WHO GUIDELINES FOR INDOOR AIR QUALITY

WORLD HEALTH ORGANIZATION
EUROPE

DAMPNESS AND MOULD

4. Health effects associated with dampness and mould

Mark J. Mendell, Anna G. Mirer, Kerry Cheung, Jeroen Douwes, Torben Sigsgaard, Jakob Bønløkke, Harald W. Meyer, Maija-Riitta Hirvonen and Marjut Roponen

This chapter contains separate reviews and a synthesis of the epidemiological, clinical and toxicological evidence on the health effects of dampness and mould.

4.1 Review of epidemiological evidence

This section summarizes the epidemiological literature on the health effects of dampness and mould and other dampness-related agents, combining the conclusions of large previous reviews with newly reviewed findings for selected health outcomes.

4.1.1 Background

Epidemiological studies provide evidence to link dampness and dampness-related exposures to human health effects; however, in interpreting these studies, consideration must be given to their inherent strengths and weaknesses. Errors such as studying only samples instead of entire populations and bias related to sampling, measurement and confounding can distort risk estimates. In addition, the evidence from a body of studies may be distorted by publication bias, which reflects a tendency not to publish or to publish more slowly negative or equivocal findings, which may therefore not be included in the review.

In interpreting epidemiological studies on dampness, the uncertainty about the causal exposures and health effects involved must also be taken into account. Microbiological organisms are considered among the most plausible explanations for the health risks associated with indoor dampness. Conventional quantitative measurements of such exposure, however, based on counts of total culturable airborne microorganisms, have shown less consistent associations with the health effects of interest than have more qualitative assessments, such as visible dampness or water damage, visible mould or mould odour. While the association between qualitative assessments of dampness and health effects does suggest preventive strategies, the demonstration of specific, quantifiable causes would allow more effective prevention.

This review combines the conclusions of a review by the Institute of Medicine (2004), covering the literature up to mid-2003, those of a quantitative meta-analysis of findings up to 2007 on dampness, mould and respiratory health effects (Fisk, Lei-Gomez, Mendell, 2007) and a new assessment of more recent

published studies on selected outcomes. The review focuses on selected categories of the outcomes included in the Institute of Medicine review (upper respiratory tract symptoms, cough, wheeze, dyspnoea, asthma symptoms in people with asthma, asthma development) and several additional categories (current asthma, respiratory infections, bronchitis, wheeze, allergic rhinitis and allergy or atopy). It excludes outcomes on which limited research has been reported (e.g. effects related to skin, eyes, fatigue, nausea, headache, insomnia, mucous membrane irritation and sick-building syndrome).

4.1.2 Methods

The online database PubMed was searched up to July 2007 for published articles, by using three groups of keywords, such as: (1) dampness, damp, water damage, moisture, humidity, fungi, fungus, mould, bacteria or microorganisms; along with (2) health, asthma, allergy, eczema, wheeze, cough, respiratory, respiratory infection, lung, skin, nasal, nose, hypersensitivity pneumonitis, alveolitis, bronchial, hypersensitivity or inflammation; and with (3) building, house, home, residence, dwelling, office, school or day-care centre. Some combinations of terms were searched separately. References in review articles or in personal databases were also included. Other references were added, as available.

For inclusion in this review, a study had to meet the following criteria:

- publication in a peer-reviewed journal (printed or online);
- reporting of original data from a study that was either an experimental intervention, a prospective cohort, a retrospective cohort or case-control, a cross-sectional or a cross-sectional case-control study;
- no minimum study size, except that if exposure was characterized only at building level, at least 10 buildings had to be included;
- it included risk factors related to dampness, fungi or microbiological components or products, other than allergens – such as those related to dust mites, cockroaches and mice;
- it included upper respiratory tract symptoms, cough, wheeze, dyspnoea, impaired lung function, allergy or atopy, asthma symptoms in people with asthma, asthma development, any diagnosis of asthma, current asthma, hypersensitivity pneumonitis, respiratory infections or bronchitis; and
- it provided adequate control, through the study design or analytical strategies, of selection bias and confounding by key variables: sex, active smoking (in studies of adults), passive smoking (in studies of children) and socioeconomic status (except in the Nordic countries).

Exposures and conditions that were not included were dust mites, humidity and measurements of moisture in mattresses. Endotoxins are components of Gram-negative bacteria that may favour moist surface conditions, but they are also

associated with agricultural settings and with pets. A full consideration of the health effects of endotoxins was not included, as they are beyond the scope of this review, although findings of indoor endotoxin concentrations in studies of damp indoor conditions were included.

From each study, a limited set of data was abstracted, including the age of the participants, study design and point estimates and confidence intervals for specific combinations of risk factors or exposures. Studies were categorized as either interventions (controlled quasi-experimental field intervention studies), prospective (prospective cohort studies), retrospective (retrospective cohort or case–control studies) or cross-sectional (cross-sectional or cross-sectional case–control studies). The findings were organized into separate tables for each health outcome, and, within each table, the findings were sorted by the age of the participants and the study design.

The findings considered in evaluating the evidence were those of associations between health effects and qualitative assessments of dampness-related factors, such as visible dampness, mould, water damage or mould odour. Analyses based on quantified microbial exposures were considered secondary, as no specific microbial metric has been shown to be a consistent risk factor for health, and the evidence that a particular outcome is associated with a specific microbial measure is poor. Microbial metrics include culture-based assays of specific or total counts of fungi and bacteria, glucans, endotoxins, ergosterol and extracellular polysaccharides.

In this review, the quality of the evidence for a relation is classified in the same way as in the review of the Institute of Medicine (2004), as shown in Box 4. For each association considered, we classified the evidence on the basis of our professional judgement about the persuasiveness of the reported findings and consideration of the strength, quality, diversity and number of studies. The strongest epidemiological evidence was considered to be that from individually randomized controlled trials in which risk factors were added or removed, followed by prospective cohort and case–control studies, retrospective cohort and case–control studies and cross–sectional studies. A set of strongly designed studies of different designs and in different populations, with findings that were generally consistent in direction and magnitude, were considered to provide the most persuasive overall evidence, especially if they were bolstered by evidence from controlled studies of exposed human beings and experimental animals that demonstrated appropriate biological mechanisms for the epidemiological findings.

4.1.3 Results

4.1.3.1. Previous reviews

The most comprehensive, relevant reviews on dampness and health are those of Bornehag et al. (2001, 2004), the Institute of Medicine (2004) and Fisk, Lei-Gomez and Mendell (2007). Other reviews, opinion pieces and position

statement on this topic were also available – for example, those of Peat, Dickerson and Li (1998), Kolstad et al. (2002), Hardin, Kelman, Saxon (2003), Douwes (2005), Hope and Simon (2007) and Mudarri and Fisk (2007).

A Nordic multidisciplinary committee (Bornehag et al., 2001) reported the findings of a review of the literature on dampness in buildings (including exposure to mites) and health, with conclusions based on 61 publications up to July 1998 that met the inclusion criteria. The review concluded that: "…dampness in buildings appears to increase the risk for health effects in the airways, such as

BOX 4 Classifying the strength of evidence

The categories in this box refer to the association between exposure to an agent and a health outcome and not to the likelihood that any individual's health problem is associated with or caused by the exposure. These categories are used for classifying the evidence in this review and that of the Institute of Medicine (2004:26–27).

Sufficient evidence of a causal relationhip
The evidence is sufficient to conclude that a causal relationship exists between the agent and the outcome; that is, the evidence fulfils the criteria for "sufficient evidence of an association" and, in addition, satisfies the following evaluation criteria: strength of association, biological gradient, consistency of association, biological plausibility and coherence and temporally correct association.
The finding of sufficient evidence of a causal relationship between an exposure and a health outcome does not mean that the exposure inevitably leads to that outcome. Rather, it means that the exposure can cause the outcome, at least in some people under some circumstances.

Sufficient evidence of an association
The evidence is sufficient to conclude that there is an association. That is, an association between the agent and the outcome has been observed in studies in which chance, bias and confounding could be ruled out with reasonable confidence. For example, if several small studies that are free from bias and confounding show an association that is consistent in magnitude and direction, there may be sufficient evidence of an association.

Limited or suggestive evidence of an association
The evidence is suggestive of an association between the agent and the outcome but is limited because chance, bias and confounding could not be ruled out with confidence. For example, at least one high-quality study shows a positive association, but the results of other studies are inconsistent.

Inadequate or insufficient evidence to determine whether an association exists
The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of an association. Alternatively, no studies of the association exist.

Limited or suggestive evidence of no association
Several adequate studies are consistent in not showing an association between the agent and the outcome. A conclusion of no association is inevitably limited to the conditions, magnitude of exposure and length of observation covered by the studies available.

cough, wheeze and asthma… [and] evidence for a causal association between 'dampness' and health effects is strong. However, the mechanisms are unknown. Several definitions of dampness have been used in the studies, but all seem to be associated with health problems. Sensitization to mites may be one but obviously not the only mechanism. Even if the mechanisms are unknown, there is sufficient evidence to take preventive measures against dampness in buildings."

Three years later, the review was updated by a European multidisciplinary committee with 40 additional publications from 1998 through 2003 (Bornehag et al., 2004). This review concluded, "Dampness in buildings is a risk factor for health effects among atopics and nonatopics both in domestic and public environments. However, the literature is not conclusive in respect of causative agents…" Thus, the second review, somewhat more cautiously, did not claim that causality had been demonstrated in the literature reviewed.

The review of the Institute of Medicine (2004) was a detailed consideration of evidence available up to mid-2003 on the health effects of indoor dampness and dampness-related agents. The studies included in the review are summarized in Table A1.1 of Annex 1. This review, by an international multidisciplinary group, resulted in a book-length publication with an 87-page summary of the epidemiological evidence alone. The group drew conclusions about two categories of risk

Table 5. Findings of the review of the Institute of Medicine (2004)

Level of confidence for an association	Risk factor	
	Exposure to damp indoor environments	Presence of mould or other agents in damp indoor environments
Sufficient evidence of a causal association	No outcomes met this definition.	No outcomes met this definition.
Sufficient evidence of an association	Upper respiratory tract symptoms Wheeze Cough Asthma symptoms in sensitized people	Upper respiratory tract symptoms Wheeze Cough Asthma symptoms in sensitized people Hypersensitivity pneumonitis
Limited or suggestive evidence of an association	Lower respiratory illness in otherwise healthy children Dyspnoea Asthma development	Lower respiratory illness in otherwise healthy children
Inadequate or insufficient evidence to determine whether an association exists	All other health effects considered	All other health effects considered

factors: exposure to damp indoor environments and the presence of mould or other agents in damp indoor environments. Table 5 summarizes the conclusions of the review with regard to the epidemiological evidence on dampness-related health effects. Sufficient evidence was considered not to be available for any of the associations. Sufficient evidence of associations with exposure to damp indoor environments and with the presence of mould or other agents in damp indoor environments was considered to exist for four outcomes: upper respiratory (nose and throat) tract symptoms, cough, wheeze and asthma symptoms in sensitized people (i.e. asthma exacerbation). Sufficient evidence was also considered to exist for an association between dampness-related agents and hypersensitivity pneumonitis in susceptible persons. Table 6 shows the numbers of published studies included in the review, by study design for the health effect categories. It also shows the numbers of studies identified in the current review that were not included in the Institute of Medicine review. The results of the newly identified studies are discussed below.

Fisk, Lei-Gomez and Mendell (2007) reported the results of a quantitative meta-analysis on residential dampness-related risks for adverse respiratory health effects, which was based on studies available through early 2006 (which included most of the studies on specific outcomes used in the current review) and which met specified inclusion criteria. They estimated summary odds ratios and 95% confidence intervals for associations between dampness-related risk factors and six types of health outcome, including some of those in the Institute of Medicine review (upper respiratory tract symptoms, cough, wheeze and asthma development) and some other categories (current asthma and ever-diagnosed asthma). All types of visible dampness, mould or mould odour, reported by either occupants or researchers, were considered to represent a single set of dampness-related risks. Studies of measured concentrations of microbiological agents or of measured or reported air humidity were excluded. The results of the meta-analysis are summarized in Table 7. The central estimates of the odds ratio ranged from 1.34 to 1.75, with a lower confidence limit exceeding 1.0 in 9 of 10 estimates (i.e. for all except asthma development). The authors also estimated the percentage increase in prevalence of each outcome in houses with dampness or mould. Fisk, Lei-Gomez and Mendell (2007) concluded that "building dampness and mould are associated with approximately 30–50% increases in a variety of respiratory and asthma-related health outcomes." In a companion paper, Mudarri and Fisk (2007) estimated that, if the reported associations were causal, 21% of the cases of asthma in the United States could be attributable to dampness and mould in housing, for a total annual national cost of US$ 3.5 billion.

4.1.3.2. Additional published studies

The search conducted for the present review resulted in an initial 153 articles published between 1986 and 2007, excluding the 45 articles in the Institute of

Table 6. Numbers of published studies on health effects of damp indoor environments

Health outcome category	Included in review of the Institute of Medicine (2004)		Additional studies	
	Study design	No. of studies	Study design	No. of studies
Asthma development	Cohort	2	Prospective	3
	Case–control	6	Retrospective	2
	Cross-sectional	0	Cross-sectional	0
Current asthma	–	–	Prospective	1
			Retrospective	0
			Cross-sectional	17
Respiratory infections (in otherwise healthy people)	–	–	Prospective	3
			Retrospective	0
			Cross-sectional	5
Upper respiratory tract symptoms	Cohort	0	Prospective	1
	Case–control	0	Retrospective	0
	Cross-sectional	14	Cross-sectional	11
Allergic rhinitis	–		Prospective	3
			Retrospective	0
		–	Cross-sectional	3
Cough	Cohort	0	Prospective	1
	Case–control	1	Retrospective	0
	Cross-sectional	20	Cross-sectional	19
Wheeze	Cohort	0	Prospective	5
	Case-control	1	Retrospective	1
	Cross-sectional	19	Cross-sectional	24
Dyspnoea	Cohort	0	Prospective	0
	Case–control	0	Retrospective	0
	Cross-sectional	4	Cross-sectional	8
Altered lung function	–	–	Intervention	1
			Prospective	2
			Retrospective	0
			Cross-sectional	7
Bronchitis	–	–	Prospective	0
			Retrospective	0
			Cross-sectional	6
Other respiratory effects	–	–	Prospective	2
			Retrospective	0
			Cross-sectional	7
Allergy or atopy	–	–	Prospective	3
			Retrospective	0
			Cross-sectional	10
Asthma, ever	–	–	Prospective	2
			Retrospective	0
			Cross-sectional	6
Asthma symptoms in people with asthma	Intervention	0	Intervention	2
	Cohort	0	Prospective	1
	Case–control	5	Retrospective	0
	Cross-sectional	18	Cross-sectional	3
Total[1]		45		68

[1] Totals are less than the sum of the numbers above, as findings for several health effects may be reported.

Table 7. Key results of the meta-analyses of Fisk, Lei-Gomez and Mendell (2007)

Outcome	Participants	No. of studies	Odds ratio (95% CI)	Estimated % increase in outcome in houses with visible dampness, mould or mould odour
Upper respiratory tract symptoms	All	13	1.70 (1.44–2.00)	52
Cough	All	18	1.67 (1.49–1.86)	50
	Adults	6	1.52 (1.18–1.96)	–
	Children	12	1.75 (1.56–1.96)	–
Wheeze	All	22	1.50 (1.38–1.64)	44
	Adults	5	1.39 (1.04–1.85)	–
	Children	17	1.53 (1.39–1.68)	–
Current asthma	All	10	1.56 (1.30–1.86)	50
Ever-diagnosed asthma	All	8	1.37 (1.23–1.53)	33
Asthma development	All	4	1.34 (0.86–2.10)	30

Note. CI, confidence interval

Medicine review but potentially including articles in other reviews. Table 6 lists the 68 studies out of the 153 that met the inclusion criteria for this review (see section 4.1.2). Table A1.2 in Annex 1 summarizes the results of these studies by category of health outcome.

After an expert assessment of all the available evidence, including the studies analysed previously by the Institute of Medicine (Table A1.1) and the new studies found for this review (Table A1.2), we summarized the level of confidence for associations between dampness or dampness-related agents and specific health outcomes (Table 8).

The overall evidence shows that house dampness is consistently associated with a wide range of respiratory health effects, most notably asthma, wheeze, cough, respiratory infections and upper respiratory tract symptoms. These associations have been observed in many studies conducted in many geographical regions (Brunekreef et al., 1989; Jaakkola, Jaakkola, Ruotsalainen, 1993; Andriessen, Brunekreef, Roemer, 1998; Nafstad et al., 1998; Peat, Dickerson, Li, 1998; Norbäck et al., 1999; Øie et al., 1999; Bornehag et al., 2001; Kilpeläinen et al., 2001; Zheng et al., 2002; Zock et al., 2002; Jaakkola, Hwang, Jaakkola, 2005). Positive associations have been found in infants (Nafstad et al., 1998; Øie et al., 1999), children (Brunekreef et al., 1989; Andriessen, Brunekreef, Roemer, 1998; Zheng et al., 2002) and adults (Norbäck et al., 1999; Kilpeläinen et al., 2001; Zock et al., 2002), and some evidence was found for dose–response relations (Engvall, Norrby, Norbäck, 2001; Pekkanen et al., 2007). The evidence for associations with measured exposures to fungi or other microorganisms or microbial agents is less consistent, probably due to uncertainties in exposure assessment (see Chapter 2). For one health outcome, asthma exacerbation, we consider the evidence to be sufficient to document an association and almost sufficient to document causal-

Table 8. Level of confidence for associations between indoor dampness-related agents and health outcomes considered in this review

Updated conclusion	Outcome (in italics if conclusion changed)	Conclusion of the Institute of Medicine (2004)	Additional evidence from new data
Sufficient evidence of a causal relationship	None	None	
Sufficient evidence of an association	Asthma exacerbation	Sufficient evidence	More studies; close to sufficient evidence of causation
	Upper respiratory tract symptoms	Sufficient evidence	Many new studies, but not of improved quality
	Cough	Sufficient evidence	Many new studies, but not of improved quality
	Wheeze	Sufficient evidence	Many new studies, some of improved quality
	Asthma development	*Limited or suggestive evidence of association*	More studies of improved quality
	Dyspnoea	*Limited or suggestive evidence of association*	More studies
	Current asthma	*Not evaluated*	First evaluation
	Respiratory infections	*Not evaluated*	First evaluation
Limited or suggestive evidence of association	*Bronchitis*	*Not evaluated*	First evaluation
	Allergic rhinitis	*Not evaluated separately*	First evaluation
Inadequate or insufficient evidence to determine whether an association exists	Altered lung function	Not evaluated	First evaluation
	Allergy or atopy	Not evaluated	First evaluation
	Asthma, ever	Not evaluated	First evaluation

ity of dampness-related factors. A number of newly available studies added to the evidence of an association between dampness and asthma exacerbation. In all the available studies (Tables A1.1 and A1.2), dampness-related factors were consistently associated with asthma exacerbation, with odds ratios (ORs) consistently exceeding 1.0. This was true for both adults (in all case-control studies the ORs were 1.7–3.3, and in all cross-sectional studies the ORs were 1.02–4.2) and children (in all intervention studies; in all prospective studies the ORs were 3.8–7.6; in all case-control studies the ORs were 1.5–4.9; and in 96% of findings in cross-sectional studies the ORs were 1.0–7.6). Kercsmar et al. (2006) conducted a well designed, controlled intervention study on asthma exacerbation in the houses of highly symptomatic children with asthma and found that comprehensive

removal of sources of dampness and mould and cleaning of visible mould dramatically reduced asthma exacerbation. This study (although of necessity unblinded) strongly suggests a causal association between indoor dampness or mould and respiratory morbidity in children with asthma.

We found that there was sufficient evidence for associations between indoor dampness and four health outcomes that were not so classified or were not evaluated by the Institute of Medicine (2004): asthma development, dyspnoea, current asthma and respiratory infections. Asthma development is a health outcome of particular public health importance. Five case–control studies included in the Institute of Medicine review (of the eight on asthma development in Table A1.1) explicitly addressed associations between dampness or mould and asthma development (Nafstad et al., 1998; Øie et al., 1999; Yang et al., 1998; Thorn, Brisman, Toren, 2001; Jaakkola et al., 2002). Four new studies were identified for the current review (Jaakkola, Hwang, Jaakkola, 2005; Matheson et al., 2005; Gunnbjornsdottir et al., 2006; Pekkanen et al., 2007), all of which were prospective or retrospective and reported effect measures exceeding 1.0 for most evaluations of dampness-related factors (see Table A1.2). Of the retrospective case–control studies of adults, 60% had ORs exceeding 1.0 (range, 0.8–2.2), and 74% of prospective or retrospective case-control studies in children had ORs exceeding 1.0 (range, 0.63–4.12). In both studies of infants, all the ORs exceeded 1.0 (range, 2.4–3.8); however, as asthma cannot be reliably assessed in infants, these findings should be interpreted with caution (Nafstad et al., 1998; Øie et al., 1999). These studies were not included in the meta-analysis of Fisk, Lei-Gomez and Mendell (2007), which found a summary OR of 1.3 (95% CI, 0.9–2.1) for asthma development and dampness factors.

The one study in which quantitative microbial measurements were used, a prospective study in adults, did not find consistent increases in risk (only 25% of ORs exceeded 1.0, ranging from 0.9 to 1.5). One of the strongest reported studies (Pekkanen et al., 2007), a retrospective case–control study of incident asthma cases, which was not included in the meta-analysis of Fisk, Lei-Gomez and Mendell (2007), showed that dampness or mould in the main living area of a house was related in a dose–response fashion to asthma development in infants and children. The multivariate-adjusted ORs for asthma incidence associated with three levels of maximum severity of moisture damage (assessed by civil engineers) were 1.0, 2.8 (95% CI, 1.4–5.4) and 4.0 (95% CI, 1.6–10.2). This well-designed study is the strongest available piece of evidence within a body of generally consistent findings that dampness-related exposure is not only associated with, but may cause, asthma in infants and children.

For dyspnoea, for which the Institute of Medicine considered there was limited or suggestive evidence of an association with dampness, the number of studies in adults and children had doubled to eight, although they were all still cross-sectional. In the available studies (Tables A1.1 and A1.2), the measures of associa-

tion between dampness-related factors and dyspnoea were predominantly (81%) greater than 1.0, ranging from 0.41 to 9.38.

Current asthma, which was not evaluated in the Institute of Medicine review, was consistently associated in the available studies (Table A1.2) with qualitative markers of indoor dampness. In three cross-sectional studies of adults and 12 of children, almost all (91%) the ORs exceeded 1.0 (range, 0.36–12.99). Of the assessments with quantitative measurements of microbial exposure, the one prospective study of adults found predominantly (75%) elevated ORs for current asthma (range, 0.92–1.54), and three cross-sectional studies of adults or children also found mainly (81%) elevated ORs (range, 0.46–8.50). In their quantitative summary of effect estimates, Fisk, Lei-Gomez and Mendell (2007) reported an OR of 1.6 (95% CI, 1.3–1.9) for current asthma and dampness factors.

Respiratory infections were also not evaluated in the Institute of Medicine review. Of the available studies of qualitative dampness-related factors and respiratory infections (Table A1.2), besides the few results for otitis media, two prospective studies of children found consistently elevated ORs, ranging from 1.34 to 5.10, and five cross-sectional studies in children found mostly (73%) elevated ORs, ranging from 0.65 to 1.85. In studies with quantitative measurements of mould levels, two prospective studies of children had mixed findings, with 67% of ORs above 1.0 but ranging from 0.46 to 6.88. The few findings on otitis media, in three studies of children, showed estimates ranging from 1.0 to 1.37 for qualitative dampness factors and 0.72 to 3.45 for quantitative mould levels.

Outcomes evaluated in this review (Table A1.2) but not in that of the Institute of Medicine include allergic rhinitis, altered lung function, bronchitis, allergy or atopy and "asthma, ever". Completely consistent associations (100%) were found for bronchitis, but the evidence was limited to five cross-sectional studies. There was some suggestion that indoor dampness was associated with allergic rhinitis, but the evidence was inconsistent. The evidence for altered lung function, allergy or atopy and "asthma, ever" was considered insufficient due to the small number of studies, inconsistencies in the data or a combination of the two.

Our review did not include studies on the health effects of moisture and microbiological growth within heating, ventilation and air-conditioning systems, although these are potentially common effects in buildings with air-conditioning systems or humidification. A number of studies suggested increased risks of building-related symptoms, including lower respiratory tract symptoms, due to poorly designed or maintained heating, ventilating and air-conditioning systems (e.g. Sieber et al., 1996; Mendell et al., 2003, 2006) or simply due to the presence of air-conditioning systems, which produce moisture on cool surfaces of the systems, over which all the ventilation air to building occupants flows (Mendell, Smith, 1990; Mendell et al., 1996; Seppänen, Fisk, 2002; Bernstein et al., 2006). These effects appeared probably to be due to uncharacterized microbiological agents. The strongest study on this question is that of Menzies et al. (2003), which

is a blinded, controlled, multiple cross-over intervention study in office buildings with no known contamination of heating, ventilation or air-conditioning systems or building-related health problems. The study showed that ultraviolet germicidal irradiation of the wet surfaces of cooling coils and condensate drip-pans in heating, ventilation and air-conditioning systems substantially reduced symptoms among the occupants. The findings suggest that microorganisms growing on moist surfaces in typical commercial air-conditioning systems can substantially increase building-related symptoms. The findings also imply that atopic people and nonsmokers are more responsive to microbiological agents in the form of increased respiratory and musculoskeletal symptoms. Thus, ultraviolet germicidal irradiation reduced the frequency of lower respiratory tract symptoms by 30% among current smokers but by 60% among people who had never smoked. This pattern of susceptibility is similar to that for hypersensitivity pneumonitis and may indicate a milder subclinical process. Hypersensitivity pneumonitis-like illness caused by indoor work environments has been reported repeatedly, generally in relation to leaks, but is considered rare (Kreiss, 1989). A possible explanation for these findings can be found in the reports of Gorny and others, who have shown that fungi and actinomycetes can emit large numbers of airborne particles smaller than spores (Gorny et al., 2002) (see Chapter 2).

4.1.3.3. Uncertainty

It is helpful to explore, as far as is feasible, how the findings of studies are influenced by various aspects of quality, including the quality of measurements of exposures and health outcomes. For instance, two reviews addressed the possibility that biased responses by building occupants in studies of dampness distort the findings. Fisk, Lei-Gomez and Mendell (2007) considered information on this question in six studies and concluded that the associations observed between respiratory health effects and dampness-related exposure were unlikely to be explained by overreporting of dampness or mould by people with respiratory symptoms. Bornehag et al. (2001) reported that the findings of studies with independent assessments of dampness and of health effects were similar to those of studies with more subjective sources of information.

The findings could also be compared on the basis of whether assessment of exposure was qualitative or quantitative. Random error in crude qualitative exposure categories tends to reduce the ability of studies to reveal true associations. The objective measures of exposure used in the studies have important limitations (see also Chapter 2). First, measurement of concentrations of culturable microorganisms is known to incur substantial error, due, for example, to short-term estimation of airborne concentrations that vary widely and rapidly over time, to the differential ability of organisms to grow on specific culture media and to the inability of culture assays to identify most bioactive microbial materials, whether intact spores or fragments. Second, the quantitative microbial meas-

urements used in some of the studies cannot be considered to be inherently more accurate for assessing exposure, as the microbes may not be relevant causal factors. The exposures that cause dampness-related illness have not yet been determined. A study of an association between health effects and the concentration of a specific microorganism or microbial compound is in fact testing a hypothesis. In the studies in our review, such hypothetical causal exposures included all culturable fungi, specific culturable fungi, all fungal spores, species-specific spores, all fungal biomass (ergosterol) (Robine et al., 2005), the total mass of specific organisms (*Aspergillus* and *Penicillium* extracellular polysaccharides) and specific toxic compounds (endotoxins, β-glucans).

Avoidance behaviour is another source of exposure misclassification; that is, people with asthma might change their living environment to reduce exposure. This is a concern mainly in cross-sectional and case-control studies, in which it can lead to an underestimate of the true effect. It is of no concern in those studies in which exposure is assessed before the onset of asthma.

Finally, the lack of standardization of the definitions of health effects can result in bias. In population studies, for instance, asthma is usually defined on the basis of self-reported (or parent-reported) asthma symptoms, which include wheeze, chest tightness, breathlessness and cough. Self-reports of doctor-diagnosed asthma are also often used. An alternative to questionnaires has been the use of more objective measures, such as bronchial responsiveness testing, either alone or in combination with questionnaires. As with measures of house dampness or fungal exposure, differences in asthma definition are likely to result in different estimates of prevalence and of relative risks. Also, as mentioned above, several studies (Nafstad et al., 1998; Øie et al., 1999) evaluated infants, in whom a diagnosis of asthma is less certain than in older children. Most of these potential sources of bias can result in underestimates of the true association between indoor damp and health effects.

4.1.4 The hygiene hypothesis

Many studies have found that health risks are increased by exposure to microbes, but others suggest that exposure in early life to endotoxins or fungal agents protects against atopy and allergic disease. This potentially protective effect is consistent with the hygiene hypothesis, which postulates that growing up in a microbiologically hygienic environment might increase the risk of developing allergies (Liu, Leung, 2006). This hypothesis was prompted by the results of epidemiological studies showing that overcrowding and unhygienic conditions were associated with lower prevalences of allergies, eczema and hay fever (Strachan, 1989). A more recent review confirmed the associations between large family size, low socioeconomic status and hepatitis A infection and decreased risks of atopy, hay fever or eczema, but not for asthma (Strachan, 2000). Exposure to certain microbial agents, including bacterial endotoxins, early in life has been proposed as an

explanation for these protective effects (Douwes et al., 2004). Several cross-sectional studies showed significant inverse associations between indoor endotoxin levels and atopic sensitization, hay fever and atopic asthma (Gereda et al., 2000; Gehring et al., 2002; Braun-Fahrländer, 2003).

A recent prospective birth cohort study showed an inverse association between the levels of both bacterial endotoxins and fungal components measured at three months on the floor and doctor-diagnosed asthma and persistent wheeze at age 4 years, confirming some of the earlier findings of the cross-sectional studies (Douwes et al., 2006). A similar birth cohort study found a protective effect against atopy in children aged 2 years (Bottcher et al., 2003); however, this was not confirmed in another birth cohort study, which showed that early exposure to endotoxins was associated with an increased risk of atopy at the age of 2 years (Bolte et al., 2003). Several studies have shown reduced risks of atopy, hay fever, asthma and eczema among farmers' children and adolescents; although the specific protective factors were not identified, the authors suggested that endotoxins and other microbial agents play an important role (Douwes, Pearce, Heederik, 2002; Douwes et al., 2004).

The evidence has not, however, been consistent (Liu, 2007; von Mutius, 2007). Several large studies showed either no protective effect or even a positive association. For instance, the National Survey of Allergens and Endotoxin in Housing in the United States showed an exposure-dependent increase in diagnosed asthma, wheeze and use of asthma medication in adults with increasing endotoxin concentrations in the bedroom floor and bedding (Thorne et al., 2005). Michel et al. (1996) showed that endotoxins in floor dust and high exposure to dust mites were positively associated with the severity of asthma in people who were also allergic to house dust mites,. Some of the inconsistency may be due to the timing of exposure, being protective in early life and being a risk factor later in life. Alternatively, exposure to endotoxins may prevent allergic asthma, but at higher exposure may cause non-allergic asthma (Douwes, Pearce, Heederik, 2002).

It is provocative that many well-conducted studies showed apparent protective effects of measured exposures to microbial agents, such as endotoxins and fungi. Douwes et al. (2006) reported the results of a prospective study that showed strongly protective associations between both endotoxins and extracellular markers of *Penicillium* or *Aspergillus* and doctor-diagnosed asthma. Other studies have shown that greater exposure to endotoxins is associated with decreased risks of atopy (Gehring et al., 2002; Bottcher et al., 2003) and asthma (Braun-Fahrländer et al., 2002). Li and Hsu (1997) reported the results of a cross-sectional study in which the concentrations of culturable *Penicillium* in indoor air were inversely associated with allergic rhinitis. Iossifova et al. (2007) reported that a prospective study showed that β-glucan was protective against recurrent wheezing in infants. The findings are not, however, consistent. For instance,

Dharmage et al. (2001) found that exposure to high indoor air levels of *Cladosporium* and *Penicillium* decreased the risk of fungal sensitization, but they found that these fungi were associated with high risks of bronchial hyperresponsiveness. Osborne et al. (2006) found that increased exposure of infants to *Cladosporium* was associated with a reduced risk of atopy, although exposure to *Penicillium*, *Aspergillus* and *Alternaria* was associated with an increased risk.

Braun-Fahrländer (2003) cautioned that "exposure to endotoxin might partly be a surrogate measure of a much broader spectrum of immunomodulatory microbial compounds." If in fact exposure to certain microbiological agents early in life protects against atopy and asthma, it is puzzling that no similar pattern is seen for dampness-related risk factors in infants. Overall, the available evidence is still inconsistent, and further research is required for clarification.

Thus, modest exposure to microbial agents may, under certain circumstances, protect against allergies and allergic disease, but the evidence is inconsistent. There is no indication that living or working in a damp building with heavy exposure to mould prevents the development of allergies and respiratory disease.

4.1.5 Conclusions

Our review of the epidemiological evidence presented in this report, the previous review by the Institute of Medicine and the quantitative meta-analysis of Fisk, Lei-Gomez and Mendell (2007) leads us to conclude that there is sufficient evidence of an association between indoor dampness-related factors and a wide range of respiratory health effects (Table 8), including asthma development, asthma exacerbation, current asthma, respiratory infections, upper respiratory tract symptoms, cough, wheeze and dyspnoea. As we did not perform a formal meta-analysis, we cannot make a quantitative assessment of the relative risk; however, the quantitative summary estimates of associations between qualitatively assessed dampness or mould in residences and selected respiratory health effects provided by Fisk, Lei-Gomez and Mendell (2007) (Table 7) are valid, as few additional studies of this type have become available. Their estimates suggest that a substantial increase in a number of important respiratory health outcomes, including a 50% increase in current asthma, is associated with dampness-related risk factors in residences. In an associated paper, it was estimated that, if these associations are causal, 21% of the cases of asthma in the United States could be attributed to residential dampness and mould (Mudarri, Fisk, 2007). As these estimates are based on limited data, broad lumping of diverse risk factors and multiple unverified assumptions, they should be interpreted cautiously; however, they do indicate that dampness-related risk factors may contribute substantially to the burden of respiratory disease.

Indoor dampness also appears to be associated with bronchitis and allergic rhinitis, but the evidence is either mixed (allergic rhinitis) or based on relatively few studies (bronchitis). For all the other health effects considered (altered lung

function, allergy or atopy and "asthma, ever"), we consider the evidence to be inadequate or insufficient to determine whether an association exists.

In agreement with the Institute of Medicine (2004), we consider that there is insufficient evidence of a causal relationship with any of the health outcomes reviewed, although for asthma exacerbation we consider that there is almost enough evidence to meet the criteria of causality for dampness-related agents. The evidence does not suggest that any one measurement of microbiological materials is demonstrably more specific or sensitive for assessing dampness-related exposure that is relevant to health effects. Thus, although it is plausible that heavy exposure to indoor mould or other microbial agents plays a causal role, this has not been established conclusively.

4.1.4.1. Limitations of the approach

The restricted scope of this review resulted in a number of limitations. The method used to evaluate the evidence was largely non-quantitative; thus, all the available tools for summarizing the scientific literature could not be used. Furthermore, the conclusions of this review were derived primarily from findings based on qualitative measures of dampness-related factors. It is thus difficult to link the conclusions to specific exposures. It is also likely that publication bias influenced the results, inflating the association between risk factors and health effects. Formal application of statistical methods for assessing bias was beyond the scope of this review. We did not search for unpublished findings, which would have decreased publication bias. The conclusions drawn from this review should thus be considered provisional until a more thorough consideration of all the available findings is possible. It is recommended that the evidence for publication bias be addressed in a future review, with updated, quantitative summary estimates of risk.

4.2 Clinical aspects of health effects

This section focuses on studies involving human volunteers or experimental animals exposed in controlled circumstances, occupational groups or clinically. Most of these studies are based on small groups of individuals, but both the exposure and the clinical outcomes are characterized better than they are in the epidemiological studies.

4.2.1 β-glucans

Numerous studies have shown that β-glucans have important effects on the human immune system. β-glucan was identified as the biologically active component of immune-stimulating yeast cell extracts in 1961 (Riggi, Di Luzio, 1961). Since then, their effects, particularly in relation to infection and cancer, have been investigated extensively. The research has focused almost entirely on orally or intravenously administered glucans, however, and few studies have been con-

ducted of experimental exposure of humans to mould or glucans; nevertheless, a series of studies was performed with healthy volunteers by Rylander and colleagues in Sweden.

In the first study, it was found that exposure to pure β-glucan (particulate curdlan at 210 ng/m^3 for 4 hours) did not significantly affect lung function (Rylander, 1996), although some exposure-related irritation in the nose and throat occurred. A second experiment, with another β-glucan (grifolan in saline at about 30 ng/m^3 for 3 hours), resulted only in an increased blood level of tumour necrosis factor alpha (TNF-α) (Beijer, Thorn, Rylander, 1998). In their third study, 125 ng of inhaled grifolan resulted in TNF-α, eosinophil cationic protein and neutrophils in sputum 24 hours later (Thorn, Brisman, Toren, 2001). These short-term exposures to pure glucan preparations cannot be compared directly with exposure to measured levels in buildings, which rarely exceed 100 ng/m^3 of β-glucan. In a Danish study of 36 volunteers exposed to dust and glucan in a climate chamber, decreased nasal volume and increased interleukin (IL)-8 indicated that glucan enhanced the inflammatory effect of the dust on the upper airways (Bønløkke et al., 2006). In an unusual method of exposure, in which aqueous solutions of β-glucan (curdlan) were instilled directly into the nostrils of recycling workers, an increase in nasal volume was observed (Sigsgaard et al., 2000). In contrast, no volume changes were observed after instillation of a solution of *A. fumigatus* or compost.

Experimental inhalation by healthy non-allergic people of *A. fumigatus* allergen extract increased the amount of nitric oxide exhaled from their lungs and in nasal lavage fluid (Stark HJ et al., 2005; Stark et al., 2006). The authors also found indications of pro-inflammatory effects in nasal lavage fluid (increases in TNF-α and IL-1β), although the latter effect was obscured by an insufficient interval between exposure to the placebo and the mould. In another study in Denmark, with double-blind, placebo-controlled exposure of people who had previously experienced building-related symptoms after exposure to spores from two different moulds, no clinical effects were observed (Meyer et al., 2005). An intriguing lack of increase in blood neutrophils on the days of exposure to mould, as compared with the normal diurnal increase after placebo, might have been due to neutrophil extravasation elsewhere in the body. 3-methylfuran, a common fungal volatile compound, was found to increase blinking frequency and enzymes from neutrophilic granulocytes and to affect lung function at a concentration of 1 mg/m^3, without causing symptoms (Walinder et al., 2005). This concentration is substantially higher than those measured in buildings.

Unfortunately, all the studies performed so far have been small and had insufficient statistical power to detect weak clinical effects. Exposure was to different components or preparations of mould, and, although concentrations were reported, none of the studies obtained sufficient information to calculate dose. In summary, mediators of inflammation and signs of inflammatory reactions tend

to appear, with little change in symptoms, after exposure to a variety of low toxicity mould components; the effects appeared to be greater after 24 hours than immediately after exposure. Although some of the studies included people who were atopic or otherwise considered susceptible, the studies provide too limited evidence to allow conclusions about differences in susceptibility among healthy people. A number of studies have been performed in which guinea-pigs were exposed to β-glucans by inhalation. β-glucans that are insoluble in water appeared not to elicit a significant inflammatory response on inhalation; however, when a water-soluble β-glucan, such as schizophyllan, was used or an insoluble β-glucan was rendered soluble by treatment with sodium hydroxide, more leukocytes appeared in bronchoalveolar lavage fluid (Fogelmark et al., 1992). Grifolan treated with sodium hydroxide was found to cause airway eosinophilia (Fogelmark, Thorn, Rylander, 2001). Milanowski (1998) reported significant acute increases in neutrophilic granulocytes (polymorphonuclear leukocytes), lymphocytes and erythrocytes in bronchoalveolar lavage fluid after inhalation of β-(1,3)-glucan from Baker yeast suspended in saline. Several β-glucans, including insoluble forms, have been shown to modulate the response to other agents, such as endotoxins (Fogelmark et al., 1992; Fogelmark, Sjöstrand, Rylander, 1994; Fogelmark, Thorn, Rylander, 2001), cigarette smoke (Sjöstrand, Rylander, 1997) and ovalbumin (Rylander, Holt, 1998; Wan et al., 1999). Concomitant exposure to endotoxins and curdlan, a (1-3)-β-glucan, was shown to diminish the acute neutrophil response but to augment chronic inflammatory effects (Fogelmark, Sjöstrand, Rylander, 1994; Rylander, Fogelmark, 1994). Thus, the effects of inhalation of β-glucans apparently depend on the type of glucan as well as on concomitant exposures.

Several studies have linked β-glucan-contaminated environments to symptoms and signs of airway inflammation. Increased airway responsiveness to methacholine (Rylander, 1997b), amplified peak flow variation in children (Douwes et al., 2000) and a higher prevalence of atopy (Thorn, Rylander, 1998) have been associated with long-term occupation of buildings with high concentrations of airborne β-glucan. In these buildings, symptoms such as dry cough (Rylander et al., 1989, 1992, 1998a), nasal and throat irritation (Rylander et al., 1989), hoarseness (Rylander et al., 1998a) and tiredness and headache (Rylander et al., 1989; Wan et al., 1999) were found to be more prevalent. A few cases have also been published in which inhabitants of houses with signs of mould and with airborne glucan levels > 100 ng/m^3 were severely afflicted with asthma-like symptoms until they moved (Rylander, 1994; Rylander et al., 1998a). These studies linking glucan with symptoms and signs of disease are insufficient to conclude that there is a causal relationship with glucan, which may be just a marker of exposure to another agent.

Waste handlers were found to have a work-related increase in polymorphonuclear leukocytes in nasal lavage fluid, which is related to biomarkers of inflamma-

tion, in particular to IL-8. The inflammatory response and swelling of the nasal mucosa were associated with exposure to glucan and fungal spores (Heldal et al., 2003). In another study, greater lymphocytosis was found at higher β-glucan levels (Thorn et al., 1998). In other occupations, no significant associations have been found between airway inflammation and exposure to β-glucans, although β-glucan levels are elevated during farming (Eduard et al., 2001; Roy, Thorne, 2003).

4.2.2 Mycotoxins

Mycotoxins are secondary metabolites produced by fungi, which can cause a toxic response in animals and human beings, often at very low concentrations. Many studies in vitro and in experimental animals have demonstrated the toxic potential of a variety of mycotoxins, including trichothecenes and sterigmatocystin (Institute of Medicine, 2004; Rocha, Ansari, Doohan, 2005).

It has been speculated that inhalation of aflatoxins and ochratoxin in industries such as peanut and livestock feed processing and industries in which exposure to grain dust occurs might increase the incidences of liver cancer, cancers of the biliary tract and salivary gland, and multiple myeloma (Olsen, Dragsted, Autrup, 1988; Selim, Juchems, Popendorf, 1998). A direct association with cancer has not been demonstrated, although adduct formation with aflatoxin has been found in such workers (Autrup et al., 1991). Inhaled mycotoxins have also been suggested to play a role in adverse reproductive outcomes among farmers (Kristensen, Andersen, Irgens, 2000).

Although mycotoxins can induce a wide range of adverse health effects in both animals and human beings, the evidence that they play a role in health problems related to indoor air is extremely weak. Nonetheless, one group of mycotoxins that has received considerable attention in this respect are the trichothecenes produced by *S. chartarum*. In a report from the Centers for Disease Control and Prevention (1994, 1997; Etzel et al., 1998), indoor exposure to these mycotoxins was suggested to be associated with acute pulmonary haemorrhage in a cluster of 10 infants presenting at the Cleveland Children's Hospital in 1993–1994. Pulmonary haemorrhage is characterized by strongly elevated levels of haemosiderin, an iron-containing pigment, in lung tissues. The condition can be fatal due to diffuse bleeding or haemorrhage in the alveoli. It is not commonly associated with bioaerosol exposure. These findings were later criticized by others on the basis of shortcomings in the collection, analysis and reporting of data and were retracted (Centers for Disease Control and Prevention, 2000), and the role of *S. chartarum* is still controversial. In view of these uncertainties, the Institute of Medicine (2004) concluded that "available case-report information, taken together, constitutes inadequate or insufficient information to determine whether an association exists between acute idiopathic pulmonary hemorrhage and the presence of *S. chartarum*." A subsequent study showed that people exposed to

satratoxin in their houses form albumin adducts in their blood, as found in rats exposed experimentally to satratoxin G. This small study shows that exposure to high levels of *S. chartarum* in houses might have a biological effect (Yike et al., 2006).

4.2.3 Allergic alveolitis

Allergic alveolitis, also known as extrinsic allergic alveolitis and hypersensitivity pneumonitis, is an inflammatory disease involving the distal proportions of the airways. The disease has an immunological component, but it has been difficult to find the immunological mechanism by which antigens create granulomatous lymphocytic inflammation in the alveoli and bordering regions.

Allergic alveolitis is diagnosed with various clinical and paraclinical tests, including pathological examination and imaging of the lungs (for a comprehensive review, see Wild, Lopez, 2001). Although IgG antibodies were initially believed to be causative, they have been shown to be only markers of exposure (Marx et al., 1990; Cormier, Belanger, 1989; Malmberg et al., 1985). There have been sporadic case reports of allergic alveolitis in indoor environments in Europe and the United States (Torok, de Weck, Scherrer, 1981; Pedersen, Gravesen, 1983; Fergusson, Milne, Crompton, 1984; Bryant, Rogers, 1991; Siersted, Gravesen, 1993; Wright et al., 1999), often in connection with the use of humidifiers (von Assendelft et al., 1979; Nordenbo, Gravesen, 1979).

In Japan, more than 60 cases of allergic alveolitis caused by the mould *Trichosporon cutaneum* occur every year during the hot and humid summer season, with an incidence of 0.5 per 10^6 person–years (Ando et al., 1991, 1995). During this season, *T. cutaneum* grows rapidly, especially in wooden houses. It occurs only south of 40° latitude. A 10-year survey was conducted of all hospital cases of this condition throughout Japan, with diagnostic criteria including comprehensive paraclinical tests and confirmation of causality by a positive challenge test either to the environment or to the mould spores themselves. The survey showed that cases were concentrated in the southern part of Japan and occurred predominantly among housewives, who therefore spent most of their time in and around a building. Similar cases have been recently reported in the Republic of Korea and in southern Africa (Swingler, 1990; Yoo et al., 1997).

A smaller series in Finland consisted of seven cases of rhinitis and four of allergic asthma, one of which included allergic alveolitis, among 14 employees at a military hospital infested with *Sporobolomyces salmonicor* (Seuri et al., 2000). The cases of asthma and allergic alveolitis were confirmed by inhalation provocation with *S. salmonicor* from the hospital.

In case series of allergic alveolitis in the home environment and in industrial cases, the affected person is often in the same environment as the general population. Therefore, susceptibility must play a significant role. Familial clustering has been found (Allen, Basten, Woolcock, 1975), but the causative factor has not

been identified (Schwarz, Wettengel, Kramer, 2000). It is striking that the vast majority of cases occur in nonsmokers.

The Institute of Medicine (2004) concluded that clinically significant allergic alveolitis occurred only in susceptible people exposed to sensitizing agents. The studies indicated that there is sufficient evidence of an association between the presence of mould and bacteria in damp indoor environments and allergic alveolitis. The only new study since that evaluation is that of Seuri et al. (2000), which does not change this conclusion.

4.2.4 Inhalation fever

Inhalation fever, also known as toxic pneumonitis, humidifier fever and organic dust toxic syndrome, is a self-limiting syndrome that occurs after inhalation of a wide range of substances, from metal fumes to bacteria and mould spores. The syndrome was first described as humidifier fever during the time when reservoir humidifiers were used. Outbreaks typically occurred during the heating season in museums, printing shops and other localities in which it was important to control the humidity in the environment (Rask-Andersen et al., 1994). Studies of farmers showed that the exposure to mould spores that leads to inhalation fever exceeds the levels that induce allergic alveolitis by one or two orders of magnitude (Rask-Andersen, 1988).

4.2.5 Infection with mould

Infection with *Aspergillus* and other fungi such as *Fusariuum* spp. is a well-known complication in the treatment of patients who are immune compromised due, for example, to treatment for cancer or infection with human immunodeficiency virus (Iwen et al., 1994, 1998; Geisler, Corey, 2002; Lednicky, Rayner, 2006). Some of these patients contract mould infection after exposure indoors, not because of water damage in the facilities where they are being treated but because an opportunistic, ubiquitous mould finds a suitable host. No studies have been conducted to link such infections to mould in the indoor environment. Furthermore, the type of disease appears to determine the type of infection, and the infecting agents are not those typically encountered in damp houses.

Aspergillus appears to be the most aggressive of these fungi, giving rise to infections also in patients with less severe airway disease, such as cystic fibrosis, asthma and chronic obstructive pulmonary disease. People who are atopic sometimes contract a severe infection in which aspergillosis causes an allergic reaction with the infection, giving the person wheeze, pulmonary infiltrates and eventually fibrosis (Kauffman, 2003). This syndrome can also be found with an aspergilloma (i.e. a tumour in a lung cavity consisting of *Aspergillus* hyphae) (Tanaka, 2004).

People with atopy sometimes develop sinus disease as a consequence of *Aspergillus* infection or presence (Dufour et al., 2006). Exposure to mould has been

proposed as the cause of chronic sinusitis, as these patients show exaggerated humoral and cellular responses, both T(H)1 and T(H)2 types, to common airborne fungi, particularly *Alternaria* (Shin et al., 2004).

4.2.6 Other effects

Several other effects of exposure to mould have been discussed in the context of indoor air, including toxic, immunological, reproductive and neuropsychiatric symptoms and syndromes. The Institute of Medicine (2004) found no evidence that the prevalences of cancer and reproductive outcomes were altered by exposure to indoor air. A search of these subjects showed no studies on this subject within the past 5 years. The Institute of Medicine (2004) included only a few case reports, with inconsistent findings for neuropsychiatric effects.

A Finnish group studied the occurrence of rheumatic diseases associated with dampness. In two studies, the authors found clustering of cases of rheumatic disease in water-damaged buildings (Myllykangas-Luosujärvi et al., 2002; Luosujärvi et al., 2003) and suggested that the symptoms could be attributed to exposure to mould spores. In a later publication (Lange, 2004), the author proposed that endotoxins and other triggers of the innate immune response might play a role, although the exposure levels are much lower than those in situations where joint pain is more prevalent, as on farms and among bird fanciers. Rheumatic diseases among people exposed in damp buildings and the possible role of endotoxins was also reported by Lorenz et al. (2006).

4.3 Toxicological mechanisms

This brief review of toxicological studies on microbial exposure in damp buildings is based mainly on studies published between 2000 and mid-2007; when appropriate, references are also be made to earlier publications, including those in the review of the Institute of Medicine (2004). The data reviewed originate from studies in vitro (i.e. experimental studies in a controlled environment outside a living organism, such as a test tube), summarized in Table A2.1, and studies in experimental animals in vivo (Table A2.2). Although direct extrapolation from experimental data to human risk is not possible, the studies that are described provide important information about the possible toxicological mechanisms behind the observed health effects in damp buildings. This review focuses on the ability of microbial exposures associated with damp buildings to activate the following potential toxicological mechanisms: immunostimulation and allergies, cytotoxicity and immunosuppression, autoimmunity, irritation, neurotoxicity, genotoxity and reproductive toxicity. Novel toxicological data on the role of microbial interactions are also included.

The variety of respiratory symptoms and diseases observed in damp and mouldy indoor environments suggests that the airways are the primary route of entry for agents. Therefore, studies in experimental animals were limited to those

in which the airways were used as the pathway of exposure, thus excluding the extensive literature on the induced toxic effects of bacterial toxins and mycotoxins, associated, for example, with ingestion of mould-contaminated food. Most of the in vitro and in vivo studies included in this review addressed the effects of microbial components found in damp buildings, such as fungal spores, bacterial spores and cells, and the toxic components or products of microbes (e.g. fungal mycotoxins and endotoxins) (see Tables A2.1 and A2.2). Possible toxic effects due to released non-microbial chemicals are not addressed, because experimental data on exposures to chemicals in damp buildings were missing or limited.

In damp buildings, people are exposed to constantly changing concentrations of different microbial species, their spores, metabolites and components, and other compounds in indoor air, including chemical emissions from building materials. This complex mixture of exposures inevitably leads to interactions, which may change the toxic characteristics of the inhaled particles, causing different outcomes in different situations. Furthermore, the effects of microorganisms, microbial substances or dampness-related chemical compounds seen in experimental animals or cells often result from exposures that are orders of magnitude higher than the average doses that reach the human lungs under normal conditions in indoor air. Nevertheless, the surface doses within the lungs of patients with respiratory conditions can vary a thousandfold, due to uneven particle deposition (Phalen et al., 2006), resulting in even larger maximal surface doses in human lungs than in those used in experimental toxicological studies. Moreover, many other factors, such as exercise, can result in larger-than-average doses in the human lung.

Thus, experimental toxicological studies are essential for clarifying cellular mechanisms and identifying causative compounds, but the dosage must be considered in interpreting the findings and attempting extrapolation to the range of human exposures indoors.

4.3.1 Immunostimulation and immunoglobulin E-mediated allergies

There is evidence that dampness in buildings increases the risks of asthma, sensitization and respiratory symptoms, as extensively reviewed by Bornehag et al. (2001, 2004). Many of the health effects may result from recurrent activation of immune defence, leading to exaggerated immune responses and prolonged production of inflammatory mediators. Overproduction of these compounds damages the surrounding tissues and may manifest itself as chronic inflammation and inflammation-related diseases, such as asthma (Martin, Frevert, 2005). The central role of inflammatory responses is corroborated by studies reporting increased levels of inflammatory mediators in nasal lavage fluid and induced sputum from the occupants of damp buildings (Hirvonen et al., 1999; Purokivi et al., 2001; Wålinder et al., 2001).

The immunostimulatory activity of Gram-negative bacterial lipopolysaccharide is well established, but several other bacteria, fungi and isolated mycotoxins associated with damp buildings have been shown to induce inflammatory responses in vitro (Huttunen et al., 2001; Nielsen et al., 2002; Huttunen et al., 2003; Pylkkänen et al., 2004; Johannessen, Nilsen, Lovik, 2005; see also Table A2.1). In line with the findings in vitro, the same microbial species activate acute (Nikulin et al., 1996; Rao, Brain, Burge, 2000; Jussila et al., 2003; Leino et al., 2003; Rand et al., 2006) and sustained inflammation in the lungs of experimental animals (Jussila et al., 2002a) (Table A2.2).

Furthermore, it has been shown in an animal model that immunological status plays an important role in airway inflammation induced by *S. chartarum*, enhancing the effects of the mould (Leino et al., 2006). The results imply that sensitized people are more susceptible to exposure to mould than nonatopic people. Different microbial species differ significantly in their immunostimulatory potency in both mouse and human cells in vitro (e.g. Huttunen et al., 2003). Furthermore, it has been clearly demonstrated that different growth conditions and competition between microorganisms for the same habitat in vitro change their inflammatory potency, protein expression and toxin production (Ehrlich, 1987; Meyer, Stahl, 2003; Murtoniemi et al., 2003).

One of the mechanisms underlying the health effects of exposure to microbial agents may be IgE-mediated allergic responses. Although many of the reported symptoms are similar to those of allergy, only a small percentage of exposed people actually develop allergies to mould (Taskinen et al., 1997; Immonen et al., 2001). The most prevalent fungal genera associated with mould allergy are *Aspergillus*, *Cladosporium* and *Penicillium* (Ledford, 1994). Sensitization to *A. alternata* has been linked to the development, persistence and severity of asthma (Zureik et al., 2002; Bush, Prochnau, 2004; Salo et al., 2006). Some fungal species can induce histamine release by other mechanisms (Larsen et al., 1996); thus, allergy-like symptoms can also occur in non-sensitized people. Some of the chemical compounds associated with damp and degrading materials, such as phthalates and their metabolites, can stimulate the immune system by acting as allergens or adjuvants (Hansen et al., 2007).

4.3.2 Cytotoxicity and immunosuppression

Increased frequencies of common respiratory infections have been observed in people living or working in damp buildings (Åberg et al., 1996; Pirhonen et al., 1996; Kilpeläinen et al., 2001), suggesting that agents present in the indoor air of these buildings can suppress immune responses, leading to increased susceptibility to infections. Several microbes originating from damp buildings or their toxins have been shown to have immunosuppressive effects in vitro mediated – for example, by impaired particle clearance (Pieckova, Jesenska, 1996, 1998) or by cytotoxicity (Huttunen et al., 2004; Penttinen et al., 2005a,b).

The immunosuppressive effects of mycotoxins have been confirmed in experimental animals. Trichothecenes T-2 and deoxynivalenol (vomitoxin) impair immune responses to respiratory virus infection, increasing the severity of infection (Li et al., 2006; Li M et al., 2007). Some airborne fungi and bacteria may act as opportunistic human pathogens, causing upper or lower airway, pulmonary and in some cases systemic infectious diseases in immunocompromised people (Bush et al., 2006).

The acute cytotoxicity of fungal strains in damp buildings has been found to be due to the metabolite profile produced in vitro, although their biological activity may not depend solely on toxin production (Nielsen et al., 2002; Huttunen et al., 2003). Fungal spores appear to have toxic effects other than those that cause the inflammatory reaction. Studies of Gram-positive and -negative bacteria (e.g. *Streptomyces californicus*, *Pseudomonas fluorescens, Mycobacterium terrae, Bacillus cereus*) have shown that the significant difference in cytoxicity among strains (Huttunen et al., 2003) is due at least partly to differences in inflammatory activity. Spores and toxins of the fungus *S. chartarum* have been shown to activate the apoptotic pathway (programmed cell death) (Islam et al., 2006; Wang, Yadav, 2006; Penttinen et al. 2007), whereas the spores of *S. californicus* induce cell cycle arrest (Penttinen et al., 2005b).

Studies in experimental animals with the same fungal or bacterial species confirm the in vitro findings for cytotoxic effects, showing increases in total protein and lactate dehydrogenase in bronchoalveolar lavage fluid from exposed animals, as well as lung tissue damage (Nikulin et al., 1996; Rao, Brain, Burge, 2000; Rao, Burge, Brain, 2000; Jussila et al., 2001, 2002a, 2002c; Yike et al., 2002; Jussila et al., 2003; Rand et al., 2006).

4.3.3 Autoimmunity

Cases of autoimmune diseases and related symptoms have been reported among the occupants of damp buildings (Myllykangas-Luosujärvi et al., 2002; Luosujärvi et al., 2003), but there are no toxicological data on autoimmune responses caused by microorganisms or microbial substances found in damp buildings. Microbial fragments can, however, cause autoimmune reactions by molecular mimicry, acting as microbial superantigens or by enhancing the presentation of autoantigens (Wucherpfennig, 2001).

4.3.4 Irritation

Spores and other particulate material, as well as volatile organic compounds produced by microorganisms, building materials, paints and solvents, are potentially irritating. In epidemiological studies, the prevalence of respiratory and irritative symptoms has been associated with perceived mould odour, possible indicating the presence of microbial volatile organic compounds (Jaakkola, Jaakkola, Ruotsalainen, 1993; Ruotsalainen, Jaakkola, Jaakkola, 1995). It has been suggested

that these compounds are present in damp buildings at levels sufficient to cause symptoms of irritation in exposed people (Hope, Simon, 2007). Furthermore, the sensation of unpleasant odours as such can cause stress responses and nonspecific somatic symptoms such as headache and nausea.

4.3.5 Neurotoxicity

Such health effects as fatigue, headache and difficulties in concentration (Johanning et al., 1996; Koskinen et al., 1999b) indicate that microbes or other agents present in damp buildings have neurological effects.

Many pure microbial toxins, such as the products of *Fusarium* (fumonisin B1, deoxynivalenol), *Stachybotrys* (satratoxin G), *Aspergillus* (ochratoxin A) and *Penicillium* (ochratoxin A, verrucosidin), have been shown to be neurotoxic in vitro and in vivo (Rotter, Prelusky, Pestka, 1996; Belmadani et al., 1999; Kwon, Slikker, Davies, 2000; Islam, Harkema, Pestka, 2006; Stockmann-Juvala et al., 2006). No study has shown, however, that people living in damp buildings who complain of nervous system symptoms are exposed to effective levels of mycotoxins.

4.3.6 Genotoxicity

Heavy occupational exposure by inhalation to mycotoxins in mouldy grain may be linked to an increased risk of cancer (Olsen, Dragsted, Autrup, 1988; Kristensen, Andersen, Irgens, 2000), but there is no epidemiological evidence for an association between exposure in damp buildings and cancer. Some of the microbial toxins produced by bacteria and fungi are known to be genotoxic and carcinogenic (IARC, 1993), but the relevance of these findings to exposure by inhalation in damp buildings is not known. It has been shown, however, that microbial isolates from damp buildings have genotoxic activity in vitro. Wang and Yadav (2006) showed that toxins of *S. chartarum* extracted from spores cause DNA damage, *p53* accumulation and apoptosis in murine alveolar macrophages. Another report suggested that the spores of *S. californicus* produce genotoxically active compound(s) that induce DNA damage, and that the production of this compound is potentiated when the microbe grows together with *S. chartarum* (Penttinen et al., 2007). Microbial compounds may not only produce genotoxic compounds but may also increase the risk of cancer through secondary mechanisms (e.g. by inducing oxidative stress in chronic inflammation) (Fitzpatrick, 2001).

4.3.7 Reproductive toxicity

No studies were found on the effects of exposure to microbes in damp buildings on reproductive responses. One group of indoor air contaminants, phthalates, are, however, potential reproductive and developmental toxicants (Lottrup et al., 2006).

4.3.8 Microbial interactions

The immunostimulatory properties of the fungal and bacterial strains typically found in moisture-damaged buildings are synergistically potentiated by microbial interactions during concomitant exposure in vitro (Huttunen et al., 2004).

These interactions are not limited to microbes grown separately (Penttinen et al., 2005a) but also occur also when microbes are cultivated together (Penttinen et al., 2005b). Interactions between *S. californicus* and *S. chartarum* during co-cultivation stimulated the production of currently unidentified cytostatic compound(s) (Penttinen et al., 2006), which significantly potentiate the abilities of the spores to cause apoptotic cell death (Penttinen et al., 2005b). Interactions during co-cultivation stimulate these microbes to produce highly toxic compounds, which can damage DNA and provoke genotoxicity (Penttinen et al., 2007). In addition, concomitant exposure in vitro with amoebae potentiates the cytotoxic and inflammatory properties of the microbial spores of *S. californicus* or *Penicillium spinolosum* isolated from damp buildings (Yli-Pirilä et al., 2007). These findings point to the importance of considering microbial interactions when investigating the causative agents and mechanisms of the adverse health effects observed in damp buildings.

4.4 Synthesis of available evidence on health effects

In this chapter, we have presented several types of evidence – epidemiological, clinical and toxicological – relevant to answering the question of whether dampness or dampness-related exposures cause adverse human health effects. This summary is based initially on the epidemiological and clinical evidence for causal relations between dampness-related factors and specific human health outcomes. Then, the available toxicological evidence is considered as either supporting or not supporting the biological plausibility of any potentially causal association. The epidemiological evidence is based on qualitative assessments of dampness-related factors, such as visible dampness, mould, water damage or mould odour, as the epidemiological findings based on quantitative measurements of specific microbial agents were too inconsistent and, for specific outcomes, too few for clear conclusions.

The epidemiological evidence is not sufficient to conclude causal relationships between indoor dampness or mould and any specific human health effect, although the findings of one strong epidemiological intervention study, in conjunction with the other available studies, suggest that dampness or mould exacerbates asthma in children.

There is sufficient epidemiological evidence of associations between dampness or mould and asthma development, asthma exacerbation, current asthma, respiratory infections (except otitis media), upper respiratory tract symptoms, cough, wheeze and dyspnoea. There is sufficient clinical evidence of associations between mould and other dampness-associated microbiological agents and

hypersensitivity pneumonitis, allergic alveolitis and mould infections in susceptible individuals, and humidifier fever and inhalation fevers. This is the only conclusion that is based primarily on clinical evidence and also the only conclusion that refers explicitly to microbial agents, as opposed to dampness-related factors.

Limited or suggestive epidemiological evidence of an association between indoor dampness or mould and allergic rhinitis and bronchitis is available.

The evidence for effects on lung function, allergy or atopy and "asthma, ever" is inadequate or insufficient. The evidence does not suggest that any specific measure of microorganisms or microbial substances results in a demonstrably more specific or sensitive assessment of a particular dampness-related exposure relevant to health effects. Nonetheless, although specific causal agents have not been identified conclusively, microbial exposure is often suggested to play a role. Further studies with valid, quantitative exposure assessment methods are required to elucidate the role of fungi and other microorganisms in damp-induced health conditions. The available epidemiological and clinical evidence suggests that both atopic and nonatopic people are susceptible to adverse health effects from exposure to dampness and mould, even if some outcomes are commoner in atopic people. Therefore, both allergic and non-allergic mechanisms may be involved in the biological response.

The mechanisms by which non-infectious microbial exposures contribute to adverse health effects associated with indoor air dampness and mould are largely unknown. It is clear, however, that no single mechanism can explain the wide variety of effects associated with dampness and mould. Toxicological studies, by investigating the ability of microbial agents associated with damp buildings to activate certain toxicological mechanisms, provide insight into the multiple biological mechanisms that might underlie the observed associations between health effects and dampness and mould. In vitro and in vivo studies have demonstrated diverse inflammatory, cytotoxic and immunosuppressive responses after exposure to the spores, metabolites and components of microbial species found in damp buildings, lending plausibility to the epidemiological findings.

Many dampness-associated conditions are likely to involve inflammation, as inflammatory responses to many microbiological agents have been found. These include histamine release by mechanisms other than those mediated by IgE, indicating a plausible mechanism for the occurrence of allergy-like symptoms even in non-sensitized people. Dampness-associated asthma, allergic sensitization and associated respiratory symptoms may result from repeated activation of the immune defences, exaggerated immune responses, prolonged production of inflammatory mediators and tissue damage, leading to chronic inflammation and inflammation-related diseases, such as asthma.

Although fungal spores associated with damp buildings produce metabolites with demonstrated acute cytotoxicity, the spores also have toxic effects other than those caused by the inflammatory reaction. The observed increase in the

frequency of respiratory infections associated with damp buildings might be explained by the immunosuppressive effects of damp building-associated microbes in experimental animals, which impair immune defences and thus increase susceptibility to infections. An alternative explanation might be that inflamed mucosal tissue provides a less effective barrier, increasing the risk of infection. Demonstration of such effects in cells or experimental animals at levels of microbial exposure similar to those in indoor environments would allow extrapolation of these results to human beings.

Various microbial agents with diverse, fluctuating inflammatory and toxic potential are present simultaneously with other airborne compounds, inevitably resulting in interactions in indoor air. Such interactions may lead to unexpected responses, even at low concentrations. Therefore, the detection of individual exposures, such as certain microbial species, toxins or chemical agents, cannot always explain any associated adverse health effects. In the search for causative constituents, toxicological studies should be combined with comprehensive microbiological and chemical analyses of indoor samples.

The synergistic interactions among microbial agents present in damp buildings suggest that the immunotoxic effects of the fungal and bacterial strains typically found can be potentiated during concomitant exposure, leading, for instance, to increased cell death or cytotoxic or inflammatory effects. Such interactions can give rise to unexpected responses, even at low concentrations of microbial (or chemical) agents, so that it is difficult to detect and implicate specific exposures in the causation of damp building-associated adverse health effects. Thus, microbial interactions must be carefully considered when evaluating the possible health effects of exposure in damp buildings. Differences in the concentrations used in studies with cell cultures or experimental animals and those that may be reached by human beings should also be kept in mind when interpreting the findings.

Most of the relevant toxicological data from studies in experimental animals refer to the immunotoxicity of fungi, especially the species *S. chartarum* and its toxins. The role of other microbial exposures, including dampness-related bacteria, requires more study. For the identification of causative constituents and risk assessment, toxicological and epidemiological data should be combined with microbiological and chemical analyses of indoor air samples. In interpreting the results of studies in experimental animals in relation to human exposures, it is important to consider differences in relative doses and the fact that the exposures used for experimental animals may be orders of magnitude higher than those found in indoor environments.

The available estimates, based on the assumption that the associations found in the epidemiological studies are unbiased and causal, suggest that dampness-related risk factors are associated with a large proportion of human respiratory disease. For instance, residential dampness is associated with a 50% increase in

current asthma and substantial increases in other respiratory health outcomes, suggesting that 21% of current asthma in the United States may be attributable to residential dampness and mould (Fisk, Lei-Gomez, Mendell, 2007; Mudarri, Fisk, 2007). These estimates, for imprecisely defined risk factors, cannot indicate true causal relationships and must be interpreted with caution, but they suggest that some dampness-related risk factors contribute substantially to the burden of human respiratory disease.

5. Evaluation of human health risks and guidelines

5.1 Summary

Sufficient epidemiological evidence is available from studies conducted in different countries and under different climatic conditions to show that the occupants of damp or mouldy buildings, both houses and public buildings, are at increased risk of respiratory symptoms, respiratory infections and exacerbation of asthma. Some evidence suggests increased risks of allergic rhinitis and asthma. Although few intervention studies are available, their results show that remediation of dampness problems can reduce adverse health outcomes.

There is clinical evidence that exposure to mould and other dampness-related microbial agents increases the risks of rare conditions, such as hypersensitivity pneumonitis, allergic alveolitis, chronic rhinosinusitis and allergic fungal sinusitis. Toxicological evidence obtained in vivo and in vitro supports these findings, showing the occurrence of diverse inflammatory and toxic responses after exposure to microorganisms – including their spores, metabolites and components – isolated from damp buildings.

While groups such as atopic and allergic people are particularly susceptible to biological and chemical agents in damp indoor environments, adverse health effects have also been found in nonatopic populations.

The increasing prevalences of asthma and allergies in many countries increase the number of people susceptible to the effects of dampness and mould in buildings.

5.2 Conditions that contribute to health risks

The prevalence of indoor dampness varies widely within and among countries, continents and climate zones. It is estimated to affect 10–50% of indoor environments in Australia, Europe, India, Japan and North America. In certain settings, such as river valleys and coastal areas, the conditions of dampness are substantially more severe than the national average.

The amount of water available on or in materials is the most important trigger of the growth of microorganisms, including fungi, actinomycetes and other bacteria. The verification code for this document is 146180.

Microorganisms are ubiquitous. Microbes propagate rapidly wherever water is available. The dust and dirt normally present in most indoor spaces provide

sufficient nutrients to support extensive microbial growth. While mould can grow on all materials, selection of appropriate materials can prevent dirt accumulation, moisture penetration and mould growth.

Microbial growth may result in greater numbers of spores, cell fragments, allergens, mycotoxins, endotoxins, β-glucans and volatile organic compounds in indoor air. The causative agents of the adverse health effects have not been identified conclusively, but an excess level of any of these agents in the indoor environment is a potential health hazard.

Microbial interactions and moisture-related physical and chemical emissions from building materials may also play a role in dampness-related health effects. Building standards and regulations for comfort and health do not sufficiently emphasize requirements for preventing and controlling excess moisture and dampness.

Apart from its entry during occasional events, such as water leaks, heavy rain and flooding, most moisture enters buildings in incoming air, including that infiltrating though the envelope, or from the occupants' activities. Allowing surfaces to become cooler than the surrounding air may result in unwanted condensation. Thermal bridges (such as metal window frames), inadequate insulation and unplanned air pathways, or cold water plumbing and cool parts of air-conditioning units can result in surface temperatures below the dew point of the air and in dampness.

5.3 Guidelines

Persistent dampness and microbial growth on interior surfaces and in building structures should be avoided or minimized, as they may lead to adverse health effects.

Indicators of dampness and microbial growth include the presence of condensation on surfaces or in structures, visible mould, perceived mould odour and a history of water damage, leakage or penetration. Thorough inspection and, if necessary, appropriate measurements can be used to confirm indoor moisture and microbial growth.

As the relationships between dampness, microbial exposure and health effects cannot be quantified precisely, no quantitative, health-based guideline values or thresholds can be recommended for acceptable levels of contamination by microorganisms. Instead, it is recommended that dampness and mould-related problems be prevented. When they occur, they should be remediated because they increase the risk of hazardous exposure to microbes and chemicals.

Well-designed, well-constructed, well-maintained building envelopes are critical to the prevention and control of excess moisture and microbial growth, as they prevent thermal bridges and the entry of liquid or vapour-phase water. Management of moisture requires proper control of temperature and ventilation to avoid excess humidity, condensation on surfaces and excess moisture in ma-

terials. Ventilation should be distributed effectively throughout spaces, and stagnant air zones should be avoided.

Building owners are responsible for providing a healthy workplace or living environment that is free of excess moisture and mould, by ensuring proper building construction and maintenance. The occupants are responsible for managing the use of water, heating, ventilation and appliances in a manner that does not lead to dampness and mould growth.

Local recommendations for different regions with different climates should be updated to control dampness-mediated microbial growth in buildings and to ensure desirable indoor air quality.

Dampness and mould may be particularly prevalent in poorly maintained housing for low-income people. Remediation of the conditions that lead to adverse exposure should be given priority to prevent an additional contribution to poor health in populations who are already living with an increased burden of disease.