

Nasal hyperresponders and atopic subjects report different symptom intensity to air quality: a climate chamber study

Abstract Short-term exposure to dust and dust added with β -(1,3)-D-glucan or aldehydes may cause sensory reactions. In random order, we exposed 36 volunteers in a climate chamber to clean air, office dust, dust with glucan, and dust with aldehydes. Three groups of subjects were exposed, eleven were non-atopic with nasal histamine hyperreactivity, 13 were non-atopic, and 12 were atopic. Subjective ratings of symptoms and general health were registered four times during four 6-h exposure sessions. Six symptom intensity indices were constructed. The nasal hyperreactive group had a high and time-dependent increase of mucous membrane irritations, whereas the atopic group had a low and stable rate of irritations with exposure time, close to the reference group ($P = 0.02$ for differences between the groups with respect to time under exposure for Weak Inflammatory Responses and $P = 0.05$ for Irritative Body Perception, significance mainly because of the nasal hyperreactive group). Exposure to dust, with or without glucan or aldehydes, showed increased discomfort measured by the index for Constant Indoor Climate, and dust with glucan had a similar effect for the index for Lower Respiratory Effects. For Psychological and Neurological Effects these were dependent on group affiliation, thus preventing a uniform statement of exposure effects for all three investigated groups.

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Practical Implications

Opportunities for identifying persons with high or low sensitivity to low-level exposures are important in preventive medicine and will reduce intra-group variability and thus increase the power of experimental and epidemiological studies searching for correlations between exposures and health effects. The contrast between nasal hyperreactive on one side and atopic and reference subjects on the other side is particularly important. The atopic group indicated a non-homogenous reaction depending on their hyperreactive status, a finding that could be important but needs further confirmation.

Background

Field investigations in non-industrial indoor units (e.g. in day care centres, offices or schools) and in homes have demonstrated frequent air pollution with dust and particulate matter in the range of 20–200 $\mu\text{g}/\text{m}^3$ with extreme values of up to 1000 $\mu\text{g}/\text{m}^3$ (Armstrong et al., 1989; Gyntelberg et al., 1994; Hodgson and Collopy,

1989; Rudblad et al., 2002; Skov et al. 1989, 1990; Smedje and Norbäck, 2001; Wallace, 1996). Often the dust is described as a relatively inert base dust with the addition of biological active compounds such as allergens or toxic chemicals.

Analyses of spreading of indoor air pollutions have indicated that for more than 50% of the air we breathe, the last contact with surfaces is with the floor close to the

person (Murakami, 2004). The reason for this is the chimney or stack effect created by the person. This stack effect is sufficiently strong to carry particles with little gravitational settling to the breathing zone (e.g. particles $< 10 \mu\text{m}$). The exposures to dust, therefore, contain a large fraction of re-suspended floor dust, and the cleanliness of the building and the intensity of housekeeping become an important factor for exposure levels to dust indoors.

It has been indicated that normal dust at $150 \mu\text{g}/\text{m}^3$ (TSP) may cause discomfort and symptoms in humans, including increased eye and nose irritation during climate chamber exposures (Mølhave et al., 2000a). Measurements of the composition of the dust used for the tests showed no known component that, by itself, could explain the responses. The analyses also indicated that an essential fraction of volatile or semi-volatile pollutants in the air might be present as adsorbed compounds on particulate matter.

Numerous investigations have shown that exposure to dust causes increased prevalence of complaints about air quality, discomfort, and symptoms related to irritation of eyes, nose or throat. (e.g. Skov et al. 1989, 1990). The Danish Town Hall study (Gyntelberg et al., 1994) showed an increased (50%) frequency of symptoms associated with sedimented dust, but not with airborne concentrations. This was interpreted as an indication of the inadequacy of short-term exposure measures. Three exposure studies of the effects of dust have previously been performed at the Department of Environmental and Occupational Medicine, the University of Aarhus, Denmark. A review is found in Mølhave, (2003). The symptoms registered in questionnaires, which were found in at least two of the studies to be related to dust exposures, were throat irritation, heavy head/sluggishness, feeling the need to cough, and general irritation in a time course registration on a potentiometer.

Respiratory effects have been associated with airborne exposures to organic dust containing pathogen associated molecular patterns. Such compounds are, for example, endotoxins (lipopolysaccharides, LPS) from Gram-negative bacteria, β -(1,3)-D-glucan from mold, and allergens in the form of proteins from the microorganisms or their spores. Also, although in the working environment, microorganisms and their endotoxins have caused adverse respiratory and systemic effects in the exposed population (Douwes et al., 1997). Furthermore, the severity of asthma symptoms has been associated with the LPS concentration in house dust (Michel et al., 1991).

Generally, the effects or symptoms observed after exposures to low-level of indoor air pollution [such as volatile organic compounds (VOCs) or dust] are unspecific, which means that the presence of a symptom does not identify a specific causality and exposure. The prevalence of an unspecific symptom may include

unpredictable contributions to effects from several exposure types (such as thermal exposures or mental strains) or from biological mechanisms not related to pollutant exposures. The mere number of possible co-variables is a possible reason why uncontrolled field investigations often fail to demonstrate clear correlations between environmental exposures and effects associated with specific types of buildings (Berglund et al., 1992; Mølhave, 1991; Wallace, 1996).

Depending on the constituents of the dust particles, the toxic effects of dust may result from a physical stimulation of nerve endings or from chemical/microbiological activity. Many of the effects are related to sensory stimulation (e.g. odors or irritation). Some are classic immunological reactions (e.g. mites). Others are more likely to be unspecific inflammatory reactions with no allergic mechanisms involved. Such inflammatory reactions might involve the release of several inflammatory markers (Sigsgaard et al., 2000). It has been found that perceived indoor air quality and general well-being is strongly correlated to both irritation and odor (Mølhave et al., 1986; Otto et al., 1990; Pan et al., 2000).

We carried out an exposure study with office dust, office dust with added β -(1,3)-D-glucan, and office dust added with aldehydes. Our main hypotheses were that, in humans, the inflammatory potential of the dust is augmented by adsorption of aldehydes and contamination of dust with β -(1,3)-D-glucan. In this paper the subjective sensory effects are in focus because they seem to be experienced at lower exposure doses (time $\times$ concentration) than the objectively measurable effects (Cometto-Muniz et al., 2004). Other results are published elsewhere (Bønløkke et al., 2006).

Study aims

The two particular hypotheses of this study were:

Adsorption of VOC (exemplified by aldehydes) on dust or contaminations of dust with β -(1,3)-D-glucan give rise to (increased) subjective sensory reactions of the upper airways.

Individuals respond differently to airborne dust exposures depending on personal factors.

This study was performed according to the Helsinki declarations and was evaluated and accepted by the local committee for ethical use of human subjects in experimentation. The reference number of the ethical evaluation is 1999/4486.

Material and methods

The main study

Details of the main study and its organization are found in (Bønløkke et al., 2006). Here a summary of the basic details of the design is given.

Working plan and organization

In a preparatory phase, volunteers were found via cohorts drawn from the student community or among those responding to announcements in the local press. To exclude subjects who may be of risk because of the exposures, a detailed medical examination was made, including the completion of an extended health questionnaire and a lung function test. The inclusion criteria were: no response to house dust-mite in a standard skin prick test (SPT), normal lung function, and no serious disease or regular medication e.g. for asthma or allergy. Additional inclusion criteria were: non-smoking for the last 6 months, age 18–64 years, and measures of histamine responsiveness in the nose.

From the pre-investigation group three sensitivity subgroups were selected by application of the SPT with 11 inhalant allergens and a nasal histamine provocation test with rhinostereometry (RSM):

Group 1 (Nasal hyperreactive group). Healthy non-atopic persons (SPT) with nasal hyperreactivity to histamine.

Group 2 (Reference group). Healthy non-atopic persons (SPT) without nasal hyperreactivity to histamine.

Group 3 (Atopic group). Atopic (SPT) persons with or without nasal hyperreactivity to histamine (no house dust allergy).

Nasal hyperreactivity to histamine was defined by the results of RSM (Hallén and Juto, 1993). The nasal hyperreactive subjects were defined by a mucosal swelling of at least 0.6 mm 2 and 5 min after a challenge to a 1 mg/ml histamine solution. In this study, the atopic persons were defined as having one or more positive responses to the SPT (but not to dust-mite). The selection was based on the diagnosis of atopy by a SPT wheal with the largest inside diameter at least 3 mm. Because of difficulties in obtaining a strictly homogenous group five atopic persons had hyperreactivity to histamine, the other seven were not nasal hyperreactive. The 36 selected subjects had an average age of 30 years (range 22–59) and the male:female ratio was 14:22. The three groups had similar distributions with respect to both age and sex.

The design was a balanced Latin-square design where each subject was exposed to all four exposures of dust in the climate chamber in sessions separated by 14 days. The timetable for exposure included 2 weeks of assessments, 1 week before and 1 week after each exposure. The exposures were arranged in daily 6 h sessions starting with 1 h of exposure to clean air for acclimatization outside the chamber, 4 h in the chamber starting with clean air (30 min), increasing exposure (30 min) and 3 h of exposure (to either clean air or

one of three types of dust), followed by 1 h of exposure to clean air outside the chamber. Three subjects, one from each of the three groups, were exposed at the same time. The order between the four exposure sessions were defined in accordance with the standard Latin-square design, and thus determined before the series of experiment started. Additional details on the design and measurements taken are found in (Bønløkke et al., 2006). In the final stage, a small imbalance in the Latin square occurred as it was difficult to find persons in concordance with the inclusion criteria, thus 11, 13, and 12 persons were included from the three sensitivity groups respectively.

The mean concentrations of dust during the exposures were $<20 \mu\text{g}/\text{m}^3$ for clean air and between 332 and $379 \mu\text{g}/\text{m}^3$ for the dusty exposures (Bønløkke et al., 2006).

Dust collection and exposures

Dust collection and exposure characterization is described in details elsewhere (Mølhave et al., 2000b); only a summary is given here. The dust used for the exposures was sampled in office buildings and institutions without known indoor climate problems. The dust was sampled outside the pollen production period. Glucan and aldehydes were added according to a well-defined scheme (Bønløkke et al., 2006).

The exposures were arranged in a 32 m^3 stainless steel chamber (Mølhave et al., 1986). The chamber, rate of dust injection, and the total dust exposure are described elsewhere (Bønløkke et al., 2006, Mølhave et al., 2002). The variation of the indoor climate factors in the chamber, such as temperature and humidity, was kept at constant values within preset ranges except for the dust concentration in the air.

Effect measurements

Subjective evaluations during the exposure sessions were carried out by a computer-based questionnaire with 29 questions (translated from Danish in Appendix 1). The questionnaire was completed in clean air before entering the chamber, after the exposure start, and twice during exposure. The subjects scored their evaluation on the screen by placing a marker on a 130-mm-long visual analogue scale (VAS). The intensity of any discomfort was registered as the length in millimeter from the left end of the scale to the marker (Mølhave et al., 1986). For each question, the ratio of response to full scale was registered.

Six indices were constructed. They represented: Environmental Perception, EP; Irritative Body Perceptions, IBP; Psychological and Neurological effects, PNE; Weak Inflammatory Responses, WIR; Lower Respiratory Effects, LRE; and Constant Indoor Climate, CIC. Appendix 1 gives details of which of the 29

questions formed the indices. They were constructed as averages of the response to the selected questions, after normalization of the VAS scale to the range 0–100 for each question, with 0 indicating no discomfort and 100 the highest discomfort. For the questions 1, 3, 4, 5, and 6 the recorded values were subtracted by 50 and then their absolute value was multiplied by 2 before normalization and formation of the index for Constant Indoor Climate. This was performed because the definition of the left end point of the scale was different from the other questions. The pre-exposure mean values for the whole study group were 17.8, 6.7, 6.3, 2.2, 16.5, and 7.7 for the EP, IBP, WIR, LRE, CIC, and PNE indices respectively. The pre-exposure readings were subtracted from the index values obtained during exposure in the chamber, i.e. all values are baseline corrected before analysis. The indices constructed as above constitute the effect measures analyzed in this report.

Statistical methods

The main outcome variables, the six indices, were analyzed with a linear mixed model with repeated measurements on each one of the 36 subjects, up to 12 measurements (under different conditions) on each subject, and in accordance with Latin square design. As defined each pre-exposure index value had been subtracted from the index values under exposure and with the mixed model for repeated measurements each subject is evaluated with differences from the subject's own pre-exposure values and with the difference from the exposure from clean air. Each analysis started with a full model where all factors and their interactions were included. The factors were Group (three levels), Exposure (four levels) and Time spent in the chamber (three levels; i.e. 70, 130 and 220 min after entering the climate chamber; thus quantifying the cumulated experience of chamber exposure). A covariate for the subject's evaluation of chamber stress was included in each analysis with the exception for the PNE index. This basic model, referred to as model I, revealed the existence of statistical interactions. In the case of no significant interactions, the next step was an analysis without interactions, model II. Statistically significant interactions led to analysis stratified for the factors involved in the interaction, model III.

To handle our difficulty of obtaining a strictly homogenous group with respect to nasal hyperreactivity for the atopic persons we performed several sensitivity analyses with (i) four groups instead of three (atopics split up into nasal hyperreactive and non-hyperreactive), or (ii) excluding either the nasal hyperreactive or the non-nasal hyperreactive atopics from the analyses, and (iii) only the two subgroups of atopics were analyzed.

The effect measure in the mixed model is expressed as the average difference between the response in the investigated category and the reference category. All estimates are supplemented with 95% confidence intervals (95% CI) and *P*-values, and the latter are given both for the factor itself and for the differences between investigated and reference categories.

SAS, version 8, (SAS Institute Inc., Cary, NC, USA), procedure MIXED, was used for the analysis.

Results

Mean values for the main outcomes are shown in Figure 1a–c. Each figure shows mean values (with SEM) of baseline subtracted indices stratified for one of the investigated factors, i.e. exposure, personal factors (group) and time spent in chamber. Exposure to dust seems to have a smaller variation in effect than personal factors; in particular the nasal hyperreactive group shows higher discomfort for the four indices EP, IBP, PNE, and WIR. With time spent in the chamber, the indices and the discomfort increase up to a maximum at 130 min after entering the chamber. The values at 220 min are lower, but still higher than those at 70 min. The Lower Respiratory Effect index has low mean values indicating that only a few symptoms were reported.

For the most general model of analysis, model I, (Table 1) Environmental Perception shows no interesting effects. For IBP and WIR there is a statistically significant interaction group $\times$ time spent in chamber, and subsequent analyses will reveal the nature of this interaction. Exposure for dust or spiked dust has a statistically significant interaction with the group factor in the analysis of Psychological and Neurological Effects. Details about this effect will be shown below.

The statistically significant exposure effect for the Lower Respiratory Effect index was because of dust added with glucan, which had a difference from clean air of 1.5 units (95% CI 0.3–2.6). The other exposures did not differ from clean air.

For Constant Indoor Climate, all exposures differed significantly from clean air; the average differences from clean air with 95% CI were for office dust 2.6 (0.9–4.2), for dust and glucan 1.7 (0.0–3.4) and for dust and aldehydes 2.1 (0.4–3.7), thus, in all cases higher than zero.

The index for Psychological and Neurological Effects was analyzed separately for each group with respect to dust exposure. The effect estimates showed that the nasal hyperreactive group had a quite different result from the atopic group with respect to exposure. For dust added with glucan, the nasal hyperreactive group had 1.9 units lower value than for clean air (95% CI–5.4, 1.7), whereas the atopic group for the same exposure had 2.8 units higher than for clean air (95% CI–1.0, 6.5). For dust, the differences were in the other

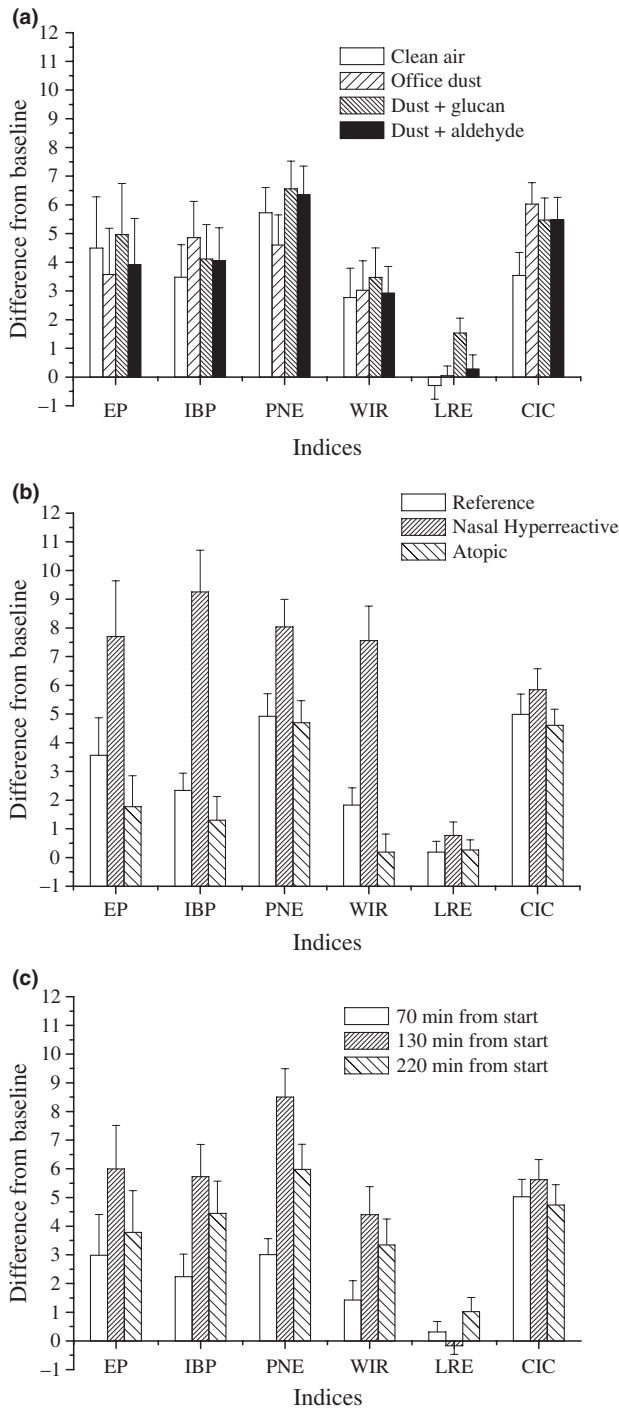


Fig. 1 (a–c) Mean values and standard error of mean (extension from bar) for the observed difference from baseline for six indices, shown stratified for exposures, groups and time from start of exposure. (Large difference = more discomfort)

direction, the nasal hyperreactive group had 3.5 units higher than clean air (95% CI–0.1, 7.1), whereas the atopic group had 2.3 units lower (95% CI–6.1, 1.4). The reference group was similar to the atopic group but with smaller deviations from clean air, except for dust. For all three groups, the exposure effect was statistically significant or almost significant (0.03, 0.05,

Table 1 Statistical significance (*P*-values) for mixed model analysis of six indices, with pre-chamber values subtracted. Group (three levels), Exposure (four levels) and Time spent in chamber (three levels) are included in the model as well as all second-degree interactions. Analysis without the covariate ‘Chamber stress’, and analysis with the covariate ‘Chamber stress’ within brackets

Index	Exposure	Group	Time in chamber	Group* exposure	Time in chamber* exposure	Group* time in chamber
EP	0.94 (0.79)	0.56 (0.53)	0.13 (0.29)	0.08 (0.15)	0.69 (0.83)	0.55 (0.52)
IBP	0.72 (0.63)	0.18 (0.10)	0.006 (0.06)	0.64 (0.39)	0.66 (0.78)	0.04 (0.02)
PNE ¹	0.39	0.50	<0.0001	0.002	0.76	0.97
WIR	0.93 (0.90)	0.12 (0.07)	0.003 (0.03)	0.59 (0.37)	0.60 (0.75)	0.07 (0.05)
LRE	0.02 (0.04)	0.86 (0.93)	0.08 (0.07)	0.36 (0.40)	0.73 (0.69)	0.96 (0.95)
CIC	0.02 (0.03)	0.81 (0.84)	0.38 (0.39)	0.18 (0.24)	0.52 (0.35)	0.97 (0.97)

EP, index for environmental perception; IBP, index for irritative body perception; PNE, index for psychological and neurological effects; WIR, index for weak inflammatory responses; LRE, index for lower respiratory effects; CIC, index for constant indoor climate. ¹Which includes chamber stress in its definition, was analyzed without chamber stress as covariate.

0.06, for the nasal hyperreactive, the atopic and the reference groups respectively) but, as stated above, in opposite directions, thus preventing a uniform statement of the exposure effect for all three groups.

Different effects of time spent in the chamber were found for WIR and IBP, and this was most pronounced with respect to group affiliation ($P = 0.02$ and $P = 0.05$ respectively). The effects for the two indices were similar and Figure 2a–c show the data for the WIR index. The figures show the adjusted index values before the subtraction of the baseline values. Consequently, baseline values are also included. Already at baseline, the nasal hyperreactive group shows higher discomfort than the other two groups. During the course of the exposure, the nasal hyperreactive group shows a steep increase in discomfort, with about two times higher values at the end of the exposure session compared with baseline. The atopic group has a different behavior with almost no change during time spent in chamber and with only a small difference to the reference group. Hence, the primary contrast is between the nasal hyperreactive group on one side and the atopic and reference groups on the other.

For the nasal hyperreactive group, increased values of WIR, as well as for IBP, were observed at the end of the exposure sessions for the spiked dust in comparison to clean air or dust, although not reaching statistical significance.

Our sensitivity analyses to investigate a further stratification of the atopic group did not show results that deviated from the main conclusions concerning the differences between non-atopic nasal hyperreactive on one side and the reference group and the atopics on the other. To draw a more definite conclusion on the difference between the two subgroups of atopic persons is difficult because of the small sample size, only a tendency can be reported. For WIR as the most

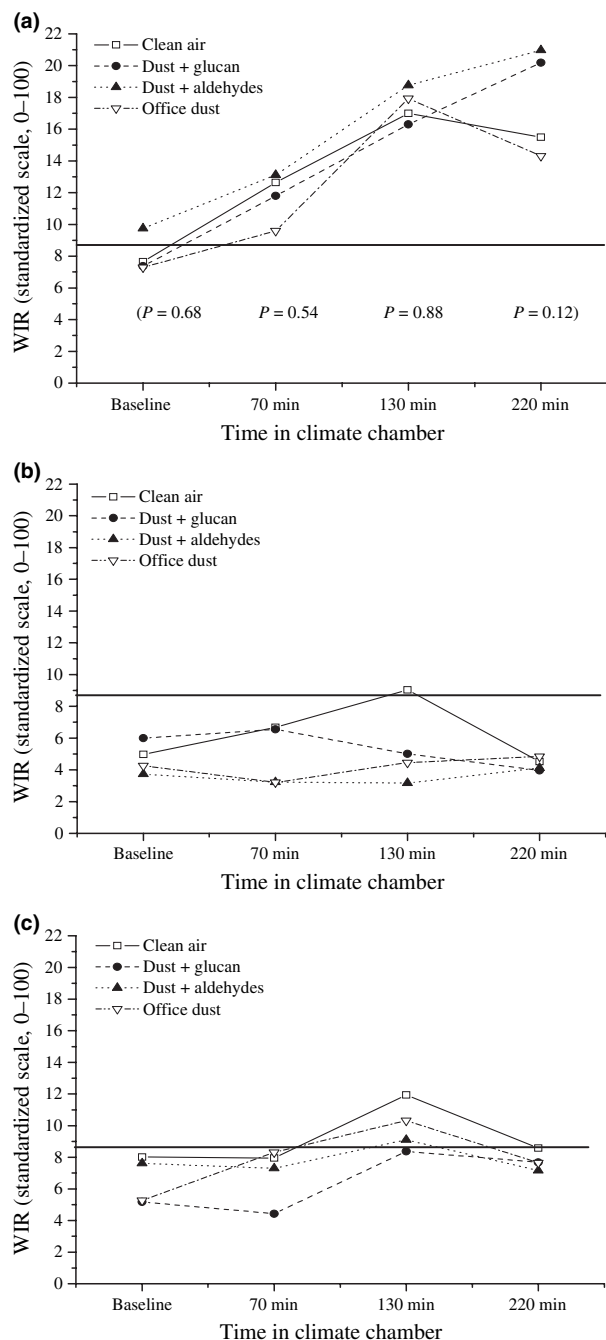


Fig. 2 (a–c) Mean values for weak inflammatory responses (WIR), Index for WIR, in the nasal hyperreactive (top), atopic (middle) and reference (bottom) groups, adjusted for chamber stress in a mixed model analysis. The thick horizontal line is the mean value for all exposures, all groups and all time-points. *P*-values for the nasal hyperreactive group indicate tests for different effect of the exposures at the specific time-points

striking observation, the nasal hyperreactive atopics had very low pre-chamber values but a substantial increase in discomfort with time that almost corresponded to that of the non-atopic nasal hyperreactive group. The non-hyperreactive atopics had higher pre-chamber values but none or almost decreasing response with time.

Discussion

The statistical analysis showed that exposure to dust and dust added with aldehydes or β -(1,3)-D-glucan in particular affected the three indices describing Psychological and Neurological Effects, Lower Respiratory Effects, and Constant Indoor Climate. Lower Respiratory Effects were seen for dust added with glucan and for Constant Indoor Climate dust exposure, with or without contamination, differed from clean air. The effect was strongest for Psychological and Neurological Effects and it interacted with the personal factors with a substantial difference between nasal hyperreactive and atopic subjects. Spiked dust in addition showed higher effects, though not statistically significant, on WIR and IBP at the end of the exposure periods compared with dust or clean air, indicating a possible (increased) sensory effect on the mucous membranes of the upper airways. Thus our first hypothesis concerning dust exposure could partly be confirmed but a unified statement for all indices and all exposures is not possible because of the effects seen on the index describing Psychological and Neurological Effects, which were in opposite directions depending on personal factors. Whether this particular result reflects a real biological effect or a chance finding is unknown. One possible biological mechanism could be that these neurological symptoms are masked or attenuated by more important inflammatory stimuli on the respiratory mucosa during the higher exposures, and that this acts differently depending on personal factors.

The second hypothesis was that the responses of individuals depend on personal factors. This was verified in the statistically significant variation of the responses to prolonged occupancy of the exposure environment. The statistical interaction between group affiliation and time under exposure was very strong for IBP and WIR. The nasal hyperreactive group had quite opposite response during the course of the experiment compared with the atopics and the reference group with increased sense of discomfort for the nasal hyperreactive group while the other two groups showed small or even no increase of discomfort with time. The result for Psychological and Neurological Effects also support the idea that responses depend on personal factors.

During the time spent in the chamber, the three indices IBP, PNE and WIR showed values that changed with time under exposure. Adjusting the regression model with the perception of subjectively reported chamber stress decreased the time effect somewhat. As a significant statistical interaction between time under exposure and group was found for the IBP and WIR indices, it is difficult to state a uniformly valid estimate of the time of exposure effect, but there are several indications that the effect of time

under exposure reached a maximum after 130 min and then starts to decline.

A possible reason for not being able to confirm some parts of our hypotheses is whether analyses of indices is less sensitive than analyses of single symptom questions, that is, those questions given in Appendix 1. We have previously conducted an analysis of a selected subset of seven of those questions (Bodin et al., 2005). The results were similar as to the group effect, as the nasal hyperreactive group had higher discomfort on all single symptoms compared with the atopics and the reference subjects. Another reason might be a possible misclassification of the person groups. However, the sensitivity analyses with different stratifications of the atopics and nasal hyperresponders do not contradict the basic results of the main analyses, as person group differences remain. Our results indicate that atopics with nasal hyperreactivity respond differently from atopics with no nasal hyperreactivity. We hypothesize that this finding indicates that there is no perfect overlap between nasal hyperreactivity and atopy, in analogy with the same lack of perfect overlap between bronchial hyperreactivity and atopy.

The limited number of subjects not only affects the statistical power of the analyses but also reduces the external validity by making it difficult to extrapolate the results to other larger populations. The general conclusion from previous studies points at a minimum of 12 to 15 subjects in each group of studied persons to obtain a reasonable power in the statistical analysis. In general, odor perceptions are considered to be the most sensitive responses to normal indoor exposures. It follows that odors will normally be obvious to the subjects in an experiment when they are exposed to

odor emitting dust. The issue of odor detection makes it difficult to conduct truly unbiased studies of this type. For example, in the study STØV95 (Mølhave et al., 2000a,b, 2002, 2004) ratings of general well-being were correlated to irritation, odor perceptions, air quality, and the need for more ventilation. Therefore, not only evaluations of odor but also all related symptoms may be biased by odor detection. Such bias was not found in another study with little odor emission from the dust in which sensory irritation was related to air quality, eye irritation, nose irritation, but not to odor intensity (Mølhave et al., 2000a). However, definitive investigations of this matter are lacking.

Few deviations occurred from the project plans and the efficiency of data collection was high. Subjects were generally selected and exposed as planned. We, therefore, conclude that the study was properly conducted according to plan.

It follows that, although the conclusions of significant findings related to each effect measure can be conclusive, the studies of overall causality and biological mechanisms in principle cannot be conclusive but only indicative.

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References

- Armstrong, C.W., Sheretz, P.C. and Lewellyn, G.C. (1989) Sick building syndrome traced to excessive total suspended particulates (TSP). In: Geshwiler, M. (ed.) *The Human Equation: Health and Comfort, Proceedings of ASHRAE-SOEH conference, IAQ 89*. San Diego, April 1989. Chapt.1: Pollutants and Health Effects. ISBN 0-910110-59-X. Atlanta, GA, USA, ASHRAE, 3-7.
- Berglund, B., Brunekreff, B., Knöffel, H., Lindvall, T., Maroni, M. and Mølhave, L. (1992) Effects of indoor air on human health, *Indoor Air*, **2**, 2-25.
- Bodin, L., Andersson, K., Mølhave, L., Bønløkke, J., Kjærgaard, S., Sigsgaard, T., Stridh, G., Juto, J.E., Bonefeld-Jørgensen, E., Pan, Z. and Jayatissa, M. (2005) Symptom reports from groups with different sensitivity of the mucous membranes in a climate chamber study *Indoor Air*, **15**(Suppl.11: 1.1-15), 7.
- Bønløkke, J., Stridh, G., Sigsgaard, T., Kjærgaard, S., Löfstedt, H., Andersson, K., Bonefeld-Jørgensen, E., Jayatissa, M., Bodin, L., Juto, J.E. and Mølhave, L. (2006) Upper airway inflammation in relation to dust spiked with aldehydes or glucan, *Scand. J. Work Environ. Health*, **32**, 374-382.
- Cometto-Muniz, J.E., Cain, W.S. and Abraham, M.H. (2004) Detection of single and mixed VOCs by smell and sensory irritation, *Indoor Air*, **14**(Suppl. 8), 108-117.
- Douwes, J., Dubbeld, H., van Zwieten, L., Wouters, I., Doekes, G., Heederik, D. and Steerenberg, P. (1997) Work related acute and (sub-) chronic airways inflammation assessed by nasal lavage in compost workers, *AAEM*, **4**, 149.
- Gyntelberg, F., Suadicani, P., Nielsen, J.W., Skov, P., Valbjørn, O., Nielsen, P.A., Schneider, T., Jørgensen, O., Wolkoff, P., Wilkins, C.K., Gravesen, S. and Norn, S. (1994) Dust and sick building syndrome, *Indoor Air*, **4**, 223-238.
- Hallén, H. and Juto, J.-E. (1993) A test for objective diagnosis of nasal hyperreactivity, *Rhinology*, **30**, 129-133.
- Hodgson, M.J. and Collopy, P. (1989) Symptoms and the micro-environment in the sick building syndrome: a pilot study. In: Geshwiler, M. (ed.) *The Human Equation: Health and Comfort, Proceedings of ASHRAE-SOEH conference, IAQ 89*. San Diego, April 1989. Chapt.1: Pollutants and Health Effects. ISBN 0-910110-59-X., Atlanta, GA, USA, 8-16.
- Michel, O., Ginanni, R., Duchateau, J., Vertongen, F., Le Bon, B. and Sergysels, R. (1991) Domestic endotoxin exposure and clinical severity of asthma, *Clin. Exp. Allergy*, **21**, 441.
- Mølhave, L. (1991) Volatile organic compounds, indoor air quality and health, *Indoor Air*, **4**, 357-376.
- Mølhave, L. (2003) Health effects of airborne dust and particulate matter indoors: a review of three climate chamber studies. (Chapter 4.2). In: Morawska, L. and

- Salthammer, T. (eds) *Indoor Dust and Particles*. Germany, Wiley – VCH, 387–406.
- Mølhave, L., Bach, B. and Pedersen, O.F. (1986) Human reactions to low concentrations of volatile organic compounds, *Environ. Int.*, **12**, 167–175.
- Mølhave, L., Kjærgaard, S.K. and Attermann, J. (2000a) Sensory and other neurogenic effects of exposures to airborne office dust, *Atmos. Environ.*, **34**, 4755–4766.
- Mølhave, L., Schneider, T., Kjærgaard, S.K., Larsen, L., Norn, S. and Jørgensen, O. (2000b) House dust in seven Danish offices, *Atmos. Environ.*, **34**, 4767–4779.
- Mølhave, L., Kjærgaard, S.K. and Attermann, J. (2002) Effects in the eyes caused by exposures to office dust, *Indoor Air*, **12**, 165–174.
- Mølhave, L., Kjærgaard, S.K. and Attermann, J. (2004) Respiratory effects of experimental exposure to office dust, *Indoor Air*, **14**, 376–384.
- Murakami, S. (2004) Analysis and design of micro-climate around the human body with respiration by CFD, *Indoor Air*, **14**(Suppl 7), 144–156.
- Otto, D.A., Mølhave, L., Rose, G., Hudnell, H.K. and House, D. (1990) Neurobehavioral and sensory irritant effects of controlled exposure to a complex mixture of volatile organic compounds, *Neurotoxicol. Teratol.*, **12**, 649–652.
- Pan, Z., Mølhave, L. and Kjærgaard, S.K. (2000) Effects on eyes and nose in humans after experimental exposure to air borne office dust, *Indoor Air*, **10**, 237–245.
- Rudblad, S., Anderson, K., Stridh, G., Bodin, L. and Juto, J.E. (2002) Slowly decreasing mucosal hyperreactivity years after working in a school with moisture problems, *Indoor Air*, **12**, 138–144.
- Sigsgaard, T., Bonefeld-Jørgensen, E.C., Kjærgaard, S.K., Mamas, S. and Pedersen, O.F. (2000) Cytokine release from the nasal mucosa and whole blood after experimental exposures to organic dust, *Eur. Respir. J.*, **16**, 140–145.
- Skov, P., Valbjørn, O., Pedersen, B.V. and DISG (1989) Influence of personal characteristics, jobrelated factors and psychological factors on the sick building syndrome, *Scand. J. Work Environ. Health*, **15**, 286–295.
- Skov, P., Valbjørn, O., Pedersen, B.V. and DISG (1990) Influence of indoor climate on the sick building syndrome in an office environment, *Scand. J. Work Environ. Health*, **16**, 363–371.
- Smedje, G. and Norbäck, D. (2001) Irritants and allergens at school in relation to furnishings and cleaning, *Indoor Air*, **11**, 127–133.
- Wallace, L. (1996) Indoor particulates, a review, *J. Air Waste Manag. Assoc.*, **46**, 98–126.

Appendix 1

DAMOS. PC-Questionnaire: Questions 1–29.

Question No	Question	Left end point	Right end point	Index
1	Illumination	Annoying weak	Annoying strong	CIC
2	Glare because of reflections	Not at all	Annoying strong	CIC
3	Sound level (noise level)	Annoying sound dead	Annoying noisy	CIC
4	Air temperature	Annoying cold	Annoying hot	CIC
5	Air humidity	Annoying dry	Annoying humid	CIC
6	Air movements	Annoying stale	Annoying draft	CIC
7	Air quality	Pleasant	Annoying unpleasant	EP
8	Odor intensity	None	Very strong	EP
9	Need more ventilation	No	Yes very much	EP
10	Eye irritation itching or stinging	Not at all	Very strong	IBP, WIR
11	Dry eyes	Not at all	Very strong	WIR
12	Runny/watering eyes	Not at all	Very strong	WIR
13	Irritation of throat	Not at all	Much	IBP, WIR
14	Irritation, itching stinging or dry nose	Not at all	Much	IBP, WIR
15	Irritation, dryness, itching or smarting skin	Not at all	Much	WIR
16	Runny/watering nose	Not at all	Very much	
17	Blocked nose	Not at all	Completely blocked	
18	Perspiration or sweating	Not at all	Much	
19	Sleepiness or drowsiness	Not at all	Much	PNE
20	Headache	Not at all	Very strong	PNE
21	Concentration difficulties	Not at all	Much	PNE
22	Nausea	Not at all	Very strong	PNE
23	Urge to cough	Not at all	Annoying strong	LRE
24	Shortness of breath	Not at all	Very strong	LRE
25	Respiratory sounds	Not at all	Very strong	
26	Fever	Not at all	Very high	
27	Tightness of breast - respiratory difficulties	Not at all	Very strong	LRE
28	General well-being	Very good	Very bad	PNE
29	Stressed by the chamber occupancy	Not at all	Ongoing strong	PNE

Indices are formed as averages of the normalized values of questions marked.