

Inflammatory and allergic responses to airborne office dust in five human provocation experiments

Abstract Five studies on the effects of dust have been published from The Department of Environmental and Occupational Medicine, the University of Aarhus, Denmark. These five studies support that exposures to normal office dust for up to 5 h at concentration ranges occasionally seen in non-occupational indoor environments may cause health effects which can be measured both objectively and subjectively in healthy subjects and subjects with different types of hypersensitivity. In these five studies, the lowest observed effect level was indicated to be $75 \mu\text{g}/\text{m}^3$ (total suspended particulates). However, dust from different buildings may have different toxicity and some of the five studies had higher No Observed Effect Level (NOEL) than this value. The studies give strong evidence that dust exposures cause decreased tear film stability or break-up time and increased number of eosinophil cells in nasal lavages. There is also strong evidence that general well-being decrease during exposures. This is also reflected in increasing general irritation in eyes, nose, and throat. The combined findings support the theory that the subjects' responses to indoor dust exposures are caused by sensory perceptions, weak allergic, or weak inflammatory responses at or in the exposed tissues. More than one biological mechanism may be involved at the same time or subsequently. No signs of effects in non-exposed organs or tissues were seen, not even in sensitive persons.

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Key words: Office dust; Air pollution; Health effect; Human provocation experiment; Inflammation; Irritation; Allergic responses.

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Practical Implications

The studies indicate that dust exposures indoors may explain many reported symptoms and health effects and underlines the importance of source reduction.

Introduction

Indoor air pollution types and exposure levels

The air of most non-industrial indoor environments is often considered clean in comparison with occupational environments. In other words, it is supposed to consist of exposures to a mixture of compounds each at low concentrations, with no single compound appearing in very high concentrations compared to the rest, and no single compound in concentrations approaching occupational guidelines. This is also the case for airborne particulate matter, which is a mixture of solids, and adsorbed gases or vapors of organic, or inorganic origin.

However, field investigations in non-industrial indoor units (e.g. in day-care centers, offices, or schools) and in homes have demonstrated frequent air pollution with

airborne dust and particulate matter in the range of $20\text{--}200 \mu\text{g}/\text{m}^3$ with extreme values up to $1000 \mu\text{g}/\text{m}^3$ (e.g. Gyntelberg et al., 1994; Rudblad et al., 2002; Skov et al., 1987; Skov et al., 1989; Skov et al., 1990; Wallace, 1996). This paper deals with such airborne dust and particulate matter as indoor air pollution in non-industrial buildings.

Most often the dust is described as an inert-base dust consisting of relative inert organic or inorganic components with the addition of biological active compounds, such as allergens or toxic chemicals. The identified compounds include gases, vapors, semi-volatile compounds (as vapors, condensed particles, or adsorbed on other particles), organic or inorganic particles (including allergens), and viable microorganisms. An essential fraction of volatile or semi-volatile pollutants in the air may be present as adsorbed compounds on particulate matter (Mølhave, 2003).

Dust from different environments has different outdoor and indoor sources (combustion, materials, furniture, etc.). These sources are affected by occupation (e.g. level of occupation (bio-effluents), processes and human activities), microbial growth, etc. No standard building dust exists and dust from different built environments may have different toxic effects for occupant health and well-being.

Analysis by computational fluid dynamics (CFD) of spreading of indoor air pollutions have indicated that for more than 50% of the air we breathe the last contact with surfaces was with the floor close to the person. The reason 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 $< 10 \mu\text{m}$) (Murakami et al., 1992). 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 exposures to dust indoors.

IAQ and health indoors

In the last 30 years, an increasing number of health effects, symptoms, or complaints related to the indoor environment has been reported (Berglund et al., 1992). More than half of the U.S. workforce is employed indoors, and estimates of the proportion of indoor workers affected by these problems range up to 30%. Among the requests received annually by National Institute for Occupational Safety and Health (NIOSH) for occupational health investigations, the proportion related to indoor non-industrial environments has increased dramatically, from 2% in 1980 to 40% in recent years (Centers for Disease Control and Prevention, DHHS/CDC, 1998). An investigation among Danes on exposures to environmental factors included three interview campaigns and three self-administered questionnaire investigations, each with 4–8000 persons (Keiding et al., 2003). From the investigation of Keiding et al., it appears that 2.1% of the persons find that building conditions affect their health or well-being. These two findings illustrate that even in normal non-problem buildings, a background prevalence of a few percent of complaints is normal while problem buildings show more than 30% or often 100% prevalence of complaints about indoor air quality (IAQ).

Most, if not all, air pollutants are expected to some degree to cause some effects at least at the higher end of the exposure levels normally found indoors (Berglund et al., 1992; Bluyssen et al., 1997), and several field and laboratory investigations have indicated that airborne particulate matter and dust affect the comfort and health of occupants of normal non-industrial buildings (Gyntelberg et al., 1994; Hedge et al., 1989; Hodgson

et al., 1991; Skov et al. 1987; Skov et al., 1989; Skov et al., 1990; Wallace, 1996).

Generally, the effects or symptoms observed after exposures to low level of indoor air pollution with particulate matter, gases or vapors, etc. are non-specific, which means that the presence of a symptom does not identify a specific causality and exposure. The prevalence of a non-specific symptom may include unpredictable contributions to effects from several concurrent exposures (such as thermal exposures) or from biological mechanisms not related to pollutant exposures (e.g. mental stress or strains). In most cases, these non-specific symptoms or reports do not identify the biological mechanisms involved and they may be considered early manifestations of objective effects, which could be seen at higher exposure levels of prolonged exposure durations. We register them at lower exposures because our senses are more sensitive than our measuring instruments.

Looking away from rare but adverse effects such as CO poisoning or cancer from indoor air exposures, the prevalent effects seem to be caused by sensory perceptions (odors or irritation of the chemical sensory organ in the nasal cavity and eyes) or classic allergic reactions (e.g. mites). Others are more likely non-specific inflammatory reactions with no allergic mechanisms involved (some types of asthmatic responses).

Such inflammatory reactions related to exposures might involve the release of inflammatory markers (Beijer et al., 1998; Heldal et al., 2003; Thorn et al., 2001). These reactions appear in the exposed body organs or are mediated to other organs through neural signals or biomarkers (e.g. cytokines). Both allergy and inflammatory reactions might involve the release of several inflammatory markers (Sigsgaard et al., 2000).

Health effects following activation of these biological mechanisms are often grouped as objective effects (such as ocular effects, nasal effects, cardiovascular effects, respiratory effects, allergic effects, neurologic effects including performance changes) and subjective effects (perceptions and symptoms). The subjective effects may be classified as sensory perceptions of the environment (e.g. odors or stimulation of the chemical sense in the nose), perceptions of body conditions all over the body, and finally generalized combined evaluations of well-being, air quality, etc.

The potential toxic effects of dust may depend on the constituents of the dust particles, which has different potential to activate biological mechanisms in the body.

Symptoms and general well-being

The most frequent effects of air pollution are sensory effects, such as unacceptable odor, bad perceived air quality, and sensory irritation from stimulation of the chemical senses including nervous trigeminus in the eyes, nose, and facial skin. The trigeminal nerve

(the fifth cranial nerve) is responsible for sensation in the face. Added to these are weak neurotoxic symptoms. The symptoms include headache, dizziness, nausea, feeling of ill health, etc. Most of the symptoms are reported as symptoms or discomfort but subtle objective neurotoxic changes for example on performance and weak inflammatory effects may also be reflected by the symptoms.

The three most frequent indoor air quality complaints found by Keiding et al. (2003) were thermal discomfort (6–7%), noise (4%), and odors (3%). Odors were more frequent in dwellings in condominiums (6%) than in single-family houses (2%). In a more detailed questionnaire on odors, 16% reported odors from smoking, 11% from traffic, and 11% from neighbours. Perceived indoor air quality and general well-being are strongly correlated to both perceived irritation and odor (Mølhave et al., 1986; Mølhave, L., Pan, Z., Kjergaard, S.K., Sigsgaard, T., Bønløkke, J., Juto, J.-E., Andersson, K., Stridh, G., Löfstedt, H. and Bodin, L., unpublished data; Otto et al., 1990; Pan et al., 2000).

Epidemiological studies on workers exposed to organic dust have shown that respiratory symptoms occur in a wide range of professions where people are exposed to dust contaminated with microorganisms and their endotoxins (lipopolysaccharides, LPS) from decaying gram-negative bacteria (Hagmar et al., 1990; Hollander et al., 1994; Iversen and Pedersen, 1990; Iversen et al., 1989; Milton et al., 1995; Vogelzang et al., 1997). These studies have revealed a range of different risk factors for respiratory diseases in the organic dust from allergens to endotoxins stemming from the ubiquitous microorganisms thriving in these surroundings.

The Danish Town Hall study (Gyntelberg et al., 1994) showed an increased (50%) frequency of symptoms associated with sedimented dust but not with airborne dust concentrations. 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 (Gyntelberg et al., 1994; Skov et al., 1987, 1989, 1990; Wallace, 1996). In the study at Dalen, in Sweden (Bornehag, 1995) a reduction in the prevalence of complaints was achieved by reduction of dust and dirt in the buildings whereas increased ventilation had no major effect.

Objective effects

Several of the IAQ-related symptoms have been associated with objective health effects for example increased numbers of inflammatory cells in eyes, lung functions among asthmatics, and airway. In Denmark, the most frequent health effects were asthma, allergy, and colds. 0.4% of the interviewed (i.e. about 20% of those reporting health effects) reported air quality problems as cause of their health effects (Keiding et al.,

2003). Several of the symptoms have been related to lung functions among asthmatics.

In a school (Norbäck and Smedje, 1996), high levels of respirable dust in the air were related to increased frequency of infections, reduced air quality, and performance changes. A Danish study showed association between sedimented dust and inflammatory cells in the eyes. Effects on respiration are not frequent at the low exposure levels encountered in non-industrial indoor environments (Berglund et al., 1992).

In addition during the past 10 years, a number of cases of respiratory illnesses and dysfunctions among employees in the occupational environment have been reported. These respiratory effects have been associated with airborne exposures to organic dust-containing microbes or to the compounds, which such organisms may emit. Candidate compounds are endotoxins, β -(1,3)-D-glucan from mold, and allergens in the form of proteins from the microorganisms or their spores. Also in the indoor 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 has been associated with the LPS concentration in house dust (Michel et al., 1991). Previous investigations using cell cultures and dust from a garbage treatment plant show that such dust is cytotoxic and that the active components of the dust may be associated with fungal growth in the dust.

Aim of this review of five studies

Five studies of the effects of dust on humans have been performed and published at Aarhus University. All five studies addressed such questions as the health effects of indoor air pollution at low levels and identification of risk groups. All included dust exposures (dust alone or in combination with other exposures). The studies used different types of building dust and subjects with different sensitivity to air pollution.

The reason for this review is to highlight repeated findings in the five studies as these should have higher weight in the scientific debate than individual findings from one study. If such health effects are found, they are discussed in the light of the hypotheses that the responses are caused by sensory perceptions, weak allergic reactions, or weak inflammatory responses in the exposed tissues and that these responses are transmitted to other organ systems through mediators or neural signals.

Materials and methods

The five studies in general

Details about the five studies are found in Table 1.

A double-blinded Latin square, cross-over design was used in most studies. In most of them, clean air

was used as a placebo exposure and subjects were their own controls through baseline measures made in the morning in clean air outside the exposure chamber. All studies were accepted by the local committee of ethical use of human subjects and followed the Helsinki declarations.

The dust used for the exposures was sampled in the Aarhus region (Denmark) from 'normal' offices. The collection of dust was organized together with the regular cleaning assistants. The offices were without known indoor air quality problems and no water damage or mold growth had been reported. The collection of dust followed an internal standardized procedure (Mølhave, 1999). Vacuum cleaner-bags were stored at 18°C in thermally sealed plastic bags without plasticizers until further processing was done. The processing of the dust from vacuum cleaner bags followed a standard procedure (Mølhave, 1995). The processing included manual removal of larger items (e.g. insects or cigarette buds), agitation in a food-processor to dispose agglomerates, sieving in a 1-mm SS-sieve, and mixing of dust from different bags.

The dust for the five studies originated from different buildings of different age and function and can for that reason have varying composition. A typical composition of the dust was reported for study 1 by Mølhave et al. (2000b). About 11 kg of dust from vacuum cleaner bags from seven Danish office buildings with about 1047 occupants (12751 m²) were processed yielding 5.5 kg of processed bulk dust. The bulk dust contained 130,000–160 000 CFU/g microorganisms and 71,000–90,000 CFU/g micro fungi. The content of culturable micro fungi was 65–123 CFU/30 g dust. The content of endotoxins ranged from 5.06 to 7.24 EU/g (1.45–1.01 ng/g). Allergens (ng/g) were from 147 to 159 (mite), 395–746 (dog) and 103–330 (cat).

The macro molecular organic compounds (the MOD content) varied from 7.8 to 9.8 mg/g. The threshold of release of histamine from basophile leukocytes provoked by the bulk dust was between 0.3 and 1.0 mg/ml. The water content was 2% (WGT) and the organic fraction 33%. 6.5 to 5.9% (dry) was water soluble. The fiber content was less than 0.2–1.5% (WGT) and the desorbable VOCs were 176–319 µg/g.

Most of the VOCs were aldehydes. However, softeners for plastic [Dibutyl Phthalate (DBP) and Bis(2-ethylhexyl) Phthalate (DEHP)] were present. The chemical composition includes human and animal skin fragments, paper fibers, glass wool, wood and textile fibers, and inorganic and metal particles. The sizes ranged from 0.001–1 mm and the average specific density was 1.0 g/m³. The bulk dust was resuspended and the airborne dust was also sampled and analyzed to illustrate the exposures that can result from sedimented dirt and dust. The airborne dust resulting from the bulk dust reached concentrations ranging from 0.26 to 0.75 mg/m³ in average contained 300–170 CFU/m³. The organic fraction was from 55 to 70% and the water content about 2.5% (WGT). The content of the dust was compared with the similar results reported in the literature and its toxic potency is estimated to be relatively low. Particle size peaked between 1 and 5 µm with few particles below or above this range (Mølhave et al., 2000b).

The exposures were arranged in a stainless steel chamber (30 or 80 m³). The generation of dust was described in (Mølhave, 1995; Mølhave et al., 2000b). The exposure generator, which was placed outside the chamber, was a slightly modified version of a previously developed acoustic generator. (Mølhave and Strien, 1993; Weyle et al., 1984). During the exposures, a number of climate and air quality factors were

Table 1 Summary of the five studies

	Study 1	Study 2	Study 3	Study 4	Study 5
Dust type/origin	Office	Office	Office	Office (Study 1)	Spiked office dust
Exposure duration	5.25 h	3 h	245 min	3 h	210 min
Maximum exposure concentration	390	394	439	75	332–379
Design	3 × 3 Latin square	2 × 2 cross-over	2 × 2 cross-over	3 × 3 Latin square	4 × 4 Latin square cross-over
Placebo/clean air exposures	y	y	y	n	y
Number of subjects	24	10	23	8	36
Average age (years)	42.6	27.5	NA	27.4	30
Sensitivity	Healthy	Healthy	12 healthy, 11 fey fever	Atopic	12 atopic, 13 non-atopic, 11 nasal hypersensitivity
Acronym	STØV-91	Pan	SURE	LEPOZ	DAMOS
References	Mølhave et al., 2000a,b, 2002a,b	Pan et al., 2000	Hauschildt et al., 1999	Mølhave et al., 2005, 2003	Mølhave et al., 2003, 2004, 2006, Mølhave, L., Pan, Z., Kjærgaard, S.K., Sigsgaard, T., Bønløkke, J., Juto, J.-E., Andersson, K., Stridh, G., Löfstedt, H. and Bodin, L., unpublished data. Bønløkke et al., 2006 Bodin et al., 2005, 2007
Notes			Risk group investigation	Ozone-dust interaction	Glucan and aldehydes

NA, not available.

maintained at stable values through technical control. The measures include ventilation, air temperature and humidity, globe temperature, sound level, air movements, light levels. The subjects were selected among responders to public announcements in local newspapers, local TV, in university journals, and by posters at local institutions and schools. The subjects were healthy and each morning examined for health conditions such as infections that could interfere with the measurements. Affected subjects were rejected.

The five studies included several measurements of effects and these methods were developed from study to study. Table 2 summarizes the measurements and shows which studies included which measurements. For further details on methods and interpretations of results see the references in each study's publications. In addition, the studies dealt with a number of

variables, which may have acted as response modifying factors in the studies. Measurements and interpretation of these variables are not the topic of this review.

In the studies (Table 2), objective ocular effects were measured in samples of tears or conjunctival fluids (cell counts, trolox equivalent antioxidant capacity (TEAC), and eosinophil cationic protein (ECP)), tear film stability (or break-up time of tear film), epidemical or conjunctival epithelial damage, eye sensitivity to CO₂, foam formation in tear film or eye cantus, and dilatation of blood vessels (eye reddening). Objective nasal effects were measured as nasal lavage [cell count, IL-1 β cytokine, IL-8 cytokine, IL-1 β and IL-8 mRNA, eosinophil cationic protein (ECP), myeloperoxidase (MPO)], rhinosteriometry (swelling, velocity, perfusion, concentration), and acoustic rhinometry (volume and minimum area). Objective respiratory effects were

Table 2 Effect measurements used in the five studies

Organ	Effects measures	Variables	Type	Study number					St.	Incl.
				1	2	3	4	5		
Objective ocular effects	Tears/conjunctival fluids	Cell counts								
		Eosinophils	1	–	0	–	0	0	–	–
		Neutrophils, lymphocytes, epithelial cells	2	+	0	?	0	0	–	+
		Trolox equivalent antioxidant capacity, TEAC	2	0	0	0	0	–	–	–
		Eosinophil cationic protein, ECP	1	0	0	0	0	–	–	–
	Tear film stability/break-up time	BUT	4	+	+	+	0	–	+	–
		Lissamin or fluorescein staining, ED	2	+	–	+	0	–	–	–
			3	–	–	+	0	0	–	–
			4	?	–	–	0	0	–	–
			2	–	0	?	0	0	–	–
Objective nasal effects	Acoustic rhinometry	Volume and minimum area, AR or NADAR	4	?	?	–	–	+	–	+
		Rhinosteriometry	2	0	0	0	0	+	–	–
		Nasal lavage								
		Cell counts								
		Eosinophils	1	0	0	0	+	+	+	–
	Rhinosteriometry	Neutrophils, lymphocytes, epithelial cells	2	0	0	0	?	–	–	–
		IL-1 β cytokine	2	0	0	0	–	?	–	–
		IL-8 cytokine	2	0	0	0	?	+	–	+
		IL-1 β mRNA	2	0	0	0	0	–	–	–
		IL-8 mRNA	2	0	0	0	0	–	–	–
Objective respiratory effects	Acoustic rhinometry	Eosinophil cationic protein, ECP	1	+	0	0	0	?	–	+
		Myeloperoxidase, MPO	2	–	0	0	0	0	–	–
		Bronchial hyper-responsiveness, BHR	4	–	0	–	–	0	–	–
		PEF	4	+	0	–	+	–	–	–
		FEV1	4	–	0	?	–	–	–	–
	Rhinosteriometry	FVC	4	–	0	0	–	–	–	–
		FEV1/FVC	4	0	0	0	0	?	–	–
		DICO and KCO	2	0	0	0	0	?	–	–
		IL-8 in alveolar liquids	2	0	0	0	0	–	–	–
		Errors and response time	4	–	0	–	0	0	–	–
Objective CNS, performance and productivity	Acoustic rhinometry									
		MOOD scales	4	+	0	?	0	0	–	+
		Temperature and humidity of skin	4	–	0	0	0	0	–	–
		Whole blood assays	2	0	0	0	0	–	–	–
		Computer-assisted questionnaire:	4	+	+	–	+	+	+	+
	Rhinosteriometry									
		26, 29 or 36 questions or six indices	4	+	+	–	+	+	+	+
Subjective symptoms and general well-being	Acoustic rhinometry									
		Time course of general feeling of irritation of eyes, nose, or throat	4	+	0	+	+	–	+	+
	Rhinosteriometry									

Outcome: +, Included, effect of exposures; –, included, no effect of exposures; ?, included, inconclusive effect of exposures; 0, not included.

Type: 1, allergic; 2, inflammatory; 3, perceptions/sensory; 4, combination of mechanisms is possible. St., strong evidence; Incl., inconclusive evidence.

measured as bronchial sensitivity to histamine/metacholine, peak flow, forced expiratory flow FEV-1 s, forced vital capacity (FVC), Teffeneaux (FEV1/FVC), CO diffusion coefficient (DICO and KCO) and IL-8 in induced sputum (alveolar liquids). Objective CNS changes, and changes of performance, and productivity were measured using additional test for distraction and MOOD scales. Other objective systemic effects were whole blood assays cytokine releases (IL-1 β , IL-8), and temperature and humidity of skin. Subjective symptoms and general well-being were measured in a computer-assisted questionnaire (26, 29, or 36 questions), six indices based on 29 questions, and as time course of general feeling of irritation in eyes, nose, and throat.

Some of the effects measured with these methods are allergic responses, some inflammatory, and some are sensory responses. Some of them may be caused by a combination of these biological mechanisms. Table 2 shows which of these measures used in the studies are predominately measuring allergic reactions (1), or inflammatory (2) responses, sensory responses (3), or responses which may be caused by combinations of these mechanisms (4).

Summary of individual studies

This chapter summarizes the five studies (see Tables 1 and 2).

Study 1. This study (Mølhave et al., 2000a,b, 2002a,b) was a first ‘fishing expedition’ aimed at testing if health effects were seen following human exposures to airborne office dust. If effects were seen, the results should ease planning of subsequent experiments to confirm the findings. Exposures consisted of clean air and office dust at 136 and 390 $\mu\text{g}/\text{m}^3$ (median level TSP) for 5.25 h in a climate chamber. The study was a 3 $\times$ 3 Latin square design. Twenty-four healthy, non-sensitive subjects were exposed. A lowest observed effect level (LOEL) of 140 $\mu\text{g}/\text{m}^3$ was indicated. The effects showed different time course and accumulation of effects during prolonged exposures as well as persisting effects 17 h after exposure stop were seen.

Study 2. This study (Pan et al., 2000) was arranged to test some of the findings of the first study that dust might cause health effects. Exposures were clean air and office dust for 3 h at 394 $\mu\text{g}/\text{m}^3$ (TSP). The design was a 2 $\times$ 2 Latin square, cross-over design. Ten non-smoking, healthy subjects were exposed. Other observations were a strong correlation between symptoms, and different time course of the effects were seen.

Study 3. The purpose of this study (Hauschildt et al., 1999) was to confirm some of the findings from the first study and to test if subjects with allergic rhinitis responded stronger than normal healthy subjects. The

exposures were to clean air and office dust at 439 $\mu\text{g}/\text{m}^3$ (TSP) for 245 min. The design was a 2 $\times$ 2 cross-over design. The subject group consisted of 23 subjects, 11 hay fever patients (suffering from allergic rhinitis) and 12 healthy subjects.

Study 4. This study (Mølhave et al., 2005) investigated the hypotheses that humans are affected by air pollution by ozone and house dust, that the effect of simultaneous exposure to ozone and dust in the air is larger than the effect of these two pollutants individually, and that the effects can be measured as release of cytokines and changes of the respiratory function. The controlled exposures were about 75 $\mu\text{g}/\text{m}^3$ total suspended particulate matter (TSP or PM), 0.3 ppm ozone, and the combination of these. The exposure duration was 3 h. The design was a 2 $\times$ 2 cross-over design and eight atopic subjects were exposed.

Study 5. This study investigated if addition of specific components (glucan and aldehydes) to normal office dust increased the toxic effects of the dust (Bodin et al., 2005, 2007; Bønløkke et al., 2006; Mølhave et al., 2003, 2004, 2006; Mølhave, L., Pan, Z., Kjærgaard, S.K., Sigsgaard, T., Bønløkke, J., Juto, J.-E., Andersson, K., Stridh, G., Löfstedt, H. and Bodin, L., unpublished data). 36 subjects were exposed to clean air, office dust, dust with glucan, and dust with aldehydes. The concentrations were 332–379 μg dust/ m^3 , with and without 5 μg glucan/ m^3 , and 300 μg aldehydes/ m^3 . Three groups of subjects were exposed. Twelve persons were atopic, 13 were non-atopic, and 11 were non-atopic with nasal histamine hypersensitivity.

Statistical methods

The purpose of this review of the five studies is to identify health effects or symptoms, which were repeatedly reported to be significantly related to dust exposure in the five studies. No new statistical analyses were made but the effects, measures are merely rated according to the number of studies in which they were significantly related to exposures compared with the number of the five studies in which they were used. In the summary, the term strong evidence of observation will be used for significant observations, which are seen in two or more of the studies and which outnumbered the negative or inconclusive observations plus one. In addition, these observations must be consistent (i.e. all increasing or decreasing effects with increasing exposures) and make biological sense regarding the mechanisms supposed to be involved. Suggestive evidence appears when the number of significant findings plus the number of inconclusive findings outnumber the negative findings plus one. All other observations are considered inconclusive.

Discussions

Conclusiveness of the studies and observations

Epidemiological field studies and experimental laboratory studies do not always agree in type, or intensity of effects seen, or in the exposure thresholds found for effects. The reasons may include:

- Definition or measuring methods for the effects differ even if the effects are called the same.
- The accumulated dose for certain types of effects may be as important as the air concentration. In addition, exposures may have to be repeated for many years to reveal time effects and accumulation of dose.
- Subjects and their sensitivity may differ between studies and thus affect the external validity of the studies.
- The response modifying variables (such as air temperature and humidity) may differ between studies.
- The dust used for the exposures may be composed by different organic, inorganic, or viable particles.
- Confounding and the present in epidemiological studies of co-pollutants (e.g. ozone or NO₂).

These factors also affect the interpretation presented here of the results from experimental exposures of humans to low levels of re-suspended house dust at or just above the threshold for effects.

In addition, several other factors related to designing and analyzing the studies affect their conclusiveness.

- Low-level exposures cause weak responses, which may be below detection limits for most objective measuring methods.
- Uncontrolled response modifying factors.
- Limited number of subjects.
- Inter-subject differences.
- Dominance of subjective effects and associated biases.
- Lack of variation data for optimization of the design in power analyses.
- Lack of an *a priori* biological model to test makes it difficult to draw definitive causal conclusions.

Generally, odor perceptions are considered to be the most sensitive responses to normal indoor exposures. It follows that odors normally will be obvious to the subjects in an experiment when they are exposed to odor-containing dust. The issue of odor detection makes it difficult to conduct truly unbiased studies of this type. For example, in study 1 ratings of general well-being were correlated to irritation, odor perceptions, air quality, and the need for more ventilation. Therefore, not only odors' evaluations but also all related symptoms may be biased by odor detection. However, definitive investigations of this matter are lacking.

The validity of the study 1 is considered fine as the design was adequate, data recovery high, and few

technical problems, etc. appeared, e.g. in relation to noise and temperature during exposures. However, the analyses are only indicative as no prior hypotheses were established. The study 2 is considered valid with respect to the conclusions made, although the small number of subjects reduces the sensitivity and increases the risk of chance findings. The study 3 included a small number of subjects and the authors judge the study to be indicative only. The findings in study 4 are based on a limited number of subjects and should therefore be considered indicative only both for the hypothesis that ozone and dust exposures interact, and for effects of dust exposures as such. No clean air exposures were included. However, baseline measures before exposures were made. In this review, results referring to dust or combined exposures are reported as indicative for effects of dust exposures. In study 5 both dust exposures and exposures to dust spiked with substances known to be biological active were used. Any observed effect may overestimate real life effects. However, the compounds are known to be present in many buildings and the added amounts were within the ranges occurring indoors. The study design and number of subjects are considered adequate for the conclusions made.

There are many confounding factors that could explain some of the findings in each of these studies. No single study can cover all the possible causes of these human responses, even if an effort was made to control most of these confounders. This should be kept in mind when using the results presented here.

Effects of experimental dust exposures

The purpose of this review of the five Danish studies was to identify health effects or symptoms which were repeatedly reported to be significantly related to dust exposure in the five different studies. The criteria for the classification of findings are given above.

Combining the five studies gives strong evidence that dust exposures cause objective effects in the form of decreased tear film stability or break-up time, and increased number of eosinophil cells in nasal lavages. There is also strong evidence that general well-being decrease during exposures. This is reflected in increasing general irritation in eyes, nose, and throat (see Table 1).

There is suggestive evidence that dust exposures increase the number of squamous plate epithelial and decrease the number of lymphocyte cells in tear fluids, increase the nasal volume in acoustic rhinometry, increase the concentration of IL-8 cytokine, and decrease eosinophil cationic protein (ECP) in nasal lavage. MOOD scales may also be affected. Several environmental perceptions (draught/air movements, odor intensity, air quality, nose and throat irritation, runny and blocked nose, sleepiness were also suggested to be affected (see Table 2).

Inconclusive evidences appeared for the remaining effects, measures as mentioned in Table 2.

The studies thus bear strong evidence of health effects of dust exposures and indicate a threshold (LOEL) of $75 \mu\text{g}/\text{m}^3$. Accumulation of effects during prolonged exposures as well as persistent effects 17 h after exposure stop were seen (Bodin et al., 2005, 2007; Mølhave et al., 2000a,b, 2002a,b; Pan et al., 2000).

It should be noted that the sampling procedure only collected ground-lying dust. Many smaller particles remain airborne and may cause 'irritation' in real life situations.

Which biological mechanisms are involved in the responses to dust exposures?

The biological model, which the researchers of the five studies in retrospective seem to have been using to interpret the observed human responses are not specifically stated in the scientific publications. The hypothesis is simply stated to be that the subjects' responses to dust exposures are caused by sensory, weak allergic or weak inflammatory responses at or in the exposed tissues, and that these responses through released mediators or neural signals are transmitted to other organ systems where subsequent effects may be found.

The spectrum of health effects and symptoms shown to be strongly associated to dust exposures (Table 2) include one irritative effect and no allergic responses. Two may be caused both by a combination of mechanisms. If the effects with indication of responses to dust exposures are added, the list includes two irritative effects and two allergic responses. Four may be caused by a combination of mechanisms. The different time courses of the effects indicate that more than one mechanism may be involved at the same time or subsequently. Such different time courses are known, e.g. from irritative effects of glucan exposures.

It is surprising that no strong evidence was found that odors are caused by the exposures. This indicates

that odor biases are less important for the dust experiments. No signs of effects in non-exposed organs were seen even in sensitive persons. This should be seen in the light of several studies using atopic subjects. The relevance of such responses can, however, not be excluded based on the five studies because the effects' measurements may not be sensitive enough to measure the effects. Cytokines may vary minute-to-minute, or even second-to-second, e.g. depending on the individual's immune status. Consistent findings of such changes in single time point measures in independent laboratory and field studies, therefore, indicate systematic cytokine levels in the body.

Conclusions

Combining the five studies support that exposures to normal office dust for up to 5 h at concentration ranges occasionally seen in non-occupational indoor environments may cause health effects, which can be measured both objectively and subjectively in healthy subjects or in subjects with different types of hypersensitivities. In these five studies, the lowest observed effect level was indicated to be $75 \mu\text{g}/\text{m}^3$. However, dust from different buildings may have different toxicity and some of the five studies had higher No Observed Effect Level (NOEL) than this value.

Combined, the five studies give strong evidence that dust exposures cause decreased tear film stability or break-up time, and increased number of eosinophil cells in nasal lavages. There is also strong evidence that general well-being decrease during exposures. This is reflected in increasing general irritation in eyes, nose, and throat. The combined findings support the theory that the subjects' responses to indoor dust exposures are caused by sensory perceptions, weak allergic, or weak inflammatory responses at or in the exposed tissues. More than one biological mechanism may be involved in parallel or subsequently. No signs of effects in non-exposed organs were seen even in sensitive persons.

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