Appendix 7B

Ambient Bioaerosols

Table of Contents

Page

7B.1 INTRODUCTION AND BACKGROUND INFORMATION ON AMBIENT BIOAEROSOLS 7B-1
7B.1.1 Plant Aerosols 7B-2
7B.1.2 Animal Aerosols 7B-3
7B.1.3 Fungal Aerosols 7B-4
7B.1.4 Bacterial Aerosols 7B-6
7B.1.5 Viral Aerosols 7B-7
7B.2 NEWLY AVAILABLE BIOAEROSOLS RESEARCH 7B-8
7B.2.1 Atmospheric Levels of Cellulose / Other Plant Debris Markers 7B-12
7B.2.2 Pollen 7B-13
7B.2.3 Fungi and Their Byproducts 7B-19
7B.2.4 Endotoxins 7B-23
7B.2.5 (1 → 3)-β-D-Glucan 7B-28
7B.3 SUMMARY 7B-30
REFERENCES 7B-31

List of Tables
(cont'd)

Number		Page
7B-1	Examples of Major Sources, Types of Particles, and Disease Agents Associated with Bioaerosols	7B-1
7B-2	Respiratory Effects of Pollen/Fungi and Particulate Matter Exposures	7B-9
7B-3	Respiratory Effects of Inhaled Endotoxin-Laden Ambient Bioaerosols	7B-11

7B.1 INTRODUCTION AND BACKGROUND INFORMATION ON AMBIENT BIOAEROSOLS

The American Conference of Industrial Hygienists defines bioaerosols as airborne particles, large molecules or volatile compounds that are living, contain living organisms, or originate from living organisms. Such particles may be suspended in the air adhered to dust particles or tiny droplets of water. Bioaerosols include fungal materials, pollen, bacteria, viruses, endotoxins, and plant and animal debris, and range in size from 0.01 µm (viruses) to well over 20 µm (pollen). They are naturally present in the environment and can pose a threat to human health, especially for sensitive individuals for whom some bioaerosols, when inhaled may cause diseases such as asthma, allergic rhinitis, and respiratory infections. The 1996 PM AQCD (U.S. Environmental Protection Agency, 1996), highlighted several examples of common bioaerosol sources, particles, and agents, as listed in Table 7B-1 and discussed in several earlier bioaerosols reviews, e.g., Cox (1987), Pope et al. (1993), Lighthart and Mohr (1994), and Cox and Wathes (1995).

TABLE 7B-1. EXAMPLES OF MAJOR SOURCES, TYPES OF PARTICLES, AND DISEASE AGENTS ASSOCIATED WITH BIOAEROSOLS

Sources	Aerosol Particles	Disease Agents
Plants	Pollen and pollen fragments, fragments of other plant parts, spores (ferns, mosses), algal cells	Glycoprotein allergens
Animals	Skin scales, secretions (saliva, skin secretions), excreta, body parts (arthropods)	Glycoprotein allergens
Fungi	Spores, hyphae, yeast cells, metabolites (toxins, digested substrate material)	Glycoprotein allergens, infectious units, glucans, mycotoxins
Bacteria	Cells, fragments, metabolites (toxins, digested substrate material)	Infectious units, allergens, endotoxin, exotoxins
Viruses	Viral particles	Infectious units

Source: Modified from 1996 PM AQCD (U.S. EPA, 1996).

7B.1.1 Plant Aerosols

Pollen. Among the best known plant aerosols are pollens produced by flowering plants, including trees (e.g., pines, cedars, birch, elm, maple, oak, etc.), weeds (e.g., ragweed, sage, etc.), and grasses (e.g., rye grass, Bermuda grass, etc.). Within these groupings, specific types are regionally more common, e.g., ragweed more so in the eastern United States, birch during the spring pollen season in New England, mountain cedar early in the year in the southwest, etc. (Lewis et al., 1983). Outdoor pollen levels are determined by numbers of plants available for pollen release, the amount of pollen produced per plant, factors controlling pollen release and dispersion from the plant, and factors directly affecting the aerosols (Edmonds, 1979). Plant numbers depend on many environmental factors (some human) that control plant prevalence, e.g., numbers of plants that produced seed in the past year, disturbed ground available for seed germination and growth, growing season (temperature, rainfall, day length, etc.), and meteorological factors. Pollen shed is controlled by temperature, humidity, wind, and rain. Air pollen levels depend on all of these factors, as well as wind and rain conditions after release and on surfaces available for impaction. Pollen grains are large complex particles that consist of cellular material surrounded by a cell membrane and a complex wall. Pollen allergens are water-soluble glycoproteins that rapidly diffuse from the grain when it contacts a wet surface and are generally specific to the type of pollen, although large groups include a single allergen (e.g., many different kinds of grasses have similar allergens in their pollen grains). Several pollen allergens have been characterized: *Amb a* I (ragweed), *Bet v* I (birch), *Par j* I (parietaria).

Other Natural Plant Aerosols. Other plant-derived particles naturally occurring in outdoor air include algal cells; spores of mosses, liverworts, club mosses, and ferns; and fragments of all kinds of plants. Very little has been reported about the prevalence or human impact of any of these aerosol particles, but they are presumed to carry allergens.

Plant-Related Bioaerosols Generated by Human Activities (Grain Dust, Latex, etc.). Human activities that accumulate plant materials, e.g., storage, handling, and transport of farm products (hay, straw, grain), composting, produce bioaerosols. Grain dusts that include respirable-size particles (< 10 μm) are of particular interest. Soybean dust aerosols released from freighters unloading the beans in port have been blamed for epidemics of asthma. Also, human uses of some plant products can result in disease-causing aerosols (Alberts and Brooks, 1992), e.g., wood trimmer's disease (from inhalation of wood dust particles released during

high-speed wood cutting); sewage composting involving use of wood chips which can release allergenic aerosols, and latex particles from automobile tires that can contaminate reentrained roadway dust.

7B.1.2 Animal Aerosols

Mammalian Aerosols. All mammals produce aerosols. Human aerosols (skin scales, respiratory secretions) generally do not cause disease except for agents of infection (see below). Other mammals release aerosols that cause hypersensitivity diseases, the most common sources being cats, dogs, farm animals, laboratory animals, and house mice — although all animals release aerosols that could be sensitizing under appropriate conditions (Burge, 1995). Mammals only cause human disease under appropriate exposure conditions, e.g., having a cat in a house or handling of any animal. Cat allergens apparently become aerosolized on very small particles (< 1 μm) shed from skin and saliva. Dog, mouse, and other rodent allergens may be borne on dried urine particles, having sizes similar to those of cat allergen. Little is known about other mammalian aerosols. Cat and dog allergens (*Fel d* I, *Can f* I) have been characterized.

Avian Aerosols. Examples of wild and domesticated birds associated with disease-causing aerosols include: starlings (histoplasmosis); pigeons (histoplasmosis, pigeon-breeders disease); parrots (psittacosis); poultry (poultry-handlers disease); etc. Only the hypersensitivity diseases (e.g., pigeon breeders and poultry handlers disease) are caused by "bird" aerosols per se. The others are infectious diseases caused by agents inhabiting the birds (see below). The avian aerosol-hypersensitivity diseases are almost exclusively confined to sites where birds are bred and handled extensively, especially in indoor environments; and birds that release antigens observed to cause human disease are those that congregate or are typically confined close to people. Relatively little is known about avian aerosols. Probably skin scales, feather particles, and fecal material are all released as antigen-containing aerosols. The antigens (allergens) responsible for avian-related hypersensitivity diseases have yet to be well characterized.

Insect Aerosols. Dust mites are arthropods (family Pyrogliphidae) that include two common species in temperate climates: *Dermatophagoides farinae*, which proliferates under relatively dry conditions; and *D. pteronyssinus*, which dominates in more humid environments (Arlian, 1989). Dust mites thrive where relative humidity consistently exceeds 60% and where skin scales and fungal spores are available as food. Bedding and carpet dust are prime exposure

reservoirs. The mite itself is about 100 µm long, but excretes 20 µm membrane-bound fecal particles that contain allergens that are a major cause of sensitization in children. The allergens are digestive enzymes that gradually diffuse from fecal particles after deposition on mucous membranes. Several dust mite allergens have been characterized, including: *Der f* I and II; and *Der p* I and II (Platts-Mills and Chapman, 1987). Cockroaches are nocturnal insects belonging to the Orthoptera (Mathews, 1989) that inhabit dark environments where food and water are available. Cockroaches are very prolific, given favorable environmental conditions, and population pressure eventually drives them into daylight in search of food. They shed body parts, egg cases, and fecal particles (all of which probably carry allergens), but little is known about the particles that actually carry the allergens. Two cockroach allergens have been characterized: *Bla g* I, and *Bla g* II; and they are likely a major cause of asthma for some populations of children. Fragments of gypsy moths and other insects that undergo massive migrations can also become abundant in ambient air. Sizes, nature, and allergen content of such particles have not been well studied; but cases of occupational asthma from exposure to insects (e.g., sewer flies) have been reported.

Other Animal Allergens. It is likely that proteinaceous particles shed from any animal could cause sensitization if exposure conditions are appropriate. For example, exposure to proteins aerosolized during seafood processing have caused epidemics of asthma.

7B.1.3 Fungal Aerosols

Fungi are primarily filamentous microorganisms that reproduce and colonize new areas by airborne spores. Most use complex non-living organic material for food, require oxygen, and have temperature optima within the human comfort range. The major structural component of the cell wall is acetyl-glucosamine polymers (chitin). Cell walls also may contain B-glucans, waxes, mucopolysaccharides, and many other substances. In the process of degrading organic material, fungi produce CO_2, ethanol, other volatile organic compounds, water, organic acids, ergosterol, and other metabolites that include many antibiotics and mycotoxins.

Fungi colonize dead organic materials in both indoor and outdoor environments. Some invade living plant tissue and cause many important plant diseases; some invade living animal hosts, including people. Fungi are universally present in indoor environments unless specific efforts are made for their exclusion (i.e., as in clean rooms). Kinds of fungi able to colonize

indoor materials are generally those with broad nutritional requirements (e.g., *Cladosporium sphaerospermum*), those that can colonize dry environments (e.g., members of the *Aspergillus glaucus* group), or organisms that readily degrade cellulose and lignin present in many indoor materials (e.g., *Chaetomium globosum*, *Stachybotrys atra*, *Merulius lacrymans*). Yeasts (which are unicellular fungi) and other hydrophilic taxa (e.g., *Fusarium*, *Phialophora*) are able to colonize air/water interfaces. Moisture, in fact, is the most important factor determining indoor fungal growth, since food sources are ubiquitous (Kendrick, 1992).

Particles that become airborne from fungal growth include spores (the unit of most fungal exposure); fragments of the filamentous body of the fungus; and fragments of decomposed substrate material. Fungal spores range from about 1.5 µm to > 100 µm in size and come in many different shapes, the simplest being smooth spheres and the most complex large multicellular branching structures. Most fungal spores are near unit density or less. Some include large air-filled vacuoles. Fungal spores form the largest and most consistently present component of outdoor bioaerosols. Levels vary seasonally, with lowest levels occurring during periods of snow. While rain may initially wash large dry spores from the air, these are immediately replaced by wet (hydrophilic) spores that are released in response to the rain.

Some kinds of spores are widespread in outdoor air (e.g., *Cladosporium herbarum*, *Alternaria tenuissima*). Others produced by fungi with more fastidious nutritional requirements are only locally abundant. Typical indoor fungal aerosols are composed of particles penetrating from outdoors, particles released from active growth on indoor substrates, and reaerosolized particles that had settled into dust reservoirs. Indoor fungal aerosols are produced by active forcible discharge of spores; by mechanisms intrinsic to the fungus that "shake" spores from the growth surface; and, most commonly, by mechanical disturbance (e.g., air movement, vibration).

Allergic rhinitis and asthma are the only commonly reported diseases resulting from fungal exposures outdoors, and which also commonly occur indoors. The allergens of fungi are probably digestive enzymes that are released as the spore germinates. Other spore components (of unknown function) may also be allergenic. Only very few fungal allergens (e.g., *Alt a* I, *Cla h* I, and *Asp f* I), out of possibly hundreds of thousands, have been characterized.

Allergic fungal sinusitis and allergic bronchopulmonary mycoses occur when fungi colonize thick mucous in the sinuses or lungs of allergic people. The patterns of incidence of allergic fungal sinusitis may be explained in part by geographic variability in ambient fungal

exposures. This disease is most commonly caused by *Bispora*, *Curvularia*, and other dark-spored fungi. Exposure patterns required for allergic bronchopulmonary mycoses are unknown. This disease is usually caused by *Aspergillus fumigatus*. Coccidioidomycoses and Histoplasmosis are infectious fungal diseases that result from outdoor exposures to *Histoplasma capsulatum* (a fungus that contaminates damp soil enriched with bird droppings) and *Coccidioides inmitis* (a fungus that grows in desert soils). Indoor aerosol-acquired fungal infections are rare and mostly restricted to immunocompromised people (Rippon, 1988).

Toxic agents produced by fungi include antibiotics, mycotoxins, and some cell-wall components that have irritant or toxic properties. The antibiotics and mycotoxins are secondary metabolites produced during fungal digestion of substrate materials, and their presence depends, in part, on the nature of the substrate. The locations of the toxins in spores or other mycelial fragments are unknown, as are the dynamics of release in the respiratory tract. Aerosol exposure to fungal antibiotics in levels sufficient to cause disease is unlikely. Mycotoxicoses have been reported as case studies from exposure to spores of *Stachybotrys atra* (Croft et al., 1986), and epidemiologically for *Aspergillus flavus* (Baxter et al., 1981).

7B.1.4 Bacterial Aerosols

Most bacteria are unicellular, although some form "pseudo" filaments when cells remain attached following cell division. The actinomycetes are bacteria that do form filaments and, in some cases, dry spores designed for aerosol dispersal. Bacteria can be broadly categorized into two groups based on a response to the Gram stain procedure. The cell walls of Gram positive (Gram+) bacteria are able to absorb a purple stain; the cell walls of Gram negative (Gram−) bacteria resist staining and contain endotoxin consisting of proteins, lipids, and polysaccharides.

Most infectious agents are maintained in diseased hosts. A few, including *Legionella pneumophila*, reside in water-filled environmental reservoirs such as water delivery systems, cooling towers, air conditioners, and lakes, streams, oceans, etc. Infectious agents are often released from hosts in droplets exhaled from the respiratory tract. Each droplet contains one or more of the infectious agent, possibly other organisms, and respiratory secretions. Most droplets are very large and fall quickly. Smaller droplets dry quickly to droplet nuclei, which range from

the size of the individual organism (< 1 μm for the smallest bacteria) to clumps of larger organisms (> 10 μm for larger bacteria).

Environmental-source aerosols are produced by mechanical disturbances that include wind, rain splash, wave action, and as occurs in air recirculation, in sprays of washes and coolants, and in humidifiers. Particle sizes from all of these activity cover a wide range from well below 1 μm to > 50 μm. The thermophilic actinomycetes produce dry aerial spores that require only slight air movements to stimulate release. Each spore is about 1 μm in diameter.

Whole living bacteria are agents of infectious disease (e.g., tuberculosis, Legionnaires' disease, etc.). For tuberculosis, a single virulent bacterial cell deposited in the appropriate part of the lung can cause disease in a host without specific immunity. For Legionnaires' disease, the number of organisms needed for disease development likely depends on how well the host's general protective immune system is operating. Some bacteria release antigens that cause hypersensitivity pneumonitis. The antigens may be enzymes (e.g., *Bacillus subtilis* enzymes used in the detergent industry) or may be cell wall components (e.g., endotoxin or glucans).

7B.1.5 Viral Aerosols

Viruses are either RNA or DNA units surrounded by a protein coat that have no intrinsic mechanism for reproduction, but rather require living cells (whose enzyme systems they utilize to make new viral particles). Viruses can be crystallized and yet remain able to reproduce and are often considered intermediates between non-life and life. Because viruses require living cells to reproduce, reservoirs for them are almost exclusively living organisms. Viruses, in rare cases, even survive (but do not reproduce) in environmental reservoirs from which they are re-aerosolized to cause disease. Hanta virus that causes severe respiratory disease in people exposed to intense aerosols of infected mouse urine is an example of this. Viral aerosols are produced when the infected organism coughs, sneezes, or otherwise forces respiratory or other secretions into the air. The viral particles are coated with secretions from the host and, as in the case for bacteria, there may be one to many in a single droplet. The size of a single viral particle is very small (a small fraction of a μm), but infectious droplets more usually occur within a larger size range (1 to 10 μm). Each kind of virus produces a specific disease, although some of the diseases present with similar symptoms. Thus, the measles virus produces measles, the chicken pox viruses produces chicken pox and shingles, etc. Influenza and common colds are

produced by a variety of viruses, all of which produce similar (but not necessarily identical) symptoms.

7B.2 NEWLY AVAILABLE BIOAEROSOLS RESEARCH

Since the 1996 PM AQCD, numerous newly available studies provide interesting new information pertinent to evaluating potential involvement of bioaerosols in contributing to health effects associated with exposures to ambient PM. Of much interest are newly published findings which (a) indicate greater contributions (than previously thought) of bioaerosols to airborne ambient PM concentrations; (b) improve our understanding of factors and mechanisms affecting release of some bioaerosol materials into ambient air; and (c) provide evidence indicative of bioaerosols contributing to ambient PM-related health effects, including contributions made in combination with other, non-biological, PM components.

The fate of bioaerosols is dependent on a number of variables: geography, time of day, moisture levels, air temperature/humidity, wind speed and direction, and seasonal variations in the latter variables. Once airborne, depending on the particle size, bioaerosols may travel great distances. As discussed in more detail below, bioaerosols generally represent a rather small fraction of the measured urban ambient PM mass and are typically present at even lower concentrations outdoors during cold seasons, when notable ambient PM effects have been demonstrated (Ren et al., 1999; Kuhn and Ghannoum, 2003). Bioaerosols tend to be in the coarser fraction of PM; but some bioaerosols (e.g., fungal spores, fragmented pollens, nonagglomerated bacteria) are found in the fine fraction as well (Meklin et al., 2002a; Schäppi, 1999) possibly due to reactions of the biological agents with ambient particles (Schäppi et al., 1999; Oikonen et al., 2003; Behrendt et al., 2001; Ormstad et al., 1998).

For the sake of bringing together information regarding bioaerosols, the following discussions include new information on bioaerosol sources and factors affecting their dispersal in ambient air as well as new studies on their health effects. The latter include not only toxicology studies, but also some studies conducted in occupational settings or results from epidemiology studies assessing health responses to airborne allergens or biological material. To the extent that other aspects of air pollution evaluated in these epidemiology studies are deemed pertinent and important, the results are discussed in Chapter 8. Tables 7B-2 and 7B-3

TABLE 7B-2. RESPIRATORY EFFECTS OF POLLEN/FUNGI AND PM EXPOSURES

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration	Particle Effects/Comments	Reference
Humans	Pollen	Ambient, London	not characterized	Not characterized	2 mo study period	ER visits increases from 2.25 patients/day to 40 patients/day following thunderstorm. Peak in pollen levels 9 h peak in ER visits.	Celenza et al. (1996)
Human, Netherlands	Ambient PM Pollens: grass; sorrel; birch; dock	Ambient (Leiden and Helmond)	Poaceae (grass) 78 pol. grains/m^3 Betula (birch) 69 pol. grains/m^3 Quercus (oak) 13 pol. grains/m^3		Deaths from 1986 to 1994 evaluated	Pollen concentrations only weakly associated with air pollution. Grass pollen levels were associated with daily deaths from pneumonia and COPD. Other pollens (birch, sorrel, dock) were also positively correlated with mortality.	Brunekreef et al. (2000)
Human, male and female, normal and allergic, ages 26-29.	Ragweed	Bronchoscopic challenge	Not characterized	Not characterized	3 h	*Nonallergic subjects:* ragweed had little effect on ciliary activity; acid reduced activity *Allergic subjects:* slight increase in albumen and 2-fold increase in BAL cell number. Allergic subjects with severe inflammatory changes had a 12-fold increase in albumin and 9-fold increase in BAL cell number	Hastie and Peters (2001)
Human, male and female, 21-49, with allergic rhinitis, nonsmoking	DPM ragweed	Intranasal spray	0.3 mg in 200 µl saline		1,4,8 days	Combined DEP/ragweed challenge induced ragweed-specific IgE, but not total IgE or IgE-secreting cell numbers. Also caused a decrease in IFNγ and IL-2 and an increase in IL-4, IL-5, IL-6, IL-10, and IL-13.	Diaz-Sanchez et al. (1997)

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TABLE 7B-2 (cont'd). RESPIRATORY EFFECTS OF POLLEN/FUNGI AND PM EXPOSURES

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration	Particle Effects/Comments	Reference
Mice, Male, BALB/c, 7 weeks old.	DPM Japanese cedar pollen OA	Intratracheal Instillation	0.3 mg/mouse 1 mg 10 µg	0.4 µm	3 times with an interval of 3 wks	IgE antibody and IL-4 production increased. Slight increase in IL-3 output. DEP affects antigen-specific IgE antibody response by enhanced IL-4 production.	Fujimaki et al. (1994)
Mice, Female, BALB/c, 6-8 weeks old.	SPM DPM (SRM1650)	Injection into mouse footpad		< 2.5 µm	20-26 days	Adjuvant activity noted on the production of IgE antibodies to OA.	Ormstad et al. (1998); Ormstad (2000)
Human, asthmatic, 9-16 years old.	PM fungal spores	Ambient, (San Diego, CA area)	24.8 ± 11.1 µg/m^3 2461 ± 1307/m^3	2.5 µm 10 µm	12 h	Inhaler puffs increased by 1.2 per 1000 fungal spores/m^3. Positive association between asthma severity and PEFR and total fungal spores. No significant relationship between asthma severity and PM_{10} or pollen exposure.	Delfino et al. (1996, 1997)
Human, children < 15, adults, adults > 59	Ascomycetes Basidiospore content in ambient TSP and PM_{10}	Ambient (Mexico City)	56-98 78-156 100-207 100-1000	10 µm spores/m^3 spores/m^3	1 y analysis	Statistically significant increase in fungal spore exposure-related asthma hospital admissions in children (but not adults and seniors) in Mexico City.	Rosas et al. (1998)
Human	Mold spores; tree, grass, and ragweed pollen	Ambient (Chicago)	Variable		7 mo periods from 1985 through 1989	On days that mold spores were > 1000 spores/m^3, death caused by asthma were 2.16 times greater than days with spores < 1000; but no increase in death seen with tree, grass, or ragweed pollen.	Targonski et al. (1995)
Chicken tracheal rings	Filamentous fungi	In vitro	N/A	N/A	24,48, 96 h	Chloroform-extractable endo- and exometabolites stopped tracheal ciliary movement.	Piecková and Kunová (2002)

SPM = suspended particulate matter DPM = diesel particulate matter

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TABLE 7B-3. RESPIRATORY EFFECTS OF INHALED ENDOTOXIN-LADEN AMBIENT BIOAEROSOLS

Species, Gender, Strain, Age, etc.	Particle	Exposure Technique	Concentration	Particle Size	Exposure Duration	Particle Effects/Comments	Reference
Humans (pig farmers), 82 symptomatic & 89 asymptomatic n = 171	Dust Endotoxin	Inhalation	2.63 mg/m^3 σg = 1.3 105 ng/m^3 σg = 1.5	N/A	5 h/day average lifetime exposure	Large decline in FEV_1 (73 mL/year) and FVC (55 mL/year) was significantly associated with estimated long-term average exposure to endotoxin at 105 ng/m^3.	Vogelzang et al. (1998)
Humans (healthy); 32 M, 32 F, 16 to 50 years old	Indoor pool water spray Endotoxin	Inhalation	N/A	0.1-7.5 µm	N/A	Recurring outbreaks of pool-associated granulomatous pneumonitis (n = 33); case patients had higher cumulative work hours. Analysis indicated increased levels of endotoxin in pool air and water.	Rose et al. (1998)
Humans (potato plant workers), low (37 M) and high (20 M) exposures	Endotoxin	Inhalation	low: 21.2 EU/m^3 σg = 1.6 high: 55.7 EU/m^3 σg = 2.1	N/A	8 h	Concentration-related decreased FEV_1, FVC, and MMEF over the work shift; endotoxin effects on lung function can be expected above 53 EU/m^3 (≈ 4.5 ng/m^3) over 8 h.	Zock et al. (1998)
Humans (healthy); 5 M, 4 F, 24 to 50 years old	LPS [1] (endotoxin)	Inhalation	0.5 µg 5.0 µg 50 µg	1 - 4 µm MMAD	30 min	Significant decrease in PMN luminal-enhanced chemiluminescence with 0.5 µg LPS; increase in blood CRP and PMNs, and increase in sputum PMNs, monocytes, and MPO with 5.0 µg LPS; increase in body blood PMNs, temperature, blood and urine CRP, sputum PMNs, lymphocytes, monocytes, TNFα, and ECP with 50 µg LPS.	Michel et al. (1997)
Rats (Fischer 344), 8 wks to 22 mo old, N = 3/group	LPS [1] (endotoxin)	Inhalation	70 EU/m^3	0.72 µm σg = 1.6	12 min	Significant increase in PMNs in bronchoalveolar lavage (BAL) in LPS exposed animals. LPS significantly affected the reactive oxygen species activity in BAL. Effects were age-dependent.	Elder et al. (2000a,b)

[1] LPS = lipopoly saccharide.

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summarize salient features of newly available studies of respiratory effects of pollens / fungi and endotoxins, respectively.

7B.2.1 Atmospheric Levels of Cellulose / Other Plant Debris Markers

Puxbaum and Tenze-Kunit (2003) investigated seasonal variations in atmospheric cellulose levels (as a "microtracer" for airborne plant debris) in and around Vienna, Austria. The 9 mo average of "free" cellulose concentrations at the downtown site was 0.374 $\mu g/m^3$ (reflective of 0.75 $\mu g/m^3$ plant debris). Given an annual average for organic carbon (OC) at the downtown site of 5.7 $\mu g/m^3$, plant debris appears to be more than a minor contributor to ambient organic aerosol at that site. Unexpectedly, size distribution determinations via impactor measurements indicated that the "free cellulose" (on a mass basis) comprised ~ 0.7% of ambient fine PM (0.1 - 1.5 µm), forming a "wetable but insoluble part of the accumulation mode aerosol," as noted by Puxbaum and Tenze-Kunit (2003). They further noted that the cellulose levels at the downtown site showed maximum concentration during the fall (probably due to increased biological activity involving seed production and entrainment of other plant cellulose materials into the air). Comparison of simultaneous measurements of cellulose at the downtown site to those from a suburban site indicated that the ambient PM cellulose did not originate in notable amounts from within the city.

The Puxbaum and Tenze-Kunit (2003) study adds further to a growing database which points toward plant debris being a significant contributor to organic aerosols present at continental sites. As discussed by Puxbaum and Tenze-Kunit, Rogge et al. (1993) and Zappoli et al. (1999) have shown a considerable portion of the organic aerosols not to be soluble in water or organic solvents, suggesting larger molecular sizes of the insoluble compounds. Also, Matthias-Maser and Jaenicke (1995) found up to 40% of the number of particles > 0.2 µm (AD) at a continental site to be of primary "biological origin". Puxbaum and Tenze-Kunit further noted that Bauer et al. (2002) found fungal spores in the 2.15 - 10 µm fraction of organic background aerosol at a mountain site to comprise on average, about 6% of the OC in the coarse PM fraction. Also, they noted that the main constituents of the organic aerosol appear to be humic-like substances (HULIS) that are present in continental aerosol samples at concentrations (HULIS-carbon) range from 7 to 24% of the OC (Havers et al., 1998; Zappoli, et al., 1999; Facchini et al., 1999). The macromolecular HULIS materials likely have many origins, e.g.,

from biomass fires (Facchini et al., 1999) or secondary atmospheric reactions (Gelenser et al., 2003). It was further noted by Puxbaum and Tenze-Kunit that cellulose is also contained in pollen at 3 - 7 % dry mass (Stanley and Linskens, 1985).

Other new studies evaluated atmospheric levels of levoglucosan (LVG) and other markers (e.g., palmitic acid, stearic acid) of biomass burning so as to investigate potential inputs of materials from that source category to ambient PM. One study (Fraser and Lakshaman, 2000), measuring effects in Texas of biomass fires in Mexico/Central America, found 0.2 - 1.2 $\mu g/m^3$ of LVG during episodes resulting from long-range transport of smoke haze. In another study, Poore (2002) reported on LVG concentrations in $PM_{2.5}$ samples taken at the Fresno, California supersite during the year 2000. Highest levels of LVG (up to 4.05 $\mu g/m^3$) were found during late fall/winter months (November - January), whereas LVG concentrations during spring/summer months were near or below the detection limit of 0.01 $\mu g/m^3$. Analogous seasonal patterns of variations in concentrations of palmitic and stearic acid were also seen for the Fresno supersite $PM_{2.5}$ samples. Given that agriculturally-related biomass burning in the Fresno area is typically completed by the end of October, the elevated LVG levels during fall/winter months were most likely derived from residential woodsmoke emissions. The same may also be true for fall/winter increases in palmitic and stearic acid levels, although as noted by Poore (2002), both of these acids are emitted from a variety of sources, including food production. In any case, these results appear to be indicative of episodic or more prolonged seasonal increases in plant-derived bioaerosol materials contributing to ambient PM levels in Texas and California, and by analogy, other areas of the western U.S. where air quality is affected by biomass burning emissions (e.g., from controlled burns on agricultural land, forest fires, or residential fireplaces/woodstoves).

7B.2.2 Pollen

With regard to pollen, important new insights are emerging with regard to: (a) factors influencing the occurrence of asthmatic or other allergic responses to certain types of common, widespread pollens; and (b) the likelihood that such bioaerosol-related asthma events are enhanced by the presence in ambient air of other types of non-bioaerosol airborne particles. More specifically, researchers in several countries have demonstrated links between epidemics of “thunderstorm asthma” (characterized by notable increases in asthma attacks and upsurges in

grass pollen allergens among respirable airborne bioaerosol components (Bellomo et al., 1992; Ong, 1994; Venables et al., 1997; Rosas et al., 1998; Newson et al., 1997; Schäppi et al., 1999; Girgis, et al., 2000).

Anemophilous plants (wind-pollinated plants) produce copious amounts of pollen, making pollen from these plants the most abundant in the atmosphere and the most important in terms of human exposure. Typically, exposure to pollen has been thought to only play a role in allergic rhinitis because they are too large to penetrate into the lower airways. However, in more recent years, there is evidence which indicates that pollen may in fact be associated with exacerbation of asthma through the release of pollen allergens small enough in size to penetrate into lower respiratory airways and/or via the binding of these allergens to other respirable size particles (Suphioglu et al., 1992; Burge and Rogers, 2000; Knox et al., 1997; Schäppi et al., 1999). More specifically, although intact (unruptured) pollen grains are typically so large (often > 10 - 20 μm) that, when inhaled, they mainly deposit in upper airways (nasopharyngeal areas), grass pollen allergens are found in the cytoplasm of the pollen grains (Taylor et al., 1994); and, upon the rupture of mature pollen grains, they are released as cytoplasmic fragments that comprise respirable (~0.1 to 5.0 μm) particles (Schäppi et al., 1999; Grote et al., 2000; Taylor et al., 2002).

The release of allergens from the pollen grains is moisture dependent (Suphioglu et al., 1992; Schäppi et al., 1997, 1999). Suphioglu et al. (1992) reported the release of a major allergen (*Lol plX*) from the intracellular starch granules of rye grass when pollen grains were ruptured during a rain storm. The allergen was small enough (< 3 μm) to penetrate the lower airways. The atmospheric concentration of the allergen showed a 50% increase on days following a rain event. Asthmatic volunteers were exposed by aerosol mask to the starch granules (volume of 1 mL nebulized, of a 0.34 $\mu g/m^3$ solution) or the pollen grain extracts. Asthmatic volunteers (n = 4) that underwent inhalation challenge showed a typical early response, described by the authors as a striking bronchial constriction following exposure to the starch granules. The effect was not noted in volunteers exposed to pollen grain extracts.

Taylor and colleagues (2002) confirmed that the key trigger for rupture of rye grass and Bermuda grass pollen is pollen grain contact with water, e.g., with the moistening of such pollen by dew, fog, rainfall, or lawn watering. They also further provided evidence on the specific sequence of events (and time periods) leading to appearance of the allergen-containing cytoplasmic material in airborne respirable aerosols. Taylor et al. (2002) reported that, upon

drying within 1 to 6 hrs after rye grass or Bermuda grass pollen were moistened with water and grain rupture occurred, allergen-containing cytoplasmic fragment particles were entrained into the air by blowing air across the grass flowers or shaking them, with many thousands of such fragments in the 0.1 to 4.7 μm size range (most below 0.4 μm) being collected by a Cascade impactor. The dispersal of such allergen-laden particles following cycles of wetting and drying of grass pollen, it was noted by Taylor et al. (a) may occur in response to such disturbances as wind, lawn mowing, and recreational activities; (b) likely account for marked increases in asthma attacks after thunderstorms; and (c) may also account for increased asthmatic symptoms during grass flowering season after any moist weather conditions. Also, more recently, Taylor et al. (2003) employed analogous experimental wetting/drying procedures, collection and measurement of wind-released cytoplasmic fragments of birch tree pollen in the 0.03 to 4 μm size range, and found them to contain *Bet v* 1 allergens.

Taylor et al. (2002) also highlighted possible bases for interactions between aerosolized allergen-laden pollen debris and other types of ambient airborne particles. They noted, for example, that diesel emission particles are a major contributor to urban respirable aerosols mass, e.g., 18% in Pasadena, CA (Schauer et al., 1996), and have been implicated as a cause of allergic rhinitis and asthma in mice and humans (Nel et al., 1998; Bayram et al., 1998; and Diaz-Sanchez, et al., 2000). Taylor et al. further noted (a) that fine combustion particles and aerosols of pollen allergens, because of their small size, may deposit in similar respiratory tract regions; and (b) that synergistic combinations of allergen-laden pollen debris and polycyclic hydrocarbons found in fine combustion aerosols may explain the notable increased prevalence of pollen-induced asthma during the past 50 years.

Further possibilities exist with regard to possible ways that the copresence of grass pollens and diesel particulate matter (or perhaps other airborne particles) may contribute jointly to enhanced probability of asthma symptoms occurring in susceptible human population groups. More specifically, the EPA Health Assessment Document for Diesel Engine Exhaust (U.S. Environmental Protection Agency, 2002) noted that Ormstad et al. (1998) investigated the potential for DPM (as well as other suspended PM) to act as a carrier for allergens into the airways and found both *Can f* 1 (dog) and *Bet v* 1 (birch pollen) on the surface of airborne PM collected inside homes. They also found that DPM adhered to polycarbonate filters could bind both of these allergens as well as *Fel d* 1 (cat) and *Der p* 1 (house mite) allergens. The authors

concluded that soot particles in indoor air house dust may act as a carrier for several allergens in indoor air. The EPA Diesel Document (2000) also noted that Knox et al. (1997) investigated whether free grass pollen allergen molecules, released from pollen grains by osmotic shock (Suphioglu et al., 1992) and dispersed in microdroplets of water in aerosols, can bind to DPM mounted on copper grids in air. Using natural highly purified *Lol p* 1 (the major grass pollen allergen), immunogold labeling with specific monoclonal antibodies, and a high-voltage transmission electron-microscopic imaging technique, Knox et al. found binding of *Lol p* 1 to DPM in vitro. They concluded that binding of *Lol p* 1 with DPM might be a mechanism by which allergens can become concentrated in air and trigger asthma attacks.

In addition to suggesting that airborne diesel exhaust particles can act as carriers of biological aerosols producing an enhanced allergic response (Knox et al., 1997; Diaz-Sanchez et al., 1997; Fujimaki et al., 1994), some studies suggest that allergen carriers (e.g., pollen grains) may incorporate other atmospheric pollutants that alter the pollen surface, leading to altered protein and allergen release (Behrendt et al., 1992, 1995, 1997, 2001). Pollen grains from an industrial region with high polyaromatic hydrocarbons were shown to be agglomerated with airborne particles. In vitro exposure of grass pollen to particles demonstrated ultrastructural changes at the surface of the pollen and within the protoplasm, such as exocytosis of granular proteinaceous material and increased allergen release (Behrendt et al., 1997).

Fujimaki et al. (1994) examined the effect of intratracheal instillation of a mixture of diesel exhaust particles and Japanese cedar pollen on IgE antibody production and lymphokine production in mice. IgE antibody production and IL-4 production in mediastinal lymph nodes were significantly increased in mice instilled with the diesel exhaust particles and the cedar pollen compared with the cedar pollen alone. There was a slight increase seen in IL-2 output. Measurable levels of birch pollen-specific human IgE were noted in hu-PBL-SCID mice previously stimulated with birch pollen. When the mice were exposed i.p. to the 25 μg birch pollen plus 500 μg of diesel exhaust particles, IgE levels were twice as high as those for birch pollen exposure only. Ormstad et al. (1998) found that *Fel d* 1 (cat), *Can f* 1 (dog), *Der p* 1 (house dust mite) and *Bet v* 1 (birch pollen) allergens bind with soot particles from diesel exhaust in the < 2.5 μm size range. When the particle mixture was injected in the footpad of mice, adjuvant activity was noted on the production of IgE antibodies to ovalbumin (Ormstad, 2000). The authors suggested that it is likely that the soot particles alone were responsible for

some of the adjuvant activity. However, the particles may increase the IgE production to allergens by modulating the immune response.

Diaz-Sanchez et al. (1997) studied possible synergistic relationships between diesel exhaust particles (DPM) and ragweed allergen. Inconsistent and low levels of mucosal cytokine mRNAs were found in ragweed sensitized subjects following intranasal challenge with ragweed allergen alone. When the subjects were challenged with ragweed allergen and DPM there was a decrease in Th1-type cytokines (IFN-γ and IL-2) expression but an elevated expression of mRNA for other cytokines (IL-4, IL-5, IL-6, IL-10, IL-13). Ragweed allergen and DPM also produced a 16-fold increase in ragweed-specific IgE but not total IgE levels or IgE-secreting cell numbers. Total and specific IgG-4 levels were enhanced , while total IgG levels were not. Subject were given short ragweed *Amb a* I allergen, starting at 10 allergen units and increasing in 10-fold units until symptoms were noted. The diesel particles were administered for a total of 0.3 mg in 200 μL of saline. Clones of deleted switch circular DNA (Sε/Sμ), representing switching from μ to ε from the nasal lavage cells, also were detected (Fujieda et al., 1998).

Brunekreef et al. (2000) suggested that airborne pollen associated with allergic responses may pose more serious effects than previously thought. They evaluated the relationship between the daily number of deaths in the Netherlands for the period of 1986 to 1994 and air pollution, meteorological factors, and airborne pollen concentrations (analyzed as categorical variables). The relationship between mortality and airborne pollen concentration was modeled using Poisson regression with generalized additive models. The pollen mortality associations were adjusted for long-term and seasonal trend, influenza morbidity, ambient temperature, humidity, and indicators for the day of the week and holidays. The average number of daily deaths for the study period was 332.5 (total), including 141.8 cardiovascular related deaths, 15.8 COPD related deaths, and 9.8 pneumonia related deaths. Pollen concentrations were only weakly associated with air pollution and there was no confounding by PM_{10}, black smoke, sulphate and nitrate aerosols, NO_2, SO_2, or O^3. *Poaceae* (grass) pollens were associated with daily deaths due to COPD and pneumonia. Other pollens, especially *Betula* (birch) and *Rumex* (sorrel, dock) were also positively correlated with mortality. Information was not included on whether this association was with daily deaths due to cardiovascular disease, COPD, and/or pneumonia. The authors suggested that acute exacerbations of allergic inflammation associated with high pollen

exposures may also precipitate death due to cardiovascular disease, COPD, or pneumonia in individuals suffering from these disorders.

Rosas et al. (1998) reported an association between asthma hospital admissions and grass pollen exposure for children, adults, and seniors in Mexico City. The effects were noted for both the wet (May through October) and dry (November through April) seasons. The number of hospital admissions increased by a factor of 2 to 3 for children and adults on day when the grass pollen concentrations were above 20 grains/m^3. There was no association between asthma exacerbation and tree pollen.

An association between asthma and emergency room visits was reported by Celenza et al. (1996). During a 2-mo study period, the daily average number of emergency room visits was 2.25 patients; but, following a thunderstorm, such visits increased to 40. There was a peak in pollen levels about 9 hs before the peak in asthma emergency room visits. Three hours after the storm, the pollen count increased from 37 to 130 grains/L. There was no evidence that vehicle exhaust pollutants were related to the increase in asthma emergency room visits.

Hastie and Peters (2001) studied the effect of in vivo ragweed allergen exposure (via bronchoscopic segmented ragweed challenge) on ciliary activity of bronchial epithelial cells harvested 24 h after challenge in human volunteers and allergic subjects with severe inflammatory response. Nonallergic subjects with mild inflammatory response showed a minimal ragweed allergen effect on ciliary activity, a slight increase in bronchoalveolar cells, and a nonsignificant increase in albumin concentration. Allergic subjects with mild inflammatory changes showed slight but significant increase in albumin concentration and a two-fold increase in bronchoalveolar cell concentration. The allergic subjects with severe inflammatory changes had a 12-fold increase in albumin concentration and a 9-fold increase in bronchoalveolar cell concentration.

Delfino et al. (1997,1996) conducted several studies evaluating the association between asthma incidence and exposure to various air pollutants and fungal spores and pollen. There was an association between exposure to air pollutants and fungal spores and symptom severity as measured by inhaler usage. Inhaler puffs increased by 1.1/100 ppb O_3 (14 to 87 ppb; 12-h daytime average) and by up to 1.2/1,000 fungal spores/m^3 (648 to 7,512 spores/m^3) depending on the species. Delfino et al. (1997) found an association between asthma severity (asthma symptom scores and inhaler use) and peak expiratory flow rate (PEFR) and total fungal spores.

Symptom severity was more strongly associated with basidiospore concentrations, especially during the period of sporulation. There was no detected association between O_3 exposure and asthma severity in the Delfino et al. (1996) study, possibly due to O^3 measurement problems (as suggested by the authors). There was also no significant relationship between asthma severity and PM_{10} or pollen exposure, but, their concentrations during the study period were low, 26 $\mu g/m^3$ and 216 grains/m^3, respectively.

In summary, newly available information indicates release of allergen-laden material from pollen-spores in respirable-sized aerosols and suggests possible ways by which binding of such material to other airborne particles (e.g., DPM) may concentrate such allergens in ambient air or, once inhaled, jointly exacerbate allergic reactions in susceptible human populations. It should also be noted that pollen itself may act as a carrier for other allergenic materials. Spiewak et al. (1996a) found Gram-bacteria and endotoxin on the surface of pollens; and Spiewak et al. (1996b) found concentrations of several immunotoxicant allergens (Gram+ and Gram− bacteria, thermophilic actinomycetes, fungi) to range from 0 to 10,000 cfu/g of pollen from several grasses or trees in Poland.

7B.2.3 Fungi and Their Byproducts

Fungal spores are known cause of allergic diseases. All fungi may be allergenic depending on the dose. Once an individual is sensitized to the fungi, small concentrations can trigger an asthma attack or some other allergic response (Yang and Johanning, 2002). Unlike fungal-induced allergic responses, fungal toxic inflammatory responses depend on airborne concentrations and are similar for most individuals. Fungi concentrations are usually higher in the indoor environment; but, as noted earlier, outdoor airborne spores are often the source of indoor fungal contamination (Koch et al., 2000).

Fungi produce a variety of byproducts, including mycotoxins and volatile organic compounds. Mycotoxins have low volatility, making inhalation of volatile mycotoxins unlikely. However, mycotoxins are an integral part of the fungus. Volatile organic compounds or VOCs (derivatives of alcohols, ketones, hydrocarbons, and aromatics) are produced when the fungi are actively growing. Concentrations of these VOCs are generally quite low and relationships between exposure and health effects are unclear (Yang and Johanning, 2002).

A number of studies have suggested a relationship between exposure to fungi and their byproducts in respiratory illnesses and immune pathology (Hodgson et al., 1998; Tuomi et al., 2000; Yang and Johanning, 2002). Some fungal byproducts have been shown to stop ciliary activity in vitro and may act to produce general intoxication of macroorganisms through the lung tissue or to enhance bacterial or viral infection (Piecková and Kunová, 2002; Yang and Johanning, 2002). Larsen et al. (1996) showed non-immunological histamine release from leukocytes exposed to a suspension of fungal spores and hyphal fragments and suggested that the fungal suspension possessed at least two histamine releasing components; an energy-dependent release process and a cytotoxic release process.

In a study by Rosas et al. (1998), there was a statistically significant increase in fungal spore exposure-related asthma hospital admissions in children in Mexico City that was not seen in adults and seniors. The highest spore (ascomycetes and basidiospore) levels were associated with a 2- to 3-fold increase in hospital admissions per day. Ascomycetes and basidiospore concentrations ranged from < 100 to 207 spores/m^3 and from < 100 to > 1000 spores/m^3, respectively. There was an association with hospital admissions during both the wet and dry season. There was no strong statistical association between asthma admissions and NO_2 (mean: 0.102 and 0.164 ppm),O_3 (mean: 0.204 and 0.187 ppm), SO_2 (mean: 0.074 and 0.081 ppm), TSP (mean: 78 and 156 μg/m^3) and PM_{10} (mean: 56 and 98 μg/m^3) concentrations during either the wet or dry seasons.

Airborne fungal concentrations of ≥1000 spores/m^3 were reportedly associated with asthma deaths among 5 to 34 year olds in Chicago between 1985 and 1989 (Targonski et al., 1995). The odds of death occurring on days with airborne fungal concentrations of ≥ 1000 spores/m^3 were 2.16 times higher than other days. Logistic regression analysis was used to compare the probability of deaths caused by asthma as the result of tree, grass, and ragweed pollen and fungal spores. Fungal spores were counted as a single group. Asthma deaths were obtained from death certificates. The deaths were also related to personal, social, and medical access factors.

Several newly-published studies have evaluated levels of fungi or their viable propagules in ambient (outdoor) and/or indoor air in various areas of the U.S. or other countries in Europe or East Asia. In an extensive 22-mo study, Cooley et al. (1998) investigated the types of fungi found in indoor and outdoor air at 48 schools in U.S. areas located along the Atlantic seaboard and Gulf of Mexico. Five fungal genera consistently found in outdoor air comprised > 95% of

the outdoor air fungi detected: *Cladosporium* (81.5%); *Penicillium* (5.2%); *Chrysosporium* (4.9%); *Alternaria* (2.8%); and *Aspergillus* (1.1%). An average of ~700 colony-forming units (CFU)/m^3 of *Cladosporium* fungi were found in outdoor air (about 3 times that found indoors); whereas relatively low concentrations of *Penicillium* (~30 CFU/m^3) and the other species (ranging from < 5 to ~40 CFU/m^3) were found in ambient air (compared to indoors, except for notably elevated average levels for samples taken from indoor "complaint areas" where markedly higher numbers of indoor air quality (IAQ)-related symptoms (nasal drainage, congestion, watery eyes, headaches, allergies, etc.) were reported among students, teachers, and other staff. The finding of *Penicillium* was most consistently found to be elevated in complaint areas, the growth of this rather ubiquitous species being optimized between 10 - 25 °C and predominating in complaint areas with a wide range (23 - 67%) of relative humidity. Cooley et al. noted: (a) that Penicillium spores are small (1 - 5 µm) and capable of entering the lower respiratory tract; and (b) that bronchial challenges with *Penicillium* species spores cause immediate and delayed-type asthma in sensitized subjects (Licorish et al., 1985).

In a detailed study of the nature and variation of fungi inside and outside homes in the greater New Haven, CT area, Ren et al. (1999) found that fungi in living room, bedroom, and outdoor air varied across seasons but did not differ seasonally in basement air. They reported that *Claudosporium spp.* dominated both indoor and outdoor air during summer months, whereas *Penicillium* and *Aspergillus* were dominant in indoor air in winter, but neither were dominant in outdoor air during any season. Ren et al. further noted: (a) the fungi isolated in their study are broadly the same as those found in European studies (Beaumont et al., 1984, 1985; Verhoeff et al., 1988; Hunter and Lea, 1994); (b) the seasonal trend found by them for fungal propagules indoors and outdoors were generally comparable with those reported by Hunter and Lea (1994) for British homes, i.e., lowest in winter, increasing in spring, reaching the maximum in summer, and decreasing in fall; (c) their results support current concepts that outdoor air may affect cultural fungal propagules indoors, but the presence of cultural molds in indoor air may not always reflect the presence of such molds in outdoor air, especially in problem indoor environments; and (d) no associations were found between fungal types and their concentrations in dust and in air, suggesting that types of fungi and concentrations measured in housedust do not necessarily reflect those in indoor air, with air samples likely providing a more direct and better measure of inhalation exposure to fungi. Lastly, Ren et al. (1999) noted that: 50% of the

342 air samples taken during the 1996-1997 study period had < 575 CFU/m^3 total cultural fungal propagules; 97% < 100 CFU/m^3 of Alternaria; < 28% > 50 CFU/m^3 of Aspergillus; and ~90% < 250 CFU/m^3 of Penicillium; and none had *Cladosporium spp.* over the 3000 CFU/m^3 level set as an allergic threshold by Gravesen (1979).

Koch et al. (2000) obtained data on fungi concentrations in a study that evaluated if differences in types of seasonal variations in concentrations of fungi in indoor and/or outdoor air occur and could perhaps account for lower prevalence of allergies and asthma in Western than in Eastern Germany. During 1995-1997, 405 homes in Hamburg (West) and Erfurt (East) Germany were visited twice and samples of settled dust taken by vacuuming from carpets in the living room. No significant differences were found between the two cities for total genera or single fungi species (*Alternaria*, *Aspergillus*, *Claudosporium*, and *Penicillium*) with regard to concentrations of viable fungi detected in settled housedust. Similar seasonal variations were observed for outdoor air and indoor dust, i.e., with a late summer peak detected in outdoor air (~2400 CFU/m^3 viable fungi in August) and a parallel peak in such concentrations in housedust. Koch et al. also noted: (a) that recent studies indicate that outdoor air spora influence the presence of fungi in indoor environments, but indoor air levels of fungi in indoor environments do not simply reflect the presence of fungi or spora in outdoor air; and (b) that the genera commonly isolated in housedust (e.g., *Claudosporium*, *Penicillium*, *Alternaria*, *Aspergillus*) reflect their relative occurrence in outdoor spore counts.

Takahashi (1997) evaluated fungal types and concentrations in indoor and outdoor air in Yokahama, Japan and found the number of outdoor total fungal colony-forming units to vary from < 13 to 2750 CFU/m^3. *Claudosporium spp.* again was found to predominate in outdoor air, followed by *Alternaria spp.* and *Penicillium spp.*, with fungal concentrations peaking in September. Outdoor fungal concentrations were significantly correlated with maximum, minimum, and average temperature of the day, as well as average wind velocity of the day, relative humidity, and precipitation for the month. The ranges of concentrations of fungi in outdoor air were reported by Takahashi to be the same as reported for many European, North American countries, and Israel — with most showing peak levels during the summer and early fall (July to October) and lowest means during winter months (January to February).

In another East Asia study, Su et al. (2001) compared concentrations of airborne fungi, endotoxin, and housedust mite allergens in the homes of asthmatic and non-asthmatic children in

southern Taiwan, where temperature and relative humidity are high throughout the year. With regard to fungi, their results paralleled those of other studies noted above in many respects, except for some differences in seasonal variations — not too surprisingly given the more constant high temperature/humidity conditions in this study area. The most predominant indoor genera were *Claudosporium*, *Aspergillus*, *Penicillium*, *Alternaria*, and yeast. *Cladosporium* ranked highest, it being in ~85% of the colonies from indoor samples and its highest CFU/m^3 concentration in winter and other seasonal variation patterns also applying for the other types of fungi. Outdoor air *Claudosporium* levels were significantly correlated with indoor air values during all seasons; and the indoor/outdoor concentrations for the other fungi were also positively correlated during the spring. This suggests that outdoor levels of fungi and/or their spores are important determinants of indoor air levels of fungi in southern Taiwan.

7B.2.4 Endotoxins

Endotoxins and lipopolysaccharides (LPS; chemically purified version of endotoxin) are present in the outer cell membrane of all Gram-negative (Gram-) bacteria. Endotoxins are toxic to most mammals. When released into the blood stream, it is thought that endotoxins/LPS interact with receptors on monocytes and macrophages and other types of receptors on endothelial cells, triggering the production of cytokines, which in turn stimulate production of prostaglandins and leukotrienes, arachidonic acid metabolites (e.g., prostacyclin and thromboxane A_2, and nitric oxide). These mediators can induce physiological changes, e.g., inflammation, smooth muscle constriction, and vasodilatation (Young et al., 1998).

Some of the more recent inhalation studies on endotoxin exposure are summarized in Table 7B-3. In vitro studies on particle-associated endotoxin are discussed in Section 7.5.2.2. Heederik et al. (2000) noted that animal feces and plant materials contaminated with bacteria contribute most to organic dust-related endotoxin exposure. Although there is strong evidence that inhaled endotoxin plays a major role in the toxic effects of bioaerosols encountered in the work place (Castellan et al., 1984, 1987; Rose et al., 1998; Vogelzang et al., 1998; Zock et al., 1998), it is not clear as to what extent typical ambient concentrations of endotoxin are sufficient to produce toxic pulmonary or systemic effects in healthy or compromised individuals.

Several new occupational exposure studies have yielded potentially useful information for estimating exposure-response relationships for health effects associated with exposure to

airborne endotoxin. For example, Vogelzang et al. (1998) evaluated exposure-response relationships for lung function decline in relation to endotoxin exposure of pig farmers in The Netherlands. Long-term average exposure to endotoxin and dust was evaluated via personal monitoring during summer and winter for a cohort of 171 pig farmers over a three-year period. Mean age at start was 39.6 yrs and mean number of years worked in pig farming was 16.7 yrs. Linear regression analyses were used to analyze relationships between declines in FEV_1 or FVC (based on measures taken in the 1st or 3rd years of the studies) and dust concentrations or endotoxin levels in the inhalable dust. Statistically significant ($p < .05$) associations (correcting for age, baseline values, and smoking) were found by regression analysis between estimated long-term average exposure (typically ≥ 5 h/day) to endotoxin (105 ng/m^3) and annual decline in $FEV_{1.0}$ (73 mL/y) and FVC (55 mL/y). The FVC, but not the $FEV_{1.0}$, declines were also significantly correlated with inhalable dust concentrations (long-term average = 2.63 mg/m^3). The $FEV_{1.0}$ annual average decline is large in relation to the expected age-related decline of 29 mL/y but equal to that of 73 mL/y reported by Iversen et al. (1994) based on a 5-y study of farmers. The least exposed pig farmers in the Vogelzang et al. study showed an average $FEV_{1.0}$ decline similar to the expected age-related decline, whereas the predicted decline for the most exposed pig farmers ranged up to 100 mL/y. The authors noted that their results support the selection of the lower of two proposed (Clark, 1986; Palchak et al., 1988) occupational exposure threshold levels of 30 or 100 ng/m^3 for airborne endotoxin.

Some health effects have been reported for occupational exposure to complex aerosols containing endotoxin at concentrations likely more relevant to ambient levels. Zock et al. (1998) reported a decline in FEV_1 (≈ 3%) across a shift in a potato processing plant with up to 56 endotoxin units (EU)/m^3 in the air. Rose et al. (1998) reported a high incidence (65%) of BAL lymphocytes in lifeguards working at a swimming pool where endotoxin levels in the air were on the order of 28 EU/m^3. Although these latter two studies may point towards possible pulmonary changes at low concentrations (~25-50 EU[3]) of airborne endotoxin, it is not possible to rule out the contribution to observed effects by other agents present in the complex airborne organic aerosols in the occupational settings studied.

In another European study, Heinrich et al. (2003) recently carried out temporal-spatial analyses of endotoxin in fine ($PM_{2.5}$) and coarse ($PM_{10-2.5}$) particle mass of ambient aerosols from two East German towns about 80 km apart. The authors noted that one town, Hettstedt, showed

consistently higher prevalence of hay fever and strong allergic sensitization for children than the prevalence rates seen in the other town, Zerbst, even into the late 1990's when levels of ambient air pollutants (TSP, SO_2) had converged in areas earlier differing in such air pollution levels (Heinrich et al., 2002a,b). From January to June 2002, weekly $PM_{2.5}$ and $PM_{10-2.5}$ samples were taken by dichotomous samplers in each of the two towns and analyzed for endotoxin in the collected ambient PM. The arithmetic mean for the $PM_{2.5}$ sample mass average 10.2 and 12.4 $\mu g/m^3$ for Hettstedt and Zerbst, respectively; and $PM_{10-2.5}$ sample mass 6.1 and 6.8 $\mu g/m^3$, respectively. Comparable ranges for Hettstedt and Zerbst were 0.3-25.8 and 4.2-26.3 $\mu g/m^3$ for $PM_{2.5}$ and 1.2-10.6 and 3.0-10.7 $\mu g/m^3$ for $PM_{10-2.5}$. Mass levels for both particle size fractions showed notable week-to-week fluctuations (mostly closely parallel for both towns), with weekly means in each town being highest in late March/early April. Airborne endotoxin levels for both towns showed strong seasonality in parallel patterns for both the fine and the coarse particle fractions, with endotoxin mass concentrations generally being low during late winter/early spring in comparison to their generally increasing from late April to highest points seen in early June (except for a brief episode of elevated endotoxin in Hettsted fine PM seen in late January/early February). Fine PM endotoxin mass concentrations for Hettstedt (1.2 EU/mg^3 arith. mean) were not significantly different from such concentrations for Zerbst (1.1 EU/mg^3 arith. mean), but endotoxin levels expressed per mg^3 were significantly higher in Zerbst, suggesting that there may be a higher biogenic content or more bioactive particles in the Zerbst fine PM fraction. The endotoxin levels in the coarse fraction were about 10 times those in the fine fraction, whether expressed in EU/mg dust or EU/m^3 air and were not statistically significantly different between the two towns. The range of endotoxin levels for Hettsted were 0.2 to 3.6 EU/mg dust and 0.002 to 0.21 EU/m^3 air for $PM_{2.5}$ versus 4.0 to 25.2 EU/mg dust and 0.01 to 0.24 EU/m^3 air for $PM_{10-2.5}$. The comparable concentrations for Zerbst were 0.2-4.3 EU/mg dust and 0.004-0.031 EU/m^3 for $PM_{2.5}$ versus 3.1-24.2 EU/mg dust and 0.02-0.17 EU/m^3 air for $PM_{10-2.5}$. The authors concluded that, given the generally similar levels and patterns in seasonal variations of endotoxin concentrations in Hettstedt and Zerbst, it was unlikely that differential exposures to endotoxin could explain differences in hay fever or allergic reaction prevalence between the two towns.

The levels of endotoxin concentrations found in Hettsted and Zerbst are similar to those reported for other ambient or rural aerosols and dusts, with those in coarse PM fractions

typically exceeding those in fine fractions, as noted by Heinrich et al. (2003). They also noted that measurements in livestock buildings (poultry, pig, cattle) often show endotoxin levels up to several thousand EU/mg dust, with levels in the inhalable PM_{10} fraction being higher by ~10-fold than in the fine PM. The finding of notably higher concentrations and absolute mass amounts of endotoxin in coarse-mode particle samples versus fine particle samples thus appears to hold, in general, across a number of geographic areas and for both occupational and environmental situations. The authors also noted the seasonal variation observed in their study with increased airborne levels of endotoxin in May and June apparently following increased growth of fungi, other plants, and presumably of microbes due to increasing outdoor spring temperatures under moderate climatic conditions in Germany. They also noted that increased levels of plant-related materials and leaf surfaces (Rylander, 2002), as well as pollen surfaces (Spiewak et al., 1996a), may provide additional sources of growth of Gram-bacteria (from which endotoxin is derived). The seasonal variation in endotoxin concentrations observed by Heinrich et al. appear to parallel those seen in other studies for ambient airborne endotoxin levels (i.e., lower in winter and high during warmer weather in late spring/summer).

Park et al. (2000) evaluated endotoxin levels in indoor dust of 20 homes, indoor air of 15 homes, and outdoor air at two locations in the Boston, MA, area. They found that endotoxin levels in indoor dust (from the bed and bedroom/kitchen floors) were not significantly associated with indoor airborne endotoxin concentrations. The airborne endotoxin levels were, however, significantly associated with absolute humidity; and a significant seasonal effect for kitchen dust (spring > fall) and indoor airborne endotoxin (spring > winter) was seen, as was a significant seasonal pattern for outdoor airborne endotoxin (summer > winter). The authors indicated that, overall, the indoor airborne endotoxin levels (geom. mean = 0.64 EU/m^3) were higher than outdoor concentrations (geom. mean = 0.46 EU/m^3); but seasonal variations were evident, in that indoor airborne endotoxin levels were generally higher than outdoor airborne endotoxin levels during September-April and lower than outdoor levels during late spring/summer (May-August). Outdoor airborne endotoxin levels showed significant seasonality, varying by more than 4-fold across seasons, with decreases in outdoor levels beginning at the end of summer/early fall and remaining at lowest levels during winter before starting to increase again with onset of the growing season in late spring. The authors noted that this pattern is consistent with data suggesting that outdoor Gram-bacteria (and thus airborne) endotoxins are shed from leaves of

growing plants (Edmonds, 1979; Andrews, 1992). Further, the overall mean outdoor airborne endotoxin levels at an urban sampling location (geom. mean = 0.51 EU/m^3) were somewhat (but not significantly) higher than at a suburban location (geom. mean = 0.39 EU/m^3).

Thorn and Rylander (1998a) examined the effect of endotoxin inhalation on inflammatory response in 21 healthy subjects from 20 to 30 years old. All subjects were known smokers, currently did not have a respiratory infection, no self-reported allergies or chronic bronchitis, and no physician diagnosed asthma. Subjects were examined before exposure to up to 40 µg LPS. The LPS was suspended in saline, aerosolized, and then delivered to the subject by a nebulizer adjusted to give a 4 µl/dose. The subjects inhaled 20 puffs of LPS at a concentration of 500 µg/mL, for a total of 40 µg. Cell counts, ECP, and MPO were monitored in the blood and sputum before and 24 h following exposure. Myeloperoxidase was significantly increased in both the blood and sputum following inhalation of the LPS. Eosinophilic cationic protein was increased but the increase was only significant in the sputum. The ratio of MPO and neutrophils was significantly decreased in blood and sputum. Spirometric testing demonstrated a significant decrease in FEV_1 and FVC values following LPS inhalation. Subjects experienced throat irritation, dry cough, breathlessness, unusual tiredness, headache, and heaviness in the head. The symptoms developed 4 to 6 h following exposure and persisted for 6 to 8 h.

Michel et al. (1997) examined the dose-response relationships for effects of inhaled lipopolysaccharide (LPS: the purified derivative of endotoxin) in normal healthy volunteers exposed to 0, 0.5, 5, and 50 µg of LPS. Inhalation of 5 or 50 µg of LPS resulted in increased PMNs in blood and sputum. At the higher concentration, a slight (3%) but nonsignificant decrease in FEV_1 was seen.

Other controlled exposure studies of laboratory animals (rat) by Elder et al. (2000a,b) indicate that priming of the respiratory tract by inhaled endotoxin increases the effect of inhaled ultrafine surrogate particles and ozone (as discussed in more detail in Section 7.6).

In vitro studies of potential endotoxin contributions to toxic effect of ambient PM are discussed in Section 7.4.2.

7B.2.5 (1 → 3)-β-D-Glucan

Studies from different countries have reported relationships between damp/humid indoor environments and various symptoms in both adults and children (Meklin et al., 2002b). Such symptoms consist of eye, nose, and throat irritation, dry cough, headache, tiredness, and sometime skin problems. Fungi and their byproducts (discussed above) and bacteria commonly present in damp/humid indoor environments contain several substances that have known inflammatory properties. Of the substances associated with these symptoms, (1→3)-β-D-glucan, a polyglucose compound in the cell walls of fungi, certain Gram+ bacteria, and plants, has begun to be accorded increasing attention.

The (1 → 3)-β-D-glucan can induce several biological responses in vertebrates, including stimulation of the reticulo-endothelial system, activation of neutrophils, macrophages, and complement, and possibly activation of eosinophils. T-lymphocyte activation and proliferation have been reported in experimental animals (Heederik et al., 2000). Rylander (1996) suggested that an acute exposure to (1 → 3)-β-D-glucan can produce symptoms of airway inflammation in normal subjects without a history of airway reactivity after exposing subjects to 210 ± 147 ng/m^3 (1 → 3)-β-D-glucan for 3 separate 4 h sessions 5 to 8 days apart. Exposure to (1 → 3)-β-D-glucan alone did not significantly impact FEV_1 values; but there was a slight decrease in FEV_1 values following administration of the two highest doses of methacholine (MCh). Methacholine was administered in increasing doses in 3 min intervals for a total of 1.25 mg. Forced vital capacity (FVC) and FEV_1/FVC were also unchanged following (1 → 3)-β-D-glucan exposure and MCh challenge. There was a significant, negative correlation between MCh-induced decrease in FEV_1 values and the intensity of throat irritation after 1 h exposure. The intensity of nasal irritation and stuffy nose and throat irritation was increased at 1 and 4 h. Dry cough, cough with phlegm, chest tightness and wheezy chest was not affected. No effects on airway responsiveness or inflammatory symptoms were noted in subjects exposed to endotoxins (9.9 ng/m^3) under the same exposure conditions.

Thorn and Rylander (1998b) examined the relationship between exposure to airborne (1 → 3)-β-D-glucan and airways inflammation. The study was conducted on a group of 75 houses in Gothenburg, Sweden where there had been numerous complaints about dampness and respiratory symptoms, fatigue, and mold odors. Measurements of (1 → 3)-β-D-glucan and endotoxins in airborne dust were made with Limulus lysates. Study participants included

67 females and 62 males 18 to 83 y old and included 34 smokers and 9 physician-diagnosed asthmatics. The average number of years the subjects lived in their house was 18 y (range 2 to 36 y). Study participants provided questionnaire information for assessment of organic dust-induced effects. The questionnaire inquired about existing diseases states; occupation; length of time the subject had lived in the house; the presence of pets; and the occurrence of cough (dry or with phlegm); shortness of breath; nose, throat, and eye irritation; nasal and chest congestion; and joint and muscle pains, headache, fatigue, and dermal disorders. Other questions addressed subjective airway reactivity, chronic bronchitis, asthma, and episodes of fever and influenza-like symptoms gone the next day. Chronic bronchitis was defined as a cough with sputum for at least 3 mo a year for a period of at least 2 y. Asthma was defined as physician-diagnosed asthma. Spirometry was performed on test subjects to exclude subjects with less than 70% of predicted values in FEV_1 and/or FEV_1/FVC. Airway responsiveness was assessed using MCh for a total of 1.2 mg MCh, administered in increasing doses at 3-min intervals. Serum eosinophilic cationic protein (ECP), myeloperoxidase (MPO), and C-reactive protein (CRP) were measured. Atopy was determined using the Phadiatop test to measure the concentration of specific IgE antibodies against airborne allergens.

No detectable levels of endotoxin were found in the homes, but (1 → 3)-β-D-glucan levels ranged from 0 to 19 ng/m^3. Of 75 homes studied, 20 had (1 → 3)-β-D-glucan concentrations below 1 ng/m^3 and 13 homes had levels above 6 ng/m^3. Twenty-four subjects had positive Phadioatop test; but there was no significant correlation between exposure and atopy. However, when evaluated by age, there was a significantly larger number of atopic subjects in the > 65 y old group exposed to > 3 ng/m^3 (1 → 3)-β-D-glucan. There was a significant inverse correlation between baseline FEV_1 and number of years the subjects lived in the house when controlled for age, gender, cigarette smoking status, asthma, atopy, and pets among male subjects < 65 y old that was not seen in the female subjects < 65 y old and in > 65 y old subjects. The relationship was present only for those male subjects exposed to > 1 ng/m^3 (1 → 3)-β-D-glucan. Atopic subjects exposed to > 1 ng/m^3 (1 → 3)-β-D-glucan had significantly higher serum MPO. Serum ECP and CRP were also higher in these subjects but not significantly so.

Douwes et al. (2000) examined the relationship between exposure to (1 → 3)-β-D-glucan and endotoxins and peak expiratory flow (PEF) in children (ages 7 to 11 y) with and without chronic respiratory symptoms. The children were monitored twice a day for PEF variability.

House dust samples from living room and bedroom floors and the children's mattresses were taken during the PEF monitoring period. As indicated by linear regression analysis (adjusting for dust mite allergen levels, the presence of pets, and the type of flooring in the home), peak expiratory flow variability in the children with chronic respiratory symptoms was strongly associated with (1→3)-β-D-glucan levels in dust from living room floors when expressed in micrograms per square meter. The association was strongest for atopic children with asthma.

7B.3 SUMMARY

Bioaerosols, from sources such as plants, fungi, and microorganisms, range in size from 0.01 to µm to > 20 µm. They comprise a small fraction of ambient PM, but have been shown to contribute to health affects associated with PM exposure.

Pollen from flowering plants, trees and grasses, deposits in upper airways to induce allergic rhinitis. Allergen-containing cytoplasmic fragments from ruptured pollen grains can enter the deep lung, where they can exacerbate asthma. Synergistic interactions between pollen debris and other ambient PM (e.g., the polycyclic hydrocarbon component of DE) are thought to be a mechanism that may explain the increased incidence of asthma morbidity and mortality. Fungal spores are the largest and most consistently present outdoor bioaerosol. They cause allergic rhinitis and asthma, which is highly dependent on seasonal variations in airborne fungi concentration (being highest in spring/summer and lowest in winter). Exposures have been linked to asthma hospitalization and death. Human handling and burning of plant material contributes to increased bioaerosol levels, which have been shown to have adverse health effects.

Animals and insects produce bioaerosols capable of producing hypersensitivity diseases. Most notably, exposure to dust mite and cockroach material has been linked to sensitization in children.

Also, bacteria and viruses are significant bioaerosols. Much of the toxicity of bacteria is due to the endotoxins present in the outer cell membrane, which trigger production of cytokines and a cascade of inflammation. Concentrations of endotoxins are seasonal (highest in warm months — lowest in cold months), and are typically associated more with coarse-mode than with fine-mode particles. Another component, (1→3)-β-D-glucan, of cell walls of fungi, certain bacteria, and plants, has also been shown to cause respiratory inflammation.

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