Chapter 8
Endotoxins, Glucans and Other Microbial Cell Wall Agents

Ioannis Basinas, Grethe Elholm and Inge M. Wouters

Abstract During the last decades an increasing interest in microbial cell wall agents has been established, since exposure to these agents has been linked to a wide range of adverse and beneficial health effects. The term microbial cell wall agents refers to a group of molecules of different composition that are integral structural components of microorganisms like gram-negative and gram positive bacteria and fungi. The available information on exposure characteristics for these cell wall agents within indoor environments and their associated health effects is summarized in this chapter.

Large variation in exposure levels of microbial cell wall agents in indoor occupational environments is documented, whereas actual airborne levels of exposures and determinants of residential indoor air are lacking. Standardisation of methods for determination is highly recommended for future studies.

Endotoxins, cell wall agents of gram-negative bacteria, are well studied and involved in the development of adverse and protective health effects, but for cell wall agents of fungi, like glucans the evidence is more limited and inconclusive. For other microbial cell wall agents, like muramic acid, EPS and ergosterol, studies have been sparse and very diverse in their design and applied methods.

Future recommendations include studies in large populations with a longitudinal design involving both exposure assessment and health effects assessment of

I. Basinas (✉)
Centre for Human Exposure Science, Institute of Occupational Medicine, Edinburgh, United Kingdom
e-mail: ioannis.basinas@iom-world.org

G. Elholm
Department of Public Health, Section for Environment, Occupation and Health, Danish Ramazzini Centre, Aarhus University, Aarhus, Denmark
e-mail: gelh@ph.au.dk

I.M. Wouters
Division of Environmental Epidemiology, Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, The Netherlands
e-mail: i.wouters@uu.nl

C. Viegas et al. (eds.), *Exposure to Microbiological Agents in Indoor and Occupational Environments*, DOI 10.1007/978-3-319-61688-9_8

distinct microbial cell wall agents and co-existent microbes, which is needed to understand the role of individual and combined exposures in health.

Keywords Cell wall agents · endotoxins · glucans

8.1 Introduction

A variety of potentially hazardous agents can be found in indoor air. Generally dusty and moist indoor environments are unpleasant to most people, but determining which components in the air are significantly associated with specific health outcomes is very challenging. Some indoor air exposures have already been found to have a negative impact on human health, others are still only under suspicion and yet some appear to even have beneficial effects. Micro-organisms, such as bacteria and fungi, and agents from a microbiological origin have been widely studied in relation to indoor air-related health outcomes. Agents composing the cell walls of microbes, such as endotoxins, glucans and extracellular polysaccharides are often implicated as risk factors or used as markers for exposure to microbiological agents. This chapter aims to summarise the available information on the exposure characteristics for these cell wall agents within indoor environments and their associated health effects.

8.2 What Are Microbial Cell Wall Agents?

Microbial cell wall agents are a group of molecules of different composition that are integral structural components of microorganisms (Fig. 8.1). They are released into the environment following replication, apoptosis, lysis or death of the microbial cell. Depending on their origin, fungal, gram positive or gram negative bacteria, microbial cell walls consist of different types of polysaccharides, proteins and acids. Although similar structures may also be present in outer layers of cereals and plant tissues, they are mostly considered to represent microbial exposures. Microbial cell wall agents are an important constituent of the so called “organic dust” arising from microbial, plant and animal origin. During the last decades an increasing interest in microbial cell wall agents has been established, since exposure to these agents has been linked to a wide range of adverse and beneficial health effects.

8.3 Why Are Microbial Cell Wall Agents Important?

Several symptoms and diseases have been associated with exposure to cell wall agents. These include systemic reactions (e.g. inflammation, fever and chills), allergies, acute respiratory symptoms, chronic respiratory disorders such as chronic bronchitis and asthma, as well as cancer (Smit et al., 2006; Madsen et al., 2012; Basinas et al., 2012a; Gladding et al., 2003; Li et al., 2006; Fang et al., 2013;

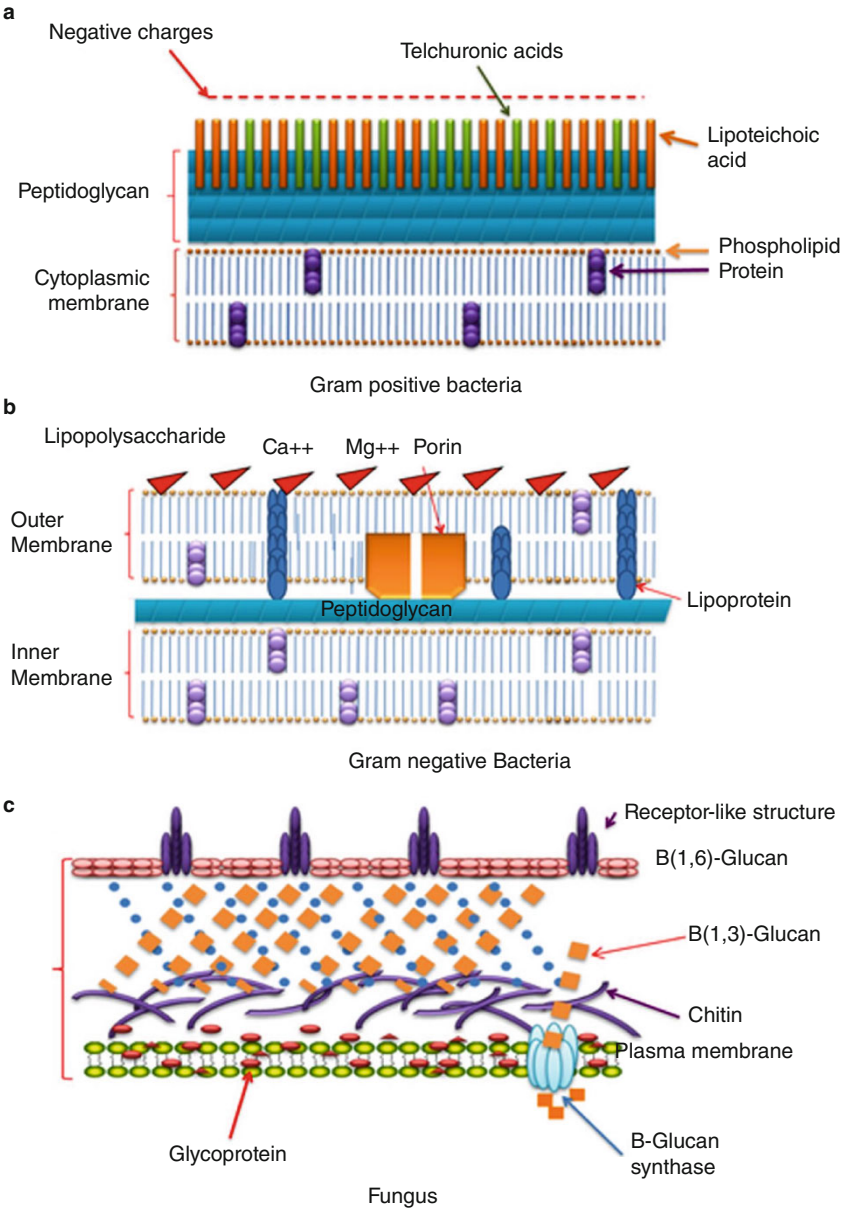

Fig. 8.1 Cell wall structures of three different types of microbial organisms. (**a**) Gram-positive bacteria which have an outer cell wall containing a thick layer of peptidoglycan. (**b**) Gram-negative bacterial cell walls which contain a thin layer of peptidoglycan and a lipid bilayer containing lipopolysaccharide. (**c**) Fungal cell walls which are composed of beta-glucan structures and chitin. (From Vatansever et al., 2013)

Eduard et al., 2004; Rylander et al., 1999; Vogelzang et al., 2000; Eduard et al., 2009; Braun-Fahrlander et al., 2002).

Bacterial endotoxins, peptidoglycans (incl. muramic acid), the fungal sourced β-D-glucans and fungal extracellular polysaccharides are all microbial cell wall agents that are either considered to have a key role in associations with health effects or, as not all necessarily have human antigenic and/or inflammatory properties themselves, being used as markers for exposure to microbes.

Once released and aerosolised, microbial cell wall agents can enter the human body mainly through inhalation. Exposure through other routes has not been thoroughly studied yet. Generally the potential for dermal absorption can be considered as rather small because of a high molecular agent weight (Bos and Meinardi, 2000), whereas direct or inadvertent (i.e. through hand to mouth contact and eating in contaminated areas) exposure by ingestion can occur (Cherrie et al., 2006; Gorman et al., 2012) but it is likely of lesser importance for respiratory diseases. After entering the human body some microbial cell wall agents may trigger a line of different receptors which evoke an increase in the release of cytokines, chemokines, adhesion molecules, and other mediators resulting in an inflammatory reaction (Reed and Milton, 2016).

The main microbiological cell wall agents that have been studied either as independent agents or as markers of exposures in relation to human health outcomes are summarized below. It is important to note that other cell wall agents of microbial origin (e.g. various types of proteins) exist but at present their immunological importance is either considered rather small or remains unknown.

8.4 Microbial Cell Wall Agents

8.4.1 Endotoxins

Endotoxins are commonly also known as Lipopolysaccharides (LPS) in reference to their purified derivative and chemical structure, which typically comprises of a long polysaccharide complex chain bound to a lipid A component (Douwes et al., 2003; Williams, 2007b). They are located at the external cell wall membrane of gram-negative bacteria and are released to the environment primarily following cell replication, death or lysis (Williams, 2007a). Endotoxin and their purified derivatives are present in the oral and nasal cavity and throughout the gastrointestinal tract of mammals, and are found ubiquitously on plant surfaces, animals, and soil (Bos et al., 2007). They are considered as one of the main and biologically most active pro-inflammatory constituents of organic dusts (Sigsgaard et al., 2010).

8.4.2 Glucans

The (1→3)-β-D-glucans are glucose polymers which are part of the cell wall structure of fungi (and of some bacteria), yeasts and mushrooms (Douwes et al., 2003;

Sigsgaard et al., 2005; Williams, 1997). They can also be present in the bran of some cereal (e.g. oat and barley) and be produced as a result of plant synthesis in response to tissue wounds (Finkelman et al., 2005; Lazaridou and Biliaderis, 2007). Their physicochemical properties vary depending on their source. Generally, they are stable molecules, non-soluble in water, and composed of a β-D-linked linear backbone containing anhydroglucose repeat units linked with a glycosidic bond between the 1 and 3 positions and sometimes bearing side chains at position 6 (Williams et al., 2005). In fungi they form the cell wall through a linkage to mannoproteins (i.e. fungal proteins linked with chains of up to several hundred mannoses), proteins, lipids and chitin and the (1→6)-β-side-branches (Miura, 2005). Their exact primary structure, solubility, degree of branching, and molecular weight play an important role in glucans biological activity (Zeković et al., 2005). Glucans are mainly studied for their immunomodulatory properties.

8.4.3 Peptidoglycans and Muramic Acid

Peptidoglycans are composed of amino acids and sugar polymers and form the backbone of the cell walls of bacteria (Figure 8.1). They are present in both gram-positive and gram-negative bacteria (Fig. 8.1). Within gram-positive bacteria, peptidoglycans form the core of the cell wall membrane comprising up to 70% of the composition, whereas in gram-negative bacteria they form only a minor part of the cell wall. Therefore peptidoglycans are considered to be a marker of exposure to gram-positive bacteria. Peptidoglycans are formed by alternating N-acetyl-muramic and N-acetylglucosamine acid residues linked by β -1→4 bonds with a pentapeptide attached to the d-lactoyl group of each combination residue (Vollmer et al., 2008; Meroueh et al., 2006). The N-acetyl-muramic acid constituent is an amino saccharide which is also commonly known as muramic acid and is measured as a marker for the presence and quantification of peptidoglycans (Poole et al., 2010; Van Strien et al., 2004; Lappalainen et al., 2012; Karvonen et al., 2014). Peptidoglycans are known to induce an inflammatory response.

8.4.4 Extracellular Polysaccharides and Ergosterol

Extracellular Polysaccharides (EPS) are stable carbohydrates that dominate the cell wall and periphery of fungal structures including septa, spores and hyphens, whereas ergosterol is a steroid alcohol (sterol) compound of the fungal cell membrane. While their immunomodulatory value is considered rather small, both ergosterol and EPS are considered as good markers for fungal exposures. Particularly EPS from *Aspergillus* and *Penicillium* spp. have been shown to correlate well with the biomass of viable fungi in house dust. On the other hand ergosterol is considered a good marker for both viable and non-viable fungal biomass (Douwes et al., 1999; Casas et al., 2016).

8.5 Methods of Quantification

Overviews of exposure measurement techniques of biological agents including microbial cell wall agents have been described previously by Douwes et al. (2003) and Casas et al. (2016). In short, quantification of microbial cell wall agents relies on the collection of dust followed by subsequent laboratory analysis of the agents within the dust. For an airborne exposure route the preferable sampling method is active airborne sampling: air is sucked though a sampling head by means of a (portable) pump in which dust is captured through filtration. Based on the sampling characteristics of the sampler specific size fractions of dust may be captured. Generally, in occupational studies the inhalable dust fraction is sampled. Alternatively passive sampling methods capturing settling airborne dust may be employed, e.g through air exposure of petri dishes, "pizza boxes" or electrostatic collectors (Frankel et al. 2012b). Instead of airborne sampling many epidemiological studies in the past have relied on dust samples of floor dust samples representing settled dust or mattress dust samples. Those dust samples are collected using a combination of regular vacuum cleaners fitted with specialised sampling devices like nozzles with collection filters or specially designed bags).

Endotoxins contained in the dust are generally measured by the Limulus Amebocyte Lysate (LAL) assay. The LAL is a biological assay which makes use of an enzyme reaction process from the horseshoe crab, Limulus Polyphymus to quantify non-cell bound endotoxins. Results are expressed in Endotoxin Units (EU), a standardized metric introduced to account for differences in biological activity (potency) per mass unit between endotoxins. The assay is very sensitive and available in several formats from which the kinetic colorimetric ones are considered as the most precise, and thus are most commonly used. Inter-laboratory variations have been described, mainly sourcing from differences in sampling and analytical methodologies between laboratories (Chun et al., 2006). To overcome the problem of batch to batch differences and interference, and to protect the horseshoe crab from extinction, an endotoxin assay has recently become available that uses recombinant Factor C (rFC) reagent produced from the cDNA of the Mangrove horseshoe crab (*Cacinoscorpius rotundicauda*) (Ding et al., 1995). Studies in livestock facilities and houses showed good correlation between results from the recombinant Factor C (rFC) assay compared to the LAL assay (Thorne et al., 2010; Alwis and Milton, 2006). However, little is still known on interference of other agents on the rFC assay results. It can be expected that the recombinant assay will be applied more and more in future studies.

Endotoxins can also be measured chemically through gas chromatography / mass spectrometry (GC/MS) to identify and quantify 3-hydroxy fatty acids (3-OHFAs) in the lipid A of endotoxin (Saraf et al., 1997). The method quantifies both cell bound and non-cell bound endotoxin with results expressed in mass concentrations, and thus cannot be compared directly to results obtained with the LAL assay. It has not been widely applied and associations with human health endpoints remain to be fully studied.

Several different assays have been applied in studies investigating (1→3)-β-D-glucans, including assays based on a modification of the Limulus amebocyte lysate (LAL) assay in which only active factor G is present. Earlier this method was referred to as the LAL assay, whereas later a commercially available Glucatell assay became available based on the same principle (Rylander, 1997; Cherid et al., 2011). A number of immunoassays to detect glucans have been developed and applied as well. Initially an inhibition immunoassay was developed (Douwes et al., 1996), which had relatively low sensitivity. More recently, several laboratories have developed more sensitive sandwich Enzyme Immunoassays (EIAs) (Noss et al., 2010b; Sander et al., 2008; Milton et al., 2001). Few data are available comparing outcomes of different (1→3)-β-D-glucans assays where results are typically expressed in units of mass. An interlaboratory comparison study showed that results of different methods were comparable in relative terms as most methods correlated moderately well with each other. Yet direct comparison of results between laboratories and assays is compromised, due to discrepancies in applied standards and extraction procedures resulting in major differences in absolute levels (Brooks et al., 2013). Available comparison data is yet to scarce to provide reliable conversion factors.

Peptidoglycans are determined through GC/MS analysis by quantification of their composite muramic acid (Poole et al., 2010; Van Strien et al., 2004; Lappalainen et al., 2012; Karvonen et al., 2014). The muramic acid content is regarded to be a measure of exposure to gram-positive bacteria. Similarly, ergosterol, which can be determined through GC/MS analyses, is a measure of fungal biomass (Saraf et al., 1997; Miller and Young, 1997). Fungal extracellular polysaccharides (EPS) are considered fungal biomarkers as well, although they allow for a certain level of differentiation of mould genera present. They are measured through a specific sandwich enzyme immunoassay (Douwes et al., 1999).

8.6 Exposure Limits

A number of countries have established occupational exposure limits for exposure to organic dust, which are commonly used as guidelines for advising and protecting workers from overexposure to microbial agents. Generally, these limits have been established based on the available information on exposure levels within certain industries and vary considerably from country to country. For example, the occupational exposure limit (OEL) for organic dust is 3mg/m^3 of ''total'' dust in Denmark and 5mg/m^3 in Norway and Sweden (Arbeidstilsynet, 2011; Arbejdstilsynet, 2007). In the US, the Occupational Safety and Health Administration (OSHA) has since 1989 advised a permissible exposure limit of 10mg/m^3 for total grain dust (OSHA, 1995). Whereas the National Health Council of the Netherlands has recommended a Health-Based OEL (HBROEL) of 1.5mg/m^3 of inhalable grain dust (DECOS, 2011).

However, despite the well-recognised strong inflammatory capability, thus far no agent- and environment-specific (i.e. residential or occupational) health-based limit values for exposure to microbial cell wall agents have been established. The only exception, to our knowledge, is the limit for endotoxin that was established by the National Health Council of the Netherlands in conjunction with the Nordic research council (DECOS, 2010). They jointly proposed a HBROEL of 90 EU/m^3, largely based on acute respiratory effects.

8.7 Exposures in Indoor and Occupational Environments

Despite the broad recognition of different cell wall agents playing a part in the development of respiratory symptoms and other health disorders, relatively little is actually known with respect to their airborne exposure levels and prevalence. Most exposure information is available for endotoxin and (1→3)-β-D-glucans airborne concentrations and an overview of measured airborne levels for these two agents across different occupational and residential environments is provided in Table 8.1. It should be noted that most data from residential environments relate to floor dust and/or mattress dust rather than airborne exposure levels. However, the focus of the current overview is on airborne levels as those are considered to be more representative of inhalation exposures.

In general, the levels of exposure to endotoxins and glucans are very varied across both occupational and residential environments. In occupational settings, levels are clearly dependent on the presence or absence of an exposure source such as manure, composted waste, animals, and/or plant materials. For endotoxin the highest levels of exposure commonly occur among workers in primary agricultural workplaces such as poultry, dairy and pig farms and among those involved in cotton processing and grain handling. Average personal concentrations measured within these industries are reported to typically range between a few hundred to many thousands of EU/m^3 (Table 8.1). Other workplaces with considerably high exposures to endotoxin include waste collection and handling, seed and paper processing and veterinary practices. The levels of exposure within these environments can be several orders of a magnitude higher than those reported within residential and office environments.

Similarly, (1→3)-β-D-glucans exposures appear to be an issue mainly in workplaces of agricultural production, waste collection and management, paper processing as well as podiatry clinics. Direct comparisons between these results however cannot be made because measured concentrations for glucans largely depend on the type and inherent sensitivity of the quantification assay applied within a study (see methods of quantification section above). The higher sensitivity of the LAL assay (Sander et al., 2008; Douwes, 2005) may, at least partly, explain the reported lower levels of exposure in studies that use this methods compared with those using the inhibition enzyme immunoassays (EIA). Other parameters such as the extraction medium, or the type of filter used and its storage or transport

Table 8.1 Overview (non-exhaustive list) of results from studies of airborne endotoxin and (1→3)-β-D-glucans levels within occupational and residential environments

Type of environment	Endotoxin (EU/m^3)					(1 → 3)-β-D-glucan (ng/m^3)				
	Measurement type	Analytical method	Range of means	Range of individual concentrations	References	Measurement type	Analytical method	Range of means	Range of individual concentrations	References
Primary animal production										
Dairy farming	P	KC/T-LAL, rFC	220–1570	<LOD–8290	(Basinas et al., 2012b; Samadi et al., 2012; Garcia et al., 2013; Spaan et al., 2006; Smit et al., 2008; Saito et al., 2009; Burch et al., 2010)	P	SI-EIA	10,300	150–232,000	(Samadi et al., 2012)
Pig farming	P	KC/T-LAL, rFC	400–6600	<LOD–374,000	(Basinas et al., 2012b; Smit et al., 2008; O'Shaughnessy et al., 2010; Simpson et al., 1999; Szadkowska-Stańczyk et al., 2010; Radon et al., 2002)	P	Glucatell	223	6–5208	(Szadkowska-Stańczyk et al., 2010)
						P	SI-EIA	4340	200–38,490	(Douwes et al., 1996)
						S	SI-EIA	NR	33–410	(Sander et al., 2008)
						S	Glucatell	NR	18–96	(Sander et al., 2008)
Poultry farming, general		KC/T-LAL	2576	190–16,348	(Radon et al., 2002)	S	Glucatell	NR	13–5000	(Sander et al., 2008)
						S	SI-EIA	NR	2–972	(Sander et al., 2008)

(continued)

Table 8.1 (continued)

Type of environment	Endotoxin (EU/m^3)					(1 → 3)-β-D-glucan (ng/m^3)				
	Measurement type	Analytical method	Range of means	Range of individual concentrations	References	Measurement type	Analytical method	Range of means	Range of individual concentrations	References
Poultry farming, layers	P	KC/T-LAL, rFC	694–7517	1162–19,745	(Basinas et al., 2012b; Spaan et al., 2006; Senthilselvan et al., 2011; Arteaga et al., 2015)					
Poultry farming, broilers	P	KC/T-LAL	596–9609	61–8120	(Spaan et al., 2006; Senthilselvan et al., 2011)					
Mink farming	P	KC/T-LAL	214	93–1050	(Basinas et al., 2012b)					
Mixed livestock production farming	P	KC/T-LAL	448	<LOD–2910	(Basinas et al., 2012b)					
Horse keeping/ farming	P	KC/T-LAL	742	92–9846	(Samadi et al., 2009)	P	SI-EIA	9500	<LOD–631,000	(Samadi et al., 2009)
Plant cultivation										
Field crops (arable)	P	KC/T-LAL	63–2700	96–41,200	(Spaan et al., 2006; Smit et al., 2008)					
Mushrooms	P	KC/T-LAL	110	10–4450	(Simpson et al., 1999)					
Flowers, greenhouses	P	KC/T-LAL	27–140	0.84–1097	(Thilsing et al., 2015; Spaan et al., 2006)					
Vegetables, greenhouses	P	KC/T-LAL	13–1180	5.4–4020	(Spaan et al., 2006; Madsen et al., 2009)					

(continued)

Table 8.1 (continued)

Type of environment	Endotoxin (EU/m^3)					(1 → 3)-β-D-glucan (ng/m^3)				
	Measurement type	Analytical method	Range of means	Range of individual concentrations	References	Measurement type	Analytical method	Range of means	Range of individual concentrations	References
Industrial processing of agriculture products										
Abattoirs	P	KC/T-LAL	28–310	27–6230	(Spaan et al., 2006)					
Seed processing, grass and cereals	P	KC/T-LAL	1160–12,869	9.1–79,900	(Madsen et al., 2012; Spaan et al., 2008a)	P	LAL	3.83	2.82–4.84	(Madsen et al., 2012)
Seed processing, vegetables	P	KC/T-LAL	22–770	25.6–42,200	(Spaan et al., 2008a)					
Fruit and vegetable preservation	P	KC/T-LAL	61	4.9–1200	(Spaan et al., 2006)					
Grain handling and animal feed industry	P	KC/T-LAL	270–628	11–80,500	(Spaan et al., 2008a; Halstensen et al., 2013)	P	SI-EIA	7400	200–1,290,000	(Halstensen et al., 2013)
Waste collection and management										
Domestic waste collection	P	KC/T-LAL	40	<4–7182	(Wouters et al., 2006)	P	SI-EIA	1220	<260–52,500	(Wouters et al., 2006)
	S	KC/T-LAL	5–7		(Thorn et al., 1998)	S	LAL	9.2–19.1		(Thorn et al., 1998)

(continued)

Table 8.1 (continued)

Type of environment	Endotoxin (EU/m^3)					(1 → 3)-β-D-glucan (ng/m^3)				
	Measurement type	Analytical method	Range of means	Range of individual concentrations	References	Measurement type	Analytical method	Range of means	Range of individual concentrations	References
Power plants (biofuel/mass)	P	KC/T-LAL	9-200	<3–2104	(Wouters et al., 2006)	P	SI-EIA	<100–290,900	<100–290,900	(Wouters et al., 2006)
Composting, domestic waste	P	KC/T-LAL	17–1038	<3–37,043	(Wouters et al., 2006)	P	SI-EIA	<600–4930	<150–206,600	(Wouters et al., 2006)
Composting, green waste	P	KC/T-LAL	6–32	<3–345	(Wouters et al., 2006)	P	SI-EIA	<600–530,000	<600–2850	(Wouters et al., 2006)
waste transferral	P	KC/T-LAL	36–520	16–3536	(Wouters et al., 2006)					
Sewage treatment	P	KC/T-LAL	15.4	0.7–214	(Cyprowski et al., 2015b)					
Wood and paper processing										
Sawmills	P	KC/T-LAL	130	10–1870	(Simpson et al., 1999)					
Sawmills	P	EC-LAL	43	1.9–784	(Mandryk et al., 1999)	P	LAL	1.37	0.16–11.74	(Mandryk et al., 1999)
Joineries	P	EC-LAL	11–24.1	1–279	(Mandryk et al., 1999; Harper and Andrew, 2006)	P	LAL	0. 43	0.11–3.6	(Mandryk et al., 1999)
Wood chipping	P	EC-LAL	32.7	20–487	(Mandryk et al., 1999)	P	LAL	2.32	0.13–10.4	(Mandryk et al., 1999)
Paper processing factories	S	KC/T-LAL	20-977	0–2200	(Rylander et al., 1999)	S	LAL	10–240	49–366	(Rylander et al., 1999)

(continued)

Table 8.1 (continued)

Type of environment	Endotoxin (EU/m^3)					(1 → 3)-β-D-glucan (ng/m^3)				
	Measurement type	Analytical method	Range of means	Range of individual concentrations	References	Measurement type	Analytical method	Range of means	Range of individual concentrations	References
Textile manufacturing and processing										
Cotton mills	P	KC/T-LAL	70–6316	10–26,300	(Simpson et al., 1999; Mehta et al., 2007; Paudyal et al., 2011)					
	S	EC-LAL	10–7500	10–17,000	(Christiani et al., 1993; Christiani et al., 1994)					
	S	KC/T-LAL	37–4556	2–18,344	(Mehta et al., 2007; Marchand et al., 2007)					
Wool mill	P	KC/T-LAL	960	10–3045	(Simpson et al., 1999)					
Hemp	P	KC/T-LAL	19,569	4734–59,801	(Fishwick et al., 2001)					
Other workplaces										
Metal working/ machining plants	P	KC/T-LAL	2	1–31	(Cyprowski et al., 2015a)					
	S	EC-LAL	25.3	<LOD–183	(Gilbert et al., 2010)					
Veterinary clinics, companion animals	P	KC/T-LAL	4.4	<LOD–75	(Samadi et al., 2011)	P	SI-EIA	3.39	<LOD–111.5	(Samadi et al., 2011)

(continued)

Table 8.1 (continued)

Type of environment	Endotoxin (EU/m^3)					(1 → 3)-β-D-glucan (ng/m^3)				
	Measurement type	Analytical method	Range of means	Range of individual concentrations	References	Measurement type	Analytical method	Range of means	Range of individual concentrations	References
Veterinary clinics, farm animals	P	KC/T-LAL	520–1498	60–49,846	(Samadi et al., 2011)	P	SI-EIA	3.10	<LOD–46.1	(Samadi et al., 2011)
Podiatry clinics	P	KC/T-LAL	9.6	0.5–32.6	(Coggins et al., 2012)					
Laboratories with animals						S	Glucatell	NR	13–5,000	(Sander et al., 2008)
						S	SI-EIA	NR	16–38	(Sander et al., 2008)
Public and social service workplaces										
Office buildings	S	EC-LAL	0.5–3		(Reynolds et al., 2001; Rylander et al., 1992)	S	LAL	<0.1–3.2		(Rylander et al., 1992; Wan and Li, 1999)
Schools without sources	S	KC/T-LAL	9.34	<2.83–>225	(Holst et al., 2015b)	S	LAL	2.9	0–6.9	(Rylander et al., 1998)
Schools with sources	S	KC/T-LAL	2.1–2.6		(Rylander et al., 1992)	S	LAL	0.49–15.3	9.2–27.4	(Rylander et al., 1998; Rylander et al., 1992)

(continued)

Table 8.1 (continued)

Type of environment	Endotoxin (EU/m^3)					(1 → 3)-β-D-glucan (ng/m^3)				
	Measurement type	Analytical method	Range of means	Range of individual concentrations	References	Measurement type	Analytical method	Range of means	Range of individual concentrations	References
Daycare centres	S	EC-LAL	24.3		(Rylander et al., 1992)	S	LAL	0.2–5.7		(Rylander et al., 1992; Wan and Li, 1999)
	S	KC/T-LAL	1.6		(Wan and Li, 1999)					
Dwellings										
Residence, general	S	KC/T-LAL	0.36–6.5	<0.005–389.2	(Noss et al., 2008; Wan and Li, 1999; Frankel et al., 2012a; Park et al., 2000; Singh et al., 2011; Dassonville et al., 2008)	S	Glucatell	1.96	0.002–41.91	(Singh et al., 2011; Thorn and Rylander, 1998)
						S	LAL	3.7		(Wan and Li, 1999)
Residence with sources	S	KC/T-LAL	22.8–64	4–256	(Semple et al., 2010; Adhikari et al., 2010)	S	LAL	3.1–15.9		(Adhikari et al., 2010)
Farm residence	S	KC/T-LAL	1.04		(Noss et al., 2008)					

EU/m^3 = Endotoxin Unit per cubic meter; ng/m^3=nanogram per cubic meter; P=personal sampling; S=Stationary/areal sampling; LAL= Limulus amebocyte lysate assay; EC-LAL=Endpoint chromogenic LAL assay; KC/T-LAL= Kinetic and/or Turbidimetric chromogenic LAL assay; rFC= recombinant Factor C Assay; SI-EIA=Specific Inhibition Enzyme-linked ImmunoAssay; Glucatell= Glucatell modification of the LAL assay; LOD=Limit of Detection; NR=Not Reported.

conditions may also play a role as has been described for endotoxin (Noss et al., 2010a; Spaan et al., 2007). However, such analytical errors are unlikely to be a major contributor to the total variability of exposure for these agents as differences in intra-laboratory variations are generally small.

Besides exposure sources, other important determinants of endotoxin exposure include the dustiness of materials handled, the production in bulk (i.e. in large quantities), and the cyclical nature of the process (Spaan et al., 2008a). Personal levels of exposure largely depend on the activities performed by the workers as well as the environmental conditions and workplace characteristics. For example, among livestock workers practices related to ventilation, animal feeding, distribution of bedding and improved building hygiene have been demonstrated as important determinants for exposure to endotoxins and (1 → 3)-β-D-glucans (Basinas et al., 2015; Samadi et al., 2009; Thilsing et al., 2015). Similarly, in sewage treatment plants higher exposures have been reported among workers performing activities related to cleaning and maintenance (Spaan et al., 2008b).

Within residential environments the level of airborne endotoxin exposure has been reported to average between 0.36-6.5 EU/m^3 in absence of an obvious exposure source (Table 8.1) which is similar to that reported for the general environment (Madsen, 2006). However, in other settings where a direct source of exposure is present, such as the burning of biomass, airborne endotoxin levels may increase to 64.0 EU/m^3 (Table 8.1). Similar differences in exposure patterns have been reported for glucan exposures with burning of biomass (Semple et al., 2010) and with the presence of moisture/mould problems within the building (Adhikari et al., 2010). The importance of mould as an exposure source for residential and public environments is well documented also from exposure studies in schools and office buildings (Rylander et al., 1998) and this is broadly supported by results from studies that utilised samples of settled house dust, like floor dust and mattress dust (Douwes et al., 1999; Douwes et al., 1998; Schram et al., 2005; Gehring et al., 2001). Very little information is available concerning other determinants of airborne levels of these agents within home environments. However, results from studies on settled house dust suggest that keeping pets, the number of occupants in the home, the flooring type, whether or not the house is a farm residence, the season and the heating system are important factors in determining the dust composition in these environments (Douwes et al., 1998; Schram et al., 2005; Giovannangelo et al., 2007; Casas et al., 2013; Abraham et al., 2005; Holst et al., 2015a).

The other microbial cell wall agents which have been reported to be elevated in settled dust from indoor environments of houses and farms include muramic acid and ergosterol (Poole et al., 2010; Van Strien et al., 2004) as well as EPS (Giovannangelo et al., 2007; Casas et al., 2013). However, little is known about actual airborne levels of these agents (Dales et al., 2006; Adhikari et al., 2014). Furthermore, it has to be noted that collection of samples and analysis of settled house dust, primarily from floor and mattresses, has been the most common approach for determination of microbial cell wall agent concentrations in epidemiological studies in the home environment. This is due to the increased

cost-efficiency of these sampling strategies compared to active airborne dust sampling, as they allow collection of dust to be performed by the participants themselves. As deposited dust is time-integrated, it is less vulnerable to short term variation in exposure and allows relative ranking of exposure levels (Douwes, 2005; Tischer et al., 2011). Nevertheless, results obtained through these methods are unlikely to be fully representative of actual airborne levels and personal exposure within indoor home environments (Adhikari et al., 2010; Adhikari et al., 2014; Noss et al., 2008; Samadi et al., 2010). Recently a simple and rather inexpensive method for passive collection of airborne dusts, the Electrostatic Dustfall Collectors (EDCs), has become available which is proving rather promising with regard to sampling efficiency for endotoxin and glucans (Noss et al., 2010a; Noss et al., 2008; Samadi et al., 2010; Frankel et al., 2012b; Jacobs et al., 2014).

8.8 Health Effects

8.8.1 Endotoxin Exposure and the Janus Faced Effect on Health

Endotoxin is a well-established pro-inflammatory agent with a broad range of health effects documented in epidemiological, toxicological, and experimental studies in humans. It is considered one of the main causes of respiratory disease in populations highly exposed to organic dusts such as farmers, cotton and grain workers (Rylander, 2006). Endotoxin can cause both acute and chronic effects. Endotoxin exposure has been linked to acute symptoms such as wheezing, dyspnea, irritation of the nose and throat, chest tightness, dry cough, fever, headache, and acute airway obstruction and inflammation (Douwes et al., 2003; Rylander, 2006; Bakirci et al., 2007; Castellan et al., 1987). High endotoxin exposure has been shown to cause organic dust toxic syndrome (ODTS) and to increase the risk of chronic respiratory diseases, including extrinsic allergic alveolitis (i.e. Famer's lung), chronic bronchitis, accelerated lung function decline, asthma and asthma-like syndrome. Endotoxin can also simply increase disease severity by causing lung function adverse effects and promoting inflammatory responses (Smit et al., 2006; Donham et al., 2000; Sigsgaard et al., 2004; Wang et al., 2002; Liu, 2002). Positive associations between endotoxin and malignant disease such as nasopharyngeal cancers have also been reported among cotton workers(Li et al., 2006; Fang et al., 2013). In contrast, more recently a protective effect of endotoxin exposure against lung cancer has also been proposed (Lenters et al., 2010). However, evidence supporting this association remains limited primarily to studies among cotton workers (Astrakianakis et al., 2007; McElvenny et al., 2011). Respiratory symptoms and bronchial hyperresponsiveness have been demonstrated among workers and healthy volunteers to initiate with exposure levels in the range of 100 to 200 EU/m^3 (Basinas et al., 2012a; Smit et al., 2008; Castellan et al., 1987; Larsson et al., 1994; Smit et al., 2010; Latza et al., 2004).

During recent decades evidence has become available for an inverse association between endotoxin exposure and atopy, allergic rhinitis and/or atopic asthma. These protective effects from endotoxin have been observed particularly among children (Braun-Fahrlander et al., 2002; Gereda et al., 2000; Douwes et al., 2006; Schram-Bijkerk et al., 2005; Von Mutius et al., 2000) but also among adults, in workers such as farmers (Eduard et al., 2004; Portengen et al., 2005) agriculture workers (Basinas et al., 2012a; Smit et al., 2008; Smit et al., 2010) and even for residential endotoxin exposures (Gehring et al., 2004; Bakolis et al., 2012). Among the adult population the protective effects of endotoxin against atopy and atopic sensitization were always observed in conjunction with a significant increase in risk for non-allergic respiratory morbidity (Basinas et al., 2012a; Eduard et al., 2004; Smit et al., 2008; Smit et al., 2010; Portengen et al., 2005) suggesting a Janus-faced (i.e. dual) role for endotoxin on the development of health symptoms among humans. For example, in a pooled analysis of four epidemiological studies from the Netherlands and Denmark including workers in farming, agricultural processing and power plants using biofuel as well as students in veterinary medicine, an inverse dose-dependent association between measured endotoxin exposure and allergic sensitization and hay fever (i.e. allergic rhinitis) was observed (Basinas et al., 2012a). However, in the same population increased endotoxin exposure was associated with an increased risk for organic dust toxic syndrome and chronic bronchitis when exposure exceeded 100 EU/m^3 (Fig. 8.2).

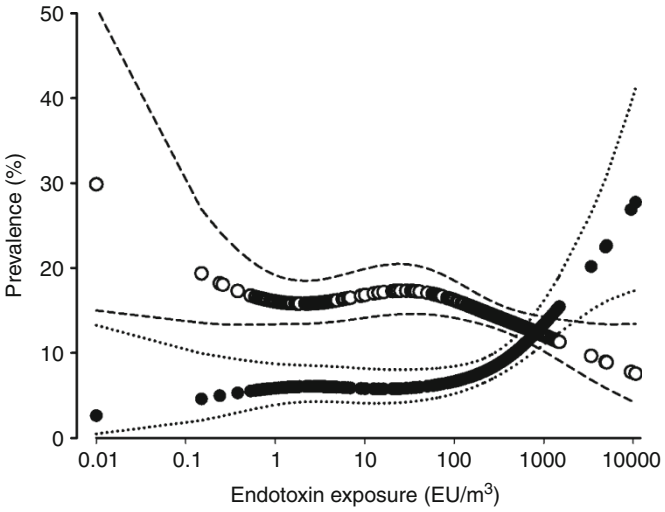

Fig. 8.2 The association between endotoxin exposure and prevalence of hay fever (circles) and chronic bronchitis (filled circles) in a population of 3883 Dutch and Danish employees in veterinary medicine, power plants using biofuel, agricultural processing, and farming. (From Basinas et al., 2012a)

These findings are in line with the hygiene hypothesis (see below) and suggest that some individuals may be more susceptible to endotoxin exposure than others. Though initial interpretation of these findings was fairly cautious, because of the cross-sectional nature of the research studies, emerging results from longitudinal studies among Danish farmers and Dutch agricultural workers seem to confirm the protective effects of adult endotoxin exposure on atopy and atopic sensitization (Elholm et al., 2011; Spierenburg et al., 2016). The individual immunological response to endotoxin exposure is determined by the interaction between dose and timing of exposure, other environmental factors and genetic predisposition (Vandenbulcke et al., 2006).

8.8.2 A Proposed Immunological Mechanism Supporting the Hygiene Hypothesis

The hygiene hypothesis suggests that exposure to microbial components like endotoxin promotes the development of a healthy immune system. The adaptive immune response is thus modified by prior events like infection (Liebers et al., 2008). The initial proposed mechanism associated with the hygiene hypothesis was that an increased microbial exposure induces a shift from atopic T-helper type 2 (Th2) responses to Th1-dominated responses through stimulation of the innate immune system. In addition, it has emerged that regulatory T cells (T_{reg}) play a crucial role in suppressing allergic and non-allergic immune responses (Schaub et al., 2006; Renz et al., 2006; Sigsgaard and Heederik, 2005). Toll-like receptors (TLRs) present on the cell surface of innate immune cells recognize microbial motifs called microbial-associated molecular patterns (MAMPs) (Sabroe et al., 2003). Following entry to the body through the airways, endotoxins/LPS will encounter alveolar macrophages carrying CD14 and LPS binding receptors (Ingalls et al., 1999). The binding of LPS to CD14 is mediated by LPS binding protein (LBP). Via toll-like receptors (TLR-3 and TLR-4) (Beutler, 2004) the alveolar macrophages will be activated, leading to the production and release of proinflammatory cytokines (Reed and Milton, 2016). Cytokines associated with endotoxin exposure are TNF-α, interleukin (IL) 1-β, IL-6, and IL-8, as well as metabolites of arachidonic acid. These cytokines will then recruit and activate neutrophils, resulting in local and systemic inflammation with leukocytosis and neutrophilia. This effect can also be seen experimentally or observationally: swine dust, cotton dust, or grain dust exposure is found to increase IL-1β, IL-6, IL-8, TNF-α, and circulating neutrophils in the airways and causes airway obstruction and methacholine responsiveness (Li et al., 1995; Schwartz et al., 1995; Wang et al., 1999; Wang et al., 1997; Senthilselvan et al., 1997; Malmberg and Larsson, 1993; Forteza et al., 1994; Jorna et al., 1994; Rylander and Bergstrom, 1993). Impairment of TLR4 has also been found to be associated with a history of atopic disease (Prefontaine et al., 2010).

8.8.3 The Role of T Regulatory Cells (T_{reg})

Lack of functional T_{reg} cells, due to a defect in T_{reg} activation is associated with insufficient repression of both Th1 and Th2 immune responses and has been found to be associated with atopic disease (Savilahti et al., 2010; Braga et al., 2011; Braga et al., 2012; O'Garra and Vieira, 2004). $Treg_s$ are a subpopulation of T cells which modulate the immune system, maintain tolerance to self-antigen, and prevent autoimmune disease. T regulatory cells are a T cell subset that produces IL-10 and TGF-β. T_{reg} cells may act both by cytokine production and by cell–cell contact signals, as programmed death-1, glucocorticoid-induced TNF receptor, membrane TGF-β, and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). T_{reg} cells contribute to the control of allergen-specific immune responses in five major ways: (1) T_{reg} cells suppress antigen-presenting cells that support the generation of effector Th2 and Th1 cells. (2) They suppress Th2 and Th1 cells. (3) They regulate B cells by suppression of allergen-specific Immunoglobulin E (IgE) antibodies and induction of Immunoglobulin G4 (IgG4), A (IgA), or both. (4) They suppress mast cells, basophils, and eosinophils. (5) They interact with resident tissue cells and remodeling (Braga et al., 2012).

8.8.4 Microbial Diversity vs. The Effect of Single Agents

Besides endotoxin, Ege et.al (Ege et al., 2011) recently argued that most likely it is the diversity and wider range of types of microbes offered by the farming environment that contributes to beneficial effects of farming exposure, rather than a single agent such as endotoxin. Other studies have tried to determine the effect of specific microorganisms on the development of allergies, and recently the effect of exposure to Acinetobacter lwoffii F78 and Lactococcus lactis G121 was investigated (Debarry et al., 2007). These two bacteria are in particular found on cattle farms. Both bacteria showed an ability to reduce allergic reactions in mice, to activate mammalian cells in vitro, and to induce a Th1-polarizing program in dendritic cells (Brand et al., 2011). Findings like these suggest that exposure to other components than cell wall agents may affect health as well, however the specific role and contribution to the health effects of the various microbial agents as well as their potential synergic effects with cell wall agents is still to be established.

8.8.5 Diverse Microbial Exposure and TLR Expression

Research has shown that prenatal and/or early life exposure to the rich microbial environment of traditional farms induces an up-regulation of innate immunity receptors that is both robust and long-lasting (Stern et al., 2007). Exposure of the mother during pregnancy to inhalant allergens is less likely to result in sensitization in the child than exposure of the child in early infancy (Kihlstrom et al., 2003; Szepfalusi et al., 2000). It has been seen that peripheral blood cells from

farm children expressed significantly higher levels of *CD14*,Toll-like receptor 2 (*TLR2*) and Toll-like receptor 4 (*TLR4*) than cells from non-farm children. Furthermore, it was indicated that it was farming exposure of the pregnant mothers that were associated with the enhanced expression (Ege et al., 2006; Lauener et al., 2002). Additionally reduced maternal T_{reg} numbers and increased *Th2* cytokine production during pregnancy has been found to influence the allergy risk of the child (Hinz et al., 2010). There is evidence that among children of farmers genetic variation in *TLR2* is a major determinant of the susceptibility to asthma and allergies (Eder et al., 2004).

8.8.6 (1 → 3)-β-D-Glucan Exposure and Known Health Effects

Indoor exposure to fungi has been associated with the development of respiratory symptoms, though the mechanisms are far from clear (Douwes, 2005). It has been shown that (1 → 3)-β-D-glucan can initiate a wide range of biological responses in vertebrates including stimulation of the mononuclear phagocyte system (Di Luzio, 1979), activation of neutrophils (Zhang and Petty, 1994), macrophages (Adachi et al., 1994; Lebron et al., 2003), complement (Saito et al., 1992) and possibly eosinophils (Mahauthaman et al., 1988). These potent biological properties of (1 → 3)-β-D-glucan are relevant irrespective of originating from either live or dead organisms. However, clarifying the health effect of (1 → 3)-β-D-glucan exposure has so far been very challenging and largely inconclusive as many studies have reported conflicting results. Some of the health effects which have been evaluated include lung function [forced expiratory volume in 1 s (FEV1) and peak flow (PEF) variability], nasal congestion, airway hyperreactivity, atopy, symptoms (upper and lower respiratory symptoms, eye irritations, head ache, fatigue/tiredness, joint pains, skin symptoms, flu-like symptoms, nausea, gastro-intestinal symptoms), inflammation characterized by inflammatory cells (T-lymphocytes, neutrophils, eosinophils, macrophages), and cytokines and other inflammatory markers –i.e interleukin (IL)-1ß, IL-4, IL-6, IL-8, IL-10, Interferon (INF)-c, Tumour necrosis factor (TNF)-a, Eosinophil cationic protein (ECP), Myeloperokidase (MPO), C-reactive protein (CRP), albumin- in blood, sputum and nasal lavage (Douwes, 2005).

In an epidemiological context positive associations with glucan exposures have been reported among both adults and children in relation to symptoms of upper airway irritation and inflammation, airway responsiveness, increased peak expiratory flow variability, systemic reactions and atopy (Gladding et al., 2003; Rylander et al., 1999; Thorn et al., 1998; Thorn and Rylander, 1998; Douwes et al., 2000; Bønløkke et al., 2006). Interpretation of the study findings though need to be made cautiously as population sizes were rather small, study designs were cross-sectional and in some cases potential interactions with other co-existing exposures were not taken into account. In a number of studies strong correlations between endotoxin and (1 → 3)-β-D-glucan levels have been reported and previously experimental studies in animals have suggested inflammatory responses to enhance in response to combinations of glucans and endotoxin exposures (Douwes, 2005). More research studies with improved and standardised

exposure assessments in longitudinal designs are warranted to provide insight on the actual health effects of exposure to glucans.

8.8.7 Health Effects of Other Cell Wall Agents

As mentioned earlier, to date only a limited number of studies addressed the health effects of cell wall agents other than endotoxins and (1 → 3)-β-D-glucans. There is some evidence for a potential and maybe even independent role for muramic acid and ergosterol in the development of health symptoms. Specifically, in a case comparison study of symptomatic and non-symptomatic workers of an office building with a history of water damage Park et al. (2008) examined the association between house dust measured fungi, ergosterol and endotoxin levels and asthma. The authors reported increased levels of ergosterol and total fungi to be associated with an increased prevalence of current asthma (Park et al., 2008). A similar association has also been reported in a cross-sectional analysis of the 1996 follow up of the European Community Respiratory Health Survey (ECRHS) cohort (Dharmage et al., 2001). However, cross-sectional studies from Canada reported no association between ergosterol and respiratory symptoms and cough among elementary school children (Dales et al., 1999), whereas neither ergosterol nor indoor moulds seem to influence the illness-associations with endotoxin exposure in infants (Dales et al., 2006). In contrast to these findings, among school-aged farm children from Austria, Germany, and Switzerland, increased levels of muramic acid were found to be associated with lower prevalence of wheezing but not with atopic sensitization (Van Strien et al., 2004). An inverse association between increased levels of muramic acid in classroom dust and the prevalence of wheeze and daytime breathlessness has been reported also among Chinese school children (Zhao et al., 2008). Based on these findings muramic acid like endotoxin has been suggested to serve as an independent marker of microbial exposure (Van Strien et al., 2004). Similar inverse associations have been found between EPS exposure in mattress dust in German school children and doctor-diagnosed asthma and rhinitis(Tischer et al., 2011). More recently, chitin, one of the earliest identified and most abundant extracellular polysaccharides in nature, has been hypothesised as playing a role in the development of asthma and allergies but the actual supporting evidence to date remains rather small (Brinchmann et al., 2011).

8.9 Conclusions and Future Directions

We spend a large proportion of our time indoors, and it is needless to say that our indoor environment will affect us for better or for worse. Indoor and occupational exposures to microbial cell wall agents and their associated health effects are far from elucidated. It is therefore of great importance to continue to improve our understanding of cell wall component agents that contaminate our indoor air and

how they affects us. It is clear that the well-studied endotoxins are involved in the development of the adverse and protective health effects, but for glucans the evidence is more limited and inconclusive. There is some evidence that other microbial cell wall agents are involved in the development of the adverse and/or protective health effects as well. However, relevant studies have been sparse and very diverse in their design and applied methods.

In addition, the literature shows large variation in exposure to microbial cell wall agents in indoor occupational environments, and we still simply lack studies of actual airborne levels of exposures and determinants of residential indoor air. The fact that many different assays and sampling methods have been deployed for evaluation of exposures and levels complicates comparison of results and affects the establishment of proper exposure limits to protect workers from excess exposure to these agents. Standardisation in methods of determination is highly recommended for future studies as well as a broader adaptation of the recently available passive airborne dust sampling methods (e.g. EDCs or dustfall collectors) for residential exposures. It has recently been suggested that both PM10 and PM>10 size fractions elicit a pro-inflammatory response in airway epithelial cells (Hawley et al., 2015), which means that dust size fractions should be taken into consideration when assessing potential risks from exposure to agricultural dusts and other microbial agents which could be found in the indoor environment.

Next to direct effects of cell-wall agents, other components and/or microbial diversity might be important with respect to both detrimental and beneficial health effects. The development and application of molecular techniques in exposure assessment – as reviewed by Casas et al. (2016) – will aid to study the role of microbial diversity and specific microbes in future studies, and may help to understand the role of the individual and combined exposures in health. Such knowledge is highly needed both for the development of targeted prevention strategies and the establishment of adequate exposure limits especially within workplaces. Further research, in particular studies in large populations with a longitudinal design involving the assessment of the health effects of both distinct microbial cell wall agents and co-existent microbes is needed to provide more in-depth insight.

References

Abraham JH, Gold DR, Dockery DW et al (2005) Within-home versus between-home variability of house dust endotoxin in a birth cohort. Environ Health Perspect 113:1516–21

Adachi Y, Okazaki M, Ohno N, Yadomae T (1994) Enhancement of cytokine production by macrophages stimulated with (1–>3)-beta-D-glucan, grifolan (GRN), isolated from Grifola frondosa. Biol Pharm Bull 17:1554–60

Adhikari A, Kettleson EM, Vesper S et al (2014) Dustborne and airborne Gram-positive and Gram-negative bacteria in high versus low ERMI homes. Sci Total Environ 482–483:92–9. doi:10.1016/j.scitotenv.2014.02.110

Adhikari A, Lewis JS, Reponen T et al (2010) Exposure matrices of endotoxin, (1→3)-β-d-glucan, fungi, and dust mite allergens in flood-affected homes of New Orleans. Sci Total Environ 408:5489–98. doi:10.1016/j.scitotenv.2010.07.087

Alwis KU, Milton DK (2006) Recombinant factor C assay for measuring endotoxin in house dust: comparison with LAL, and (1 –> 3)-beta-D-glucans. Am J Ind Med 49:296–300. doi:10.1002/ajim.20264

Arbejdstilsynet (2007) At-vejledning. Grænseværdier for stoffer og materialer. [Danish Working Environment Authority. Limit values for substances and materials]. Publication no. C.0.1. The Danish Working Environment Authority, Copenhagen, Denmark. https://arbejdstilsynet.dk/da/regler/at-vejledninger/g/c-0-1-graensevaerdi-for-stoffer-og-mat. Accessed 05 Jan 2016

Arbeidstilsynet (2011) Veiledning om administrative normer for forurensning i arbeidsatmosfære. [The Norwegian Labour Inspection Authority. Guidance for administrative standards for contamination of the work environment]. Manual no. 361. Trondheim, Norway. Available via http://www.arbeidstilsynet.no/binfil/download2.php?tid=77907. Accessed 05 Jan 2016

Arteaga V, Mitchell D, Armitage T et al (2015) Cage versus noncage laying-hen housings: respiratory exposures. J Agromed. 20:245–255. doi:10.1080/1059924X.2015.1044681

Astrakianakis G, Seixas NS, Ray R et al (2007) Lung cancer risk among female textile workers exposed to endotoxin. J Natl Cancer Inst 99:357–364. doi:10.1093/jnci/djk063

Bakirci N, Kalaca S, Francis H et al (2007) Natural history and risk factors of early respiratory responses to exposure to cotton dust in newly exposed workers. J Occup Environ Med 49:853–61. doi:10.1097/JOM.0b013e3180dca598

Bakolis I, Doekes G, Heinrich J et al (2012) Respiratory health and endotoxin: associations and modification by CD14/-260 genotype. Eur Respir J 39:573–81. doi:10.1183/09031936.00164410

Basinas I, Schlunssen V, Heederik D et al (2012a) Sensitisation to common allergens and respiratory symptoms in endotoxin exposed workers: a pooled analysis. Occup Environ Med 69:99–106. doi:10.1136/oem.2011.065169

Basinas I, Sigsgaard T, Heederik D et al (2012b) Exposure to inhalable dust and endotoxin among Danish livestock farmers: results from the SUS cohort study. J Env Monit 14:604–614. doi:10.1039/c1em10576k

Basinas I, Sigsgaard T, Kromhout H et al (2015) A comprehensive review of levels and determinants of personal exposure to dust and endotoxin in livestock farming. J Expo Sci Env Epidemiol 25:123–137. doi:10.1038/jes.2013.83

Beutler B (2004) Inferences, questions and possibilities in Toll-like receptor signalling. Nature 430:257–263

Bønløkke JH, Stridh G, Sigsgaard T et al (2006) Upper-airway inflammation in relation to dust spiked with aldehydes or glucan. Scand J Work Environ Health 32:374–82

Bos JD, Meinardi MM (2000) The 500 Dalton rule for the skin penetration of chemical compounds and drugs. Exp Dermatol 9:165–9

Bos MP, Robert V, Tommassen J (2007) Biogenesis of the Gram-Negative bacterial outer membrane. Ann Rev Microbiol 61:191–214. doi:10.1146/annurev.micro.61.080706.093245

Braga M, Quecchia C, Cavallucci E et al (2011) T regulatory cells in allergy. Int J Immunopathol Pharmacol 24:55S–64S

Braga M, Schiavone C, Di Gioacchino G et al (2012) Environment and T regulatory cells in allergy. Sci Total Environ 423:193–201. doi:10.1016/j.scitotenv.2010.08.015

Brand S, Teich R, Dicke T et al (2011) Epigenetic regulation in murine offspring as a novel mechanism for transmaternal asthma protection induced by microbes. J Allergy Clin Immunol 128:617–618. doi:10.1016/j.jaci.2011.04.035

Braun-Fahrlander C, Riedler J, Herz U et al (2002) Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med 347:869–877. doi:10.1056/NEJMoa020057

Brinchmann BC, Bayat M, Brøgger T et al (2011) A possible role of chitin in the pathogenesis of asthma and allergy. Ann Agric Environ Med 18:7–12

Brooks CR, Siebers R, Crane J et al (2013) Measurement of β-(1,3)-glucan in household dust samples using Limulus amebocyte assay and enzyme immunoassays: an inter-laboratory comparison. Environ Sci Process Impacts 15:405–11. doi:10.1039/c2em30749a

Burch JB, Svendsen E, Siegel PD et al (2010) Endotoxin exposure and inflammation markers among agricultural workers in Colorado and Nebraska. J Toxicol Env Heal A 73:5–22. doi:10.1080/15287390903248604

Casas L, Tischer C, Täubel M (2016) Pediatric asthma and the indoor microbial environment. Curr Environ Heal Reports 3:238–249. doi:10.1007/s40572-016-0095-y

Casas L, Tischer C, Wouters IM et al (2013) Endotoxin, extracellular polysaccharides, and beta (1-3)-glucan concentrations in dust and their determinants in four European birth cohorts: results from the HITEA project. Indoor Air 23:208–218. doi:10.1111/ina.12017

Castellan RM, Olenchock SA, Kinsley KB, Hankinson JL (1987) Inhaled endotoxin and decreased spirometric values. An exposure-response relation for cotton dust. N Engl J Med 317:605–610. doi:10.1056/NEJM198709033171005

Cherid H, Foto M, Miller JD (2011) Performance of two different Limulus amebocyte lysate assays for the quantitation of fungal glucan. J Occup Environ Hyg 8:540–3. doi:10.1080/15459624.2011.601994

Cherrie JW, Semple S, Christopher Y et al (2006) How important is inadvertent ingestion of hazardous substances at work? Ann Occup Hyg 50:693–704. doi:10.1093/annhyg/mel035

Christiani DC, Velazquez A, Wilcox M, Olenchock SA (1993) Airborne endotoxin concentrations in various work areas within a cotton mill in Central America. Environ Res 60:187–192. doi:10.1006/enrs.1993.1026

Christiani DC, Ye TT, Wegman DH et al (1994) Pulmonary function among cotton textile workers. A study of variability in symptom reporting, across-shift drop in FEV1, and longitudinal change. Chest 105:1713–21. doi:10.1378/chest.105.6.1713

Chun DT, Bartlett K, Gordon T et al (2006) History and results of the two inter-laboratory round robin endotoxin assay studies on cotton dust. Am J Ind Med 49:301–306. doi:10.1002/ajim.20266

Coggins MA, Hogan VJ, Kelly M et al (2012) Workplace exposure to bioaerosols in podiatry clinics. Ann Occup Hyg 56:746–753. doi:10.1093/annhyg/mer124

Cyprowski M, Ławniczek-Wałczyk A, Górny RL (2015a) Airborne peptidoglycans as a supporting indicator of bacterial contamination in a metal processing plant. Int J Occup Med Environ Health 29:427–437. doi:10.13075/ijomeh.1896.00594

Cyprowski M, Sobala W, Buczyńska A, Szadkowska-Stańczyk I (2015b) Endotoxin exposure and changes in short-term pulmonary function among sewage workers. Int J Occup Med Environ Health 28:803–811. doi:10.13075/ijomeh.1896.00460

Dales R, Miller D, Ruest K et al (2006) Airborne endotoxin is associated with respiratory illness in the first 2 years of life. Environ Health Perspect 114:610–614. doi:10.1289/ehp.8142

Dales RE, Miller D, White J (1999) Testing the association between residential fungus and health using ergosterol measures and cough recordings. Mycopathologia 147:21–7

Dassonville C, Demattei C, Vacquier B et al (2008) Indoor airborne endotoxin assessment in homes of Paris newborn babies. Indoor Air 18:480–7. doi:10.1111/j.1600-0668.2008.00549.x

Debarry J, Garn H, Hanuszkiewicz A et al (2007) Acinetobacter lwoffii and Lactococcus lactis strains isolated from farm cowsheds possess strong allergy-protective properties. J Allergy Clin Immunol 119:1514–1521. doi:10.1016/j.jaci.2007.03.023

DECOS (2010) Endotoxins: health based recommended exposure limit. A report of the Health Council of the Netherlands, publication no. 2010/04OSH. Health Council of the Netherlands, The Hague.

DECOS (2011) Grain dust: Health-based recommended occupational exposure limit. A report of the Health Council of the Netherlands, publication no. 2011/13. Health Council of the Netherlands, The Hague.

Dharmage S, Bailey M, Raven J et al (2001) Current indoor allergen levels of fungi and cats, but not house dust mites, influence allergy and asthma in adults with high dust mite exposure. Am J Respir Crit Care Med 164:65–71. doi:10.1164/ajrccm.164.1.9911066

Di Luzio NR (1979) Lysozyme, glucan-activated macrophages and neoplasia. J Reticuloendothel Soc 26:67–81

Ding JL, Navas MA, Ho B (1995) Molecular cloning and sequence analysis of factor C cDNA from the Singapore horseshoe crab, Carcinoscorpius rotundicauda. Mol Mar Biol Biotechnol 4:90–103

Donham KJ, Cumro D, Reynolds SJ, Merchant JA (2000) Dose-response relationships between occupational aerosol exposures and cross-shift declines of lung function in poultry workers: recommendations for exposure limits. J Occup Environ Med 42:260–269

Douwes J (2005) (1–>3)-Beta-D-glucans and respiratory health: a review of the scientific evidence. Indoor Air 15:160–169. doi:10.1111/j.1600-0668.2005.00333.x

Douwes J, Doekes G, Heinrich J et al (1998) Endotoxin and β(1→3)-glucan in house dust and the relation with home characteristics: a pilot study in 25 german houses. Indoor Air 8:255–263. doi:10.1111/j.1600-0668.1998.00006.x

Douwes J, Doekes G, Montijn R et al (1996) Measurement of beta(1–>3)-glucans in occupational and home environments with an inhibition enzyme immunoassay. Appl Env Microbiol 62:3176–3182

Douwes J, Thorne P, Pearce N, Heederik D (2003) Bioaerosol health effects and exposure assessment: progress and prospects. Ann Occup Hyg 47:187–200. doi:10.1093/annhyg/meg032

Douwes J, van der Sluis B, Doekes G et al (1999) Fungal extracellular polysaccharides in house dust as a marker for exposure to fungi: relations with culturable fungi, reported home dampness, and respiratory symptoms. J Allergy Clin Immunol 103:494–500. doi:10.1016/S0091-6749(99)70476-8

Douwes J, van Strien R, Doekes G et al (2006) Does early indoor microbial exposure reduce the risk of asthma? The prevention and incidence of asthma and mite allergy birth cohort study. J Allergy Clin Immunol 117:1067–1073. doi:10.1016/j.jaci.2006.02.002

Douwes J, Zuidhof A, Doekes G et al (2000) (1–>3)-beta-D-glucan and endotoxin in house dust and peak flow variability in children. Am J Respir Crit Care Med 162:1348–54. doi:10.1164/ajrccm.162.4.9909118

Eder W, Klimecki W, Yu L et al (2004) Toll-like receptor 2 as a major gene for asthma in children of European farmers. J Allergy Clin Immunol 113:482–8. doi:10.1016/j.jaci.2003.12.374

Eduard W, Douwes J, Omenaas E, Heederik D (2004) Do farming exposures cause or prevent asthma? Results from a study of adult Norwegian farmers. Thorax 59:381–386

Eduard W, Pearce N, Douwes J (2009) Chronic bronchitis, COPD, and lung function in farmers: the role of biological agents. Chest 136:716–725. doi:10.1378/chest.08-2192

Ege MJ, Bieli C, Frei R et al (2006) Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. J Allergy Clin Immunol 117:817–823. doi:10.1016/j.jaci.2005.12.1307

Ege MJ, Mayer M, Normand A-C et al (2011) Exposure to environmental microorganisms and childhood asthma. N Engl J Med 364:701–709. doi:10.1056/NEJMoa1007302

Elholm G, Omland Ø, Sigsgaard T et al (2011) Endotoxin exposure protects against new onset of pollen sensitisation. Eur Respir J 38:p3315

Fang SC, Mehta AJ, Hang JQ et al (2013) Cotton dust, endotoxin and cancer mortality among the Shanghai textile workers cohort: a 30-year analysis. Occup Environ Med 70:722–9. doi:10.1136/oemed-2012-100950

Finkelman MA, Tamura H (2005) Detection and measurement of (1→3)-beta-D-glucan with Limulus amebocyte lysate-based reagents. In: Young S-H, Castranova V (eds) Toxicology of 1→3-Beta-Glucans: Glucans as a Marker for Fungal Exposure. CRC Press, Taylor & Francis Group, Boca Raton, USA, pp 179–198

Fishwick D, Allan LJ, Wright A, Curran AD (2001) Assessment of exposure to organic dust in a hemp processing plant. Ann Occup Hyg 45:577–83

Forteza R, Lauredo IT, Burch R, Abraham WM (1994) Extracellular metabolites of Pseudomonas aeruginosa produce bronchoconstriction by different mechanisms. Am J Respir Crit Care Med 149:687–693. doi:10.1164/ajrccm.149.3.8118638

Frankel M, Bekö G, Timm M et al (2012a) Seasonal variations of indoor microbial exposures and their relation to temperature, relative humidity, and air exchange rate. Appl Environ Microbiol 78:8289–8297. doi:10.1128/AEM.02069-12

Frankel M, Timm M, Hansen EW, Madsen AM (2012b) Comparison of sampling methods for the assessment of indoor microbial exposure. Indoor Air 22:405–414. doi:10.1111/j.1600-0668.2012.00770.x

Garcia J, Bennett DH, Tancredi D et al (2013) Occupational exposure to particulate matter and endotoxin for California dairy workers. Int J Hyg Environ Health 216:56–62. doi:10.1016/j.ijheh.2012.04.001

Gehring U, Bischof W, Schlenvoigt G et al (2004) Exposure to house dust endotoxin and allergic sensitization in adults. Allergy 59:946–952. doi:10.1111/j.1398-9995.2004.00551.x

Gehring U, Douwes J, Doekes G et al (2001) Beta(1–>3)-glucan in house dust of German homes: housing characteristics, occupant behavior, and relations with endotoxins, allergens, and molds. Environ Health Perspect 109:139–44

Gereda JE, Leung DY, Thatayatikom A et al (2000) Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. Lancet 355:1680–1683. doi:S014067360002239X. [pii]

Gilbert Y, Veillette M, Mériaux A et al (2010) Metalworking fluid-related aerosols in machining plants. J Occup Environ Hyg 7:280–289. doi:10.1080/15459621003680227

Giovannangelo M, Gehring U, Nordling E et al (2007) Determinants of house dust endotoxin in three European countries – the AIRALLERG study. Indoor Air 17:70–79. doi:10.1111/j.1600-0668.2006.00461.x

Gladding T, Thorn J, Stott D (2003) Organic dust exposure and work-related effects among recycling workers. Am J Ind Med 43:584–91. doi:10.1002/ajim.10220

Gorman NgM, Semple S, Cherrie JW et al (2012) The relationship between inadvertent ingestion and dermal exposure pathways: a new integrated conceptual model and a database of dermal and oral transfer efficiencies. Ann Occup Hyg 56:1000–12. doi:10.1093/annhyg/mes041

Halstensen AS, Heldal KK, Wouters IM et al (2013) Exposure to grain dust and microbial components in the norwegian grain and compound feed industry. Ann Occup Hyg 57:1105–1114. doi:10.1093/annhyg/met036

Harper M, Andrew ME (2006) Airborne endotoxin in woodworking (joinery) shops. J Env Monit 8:73–78. doi:10.1039/b508065g

Hawley B, Schaeffer J, Poole JA et al (2015) Differential response of human nasal and bronchial epithelial cells upon exposure to size-fractionated dairy dust. J Toxicol Environ Heal Part A 78:583–594. doi:10.1080/15287394.2015.1015699

Hinz D, Simon JC, Maier-Simon C et al (2010) Reduced maternal regulatory T cell numbers and increased T helper type 2 cytokine production are associated with elevated levels of immunoglobulin E in cord blood. Clin Exp Allergy 40:419–426. doi:10.1111/j.1365-2222.2009.03434.x

Holst G, Host A, Doekes G et al (2015a) Determinants of house dust, endotoxin, and beta-(1–>3)-D-glucan in homes of Danish children. Indoor Air 25:245–259. doi:10.1111/ina.12143

Holst GJ, Høst A, Doekes G, et al (2015b) Allergy and respiratory health effects of dampness and dampness-related agents in schools and homes: a cross-sectional study in Danish pupils. Indoor Air 1–12. doi:10.1111/ina.12275

Ingalls RR, Heine H, Lien E et al (1999) Lipopolysaccharide recognition, CD14, and lipopolysaccharide receptors. Infect Dis Clin North Am 13:341–53. vii

Jacobs JH, Krop EJM, Borras-Santos A et al (2014) Endotoxin levels in settled airborne dust in European schools: the HITEA school study. Indoor Air 24:148–57. doi:10.1111/ina.12064

Jorna TH, Borm PJ, Valks J et al (1994) Respiratory symptoms and lung function in animal feed workers. Chest 106:1050–1055

Karvonen AM, Hyvärinen A, Rintala H et al (2014) Quantity and diversity of environmental microbial exposure and development of asthma: a birth cohort study. Allergy 69:1092–101. doi:10.1111/all.12439

Kihlstrom A, Lilja G, Pershagen G, Hedlin G (2003) Exposure to high doses of birch pollen during pregnancy, and risk of sensitization and atopic disease in the child. Allergy 58:871–877

Lappalainen MHJ, Hyvärinen A, Hirvonen M-R et al (2012) High indoor microbial levels are associated with reduced Th1 cytokine secretion capacity in infancy. Int Arch Allergy Immunol 159:194–203. doi:10.1159/000335596

Larsson KA, Eklund AG, Hansson LO et al (1994) Swine dust causes intense airways inflammation in healthy subjects. Am J Respir Crit Care Med 150:973–977. doi:10.1164/ajrccm.150.4.7921472

Latza U, Oldenburg M, Baur X (2004) Endotoxin exposure and respiratory symptoms in the cotton textile industry. Arch Env Heal 59:519–525. doi:10.1080/00039890409605168

Lauener RP, Birchler T, Adamski J et al (2002) Expression of CD14 and Toll-like receptor 2 in farmers' and non-farmers' children. Lancet 360:465–466. doi:10.1016/S0140-6736(02)09641-1

Lazaridou A, Biliaderis CG (2007) Molecular aspects of cereal β-glucan functionality: physical properties, technological applications and physiological effects. J Cereal Sci 46:101–118. doi:10.1016/j.jcs.2007.05.003

Lebron F, Vassallo R, Puri V, Limper AH (2003) Pneumocystis carinii cell wall beta-glucans initiate macrophage inflammatory responses through NF-kappaB activation. J Biol Chem 278:25001–8

Lenters V, Basinas I, Beane-Freeman L et al (2010) Endotoxin exposure and lung cancer risk: a systematic review and meta-analysis of the published literature on agriculture and cotton textile workers. Cancer Causes Control 21:523–555. doi:10.1007/s10552-009-9483-z

Li D, Zhong YN, Rylander R et al (1995) Longitudinal study of the health of cotton workers. Occup Environ Med 52:328–31

Li W, Ray RM, Gao DL et al (2006) Occupational risk factors for nasopharyngeal cancer among female textile workers in Shanghai, China. Occup Environ Med 63:39–44. doi:10.1136/oem.2005.021709

Liebers V, Raulf-Heimsoth M, Brüning T (2008) Health effects due to endotoxin inhalation (review). Arch Toxicol 82:203–210. doi:10.1007/s00204-008-0290-1

Liu AH (2002) Endotoxin exposure in allergy and asthma: reconciling a paradox. J Allergy Clin Immunol 109:379–392

Madsen AM (2006) Airborne endotoxin in different background environments and seasons. Ann Agric Environ Med 13:81–86

Madsen AM, Hansen VM, Nielsen SH, Olsen TT (2009) Exposure to dust and endotoxin of employees in cucumber and tomato nurseries. Ann Occup Hyg 53:129–138. doi:10.1093/annhyg/men073

Madsen AM, Tendal K, Schlunssen V, Heltberg I (2012) Organic dust toxic syndrome at a grass seed plant caused by exposure to high concentrations of bioaerosols. Ann Occup Hyg 56:776–788. doi:10.1093/annhyg/mes012

Mahauthaman R, Howell CJ, Spur BW et al (1988) The generation and cellular distribution of leukotriene C4 in human eosinophils stimulated by unopsonized zymosan and glucan particles. J Allergy Clin Immunol 81:696–705

Malmberg P, Larsson K (1993) Acute exposure to swine dust causes bronchial hyperresponsiveness in healthy subjects. Eur Respir J Off J Eur Soc Clin Respir Physiol 6:400–404

Mandryk J, Alwis KU, Hocking AD (1999) Work-related symptoms and dose-response relationships for personal exposures and pulmonary function among woodworkers. Am J Ind Med 35:481–90

Marchand G, Lalonde M, Beaudet Y et al (2007) Documentation of the endotoxins present in the ambient air of cotton fiber textile mills in Québec. J Env Monit 9:869. doi:10.1039/b704087c

McElvenny DM, Hurley MA, Lenters V et al (2011) Lung cancer mortality in a cohort of UK cotton workers: an extended follow-up. Br J Cancer 105:1054–1060. doi:10.1038/bjc.2011.312

Mehta AJ, Wang XR, Eisen EA et al (2007) Work area measurements as predictors of personal exposure to endotoxin and cotton dust in the cotton textile industry. Ann Occup Hyg 52:45–54. doi:10.1093/annhyg/mem061

Meroueh SO, Bencze KZ, Hesek D et al (2006) Three-dimensional structure of the bacterial cell wall peptidoglycan. Proc Natl Acad Sci U S A 103:4404–9. doi:10.1073/pnas.0510182103

Miller JD, Young JC (1997) The use of ergosterol to measure exposure to fungal propagules in indoor air. Am Ind Hyg Assoc J 58:39–43. doi:10.1080/15428119791013062

Milton DK, Alwis KU, Fisette L, Muilenberg M (2001) Enzyme-linked immunosorbent assay specific for (1–>6) branched, (1–>3)-beta-D-glucan detection in environmental samples. Appl Environ Microbiol 67:5420–4. doi:10.1128/AEM.67.12.5420-5424.2001

Miura NN (2005) Fate of β-glucans in vivo. In: Toxicol. 1 - 3-beta-glucans. Informa Healthcare, pp 109–126

Noss I, Doekes G, Sander I et al (2010a) Passive airborne dust sampling with the electrostatic dustfall collector: optimization of storage and extraction procedures for endotoxin and glucan measurement. Ann Occup Hyg 54:651–658. doi:10.1093/annhyg/meq026

Noss I, Wouters IM, Bezemer G et al (2010b) beta-(1,3)-Glucan exposure assessment by passive airborne dust sampling and new sensitive immunoassays. Appl Environ Microbiol 76:1158–1167. doi:10.1128/AEM.01486-09

Noss I, Wouters IM, Visser M et al (2008) Evaluation of a low-cost electrostatic dust fall collector for indoor air endotoxin exposure assessment. Appl Environ Microbiol 74:5621–7. doi:10.1128/AEM.00619-08

O'Garra A, Vieira P (2004) Regulatory T cells and mechanisms of immune system control. Nat Med 10:801–805. doi:10.1038/nm0804-801

O'Shaughnessy PT, Donham KJ, Peters TM et al (2010) A task-specific assessment of Swine worker exposure to airborne dust. J Occup Env Hyg 7:7–13. doi:10.1080/15459620903327970

OSHA (1995) Subpart Z - Toxic and hazardous substances. Standard Num: 1910.1000. In: Occupational Safety and Health Standards part 1910. Available via https://www.osha.gov/pls/oshaweb/owasrch.search_form?p_doc_type=STANDARDS&p_toc_level=1&p_keyvalue=1910. Accessed 05. Occupational Safety and Health Administration.

Park JH, Cox-Ganser JM, Kreiss K et al (2008) Hydrophilic fungi and ergosterol associated with respiratory illness in a water-damaged building. Environ Health Perspect 116:45–50. doi:10.1289/ehp.10355

Park J-H, Spiegelman DL, Burge HA et al (2000) Longitudinal study of dust and airborne endotoxin in the home. Environ Health Perspect 108:1023–1028. doi:10.1289/ehp.001081023

Paudyal P, Semple S, Niven R et al (2011) Exposure to dust and endotoxin in textile processing workers. Ann Occup Hyg 55:403–409. doi:10.1093/annhyg/meq084

Poole JA, Dooley GP, Saito R et al (2010) Muramic acid, endotoxin, 3-hydroxy fatty acids, and ergosterol content explain monocyte and epithelial cell inflammatory responses to agricultural dusts. J Toxicol Env Heal A 73:684–700. doi:10.1080/15287390903578539

Portengen L, Preller L, Tielen M et al (2005) Endotoxin exposure and atopic sensitization in adult pig farmers. J Allergy Clin Immunol 115:797–802. doi:10.1016/j.jaci.2004.11.046

Prefontaine D, Banville-Langelier A-A, Fiset P-O et al (2010) Children with atopic histories exhibit impaired lipopolysaccharide-induced Toll-like receptor-4 signalling in peripheral monocytes. Clin Exp Allergy 40:1648–1657. doi:10.1111/j.1365-2222.2010.03570.x

Radon K, Danuser B, Iversen M et al (2002) Air contaminants in different European farming environments. Ann Agric Env Med 9:41–48

Reed CE, Milton DK (2016) Endotoxin-stimulated innate immunity: a contributing factor for asthma. J Allergy Clin Immunol 108:157–166. doi:10.1067/mai.2001.116862

Renz H, Blumer N, Virna S et al (2006) The immunological basis of the hygiene hypothesis. Chem Immunol Allergy 91:30–48. doi:10.1159/000090228

Reynolds SJ, Black DW, Borin SS et al (2001) Indoor environmental quality in six commercial office buildings in the midwest United States. Appl Occup Env Hyg 16:1065–1077. doi:10.1080/104732201753214170

Rylander R (1997) Airborne (1→3)-β-d-glucan and airway disease in a day-care center before and after renovation. Arch Environ Heal An Int J 52:281–285. doi:10.1080/00039899709602199

Rylander R (2006) Endotoxin and occupational airway disease. Curr Opin Allergy Clin Immunol 6:62–66. doi:10.1097/01.all.0000202356.83509.f7

Rylander R, Bergstrom R (1993) Bronchial reactivity among cotton workers in relation to dust and endotoxin exposure. Ann Occup Hyg 37:57–63

Rylander R, Norrhall M, Engdahl U et al (1998) Airways inflammation, atopy, and (1–> 3)-beta-D-glucan exposures in two schools. Am J Respir Crit Care Med 158:1685–7. doi:10.1164/ajrccm.158.5.9712139

Rylander R, Persson K, Goto H et al (1992) Airborne beta-1,3-glucan may be related to symptoms in sick buildings. Indoor Built Environ 1:263–267. doi:10.1177/1420326X9200100502

Rylander R, Thorn J, Attefors R (1999) Airways inflammation among workers in a paper industry. Eur Respir J Off J Eur Soc Clin Respir Physiol 13:1151–1157

Sabroe I, Read RC, Whyte MKB et al (2003) Toll-like receptors in health and disease: complex questions remain. J Immunol 171:1630–1635

Saito K, Nishijima M, Ohno N et al (1992) Activation of complement and limulus coagulation systems by an alkali-soluble glucan isolated from omphalia lapidescens and its less-branched derivatives. (studies on fungal polysaccharide. XXXIX). Chem Pharm Bull (Tokyo) 40:1227–1230

Saito R, Cranmer BK, Tessari JD et al (2009) Recombinant factor C (rFC) assay and gas chromatography/mass spectrometry (GC/MS) analysis of endotoxin variability in four agricultural dusts. Ann Occup Hyg 53:713–722. doi:10.1093/annhyg/mep052

Samadi S, Heederik DJ, Krop EJ et al (2010) Allergen and endotoxin exposure in a companion animal hospital. Occup Env Med 67:486–492. doi:10.1136/oem.2009.051342

Samadi S, Rietbroek NNJ, Dwars RM et al (2011) Endotoxin and beta-(1 –> 3)-glucan exposure in poultry and ruminant clinics. J Environ Monit 13:3254–3261. doi:10.1039/c1em10566c

Samadi S, van Eerdenburg FJ, Jamshidifard AR et al (2012) The influence of bedding materials on bio-aerosol exposure in dairy barns. J Expo Sci Env Epidemiol 22:361–368. doi:10.1038/jes.2012.25

Samadi S, Wouters IM, Houben R et al (2009) Exposure to inhalable dust, endotoxins, beta(1->3)-glucans, and airborne microorganisms in horse stables. Ann Occup Hyg 53:595–603. doi:10.1093/annhyg/mep040

Sander I, Fleischer C, Borowitzki G et al (2008) Development of a two-site enzyme immunoassay based on monoclonal antibodies to measure airborne exposure to (1–>3)-beta-D-glucan. J Immunol Methods 337:55–62. doi:10.1016/j.jim.2008.05.010

Saraf A, Larsson L, Burge H, Milton D (1997) Quantification of ergosterol and 3-hydroxy fatty acids in settled house dust by gas chromatography-mass spectrometry: comparison with fungal culture and determination of endotoxin by a Limulus amebocyte lysate assay. Appl Env Microbiol 63:2554–2559

Savilahti EM, Karinen S, Salo HM et al (2010) Combined T regulatory cell and Th2 expression profile identifies children with cow's milk allergy. Clin Immunol 136:16–20. doi:10.1016/j.clim.2010.02.011

Schaub B, Lauener R, von Mutius E (2006) The many faces of the hygiene hypothesis. J Allergy Clin Immunol 117:969–77. doi:10.1016/j.jaci.2006.03.003. quiz 978

Schram D, Doekes G, Boeve M et al (2005) Bacterial and fungal components in house dust of farm children, Rudolf Steiner school children and reference children–the PARSIFAL Study. Allergy 60:611–618. doi:10.1111/j.1398-9995.2005.00748.x

Schram-Bijkerk D, Doekes G, Douwes J et al (2005) Bacterial and fungal agents in house dust and wheeze in children: the PARSIFAL study. Clin Exp Allergy 35:1272–1278. doi:10.1111/j.1365-2222.2005.02339.x

Schwartz DA, Thorne PS, Yagla SJ et al (1995) The role of endotoxin in grain dust-induced lung disease. Am J Respir Crit Care Med 152:603–608. doi:10.1164/ajrccm.152.2.7633714

Semple S, Devakumar D, Fullerton DG et al (2010) Airborne endotoxin concentrations in homes burning biomass fuel. Environ Health Perspect 118:988–91. doi:10.1289/ehp.0901605

Senthilselvan A, Beach J, Feddes J et al (2011) A prospective evaluation of air quality and workers' health in broiler and layer operations. Occup Env Med 68:102–107. doi:10.1136/oem.2008.045021

Senthilselvan A, Zhang Y, Dosman JA et al (1997) Positive human health effects of dust suppression with canola oil in swine barns. Am J Respir Crit Care Med 156:410–417

Sigsgaard T, Bonefeld-Jorgensen EC, Hoffmann HJ et al (2005) Microbial cell wall agents as an occupational hazard. Toxicol Appl Pharmacol 207:310–319. doi:10.1016/j.taap.2004.12.031

Sigsgaard T, Heederik D (2005) On the hygiene hypothesis: regulation down, up, or sideways? J Allergy Clin Immunol 115:1325–6. author reply 1326

Sigsgaard T, Jensen LD, Abell A et al (2004) Endotoxins isolated from the air of a Danish paper mill and the relation to change in lung function: an 11-year follow-up. Am J Ind Med 46:327–332. doi:10.1002/ajim.20068

Sigsgaard T, Omland Ø, Thorne PS, Parnham MJ (2010) Asthma-like diseases in agriculture. In: Sigsgaard T, Heederik D (eds) Occup. Asthma. Birkhauser Basel, Basel, Switzerland, pp 163–183

Simpson JC, Niven RM, Pickering CA et al (1999) Comparative personal exposures to organic dusts and endotoxin. Ann Occup Hyg 43:107–115. doi:S0003487898000830 [pii]

Singh U, Levin L, Grinshpun SA et al (2011) Influence of home characteristics on airborne and dustborne endotoxin and β-D-glucan. J Environ Monit 13:3246–53. doi:10.1039/c1em10446b

Smit LA, Heederik D, Doekes G et al (2008) Exposure-response analysis of allergy and respiratory symptoms in endotoxin-exposed adults. Eur Respir J 31:1241–1248. doi:10.1183/09031936.00090607

Smit LA, Wouters IM, Hobo MM et al (2006) Agricultural seed dust as a potential cause of organic dust toxic syndrome. Occup Env Med 63:59–67. doi:10.1136/oem.2005.021527

Smit LAM, Heederik D, Doekes G et al (2010) Occupational endotoxin exposure reduces the risk of atopic sensitization but increases the risk of bronchial hyperresponsiveness. Int Arch Allergy Immunol 152:151–158. doi:10.1159/000265536

Spaan S, Heederik DJ, Thorne PS, Wouters IM (2007) Optimization of airborne endotoxin exposure assessment: effects of filter type, transport conditions, extraction solutions, and storage of samples and extracts. Appl Env Microbiol 73:6134–6143. doi:10.1128/AEM.00851-07

Spaan S, Schinkel J, Wouters IM et al (2008a) Variability in endotoxin exposure levels and consequences for exposure assessment. Ann Occup Hyg 52:303–316. doi:10.1093/annhyg/men024

Spaan S, Smit LAM, Eduard W et al (2008b) Endotoxin exposure in sewage treatment workers: investigation of exposure variability and comparison of analytical techniques. Ann Agric Environ Med 15:251–261

Spaan S, Wouters IM, Oosting I et al (2006) Exposure to inhalable dust and endotoxins in agricultural industries. J Env Monit 8:63–72. doi:10.1039/b509838f

Spierenburg A, Smit L, Robbe P, et al (2016) Occupational endotoxin exposure dose-dependently protects against atopy and hay fever: Results of a longitudinal study. Eur. Respir. J. 48:

Stern DA, Riedler J, Nowak D et al (2007) Exposure to a farming environment has allergen-specific protective effects on TH2-dependent isotype switching in response to common inhalants. J Allergy Clin Immunol 119:351–358. doi:10.1016/j.jaci.2006.10.013

Szadkowska-Stańczyk I, Bródka K, Buczyńska A et al (2010) Exposure to bioaerosols among CAFO workers (swine feeding). Med Pr 61:257–69

Szepfalusi Z, Loibichler C, Pichler J et al (2000) Direct evidence for transplacental allergen transfer. Pediatr Res 48:404–407

Thilsing T, Madsen AM, Basinas I et al (2015) Dust, endotoxin, fungi, and bacteria exposure as determined by work task, season, and type of plant in a flower greenhouse. Ann Occup Hyg 59:142–57. doi:10.1093/annhyg/meu090

Thorn J, Beijer L, Rylander R (1998) Airways inflammation and glucan exposure among household waste collectors. Am J Ind Med 33:463–470. doi:10.1002/(SICI)1097-0274(199805)33:5<463::AID-AJIM5>3.0.CO;2-T [pii]

Thorn J, Rylander R (1998) Airways inflammation and glucan in a rowhouse area. Am J Respir Crit Care Med 157:1798–803. doi:10.1164/ajrccm.157.6.9706081

Thorne PS, Perry SS, Saito R et al (2010) Evaluation of the Limulus amebocyte lysate and recombinant factor C assays for assessment of airborne endotoxin. Appl Env Microbiol 76:4988–4995. doi:10.1128/AEM.00527-10

Tischer C, Gehring U, Chen CM et al (2011) Respiratory health in children, and indoor exposure to (1,3)-β-D- glucan, EPS mould components and endotoxin. Eur Respir J 37:1050–1059. doi:10.1183/09031936.00091210

Vandenbulcke L, Bachert C, Van Cauwenberge P, Claeys S (2006) The innate immune system and its role in allergic disorders. Int Arch Allergy Immunol 139:159–165. doi:10.1159/000090393

Van Strien RT, Engel R, Holst O et al (2004) Microbial exposure of rural school children, as assessed by levels of N-acetyl-muramic acid in mattress dust, and its association with respiratory health. J Allergy Clin Immunol 113:860–867. doi:10.1016/j.jaci.2004.01.783

Vogelzang PFJ, Van Der Gulden JWJ, Folgering H et al (2000) Longitudinal changes in bronchial responsiveness associated with swine confinement dust exposure. Chest 117:1488–1495. doi:10.1378/chest.117.5.1488

Vollmer W, Blanot D, De Pedro MA (2008) Peptidoglycan structure and architecture. FEMS Microbiol Rev 32:149–167. doi:10.1111/j.1574-6976.2007.00094.x

Von Mutius, Braun-Fahrländer, Schierl et al (2000) Exposure to endotoxin or other bacterial components might protect against the development of atopy. Clin Exp Allergy 30:1230–1234. doi:10.1046/j.1365-2222.2000.00959.x

Wan GH, Li CS (1999) Indoor endotoxin and glucan in association with airway inflammation and systemic symptoms. Arch Environ Health 54:172–9. doi:10.1080/00039899909602256

Wang XR, Pan LD, Zhang HX et al (2002) Follow-up study of respiratory health of newly-hired female cotton textile workers. Am J Ind Med 41:111–118. doi:10.1002/ajim.10042

Wang Z, Larsson K, Palmberg L et al (1997) Inhalation of swine dust induces cytokine release in the upper and lower airways. Eur Respir J Off J Eur Soc Clin. Respir Physiol 10:381–387

Wang Z, Malmberg P, Ek A et al (1999) Swine dust induces cytokine secretion from human epithelial cells and alveolar macrophages. Clin Exp Immunol 115:6–12

Williams DL (1997) Overview of (1→3)-β-D-glucan immunobiology. Mediators Inflamm 6:247–250. doi:10.1080/09629359791550

Williams DL, Lowman DW, Ensley HE (2005) Introduction to the chemistry and immunobiology of _ -glucans. In: Toxicol. 1 - 3-Beta-Glucans. Informa Healthcare, pp 1–34

Williams KL (2007a) Endotoxin relevance and control overview. In: Williams KL (ed) Endotoxins pyrogens, LAL test. depyrogenation. Informa Healthcare, New York, USA, p 27–45

Williams KL (2007b) Endotoxin structure, function, and activity. In: Williams KL (ed) Endotoxins pyrogens, LAL test. depyrogenation. Informa Healthcare USA, Inc, New York, USA, p 67–90

Wouters IM, Spaan S, Douwes J et al (2006) Overview of personal occupational exposure levels to inhalable dust, endotoxin, beta(1–>3)-glucan and fungal extracellular polysaccharides in the waste management chain. Ann Occup Hyg 50:39–53. doi:10.1093/annhyg/mei047

Zeković DB, Kwiatkowski S, Vrvić MM et al (2005) Natural and modified (1→3)-β-d-glucans in health promotion and disease alleviation. Crit Rev Biotechnol 25:205–230. doi:10.1080/07388550500376166

Zhang K, Petty HR (1994) Influence of polysaccharides on neutrophil function: specific antagonists suggest a model for cooperative saccharide-associated inhibition of immune complex-triggered superoxide production. J Cell Biochem 56:225–35

Zhao Z, Sebastian A, Larsson L et al (2008) Asthmatic symptoms among pupils in relation to microbial dust exposure in schools in Taiyuan, China. Pediatr Allergy Immunol 19:455–65. doi:10.1111/j.1399-3038.2007.00664.x