechanical ventilation ducts were higher than the PF of dust from natural ventilation ducts ($p < 0.0001$, Mann-Whitney test).

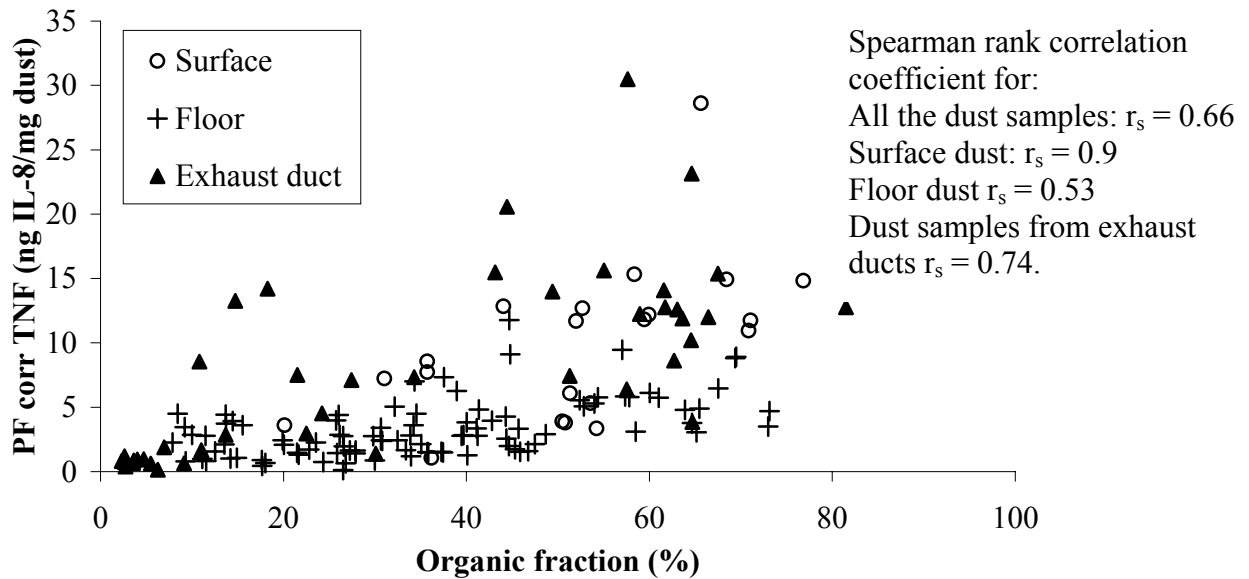


Figure 2: The PF corrected with TNF of dust samples tested in the A549 bioassay versus the organic fraction in the sample.

A significant positive correlation was found between the TNF corrected PF of all the dust samples in the A549 bioassay and the content of organic dust, with a Spearman rank correlation coefficient $r_s = 0.66$ (figure 2). 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 schools with the lowest symptom prevalence had a significantly lower median PF of dust from floors (Table 3).

Table 3. Inflammatory potential (PF) corrected by the TNF control of sampled dust from selected rooms of the 10 schools with low prevalence of BRS and the 10 schools with high.

Dust samples from	10 low prevalence schools Inflammatory potential (ng IL-8/mg dust) median (min.- max.)	10 high prevalence schools Inflammatory potential (ng IL-8/mg dust) median (min.- max.)
Surface ($p=0.8$)	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 ducts ($p=0.19$)	3.8 (0.2 - 30.5) [n=23]	10.2 (0.7 - 20.6) [n=19]

Figure 3 shows that the PF values of the tested floor dust samples fall in three categories: below cut point 1 (CP1), above CP2 and between CP1 and CP2. 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 exhaust ducts (table 3).

CP2 was used to see if any difference appeared from high prevalence schools above and below the CP2. A significant difference in distribution of low and high prevalence schools above and below the CP2 was found with the schools with low prevalence of BRS predominantly below and the high prevalence schools above the CP2 ($p=0.0001$).

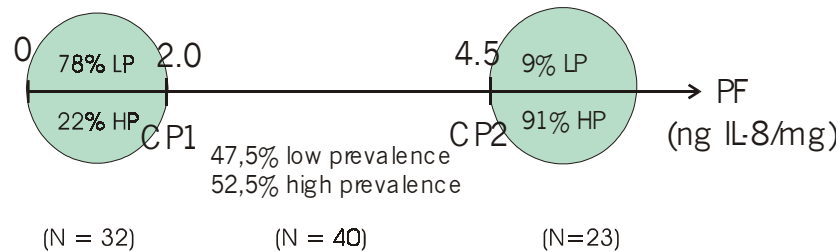


Figure 3: Grouping the PF values (ng IL-8/mg dust) regarding the origin of the dust sample from schools with low prevalence (LP) and high prevalence of symptoms (HP). The cut point values (CP1 and CP2) were chosen arbitrary. About the same number of samples comes from schools with low and high prevalence of symptoms. Of the samples below the CP1 78% are from low prevalence schools and 22% from high prevalence schools (32 samples from 10 low and 5 high prevalence schools). In the area above CP2 9% of the samples comes from schools with low prevalence of symptoms and 91% from schools with high prevalence (23 samples from 2 low and 8 high prevalence schools). In the between area ($>CP1$ and $<CP2$) the distribution of schools with low and high prevalence of BRS are about half of each.

DISCUSSION

Stimulation of the lung epithelial cells with dust gave biphasic course of the dose response curves. This could be explained by the cytotoxic effect on the cells as found for some samples at the higher concentrations of dust or an interference of the secreted IL-8 with the dust. Since the potency factor (PF) was calculated from the initial linear part of the dose response curve, this interference or cytotoxic effect had limited impact on the calculation of the PF.

The PF of floor dust could in this study clearly differentiate between schools with a low and a high prevalence of building related symptoms. 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. It should, be emphasized 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 e.g. reactive VOC's can make the dust more potent.

The endotoxin concentration in the different dust samples (surface, floor and exhaust duct) of this study was not significantly different in schools with low and high prevalence of symptoms (Meyer, 2000). However, a weak correlation between PF of surface dust and endotoxin was found (Allermann and Poulsen, 1999). For viable counts only a weak correlation was found between the PF and moulds in dust from exhaust dust (Allermann and Poulsen, 1999). Thus, the concentration of endotoxin and viable microorganisms does not seem to contribute significantly to the inflammatory potential of dust in the schools included in this study.

The active components responsible for the difference in PF of dust from schools are still unknown. One hypothesis may be that the potency of the dust is linked to the organic fraction of the dust. The organic fraction of the dust sample correlated with the PF of the dust,

indicating that the inflammatory agent was to be found in the organic fraction of the dust. Dust from mechanical ventilation ducts contained a higher content of organic dust than dust from the natural ventilated ducts, and a significant higher PF were found from the dust samples from mechanical ventilation ducts compared with the samples from natural ventilation ducts. Also schools with high prevalence of symptoms had significantly more rooms with mechanical ventilation (Meyer, 2000).

Correcting the PF with the organic fraction did generally not result in better correlations with the microbiological data (unpublished results), indicating that none of the tested parameters are quantitatively dominating factors in the dust.

CONCLUSION

The present study demonstrate that the inflammatory potential of floor dust can differentiate between schools with low versus high prevalence of BRS, i.e. dust from the schools with high prevalence of symptoms having a higher PF. This may indicate that measurements of the PF of dust may be a useful screening tool for evaluation of heavily populated buildings as schools. However, no single factors could explain the observed differences between the PF of dust samples from the schools with low and high prevalence of BRS. The possible presence of one still unknown causal agent cannot be ruled out, but the inflammatory potential of the dust may have multifactorial causes with no known single parameter being of major importance.

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