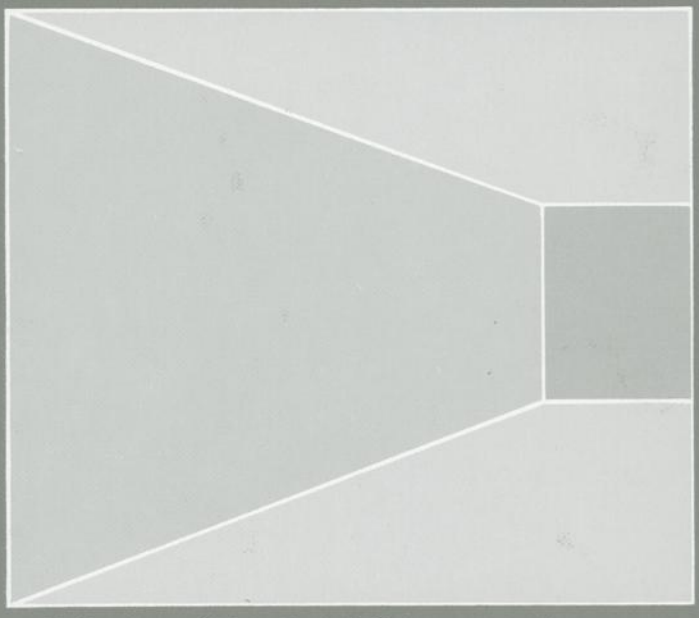
BIOLOGICAL CONTAMINANTS IN INDOOR ENVIRONMENTS



Morey/Feeley/Otten, editors

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Biological Contaminants in Indoor Environments

Philip R. Morey, James C. Feeley, Sr., and James A. Otten, editors

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Peer Review Policy

Each paper published in this volume was evaluated by three peer reviewers. The authors addressed all of the reviewers' comments to the satisfaction of both the technical editor(s) and the ASTM Committee on Publications.

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Foreword

This publication, *Biological Contaminants in Indoor Environments*, contains papers presented at the symposium of the same name held in Boulder, CO, on July 16–19, 1989. The symposium was sponsored by ASTM Committee D-22 on Sampling and Analysis of Atmospheres and its Subcommittee D22.05 on Indoor Air, Section .06 on Biological Aerosols. Dr. Philip R. Morey of Clayton Environmental Consultants, James A. Otten of Martin Marietta Energy Systems, Inc., and the late James C. Feeley, Sr. of Pathogen Control Associates, Inc., presided as symposium chairman. They also served as editors of this publication.

James C. Feeley, Sr.

1937-1989

Dedication

The proceedings of this symposium are dedicated to the memory of James C. Feeley, Sr. (1937-1989). Jim was involved in the planning and organization of this symposium and is listed as one of the editors for this important work on biological contaminants in indoor environments.

Jim gave abundantly of his time, talent, and knowledge to further the science of microbiology and the emerging field of indoor air quality. Many of us are beneficiaries of his constant efforts to strive for excellence; the science of microbiology is his debtor.

Jim Feeley received his Ph.D. in microbiology from the University of Georgia, Athens in 1978. Jim had already begun his distinguished career with the Centers for Disease Control (CDC) in Atlanta which continued until his retirement in 1986. Jim began his career as a microbiologist with CDC in 1964 and at the time of his retirement he was Chief of the Field Investigations Laboratories, Respiratory and Special Pathogens Branch. During this time, Jim authored or co-authored over 100 publications. His earlier publications included work on Bacillus anthracis. In 1969 Jim was part of a team that performed an epidemiologic study of inhalation authrax. Later publications were centered around Yersinia enterocolitica.

Following the outbreak of Legionnaires' Disease in 1976, Jim played an active role in studying the epidemiology of the disease and the growth characteristics of Legionella pneumophila. Jim received a special citation for his work on Legionella by the Infectious Disease Society of America and in recognition for his work with Legionnaires' Disease, the species Legionella feeleii was named in his honor. In addition to these honors, Jim also received six special commendations from the directors of the CDC.

Following his retirement from CDC, Jim formed his own company, Pathogen Control Associates, Inc., where he remained until his sudden death from a heart attack on September 15, 1989. Jim's company provided a valuable consulting service to other organizations in the identification of microbial contaminants and their subsequent remediation. It is an indication of Jim's foresight that this company continues to provide this service today.

Jim always found time to give sage counsel to professional societies. He was a charter member of the American Conference of Governmental Industrial Hygienists' (ACGIH), Bioaerosols Committee. His thinking on "reservoirs, amplifiers, and disseminators" is evident in the latest "Guidelines for the Assessment of Bioaerosols in the Indoor Environment" published by the ACGIH. Jim's counsel will be missed by ventilation engineering colleagues. He was a charter member of the American Society of Heating, Refrigerating, and Air-conditioning Engineers', Inc. (ASHRAE), Environmental Health Committee. The latest ASHRAE position paper on Legionnaires' Disease largely derives from Jim's thoughts and input.

We honor and cherish the memory of our departed colleague. We will miss his encouragement, counsel, hearty laughter, and good nature. We dedicate ourselves and this volume to his memory and to the attainment of the objectives that Jim held so dear.

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Contents

Overview

The purpose of this symposium on biological contaminants in indoor environments was to develop and explore sampling and analytical protocols for microbial agents that may be commonly or uncommonly found indoors. Classes of microbial agents considered in this symposium were:

- Viruses
- Bacteria including, gram negative, gram positive, Legionella, and mycobacteria
- Specialized bacteria including Chlamydia and rickettsia
- Fungi including saprophytes and pathogens
- Protozoa
- New microorganisms
- Mycotoxins and endotoxins

This symposium was organized by ASTM Committee D22.05.06 (Indoor Air, Biological Aerosols) because of increasing concern over possible involvement of microorganisms in building related illnesses and sick building syndrome. The extent to which microorganisms are involved in building sicknesses is still unknown. Sampling and analytical protocols for most types of microbial agents are poorly developed and in those few instances where protocols are available, interpretation of analytical results is inconsistent.

The volume begins with a review by Morey and Feeley on the importance of microbial agents in indoor air quality issues. Twelve chapters follow, each on a specific class of microorganism or microbial agent. Emphasis in these chapters is placed on sampling and analytical protocols, case studies, and data interpretation. An important aspect of each of these 12 chapters is the discussion and closure session where authors responded to the specific questions of the attendees of the conference held in Boulder, Colorado on July 16-19, 1989. The last three chapters provide viewpoints on future developments in the field of indoor bioaerosols research and the likely future involvement of bioaerosols in building performance.

Most of the technical data presented at this conference is primarily of use to microbiologists and professionals who investigate environmental problems in indoor environments. Each of the 12 chapters on specific types of microbial agents provides information on reservoirs where the agent may accumulate, conditions under which the agent may grow or amplify, and examples where dissemination to the breathing zone occurs. Some emphasis is also given to the possible adverse health effects caused by each agent as well as to interpretation of analytical results. Many of the questions on microbial contaminants that are often asked by occupants and owners of buildings are answered in the discussion and closure section at the end of each chapter.

Hierholzer discussed the biological properties and the diseases caused by viruses. The diseases involve mainly the respiratory, conjunctivial, and gastrointestinal tracts of affected individuals.

The physical makeup of viruses determines their ability to survive in the environment with the majority being highly susceptible to desiccation. Viruses can be collected from the air by impaction onto agar surfaces or into liquid media with subsequent growth in cell culture. In addition to cell culture, some viruses can be detected directly by fluorescent antibody tests, enzyme assays, and DNA probes.

LaForce, Hood, Falkinham, and Dennis described gram positive, gram negative, acid fast, and legionella bacteria. Several gram positive bacteria can be recovered from room air, but in cases involving micrococci and staphylococci, the relevance of their role as pathogens has not been established. The pathogenicity of Bacillus anthracis, the causative agent of anthrax, is well known and is used as a case study in describing the transmission of gram positive organisms by the aerosol route. Gram positive bacteria can be collected by impaction on agar and by impingement in liquid media with subsequent incubation at 37C or at room temperature for 48 hours. Filter cassettes can also be used to collect gram positive bacteria, but care must be taken to prevent desiccation which destroys viability in most cases.

Gram negative bacteria have been associated with respiratory diseases and other syndromes where exposure involved bioaerosol emissions from heating, ventilation, and air-conditioning and humidifying systems. An outbreak of hypersensitivity pneumonitis, possibly caused by Cytophaga, is presented as a case study. All gram negative bacteria possess an endotoxin (see chapter by Olenchock) in their cell wall and endotoxin is an important factor in respiratory diseases. Gram negative bacteria can be collected by impaction on agar, in liquid media, and also by filter cassettes. As with gram positive bacteria, gram negative bacteria can be destroyed by desiccation on filters. The organisms are grown on agar media at room temperature, 35C, or 45-50C for evaluation. There is still a lack of information regarding the exact role of these bacteria in respiratory diseases.

Mycobacteria are known to cause disease by the respiratory route; especially well studied is tuberculosis caused by Mycobacterium tuberculosis. Other mycobacteria occurring in natural aerosols do cause human infection, but their epidemiology and route of human infection is less well understood. Mycobacteria can be recovered using impaction on specialized media with subsequent growth at 37C for up to eight weeks.

Legionella pneumophila, causative agent of Legionnaires' disease, is a common aquatic organism which is able to colonize manmade water systems. It is classed as an opportunistic pathogen for man and causes a pneumonia which can be fatal. Pontiac fever, a non-pneumonic disease, has also been linked to Legionellae but is more influenza like. Legionellae can be isolated from a wide range of habitats, especially from warmer waters (35-60C). The Legionellae are generally collected in bulk water samples, concentrated, and their presence detected by fluorescent antibody tests. Legionellae can be identified by growth on special media by

incubation at 35C for several days. Unlike most other airborne organisms, Legionellae are difficult to collect in aerosols.

Cole and Regnery discussed two specialized bacterial groups, the Chlamydia and rickettsia. The Chlamydia consist of three species of organisms causing diseases including pneumonia, conjunctivitis, and diseases of the reproductive and urinary tracts. The organisms replicate only in the cytoplasm of eucaryotic cells and are harbored by man and other animals, especially birds. All three species can transmit disease by the aerosol route. Recommended sampling methods include the liquid impinger and membrane filtration. Chlamydia are analyzed by utilizing a direct fluorescent antibody test or grown in cell culture. Rickettsia are thought to be transmitted between vertebrate hosts exclusively by arthropod vectors. However, the agent responsible for Q fever, Coxiella burnetii, can occur in aerosols and the organism is extremely infectious by inhalation. Most disease in humans is inapparent or self-limiting. However, life-threatening endocarditis can occur. Evidence suggests that Coxiella burnetii may be a common environmental contaminant when appropriate animal reservoirs are present. Cattle, sheep, goats, and several wild animal species harbour the agent. Coxiella burnetii can only replicate within living cells. The agent is sensitive to tetracycline and vaccines are available. The agent can be collected easily using liquid impingers or cotton filters. Coxiella burnetii is metabolically inactive at any pH other than the acid environment associated with the phagolysosome (pH 4.5).

Burge described the saprophytic and pathogenic fungi. Fungi are heterogenous organisms grouped by structure, biochemistry and physiology. Most fungi are able to use non-living organic material as a substrate, but a few are pathogenic and will invade human tissues. Fungi can cause human hypersensitivity, infections, and toxic diseases. Infectious diseases are generally of four types: cutaneous, subcutaneous, systemic, and those that cause opportunistic infections when host defenses are impaired. Cutaneous and subcutaneous mycoses are not considered airborne diseases. Fungi produce a variety of antigenic materials that are spread by the aerosol route. Sensitization to the antigens can occur and result in allergenic diseases such as asthma and hypersensitivity pneumonitis. Fungi need not be viable to be antigenic. In addition, fungi can produce mycotoxins (see chapter by Jarvis) and irritants (for example, volatile organic compounds). Assessment of the indoor environment for fungi should take into account the following: a reservoir that contains living fungi, an amplifier, and a means of dissemination. During an evaluation of a building for indoor environmental problems, bulk samples can be taken to confirm the presence of fungal reservoirs and amplifiers. Air sampling, in general, should only be done to confirm that amplifiers are producing aerosols. Fungi can be collected by impaction onto agar surfaces, into liquids (use a wetting agent because of hydrophobicity), or the spores can be collected on a sticky surface for enumeration. Sample analysis depends on the mode of collection with culture plate samples being incubated for at least 5 days at room temperature for saprophytes and 37C for human pathogens. Spores collected on sticky surfaces are enumerated by examining them

microscopically. Fungi can also be collected on filters and the material enumerated by culture on suitable media or by counting the spores directly. Antigens are assayed using immunological methods. At the present time it is impossible to assess numeric risk for airborne fungi.

Tyndall discussed the health concerns associated with protozoa in indoor environments. Free living amoeba are the likely protozoa that could be implicated in health concerns in the indoor environment. Amoeba can cause allergic reactions, eye infections, and encephalitis. Amoeba can support the multiplication of Legionella. Because of their large size, free living amoeba are not easily aerosolized so they are usually isolated in bulk samples. If air sampling is done, impaction onto agar or collection into liquid is used. Detection methods include plating on agar with E. coli or inoculation into animals. These methods are laborious and time consuming, but new techniques such as monoclonal antibodies and gene probes exist for rapid detection.

Plouffe presented a historical review of organisms causing respiratory infections, especially pneumonia. This discussion centered around the fact that respiratory infection in each recent decade was associated with a particular class of organism and the realization that new organisms would arise as causative agents of pulmonary infections. Future research should concentrate on new culture media, immunocompromized patients, epidemics, and environmental isolates.

Olenchock discussed endotoxins which are an intergral component of the outer membrane of gram negative bacteria. Endotoxins are potent biological agents that can be associated with acute lung reactivity in exposed individuals. They are found in the soil, water, and in other living organisms around the world. Agricultural environments are a prime source, but they can also be identified in office buildings with humidification systems that contain water sumps. Endotoxins can be sampled from bulk materials, water, and airborne dusts. Filters are used commonly to collect dusts and the sample is analyzed using the Limulus amebocyte lysate gelation test. There are newer test methods under development.

Jarvis described mycotoxins and their association with indoor air quality. Mycotoxins are secondary metabolites produced by fungi. Cases of intoxication through ingestion of food have been reported; however, little is known about the threat of airborne toxigenic fungal spores. Mycotoxins may play a role in the symptoms experienced by occupants of buildings that are heavily contaminated by certain species of fungi. Fungal spores need not be viable to elicit a toxic effect so sampling techniques should employ samplers that collect viable spores (impaction onto agar with subsequent incubation) and non-viable spores (filters, spore traps). Species identification is important because of the large variation among taxa of the same genus in the production of mycotoxins. Air samples must be collected on several different occasions in areas where complaints are the highest and also in areas where there are no complaints. Analysis can take the form of injection of material

into susceptible animals or if the microbial and toxicological analysis point to a particular fungus whose toxins are known and available, as standards, the samples can be analyzed by a variety of chromatographic, spectometric, and immunoassay techniques.

In the last 3 chapters in this volume Burge, Feeley, Morey, and Levin describe future bioaerosol problems in buildings and the intervention actions and studies that will likely result from attempts to reduce indoor microbiological pollution. Feeley and Morey describe how bioaerosol problems indoors will likely increase in importance in the future. Poor preventive maintenance of building systems is often cited as the major reason for the increase of microbial contamination indoors. Studies will be carried out in the future to determine if intervention techniques such as more effective filtration, increased outdoor air ventilation, and better accessibility of ventilation system components for purposes of maintenance result in reduced concentrations of indoor microbial contaminants. The incorporation of documented microbial intervention techniques into building codes will be the likely result of these research efforts. As discussed by Levin, architects, building owners, and tenants will consider the cost of microbial intervention procedures versus the possible adverse health effects and productivity losses associated with exposure to microbial agents. Burge describes how a significant amount of future study will be directed toward establishment of exposure/dose-response relationships for common diseases caused by bioaerosols indoors. The possible development of bioaerosol exposure guidelines will result from these efforts.

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