Proceedings 1994 International Conference
FUNGI AND BACTERIA IN INDOOR AIR ENVIRONMENTS

PROCEEDINGS

of the
International Conference
Saratoga Springs, New York

October 6–7, 1994

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Health Effects, Detection and Remediation

EDITORS
Eckardt Johanning, M.D., M.Sc.
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EDITORIAL PREFACE

We have received many requests to publish the proceedings of the recent conference on *"Fungi and Bacteria in Indoor Air Environments—Health Effects, Detection and Remediation,"* held October 6 and 7, 1994 in Saratoga Springs, New York. This conference brought together a wide field of experts on indoor air quality and laboratory testing, as well as microbiologists, mycologists, toxicologists, occupational and environmental physicians, industrial hygienists, public health officials and legal consultants—more than 150 participated. There seemed to be a great desire and need to exchange new information, experiences, data and guidelines for diagnostic criteria, testing, remediation and prevention measures.

Well, we have asked all the presenters to provide us with a written summary of their presentations. Please bear in mind that most of the presentations were accompanied by very informative slides and graphic material, all of which we cannot reproduce here. We are happy to present to the interested reader a collection of papers, thoughts and conclusions based on newest research results and multidisciplinary investigations, often of "real world", practical indoor air quality problems, in the U.S., Canada and Europe. Derived from this, new hypotheses and "exposure limits" have been proposed or defended, which may be important for public health policy decisions regarding risk assessment, medical diagnosis, remediation of contaminated buildings and pro-active control of biological building contamination.

Two presentations on large scale municipal waste treatment using new composting technology can only be a very limited introduction to related health problems and growing public concern. Workers involved in handling municipal biological waste products may be at often unrecognized health risk. This topic probably deserves its own forum, similar to the one sponsored by the German government held parallel to the Saratoga Springs Conference.[1]

The general reader should know that this collection of presentations does not claim to be the only authority on the issues; we continue to learn—practically daily—as new information, technology and patients' (building occupants) histories become available. This may render some advice and guidelines given here either too restrictive, too permissive or already obsolete. But we tried to carefully

[1]Nachweis und Bewertung von Keimemissionen bei der Entsorgung von kommunalen Abfällen sowie spezielle Hygieneprobleme der Bioabfallkompostierung (Detection and assessment of bioaerosols in municipal waste management and special hygiene problems in bio-waste composting) October 5 & 6, 1994. Deutsche Veterinärmedizinische Gesellschaft e.V., Frankfurt, Germany.
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present the current "best evidence and judgement" by all presenters, who are subject to constant peer-review.

The goal is to give the medical and non-medical practitioners from many disciplines a tool to review the latest knowledge, to "get up to speed"—and act responsibly, effectively and efficiently to improve the air quality at work and at home impacted by biological contaminants.

The lay reader may please consider, that fungi and bacteria are part of our "normal" life and their ubiquitous presence does not necessarily mean adverse health consequences in most situations. Rather we need to learn and recognize the atypical, abnormal circumstances and conditions under which biological material can turn out to be a health risk to humans and animals requiring our intervention.

We wish to specially thank the U.S. Public Health Service, Division of Federal Occupational Health, Philadelphia and the Department of Community Medicine, Mount Sinai Medical School, New York for their co-sponsorship and institutional support, Ms. Chris Grosse (ENYOHP), Ms. Marise Burger (Editor, American Journal Of Industrial Medicine), Ms. Donna Regalbuto (Federal Occupational Health—Philadelphia), John VanRaalte C.I.H. (ENYOHP) and the staff of the Eastern New York Occupational Health Clinic, Albany, New York—Anne, Donna, Nicki, Sue, Pat and Wanda, for their special efforts to make this conference successful and these proceedings possible.

As the famous pathologist, Rudolf Virchow, said last century, *"politics is medicine on a larger scale"* advocating better housing conditions to control infectious disease outbreaks, we ought to develop public health standards and rules to establish safe living and working conditions at home and in the office buildings. We hope the conference and proceeding papers are a contribution to this collective preventive effort.

THE EDITORS:

Eckardt Johanning, M.D., M.Sc.
Chin S. Yang, Ph.D.

CONFERENCE INTRODUCTION

ECKARDT JOHANNING, M.D., M.Sc.

Welcome, Good morning, Guten morgen, bon jour, god dag, hyvää päivää.

The topic of the next two day's presentations and discussions will be indoor air quality—building problems and health, especially bioaerosols in offices, homes, schools, hospitals and other work locations.

We have brought together a wide spectrum of international, leading experts from research centers, universities, government agencies, hospitals and environmental hygiene and safety firms. As you will see, there are people with many different accents and experiences in many fields and disciplines.

It is our intention—not without selfish interest, as practicing health care providers—to present to you a broad spectrum of current and new methods to investigate, diagnose, treat and remediate biological problems in and from buildings. I know in the audience we have many people also with great expertise and practical experience in this matter; physicians, occupational experts, industrial hygienists, public health administration officials, ventilation specialists, lawyers and building specialists, altogether more than 150 participants from all around the country, Europe, and Canada.

When I started my training in occupational medicine at Mount Sinai Medical center I thought I would often diagnose coal workers' pneumoconioses. Practicing in New York, I have yet to see in my office a coal miner like the one shown on the slide. Coal dust inhalation problems and disease did pose great challenges to occupational physicians for many years. However, today, at least in Europe, coal miners have statistically speaking a longer life expectancy than the average population. This was felt to be due to greater efforts in reducing hazardous coal dust exposure and improved medical diagnosis and rapid treatment of complications. Paradigm changes are not uncommon in occupational medicine, as technology and the physical work environments change. Shifts in risk assessments, occupational health perspectives and governmental responses have been recently reviewed.[1]

Today the major challenge for many of us are calls to our office and frantic cries for help because the patient or building occupants relate their health problems to certain building conditions and bad air quality. Often these buildings are then called "sick buildings" if environmental testing did not show any abnormalities and no one really knows what is going on.

Address correspondence to: Eckardt Johanning, M.D., M.Sc., Medical Director, Eastern New York Occupational Health Program, 1 CHP Plaza, Latham, NY 12110.

Well it is a fact that more and more people spend more and more time indoors. EPA estimates that in Europe and the U.S.A. people spend more than 90% of their time indoors. Infants, the elderly, persons with chronic diseases, and most urban residents of any age spend even more time indoors. Several studies have shown that the concentrations of many air pollutants are often higher indoors than outdoors. Environmental tobacco smoke is a major contributor to poor air quality, besides malfunctioning ventilation equipment and building material or furniture. Therefore indoor environments may pose a new kind of "exposure situation" compared with traditional industrial exposures.

The lung is most commonly the site of the injury of airborne pollutants. Many industrial hazards are generally regulated and dealt with by experienced safety and health specialists. Not so, for many air contaminants found in daily life, such as cigarette smoke, mixed dust, mites or biological contaminants. The etiology and association with health problems can be very difficult to establish because many medical signs and symptoms are nonspecific. Multiple factors may be involved. Pre-existing problems, such as allergies, immune system disorders, chronic respiratory or metabolic disease may influence the well being and modify health effects from air pollutants.

Because "quality of life" expectations have been rising and because of financial reasons, more and more room air is "handled" by mechanical units at home or at work—especially in the U.S., it seems to me. Windows are closed shut and a "tight building" situation is created to conserve energy and cost. In these heating-ventilation-air conditioning (HVAC) systems, air is filtered, dried, moisturized, cooled and heated—often according to "one size fits all" even in the very individualistic society of the United States. Often my patients tell me that they have no access to room unit controls to raise or lower temperature and control fresh air supply as they feel they need. This can also have a psychological effect on building occupants.

Again, it is most often economics which drives this. All air handling and processing costs money to operate, to maintain, to repair and clean. Beyond that, there is not infrequently a problem with air contamination from bio-aerosols, fungal or bacterial contamination, because of system failures, roof leaks or operation personal neglect. We are beginning to learn and understand, that this is can pose new and far too often unrecognized public health problem.

There is still a great knowledge deficit: Untrained physicians have problems recognizing airborne fungal symptoms and disease—signs are often not specific and medical tests are not easy. Building engineers and managers are commonly not aware of the nature of the potential health hazards. Industrial hygienists have problems measuring and characterizing the exposure sufficiently to direct medical care of occupants and control or remediate the biological hazards.

Indeed the "official" recommendation is not to engage in sampling of biological contaminants such as fungi because of previous methodological problems and concerns. Well, I think it is "high time" to change this and to consider new approaches to quantify and qualify environmental conditions. We will hear many

presentations which will illustrate this and show that proper building and HVAC care can prevent "sick buildings" and building related disease.

There are many facets to the problem of biological air contamination in homes and buildings. Just a few examples: (slides)

1) Here is a university hospital in Frankfurt, Germany, with cases of complications and deaths of transplant unit patients related to aspergillus infections. The state attorney investigated 'malpractice', because it was thought that during the asbestos remediation work, careless workers—not trained and instructed—disturbed the normal environment and caused a contamination of the transplant unit with *Aspergillus* mold.
2) Here is an office building in New York City where recurrent major water leaks and floods led to biological growth of highly toxigenic mold on HVAC-units, pipe-insulation, gypsum-board walls, rugs and paper materials. Subsequently building occupants complained about mysterious and unusual health problems, which they related in time and place to the office.
3) Chicago: a private home with recurrent flood damage from a drain pipe causing fungal growth on the walls, rugs, and wooden furniture. Occupants, including children, complained of upper and lower respiratory problems, skin irritation and the death of pet animals (hamster and guinea pig).
4) Oakland, California: Similar situation. Massive fungal growth on dry wall in crawl-space beneath the living room and bedrooms. Occupants complained about stinging skin reactions and rashes, excessive fatigue, memory and concentration problems related to presence in this environment.
5) Tempe, a dry desert town in Arizona—who would expect any big mold problem there? Still, inside a modern private home, a malfunctioning water supply tube led to heavy fungal growth and subsequently to ill-health problems of the occupants.
6) Composting. We recently investigated the exposure situation of a municipal compost worker, who developed pneumonitis and lung fibrosis after just working two years in a new facility. Fungal air testing revealed extremely high levels of typically allergenic or toxigenic fungi and actinomyces, however no endotoxins were detected. In addition the facility had inadequate ventilation, inadequate and inappropriate respiratory protection.

All these examples have one thing in common, what is felt to be atypical, high air-borne levels of fungi species, known to be implicated in poor animal and human health.

Parallel to our conference today, a meeting is taking place in Germany sponsored by the German Government to develop safety and medical surveillance rules for workers and the public using "Bio-garbage" recycling technology. Such a conference was prompted by recent investigations revealing a consid-

erable risk of exposure to bacteria, fungi, actinomyces and viral organisms for users and municipal handlers. We have two presentations on this matter later today.

Two weeks ago an important philosopher and science critic died, his name was Charles Popper, from Great Britain. You may have heard about him. He established an important principle and point of critical reflection, which may apply especially to our professional field of bio-science and medical dogma—so-called current wisdom. He postulated that any science—(in my words)—needs to formulate correct statements/rules about the reality of our world. These often become exclusive paradigms or sometimes called "medical or technical standards". Although these often look like they have an inherent tendency to have eternal validity, these are only correct until we find and acknowledge that reality is in conflict with these rules. His examples are swans—we all know that *all* swans are white, that is, *until* we see a black swan. Surely rare, but "normal".

Of all the biological contaminants, fungi become more and more recognized. There is a vast variety of them in our environment, some are felt to be "normal" in their indoor presence, others are considered "atypical". A few have been related to human infectious or allergic diseases. Rarely do we hear of poisoning effects not related to ingestion of contaminated food stuff. However, inhalation of the fungal material—invisible in size, often small enough to enter deep into the lungs—may pose an even greater health risk as experience and research have shown.

One of the perhaps new bio-hazards we hear lately more often about is *Stachybotrys chartarum (atra)*, a black-sooty looking fungi, which can under particular conditions produce very potent toxins. These fungal chemical metabolites have been researched for use ranging from chemotherapy to biological-chemical warfare.

The earliest account of *Stachybotrys* fungi-related health problems I could find came from a German article from 1929 by a Hungarian veterinarian, Prof. Dr. Jarmai. He described in great detail several cases of lethal horse poisoning, called at the time *"Viscosus-sepsis"*. He thought—as did other scholars of his time —that it was related to a bacterium—called *pyosepticum viscosum or equii.* Today we think these were clear cases of trichothecene poisoning, a toxin produced by the fungus *Stachybotrys chartarum.*[2,3,4] The first English account about this unique disease of unknown etiology among horses and some farm workers came 43 years later, 1972 by Forgacs.[5] He described the investigations of the massive horse killing in the Ukraine. Stalin thought someone was out to kill all his cavalry horses, until an epidemiological study by Russian vets indicated that the disease was related to Stachybotrys infected wet straw fed to the horses. The first published report about airborne *Stachybotrys* health problems in a private home was published by Drs. Croft, Jarvis and Yatawara in 1982.[6] We will hear a lot more about this and other similar fungi over the next two days.

And so, here we are today. We need to review and assess the information and research from many disciplines, including agriculture, veterinary, food safety, toxicology and the many new medical cases and cluster investigations by indoor

air specialists, in order to establish diagnostic criteria and practical guidelines to provide good and realistic advice for our patients and building managers. With this in mind, we will present to you results of new studies or field investigations, and discuss with you medical, legal, public health and social implications.

Before we start with our key-note speakers, I would like to specially thank Dr. Frank Zampiello, Frank Lewis from the U.S. Public Health Service - Federal Occupational Health Division (FOH) in Philadelphia, Pa. and Dr. Philip J. Landrigan, my chairman from the Department of Community Medicine, Mount Sinai School of Medicine, for their practical and institutional support and last not least the three other members of the scientific organizing committee, Pierre L. Auger, M.D., Direction de Sante Publique de Quebec, Canada, Ed Olmsted, MS, CIH, Olmsted Environmental Consultant, Chin Yang, Ph.D, P&K Microbiology Services.

Thank you.

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SETTING THE STAGE FOR CHANGE MULTIDISCIPLINARY APPROACHES TO IAQ

FRANCIS A. ZAMPIELLO, M.D.

INTRODUCTION

The Public Health Service, Division of Federal Occupational Health is proud to be a sponsor of this conference addressing many cutting edge issues in indoor air environments.

For many years we have been advocating to our client federal agencies a comprehensive approach to occupational health—one that emphasizes prevention, promotes wellness, and recognizes the interaction between people and the environment in which they work.

This proactive approach requires a multi-disciplinary team. A team that brings to bear the scientific analysis of environmental hygiene professionals, the operations and maintenance expertise of building management professionals, and the clinical expertise of occupational health professionals—physicians and nurses.

Today, this conference will expand that circle of disciplines to encompass epidemiologists, microbiologists and other scientific investigators that have added to the knowledge base upon which this program was conceived—that microbials, and fungi in particular, play a significant role in the health effects of indoor environments. Indeed, our own work confirms the current impression that well over one third of such problems may be rooted in such bioaerosol exposures.

We anticipate that the discussions held throughout the next two days will affirm the need to increase such awareness among all disciplines. These presentations will form the basis upon which to understand the role indoor microbial contamination plays in occupational disease, the practical approach to investigation of such possible exposures, and sound principles upon which to address remediation.

In our experience a multi-disciplinary approach to IAQ problems yields the optimum results when a clear strategy is utilized. Such an investigation should generally follow logical steps;

- Review of available records. This would include an analysis of the building design and operation, and a medical review of the history of health complaints or problems.

Address correspondence to: Francis A. Zampiello, M.D., Division Director, USPHS, Federal Occupational Health Region III, 3535 Market St., Room 1310, Philadelphia, PA 19104.

- Initial Walk-through. To inspect source areas of complaints and interview key operators and complainants.
- Hypothesis Formation. Propose plausible cause-and-effect relationships.
- Initial Complaint Screening. Identify possible patterns by determining the location and occurrence of occupant concerns.
- Medical Evaluation - including appropriate histories and examination.
- HVAC Evaluation. Surveying building mechanical systems and measuring routine IAQ indicators (CO2, temperature, humidity, air movement).

The next steps include qualitative and quantitative monitoring, testing or measurements. We acknowledge the controversy around biological sampling on a routine basis. However, it is our belief that the application of the current state of knowledge and appropriate investigative tools and strategies will allow for the establishment of baseline data, development of acceptable concentration levels and eventual standards and criteria— all scientifically sound reasons for including such sampling in most studies. These are some of the issues that I challenge all of you to address in the next several days.

- Source Assessment. Identify conditions of equipment or activity that could contribute to airborne contaminants. Factors that must be considered extend to the surrounding outdoor environment and the controlling interface areas.
- Pollutant Sampling. Such analysis to be done only when steps 1-7 are complete.
- Pattern Analysis. Determine if observation support or dispute the initial hypotheses.
- Recommend corrective measures or collection of additional data.
- Validate Effectiveness. Do complaints and/or building condition change consistent with survey conclusions and subsequent response measures?

We recognize the need to train professionals in such a collaborative approach to indoor work environments, and have joined in partnership with the EPA and the University Science Center in Philadelphia, to establish the Mid-Atlantic Environmental Hygiene Resource Center.

It is in that same spirit of cooperation and the desire to continue to encourage cost effective and practical solutions to indoor environmental concerns that we support the agenda of this meeting.

We strongly believe that many indoor air quality problems yield to simple solutions—solutions that require an understanding of the appropriate application of basic building operation and maintenance principles. To obtain quality outcomes requires a commitment to excellence—to the anticipation of problems. That proactive approach to building wellness will be demonstrated in the technical sessions and discussions at this symposium during the next two days.

We hope you come away from this experience with an understanding of what is required to keep facilities operating in their most environmentally

sound condition. We encourage you to learn to do *the right things the right way,* and join the US Public Health Service and the Division of Federal Occupational Health, in the forefront of building wellness.

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REGULATING INDOOR MICROBES THE OSHA PROPOSED RULE ON IAQ A FOCUS ON MICROBIAL CONTAMINATION

FRANK A. LEWIS, M.Sc.

Abstract: *OSHA's proposed IAQ standard provides for a performance-related approach to resolving indoor air quality issues. This approach relies primarily upon implementing proper building operations and maintenance procedures. OSHA recognizes that poorly designed building systems and procedures may result in indoor microbial contamination problems which pose potential health problems to building occupants. The standard further addresses microbial problems through moisture control and contaminant removal.*

Key words: bioaerosols, building systems, exposure, health effects, inspection, IAQ Proposed Rule, IAQ Standard, maintenance, microbes, moisture

INTRODUCTION

The Division of Federal Occupational Health's (FOH) strategy on indoor air quality has always been a proactive, interdisciplinary and multi-phased approach. FOH terms this approach "building wellness". FOH's Office of Environmental Hygiene and EPA's Office of Radiation and Indoor Air have stated that "we know what to do and how to do it—the choice for building owners and managers is whether it will be done voluntarily or through regulations".

The regulatory route became more of a reality in late March '94 when OSHA announced its proposed rule on IAQ. The proposed regulation would require employers to ensure that employees have acceptable IAQ in the non-industrial work environment. The basis for this action is a determination by OSHA that employees face a significant health risk due to poor IAQ and that compliance with the proposed IAQ standard will substantially reduce this risk.

Address correspondence to: Frank A. Lewis, B.A., M.Sc., Director, Office of Environmental Hygiene, U.S. Public Health Service, Division of Federal Occupational Health, 3535 Market Street, Room 1310, Philadelphia, PA 19104.

Highlights of OSHA's Indoor Air Quality Proposed Rule 29CFR Parts 1910.1915, 1925 and 1928

Employers are required to provide:

- A written IAQ compliance plan which is implemented through inspection and maintenance of the building systems.
- Controls for specific air contaminants (e.g., microbes, cleaning chemicals, pesticides, ETS).
- Designated smoking areas (if smoking is allowed indoors)—separate, enclosed and exhausted to the outdoors.
- Action plans to minimize IAQ problems resulting from remodeling and renovation activities.
- Information and training for building maintenance and operations personnel as well as other key employees within the facility.
- Inspection and maintenance records, written compliance programs and employee complaint records which would be subject to retention, availability and transfer to authorized parties.

The entire proposal contains a history of the events leading to this action, health effects, exposure, preliminary quantitative risk assessment, significance of risk, preliminary regulatory impact analysis, summary and explanation, state-plan standards, federalism, information collection requirements, public participation, authority and signature and the proposed standard.

Evolution of the OSHA Proposed Rule on IAQ

May 1987—OSHA petitioned for an Emergency Temporary Standard by The American Public Health Association and Public Citizen *and* by the Action on Smoking and Health to prohibit smoking in most indoor workplaces. Outcome: insufficient data to demonstrate a "grave danger".

October 1989—ASH files suit against OSHA; court upholds OSHA and denies ASH's petition for review in May '91.

September 1991—OSHA issues a Request for Information on IAQ problems; Info on specific contaminants such as ETS and bioaerosols is also requested.

March 1992—AFL-CIO petitions OSHA to promulgate an overall IAQ standard. RFI response—1,200 comments. Wide support for regulatory approach which focuses on performance based standards; this would also take care of ETS.

March 25, 1994—IAQ Proposed Rule announced.

April 5, 1994—IAQ Proposed Rule issued for comments and hearings.

June 6, 1994—comment period extended to Aug 13, notices of intention to appear at hearing by Aug 5 and hearings scheduled for Sept. 20–Oct. 14 in D.C.

The hearings have been extended to December 5, 1994. More than 100,000 comments have been received by OSHA and approximately 800 speakers are expected to present their views on the proposed standard. A significant portion of the letters received came from the tobacco industry letter writing campaign. Responses also came from the three most active non-governmental IAQ groups in the U.S.—Building Owners and Managers Association (BOMA), American

Society for Refrigerating and Air Conditioning Engineers (ASHRAE) and the American Industrial Hygiene Association (AIHA).

DISCUSSION—IAQ & MICROBIAL CONTAMINATION

Building Wellness—A New Paradigm

Public health concerns related to building systems first surfaced in the mid-1970's when Legionnaire's Disease, at the Bellevue-Stratford Hotel in Philadelphia, made national headlines. It is interesting to note that the idea of a building-related illness (BRI) was, at the time, not readily acknowledged. The first investigative hypotheses, by the news media and the health professionals, focused on chemical agents and terrorist activity.

If you look at the background of scientists and medical professionals at that time, one can see that this was a problem of the existing paradigm. For example, many industrial hygienists had an extensive chemistry background but no solid microbiology background. Universities had environmental chemistry courses but no environmental microbiology courses, and certainly not indoor environmental microbiology courses. And there was minimal interaction between industrial hygienists and microbiologists. The majority of microbiologists being medically-oriented bacteriologists with little experience in environmental issues or with fungi.

This was also reflected in the approximately 500 IAQ investigations conducted by NIOSH industrial hygienists and medical professionals during the 1970s and 1980s. Only 5% of these IAQ problems were identified as being caused by microbial contamination. The other IAQ problems were characterized as due to inadequate ventilation, contamination outside the building, contamination from building fabric and unknown sources.

However, the current view on microbe-related IAQ problems reflects the paradigm shift towards an interdisciplinary, building wellness approach to public health and building problems. Media attention, research into the potential health hazards inherent in today's buildings, absenteeism, decreased worker productivity and morale and potential litigation have increased both occupant and building management/owner concerns for the indoor environment. There have been a number of IAQ investigations and many case studies which clearly demonstrate the impact of microorganisms upon the quality of the indoor environment.

- Recent studies say that microorganisms are the primary source of symptoms in as many as 35-50% of IAQ cases.
- The Minnesota Department of Employee Relations—reports that approximately 33% of IAQ are problems related to microbes.
- FOH's IAQ Investigations over a 10 year span show 33% due to microbial contamination.
- EPA's Building Air Quality Guide—5 of the 15 (33%) "Sample Problems and Solutions" are microbial examples.

- *OSHA's IAQ Proposed Rule*—24 of the 74 pages (33%) of the preamble and standard contain references to microorganisms.

OSHA's IAQ Proposed Rule—Microbial Contaminants

The following are microbe-related key words which appear throughout the IAQ preamble and standard:

- *Health Effects*—Microbial Contaminants:
  bioaerosols, respiratory allergies, asthma, nosocomial infections, humidifier fever, hypersensitivity pneumonitis, Legionnaires' disease, endotoxins, mycotoxins, viruses, fungi, bacteria, protozoans.
- *Exposure*—Microbial Contamination:
  humidity, temperature, media, HVAC, reservoirs, cooling tower, plumbing, cooling coils, condensate drip pans, moisture, building materials, biologicals, indoor environments, indoor air allergens, asthma, hypersensitivity pneumonitis, bioaerosols, *Legionella.*

The microbial section of the proposed standard appears under section (e)(3) as follows:

(e) *Controls for specific contaminant sources.*
(1) *Tobacco smoke*
(2) *Other indoor air contaminants.*
(3) *Microbial contamination.*
(4) *Use of cleaning and maintenance chemicals, pesticides, and other hazardous chemicals in the workplace.*

The specific requirements addressing microbial contamination are as follows:

(3) *Microbial contamination.*

(i) The employer shall control microbial contamination in the building by routinely inspecting for, and promptly repairing, water leaks that can promote growth of biologic agents;
(ii) The employer shall control microbial contamination in the building by promptly drying, replacing, removing, or cleaning damp or wet materials; and
(iii) The employer shall take measures to remove visible microbial contamination in ductwork, humidifiers, other HVAC and building system components, or on building surfaces when found during regular or emergency maintenance activities or during visual inspection.

CONCLUSION

The extent of IAQ problems related to microbial contamination has previously been quite underestimated. The author believes this to be attributed to a chemical materials bias and to a lack of interaction and understanding

between the environmental health and microbiology communities. Recent media attention, IAQ research and investigations and litigation activities have focused in on the microbials. A shift towards recognizing microbes as one of the major causes of building-related illness has occurred. OSHA is addressing this new concern by proposing a performance-based standard which stresses adherence to proper operations and maintenance procedures for moisture and source control of microbes.

ACKNOWLEDGMENTS

Many thanks to Chin S. Yang, Ph.D., consultant to the Division of Federal Occupational Health and President, P&K Microbiology Services, Inc. and to Ken Gerecke, CIH, Deputy Director, OSHA Region III, Philadelphia for their valuable feedback.

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THE CLINICAL RECOGNITION OF OCCUPATIONAL AND ENVIRONMENTAL DISEASE*

PHILIP J. LANDRIGAN, M.D., M.Sc.

INTRODUCTION

It is a great pleasure to be at this splendid conference organized by the Eastern New York Occupational Health Program. Moreover, it is a source of great gratification for me to honor the work of Dr. Eckhardt Johanning, who was previously one of our trainees in occupational medicine at the Mount Sinai School of Medicine.

Most persons spend many years of their lives at work, and the evidence is persuasive that chemicals and processes encountered at work are responsible for many cases of illness.[1] It is important therefore that physicians, particularly those engaged in primary care, have a basic understanding of occupational and environmental medicine. Physicians need to be able to recognize and diagnose diseases that are caused or exacerbated by occupational and environmental factors.[2] The American College of Physicians has recommended that medical training at every level place increased emphasis on occupational and environmental medicine. Also the American College of Physicians has recommended that physicians routinely inquire in the diagnostic interview about patients' occupational and environmental exposures.[3-4]

Occupational and environmental toxins can cause a broad range of illnesses, and these diseases can involve virtually every organ system in the human body.[1] They include classic, well described diseases such as lung cancer and malignant mesothelioma in workers exposed to asbestos; cancer of the bladder in dye workers; pneumoconiosis in coal miners; leukemia and lymphoma in people exposed to benzene; skin cancer in farmers and sailors chronically exposed to the sun; and chronic bronchitis in workers exposed to dusts. They also include newer entities recognized only in recent years such as dementia in persons exposed to solvents; sterility in man and women exposed to certain pesticides; and asthma and bronchitis in children and adults chronically exposed to air pollution. Some of these diseases are acute; others are chronic. Some are manifest though obvious symptoms, while others involve more subtle degrees of dysfunction.

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*Based on a report published in the *Mount Sinai Journal of Medicine*, 1995.

In the United States occupational exposures account each year for an estimated 50,000 to 70,000 deaths and for 350,000 new cases of illness.[5-6] An additional 10 million people suffer traumatic injuries on the job each year and 10,000 die of occupational trauma. In the environment, the Centers for Disease Control and Prevention estimate that at least 2 million children suffer from lead poisoning and that tens of thousands have asthma induced by air pollution. Occupational and environmental exposures always need to be considered in formulating a differential diagnosis. Because of the enormous numbers of persons exposed and the wide range of illnesses, occupational and environmental exposures need to be sought in every patient.

THE CLINICAL DIAGNOSIS OF OCCUPATIONAL AND ENVIRONMENTAL DISEASE

Occupational and environmental diseases are underdiagnosed. Many are incorrectly attributed to other causes because frequently these diseases are not distinct in their clinical presentations and can closely resemble chronic diseases caused by other factors.[7] Examples include: (1) Lung cancer caused by asbestos, radon, or beryllium is commonly attributed to cigarette smoking. (2) Severe abdominal pain caused by lead poisoning may be erroneously diagnosed as acute appendicitis; such cases have resulted in unnecessary surgery. (3) Dementia caused by organic solvents has been attributed to "old age" or to ethanol ingestion. (4) Renal failure caused by chronic exposure to cadmium has been ascribed to "idiopathic factors."

Only if a careful history of toxic occupational and environmental exposures is taken in such cases can a correct diagnosis be made. A barrier to accurate diagnosis is that a long latency interval frequently elapses between occupational and environmental exposure and the appearance of disease. For some occupational and environmental cancers (e.g., mesothelioma caused by asbestos or lymphoma caused by benzene) this latency may span decades.[8] Another impediment to diagnosis is that many people have had multiple toxic exposures at work or in the environment over the course of their lifetime. Also, at least until recently, workers were often not given the names of the materials with which they worked, nor provided with adequate information about the hazards of these materials. For these reasons it is often difficult for the physician to accurately ascertain the nature of a patient's past exposures.

The keys to properly diagnosing occupational and environmental disease are: (1) *obtaining an adequate history of occupational and environmental exposure for every patient.* (2) *possessing basic knowledge about the pathogenesis and clinical presentation of the major types of occupational and environmental disease; and* (3) *knowing how to report suspect cases of occupational and environmental illness to public health authorities so that additional cases caused by the same exposures can at least be recognized and at best prevented.*

Physicians should be especially knowledgeable about the occupational and environmental diseases that occur commonly in their practice areas, such as asbestosis and malignant mesothelioma in port cities with shipyards, pesticide intoxication in agricultural areas, and poisonings from solvents and exotic metals in regions that produce microelectronics.[9-10]

THE OCCUPATIONAL AND ENVIRONMENTAL HISTORY

The history is the single most important instrument for obtaining information on the role of occupational and environmental factors in causing disease.[7] Information about current and past exposures should routinely be sought at several logical points in taking the history on every patient. At each juncture, a few brief screening questions need systematically to be asked. Then if suspicious information is elicited, more detailed follow-up questions are needed. An efficient, routine screen for occupational and environmental disease consists of the following items:

(1) In *the history of the present illness*, pay attention to any temporal relationship between onset of illness and introduction of toxic exposures in the workplace or the environment. For example, did symptoms begin shortly after the patient started a new job? Did they abate during vacation and then recrudesce after the patient resumed work? Were they related to the introduction of a new chemical or process? Did they correlate with episodes of pollution? Were there similar illnesses among coworkers or neighbors? Were the individuals who were more heavily exposed the more severely affected?

A possible occupational etiology should be sought in every case of acute trauma (in children and adolescents as well as adults because too many children work at dangerous jobs) and in every case of repetitive trauma, e.g., carpal tunnel syndrome.

(2) In *the past medical history*, obtain a list of current and principal past occupations and of industries of employment. Each patient should be asked whether he or she ever developed illness as a consequence of work.

(3) In *the review of systems*, routinely ask every patient: "Do you now or have you previously had occupational and environmental exposures to asbestos, lead, fumes, chemicals, dusts, loud noise, radiation, or other toxic factors?" Also ask every patient whether he or she believes that any of these factors may have caused or contributed to his or her illness. Even if a postulated connection between exposure and disease initially appears tenuous, such suspicions always need to be carefully considered.

Detailed Exposure History

If information from the routine interview suggests an occupational and environmental etiology, the physician should obtain a more detailed history of toxic exposures. Duration and intensity of exposures are particularly important. It is necessary to learn how the patient worked with the suspected toxin and to consider how he or she may have absorbed the material; information should be obtained on all jobs ever held, places of employment, products manufactured, and materials with which the patient worked.

If toxic exposures are identified or strongly suspected and an occupational and environmental cause seems likely, further follow-up inquiries may need to be made through the patient's labor union, the companies where he/she has been employed, company physicians, or through the state or local health departments. Information on toxic substances used in a workplace may be legally available to patients under the Records Access Standard and Hazard Communication Standard of the Occupational Safety and Health Administration and under state and local "right-to-know" laws.

Reporting and Referral

If the diagnostic interview indicates or raises strongly the suspicion that disease is due to toxic occupational and environmental exposures, it is imperative that the physician report the case to state or local public health authorities. Many episodes of these diseases are in essence common-source outbreaks of highly preventable illness. Prompt reporting can lead to identifying additional cases earlier and to prevention by abating a common exposure source.

The physician may require access to specialized referral sources in occupational and environmental medicine. Two national organizations that maintain listings of occupational and environmental specialist physicians are the American College of Occupational and Environmental Medicine (Arlington Heights, Illinois) and the Association of Occupational and Environmental Clinics (Washington, D.C.). Another valuable resource is the U.S. Public Health Service's National Institute for Occupational Safety and Health (Cincinnati, Ohio).

At Mount Sinai, expert diagnostic and evaluation services in occupational and environmental medicine are available through the Mount Sinai-Irving J. Selikoff Clinical Center in Occupational and Environmental Medicine. This Center is named in memory of the late Dr. Irving J. Selikoff, a pioneering physician in occupational medicine best known for his seminal work on the carcinogenesis of asbestos.[8] The Center is directed by staff of the Department of Community Medicine and is supported by a generous grant from the New York State Department of Health.

ESTABLISHING THE DIAGNOSIS OF OCCUPATIONAL OR ENVIRONMENTAL ILLNESS

If the history suggests an occupational or environmental etiology, the following fundamental principles help make a diagnosis of occupational or environmental disease:

(1) **Biological Plausibility.** The likelihood that a disease is of occupational or environmental origin increases if the disease has previously been seen in other patients with the same or similar exposures, if a biologic mechanism is known, or if the disease has been seen in laboratory animals exposed experimentally to the chemical or to a similar chemical. Bear in mind, however, that many thousands of chemicals to which workers are exposed regularly in industry and that have been dispersed into the environment have never been laboratory tested for their toxicity. Therefore, the possibility always exists of diagnosing a disease entity that has never previously been recognized, e.g., malignant mesothelioma in workers exposed to asbestos, hepatic angiosarcoma in workers exposed to vinyl chloride, and cancer of the bladder in aniline dye workers.

(2) **Dose-Response.** The likelihood of occupational or environmental causation increases if the disease occurs more commonly and more seriously in the more heavily exposed members of a population. Bear in mind, however, that in the case of occupational and environmental carcinogens, there are no threshold levels of exposure below which safety is assured; any exposure to these agents is potentially carcinogenic, although heavier exposures carry greater risks. Also, agents that are allergens or chemical sensitizers can cause symptoms at very low exposure levels.

(3) **Sentinel Health Events.** To assist physicians to establish linkages between occupational exposures and disease, Rutstein and colleagues developed the concept of the sentinel health event.[11] It is defined as "an unnecessary disease, disability, or untimely death whose occurrence signals a failure of prevention." Examples include unnecessary maternal deaths, an outbreak of cholera or a single case of poliomyelitis.

Extending the concept of "sentinel health events" to occupational and environmental exposure, Rutstein and colleagues defined a "sentinel health event (occupational)" as "an unnecessary disease, disability or untimely death which is occupationally related."[12] A selected list of these events is presented in Table I. By scanning the table, physicians can identify work-related illnesses or exposures that may occur in their patients. Also they can identify occupations and industries that may be pertinent to their local practice areas. This list represents an accessible starting point for developing competence in the differential diagnosis of occupational and environmental disease.[13]

Table I. Abbreviated List of Sentinel Health Events (Occupational)—Occupationally Related Unnecessary Disease, Disability, and Untimely Death.

Condition	Industry/Occupation	Agent
Pulmonary tuberculosis	Physicians, medical personnel	Mycobacterium tuberculosis
Plague, tularemia, anthrax, rabies, and other infections	Farmers, ranchers, hunters veterinarians, laboratory workers	Various infectious agents
Rubella	Medical personnel, intensive care personnel	Rubella virus
Hepatitis	Day-care center staff, orphanage staff, medical personnel	Hepatitis A virus, hepatitis B virus
Ornithosis	Bird breeders, pet shop staff, poutry producers, veterinarians, zoo employees	Chlamydia psittaci
Malignant neoplasm of nasal cavities	Woodworkers, cabinet, furniture makers,	Hardwood dust
	Radium chemists and processors	Radium
	Nickel smelting and refining	Nickel
Malignant neoplasm of larynx	Asbestos industries and utilizers	Asbestos
Malignant neoplasm of trachea, bronchus, and lung	Asbestos industries and utilizers	Asbestos
	Topside coke oven workers	Coke oven emissions
	Uranium and fluorspar miners	Radon daughters
	Smelters, processors, users	Chromates, nickel, arsenic
	Mustard gas formulators	Mustard gas
	Ion exchange resin makers, chemists	Bis(chloromethyl)ether
Mesothelioma	Asbestos industries and utilizers	Asbestos
Malignant neoplasm of bone	Radium chemists and processors	Radium
Malignant neoplasm of scrotum	Automatic lathe operators, metal workers	Mineral/cutting oils
	Coke oven workers, petroleum refiners	Soots and tar
Malignant neoplasm of bladder	Rubber and dye workers	Benzidine, naphthylamine, auramine, 4-nitrophenyl
Malignant neoplasm of kidney	Coke oven workers	Coke oven emissions
Acute lymphoid leukemia	Radiologists, rubber industry	Ionizing radiation
Acute myeloid leukemia	Occupations with exposure to benzene	Benzene
	Radiologists	Ionizing radiation
Erythroleukemia	Occupations with exposure to benzene	Benzene

Condition	Industry/Occupation	Agent
Nonautoimmune hemolytic anemia	Whitewashing and leather industry	Copper sulfate
	Electrolytic processes, smelting	Arsine
	Plastics industry	Trimellitic anhydride
Aplastic anemia	Explosive manufacture	TNT*
	Radiologists, radium, chemists	Ionizing radiation
Agranulocytosis or neutrophenia	Explosives and pesticide industries	Phosphorus
	Pesticides, pigments, pharmaceuticals	Inorganic arsenic
Toxic encephalitis	Battery, smelter, and foundry workers	Lead
Parkinson's disease (secondary)	Manganese processing, battery makers, welders	Manganese
Inflammatory and toxic neuropathy	Pesticides, pigments, pharmaceuticals	Arsenic and arsenic compounds
	Furniture refinishers, degreasing operations	Hexane
	Plastics, rayon industries	Methyl butyl ketone copper disulfide, other solvents
	Explosives industry	TNT*
	Battery, smelter, and foundry workers	Lead
	Dentists, chloralkali plants, battery makers	Mercury
	Plastic industry, paper manufacturing	Acrylamide
	Microwave and radar technicians	Microwaves
	Radiologists	Ionizing radiation
	Blacksmiths, glass blowers, bakers	Infrared radiation
	Moth repellent formulators, fumigators	Napthalene
Noise effects on inner ear	Many industries	Excessive noise
Raynaud's phenomenon (secondary)	Lumberjacks, chain sawyers, grinders	Whole body, segmental vibration
	Vinyl chloride polymerization industry	Vinyl chloride monomer
Extrinsic asthma	Jewelry, alloy, and catalyst makers	Platinum
	Polyurethane, adhesive, paint workers	Isocyanates
	Plastic, dye, insecticide makers	Phthalic anhydride
	Foam workers, latex makers biologists	Formaldehyde

Condition	Industry/Occupation	Agent
Extrinsic asthma (con't)	Bakers	Flour
	Woodworkers, furniture makers	Red cedar and other wood dust
Pneumoconiosis of coal workers	Coal miners	Coal dust
Asbestosis	Asbestos industries and utilizers	Asbestos
Silicosis	Quarrymen, sandblasters, silica processors, mining, ceramic industries, and foundries	Silica
Talcosis	Talc processors	Talc
Chronic beryllium disease of the lung	Beryllium alloy workers, ceramic cathode ray tube makers, nuclaer reactor workers	Beryllium
Byssinosis	Cotton industry workers	Cotton, flax, hemp, and cotton-synthetic dusts
Acute bronchitis, pneumontis and pulmonary edema due to fumes and vapors	Alkali and bleach industries	Chlorine
	Silo fillers, arc welders	Nitrogen oxides
	Paper, refrigeration, oil industries	Sulfur dioxide
	Plastics industry	Trimellitic anhydride
Toxic hepatitis	Solvent utilizers, dry cleaners plastics industry	Carbon tetrachloride, chloroform trichloroethylene
	Explosive and dye industries	Phosphorus, TNT*
	Fumigators, fire extinguisher formulators	Ethylene dibromide
Acute or chronic renal failure	Battery makers, plumbers, solders	Inorganic lead
	Electrolytic processes, smelting	Arsine
	Battery makers, jewelers, dentists	Inorganic mercury
	Fire extinguisher makers	Carbon tetrachloride
	Antifreeze manufacturers	Ethylene glycol
Male infertility	Formulators and applicators	Dibromochloro-propane
Contact and allergic dermatitis	Leather tanning, poultry dressing plants, packing, adhesives and sealant industry, boat building and repair	Irritants (e.g. cutting fish oils, solvents, acids, alkalis, allergens)

*TNT indicates 2, 4, 6–trinitrotoluene

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BIOLOGICAL PARTICLES IN THE AIR OF INDOOR ENVIRONMENTS

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Abstract: *In indoor work environments, bacteria and fungi are usually the most significant airborne bioparticles affecting health. They can grow as saprotrophs in damp dust, on surfaces of structural materials and in humidifiers and HVAC systems and add to those derived from outdoor air. The bacterial flora is usually dominated by micrococci from human sources, and high counts indicate poor ventilation. Pseudomonas and other Gram-negative bacteria may occasionally be prominent as a result of uncontrolled growth in stagnant water. The mold flora of indoor air should be expected to reflect outdoor air, with phylloplane fungi such as Alternaria, Cladosporium and Epicoccum dominating, but growth in damp amplification sites can lead to predominance of Penicillium, Aspergillus and Eurotium. Prominence of yeasts and hydrophilic molds such as Stachybotrys, Trichoderma and Ulocladium indicates extremely wet sites within buildings. The opportunistic pathogens Aspergillus fumigatus and Legionella pneumophila are seldom abundant. Numbers of bacteria in indoor air samples usually appear to be greater than fungi, but for many reasons comparisons of numbers in different studies are likely to be futile.*

Key words: hydrophilic molds, phylloplane fungi, airborne bioparticles

INTRODUCTION

Although this meeting is concerned with bacteria and fungi, we cannot ignore the fact that there is a range of other particles of biological origin (bioparticles) in indoor air, particularly as we can now recognize that the health outcome of exposure to these other particles may be modified by bacterial and fungal exposure, and vice versa. In addition to microorganisms, including viruses and Protozoa as well as bacteria and fungi, indoor air may also carry pollen grains, animal dander, and fragments and excretory products of insects and mites (Wanner et al., 1993). In most indoor work environments, it is microorganisms that are the bioparticles of greatest significance for health.

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However, in homes, mites may well be of greater significance than microorganisms for atopic individuals. In addition to particulate bioaerosols, the air will also contain volatile organic compounds originating from living organisms such as indoor plants and microorganisms.

NON-MICROBIAL PARTICLES

Pollen grains which contain allergens causing 'hay fever', or rhinitis, are primarily associated with the outdoor environment; in indoor air, pollen concentrations are usually much lower. The difference between outdoor and indoor air is greatest in buildings where heating ventilation and air-conditioning (HVAC) systems have efficient filtration at the intake. Window air-conditioning units also give low indoor pollen levels. The air of some indoor work environments may have high pollen counts, e.g. where flowering plants are present for aesthetic reasons.

Dander, consisting of fine skin and hair/feather particles (and associated dried saliva and urine), contains potent allergens, such as Fel d 1 (cat) and Can f 1 (dog) which can cause rhinitis or asthma in the susceptible. Cats and dogs are most often the main sources of dander in indoor environments, but rats and mice, hamsters, gerbils (desert-rats), guinea pigs and cage-birds may also contribute. Although dander from such animals and from farm and recreational animals can be brought into buildings on clothes, in work environments the greatest concentrations of dander are likely to be found in animal-rearing facilities and laboratories, or in vermin-infested buildings.

Insects and their excretory products may also cause respiratory and other allergies, but do not appear to contribute significantly to the airborne bioburden in most situations. Particles from *Blatella germanica and Periplaneta americana* and other cockroaches may be present in significant amounts in unsanitary, hot and humid indoor environments. As with dander from experimental mammals, particulates from cockroaches, locusts, fruit flies and other insects can be a cause of ill-health in staff working in rearing facilities and laboratories.

Mites are particularly associated with dust, but mite fragments and fecal particles may be present in indoor air. The most important species is the house dust mite, *Dermatophagoides pteronyssinus*. It is a major cause of respiratory allergy in the home environment, being particularly abundant in bedding, but also present in upholstered furniture and other soft furnishings. Indoor air may also contain allergenic fragments of storage mites such as *Acarus, Glycyphagus and Tyrophagus*, which are associated with stored foods and animal feedstuffs. They are most likely to affect farmers and workers handling bulk foods, but they can exist in dust in buildings along with house dust mites, particularly under warm humid conditions.

MICROBIAL PARTICLES AND THEIR SOURCES

Viruses are principally transmitted by person-to-person contact and by inhalation at short—range from the human source of aerosols generated by coughing or sneezing, e.g. of rhinoviruses (common cold) and the influenza virus. Rates of infection with viruses are therefore likely to be higher in crowded premises. However, rhinoviruses can be spread via recirculating air.

Bacteria in indoor air (see Flannigan et al., 1991) are predominantly Gram-positive types originating from the mouth, nose, nasopharynx and skin usually, viz. species of *Staphylococcus, Micrococcus* and *Streptococcus* (Table I). Although Gram-negative bacteria are not usually abundant, occasionally *Acinetobacter, Aeromonas, Flavobacterium* or especially *Pseudomonas* species may be prominent. Their presence usually indicates growth in sites where there is an abundant source of water, e.g. humidifier drainage pans or reservoirs, drains and saturated surfaces. *Legionella pneumophila,* the cause of Legionnaires' Disease and Pontiac Fever, may be present as a result of spread of aqueous aerosols from contaminated air-conditioning humidifiers, shower stalls, spas, jacuzzis and even respiratory therapy equipment, but may also enter buildings in aerosols from nearby cooling towers. However, *L. pneumophila* appears to survive in indoor air normally for no more than 15 minutes.

Table I. Viable bacteria isolated from indoor air during an investigation of sick buildings (after Austwick et al., 1989).

Actinobacter calco var. *lwoffi*	*Ps. cepacia*
Aeromonas hydrophila	*Ps. fluorescens*
Flavobacterium sp.	*Ps. paucimobilis*
Micrococcus spp.	*Ps. vesicularis*
Moraxella sp.	*Staphylococcus aureus*
Pasteurella haemolytica	*Staph. epidermidis*
P. pneumotropica	*Streptococcus* spp.
Pseudomonas aeruginosa	*C.D.C. Group VE*

As well as these various unicellular bacteria, spores of filamentous types, i.e. Actinomycetes, may also be present in indoor air. These actinomycetes appear to be associated with damp structural materials, often giving a characteristic earthy odor. Two which are able to grow at 60°C, *Faenia rectivirgula* (formerly *Micropolyspora faeni*) and *Thermoactinomyces vulgaris,* may be found in humidifiers and other HVAC equipment.

Fungi in indoor environments comprise microscopic yeasts and molds, known as microfungi, and plaster and wood-rotting fungi, referred to as macrofungi because they produce sporing bodies that are visible to the naked eye. Apart from unicellular yeasts, fungi colonize substrates as a network (mycelium) of filaments (hyphae) and produce numerous aerially dispersed spores.

Indoor air contains spores and hyphal fragments of many different molds, but the most common are likely to be species of *Cladosporium, Penicillium, Aspergillus* and *Eurotium*. Some molds in indoor air, such as *Cladosporium, Alternaria* and *Aureobasidium*, are abundant on leaf surfaces (consequently often being referred to as phylloplane fungi) and other plant parts outdoors, particularly in summer. However, although *Cladosporium* and *Aureobasidium* spores in indoor air may have an outdoor origin, these fungi are also able to grow and produce spores on damp surfaces indoors. In fact, together with *Penicillium* and yeasts, *Cladosporium* spp. were among the most common molds isolated from damp walls of homes by Hunter et al. (1988). In winter, when counts of airborne fungi are extremely low outdoors, the indoor spora may be boosted as a result of growth of *Cladosporium* on damp surfaces within buildings. As with *Aspergillus* and *Eurotium*, the various species of *Penicillium* in indoor air are regarded as originating principally indoors, although there is considerable variation in the relative abundance in outdoor and indoor air between different species (Fradkin et al., 1987). It should be noted that there are also differences in the relative outdoor/indoor abundance of different *Cladosporium* spp.; for example, *C. herbarum* is more prevalent outdoors than other species (Verhoeff et al., 1992). Although not usually prominent, yeasts may occasionally be present in large numbers, with pink yeasts in the genera *Rhodotorula* or *Sporobolomyces* being conspicuous. Yeasts are primarily thought of, like bacteria, in connection with stagnant water in humidifiers or drains, but they can also be isolated from wet, mold-affected surfaces.

Most bacteria and fungi found in indoor air are saprotrophic, being able to obtain the nutriment they need from dead organic material. Wood, paper, surface coatings such as paint, soft furnishings, soil in plant pots, dust and shed skin scales, and cooked and raw foods and their ingredients all provide materials which can be metabolized by saprotrophic microorganisms. These materials cannot be utilized and growth cannot occur unless they are sufficiently moist, but when moisture is available they provide a variety of niches in buildings which can act as amplifiers for bacteria and fungi. Moisture-loving (hydrophilic) molds, and nearly all bacteria and yeasts, need conditions close to saturation, but some molds are also able to grow under conditions that are damp rather than saturated. Having an ability to grow under "drier" conditions than hydrophilic fungi, these species are said to be xerophilic. Settled dust acts as a store of bacteria and fungi, and, if it is sufficiently moist, an amplifier. Disturbed dust can therefore be an important source of airborne bacteria, yeasts and mold spores.

Protozoa such as *Acanthamoeba* and *Naegleri* can proliferate in humidifiers, reservoirs and drain pans, feeding on bacteria and other organic particles. Particles of these microscopic unicellular animals may be aerosolized and have been cited as possible causes of humidifier fever.

BACTERIA AND FUNGI IN INDOOR AIR

The composition of the fungal air spora in buildings in North America and Europe is similar (Hunter et al., 1988; Flannigan et al., 1991; Miller et al., 1988; Verhoeff et al., 1990, 1992) and is expected to broadly reflect that of outdoors (Flannigan et al., 1991; Miller, 1992). From studies of the viable air spora indoors it has been concluded that the predominant fungi in homes and non-industrial workplaces (Tables II and III) are generally species of *Penicillium* and *Cladosporium*, although the basidiomycete *Sistotrema brinkmannii* appears to be a major component of the air spora in UK homes (Hunter et al., 1988). *Aspergillus* spp. usually comprise a smaller but nevertheless notable part of the air spora. They include those xerophilic species which are often referred to as being in the *A. glaucus* group but are now allocated to the Ascomycete genus *Eurotium*. *A. versicolor* is frequently reported as being the most prominent species of *Aspergillus* present (see Hunter et al., 1988), but Verhoeff et al. (1992) found that the strongly xerophilic species *A. penicillioides* could be more prevalent. Another xerophilic mold which can occasionally also be prominent is *Wallemia sebi*. It has apparently been found growing with various xerophilic *Aspergillus* spp. in damp air-conditioning filters (Elixmann et al., 1990).

Although the opportunistic pathogen *Aspergillus fumigatus* is listed as a component of the air spora in Table II, fungal pathogens are rarely abundant in indoor air. However, *A. fumigatus* and some other *Aspergillus* spp. that can

Table II. Viable airborne fungi, expressed as counts of colony-forming units (CFU), at air-conditioned (AC) and naturally ventilated (NV) sites in two UK buildings (after Austwick et al., 1989).

	CFU/m³ air					
	Building 1 (June)			Building 2 (November)		
Type of fungus	AC		NV	AC		NV
	(1)	(2)		(1)	(2)	
Alternaria sp.	-	-	4	-	-	6
Aspergillus fumigatus	45	-	10	-	-	8
A. versicolor	2	-	-	81	122	39
Aureobasidium pullulans	-	-	-	6	-	4
Botrytis cinerea	-	-	4	-	-	2
Cladosporium spp.	30	45	209	2	2	39
Phoma fimeti	-	8	12	-	-	-
Penicillium spp	2	4	12	31	64	2
Yeasts	4	-	12	-	-	6
Unknown small	-	-	-	-	-	24
Unknown white	12	20	8	1	-	22
Basidiomycetes	2	4	2	-	-	-

become invasive of humans may grow in the soil of potted plants. *Exophiala jeanselmei* is able to grow in drains. In the normal run of events, the spores of these and other opportunistic pathogens such as *Fusarium solani* and *Pseudallescheria boydii* are unlikely to present a health hazard, except to immunologically compromised individuals. As far as pathogenic bacteria are concerned, it has been noted that *Mycobacterium tuberculosis* in droplet nuclei from infected individuals can be dispersed by recirculation systems to all parts of an enclosed environment. Although the pathogen *Legionella pneumophila* has been isolated from humidifiers and airconditioners, as mentioned previously its survival time in indoor air is very short; most outbreaks of Legionellosis have been attributable to aerosols in showers or from adjacent cooling towers.

Table III. Principal fungi in air of naturally ventilated and air-conditioned summer offices in Edinburgh, UK (after Flannigan, 1992).

	Composition of air spora (%)				
	Naturally ventilated building	Air-conditioned building			
		Office 1.06		Office 3.29	
Type of fungus	Monday	Monday	Friday	Monday	Friday
Alternaria alternata	5	-	3	9	2
Aspergillus versicolor	-	1	-	-	-
Cladosporium spp.	59	71	77	73	42
Epicoccum purpurascens	<1	-	-	-	-
Mucor spp.	1	1	-	-	-
Penicillium spp.	29	18	5	2	54
Non-sporing isolates	<1	9	6	16	2
Yeasts	3	-	9	-	-

In summer, when the growth of plant and associated microorganisms outdoors is greatest, counts of airborne mold indoors are usually, and should be, markedly lower than outdoors (see Flannigan et al., 1991). In naturally ventilated buildings, keeping windows and doors shut reduces the numbers of phylloplane fungi entering. Air-conditioning also reduces the contribution from outdoors of, for example, *Cladosporium* (Table II), which can account for as much as 90% of spores in outdoor air during summer in some areas. The phylloplane fungi *Alternaria* (Tables II and III) and *Epicoccum* (Table III) are present in indoor air throughout the year, but in UK even in summer, when they are relatively abundant in outdoor air, they are only minor components of the indoor air spora. In air-conditioned buildings in North America, these phylloplane fungi are the dominant part of the air spora (Miller, 1992).

It is patent that there should be a link between mold growth in a building and the air spora in that building, and that counts are likely to be higher nearer to the focus of the growth. However, the relationship is complicated by the effect of activity on the air spora. Routine activities in the home or workplace, cleaning and constructional work and any other operations that raise dust throw spores into the air (Hunter et al., 1988). Ventilation also creates air currents which affect spore clouds, so that there are large fluctuations in numbers, even over very short periods (Verhoeff et al., 1990). Mouilleseaux et al. (1993) observed fluctuations in numbers of both viable airborne bacteria (from <100 to >20000) and fungi (from <100 to >15000 m^{-3} air), but not necessarily occurring at the same time, during the course of an afternoon in one room of a nursery school.

One of the clearest demonstrations that mold growth in a building directly affects air quality occurs when species that are uncommon in outdoor air but are able to grow in damp locations indoors are prominent in the air spora. Large numbers of hydrophilic fungi, and/or Gram-negative bacteria and actinomycetes, are indicators of extremely wet amplification sites (visible or hidden), and therefore poor indoor air quality. Such hydrophilic fungi include yeasts, and molds in the genera *Fusarium, Phoma, Stachybotrys, Trichoderma* and *Ulocladium,* and very occasionally opportunistic pathogens like *Aspergillus fumigatus* and *Exophiala jeanselmei.* High levels of molds which are, to varying extents, xerophilic can indicate the existence of amplification sites which are damp enough to allow growth of xerophiles, but not hydrophilic organisms. An abundance of these species in the air can also indicate a dusty atmosphere, however, as xerophilic fungi are a characteristic feature of the mycoflora of house dust. Yeasts appear to be particularly abundant in house dust and, as in indoor air (Fig. 1), *Cladosporium* spp. and *Penicillium* spp. are prominent. *Cladosporium*

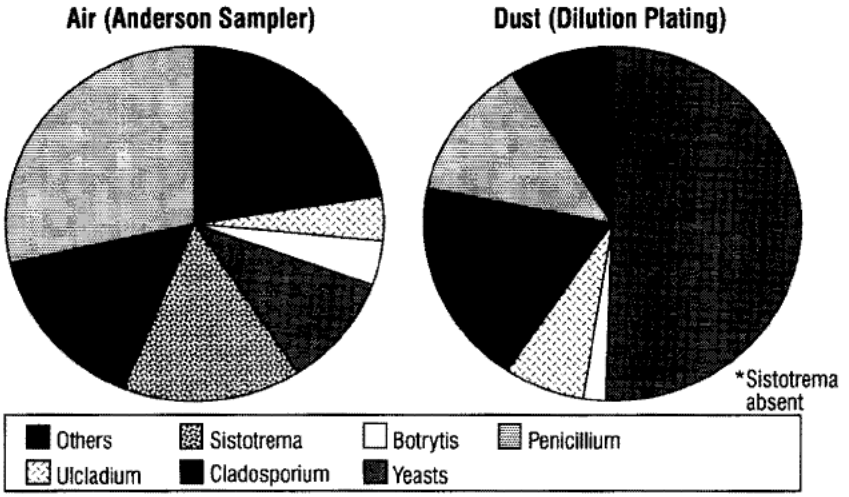

Fig. 1. Principal fungi in the indoor air and house dust of 35 naturally ventilated UK homes during summer.

spp. are slightly xerophilic and the penicillia include moderately xerophilic species such as *P. aurantiogriseum* and *P. chrysogenum*. *A. versicolor* is another moderately xerophilic species present, and the extremely xerophilic *Eurotium* spp., *A. penicillioides* and *W. sebi* are also notable.

Finally, as far as the magnitude of the airborne bioburden is concerned, only a few counts have been quoted in figure 1. As indicated by those of Mouilleseaux et al. (1993), numbers of airborne viable bacteria in indoor air are generally greater than fungi. Since many of the bacteria are shed by the occupants, bacterial numbers are likely to be particularly high where there is overcrowding and/or poor ventilation (Mouilleseaux et al., 1993). There *are* numerous other papers to quote and numbers to be compared, but to do this would be a largely futile exercise. There are various reasons why this is so. For example, the conditions under which sampling is carried out, the type of sampler, the sampling time, and the agar medium used all affect the numbers and species of viable microorganism isolated, and they differ from one investigation to another. Also, some bacteria and fungi are much less robust than others. For example, the spores of the molds *Aspergillus* and *Penicillium* appear to retain their viability during exposure to the environment better than *Stachybotrys*. The microbial burden in the air is therefore underestimated to varying degrees by sampling only for viable organisms. This disparity between viable and total counts will be raised later when evaluation of the air spora is considered.

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AEROSOL MYCOTOXINS: A VETERINARY EXPERIENCE AND PERSPECTIVE

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MARJO NIKULIN, M.Sc.

Abstract: *A membrane filter sampling method for airborne stachybotryotoxins was developed, with toxic strains of Stachybotrys atra used as the toxin source on straw, hay and wallpaper. A cytotoxicity test was used to screen filter samples for their toxicity. Cytotoxic samples were further analyzed for trichothecene mycotoxins by chemical methods. These methods were used to screen air samples from homes with mold or disease problems. Of ten air samples, four were slightly cytotoxic, but no trichothecenes were found by chemical methods at detectable levels.*

Key words: stachybotryotoxins, cytotoxicity, air sampling

INTRODUCTION

At the beginning of our knowledge of aflatoxin, animals, especially poultry in England, played an important role. A large epidemic among turkeys and chickens led to the discovery of aflatoxin, a carcinogenic mycotoxin. Research on aflatoxin began as a study of an animal disease problem, which later became recognized as a problem of human health as well. This same pattern has repeated itself with several mycotoxins.

Domestic animals are far more often exposed to the mycotoxin hazard than are people, since they live in surroundings often heavily contaminated with bacteria and fungi. Moldy grain is fed to the animals, and the grain of better quality is consumed by people. Especially hay, straw and silage can be moldy and therefore a source of the problem (Ueno, 1983).

The most common way for mycotoxins to enter the human or animal body is by ingestion. This topic is thoroughly studied in veterinary medicine. Another important route is exposure through the skin, likely to be more common among animals than among people. The third means of mycotoxin exposure is inhala-

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tion of mycotoxins in aerosol form. This paper describes a method to measure mycotoxins directly from an air sample.

MATERIAL AND METHODS

A small-scale laboratory model for measuring aerosol mycotoxins was developed, with *Stachybotrys atra* used as a model fungus for toxin production, its spores being collected on a membrane filter.

Toxigenic *S. atra* strains were cultivated on grains, straw, haw, or some building materials like gypsum board or on wallpaper. Straw and hay are excellent materials for stachybotrys toxin production, but *S. atra* is able to produce toxins also on wallpaper and gypsum board.

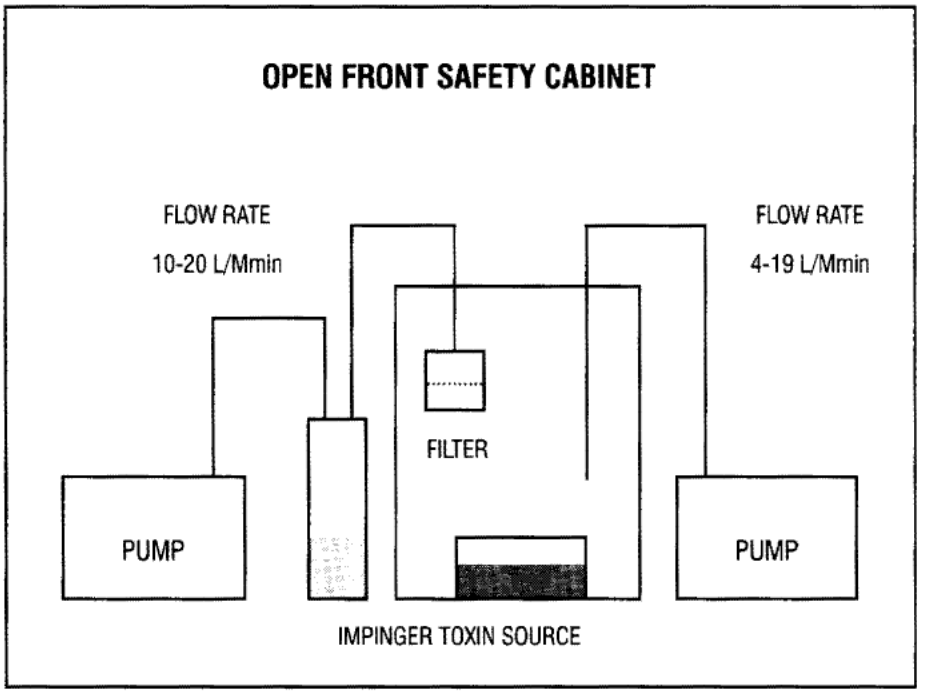

Fig. 1. Experimental set-up for collection of airborne stachybotryotoxins on membrane filters (cellulose nitrate and polycarbonate filters, 37 mm dia.) and in methanol.

Our laboratory model (Fig. 1) consisted of 1-liter chambers in which toxin sources were placed. Air samples were collected on open-faced membrane filters at a flow-rate of 10-20 l/min. Sampling time varied from half an hour to several hours, and the corresponding air volumes were 0.3-3.6 m^3. Turbulent air flow

inside the chamber was directed towards the toxin source to release *S. atra* spores effectively into the air. Stachybotryotoxins were analyzed from the filters by biological toxicity tests and/or chemical methods. Cell culture tests were used as screening tests to pick up among a larger amount of samples those samples in which a toxic effect was detected. A continuous cell line (feline fetus lung cells) was used in the assay; FL-cells are rapidly growing fibroblast-type cells, sensitive to small amounts of trichothecene mycotoxins and easy to work with. A standard cell culture medium was used. The assay was carried out on microwell plates. Diluting the sample into a series allows one to estimate the amount of toxin. The cell cultures were incubated in normal cell culture methods for 5-7 days, with cell-death indicating toxicity of the sample (Fig. 2). The details of the cell culture method were published earlier (Pasanen et al. 1993). HPLC- and GC-MS-methods were used to quantify the toxins (Pasanen et al. 1993).

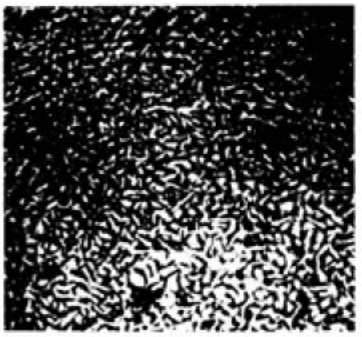

Fig. A

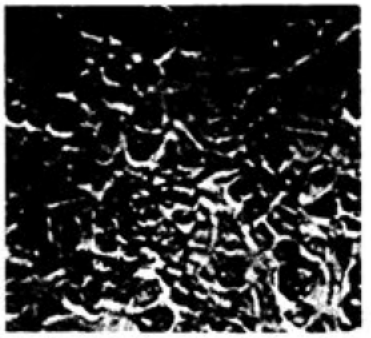

Fig. B

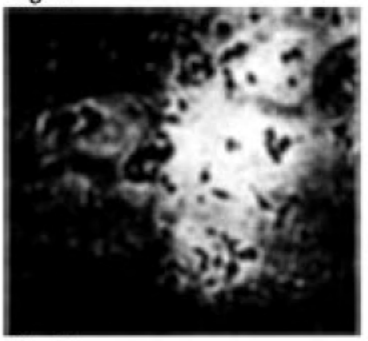

Fig. C

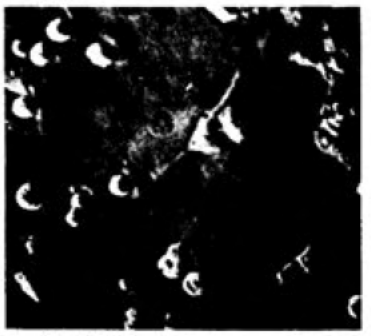

Fig. D

Fig. 2. A. Feline fetus lung cells (control) after a four-day incubation, x 100.
B. as A, x 400.
C. Cells after a four-day incubation with air filter sample extract. Sample from house heavily contaminated with *S. atra*, x 100.
D. as C, x 400.

RESULTS

By use of these methods, it is possible to measure stachybotryo toxins in the air samples (Pasanen et al. 1993), respirator filters (Pasanen et al. 1994) and in building material and animal fodder samples (Nikulin et al. 1994).

The same methods were also used to detect possible toxicities of a few air samples from homes in the USA which had mold problems or disease problems suspected to be caused by fungi or mycotoxins. According to very preliminary results, in four out of ten air samples, a slight cytotoxic effect was found, but no macrocyclic trichothecenes were found at detectable levels in those samples.

DISCUSSION AND CONCLUSIONS

Much work has been done in qualitative and quantitative mycological studies of indoor and outdoor air. Often the number of colony-forming units of fungi (CFU) per cubic meter of air is used to describe the mycological quality of the air. The Andersen sampler or some other methods are used to give the exact number of different fungal spores in the air. However, using this mycological cultivation method to describe air quality presents an unsolved problem possibly leading to a major error. The mycological method naturally can reveal the presence of only those fungal spores able to germinate by the method used. Those "dead" spores which cannot grow on the media often go unnoticed, but these spores as well can contain mycotoxin. Often less than 10% of the spores in the air sample are able to grow on the media used (Heikkilä et al. 1988).

The presence of mycotoxins in the air sample depends on several factors such as humidity of the air, air circulation and water content of the substrate on which the fungus is growing.

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MYCOTOXINS IN THE AIR: KEEP YOUR BUILDINGS DRY OR THE BOGEYMAN WILL GET YOU

BRUCE B. JARVIS, Ph.D.

Abstract: *An overview is presented of mycotoxins focusing on their role as potential toxic agents in fungal-contaminated buildings.*

Key words: *Stachybotrys atra*, trichothecenes, aflatoxins, immunosupression, mycotoxins, inhalation

INTRODUCTION

The overall purpose of this Conference is to discuss the role of bioaerosols on the health of occupants in buildings contaminated by bacteria and fungi. It has long been appreciated that certain microorganisms cause disease, but it was only with the elucidation of the etiology of Legionnaires' disease that there arose a general awareness that improperly maintained buildings, particularly the HVAC systems, could result in serious risk to building occupants because of the presence of dangerous microorganisms. A more common problem for people in fungal-contaminated buildings are the allergenic responses. Another problem often encountered, though usually more of a nuisance rather than a real health hazard, are complaints of the odors produced by the volatile chemicals given off by the fermenting microorganisms. A less well appreciated hazard is the danger posed by non-volatile chemicals produced by microorganisms, most often fungi, which are toxigenic. These chemicals are known as mycotoxins, and unlike the allergens, elicit toxic responses from virtually all people with whom they come into contact. It is the purpose of this paper to describe the overall nature of these chemicals and to discuss some examples of where they appear to have played an adverse role in the health of occupants of fungal-contaminated buildings.

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Secondary Metabolites

Metabolism falls into two broad classes: primary and secondary. The former is the purview of biochemistry and is characterized by its generality, i.e. essentially all living organisms share fundamentally the same core of primary metabolism. All organisms on this planet have basically the same chemical processing mechanisms involving the four great classes of primary metabolites: proteins, nucleic acids, carbohydrates, and lipids (e.g. steroids, fats, and oils). Secondary metabolites, on the other hand, tend to be idiosyncratic in that their appearance is often highly restricted, sometimes to a single species. Furthermore, secondary metabolites are low molecular weight (<3000 Daltons) organic compounds that appear "to have no explicit role in the internal economy of the organism that produces it" (Williams et al. 1989). Many secondary metabolites are well known by the general public, e.g. plant alkaloids (strychnine, quinine, morphine, etc.), antibiotics, essential oils, active principles in spices, etc. Although humans have made great use over the years of these secondary metabolites (mainly from plants), we should keep in mind that this has little or no bearing on the question as to why plants and microorganisms produce such compounds in the first place. Although this is interesting, it is beyond the scope of this paper to take up this question. Suffice it to say that one principal purpose that plants and microorganisms have for producing such compounds, many of which are highly biologically active, is for defensive purposes. That mammals, particularly humans, appear to lack what might be thought of as classical secondary metabolites is a reflection of the fact that we have evolved a far more elegant system of protection (the immune system) from attack by pathogens and that we are able to monitor and control our environment through the activities of the most sophisticated organ known, the human brain. However, even adopting a more conservative notion of what constitutes a secondary metabolite can lead one to conclude that we too employ such weapons in our arsenal. The recent finding that nitric oxide is released by macrophages as cytotoxins in response to bacterial infections would appear to be such an example—though Nature, always the opportunist, has found additional uses for this highly reactive chemical (Stramler et al. 1992).

The discovery of penicillin ushered in the modern era of antibiotics and perhaps left the impression that with respect to secondary metabolites, fungi (and certain bacteria such as the streptomycetes and actinomycetes) were the "good guys"—resoundingly not so. All clinically useful antibiotics are toxic at higher levels, and from a chemical viewpoint, there is no fundamental biochemical difference between a medicinally useful antibiotic (e.g. penicillin) and the active principles found in poison mushrooms (e.g. the potent neurotoxin, muscarine). The fact that we find penicillin medically useful and that the unwary die from eating muscarine-containing mushrooms is not a reflection of any fundamental biochemical difference between these two fungal secondary metabolites.

There is a great body of literature about the role of fungal secondary metabolites on the health of animals and humans that come into contact with these fungi, usually through ingesting fungal-contaminated feed and grains.

These reports most often arise from an agricultural setting, and the typical veterinarian is ignorant of the true etiology of the animal toxicoses—physicians are even less aware. Although certainly far less common, some of these fungi, under favorable growth conditions, are found growing in water-damaged offices, schools, and homes.

Before I discuss the role of specific fungi and their metabolites on the health of those exposed to these fungi in indoor air environments, it is useful to elaborate on how truly complex the situation can be. There are hundreds of known or suspected mycotoxins, but actually the list in practice could easily be in the thousands. Furthermore, the list of structure types also is large (see Figure 1 for a few examples). To complicate matters further, the various structural classes of mycotoxins have different biological properties. Although certain structure types often are restricted to one fungal genus (e.g. aflatoxins produced by *Aspergillus*), a genus of fungus may produce a hundred or more different classes of mycotoxins (e.g. *Penicillium*).

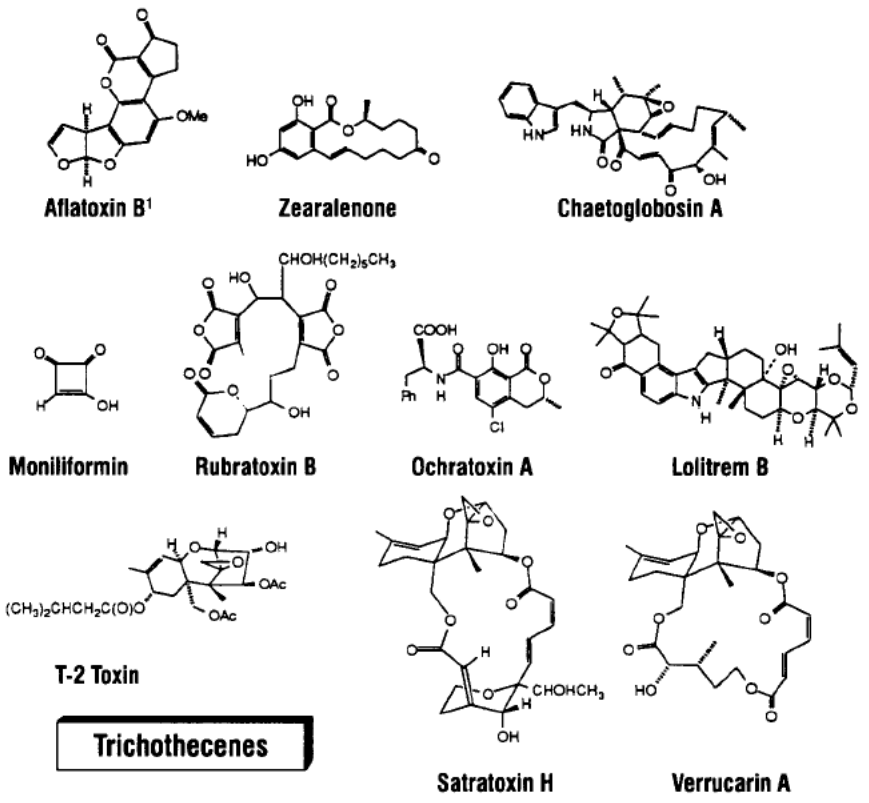


Fig. 1. Selected Examples of Mycotoxins.

In real life situations, one never finds monocultures; there are always many different fungi present. However, often one species will predominate, and because of past experience, one could make an "educated guess" as to whether exposure to this organism is likely to put exposed people at risk. For example, buildings contaminated by *Aspergillus flavus* must be considered a serious hazard to the occupants since nearly all isolates of *A. flavus* will produce aflatoxins, which are among the most potent carcinogens known. However, in this case there is a further and very common complication. The level of mycotoxin production by a toxigenic fungus depends very much on the specific isolate and the conditions of its growth. One isolate of *A. flavus* will give high levels of aflatoxins under certain culture conditions while another isolate under the same or different growth conditions may give no detectable aflatoxins. Thus the isolation of a toxigenic fungus from a building cannot be taken as proof that the occupants were exposed to mycotoxins. The fact that the particular fungus may produce mycotoxins in laboratory cultures is suggestive of mycotoxin production in the native environment (e.g. in the contaminated building) but is not proof positive; the alternative is also the case: lack of mycotoxin production in laboratory cultures does not mean that the fungus is not producing mycotoxins in the contaminated building. Another sticky question has to do with mode of exposure. The usual cases of mycotoxicoses in humans and animals are the result of eating contaminated food and feed. Exposure to mycotoxins in indoor air environments is almost exclusively through inhalation. The bronchial and lung tissues appear to be particularly sensitive to chemical insult, and the mucosa of these tissues exhibit strong responses to immunoactive agents. Although 70% of the antibodies of the immune system are found in the mucosal, relative to blood-circulating immunology, little is known about the mucosal immune system (Service 1994).

Very little has been published on the natural occurrence of mycotoxins in the air. Only a limited number of mycotoxins (e.g. aflatoxins, trichothecenes, zearalenone, and secalonic acid D) have been found in dust to which people have been exposed (Hendry and Cole, 1993). Studies have been carried out with aerosolized particles of pure trichothecene mycotoxins in connection with their evaluation as potential chemical warfare agents (Pang et al. 1988, Creasia and Lambert 1989). Inhalation of aflatoxins in rats led to cancers of the liver, kidney, and intestines (Northrup and Kilburn, 1978), and inhalation exposure to aflatoxin-containing spores has been shown to result in elevated liver cancer for those who handle such material (Wicklow and Shotwell 1983): safety procedures for those handling such material have been published (Anon. 1979). The above case with *A. flavus* is a clear indication that *A. flavus* (and the less commonly encountered *A. parasiticus*) must always be treated as a threat to human health. Whether such studies have practical relevance to natural causes where humans and animals might respire dust particles (or spores) which contain trichothecene mycotoxins is unclear.

There are few if any documented cases of the direct involvement of mycotoxins on the health of occupants of fungal-contaminated buildings (Hendry and Cole 1993). There may be a variety of reasons why this is the case, including the

possibility that mycotoxins in fact have no significant effect on these people. However, it is clear that the major responses of people to the fungi in these buildings are allergenic and inflammatory. Having introduced all these caveats, it is nonetheless useful to discuss a specific fungus that is sometimes, though not commonly found in water-damaged buildings: *Stachybotrys atra* (also known as *S. chartarum*).

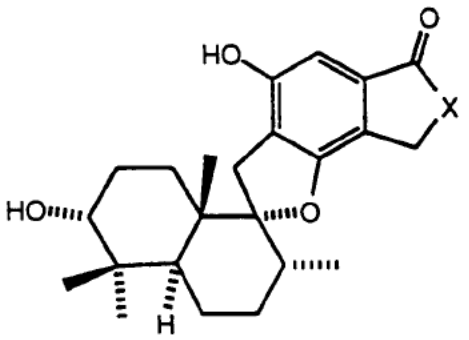
S. atra is the most important macrocyclic trichothecene-producing fungus (e.g. satratoxin H, see Fig. 1) with respect to posing a health hazard to humans and animals. Stachybotryotoxicosis was first reported around 1931, in the Ukraine (Forgacs 1972), but since that time has been reported in many parts of the world, especially in eastern Europe and the former Soviet Union. It is likely that stachybotryotoxicosis has a much longer history but went unrecognized. Horses appear to be particularly susceptible to this intoxication, and it has been suggested that as little as "1 mg of the pure toxin will cause death in a horse" (Forgacs 1972). There is an interesting reference by Khrushchev (1970) to an episode where Stalin threatened to shoot some veterinarians whom he was convinced were poisoning Ukrainian horses during the late 1930's. As it turned out, the horses had eaten hay heavily infested with a sooty black fungus, which most certainly was *S. atra*.

S. atra is a fungus which is able to subsist on low nitrogen high cellulose media. It requires high moisture, and therefore, typical natural substrates are wet straw, hay, and paper. In homes and buildings with heavy water damage, *S. atra* can be found growing on wallpaper, ceiling tile, carpets (especially the jute backings), insulation material (e.g., urea-formaldehyde foam, Bissett 1987), and general debris (Hunter et al. 1988).

Field cases of stachybotyotoxicosis have been reported in a number of European countries (Servantie et al. 1985), India (Rajendran et al. 1975), and in South Africa (Schneider et al. 1979) and involving a wide variety of livestock including poultry, cattle, horses, swine, sheep, and zoo animals (Hintikka 1978b; Harrach et al. 1983, 1987). A common source of this problem is bedding straw that the animals tend to consume in the winter months when their normal feed is not available in sufficient quantity. Cases also occur when the animals are fed damp straw in which the fungus has had sufficient time to form a luxuriant growth. Interestingly, animals appear to show a preference for *Stachybotrys*-infested straw, which increases the danger since the straw is usually quite heterogeneous with respect to fungal contamination. The syndrome also is reported in man (Hintikka 1978a, Andrássy et al. 1980) though the vast majority of cases are associated with handling contaminated straw used for farm animals. The only reported case of apparent stachybotryotoxicosis in the United States occurred in people living in a water-damaged home with a heavy infestation of *S. atra* (Croft et al. 1986).

Stachybotryotoxicosis has been most extensively studied in horses, which appear to be the most sensitive of animals to this toxicosis. The clinical signs include initially rashes and necrotic lesions near the mouth, rhinitis, conjunctivitis, excessive salivation, and elevated temperature. If the animals continue

to ingest contaminated material, the disease proceeds to a more serious stage where the animal suffers serious hemorrhaging both internally and externally. Animals whose dosage has been less acute become susceptible to opportunistic infections due to a general lowering of their immune functions. These animals do not respond to antibiotic treatment, and the only cure is to be placed on uncontaminated feed. If the animal has not been sufficiently compromised by this toxicosis, it will recover and show no apparent long-term ill effects. The most striking feature revealed upon autopsy is the massive internal hemorrhaging that has occurred especially in the mucous membranes. These effects can be reproduced with laboratory-grown cultures of *S. atra* (Schneider et al. 1979) and are very similar in nature to those observed in animals treated with verrucarin A (Fig. 1) (Mortimer et al. 1971). However, no livestock feeding studies have been conducted with pure satratoxins since these compounds have never been isolated in sufficient quantity to allow such studies. Although a South African toxigenic isolate of *S. atra* was shown to be lethal at a level of 5 grams of rice culture in a 40 kg ram, this sample was shown to contain less than 1 mg total of macrocyclic trichothecenes (Jarvis et al. 1995), an amount difficult to believe sufficient to cause death in this animal. Clearly, there are additional toxins produced by this organism. One class of such toxins which may play a role in the etiology of stachybotryotoxicosis are the anticomplement spirolactones, previously reported to be reproduced by a related organism, *S. complementi* (Kaise et al. 1979, Miyazaki et al. 1980). We have isolated a series of related spirolactones and lactams (e.g. stachybotrylactone and stachybotrylactam), from several toxigenic isolates of *S. atra* which also produce the satratoxins (Jarvis et al. 1995). At this time, the possible synergy between the macrocyclic trichothecenes and these spirolactones has not been evaluated.



Stachybotrylactone: X = 0
Stachybotrylactam: X = NH

There is an increasing concern with indoor air quality and the roles played by fungi in so-called "sick buildings" (Samson 1985, Tobin et al. 1987, Hunter et al. 1988). Although the vast majority of complaints from occupants of such buildings can be attributed to allergic responses to fungal spores, mycotoxins also have been suggested to play a role in some cases (Flannigan 1987). *Stachybotrys atra* has been found in indoor environments (Kozak et al. 1985) but only under very damp conditions. Inhalation of trichothecene mycotoxins poses a substantial risk since the potency of these toxins administered by this route is an order of magnitude higher than the toxicity observed upon either oral or IV dosage, though these data are species-dependent (Pang et al. 1988, Creasia and Lambert 1989). Inhalation of *S. atra* spores poses a risk to those handling straw and hay contaminated by this fungus (LeBars and LeBars 1985), but the only reported case of this organism being involved in an urban setting occurred in a Chicago suburb, where a badly water-damaged home had become heavily infested with *S. atra*. The occupants complained of numerous symptoms (headaches, sore throats, hair loss, flu symptoms, diarrhea, fatigue, dermatitis, and general malaise), which in view of the heavy and long standing growth of *S. atra* in the home could be attributed to stachybotryotoxicosis (Croft et al. 1986). Chemical analysis of *S. atra* contaminated material from this home led to the isolation and identification of verrucarins B and J, satratoxin H and trichoverrins A and B (Croft et al. 1986).

S. atra is certainly capable of producing potent toxins, but perhaps the most important concern should center on the immunosuppressive properties of some of its metabolites such as the stachybotrylactones and stachybotrylactams (Jarvis et al. 1994; Ayer and Miao 1993) and the cyclosporins, also produced by *S. atra* (Sakamoto et al. 1993). It is worth noting that the principal effect of chronic intoxication by *S. atra* in farm animals is immunosuppression which results in life-threatening opportunistic bacterial infections. Recurring colds were a common complaint of the occupants of the *S. atra*-contaminated house in Chicago (Croft et al. 1986). It may be that the inhalation of the *S. atra* spores (and hence *S. atra* metabolites) caused a local suppression of the mucosal immune system in the respiratory track which allowed the infecting bacteria to gain a foothold.

The old adage, "the dose makes the poison" needs to be emphasized. Patches of *S. atra* growing in your basement are not a risk. However, such fungal growth may be a harbinger of significant *S. atra* growth elsewhere in the building that may indeed pose a serious problem, especially if this growth is located in the HVAC system.

ANALYSIS FOR MYCOTOXINS

There is a great body of literature on the isolation and characterization of secondary metabolites (Luckner 1990). Particular attention has been paid to the characterizations of mycotoxins and to their quantitation in food products and animal feed (Cole 1986). A large number of standard analytical procedures for the analysis of mycotoxins have been published by the American Organization

of Analytical Chemists (AOAC), but few of these standard procedures are apropos to the analysis of fungal-contaminated buildings. The principal reason for this is that few of the fungi involved in cases of mycotoxin-contaminated food and feed intoxications are ever found in water-damaged buildings. The basic reason for this is that food products are quite different, as fungal substrates, from material (e.g. damp rugs, wet wall board and ceiling tiles, etc.) found in buildings. Most fungi that grow well on rich substrates such as corn, do not do well on nutrient-poor substrates such as paper and insulation.

Evidence to date suggests that only two fungi, *Stachybotrys atra* and *Aspergillis versicolor* are likely to pose a serious mycotoxin problem with respect to water-damaged buildings. The latter is a producer of sterigmatocystin, a mycotoxin closely related in chemical structure and biological activity to the aflatoxins, and there are standard procedures for the analysis of sterigmatocystin (Shotwell 1986). However, as discussed above, *S. atra* poses a challenging problem with respect to the analysis of its toxins, and what is needed in the case of *S. atra* are rapid and reliable assays for the metabolites being produced and their toxic potentials.

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RESPIRATORY DISEASE CAUSED BY BIOAEROSOLS—EXPOSURE AND DIAGNOSIS

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Abstract: *This presentation reviews the conditions for microbial growth indoors with special emphasis on humid buildings. Various microbial species commonly found indoors are discussed as well as two specific agents found in these—bacterial endotoxin and (1→3)ß-D-glucan from molds. The different symptoms and diseases related to bioaerosol exposure are described and diagnostic procedures are reviewed. It is suggested that bioaerosols account for a majority of the symptoms observed in indoor air environments.*

Key words: Endotoxin, (1→3)ß-D-glucan, molds, inflammation, microbes, pulmonary diseases

INTRODUCTION

Bioaerosols are particles of a biological origin and aerosols referred to as organic dusts, comprising material of vegetable, animal and microbial origin (Rylander and Peterson 1990; Rylander and Jacobs 1994). In the present context, the term will be restricted to airborne microbes.

Bacteria and molds are ubiquitous in man's environment and, at lower exposure levels, are usually tolerated without adverse reactions. When levels increase, however, effects become observable, in terms of infection, inflammation and sensitization. The first recorded observation of ill effects related to microbes in an indoor environment is found in the Bible, where a caution against what was probably mold in houses is found in the book of Leviticus (Leviticus~200 BC). Sensitivity to molds was first reported by Floyer (1726), who described an asthmatic reaction after inhalation of molds. Blackley (1873) described his own reactions after inhaling *Penicillium* spores as follows:

> *"The spores of the microscopic fungi I have reason to believe will, when brought into contact with the respiratory mucous membrane, generate symptoms not unlike those of hay fever in some respects but differing materially in others—being much like those of ordinary influenza."*

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Blackley, who had suffered from hay fever since childhood, afterwards developed a severe attack of hoarseness leading to complete aphonia, which lasted for a period of about two days and ended with a "bronchial catarrh". He declined to repeat the experiment.

These old accounts demonstrate nearly all of the features that are currently associated with exposure to molds. Had this knowledge not been forgotten by the 1970s and 1980s, we would probably have solved many problems related to indoor air problems (Burge et al. 1987; Finnegan et al. 1984; Robertson et al. 1985) much sooner.

In the following, I will review the diseases that may be caused by microorganisms, report data on the effects of two cell wall constituents of microbes—bacterial endotoxin and (1→3)ß-D-glucan—and suggest appropriate diagnostic procedures.

THE EXPOSURE

A number of studies have been published in which the indoor air flora of microbes has been well characterized and the conditions under which growth occurs determined (Burge et al. 1990; Flannigan et al. 1991). A review of major microbial agents in indoor air is reported in Table I.

Table I. Major microbial agents in indoor air.

Gram-negative bacteria
Enterobacter agglomerans
Pseudomonas syringae
Klebsiella pneumoniae
Fungi
Cladosporium
Alternaria
Penicillium
Trichoderma
Aspergillus
Stachybotrys atra
Mucor

Gram-negative bacteria grow in pools of water such as the reservoirs of humidifiers. In order for them to become aerosolized, physical forces are needed such as vacuum cleaning or—most commonly—operation of a humidifier.

Molds grow at humidities lower than 100%—some species thrive at levels of only 70% (Gravesen, 1979). This means that they will grow on cold walls on which humidity in indoor air condenses, in humid carpets and between inner and outer walls. Other important locations are around deficient window frames, along leaking water pipes and other sites of humidity.

Molds indoors have no special mechanism for the liberation of their spores and are dependent on some type of disturbance for the dispersal of large numbers (Lacey 1994). The number of mold spores found indoors is thus closely related to activity in the room—a sampling might yield no or only a low number of molds in an empty room, but when persons enter and move around, the levels increase. Particularly high levels are generated when renovating buildings or changing carpets or flooring materials.

Specific Agents

There are several specific agents in microbes that have important biological effects. The most studied of these agents in indoor air are bacterial endotoxin and (1→3)ß-D-glucan.

Endotoxins are lipopolysaccharide compounds present in the outer cell wall of gram-negative bacteria and blue-green algae. They cause inflammation in the lung when inhaled and affect the immune system (Rylander and Snella 1983). Endotoxins are often present in the water reservoir of humidifiers when the water is contaminated with gram-negative bacteria. They can liberate their endotoxin into the water, where levels up to 3 µg/ml have been recorded. In the air, levels of 0.5-0.8 µg/m3 have been measured when contaminated humidifiers have been operating (Rylander and Haglind 1984).

(1→3)ß-D-glucans are polyglucose compounds that are part of the structure of the cell wall of molds and certain bacteria. They have important immunomodulating properties (Di Luzio 1985) and particularly influence macrophages, causing a delayed effect. After exposure, an increased secretion of lysosomal enzymes can persist up to 30 to 40 days (Lew et al. 1986). A synergistic effect between inhaled endotoxin and (1→3)ß-D-glucan has been demonstrated (Fogelmark et al. 1994). Indoor levels up to 100 nanograms/m3 have been reported (Rylander et al. 1994).

Endotoxin and (1→3)ß-D-glucan are still active after the death of the host. An appropriate dose description is thus not obtained by determining the number of viable, airborne organisms. An estimate of the total number of cells or the amounts of the specific substances must be made.

Bioaerosols contain many antigenic materials, particularly in fungi. the most important fungal allergens are found among the saprophytic microfungi, e.g. *Mucor, Rhizopus, Cladosporium and Aspergillus.* Endotoxins and (1→3) ß-D-glucan, although not primarily allergens, are potent modulators of the immune system and may enhance or depress the reaction to antigens (Rylander and Holt 1995).

EFFECTS

The major exposure route for bioaerosols is by inhalation and thus effects on the lungs have received the greatest attention. In addition, bioaerosols can cause *systemic symptoms* in terms of headache, fatigue, joint pain and nerve symp-

toms. It is likely that several of these effects are caused by inflammatory mediators, produced in the lungs after inhalation of organic dusts and distributed to different parts of the body via the blood (Dunn 1992; Michel et al. 1994).

An overview of the different types of pulmonary diseases related to bioaerosol exposure is given in Table II.

Table II. Different pulmonary diseases related to exposure to microorganisms with corresponding ICD numbers, whenever applicable.

Infection
Inflammation/Sensitization
Bronchitis and pneumonitis (ICD J40)
Toxic pneumonitis (inhalation fever)
Airways inflammation (nonallergic asthma)
Chronic bronchitis (ICD J42)
Hypersensitivity pneumonitis (allergic alveolitis) (ICD J67)
Rhinitis, conjunctivitis
Asthma (ICD J45)

INFECTION

Infection caused by airborne microbes in organic dusts can arise either by human pathogens or by organisms that are not true pathogens but that invade the tissue of particularly sensitive individuals.

Examples of pathogens are tuberculosis, viruses and Legionella. Among the non-pathogenic organisms, *Aspergillus fumigatus* is of particular interest. This is a common organism in the general environment—levels up to several hundred/m3 have been reported in humid homes (Lacey 1994).

The lung has a great capacity to defend itself against *Aspergillus* spores. Occasionally, however, *A. fumigatus* may cause widespread invasion in the tissue of immunocompromised persons.

INFLAMMATION/SENSITIZATION DISEASES

Among the different entities of inflammation, reported in Table II, we recognize the symptoms described by Floyer (1726) and Blackley (1873).

An acute exposure to biogenic dust can cause a *toxic pneumonitis* (inhalation fever), which is characterized by an increase in body temperature, shivering and muscular and joint pain—symptoms resembling influenza. The disease is short-lived, and the symptoms disappear within a few days (Von Essen et al. 1990). In one study in a printing factory, 20 of 50 workers reported toxic pneumonitis in connection with work. The levels of airborne endotoxin were 0.13 to 0.39 µg/m3 when the humidifiers were operating (Rylander and Haglind 1984).

Repeated exposures over longer time periods may cause inflammation in the airways (*airways inflammation*). The symptoms are initially a dry cough and some impairment of lung function, usually measured as the decrease in forced expiratory volume during one second (FEV1). An accompanying symptom is increased airway responsiveness, which can be measured with a methacholine or histamine test. The subjects experience irritation in the airways, particularly in dusty environments, and shortness of breath during physical exercise. The symptoms gradually disappear when leaving work. The incidence of the disease is high—figures up to 50-60% of the exposed populations have been reported (Donham et al. 1989; Sigsgaard et al. 1994).

A special form of bronchitis—*chronic bronchitis*—is characterized by an increased production of mucus in the airways, a continuous, productive cough and breathlessness. It is operationally defined as a persistent cough for more than three months, lasting over a period of two years.

There are a number of agents in organic dusts that can cause the above inflammatory diseases. A common denominator is that they are all powerful inflammatory agents. In particular, bacterial endotoxin has been studied in this respect. Tentative threshold values for toxic pneumonitis are 1-2 µg endotoxin/m3, and for airways inflammation 0.02 µg/m3 (Rylander 1987).

A special inflammatory effect may develop after prolonged exposure. This disease—*hypersensitivity pneumonitis*—was first described among farmers, hence the name farmer's lung. It is now known that it appears among many different kinds of environments where exposure to organic dusts take place. It is characterized by a modest increase in body temperature, breathing difficulties and fatigue. There are pathological changes in the lungs in the form of lymphocytosis and granulomas. The incidence of the disease is low—in the order of 1/10,000-1/100,000 exposed.

There are probably several agents that can cause hypersensitivity pneumonitis by virtue of their capacity to influence the activity of T lymphocytes, probably through macrophages (Schuyler et al. 1994). The most widely recognized of these agents is molds.

Rhinitis, conjunctivitis and *asthma* are the classical sensitizing reactions seen after exposure to specific allergens, such as animal protein or pollen.

Asthma is characterized by variable airflow limitation with accompanying subjective symptoms of breathlessness. Important from a clinical point of view is the chronic inflammation which develops in the airways, probably as a result of several acute reactions. For two of the common molds regarded as the main allergenic fungi, threshold concentrations for evoking allergic symptoms have been estimated to be 100 *Alternaria* spores/m3 and 3000 *Cladosporium* (Gravesen 1979).

Epidemiological Evidence

Medical problems related to microbes in buildings were first described by van Leeuwen (1924), who suggested that asthma symptoms that were prevalent in humid areas in the Netherlands were related to the presence of molds indoors.

Several epidemiological studies have evaluated the relation between the effects reported above and bioaerosols in indoor air, and reviews have been presented previously (Burge 1990; Flannigan et al. 1991). Relations have been reported between the presence of molds and the extent of wheezing (Strachan et al. 1990), the presence of asthma (Strachan and Sanders 1989) and subjective respiratory symptoms (Platt et al. 1989; Waegemaekers et al. 1989; Dales et al. 1991).

Regarding specific microbial agents indoors, some previous studies have evaluated the relation between airborne (1→3)ß-D-glucan and endotoxin indoors and the extent of subjective symptoms. One study was made in a series of apartment buildings, where the amounts of airborne endotoxin and (1→3)ß-D-glucan were measured and the extent of symptoms was studied using a questionnaire. The results demonstrated a relation between the amount of airborne (1→3)ß-D-glucan and the extent of nasal irritation and hoarseness and between the amounts of airborne endotoxin and symptoms of cough and itchy eyes (Rylander et al. 1989). A subsequent study examined day-care centers, a post office and some apartments (Rylander et al. 1992). Again, a relation was found between the amount of airborne (1→3)ß-D-glucan and the extent of irritation in the throat.

In a private house with airborne (1→3)ß-D-glucan at levels up to 100 ng/m3, two boys developed airways inflammation, and one became sensitized to house dust mite (Rylander et al. 1994).

DIAGNOSTIC TOOLS

General Considerations

The diagnostic tests to be used should be thoroughly evaluated for each study of the diseases caused by aerosols that are reported in Table II. When more advanced diagnostic tools are applied, a consideration must always be whether they are for the benefit of the investigator or for the persons examined. Advanced tests usually require sampling for biological material and, while blood sampling does not present a large problem, lung biopsies and lung lavage are procedures that place the person investigated at a certain risk. If the investigated person is a participant of a research project, adequate information should be given and written consent obtained. The persons should also be informed as to his or her right to withdraw from the test at any time.

Spirometry

Effects of bioaerosols on lung function are usually evaluated as the forced expiratory volume in one second (FEV1), and standard procedures for the test have been published by several organizations. This measure can be used to detect differences in baseline values, comparing exposed and non-exposed populations. It can also be used to determine the change over a workshift or a larger than normal decline with age.

Experience from several studies on organic dust-exposed populations show that spirometry is a rather insensitive measure of effects. Widespread subjective symptoms indicative of airways inflammation and related to the exposure may be present even when spirometry is negative (Donham et al. 1989). Where advanced effects are suspected, however, spirometry is a useful tool for detecting exposure effects.

Questionnaires

Questionnaires are a most useful tool for detecting effects of bioaerosol exposure. Over the years, a number of questionnaires for pulmonary diseases have been proposed such as the British Medical Council questionnaire on chronic bronchitis, the American Thoracic Society questionnaire on asthma, etc. It must be realized that none of these questionnaires are satisfactory for studies on organic dust-induced effects, as they do not incorporate important effects such as toxic pneumonitis and airways inflammation. A questionnaire for organic dust exposure effects has been presented (Rylander et al. 1990).

Airway Responsiveness

An increased airway responsiveness indicates the presence of inflammation. By tradition, measurements of airway responsiveness have been used in the diagnosis of asthma. With the growing appreciation that inflammation can also be induced by organic dusts and bioaerosols, measurements of airway responsiveness can be used to assess airways inflammation in persons with such exposure.

The traditional method to measure airway responsiveness is to expose persons to increasing doses of methacholine or histamine, and determine the dose at which a 20% reduction of FEV1 is obtained. This method is difficult to use under field conditions, as it requires a considerable amount of time and interferes with work.

An alternative to this method was proposed by Yan et al. (1983). The procedure is to administer small doses of the agent rapidly, up to a maximum dose. For methacholine, this is 1.25 mg. The result is then read as the decrease in FEV1. This method is suitable for field conditions, and effects caused by organic dust exposure have been shown in several studies (Carvalheiro et al. 1995; Rylander and Bergström 1993; Pattermore et al. 1990).

Cells, Cell Reactivity and Mediators

Determination of cell numbers, cell reactivity and cell-derived mediators represent techniques which are still at a research stage. Data have been published on increases in cell numbers and types in lung lavage fluid and nasal lavage, and on cell reactivity in blood of persons exposed to organic dusts (Jacobs et al. 1993; Sandström et al. 1992; Beijer et al. 1990). In view of the importance of airways inflammation likely to be caused by cell activation, these tests are of great interest, although they cannot as yet be applied on a routine basis.

As regards inflammatory mediators, these are secreted by effector cells such as macrophages. Inflammatory mediators have been measured in blood after exposure to endotoxin (Michel et al. 1992). As they are secreted only during limited time spans, it is difficult to assess their value in a diagnostic framework.

Secondary indicators of inflammation are more promising as diagnostic tools. It has been shown that fibrinogen degradation products (FDP) and C-reactive protein (CRP) are elevated in persons exposed to organic dust and endotoxin (Mattsby and Rylander 1978; Michel et al. 1994). When evaluating these results, it must, however, be remembered that they are nonspecific indicators of an effect and that infection and other environmental agents may also influence the levels of these secondary indicators of mediator secretion. Appropriate control groups would offer one way to reduce this source of error.

Antibodies/Precipitins

To diagnose inflammatory diseases caused by bioaerosols, the determination of antibodies/precipitins is of limited value. The presence of specific antibodies to a particular agent in the indoor environment indicates exposure but this is seldom specific and similar antibodies are also present among persons without disease. Furthermore, the absence of antibodies does not mean the absence of a pathological process. In some organic dust environments containing bioaerosols, there is also a general increase of antibodies that are nonspecific for the environment (Mattsby and Rylander 1978). Antibodies are not related to the disease pathology.

To diagnose sensitization, the presence of IgE antibodies can be determined using the skin prick test, which reveals IgE-related activity to the particular antigen used. Care must be taken to work with relevant and well-defined preparations. Some of these have been found to be contaminated with bacterial endotoxin or (1→3)ß-D-glucan, which by themselves produce inflammatory reactions, as described above.

Total IgG, IgM and IgE

Determinations of total levels of IgG, IgM and IgE are seldom useful. Although an increase may be present, it is nonspecific, and an absence of it does not exclude disease. Inflammatory agents such as tobacco smoke can influence total immunoglobulin levels and act as confounders.

Inhalation Provocation

Provocation with a bioaerosol or an extract thereof to prove that the particular environment causes the observed effects is not a recommended procedure for diagnosis. The risk of anaphylactic reactions or an acute fibrosis in light cases of hypersensitivity pneumonitis does not justify the small increase in diagnostic precision.

CONCLUSION

This review demonstrates that bioaerosols are a common pollution in certain indoor environments, particularly in humid buildings. The relevant effects are infection—which is rare—and inflammatory diseases in the airways. Sensitizing reactions in terms of asthma may also develop. Two major cell wall constituents of microbes seem to be of primary importance for the development of the effects and these agents are also active when the hosts are no longer viable. The variety of pulmonary disease associated with exposure to bioaerosols demonstrates that efficient preventive measures must be taken to avoid bioaerosol exposure indoors and that once they are present, vigorous actions must be undertaken to destroy the source of pollution.

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AEROSOLIZED MYCOTOXINS: IMPLICATIONS FOR OCCUPATIONAL SETTINGS

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Abstract: *It has been realized for some time that mycotoxins represent a threat to human health, but until recently this threat was believed to be exclusively or nearly exclusively due to ingestion. Evidence is accumulating that biologically significant exposure to mycotoxins can occur via the pulmonary route. Since the vast majority of known mycotoxins are nonvolatile, this exposure must occur primarily via spores, which carry either preformed mycotoxins or the genetic potential for the production of mycotoxins in the tissues of the host. There is also increasing evidence that ß1, 3-glucans, although not mycotoxins in the classical sense, can produce disease as a result of stimulation of inflammation. The mechanism(s) of their action is markedly different from those of the classical mycotoxins. Diseases associated with inhalation of fungal spores along with the ß1, 3-glucans and/or mycotoxins include organic dust toxic syndrome, hypersensitivity pneumonitis, tremors, chronic fatigue syndrome, kidney failure and cancer.*

Key words: Mycotoxins, spores, inhalation, ß1, 3-glucans, inflammatory disease, ODTS, hypersensitivity pneumonitis, cancer

INTRODUCTION

Fungi are heteotrophic, filamentous organisms which, by virtue of their dependence on external sources of organic carbon and their rigid cell walls, are confined to a saprobic and/or parasitic life style in which they absorb soluble nutrients through the cell membrane. The fungi, together with the bacteria, are responsible for decay of organic matter, and the fungi have been estimated to comprise ca. 25% of the biomass of the earth (Miller, 1992). As such, they are among the principal microorganisms involved in biodeterioration and are found in many substrates and occupational settings. Occupations and/or workplaces that may involve exposure to fungi include grain harvesting, stor-

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age and processing, sawmills and wood pulp mills, mushroom cultivation, waste treatment, and even offices, libraries, and museums. In fact, virtually any occupation which brings workers into close association with biodeterioration processes offers the possibility of exposure to fungi and their spores.

As might be expected, a very large number of workers are potentially at risk. For example, over 5 million U.S. grain handlers, lumberworkers, woodworkers and farm workers are believed to be at risk of developing occupational asthma and rhinitis (Salvaggio et al. 1986). Similarly, workers at risk of various hypersensitivity pneumontides involving fungi include approximately 3 million farm workers, mushroom workers, and malt workers combined (Fink, 1986). These numbers do not include those individuals at risk of exposure to fungi in offices and homes. Evidence for a link between inhaled fungal spores and the production of lower respiratory disease is limited, but recently several studies have noted a relationship between reported mold growth and respiratory symptoms in houses (Strachan, 1988; Brunekreef et al. 1989; Dales et al. 1991). Although spore concentrations are usually much lower in homes than in agricultural work places, concentrations as high as 450,000 cfu/m^3 have been reported (Hunter et al. 1988). In some of these homes, the toxigenic *Stachybotrys atra* was prominent on the walls and in the air.

INFLAMMATORY DISEASE IN AGRICULTURAL WORKERS

As noted above, workers in many occupations may be at risk of exposure to fungi and their products, but this is especially true in agriculture where spore concentrations on the order of 200 million spores/m^3 have been reported (Darke et al. 1976). Relatively little has been published on health effects due to inhalation of mycotoxins, but the role of agricultural dust (which includes fungal spores) was recognized at least as early as 1555 (Rylander, 1994).

Two important disease entities associated with the inhalation of organic dust containing fungal spores are organic dust toxic syndrome (ODTS) and hypersensitivity pneumonitis (HP), the latter also known as extrinsic allergic alveolitis (EAA). Rylander (1994) has suggested that these diseases be termed "toxic pneumonitis" and "granulomatous pneumonitis" respectively. Both are associated with inhalation of high concentrations of organic materials, particularly agricultural materials such as grain dust, hay or silage contaminated with microorganisms (Lecours et al. 1986; May et al. 1986; Pratt and May, 1984; Pratt et al. 1990). HP has been recognized for a very long time and occurs in a variety of occupational settings (Parker et al. 1992). Farmer's lung disease (FLD) is the most familiar form of HP in agriculture. ODTS is a noninfectious illness resembling the "flu" and is characterized by fever, malaise, myalgia and a neutrophilic inflammation of the lower respiratory tract (Lecours et al. 1986; Parker et al. 1992). The presence of high levels of fungi and bacteria in dust associated with ODTS has been found to be a hallmark of the syndrome (Dutkiewicz et al. 1989; Olenchock et al. 1990). These diseases have many features in common including similar exposure settings and clinical symptoms (Emmanuel et al. 1975;

Pratt and May 1984; Parker et al. 1992). Outbreaks of ODTS are characterized by much higher "attack rates" than is observed in farmer's lung disease; there is usually no correlation between presence of precipitating antibodies and illness; and clinical findings suggest that ODTS results from nonspecific immune mechanisms (Von Essen et al. 1990). A number of possible mechanisms have been suggested for ODTS including direct activation of complement (Olenchock and Burrell 1976; Olenchock et al. 1990) and the presence of "polyclonal cell activators," i.e., agents which stimulate cells of the immune system in a nonspecific way, resulting in production of various mediators of inflammation (Willoughby et al. 1985). Polyclonal cell activators (PCAs) may stimulate as much as 10^6 times the number of cells with 60-fold greater mediator release than by antigen stimulation (Willoughby et al. 1985). PCAs may be especially significant with respect to macrophages, because whereas antigen can only stimulate these cells indirectly via lymphokines from sensitized lymphocytes, most PCAs can activate macrophages both directly and indirectly (Willoughby et al. 1985). Activation of macrophages can result in enhanced glucose oxidation, secretion of enzymes, synthesis and release of complement components, release of prostaglandins and reactive oxygen intermediates, release of cytokines and enhanced phagocytosis, as well as enhanced bactericidal and tumoricidal activities (Willoughby et al. 1985).

Sorenson et al. (1994, 1995) demonstrated that conidia of several fungi have the ability to activate complement and stimulate production of LTB_4 and superoxide anion. Complement activation has several important consequences including facilitation of phagocytosis with the production of complement factor C3b and stimulation of chemotaxis with the production of components C3a and C5a which are potent chemotactic agents and lead to recruitment of large numbers of PMN to the site of activation. LTB_4 possesses potent calcium ionophore activity and, in addition to recruiting inflammatory cells to the lung, it can upregulate their function and modulate the immune response. Production of reactive oxygen intermediates has been postulated to contribute to the tissue damage seen in pulmonary inflammation. Fungal spores alone were found to stimulate a modest increase in O_2 production. However, if macrophages were primed by pretreatment with lipopolysaccharide (LPS) and then exposed to the fungal spores, significant enhancement of O_2 production was observed (Shahan et al. 1994). Tai and Pestka (1988) reported increased endotoxin sensitivity in mice following exposure to T-2 toxin leading to increased mortality; Taylor et al. (1991) showed that simultaneous exposure of mice to endotoxin and T-2 toxin resulted in increased mortality, hypothermia, TNFα production, and thymic atrophy compared to treatment with either agent alone. These results support the concept that the combined exposure to both bacterial and fungal agents may be important in the pathogenesis of ODTS.

ß1, 3-glucans are major components of the fungal cell wall and are glucose polymers consisting predominantly of chains of glucopyranosyl rings joined by ß1, 3 linkages with varying degrees of ß1, 6 side chains. It is believed that macrophage activitation is the central event in the ß-glucan immune stimulatory

effect (Williams et al. 1985). Glucans enhance both the number and function of macrophages (Patchen et al. 1984; Williams et al. 1987), including release of hemopoietic growth factors and stimulating proliferation of hemopoietic progenitors (Patchen et al. 1984). Soluble glucans also enhance production of IL-1 and IL-2 by splenic macrophages (Sherwood et al. 1987) and stimulate production of monocyte chemoattractant protein 1 (MCP-1), which was preceded by an early, transient rise in TNF (Jones and Warren, 1992).

Fogelmark et al. (1992) compared the effects of inhaled endotoxins and ß1, 3-glucan, individually and in combination, on the number of inflammatory cells in lung walls and airways. Endotoxin caused an increase in lung lavage leukocytes (neutrophils, macrophages, and eosinophils) at 4 and 24 hours after exposure whereas no significant increase in lung lavage neutrophils were found after exposure to an equal concentration of glucan. The number of all cell types in lung lavage was less after exposure to a combination of endotoxin and glucan than after exposure to endotoxin alone. Hoffman et al. (1993) demonstrated that fungal ß-glucans stimulate rat macrophage release of tumor necrosis factor-α (TNFα) at concentrations of less than 500 µg/ml of ß-glucan but that higher concentrations of ß-glucan resulted in TNF release comparable to untreated cells with no effect on cell viability. These results suggest an immunomodulating role for fungal ß-glucans.

The term "pulmonary mycotoxicosis" was applied to ODTS to differentiate it from FLD and to underscore the apparent importance of fungi and/or their metabolic products (Emanuel et al. 1975). Attempts to implicate mycotoxins in the syndrome were limited, both in number and in scope, and failed to reveal significant amounts of those few mycotoxins which were sought in dust samples collected from outbreaks of ODTS (May et al. 1986). This is not to say that mycotoxins are not present in these exposures or that they could not contribute to the pathogenesis. It should be recognized that new mycotoxins continue to be described and the fumonisins, for example, now considered an especially important group because of the frequency of their occurrence and the severity of their toxicity, were not known until 1988. Even if mycotoxins are not part of the "normal etiology" of ODTS, their presence in organic dust may exacerbate the disease outcome.

Mycotoxins and Mycotoxigenic Fungi

Bennett (1987) defined mycotoxins as "...natural products produced by fungi that evoke a toxic response when introduced in low concentrations to higher vertebrates by a natural route." At present, approximately 350 to 400 fungal metabolites, excluding mushroom toxins, are considered to be toxic, and most of these are relatively small molecules of greater than 200 but less than 500 mass units (Samson, 1992) although the fumonisins are greater than 750 mass units. Perhaps the most important mycotoxins in agriculture are the aflatoxins, the 12,13-epoxytrichothecenes, ochratoxin, and the fumonisins. The most important mycotoxigenic species belong to the general *Aspergillus, Penicillium,* and *Fusarium* (Samson, 1992). For example, the *Penicillium verrucosum*-complex (*P. ver-*

rucosum, P. aurantiogriseum, P. viridicatum, P. crustosum, and *P. solitum*) have been shown to produce nearly 20 different mycotoxins (Frisvad and Filtenborg, 1989). These fungi are common contaminants of agricultural commodities, and some of the mycotoxins produced by these species are known to be produced by fungi common in house dust (Tobin et al. 1987). Other toxigenic fungi include species of *Alternaria, Paecilomyces, Rhizopus, Trichoderma,* and *Trichothecium*. All of these fungi are commonly found in soil, agricultural products, grain dust, and house dust (Tobin et al. 1987).

Mycotoxins in Spores

Although few investigators have examined spores of various fungi for mycotoxins, the presence of mycotoxins has been demonstrated in the spores of several species of toxigenic fungi, including *Alternaria alternata* (Häggblom, 1987), *Aspergillus fumigatus* (Parker and Jenner, 1968; Palmgren and Lee, 1986; Land et al. 1994), *Aspergillus flavus* and *Aspergillus parasiticus* (Wicklow and Shotwell, 1983), *Fusarium graminearum* (Miller, 1992), *Fusarium sporotrichioides* (Miller, 1992) and *Stachybotrys atra* (Sorenson et al. 1987). Mycotoxins found in spores include deoxynivalenol (Miller, 1992), fumitremorgen and verruculogen (Land et al. 1995), fumigaclavine C (Palmgren and Lee, 1986), T-2 toxin (Miller, 1992), tryptacidin (Parker and Jenner, 1968), alternariol and alternariol monomethylether (Häggblom, 1987), and the macrocyclic trichothecenes satratoxins G and H (Sorenson et al. 1987). Gliotoxin, a known metabolite of *A. fumigatus* which has been demonstrated in tissues infected by *A. fumigatus* (Bauer et al. 1989) and *Candida albicans* (Shah and Larsen, 1993), was not detected in spores of *A. fumigatus* (Land et al. 1989). The fact that several mycotoxins have been found in spores—in a high proportion of species in which attempts were made to find them—suggests that their presence in spores of toxigenic species is much more common than is currently appreciated. Smith et al. (1992) reported that extracts from spores of 47% of a group of 83 isolates collected from damp public sector housing in Scotland were cytotoxic to the human embryonic hybrid fibroblast lung cell line MRC-5. These findings seem to support earlier findings of health hazards in epidemiological studies of the inhabitants of damp, moldy houses (Strachan, 1988; Tobin et al. 1987). Macgeorge and Mantle (1990) have shown that spores of *Penicillium aurantiogriseum,* which contain the benzodiazepine metabolite auranthine, cause nephrotoxicity and pathology typical of Balkan endemic nephropathy when mixed with feed and fed to rats. These findings demonstrate the presence of auranthine in the spores and suggest that workers and others who handle infected grain may be at risk of exposure by inhalation. The vast majority of mycotoxins are nonvolatile and therefore mycotoxin exposure by inhalation is most likely to occur via inhalation of spores.

Effects of Mycotoxins on Alveolar Macrophages and Immune Function

T-2 toxin, patulin, and penicillic acid were shown to be acutely toxic to rat alveolar macrophages in vitro, causing membrane damage, inhibition of protein

and RNA synthesis, inhibition of phagocytosis, and inhibition of the ability of AM to respond to lymphokines (Gerberick and Sorenson, 1983; Gerberick et al. 1984; Sorenson et al. 1985; Sorenson et al. 1986). Ayral et al. (1992) showed that the trichothecenes diacetoxyscirpenol (DAS) and deoxynivalenol (DON) reduce phagocytosis, suppress microbicidal activity, and inhibit superoxide anion production and phagosome-lysosome fusion of peritoneal macrophages at concentrations that did not affect cell viability. Similarly, Vidal and Mavet (1989) demonstrated inhibition of phagocytosis of *Pseudomonas aeruginosa* by murine peritoneal macrophages in the presence of 0.001 μM of T-2 toxin. The trichothecene mycotoxins are immunotoxic in rats and mice, causing acute inhibition of antibody and delay of skin graft rejection (LaFarge-Frayssinet et al. 1979; Rosenstein et al. 1979).

Gliotoxin has anti-phagocytic and immunomodulating activity, it is produced by *A. fumigatus* (Eichner et al. 1986), and it has been demonstrated in tissues infected by *A. fumigatus* (Bauer et al. 1989). Gliotoxin has also been shown to contribute to the pathogenesis of vaginal candidiasis (Shah and Larsen, 1993). Jakab et al. (1994) confirmed earlier reports that dietary exposure to aflatoxin B_1 impairs innate and acquired host defenses. Subsequent work by these authors also showed that alveolar macrophage phagocytosis was suppressed for approximately two weeks following nose-only inhalation exposure to an estimated dose of 16.8 μg/kg. Exposure using intracheal instillation (IT) of aflatoxin B_1 also suppressed AM phagocytosis in a dose-dependent manner but ca. 10-fold higher doses were required than for inhalation. Animals exposed by IT administration also had impaired release of TNFα, primary splenic antibody response and peritoneal macrophage phagocytosis.

These studies show that experimental respiratory tract exposure can suppress pulmonary and systemic host defenses and that inhalation exposure to AFB_1 could lead to increased susceptibility to infection. Richard et al. (1983). used killed spores of *Aspergillus fumigatus* as carrier vehicles for AFB_1 in studies of the effect of long-term exposure to aflatoxin. Lung lesions were not observed in unexposed animals, but lesions of varying severity were observed in the lungs of rats exposed to aflatoxin. The authors reported that the general response of the exposed animals appeared to be that of a compromised host.

Although the effects of mycotoxins as a result of dietary exposure is beyond the scope of the present discussion, a number of mycotoxins—especially aflatoxin, the trichothecenes, ochratoxin A, patulin, citrinin and zearalenone — experimentally alter immunity causing inhibition of natural killer cell activity, impaired resistance to pathogenic microorganisms, suppression of antibody response, lymphoid depletion of the thymus associated with delayed hypersensitivity and functional alteration of bone marrow cells. This subject has been extensively reviewed by Richard et al. (1978), Thurston et al. (1986), and Pestka and Bondy (1990).

In studies of the acute inhalation toxicity of T-2 toxin in mice, Creasia et al. (1987) demonstrated that T-2 toxin was at least 10 times more toxic than systemic administration and at least 20 times more toxic than dermal administration.

Health Effects Linked with Inhalation of Mycotoxins

Although extensive literature has been developed since the discovery of the aflatoxins, relatively little is known of the occurrence of these substances in airborne grain or other organic dust or of the inhalation hazard to workers and others exposed to contaminated airborne dust. Recent studies have provided circumstantial evidence for the association of cancer in humans with inhalation of aflatoxin-contaminated dust. Dvorackova (1976) reported that two chemical engineers working with contaminated peanut dust developed alveolar cell lung carcinoma and Deger (1976) described cases of two biochemists who developed colon carcinoma while working to purify aflatoxins by preparative thin-layer chromatography. Van Nieuwenhuize et al. (1978) reported an epidemiological study of workers in a peanut-processing plant in the Netherlands in which rates of multiple kinds of cancer were more than 3 times those reported in the matched group.

In a follow-up study in the same plant, Hayes et al. (1984) demonstrated that mortality for total cancer and respiratory cancer in the aflatoxin-exposed group of peanut-oil-press workers was higher than expected based on standardized mortality ratio (SMR) analysis. Workers at this plant admitted occasionally eating peanuts during work shifts so their exposure was not totally via inhalation.

Sorenson et al. (1981) and Burg et al. (1982) have reported the presence of aflatoxins in respirable airborne corn dust and Sorenson et al. (1984) showed that airborne peanut dust from contaminated lots of peanuts contained up to 612 ppb aflatoxin B_1 (AFB_1). Workers inhaling 50 mg/m^3 containing 100 ppb aflatoxin B_1 could inhale 120 ng in an 8-hour workshift. Olsen et al. (1988) did a retrospective study of cancer risk and occupational exposure to aflatoxin among livestock feed processing workers in Denmark. Their study was based on a data linkage system which allows linkage of personal identity numbers for individual workers, companies, employment histories back to 1964, and cases of cancer reported to the Danish Cancer Registry. The average concentration of organic dust in these companies was ca. 100 mg/m^3; crops imported for feed production have been highly contaminated (average level of 140 ppb in prepared cattle feed); and the estimated daily pulmonary exposure was ca. 170 ng.

The Danish investigators noted elevated risks for liver cancer and cancers of biliary tract in their work population, which increased by two-three-fold significance after a 10-year latency, and they reported that exposure to aflatoxins in the imported feed is the most likely explanation for their findings. Our estimate of a possible daily exposure to 120 ng AFB_1 by the pulmonary route is consistent with the Danish estimate and is conservative (Sorenson, 1990). Autrup et al. (1991) used measurements of aflatoxin bound to serum albumin as an index of exposure and showed that seven of 45 workers exposed to feed contaminated with low levels of aflatoxin B_1 (0-26 μg/kg) had detectable levels of aflatoxin B_1 bound to serum albumin, confirming systemic exposure. In addition, there appeared to be a good association between the level of aflatoxin B_1 adducts to albumin and exposure.

Two other recent reports of human disease thought to be due to inhalation of mycotoxins are noteworthy. Di Paolo et al. (1993) reported acute renal failure in a female agricultural worker exposed to grain dust in an enclosed granary which they stated was "...undeniably due to inhalation of ochratoxin of *Aspergillus ochraceous*." Although the authors did not detect ochratoxin in airborne dust in the granary, they were able to isolate *A. ochraceous* from a sample of wheat from the granary, extracts of ground, moldy grains contained material which was identical to authentic ochratoxin A on thin-layer chromatography, and they were able to demonstrate acute kidney failure in experimental animals (rabbits and guinea pigs) exposed for 8 hours to aerosols generated by their natural movement on moldy wheat in their cages.

Gordon et al. (1993) reported tremorgenic encephalopathy in a young man exposed to high concentrations of grain dust contaminated with several species of fungi known to be capable of producing tremorgenic mycotoxins. Because of the circumstances of the young man's exposure, the similarity of his syndrome to that of an animal model, and the lack of an alternative explanation despite extensive testing, the authors proposed that his illness may have resulted from inhalation of tremorgenic mycotoxin(s).

Auger et al. (1994) suggest that ß1, 3-glucans and mycotoxins in the spores of toxigenic fungi present in homes may explain the association between exposure to mold and dampness and various respiratory and nonrespiratory symptoms. This is based on the known properties of these substances and the repeated observation that removal of these fungi from homes has led to substantial reduction or elimination of symptoms (Croft et al. 1986; Holmes et al. 1988).

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STACHYBOTRYS ATRA: IS THE CLINICOPATHOLOGICAL PICTURE CHANGING?

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Abstract: *In a review of stachybotryotoxicosis, only three publications were located which described the disease in North America. This might be due to a failure to call the disease by its name: stachybotryotoxicosis. Symptoms of the disease include coughing, rhinitis, sore throat, nose bleeding, moderate fever, headaches, dermatitis, fatigue and general malaise. It is suggested that more publications on instances of this disease should be encouraged. In all cases of sick building syndrome, the presence of* Stachybotrys atra *should be considered.*

Key words: *Stachybotrys atra*, stachybotryotoxicosis, mycotoxins, trichothecenes

INTRODUCTION

In a 1980 paper, Mirocha described stachybotryotoxicosis in people as follows: "Human beings were affected by aerosols of the toxic fungi or their toxic substrata in the area where the disease was rampant among animals. Workers who handled fodder developed a toxicosis as well as those who used infected straw for fuel or slept on mattresses made of infected straw. A dermatitis localized chiefly on the scrotum and less frequently on the hands or other parts of the body. The dermatitis was characterized by hyperemia, serum exudation, encrustations, and necrosis. The symptoms in man included a catarrhal angina, bloody rhinitis, cough, pain in the throat, tightness in the chest and a burning sensation in the nasal passages."

More recently, Andrassy et al. presented a detailed account of the effect on farm workers in the Debrecen area of Hungary, where this disease is a common occurrence. Twenty-three persons were affected; the most general symptoms were observed in the respiratory tract. Those affected complained of dyspnea, shortness of breath, sore throat, rawness in the throat, nose bleed, bloody discharge from the nose, and burning sensation in the eyes. The skin of the face around the nose and eyes was inflamed, the eyelids swollen, the nostrils encrusted. The mucous membranes were inflamed. Weakness, exhaustion and

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perspiration completed the symptoms as offered by the workers. Abstinence from working with affected straw and treatment with an antiphlogistic preparation resulted in remission of symptoms in a week, or in an elderly asthmatic, two weeks. The mycotoxicosis was caused by inhalation and skin contact of infected litter and fodder."

It is important to point out that at the time of this report *Stachybotrys atra* was unknown amongst animals in North America, and to the best of our knowledge no such disease occurred in people.

MATERIALS AND METHODS

The literature was searched from 1966 to 1995; however, references to the European literature were not included since only North American were used. The search included *Stachybotrys atra* and related compounds. Papers which dealt with aspects other than human health effects were also excluded.

RESULTS

In the first paper, dealing with stachybotryotoxicosis in a house in Chicago, Illinois, Croft et al. (1986) reported on the events over a five-year period. Besides the parents, two children and a maid were affected. The symptoms included signs of cold and flu, sore throat, diarrhea, headaches, fatigue, dermatitis, intermittent focal alopecia and generalized malaise.

Investigation of the house revealed a few spots under the roof where it had leaked when it rained, and circumscribed areas of *Stachybotrys atra* were located there and in the air ducts. It was found that the dark brown spots contained *Stachybotrys atra* which included various trichothecenes, i.e., verrucarol, verrucarin B, verrucarin J, satratoxin H and trichoverrins A and B. An extract given to rats resulted in death within 24 hours. The cleaning of the air ducts caused respiratory tract distress and skin irritation among the workers.

The second report is from Quebec City, Canada, and was written by Mainville et al. (1988). In this paper, the authors reported generalized fatigue and, in some cases, impairment of immune function. A large number of persons were involved. The event took place in a large hospital.

The third report, by Johanning et al. (1993a) describes a large renovated building in New York City which had been unoccupied for a number of years. After the former garment factory was converted to an office building, the employees reported adverse effects of the central nervous system and upper and lower respiratory tract, eye and skin irritation and excessive chronic fatigue. Investigators found *Stachybotrys atra*, specifically satratoxin H.

From 43 employees tested, four had a positive *Stachybotrys atra* 1gE-antibody test. These four employees were clustered in one office area.

DISCUSSION

What has happened with a disease which was first described in 1837 when a strain of *Stachybotrys* was defined by Corda in a house in Prague where the fungus was growing under the wallpaper (Forgacs, 1972)?

The paper by Forgacs (1972) demonstrates the classical picture of "rural areas" of the times: farmers were exposed to the organism in the fodder they fed their livestock and in the houses they lived in. A number become seriously ill and some died. It is obvious that this picture no longer applies to our condition. We live, to a large degree, in big cities, work in various places, and *stachybotryotoxicosis* does not even occur amongst the farm animals in North America.

It is necessary to look at the situation in North America. The clinical picture is defined by coughing, rhinitis and nose bleeding, throat irritation, moderate fever, headaches and general fatigue. Death does not occur. What is different?

Jarvis (1990) gave an excellent review of the condition which resulted in more or less admitting that most examples had not produced convincing evidence. In contrast to these findings, however, is the strong evidence that "something is happening" when the disease affects people. For instance, one would have to look at the white blood cell count repeatedly over several days because the typical leucopenia (Forgacs, 1972) may occur only sporadically and may not differ much from the norm. Therefore, if a patient's blood cell count was checked only once, there would be no evidence of the disease.

Other authors are less certain. While they have found *Stachybotrys atra* in some cases and in other instances *Stachybotrys* spp, they list these agents as potentially causing the disease (Miller et al., 1988; Flannigan et al., 1991) or report outbreaks of stachybotryotoxicosis in homes (Sorenson et al., 1987) without further describing the course of the disease. It has been reported also, in a study of filter material, that *Stachybotrys atra* can grow only in filter material with high cellulose content (Pasanen, 1994).

The excellent paper by Ueno (1983) gives all potentially incriminating trichothecenes, listing them by type A to D. The agents causing the disease which is discussed in this paper are found in type D and it is noteworthy that these agents are ten times more toxic than the other types.

It is time to recognize this disease as health-endangering, not necessarily as life-threatening, with the exception of very young (less than one year old) children, who may be in danger of dying.

Johanning et al. (1993b), in a further report on the case cited earlier as the third report (Johanning et al., 1993a), recommends classifying the mycotoxin *Stachybotrys atra* as a hazardous substance requiring remediation techniques similar to asbestos abatement.

CONCLUSIONS

Stachybotrys atra is not behaving in a novel manner. Death due to the actions of this organism is very unlikely both in Europe and in North America. The mobility of modern people makes it unlikely that anyone would sustain the constant level of exposure necessary to become seriously ill. Flu-like symptoms, possibly attributable to *Stachybotrys atra* in some instances, are seldom recognized as such.

Thus, one can conclude that the clinicopathological picture has not changed when compared with the picture which was presented in the period before stachybotryotoxicosis was found to occur in North America. It will be interesting to watch the North American literature during the next few years to see if our physicians and veterinarians will recognize this disease, learn to diagnose it, and publish their findings.

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PANEL DISCUSSION 10/6/94

This discussion was transcribed and edited from the videotape of the conference. We apologize in advance for any inaccuracies due to technical difficulties. —The Editors.

Panelists: Flannigan, Gareis, Hintikka, Jarvis, Sorenson

Q. *On the frequency of organisms found in the lungs of autopsy victims in Japan—were these parasitic colonies that were found? How was the determination made that these organisms were present?*

A. *Brian Flannigan:* No, these were not parasitic colonies, as far as I remember. The slides are something to see, I'll tell you. They sliced open the lungs and then they swabbed out material from the lungs.

Q. *So it was not cultured? It was just a tissue section that was put under a microscope?*

A. *Brian Flannigan:* No, they took the swabs and then they stripped the swabs out on plates and these were the principle organisms that came out. You had *Penicillium, Aspergillus* and way, way down on the scale was *Cladosporium.* These were people who had died suddenly in road accidents and things like that. They were healthy but dead. (laughter)

Q. *These were very low counts of organisms that were found?*

A. *Brian Flannigan:* I can't remember that they actually quantified the counts so it was a plus or minus whether these fungi were present or not in the lung tissue. I should say that in people who died of respiratory diseases, you find things like *Aspergillus fumigatus* with a slightly different range of fungi, but in the healthy people, these were the predominant organisms.

Q. *I read an article recently in my favorite journal, I think it was the Seattle Times; there was a publication that suggested food additives prevented cancer. My immediate reaction was it was probably because of preventing fungal growth and now that I have heard all these lectures, I am even more convinced that that is probably the case. Does anyone want to comment on that or has anyone responded to that offer? I don't know the author; AP may have put the story on the national wire.*

A. *Bruce Jarvis:* Well, I'll take a crack at it. As I understand the question there are certain foods or food additives that are supposed to lower the incidence of cancer in humans? Is that it?

Questioner: *Yes, somebody did a big study and showed that people that ate more food additives had less cancer, in opposition to the theory that everything chemical that is put in food is bad.*

Panelist: BHA and BHT are antioxidants.

A. ***Bruce Jarvis:*** We have a law called the Delaney clause which prohibits the purposeful addition of known carcinogens that are not naturally occurring into our food. They neglect to tell the general public that there are a lot of known carcinogens that occur naturally; chemicals that do not seem to bother them. The Delaney clause does not prohibit that. In any event, there is this whole flurry of antioxidants doing all kinds of good things for us, but if you wait long enough you will find out they do bad things. So it is a cyclical thing. I do not know anything specific about those trials but my experience with reading the popular press and even reading the professional journals, from time to time on this subject people seem to change their minds about what is good for them. For instance, I have now lost track whether polyunsaturates are good for us or bad for us; it depends on what you plan on dying of. (laughter)

A. ***W.G. Sorenson:*** I don't know how relevant this is, but I have seen reports that certain substances in cauliflower and a few foods like that are antimutagenic. I don't know exactly what they do, whether they are antioxidants but they seem to reverse the effect of some of the mutagenic agents, chlorophyll, for example has been shown to have this kind of effect.

Q. *I have a question for all of the panelists from a physician in the audience. With the exception of endotoxins where human controlled exposures have been undertaken, many of the toxins that you are talking about have not to my knowledge been studied in humans in a controlled planned way. How do the individual scientists out there feel about controlled exposures in humans with any one of the agents besides endotoxins?*

A. ***Bruce Jarvis:*** You are right, there are very few. The only one that comes to my mind is the Phase one and Phase two clinical trials of diacetoxyscirpenol which is in fact a mycotoxin, which was used potentially as an anti-cancer treatment. So there is a great deal known about the pharmacokinetics of that compound and as far as I know it parallels reasonably well animal studies. You know as a physician, and I guess other people realize this too, that animal studies are done on a highly homogenous group of animals. A lot of people get upset when we don't get good data from animals, because we don't get a spectrum of animals with different properties like humans have. The reason is that it is extremely expensive to do animal studies but it is a lot cheaper to do them on 12 rats than 120,000 rats. The problem is you can't do this with humans because humans are so diverse, besides the legal problems. (laughter) Known toxins and people—that simply can't be done. So other than a retrospective study in which you have good data, and you know there is exposure, you look backwards to see what the result of that is. I don't know of any such studies as you ask about.

Any other comments?

A. ***Brian Flannigan:*** I was at a meeting last week at which somebody reported work done in Europe in which six individuals who worked in a school where *Stachybotrys* was a problem had volunteered to undergo an inhalation test and inhaled *Stachybotrys atra* from contaminated gyprock and had suffered a variety of different symptoms. To be fair the experimenter did this also and suffered symptoms, but there were no controls in this. It does seem that some of the findings that Bill Sorenson has made on the effects of certain satratoxins on interleukin levels would be borne out by this. But I think medical ethics people might have something to say about experiments like this. (laughter)

Q. *I am on our institutional review board, which is why I am asking these questions. For the* Stachybotrys *controlled exposure just mentioned, were exposures actually measured? Did they have some measure of the actual does?*

A. ***Brian Flannigan:*** No, I don't think so. I think the stuff was put in a large plastic bag and they inhaled. (laughter)

Q. *We are officially against using this on human subjects. Question for Dr. Jarvis, I am neither an immunologist nor a chemist, but I fear neither. (laughter) You spoke briefly of an anti-complement class of mycotoxins. I have read of cytotoxic mycotoxins, I have read of genotoxic mycotoxins, but specific anti-complement, I wish you would comment on that a little more.*

A. ***Bruce Jarvis:*** Well, I think an immunologist ought to speak. The immune system is extremely complex. I am not an immunologist. The complement system has to do with blood and is part of the immune system. When I think of immunology, I usually think of blood immunology, things zipping around in our blood and eating up bacteria, but it turns out that the preponderance of immunology occurs in the mucosal system, which is exactly what we are concerned with here, particles hitting the mucosal system. I have some anecdotal stories that I can relate to people over beer where I think that we have evidence that the exposure to *Stachybotrys atra* causes local suppression of the immune system, allowing opportunistic bacterial infections that present real problems in terms of getting rid of them. Now it turns out that it wasn't a lethal case for the poor students who went through this, but it was a little scary when they did this. But perhaps someone else can answer your question specifically about what anti-complement is.

Q. *Maybe I can put it more in specific terms. Anti-complement, that is part of the innate immune system. When you say anti-complement what comes to mind is something that binds to complement receptors and prevents them from acting the way they normally act with other cells...*

A. ***Bruce Jarvis:*** Yes, it does that with it as far as I know, I don't know the exact mechanism you spoke about.

Q. *So it actually binds with the complement receptors that are found in cells?*

A. ***Bruce Jarvis:*** I can't answer that question. I have never seen a description of the mechanism by which these act.

Q. *Dr. Gareis may entertain this question. I was struck by your discussion on how the substrate may affect the mycotoxin development and would you know of any information that may pertain to artificial man-made substrates like binders and adhesives that may be used in fiber board and insulation.*

A. ***Manfred Gareis:*** This is a general observation everyone who has worked with mycotoxins and fungi has made. If you culture toxigenic fungi over weeks on agar plates the fungi will lose their ability to produce mycotoxins. The mycotoxins are ecologic metabolites; they use these metabolites as "chemical weapons." These "chemical weapons" are not necessary; they are used on competitors. They do not produce the mycotoxins on culture plates because they "cost" too much to produce and there are no competitors. In every natural environment you will find that the fungi begins again to produce mycotoxins. I have no information on the second part of your question.

Panelist: I think perhaps the best one to answer that question is standing before the microphone at this very minute.

David Miller, Agriculture Canada: I wanted to comment on human exposure to mycotoxins because it is not really fair to leave it that we don't know very much about that. (laughter) I feel compelled to do this in interest of the Food and Drug Administration and the Department of Health. It is true that there are hundreds of mycotoxins and this is a point that Bruce and Manfred made clear and this is a point that we are going to have to wrestle with in this paradigm. But actually, there are only five that really count in real life, in the world: aflatoxin, ochratoxin, deoxynivalenol, fumonisin, zearalenone. As a consequence, we know a huge amount about those five, excepting fumonisin, which we only discovered in 1988. But I wanted just to address for Michael that for aflatoxin, because we can measure aflatoxin adducts, as Bill Sorenson mentioned in his presentation, we know a great deal about the exposure outcome of aflatoxin in humans. Another mycotoxin that we can measure in humans and have an immense amount of data on is ochratoxin, so we don't understand as well the exposure outcome, but we know a lot about the exposure in some parts of the world where it occurs. Ochratoxin doesn't occur in North America. Fumonisin, the third very important mycotoxin, we can determine exposure in humans.

There are a couple of points here. One point is that there are mycotoxins for which there are lots of data, but they are only in this group of five for which governments have invested huge amounts of money to acquire. Even though there are five listed, there are only really two that we can actually determine exposure by molecular dosimetry and that is after much expense. So there are hundred of kinds of toxins that might be present in the various and sundry species that occur in building materials. We may know the structure of these compounds and have some rudimentary idea of what their cytotoxicity is, but we don't know very much about their toxicity in general. Even in, repeat, the compounds that we

understand really well, we can only determine human exposure in precise terms in two of them.

You have a comment on the problem of whether there is something magic about the fungus making mycotoxins so another point I want to draw from this slide is that scientists said every one of those toxins on that list could not be made in synthetic media in the lab. But in fact that has been universally proven to be wrong. The main point is that in nature, in every case where the fungi that produce those toxins occur, they are able to produce toxin with alacrity. So I think that the issue is that if it can grow it is going to make the toxins.

Q. *You were talking about a stimulation of the macrophage. Are any other cells involved, especially the lymphocyte? Have any other studies been done on cytokines or interleukin, especially interleukin-6, tumor necrosis factor?*

A. ***Bill Sorenson:*** I think perhaps we have less information for lymphocytes and these other cells. I remember seeing some reports in which they were able to demonstrate stimulation. Our work has been with macrophages and that is what I'm concentrating on, so I can't talk about lymphocytes.

Q. *Is this by tissue culture, your macrophages?*

A. It was done in vitro, but I think there was some studies in the literature with beta glucan in vivo, after exposure they took cells out and demonstrated enhanced activities.

Pierre Auger: Is Dr. Rylander in the audience? Somebody has a written question for you here.

Q. *Please describe the human experiments with the four hour exposure to beta 1, 3, glucan.*

A. ***Ragnar Rylander:*** This refers to one of the previous comments here, what can you do in the human exposures? We are limited; we can only study physiological changes. We can only do exposures one, maybe two times, not more than that. But I think one has to realize that most of the things we are interested in, including carcinogenicity, requires a very long exposure period. As a general tendency we should try much more to go over to the long term exposure in understanding what happens after a longer time. So having pulled the rug out from under inhalation experiments I'll describe how we do them. We use a chamber, a very simple chamber where people sit and we have controlled ventilation from ducts and there is an aerosol of endotoxin glucan coming into that chamber. This is for four hours and we do various types of measurements, function measurements and several other measurements.

There are two other points I would like to come back to which were raised during the discussions. Someone made the comment concerning antioxidant and there is now tremendous information showing that consumption of vegetables and fruit protect against all kinds of pulmonary disease, not only lung cancer, but also chronic bronchitis and changes in respiratory function. I think that is

very solid. The interesting thing that is coming out is that other things are deleterious, like fat intake. With all these studies coming out at some point we will probably have to take diet into account because it has such a very strong effect.

STUDIES ON FUNGI IN AIR-CONDITIONED BUILDINGS IN A HUMID CLIMATE

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Abstract: *Fungi can grow in air-conditioned buildings in Florida for a variety of reasons including water disasters and defects in building design, operation, and maintenance. The objectives of this study are to describe (1) fungal contamination in buildings including both those with and without building-related allergic respiratory disease, (2) methods used in fungal sampling, and (3) guidelines for removal of visible mold contamination. Reasons for fungal problems in air-conditioned Florida buildings include chronic flooding, condensation, dirty wet materials in ventilation systems, elevated relative humidity and use of wet building materials in construction. A combination of bulk and air sampling works best to document fungal contamination in most buildings. Using the principles of asbestos abatement technology, visible mold can be removed from buildings so that spores and hyphae are not disseminated into occupied or cleaned areas.*

Key words: air-conditioned buildings, *Aspergillus*, Florida, fungi, hypersensitivity pneumonitis, moisture problems, molds, remediation of mold contamination, sampling methods for fungi, *Stachybotrys*

FUNGAL PROBLEMS IN FLORIDA BUILDINGS

Warm, humid, environmental conditions, such as those that exist in central and south Florida, are conducive to fungal growth in air-conditioned buildings with design, operation, and maintenance problems. Analytical results of sampling for fungi are presented for a moldy building with an outbreak of building-related hypersensitivity pneumonitis and asthma (Table I), for buildings with visible fungal contamination, but without any formal epidemiological or medical surveillance program (Table II), and for a building that was initially thought to be free of mold contamination (Table III). The primary

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objectives of sampling in these buildings were to determine if indoor mycoflora were normal and typical of those commonly found outdoors and in the indoor air of buildings without mold problems and to provide background data for occupational physicians investigating occupant health complaints that might be building-related.

Table I. Air Sampling Where Extensive Visible Fungi Were Present in Occupied Spaces and Where Building-Related Allergic Respiratory Disease Occurred Among Several Occupants.[a]

Type of Sample	Number of Samples	Average cfu/m³ (range)	Taxa
Indoor air, quiescent	15	250 (30-970)	*Aspergillus sydowi* group dominated 11 of 15 samples; *Cladosporium* dominated 4 of 15 samples; *Aspergillus versicolor* group present in 7 of 15 samples
Outdoor air	6	5,800 (530 to 22,000)	*Cladosporium* dominates 5 of 6 samples; nonsporulating fungi dominate one sample; minor isolates include *Penicillium*, yeasts, *Aureobasidium*, *Periconia*, and unidentified *Aspergillus* species.

[a]Air samples were collected using a single stage volumetric culture plate (malt extract agar) impactor operating at a flowrate of approximately 0.18 m³ per minute (sampling times 20 seconds to 2 minutes). Visible fungi present on gypsum board of perimeter walls. *Aspergillus versicolor* is dominate isolate from gypsum board.

Table I presents analytical results of air sampling for fungi in a new building where epidemiological data suggested that several occupants had building-related hypersensitivity pneumonitis and asthma. The building was characterized by constructional and design defects including a leaking roof, inadequate window flushing that led to chronic flooding, and a HVAC system with oversized air handling units that resulted in an inability to remove latent heat (moisture) from ventilation air. Extensive visible mold contamination was observed on and behind (on gypsum board) vinyl wall covering found on perimeter walls. The vinyl wall covering had acted as a vapor diffusion retarder and a locus of condensation because of overall negative pressurization in the building and an absence of a vapor diffusion retarder on external surfaces of perimeter walls.

Air sampling performed quiescently in occupant areas (Table I) showed that *Aspergillus sydowi* dominated 11 of 15 samples. This *Aspergillus* species and *Aspergillus versicolor* present in indoor air samples were not detected in outdoor

air control samples. Even though the average concentration of fungi indoors (250 cfu/m^3) was at least an order of magnitude lower than that outdoors (5,800 cfu/m^3) the dominating presence of *Aspergillus* species indoors was a clear indication that indoor mycoflora were atypical and that indoor fungal amplifiers and reservoirs were present. *Aspergillus versicolor* and *sydowi* were the dominating fungal contaminants subsequently found on vinyl wall coverings and gypsum board of the building's moldy perimeter walls (Table IV).

Table II. Sampling in Buildings Where Extensive Visible Fungal Contamination Occurs but Where There is an Absence of Medical Opinion on Presence of Allergic Respiratory Disease.

Building and Kind of Sample	Analytical Results
10 bulk insulation samples from air handling unit where at least 1 m^2 of porous insulation in cooling coil or fan plenum is covered with visible fungi	Hyphae[a] entwined among manmade insulation fibers in all samples. Concentration[b] of viable fungi per cm^2 equals 5×10^3 to 5×10^5 cfu/cm^2 on malt extract agar. Dominant taxa are *Fusarium* (3 of 10), *Cladosporium* (3 of 10), *Acremonium* (1 of 10), *Sterigmatomyces* (1 of 10), *Ulocladium* (1 of 10), *Tritirachium* (1 of 10)
Indoor air, quiescent, 20 samples	Concentration[c] of fungi ranged from 5 to 90 cfu/m^3 (average=40 cfu/m^3). Dominant taxa are *Penicillium* 11 of 20; *Verticillium* 3 of 20; 1 of 20 for *Cladosporium, Rhizopus, Acremonium;* nonsporulating 3 of 20
Outdoor air, 3 samples	Concentration[c] of fungi ranges from 100 to 400 cfu/m^3 (average=200 cfu/m^3) *Cladosporium* dominates all samples

[a]Direct microscope observation.
[b]Serial dilution, malt extract agar.
[c]Air samples collected in manner as in Table I.

The analytical results presented in Table II were obtained from Florida buildings where visible mold was present in HVAC system components and/or in occupied spaces but where there was an absence of epidemiological or medical evaluation to detect the presence of building-related allergic respiratory disease.

Samples were cut out of porous insulation in cooling coil and fan plenums of air handling units in one building where musty odors were present in occupied spaces served by the same units (Table II). Direct microscope observation of the airstream surface of all insulation samples indicated that hyphae were entwined among manmade insulation fibers. Culture studies showed a diversity of fungi growing on insulation including *Fusarium, Cladosporium,*

Acremonium, Sterigmatomyces, Ulocladium, and *Tritirachium.* The recommendation was made to remove insulation lining cooling coil and fan plenums so as to eliminate the possibility of dissemination of biocontaminants into occupied spaces.

The air sampling data in Table II was obtained in another building characterized by occupant complaints of allergies, sinus and nasal congestion, eye irritation, and elevated relative humidity. Moisture incursion had occurred subsequent to new construction and fungi were visible on some vinyl wall covering and gypsum board surfaces. Analytical results showed that only 1 of 20 indoor samples was dominated by *Cladosporium* while all the outdoor samples were dominated by this fungus. The dominant presence of *Penicillium* and *Verticillium* indoors was considered atypical. The recommendation was made to remove moldy interior finishes under containment.

Table III. Air Sampling in Control Building with Absence of Visible Fungi in Occupied Space.

Type of Sample	Number of Samples	Average cfu/m^3	Taxa
Indoor air, quiescent[a]	1	600	*Stachybotrys* (99%)
Indoor air, quiescent[b]	8	40 (range 20 to 80)	*Cladosporium* dominates 3 of 8; *Penicillium* dominates 2 of 8; *Aureobasidium* dominates 1 of 8 ; 1 sample co-dominated by *Aspergillus* and *Stachybotrys;* 1 sample contains non-sporulating fungi
Outoor air[b]	5	300 (range 80 to 650)	*Cladosporium* dominates 3 of 5; *Penicillium* dominates 2 of 5

[a]Collected at same time as samples in Table I.
[b]Collected 2 months later.

As a control for air samples collected in the building in Table I, one air sample was obtained in another building in which there was, according to the operator, an absence of both visible mold and occupant health complaints. *Stachybotrys* at a concentration of almost 600 cfu/m^3 was found in the control building (Table III). During a subsequent evaluation of the control building, *Stachybotrys* was found in only one of eight air samples. Discussion with occupants of the building subsequently revealed that renovation had occurred several years in the past at a time when occupied spaces were not ventilated and relative humidity indoors approached conditions similar to those in outdoor Florida air. Presumably the *Stachybotrys* in Table III resulted from residual spores from earlier mold growth in occupied spaces. Table III shows that it is difficult to choose a suitable control building without a thorough knowledge of the moisture history of the building.

Reasons for fungal problems in air-conditioned Florida buildings include the following:

- Chronic flooding or leaks in indoor environments.
- Condensation on cold surfaces on the occupied side of the walls subject to infiltration and diffusion of water vapor.
- Dirty, wet porous materials on the air stream surfaces of air handling equipment.
- Relative humidity in occupied spaces consistently (for weeks or months) in the 70 to 75% range.
- Use of wet construction and finishing materials without adequate provision for dehumidification.

SAMPLING METHODS FOR FUNGI

Methods of sampling for fungi are highly varied and are limited only by the objectives and imagination of the investigator. All examples illustrated in Tables IV and V are based on culture or direct microscope observation methods and are taken from case studies in Florida buildings.

The choice of sampling methodology for fungi should primarily be determined by the objectives of the microbial evaluation. Once the objective of the evaluation is clear, technical strategy can be determined, such as use of bulk and/or air samples, choice of culture media (for example, for xerophilic or hydrophilic fungi), number of repetitions required, and directions to be given to the laboratory processing the samples. Some analytical results with brief interpretations are presented for various bulk (Table IV) and air (Table V) sampling methods described.

Table IV. Bulk/Surface Samples for Fungi in Florida Buildings.

Building and Description of Sample	Analytical Results
VINYL WALL COVERING[a]	
Perimeter walls, both discolored and non-discolored	14 samples; average concentration of fungi was 1.7×10^6 cfu/cm^2; *Aspergillus versicolor* dominates 9 of 14; *Aspergillus sydowi* dominates 4 of 14; *Scopulariopsis* dominates 1 sample
Interior walls, none discolored	Fungi detected in 1 of 3 samples at concentration of 40 cfu/cm^2; fungi not detected in other samples (LOD was 40 cfu/cm^2)

Building and Description of Sample	Analytical Results
CEILING TILE[a]	
Lower surface of moldy tile, location #1	1×10^7 cfu/cm^2 *Stachybotrys* 1×10^5 cfu/cm^2 *Penicillium*
Upper surface of moldy tile, location #2	6×10^6 cfu/cm^2 *Stachybotrys* 2×10^8 cfu/cm^2 *Pseudomonas fluorescens* 2×10^8 cfu/cm^2 other Gram-negative bacteria
DUST COLLECTED BY MINIVACUUM[b]	
Settled dust, upper surface of undamaged tile 5 m from moldy tile at location #1	*Stachybotrys* was dominant morphological spore type by direct microscope observation of dust.
Dust in carpet located beneath moldy tile at location #2	Total fungal concentration was 1.9×10^5 cfu/g; (*Stachybotrys*=83%, *Aspergillus versicolor*=10%, non-sporulating fungi =5%, *Penicillium*=2%)
Settled dust, upper surface of undamaged tile, at least 10 m from perimeter wall and any visible evidence of water damage	2.5×10^6 cfu/g; (*Aspergillus versicolor* =95%, *Penicillium*=5%)
DUST COLLECTED BY STICKY TAPE[c]	
Dust in fissure of stone veneer, location #1	1.1×10^3 spores/cm^2, *Penicillium-Aspergillus* dominates by direct microscope observation
Dust in fissure of stone veneer, location #2	1.5×10^3 spores/cm^2, *Cladosporium* dominates by direct microscope observation
DUST COLLECTED BY SWAB[d]	
Supply vent of unit ventilator, dirtiest location visually	17,700 cfu/cm^2; 95% *Cladosporium*, 5% *Penicillium*
Supply vent of unit ventilator, cleanest location visually	5 cfu/cm^3 (all *Penicillium*)

[a]Samples removed with disinfected knife; homogenized in sterile water; serially diluted and plated on malt extract agar.

[b]Dust collected in a closed-face three piece filter cassette (minivacuum) with polycarbonate filter, 0.8 micrometer pore size, with inlet orifice open. Dust on preweighed filter was mixed with sterile water, serially diluted, and plated onto malt extract agar.

[c]Dust collected by clear sticky tape pressed onto surfaces. Observations made by direct microscope examination at a magnification of approximately 1,000 times.

[d]Dust collected by wet (sterile water) swabs stroked in one direction over 1 x 5 cm surface outlined by sterile template. Up to four (4) swabs used to remove all dust. Dust from swabs extracted in sterile water. Serially diluted, and plated on DG-18 medium. LOD was 5 cfu/cm^2.

Pieces (approximately 10 cm^2) of vinyl wall covering were cut out of discolored (moldy) and visually non-moldy perimeter walls at various locations in a building and compared for fungal content with wall coverings from interior walls. The objective of sampling (Table IV) was to provide guidance on which walls should be demolished during bioremediation. The analytical results showed that the concentration of fungi in vinyl wallcovering of perimeter walls was approximately 1.7×10^6 cfu/cm^2. *Aspergillus* species dominated 13 of 14 perimeter wall samples. Only 1 colony of *Fusarium* (limit of detection was 40 cfu/cm^2) was detected in the three (non-discolored; control) interior wall samples. These analytical results showed that growth sites of *Aspergillus* were generally present on surfaces of perimeter but not interior walls. This led to the recommendation to demolish only perimeter walls.

In another building, pieces of moldy ceiling tiles and dusts from surfaces of non-moldy ceiling tiles and carpet were collected to determine if fungal spores had disseminated into porous interior finishes. Analytical results showed that *Stachybotrys* dominated the fungi in moldy ceiling tiles at two locations (Table IV). Dusts collected from the carpet beneath one moldy ceiling tile and from the upper surface of an undamaged tile 5 m away from the second moldy tile were also dominated by *Stachybotrys*. In addition, settled dust from the upper surface of a ceiling tile in an entirely different area of the building contained 1×10^6 cfu/g of *Aspergillus versicolor*. These analytical results showed that spores from fungal growth sites had been widely disseminated into porous finishes throughout the building. Because medical opinion indicated that several occupants had developed hypersensitivity pneumonitis, the recommendation was made to remove all porous finishes throughout the building including areas from which visible fungal contamination was absent.

Dust present on stone veneer in a corridor of a building that had been affected by considerable flooding was collected by application of clear sticky tape into fissures in the stone. The objective was to determine if underlying gypsum board (stone attached to gypsum board on metal studs) was contaminated by *Aspergillus*. Result presented in Table IV showed that while *Penicillium-Aspergillus* spores (direct microscope observation) were present at location #1, *Cladosporium* dominated the spores present at location #2. Subsequent analysis showed that the gypsum board behind the stone veneer was heavily contaminated by *Aspergillus versicolor*. Thus, the sticky tape sample collected at location #2 yielded a false negative result, a possibility that should be considered in interpretation of any set of analytical results.

Swab (surface) sampling as illustrated in the last example in Table IV can yield quantitative analytical results. In the example in Table IV, the fungal content on the visually dirtiest surface of a unit ventilator supply air vent in a hotel guest room was compared to the cleanest surface on the same supply air vent. *Cladosporium* dominated the fungi found on the dirtiest (concentration 17,700 cfu/cm^2; 95% *Cladosporium*) portion of the air supply vent and only a few

fungi (concentration 5 cfu/cm^2; Penicillium) were found on the cleanest surface. This orders of magnitude variability between the dirtiest and cleanest areas on surfaces of one supply vent indicates that many samples would need to be collected to assess the overall mycological status of supply air vents in guest rooms in the hotel.

Table V. Air Sampling in Florida Buildings.

Type of Sample	Number of Samples	Average cfu/m^3	Taxa
QUIESCENT SAMPLING[a]			
Air sampling indoors yields false negative data, June	17	85 (range 30 to 250)	*Cladosporium* dominates 14 of 17; *Penicillium* dominates 2 of 17; *Curvularia* dominates 1 of 17; *Aspergillus versicolor* absent from all samples
Air sampling indoors detects source, October	27	80 (range 15 to 250)	*Cladosporium* dominates 12 of 27;*Penicillium* dominates 9 of 27 ; *Aspergillus versicolor* dominates 5 of 27; one sample dominated by non-sporulating fungi.
SEMI-AGGRESSIVE OR STIR-UP-THE-DUST SAMPLING[b]			
Air sampling in vacated building	10	1,900	*Aspergillus versicolor* dominates 6 of 10 samples; other *Aspergillus* species dominate rest of samples
AGGRESSIVE SAMPLING[c]			
Air sampling in vacated building	7	>3 x 10^5	*Aspergillus versicolor* and *Penicillium* at each impaction site

[a]Air samples collected using single stage volumetric culture plate (malt extract agar) impactor operating at a flowrate of approximately 0.18 m^3 per minute (sampling times 1 to 2 minutes). Fungi (mainly *Aspergillus versicolor*) visible on perimeter walls (vinyl wall covering/gypsum board).

[b]Same as "a" except malt extract agar with 20% sucrose used (sampling time 1 minute). Small desktop fan used to simulate air currents that would be brought about by normal occupant activities.

[c]Cascade impactor (400 jets per plate) operating at a flowrate of 0.027 m^3 per minute used. Sampling times approximately 15 seconds. Vinyl wall covering (10 x 10 cm) pulled off moldy gypsum board during sampling.

Table V presents the analytical results of quiescent, semi-aggressive, and aggressive air sampling performed in Florida buildings in which visible mold was evident indoors as a result of chronic water damage. During quiescent sam-

pling, air is collected under normal operating conditions, that is, without any abnormal disturbance of HVAC system components or objects in the occupied spaces. In one of the two examples of quiescent sampling in Table V, *Aspergillus versicolor* was totally absent from all 17 air samples even though visible mold (*Aspergillus versicolor*) was present on vinyl wall covering of perimeter walls throughout the building. This is a clear example of false negative analytical results. The second quiescent data set (27 air samples, Table V) showed that there was a source of *Aspergillus versicolor* in the building, a conclusion which was self evident because of the presence of this mold on vinyl wall covering of perimeter walls (see Table IV).

Semi-aggressive or stir-up-the-dust air sampling is performed coincident with some activity such as operation of a small office desk fan to aerosolize settled dust that might be present on interior surfaces. The analytical results in Table V showed that *Aspergillus*, especially *Aspergillus versicolor* dominated all semi-aggressive air samples. This data set indicated that interior surfaces in the building contained fungal reservoirs that had likely disseminated from growth sites on perimeter walls.

Aggressive air sampling occurs when a building component is mechanically disturbed or pounded coincident with sample collection. In the example in Table V, a small piece (10 x 10 cm) of moldy vinyl wall covering on a perimeter wall was peeled off its underlying gypsum board. *Aspergillus* and *Penicillium* colonies were present in room air 3 m away from the wall at concentrations exceeding 3×10^5 cfu/m^3. This kind of sampling shows that even a small disturbance of the moldy wall results in massive emission of spores into occupied spaces.

Quiescent air sampling is useful in investigating fungal problems in buildings provided proper precautions are taken to reduce the possibility of false negative results. Collection of semi-aggressive or aggressive air samples subsequent to quiescent sampling can provide adequate controls to reduce the possibility of false negative results. Collection of aggressive air samples must always be performed with care to exclude possible hazardous exposure of both occupants (occupants should be absent) and the sample collector (use personal protective equipment including a respirator with a HEPA filter; see Morey, 1992).

GUIDELINES AND SPECIFICATIONS FOR FUNGAL REMEDIATION

The presence of visible fungal growth in buildings including their HVAC systems should not be tolerated. Extensive visible growth for one kind of fungus, *Stachybotrys atra*, is defined as more than 30 square feet of surface area (NYC, 1994), and this mold should **immediately** be removed under full containment. Additional toxigenic fungi including some *Aspergillus* and *Fusarium* species (*Aspergillus versicolor* and *flavus; Fusarium moniliforme*) should probably also be included in the same category as *Stachybotrys*. For other kinds of fungi that may dominate moldy surfaces in occupied spaces, extensive is still arbitrarily defined as more than 30 square feet because all molds can cause allergy and most molds can, at some time in their life cycle, produce toxins (see Table VI).

Visible fungal growth is defined as mold or mildew covering the surface or the presence of hyphae or a mycelium as seen by direct microscopic examination of the surface.

Table VI. Guidelines for Removal of Toxigenic[a] Fungi Visible[b] in Occupied Spaces.

Extent of Visible Contamination[c]	Type of Containment
<2 ft² in occupied space	Remove with minimum dispersal of dust and spores; place contaminated material in plasic bag during removal; local use of disinfectant
2 to 30 ft² in occupied space	Local containment required; use HEPA vacuum cleaner to contain dusts and spores
>30 ft² in occupied space	Full containment required; negative pressurization required to contain dust and spores; use personnel trained in handling of hazardous materials

[a]Toxigenic fungi, including following taxa: *Stachybotrys atra, Aspergillus flavus, Aspergillus versicolor, Fusarium moniliforme,* etc.

[b]Visible contamination means that mold is visible on surfaces in occupied space. The presence of hyphae and mycelium growing on or in materials as seen by direct microscope observation is verification of visible contamination.

[c]Visible toxigenic fungi, such as those in "a", must be removed **immediately** from a building. All other visible fungi should then be removed.

Table VI summarizes remedial actions recommended (NYC, 1994) to remove visible *Stachybotrys atra* that may contaminate surface areas from less than 2 to more than 30 square feet in occupied spaces. For surface areas up to the approximate size of one gypsum wall panel (30 square feet) local containment actions performed by building maintenance personnel with a proper respiratory program are sufficient to remove moldy materials and prevent dissemination of spores. When molds contaminate surface areas larger than 30 square feet, a large-scale remediation with full containment similar to that used during asbestos abatement is recommended.

The growth of mold in HVAC system components is potentially more serious than that in occupied spaces because of the possibility of direct transmission of spores to occupant breathing zones through ventilation air and because the contamination is hidden from view due to the limited accessibility inherent within HVAC system components.

When non-porous (for example, sheetmetal) surfaces in HVAC systems are contaminated with mold growth, the general procedures in Table VI (depending on the extent of contamination) provide a basis for biological remediation guidelines. If disinfectants are used, care must be taken to prevent dissemina-

tion of chemicals into occupied spaces (Morey, et al., 1984). Removal of the mold is required even if disinfectants are used because potential allergic or toxic responses are not related to viability.

When porous materials in HVAC system components are contaminated with mold growth, source removal to bare (underlying) metal or replacement of the HVAC component is required regardless of the extent of contamination. For small HVAC system components, such as fan coil units, induction units, and unit ventilators, local containment and use of a HEPA vacuum cleaner is probably adequate when contaminated porous materials are removed. Full containment is necessary when contaminated porous materials are removed from larger HVAC system components such as air handling unit plenums (Table II).

As a general principle, spores, hyphae, dusts, components of porous materials, and disinfectant/biocides should not be disseminated into occupied spaces during remediation of mold growth in HVAC systems.

SPECIFICATIONS FOR LARGE SCALE FUNGAL REMEDIATION

The following general principles have been used in specifications for large scale remediation of mold in contaminated Florida buildings.

Determine The Containment Strategy. The strategy to be used in construction of critical containment barriers must be identified early in the project. Maximum isolation (2 layers of polyethylene) or Level 1 containment is required for areas with visible mold growth. A lesser degree of isolation (one layer polyethylene) or Level 2 containment may be required for areas where visible mold growth is absent but where fungal spore reservoirs may occur because of dispersal from moldy areas in other parts of the building (see Table IV). The extent and severity of remediation is highly influenced by medical considerations such as potential reoccupancy by individuals sensitized during prior occupancies or, in medical centers, by the presence of nearby patients with highly suppressed immune systems.

Isolation Of Areas To Be Remediated. Isolate the Level 1 or 2 work areas prior to removal of contaminated finishes or construction materials. Isolation means that the work area is sealed from all occupied or cleaned areas in the building by blocking off all openings, fixtures, and HVAC system components in the work area so that spores cannot be carried on air currents into areas external to the work area. Care must be taken during construction of critical barriers (2 layers polyethylene for Level 1; one layer for Level 2) not to disturb moldy materials or dust reservoirs in order to prevent the aerosolization of fungal spores.

Personal Protective Equipment. A full respiratory protection program is required. Ideally, respiratory protection should consist of a full-faced powered air purifying respirator with HEPA filters. Respirator filters should be changed daily to preclude the possibility of germination of fungal spores on moist filters. Protective disposable clothing consisting of full-body coveralls, head covers,

gloves, and boot type covers should be worn in work areas. Skin protection is essential to prevent contact with mycotoxins that may be present on fungal-contaminated materials. Workers performing fungal remediation should be warned of the special risks of fungal aerosols for people with immunodeficiency disease, cancer, disorders of immune regulation, or allergic or hypersensitivity disease including atopic conditions.

Negative Pressure In Work Area. A negative air pressure differential should be established inside the enclosed work area relative to interior areas outside the containments before remedial operations begin. An air filtration device (AFD) with HEPA filters as part of the exhaust ventilation system is used to maintain the air pressure differential. Ideally, the exhaust ventilation system should be capable of maintaining a minimum differential pressure of 0.02 inches of water gauge and a minimum of four air changes per hour in the work area. The guiding principle for use of AFDs in establishing air pressure differentials is to prevent spores and hyphal fragments from fungal growth sites or reservoirs from entering occupied spaces or cleaned areas in the building.

Decontamination Unit System. A decontamination system consisting of three chambers (clean room, equipment room, and airlock) should be used for entry into and exit from Level 1 work areas. A one-chamber air lock is adequate for entry and exit from Level 2 work areas. Bags or wrappings containing moldy materials should be passed through the decontamination unit where they are thoroughly cleaned with a HEPA vacuum before transport to interior areas of the building outside of the work area containment. Bags containing moldy materials should be removed from the building and disposed in a landfill as if the contents were moldy compost. Care must, however, be taken during removal and disposal to make sure that workers handling the bags are not exposed to fungal particulate as might occur if bags rupture.

Removal Of Debris And Dusts Contaminated By Fungi. All contaminated materials should be double bagged in 6-mil polyethylene for removal from Level 1 work areas (single bag for Level 2 areas). Finishes visually contaminated by mold should be taken down or cut out intact or in large pieces so as to reduce the amount of spores released during demolition. Use HEPA vacuums and damp wiping to remove settled microbial particulate in both the work area and decontamination unit prior to removal of containment barriers.

In order to reduce the potential for new fungal growth, only limited use of water for cleanup and containment of dust is allowed. This is especially important in Florida where soaked interior surfaces and elevated relative humidity (>70%) will almost certainly lead to new fungal growth (Block, 1953). Disinfectants such as hypochlorite and chlorine dioxide may be applied locally to nonporous surfaces such as concrete (for example, where mold is attached to residual carpet adhesive) providing that adjacent porous surfaces are not wetted. Workers applying disinfectants must be provided with proper respiratory protective equipment necessary to prevent inhalation of aerosolized disinfectant.

Compliance Air Sampling By Spore Trap And Sticky Tape. During removal of debris and dusts from contained work areas, air sampling by spore trap is per-

formed in adjacent occupied or clean areas. Table VII shows analytical results of samples collected in work areas during microbial remediation and in adjacent occupied areas. *Penicillium-Aspergillus* or *Stachybotrys* spores dominated air samples collected in Level 1 or Level 2 work areas during demolition, whereas air samples collected in adjacent occupied spaces were dominated by phylloplane (for example, *Cladosporium*) fungi. Demolition work activities would be halted if air sampling revealed that *Penicillium-Aspergillus* spores were escaping into occupied spaces.

Air sampling for spores is performed in Level 1 and 2 work areas after visual inspection has determined that all debris and settled dust has been removed by HEPA vacuum cleaning and damp wiping. Table VII presents sampling data in a cleaned Level 1 work area prior to removal of the final containment barrier. The total concentration of spores in the cleaned Level 1 work area was only 550/m^3 compared with 11,000/m^3 in the outdoor air. Since *Penicillium-Aspergillus* dominated the kinds of spores in both samples, and the total concentration of spores in the cleaned work area was low, the work of the contractor performing the cleanup would be considered acceptable. However, if sticky tape samples also collected in the same work area had demonstrated the presence of residual fungal contamination (for example, 1×10^3 *Stachybotrys* spores per cm^2) on "cleaned" interior construction materials such as metal studs or concrete, the work of the contractor would be considered unacceptable and the contained work area would be revacuumed or damp wiped.

Table VII. Airborne Fungal Spores During and After Microbial Remediation.

Type of Sample	Spores/m^3[a]	Taxa
SAMPLE DURING DEMOLITION		
Level 1, work area	1.8×10^7	>99% *PEN-ASP*[b]
Level 1, work area	1.2×10^6	95% *PEN-ASP* 3% *Stachybotrys*
Level 2, work area	7.2×10^4	33% *PEN-ASP* 33% *Stachybotrys*
SAMPLE IN OCCUPIED ROOM		
Quiescent, sample #1	1.9×10^3	31% *Cladosporium* 8% *PEN-ASP*
Quiescent, sample #2	7×10^2	40% *Cladosporium* 20% *Aureobasidium* 20% Basidiospores
SAMPLE BEFORE CONTAINMENT BARRIER REMOVED		
Cleaned, Level 1, work area	5.5×10^2	45% *PEN-ASP* 9% *Cladosporium*
Outdoor air, control	1×10^4	53% *PEN-ASP* 29% Basidiospores 6% *Cladosporium*

Type of Sample	Spores/m^{3a}	Taxa
SAMPLES FROM ONE BUILDING AFTER CONTAINMENT BARRIERS REMOVED		
Former work areas, N=60	15[c]	*PEN-ASP*
Indoor control areas, N=50	10[c]	*PEN-ASP*
Outdoor air controls, N=50	5[c]	*PEN-ASP*

[a]Fungal spores collected on greased glass slide in spore trap operating at a flowrate of approximately 0.01 m^3/min. Sampling times 2 seconds (demolition samples) to 12 minutes (some outdoor air samples). Taxa identified by direct microscope observation at a magification of approximately 1,000 times.
[b]*Penicillium-Aspergillus*.
[c]Average percentage of *Penicillium-Aspergillus* spores in samples. *Aspergillus versicolor* was dominant mold removed during bioremediation.

Table VII shows air sampling data from cleaned former work areas after all containment barriers were removed. *Aspergillus versicolor* was the dominant mold that had been present before remediation. *Penicillium-Aspergillus* spores accounted for 15% of the spores collected in cleaned former work areas but only 10% of the spores in indoor control areas and 5% of the spores in outdoor air. These analytical results suggest that a small amount of residual spore contamination exists in formerly moldy zones subsequent to biological remediation.

Experience in mold contaminated Florida buildings, shows that biological remediation is effective in removing the vast majority of existing fungal contamination. Assuming that the building in the future is kept dry, the indoor mycoflora should mirror that present in the outdoor air.

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INDUSTRIAL HYGIENE SURVEY: SEWAGE TREATMENT COMPOSTING FACILITY

EDWARD OLMSTED, M.Sc., CIH, CSP

Abstract: *An industrial hygiene investigation was made at the above referenced facility on June 30 and July 15, 1994 by Eckardt Johanning, M.D. and Edward Olmsted, CIH, CSP. The survey was conducted in order to investigate worker exposure to bioaerosols, endotoxin, airborne particulate material, and ammonia. A medical evaluation of one of the plant operators revealed serious respiratory illness.*

Key words: *Aspergillus-fumigatus*, asthma, respiratory-hypersensitivity, compost, bioaerosols, rhinitis, *Stachybotrys atra*

BACKGROUND

The composting facility is located in a municipality in upstate New York at a sewage waste water treatment plant. The solids that settle out of the waste water treatment process are composted for purposes of disposal of the material as non-pathogenic compost material. The composting process is as follows:

(1) The facility is used to treat sewage that is produced in the town. This consists of primarily residential and not industrial waste water. During the treatment process waste water is held in settling tanks and solids settle out. These solids are pumped to the composting building.
(2) The solids are received in a large tank in the de-watering area. A flocculent is added to the sludge to help separate water from the material. The flocculent is piped in as aqueous solution from a tank located in the de-watering area.
(3) The sludge is moved from the tank along a conveyor belt through two rollers squeezing out the water and producing a cake approximately 1 inch in thickness.
(4) The solids are conveyed to a hopper approximately 10 feet above the floor where they are discharged into a mixing truck. 3,200 pounds of sludge are dumped into the truck.

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(5) The truck is driven to the outdoor storage shed where 1,700 pounds of shredded yard waste is dumped into the bed using a front end loader. 750 pounds of finished compost material from a previous run is also dumped into the truck.
(6) The bed of the truck has two augers which are used to mix the sewage and compost. The mixing is done in the composting building and is conducted for approximately 2 minutes.
(7) The mixed material is discharged onto the floor of the composting building. A Bobcat front end loader is used to move the mixed material into one of six bays (windrows) which are 150 feet long and approximately 10 feet wide. Fans blow air up from slots in the floor through the composting sludge. The sludge must be maintained at a temperature of 55 degrees Centigrade. Thermocouples continuously monitor the temperature.
(8) The sludge is composted for 21 days. Each day an agitator mixes the sludge and moves it down the bay approximately ten feet. The agitator moves along rails above the compost.
(9) Finished compost is moved to a pile outside the building.

The process requires approximately 45 minutes from loading the truck to putting the mixed material into the bay. A typical day involves the processing of five loads of sludge. The work is done by two workers. Personal protective equipment worn by the workers was limited to a paper disposable respirator and hard hats. Workers did not wear gloves, or other PPE.

The building ventilation consists of five exhaust fans which pull air through hoods located at the roof of the composting winnows. The air is discharged outdoors under the soil surface. There have been problems with these fan units and during this survey only three of the fan units were operating.

This assessment was limited to the monitoring of bioaerosol levels including fungi and bacteria, ammonia levels, respirable dust and total suspended dust. Airborne dust samples were also analyzed for the mycotoxin trichothecenes and endotoxin. An assessment of machine guarding or other safety issues was not included in this survey nor was an assessment of noise levels.

METHODS

Sampling for total dust and respirable dust was conducted using personal sampling pumps attached to the workers belt with the sample collected in the workers breathing zone. Respirable dust was sampled using a 20 mm nylon MSA cyclone with a flow rate of 1.7 liters per minute (LPM). Samples were collected on 5 micron PVC filters preweighed and analyzed by an American Industrial Hygiene Association (AIHA) accredited lab. Particulate samples were also collected on 0.2 micron polycarbonate filters at a flow rotate of 10 liters per minute. These samples were taken as area samples using BGI high volume sampling pumps. All samplers were calibrated using rotameters calibrated Buck calibrator bubble meter.

Sampling for bioaerosols was conducted using an Anderson N6 single stage sampler operated at a flow rate of 28.3 LPM. The sampler was calibrated using an in line rotameter calibrated against a Gilibrator bubble meter. Samples in each location were collected on 2% malt extract agar (MEA) and cornmeal agar (CMA) for total fungi. Sampling for bacteria was conducted using tryptose soy agar (TSA). All Petri dishes were prepared and analyzed by P&K Microbiology Services of Cherry Hill, NJ.

Ammonia sampling was conducted using a Draeger bellows pump and ammonia length of stain tubes.

Endotoxin exposure was measured using the total dust filter samples described above. After gravimetric analyses the filters were shipped to the Associates of Cape Cod, Inc. Samples were analyzed using the Limulus Amebocyte Lysate test using the gel-clot method.

RESULTS

The air sampling results for the microbiological organisms are summarized below:

Fungi

Airborne levels of mold and bacteria are reported in colony forming units per cubic meter of air (CFU/M^3). The levels can be used as a general basis of comparison between different areas. Levels of fungi inside the composting facility were similar to those levels measured outside the building. But, the species of mold indoors included *Stachybotrys atra* at the finish end of the windrows. The *S. atra* was only evident in airborne samples after agitating the compost. Because the outdoor sample was taken near the shredded yard waste facility, measured outdoor fungi levels were moderately elevated. The types of fungi species are generally common outdoor molds. Of particular interest is the presence of the opportunistic fungi *Aspergillus fumigatus*. This is a mycotoxin producing species that is frequently associated with wet cellulose materials such as shredded yard waste. Elevated fungi levels relative to the outdoors are evident in the finish end of the compost bays both before and after agitating. The mycotoxin producing species *Stachybotrys atra* is prevalent in the air after agitating. This species produces a class of compounds called trichothecenes, which have been shown to cause significant health effects in exposed persons. Other species of fungi found on both outdoor and indoor samples include *penicillium, cladosporium, basidiomycetes and alternaria.*

Bacteria

Levels of airborne bacteria were significantly elevated inside the composting facility as compared to outdoors. The average indoor level of bacteria was 28,000 colony forming units per cubic meter of air as compared to an outdoor level of 777. Bacteria were identified as actinomycetes and gram negative bacteria. The highest level of airborne bacteria were measured in the finish end of

the compost pile. Identification of the species of bacteria was not possible through this sampling method. Identification of bacteria species would be time consuming and expensive.

Dust Exposure

Airborne dust exposure levels are summarized in the attached sample data sheet. Two workers were sampled for both total dust and respirable dust levels. Samples represent time weighted averages. The sampling was conducted during the course of a morning where four truck loads of compost were mixed and loaded into the bays. Work included operating the front end loader, filling the mixing truck with yard waste and sewage solids, running the mixing truck, and moving the compost to the bays. Agitation of the compost pile was not conducted during these samples. The outdoor work involving loading of the truck with yard waste was conducted in a heavy rain. Average total dust exposure levels were 0.27 milligrams per cubic centimeter. Respirable dust levels averaged at 0.14 $mg/m.^3$

Endotoxin Exposure

The samples analyzed are the two total dust samples taken on PVC filters referred to above in the dust sampling section. The levels of endotoxin detected on each sample was found to be at the level of a blank. This indicates that endotoxin levels at the time of this survey were negligible.

Ammonia

Ammonia levels were tested in three locations and are reported in parts per million. The test results are as follows:

- Area proximal to the front end of the compost bay 1 ppm
- At the finish end of the compost bay 10 ppm
- Inside the Bay above the compost pile 25 ppm

Workers generally spend most of the work day at the in areas at 1 ppm of ammonia.

Worker Protection

Workers were wearing single use paper nuisance dust half face respirators. We also noted 1/2 face dual cartridge respirators with ammonia cartridges stored on a table in the de-watering room of the plant. These respirators were not stored properly.

CONCLUSIONS

Airborne levels of bacteria were extremely high throughout the facility. There are no numeric standards or guidelines set for evaluating airborne bacteria, but studies have shown that exposure to airborne bacteria and mold may cause hypersensitivity diseases such as asthma, allergic rhinitis, and hypersensitivity pneumonitis. Actinomycetes, which were identified on all of the samples, have been shown to cause Farmer's lung disease.

Although the levels of fungi detected in the air are not significantly higher than outside levels, the presence of *Stachybotrys atra* is of concern. This fungi produces a class of mycotoxins that are carcinogenic and can affect the exposed individual's immune system. Even low levels of exposure to this species of fungi may cause health effects.

Exposure to ammonia has been shown to cause respiratory effects for both chronic and acute exposures. Some animal studies have shown ammonia gas to temporarily limit the effectiveness of the mucociliary escalator of the airways in the respiratory system. This ultimately affects the lung's ability to clear inhaled particulate matter.

This study provides further evidence that working in compost facilities results in worker exposure to airborne microbiological contaminants far in excess of normal outdoor exposure that are likely to cause both acute and chronic respiratory disease.

Table I. Compost Plant/Fungi in Air Sampling.

Sample Location and Description	CFU/M³	Predominant Taxa
Outside the building at the shredded yard waste shed	1978	*Cladosporium* *Penicillium* *Asp Fumigatus*
Inside the compost building at the mixing truck	2183	*Cladosporium* *Penicillium* *Asp Fumigatus*
At the front end of the composting windrows	4366	*Cladosporium* *Penicillium* *Asp Fumigatus*
In the office inside the compost building	985	*Cladosporium* *Penicillium* *Asp Fumigatus*
At the finish end of the compost windrow before agitating	2112	*Cladosporium* *Penicillium* *Asp Fumigatus* *S. Chartarum*
At the finish end of the windrow after agitating	12,957	*Penicillium* *S. Chartarum* *Cladosporium*

Table II. Compost Plant/Bacteria in Air Sampling.

Sample Location and Description	CFU/M³	Predominant Taxa
Outside the building at the shredded yard waste shed	29,915	*Actinomycetes* gram-negative bacteria
Inside the compost building at the mixing truck	24,366	*Bacillus* *Actinomycetes*
At the front end of the composting windrows	26,619	*Actinomycetes* gram-negative bacteria
In the office inside the compost building	7,746	*Actinomycetes* gram-negative bacteria
At the finish end of the compost windrow before agitating	27,605	*Bacillus* *Actinomycetes* gram-negative bacteria
At the finish end of the windrow after agitating	56,338	gram-negative bacteria

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A STUDY OF COMMUNITY HEALTH SYMPTOMS AND BIOAEROSOL LEVELS NEAR A YARD WASTE COMPOSTING FACILITY

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Abstract: *Bioaerosols from a large, open yard-waste composting facility were investigated as a source of allergic and respiratory irritation symptoms in a nearby residential neighborhood. Counts of airborne* Aspergillus fumigatus *spores were highly variable. Average background spore counts were about 50 spores/m³. A.* fumigatus *spores averaged about 100 spores/m³ in the study neighborhood and about 500 spores/m³ at the compost facility. Elevated A.* fumigatus *levels (≥300 spores/m³) were more frequent in the study neighborhood than in either background site.*

The incidence of asthma and allergy symptoms declined over the 72-day study period. The incidence rates of allergy and asthma symptoms were not associated with airborne A. fumigatus *or other molds. However, the occurrence of these symptoms was associated with ragweed pollen, ozone, temperature and day of the study.*

Key Words: composting facility, fungus spores, community health symptoms, *Aspergillus fumigatus*, bioaerosols

INTRODUCTION

Composting is fast becoming a common method of diverting yard waste, sewage sludge and other organic wastes from landfills and incinerators to beneficial use. Compost facilities are often located close to residential neighborhoods, particularly in heavily populated areas, and health agencies are receiving numerous complaints about odors, respiratory irritation and other health effects.

Soon after a large, yard-waste composting facility on Long Island (New York, USA) began operation in 1988, residents living mainly north-northeast of the facility (in the Town of Brookhaven) complained of odors and debris from the facility. By late 1991, some residents and two local physicians associated various

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health symptoms, including respiratory infections, asthma, hayfever symptoms and skin rashes, with the presence of the facility and exposure to *Aspergillus fumigatus* or other bioaerosols released from the composting site.

Several investigators have measured elevated levels of *Aspergillus fumigatus* and thermophilic actinomycetes in the air near compost piles and sewage sludge compost facilities (Millner et al., 1977; Millner et al., 1980; Millner, 1982; Clark et al., 1983; Passman, 1983). However, little data are available to assess the potential for significantly elevated levels of airspora downwind of a compost facility, and health effects on nearby residential communities have not been investigated. This study was undertaken to estimate daily levels of airborne spores in the neighborhood near the facility (the study neighborhood), two reference neighborhoods and at the compost facility, and to relate symptom incidence in the neighborhood to air quality measures.

METHODS

Study Populations and Health Diary Survey

The Islip Composting Facility (ICF) is located at the northern end of the MacArthur Airport property in west-central Suffolk County (Fig. 1). A residential area is located to the north of the facility with the closest houses about 280 meters (915 feet) from the ICF boundary. The ICF site is 40 acres in area, of which approximately 33 acres are used for compost windrows (long piles of composting material).

The study area was the neighborhood nearest to the compost facility from which most of the odor complaints had been received. The reference community chosen was about eight kilometers (five miles) from the facility, not in the direction of the prevailing winds and with demographic characteristics similar to the study neighborhood. After a health survey of the two communities, 237 candidates (116 from the study area and 121 from the reference area) were selected to participate in the symptom diary study. All participants were asked to complete daily diaries from August 21 through October 31, 1992.

A one-page symptom diary was used consisting of daily checklists for a one-week period. Participants were asked to check "no", "slight" or "moderate/severe" for each of ten health symptoms: nasal congestion, eye irritation, sore throat, coughing, wheezing, difficulty breathing, skin rash, nausea/upset stomach, joint pain and cold or flu. Several additional questions regarding activities and use of medications were included. A general background questionnaire requesting additional information including residential characteristics and occupational history was also completed by study participants.

Individuals may experience different allergy symptoms (eye irritation, stuffy nose) in response to the same exposure. For this reason, a report of eye irritation, stuffy or runny nose, sore throat, coughing, or an allergy symptom written in the 'other' category (postnasal drip, sneezing), was counted as

Sampling Locations

Four sampling stations were established for environmental monitoring (Fig. 1). Two sampling stations were located in the predominant downwind direction relative to the compost windrows, one at the northeast corner of the ICF property about 30 m (100 ft) from the nearest windrow and the other on Union Avenue in the study neighborhood about 540 m (1775 ft) north-northeast of the ICF. A third sampling station was in the reference neighborhood (on Fisher Avenue in the community of Islip Terrace). Air levels of *A. fumigatus* and thermophilic actinomycetes at this site were assumed to be unrelated to ICF spore emissions, and no other obvious large source of *A. fumigatus* and thermophilic actinomycetes was found near this site. The other reference site was located on the MacArthur Airport property about 460 m (1500 ft) southwest (in the predominant upwind direction) from the ICF. Airborne spore levels of *A. fumigatus* and thermophilic actinomycetes at this site were assumed to be unrelated to ICF spore emissions except on days when the wind direction was from the northeast.

At each location, the sampling equipment was mounted on a platform 1.2 x 2.4 m (4 x 8 ft) in depth and width and 1.2 m (4 ft) high (except at the ICF which was 1.5 m or 5 ft high). The ICF sampling platform was located on the top of a finished compost berm roughly 10 m (33 ft) above ground level. Platforms at the other sites were located at ground level.

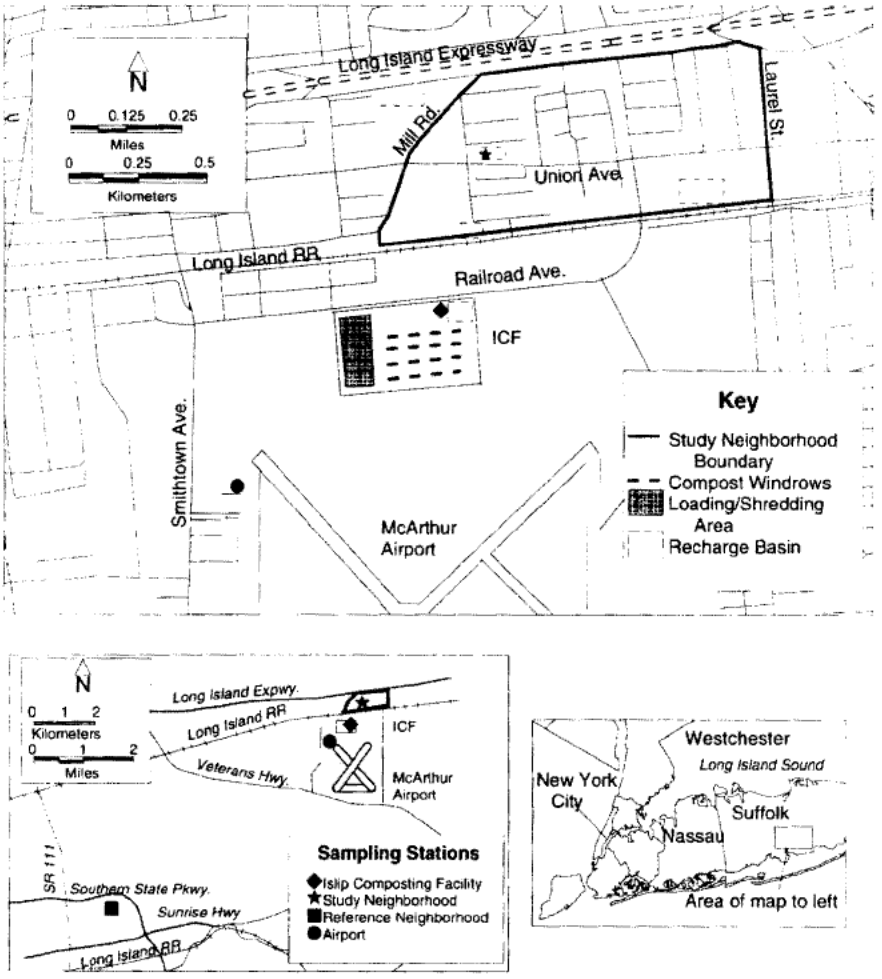
Spore Counts

A Burkard seven-day recording spore trap was operated at each site to collect samples for counts of airborne fungal spores. In a Burkard sampler, fungal spores and other particulate matter are captured on a slowly moving (2 mm per hour), sticky tape which is removed from the sampling drum, cut into convenient lengths and mounted on a microscopic slide. Spores were identified and counted under phase-contract microscopy. Counts were made by counting across the tape at a particular distance from the origin, equivalent to 6 AM and 6 PM each day. The field of view in the microscope was equivalent to a sampling time of four to five minutes. The detection limit was about 20 spores/m^3.

Along with the Burkard continuous samplers, samples of viable *A. fumigatus* and viable thermophilic actinomycetes were collected every sixth day at each site using a portable Reuter Centrifugal Sampler (Biotest Diagnostics model RCS Plus; Placencia et al., 1982, US FDA, 1984; Shane, 1988). Samples were typically collected for less than 10 minutes and had a detection limit of about 2 colony-forming units/m^3. These samples only enumerated airborne *A. fumigatus* and thermophilic actinomycete spores. The *A. fumigatus* were collected and cultured on Rose Bengal medium at 45°C for 72 hrs. The thermophilic actinomycetes were collected and cultured on trypticase soy agar and incubated at 52°C for 48-72 hrs.

an allergy symptom. The incidence rates were computed as the number of symptom events divided by the number of participants eligible to report each symptom.

Similarly, wheezing and difficulty breathing were grouped as asthma symptoms. Incident symptoms or events were tallied each time a person's reporting status increased in severity.



Community Health and Bioaerosols Near Compost

Fig. 1. Location of the Islip Composting Facility (ICF). Sampling sites for the environmental monitoring and neighborhoods where symptom diaries were maintained are identified.

Pollen Data

Ragweed (*Ambrosia spp.*) pollen counts were obtained from Deer Park, NY, the nearest pollen reporting station to Islip, for the period August 1, 1992 through October 31, 1992. Pollen counts were made with a Rotorod sampler following standard protocols (Ogden et al., 1974). General seasonal pollen-level patterns remain roughly uniform over distances of at least 80 to 160 kilometers (50 to 100 miles) due to the consistency of plant communities and to transport of pollen across these distances (Bielory, pers. comm.). Deer park is about 20 kilometers (12 miles) from Islip and 12 kilometers (7.5 miles) from the reference neighborhood in Islip Terrace, so these ragweed counts can be assumed to be quite similar to ragweed levels in the study areas.

Meteorological Data and Miscellaneous Air Quality Data

Two sources of meteorological data were used. At the MacArthur Airport, temperature, wind speed, wind direction, and weather conditions are recorded once per hour by an observer. In addition, a continuous weather monitoring station, operated in Hempstead (about 40 km (25 mi) west of the ICF), provided 60 temperature, wind speed and direction observations per hour. The wind parameters were reported as hourly means and hourly standard deviations (sigmas) about those means.

Ozone, sulfur dioxide and nitrogen dioxide air concentration data were obtained from continuous air-monitoring stations in Babylon, NY (ozone and sulfur dioxide) and Hempstead, NY (nitrogen dioxide). These were the closest air-monitoring stations to Islip following US EPA—approved methods for air-contaminant monitoring. Ozone was analyzed using a Thermo Electron 49 UV spectrophotometric analyzer (USEPA equivalency number EQOA-0880-047. Sulfur dioxide was analyzed with a Thermo Electron 43 pulsed fluorescence analyzer (USEPA equivalency number EQSA-0276-009). Nitrogen dioxide was analyzed with a Columbia Scientific Inc. 1600 gas-phase chemiluminescence analyzer (US EPA equivalency number RFNA-0977-025). Standard methods for data quality were followed (USEPA, 1993).

RESULTS

Burkard Spore-trap Counts

Forty-one different categories of airborne fungus spores were recognized and counted. Total spore counts ranged from 119 to 194,000 spores/m^3 at the four locations. Mean levels of total fungal spores were very similar at each location (7,340-13,000 spores/m^3). Except for *Aspergillus fumigatus*, no other category of airborne fungus spores were associated with the ICF.

Individual counts of *A. fumigatus* ranged from non-detect at all sites to about 1,000 spores/m^3 at the airport and Fisher (the reference sites), to 14,000 spores/m^3 at Union (the study neighborhood) and to 22,000 spores/m^3 at the ICF

(Table I). At Union, Fisher and the airport, *A. fumigatus* was not detected in more than half of the counts, i.e. median spore counts were zero (<20 spores/m^3). However, at the ICF the median *A. fumigatus* count was 56 spores/m^3. The mean Burkard *A. fumigatus* count during the study period was significantly higher at the ICF compared to the other three sites. The mean *A. fumigatus* level at Union was higher than the mean levels at Fisher and the airport, but these differences were not statistically significant. The mean *A. fumigatus* level at Union was still two-to-three times higher than the background levels after removing the highest observation from Union, which might be considered an outlier with a disproportionate influence on the mean (Table I).

Table I. Summary statistics for *Aspergillus fumigatus* spore levels from Burkard samples.

Site	Spore Concentration (spores/m^3)				N
	Mean*	Minimum	Maximum	SD	
Time-points common to ICF, Fisher and Union:					
Airport	38[1]	0	974	121	85
Fisher	55[2]	0	685	114	157
ICF	438[1,2,3]	0	22,100	1980	157
Union	188[3]	0	14,200	1140	157
Union (censored)**	116[3]	0	1,600	245	156
All time-points:					
Airport	38	0	974	121	85
Fisher	55	0	685	114	158
ICF	457	0	22,100	1760	216
Union	194	0	14,200	1010	208

*Mean values with matching superscripts are significantly different ($P<0.05$) by post-hoc multiple comparison.
**The highest value (14,200 spores/m^3) was deleted from the calculations (censored).

Table II. Frequency of elevated (≥300 spores/m^3) Burkard AF counts.

Site	Count Frequency (%)		N
	<300 spores/m^3	≥300 spores/m^3	
Airport	83(98)	2(2)	85
Fisher	149(95)	8(5)	157
ICF	127(81)	30 (19)*	157
Union	134(85)	23(15)	157

*$P<0.05$ by X^2 compared to airport and to Fisher.

Although the overall mean or median *A. fumigatus* levels were not significantly different between Union and the reference locations, the frequency of elevated spore counts was significantly different. Elevated counts were defined as counts that were approximately two standard deviations above the mean *A. fumigatus* level at the Fisher Avenue reference site (300 spores/m^3). Elevated *A. fumigatus* counts were significantly more frequent at the ICF and at Union (19% and 15% of the counts, respectively) compared to the reference sites (2% and 5% of the counts) (Table II).

Viable Spore Counts

As with the Burkard samples, average viable *A. fumigatus* and thermophilic actinomycetes counts were significantly higher at the ICF than at any of the other sites (Table III). Levels at the ICF averaged roughly 10 times the background levels at the reference sites, and *A. fumigatus* was present in every sample at that site. Mean viable *A. fumigatus* and thermophilic actinomycete levels at Union averaged 2- to 3-fold higher than average background levels, but these differences were not statistically significant. The mean viable *A. fumigatus* counts and the Burkard *A. fumigatus* counts were remarkably similar at each of the sites (Tables I and III).

Table III. Summary statistics for viable *A. fumigatus* and thermophilic actinomycete counts.

Category/Site	Spore Concentration (spores/m^3)				N
	Mean*	Minimum	Maximum	SD	
A. fumigatus					
Airport	20^{1}	0	296	65	20
Fisher	46^{2}	0	894	198	20
ICF	$603^{1,2,3}$	12	6000	1340	20
Union	81^{3}	0	740	170	20
Thermophilic actinomycetes					
Airport	33^{1}	0	239	52	20
Fisher	36^{2}	5	119	35	20
ICF	$471^{1,2,3}$	30	2600	634	20
Union	109^{3}	2	610	148	20

*Mean values with matching superscripts are significantly different ($P<0.05$) by post-hoc multiple comparison.

Wind Direction and Spore Counts

The two meteorology data sets were not very different in their overall characterization of wind directions during the study period. The Spearman correlation between associated wind-direction observations at the MacArthur airport at Hempstead for times when 4-minute Burkard counts were made (6 AM, 6 PM and some noon counts) was significant ($r=0.8$, $P<0.001$, $N=429$).

Data pairs were eliminated from this correlation estimate if either of the observations was a "calm" or a "variable". Given this strong correlation, count data in subsequent sections are only discussed in relation to the Hempstead weather data, because the variability in wind direction and velocity was better characterized there than in the airport weather data. Hourly wind direction observations which were "calm" or "variable" were excluded from the wind-direction analysis.

Table IV. Summary statistics for *Aspergillus fumigatus* spore levels from Burkard samples stratified by wind quadrant.

Wind Quadrant/ Site	Spore Concentration (spores/m³)					N
	Mean	Median	Minimum	Maximum	SD	
Airport						
N-E	36	12	0	95	47	6
E-S	26	0	0	238	64	19
S-W	8	0	0	71	20	19
W-N	97	0	0	974	267	13
Fisher						
N-E	37	0	0	333	111	9
E-S	52	0	0	310	92	24
S-W	51	0	0	278	79	36
W-N	65	0	0	685	150	32
ICF						
N-E	77	0	0	619	169	14
E-S	158	30	0	1020	254	28
S-W	1260*	239	0	22,100	3250	50
W-N	112	36	0	702	166	50
Union						
N-E	51	0	0	476	135	14
E-S	66	0	0	398	113	28
S-W	228	95	0	2210	373	47
W-N	401	0	0	14,200	2020	49
W-N**	114	0	0	1390	239	48

*Mean significantly higher ($P<0.05$) than means for other wind categories at each site by post-hoc multiple comparison.
**The highest value (14,200 spore/m³) was deleted (censored) from the calculations.

At all the sites, winds from the northeast quadrant were relatively rare, and therefore few counts were available for northeast winds (Table IV). Burkard 4-minute *A. fumigatus* counts were more equally divided among the other three quadrants, although somewhat fewer counts were available for southeast winds at the ICF, Union and Fisher. The mean *A. fumigatus* level at the ICF with south to west winds was significantly greater than the mean *A. fumigatus* level for the other three wind quadrants at the ICF (multiple comparison, $P<0.05$; Table IV). The mean *A. fumigatus* levels at Union for the south to west and west to north wind quadrants were greater than the mean *A. fumigatus* levels for the other two

quadrants at Union, but only the comparison between the south to west mean and the north to east mean approached significance (P=0.08; Table IV). The other pairwise comparisons had P values well in excess of 0.1. Removing the highest observation at Union, which might be considered an outlier with a disproportionately strong influence on the mean, caused the mean *A. fumigatus* level for the west to north quadrant to decrease by roughly a factor of four, but did not change the significance level.

The mean Burkard *A. fumigatus* levels at ICF and Union associated with each of the wind quadrants were also tested against the mean level at Fisher (all wind directions combined), i.e. background. The mean *A. fumigatus* levels with S-W winds at the ICF was significantly higher than the Fisher mean (Mann-Whitney U=969, P<0.001) as was the S-W mean at Union (Mann-Whitney U=1600, P<0.001).

Table V. Frequency of high Burkard AF counts by wind category.

Site/Wind Quadrant	Count Frequency (%)		N
	<300 spores/m³	≥300 spores/m³	
Airport			
N-E	6 (100)	0 (0)	6
E-S	19 (100)	0 (0)	19
S-W	19 (100)	0 (0)	19
W-N	12 (92)	1 (8)	13
Fisher			
N-E	8 (89)	1 (11)	9
E-S	23(96)	1(4)	24
S-W	36 (100)	0 (0)	36
W-N	29 (91)	3 (9)	32
ICF			
N-E	13 (93)	1 (7)	14
E-S	22 (79)	6 (21)	28
S-W	27 (54)	23 (46)*	50
W-N	44 (88)	6 (12)	50
Union			
N-E	13 (93)	1 (7)	14
E-S	26 (93)	2 (7)	28
S-W	32 (68)	15 (32)*	47
W-N	42 (86)	7 (14)	49

*Frequency significantly higher than for other groups combined (P<0.005) by X^2.

To further examine the relationship between Burkard *A. fumigatus* counts and wind direction, the proportion of *A. fumigatus* observations at or above 300 spore/m³ at each site relative to wind direction was tested using contingency table analysis (Steele and Torrie, 1980). The frequency of high counts at the ICF and at Union associated with winds from the south to west (S-W category) was compared to the frequency of high counts from all other wind directions combined. There were no significant differences in the proportion of high *A. fumi-*

gatus counts among the four wind quadrants at the airport or at Fisher (Table IV). A much higher proportion of Burkard *A. fumigatus* counts associated with S-W winds at ICF was elevated compared to all other wind quadrants combined (46% versus 10%). The proportion of elevated *A. fumigatus* counts from Union samples associated with S-W winds was also significantly higher than for samples associated with the other wind quadrants (32% versus 9%). Moreover, a disproportionate number of the high counts at the ICF (23/36 or 64%) and at Union (15/25 or 60%) occurred when winds were from the southwest quadrant (Table V).

Symptom Diary Study

After a two-week pilot study was conducted to test the design of the diary, 237 candidates (116 from the study neighborhood and 121 from the reference neighborhood) were invited to participate in the symptom diary study. At initial telephone follow-up, eight refusals were identified, five of whom were no longer available due to a change in residence. During the course of the study, 24 candidates (21%) from the study area and 15 (12%) from the reference area refused participation or never returned a completed diary. Only respondents providing at least four diaries (28 days of data) during the study period with at least three diaries in sequence were considered to be participants in the study and were included in the analysis. A total of 63 candidates (54%) from the study area and 82 candidates (68%) from the reference area provided sufficient data to be considered participants in the study.

Participants' chronic health conditions and medication usage were reviewed to determine possible influence on the symptoms being evaluated in this study. One participant from the reference area was excluded from the analysis due to serious chronic disease. Medications taken on a short-term basis were reviewed. None were listed as having upper respiratory symptoms as a side effect. Each report of short-term medication use was checked to determine whether a skin rash was reported during the period that the medication was taken. In one case, onset of hives was reported coincident with medication usage and was excluded from the analysis of skin rash during the period of medication use.

In general, the demography of participants from the study and reference areas was similar (Table VI). The age distribution was somewhat different for participants in the study area versus the reference area due to a higher dropout rate of adults in the study area group. In the study community, 47% of adult candidates completed the study compared to 80% of children originally selected. The sex distribution and proportion of participants reporting history of physician diagnosis of allergic rhinitis and/or asthma were similar for the two study areas. However, a higher proportion of males in the study area were less than 18 years of age, 44% versus 20% of males in the reference area group. A somewhat higher proportion of reference area participants were current smokers.

In general, incident symptoms tended to be reported somewhat more frequently by participants from the study area (Fig. 2) Among both study and ref-

erence area participants, incidence rates for both allergy and asthma declined over the course of the study period.

Table VI. Age, sex, smoking status and medical history for participants in the Symptom Diary Study.

	Study Area Frequency (%)	Reference Area Frequency (%)
Number of Participants	63	81
Age		
Children	20 (31.8)	17 (21.0)
Adults	43 (68.2)	64 (79.0)
Sex		
Male	34 (54.0)	39 (48.2)
Female	29 (46.0)	42 (51.8)
Smoking Status		
Current	3 (4.8)	11 (13.6)
Never	50 (79.4)	58 (71.6)
Past	10 (15.9)	12 (14.8)
History of Allergy/Asthma*		
Allergic rhinitis	34 (54.0)	42 (51.8)
Asthma	13 (20.6)	18 (22.2)
Neither allergic rhinitis/asthma	16 (25.4)	21 (25.9)

*Those reporting asthma may also have reported a physician diagnosis of allergic rhinitis but were included only in the asthma category.

Temperature, ozone, sulfur dioxide, nitrogen dioxide, ragweed pollen and time since start of the study period were all considered as potential predictors of symptom occurrence. The distribution of daily ozone, sulfur dioxide, nitrogen dioxide, temperature and ragweed pollen for the entire study period is presented in Table VII.

During the study period, ambient levels of sulfur dioxide and nitrogen dioxide levels were less than levels reported to result in respiratory effects and were not associated with increased symptom incidence. Ozone, ragweed, temperature and time since start of the study were all intercorrelated ($r=0.66$ to 0.83). Each of these variables was significantly ($P<0.05$) associated with allergy and asthma symptom incidence in separate Poisson regression models; however, due to intercorrelations, use of all four factors in the same statistical model would have been inappropriate. A factor analysis was used to compute a single "combined factor" to account for the influence of ozone, ragweed, temperature and time since start of the study for use in the Poisson regression model. Among study area participants, the combined factor was significantly associated with the frequency of incident allergy and asthma symptoms. For the reference area, a significant positive relationship was observed between the combined factor and

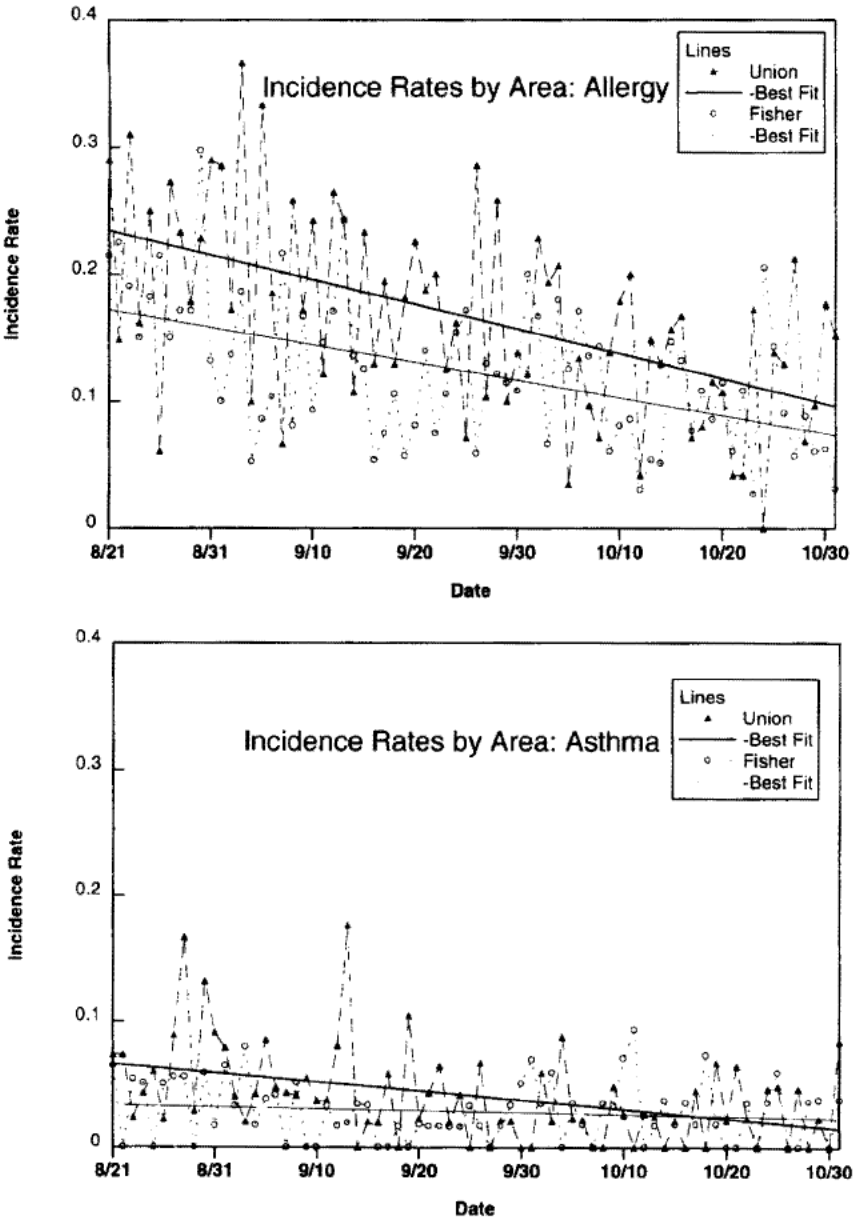


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Fig. 2. Allergy and asthma incidence rates at Union and Fisher.

Table VII. Summary statistics for ragweed, temperature and chemical air contaminants, for the entire study period (72 days). Ragweed values are 24-hour time-weighted averages. Temperature and chemical values are daily maximum observations.

	Mean	Range	Standard deviation
Maximum temperature (°F)	71	45-95	11
Maximum ozone (ppm*)	0.044	0.016-0.126	0.021
Maximum nitrogen dioxide (ppm)**	0.046	0.007-0.113	0.02
Maximum sulfur cioxide (ppm)	0.013	0.001-0.037	0.008
Ragweed pollen (grains/m^3)	7.7	0-67	12.2

*ppm=parts per million.
**Nitrogen dioxide data were available for 62 days.

the incidence of allergy symptoms; however, the relationship was not significant for the incidence of asthma symptoms. The rate ratios tend to increase for the higher quartiles of the combined factor in a dose-response fashion (Table VIII).

Table VIII. Symptom incidence rate ratios for quartiles of combined factor representing ragweed, ozone, temperature and time since start of study using data for the entire study period.

	Study Area		
Symptoms	# events/ # eligible	Quartile	Rate ratio (confidence interval)
Allergy	367/1808	4	2.00 (1.43-2.80)*
		3	1.83 (1.30-2.58)*
		2	1.32 (0.92-1.90)
		1	1.0
Asthma	134/3170	4	2.70 (1.62-4.51)*
		3	1.04 (0.60-1.80)
		2	1.04 (0.57-1.92)
		1	1.0

Symptoms	Reference Area # events/ # eligible	Quartile	Rate ratio (confidence interval)
Allergy	337/2358	4	2.04 (1.46-2.86)*
		3	1.35 (0.94-1.93)
		2	1.49 (1.04-2.13)*
		1	1.0
Asthma	115/3997	4	1.20 (0.70-2.05)
		3	1.04 (0.60-1.80)
		2	1.23 (0.72-2.09)
		1	1.0

*P<0.05, statistically significant.
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Poisson regression analysis was used to look at the relationship between allergy and asthma symptom incidence and *A. fumigatus* spore levels. Both the unadjusted model and the model adjusting for the combined factor (ozone, ragweed, temperature and time) failed to show a positive association (data not shown).

Since fungal spores other than *A. fumigatus* are known to be allergenic, analysis were performed to evaluate associations between symptom incidence and categories of fungal spores other than *A. fumigatus* for which a sufficient number of detectable counts were available. Total spores and individual spore categories were analyzed using Poisson regression analysis. The combined factor was included as a potential confounder. No significant positive associations were observed between fungal spore categories and incident symptoms for study area or reference area participants.

DISCUSSION

The purpose of this study was to determine whether exposures to bioaerosols originating from the Islip Composting Facility (ICF), particularly *Aspergillus fumigatus* spores, were affecting the health of residents living near the facility.

Bioaerosols

No published studies were found that reported airborne *A. fumigatus* levels using the Burkard sampler for comparison to the findings of this study. However, the viable spore counts observed in this study (with a median of 10 CFU/m^3 and a mean of 46 CFU/m^3), and the mean Burkard *A. fumigatus* count (55 spores/m^3) are quite similar to background levels reported in the literature.

A background range of 0 to 181 CFU/m^3 was observed in a study of airborne viable *A. fumigatus* levels at three sewage-sludge composting facilities in Beltsville, MD and Washington, DC (Miller et al., 1980). Background samples in this study were collected outdoors immediately upwind from the composting area and at sites well removed from the composting facilities. Passman (1983) found background levels ranging from 0 to 54 CFU/m^3 in a study of airborne viable *A. fumigatus* prevalence near three sewage-sludge composting facilities in Maine. Viable *A. fumigatus* counts ranged from 0 to 71 CFU/m^3 in a two-year study of outdoor ambient bioaerosol levels in a Washington, DC suburb (Jones and Cookson, 1983). Samples in this study were collected near the site of a proposed sewage-sludge composting facility; however, there were no bioaerosol point sources near the sampling area during the study. Clark et al. (1983) reported viable *A. fumigatus* air levels from several facilities in Sweden which composted sewage sludge or sewage sludge mixed with municipal solid waste. They measured presumed background levels ranging from 100 to >630,000 viable *A. fumigatus* CFU/m^3. However, the locations they chose as putative background sites were generally indoor areas within the composting facility such as operation control rooms or offices which may have been influenced by bioaerosol emissions from the composting operation.

Thermophilic actinomycetes averaged 36 CFU/m^3 at the Fisher Avenue and 33 CFU/m^3 at the airport. The 40 samples ranged from 0 to 240 CFU/m^3. These values are at the low end of the range reported elsewhere. Millner et al. (1980) reported background viable thermophilic actinomycetes levels ranging from 0 to 7,100 CFU/m^3. Twelve of 14 background viable thermophilic actinomycetes observations were less than 500 CFU/m^3 in that study.

Thus, background levels of *A. fumigatus* and thermophilic actinomycetes bioaerosols at the comparison sites in this study are similar to those found in other studies (Millner et al., 1980; Passman, 1983 and Jones and Cookson, 1983) which suggest that outdoor, background viable *A. fumigatus* levels are generally in the range of 0 to 200 CFU/m^3 with background viable thermophilic actinomycetes possibly ranging somewhat higher.

In the studies discussed above, viable airborne *A. fumigatus* were enumerated using an Andersen volumetric sampler instead of the RCS Plus sampler used in this study. Leong et al. (1989) sampled bacterial bioaerosol concentrations side-by-side with these two samplers. The estimates obtained with the two samplers were generally quite close with a consistent bias of slightly higher values obtained from the RCS Plus samples. This suggests that the background estimates obtained with Andersen samplers might slightly underestimate viable bioaerosol levels compared to background measurements made using the RCS Plus sampler.

Viable *A. fumigatus* spore levels from Fisher and the airport ranged from 0 to 894 CFU/m^3 with only 2 observations out of 40 exceeding 100 CFU/m^3. One elevated observation was from the airport site and was associated with a

northeasterly wind. Under this condition, bioaerosol levels at the airport might be influenced by ICF spore emissions and would, therefore, not be representative of background. The single elevated value from Fisher may have been due to inadvertent contamination of that sample or may indicate a transient release of spores from a point source located near the Fisher Avenue sampling station. Viable thermophilic actinomycetes bioaerosol levels from the background sites in t his study ranged from 0 to 239 CFU/m^3. Only 3 out of 40 thermophilic actinomycetes observations were greater than 100 CFU/m^3, one of which was an airport observation associated with a northeasterly wind (239 CFU/m^3). Overall, viable counts from Fisher and the airport were well within the background ranges reported by Millner et al. (1980), Jones and Cookson (1983) and Passman (1983).

The strong similarities between the viable and total bioaerosol values from our reference sites with background counts reported previously suggest that counts from the Fisher Avenue and airport sites are representative of typical background bioaerosol levels in the Islip area, particularly for the groups of microorganisms discussed above. Bioaerosol (*A. fumigatus* and thermophilic actinomycetes) counts at the ICF or Union elevated above these background levels, particularly when associated with southwest winds, are most likely to be from ICF emissions.

Average and maximum levels of *A. fumigatus* spores at the ICF were markedly higher than corresponding *A. fumigatus* levels observed at the background sites. Elevated *A. fumigatus* levels were much more frequent at the ICF and Union than at the reference sites. The strong increase in viable and total *A. fumigatus* levels seen at the ICF with winds from the south to southwest is consistent with a direct influence of compost-pile spore emissions on ICF and Union bioaerosol levels. Viable thermophilic actinomycetes air levels were also elevated at the ICF compared to the background thermophilic actinomycetes levels. This observation, and the elevated viable thermophilic actinomycetes counts with southerly winds at the ICF further support the hypothesis that the composting process increases *A. fumigatus* and thermophilic actinomycetes concentrations in air at the facility.

A. fumigatus levels at the Union Avenue site were somewhat elevated compared to Fisher Avenue and airport levels, although this difference was not statistically significant. Elevated Burkard *A. fumigatus* counts were found at a significantly higher frequency in Union samples than in Fisher samples, and a majority of the highest Burkard and viable *A. fumigatus* counts observed at Union were associated with south-to-southwest winds. Conversely, Burkard and viable counts from Union associated with north-to-east winds were nearly all zeros (non-detects). These findings are consistent with some influence of ICF compost-pile emissions on Union bioaerosol levels particularly when winds are blowing from the south or southwest. However, some high *A. fumigatus* counts at Union were associated with northwest winds. The presence of a small pigeon enclosure at a residence northwest of the Union Avenue sampling station might have been a possible source of these elevated observations. A few birds (<10)

were housed in this enclosure for at least part of the study period, although no activity was observed at the enclosure during field visits made to the Union Avenue site. Pigeons feces are a known source of *A. fumigatus* spores (Walsh and Dixon, 1989).

Viable *A. fumigatus* counts at the ICF ranged from 12 to 6,000 CFU/m^3 and Burkard counts ranged from 0 to 22,100 spores/m^3 during the study period. This range is similar to the range of values reported by Passman (1983), who found levels up to 8,000 CFU/m^3 viable *A. fumigatus* near composting sewage sludge, and Millner et al. (1980), who reported viable *A. fumigatus* counts as high as 55,000 CFU/m^3 immediately downwind from the compost piles in their study. While our study and the Passman study had fixed sampling stations, sampling locations in the Millner study were selected during each sampling session to be directly downwind from the composting operation. This sampling strategy should yield the highest possible counts during each sampling event and would explain some of the difference seen between viable *A. fumigatus* counts in their study and counts in our study. A number of operational differences between the ICF and the facilities studied by Millner et al. exist which could also account for these differences in bioaerosol levels. Among these differences are the waste materials being composted (yard waste at ICF, sewage sludge and wood chips at the MD sites), the composting process being used (actively turned windrows at ICF, aerated static piles at the MD sites) and possible differences in the quantities of material being composted and in seasonal *A. fumigatus* production patterns at the two sites. Further investigation would be required to assess which of these factors or others are most important in determining the level of bioaerosol production that occurs in a municipal composting operation.

Consistent differences were not seen between the ICF or Union and the reference sites in levels of other potentially allergenic fungal spores. Fungal groups, other than *A. fumigatus*, which were enumerated from Burkard samples are commonly occurring species which were not anticipated to be associated with the composting process. Their presence in samples from all four sites represents the typical background exposure levels for these fungal spores.

The extreme variability in Burkard *A. fumigatus* counts, even within a single day, complicates interpretation of the bioaerosol data. In order to assess whether *A. fumigatus* spore levels might be related to allergy or asthma symptoms reported in the diary study, a measure of overall daily exposure to *A. fumigatus* spores was needed. When the study was designed, it was believed that four counts per day would provide a reasonable estimate of daily average or maximum exposure. This was based on an assumption of fairly gradual increases and decreases in spore levels taking several hours to peak or fall off. However, the 4-minute counts within each day were highly variable, and more frequent counts over eight days confirmed that this variability was common. To better estimate daily average *A. fumigatus* levels, hourly counts were made at the study area for a 20-day period (data not presented). The daily averages calculated from the 4-minute and 1-hour counts did not compare very well with one another. We

believe that the large number of 4-minute samples throughout the study adequately measured the overall average and variability at the different sampling locations throughout the study period. However, the daily average spore levels could not be adequately measured without resorting to the 1-hour counts which were very labor-intensive. Future studies should carefully evaluate spore counting methods which can measure average and peak spore levels.

Health Diaries

Diary studies have gained increased attention over the past 10 years as new statistical methods have been developed for analyzing longitudinal or time-series data. The use of a diary study can reduce the possibility of confounding by other factors that would have been difficult to control for in a cross-sectional comparison study. In this diary study, it was intended that the population being surveyed in the study area act as its own control. With this approach, variation in reporting rates due to subjective factors or differences in susceptibility would not confound the relationship under examination. A reference community was included in the study in order to provide information on expected seasonal trends in symptom incidence and to assess the effects of fatigue and sensitization (described below) on the reporting of symptoms by study participants.

There are two problems inherent in conducting a diary study. The first problem, fatigue, refers to the burden placed upon participants to recall and record their health symptoms on a daily basis. After a period of time some people will be less attentive in recalling and recording symptoms. The second problem, sensitization, refers to the possibility that simply by participating in a diary study individuals may change their attitudes and behaviors with respect to their health. Fatigue will act to reduce symptom reports over time while sensitization will increase awareness of symptoms by participants. It is difficult to determine the combined effect of these two influences.

During the study period, a wide range in spore counts was observed. However, increases in allergy and asthma symptom incidence were not associated with the elevations in spore levels among participants. Thus, although the levels of *A. fumigatus* spores observed in the community near the ICF were somewhat higher than those for the reference community, increased rates of allergy and asthma incidence in response to elevated *A. fumigatus* counts were not demonstrated in this study. However, we cannot conclude that exposures from the ICF are not affecting the health of nearby residents. The analysis are based on fewer data than would have been preferred. The measured levels of airborne *A. fumigatus* spores may not have adequately estimated the individual's exposure to *A. fumigatus*.

In addition, only 54% (63) of persons from the study area who originally agreed to take part in the symptom diary provided sufficient data to be considered participants. There were many gaps in the records for those who did participate, further limiting the data available. Thus, a method of data analysis that has been suggested for use in diary studies (Korn et al., 1979) could not

be applied to our data. Using Poisson regression analysis of incidence rates, participants do not serve as their own controls. Therefore, there is the potential for differences in individual susceptibility to confound the relationship being evaluated.

Many participants from both the study area and the reference area reported persistent symptoms ranging from several days to the whole study period in duration. It is possible that participants who were allergic to *A. fumigatus* were sensitized earlier in the season, causing them to experience chronic symptoms in response to lower levels of *A. fumigatus* during the later part of the summer and early fall. These prolonged responses would reduce our ability to observe a direct relationship between changes in spore levels and symptom events.

Inaccuracies in record keeping might also have limited our ability to associate day-to-day changes in symptom incidence with variations in spore levels. Some participants admitted at least occasional retrospective completion of diaries, introducing the possibility of recall errors. In some instances, reporting patterns appeared to change from Saturday to Sunday, possibly reflecting the start of a new diary rather than the exact timing of symptom events.

For the purpose of this study, it was necessary to measure daily exposure to *A. fumigatus* spores. The 4-minute counts may not represent average daily exposure. Also, individual exposure to fungal spores may not be strongly associated with observed spore counts for a number of reasons. Indoor spore levels may differ significantly from levels measured outdoors, and many people spend most of their time indoors. In addition, other indoor and outdoor environments away from home may have an effect on exposure.

The variation of allergy and asthma incidence that was observed was associated with ragweed, ozone, temperature and time since start of the study. This was true for participants living near the ICF and, to a lesser degree, participants from the reference community. The presence of a dose response trend further indicates that changes in one or more of these variables are having an effect on symptom incidence. If ragweed, ozone and temperature are independently related to symptom incidence (and not just in association with time since the start of the study), our ability to assess the effect of environmental influences on allergy and asthma symptom occurrence would be supported. However, time since start of the study was strongly correlated with ragweed, ozone and temperature and could provide an alternative explanation for the decrease in symptom incidence observed over the course of the study. A general tendency to report fewer symptoms as a study progresses, termed a "fatigue effect", has been noted in other studies using diary data (Abramson, 1990). Separating the relative importance of the environmental factors and time since the start of the study was not possible due to their strong intercorrelations.

A high proportion of people reporting a history of allergy or asthma (3 to 1) were selected to participate in the symptom diary study. Therefore, the rates of allergy and asthma symptom occurrence among participants in the symptom

diary study would be expected to be higher than those for community residents in general. However, allergic reactions are not the only health effects that can result from exposure to *A. fumigatus*. While *A. fumigatus* may colonize air passages without causing harm, among susceptible individuals including asthmatics, a severe asthmatic reaction can occur known as allergic bronchopulmonary aspergillosis (ABPA). Growth of *Aspergillus* into lung tissues (invasive aspergillosis) is a life-threatening condition which is rare and usually occurs only among immuno-suppressed individuals. Questions regarding risk of ABPA or invasive aspergillosis could not be addressed in this study.

Hospital outbreaks of invasive aspergillosis have been observed among severely immuno-compromised patients exposed to *A. fumigatus* levels as low as 1-15 CFU/m^3 (Opal et al., 1986; Arnow et al., 1991; Rhame, 1991). These levels are well within the background levels observed in this and other air-monitoring studies. Rhame (1991) states that nosocomial (hospital-acquired) aspergillosis incidence is directly proportional to hospital *A. fumigatus* air levels. This may be true among patients predisposed to *A. fumigatus* infection, although a simple dose-response relationship is less likely to exist for the general population (reviewed in Marsh et al., 1979). The finding that viable *A. fumigatus* spore levels were, at least occasionally, elevated above background levels at the study neighborhood site, about 540 m (1,775 ft) distant from the ICF, suggests that particular caution should be taken in siting yard-waste composting facilities (and probably facilities composting other wastes) near hospitals or other health-care facilities where immuno-suppressed patients spend considerable periods of time.

CONCLUSIONS AND RECOMMENDATIONS

Average levels of airborne *Aspergillus fumigatus* spores and thermophilic actinomycetes at the Islip Composting Facility (ICF) were significantly elevated above background during the study period of August 21 to October 31, 1992. *A. fumigatus* spore levels in the study neighborhood, 540 meters (1,775 feet) away from the ICF, averaged about 100 spore/m^3 which was about twice the average level in a reference community eight kilometers (five miles) away, although this increase was not statistically significant. However, when the study neighborhood was downwind of the ICF, the average *A. fumigatus* spore count was four times the average background level, and this difference was statistically significant. Elevated spore counts were more frequent at the ICF and in the study neighborhood than in the reference neighborhood, particularly on days when the community was downwind of the facility. Elevated *A. fumigatus* spore levels were also somewhat more frequent in the study neighborhood on days when the facility was in operation than on days when it was closed, although this difference was not statistically significant.

In this study, *A. fumigatus* and other mold spores were not observed to be associated with increased allergy and asthma symptom reporting among residents living near the Islip Composting Facility. However, the occurrence of these

symptoms was associated with ragweed pollen, ozone, temperature and time since start of the study period. These factors were taken into account in analysis looking at the effect of *A. fumigatus* levels on symptom incidence. Allergy and asthma symptoms could also have been influenced by exposures that were not measured and accounted for in the analysis. Exposures to molds, other allergens and irritants may occur in a variety of settings including homes, schools and workplaces (Hirsch and Sosman, 1976; Flannigan et al., 1991). For example, gardens and home environments can have *A. fumigatus* spores levels much higher than the background levels observed in this study (Sikora et al., 1985).

Along with the symptoms evaluated in this study, *A. fumigatus* can cause unusual, but severe illnesses including allergic bronchopulmonary aspergillosis, aspergilloma, and invasive aspergillosis. These illnesses occur very infrequently and for this reason were not evaluated in this study.

Although this study was not able to evaluate the risk of serious *A. fumigatus* infections, its results suggest that extreme caution should be exercised when considering the siting of compost facilities near certain health care facilities. Hospital outbreaks of invasive aspergillosis have been observed among severely immuno-compromised patients exposed to *A. fumigatus* levels as low as 1-15 CFU/m^3 (Opal et al., 1986; Arnow et al., 1991; Rhame, 1991). These levels are well within the background levels observed in this and other air-monitoring studies. Hospitals with the most severely immuno-compromised patients (e.g., bone-marrow transplant wards) must take extreme precautions to prevent infection of these patients. If outdoor *A. fumigatus* spore levels at such a hospital were more frequently elevated than normally occurs, the risk of life-threatening infection could be increased. Thus, composting facilities should not be sited close to hospitals or other health care facilities where extreme precautions are being taken to prevent infection of severely immuno-compromised patients, unless bioaerosol emissions can be controlled.

The ability of operational practices at the Islip Composting Facility and other yard-waste composting facilities to reduce the production or migration of bioaerosols should be assessed. This study and others (Millner et al., 1980; Passman, 1983) suggest that activities at the site (shredding, turning windrows, moving woodchips, traffic, etc.) are more important than the presence of the windrows per se.

The potential for bioaerosols from compost facilities to trigger or exacerbate allergy and asthma symptoms needs further evaluation. Although this study did not find an association of these symptoms with *A. fumigatus* or other mold spores, a number of study limitations warrant further evaluation, particularly at sites where more extensive or serious exposure might be occurring. Other bioaerosols should also be considered.

Studies are needed to better assess bioaerosol exposures from various sources, including residential composting. More important sources of exposures to *A. fumigatus* and other bioaerosols may exist apart from compost facilities.

Techniques need to be developed to better estimate bioaerosol levels and their effects. Bioaerosol levels are highly variable even over relatively short time

scales (hours or minutes). Present techniques are labor intensive, require skilled technicians and are most suited to sampling for very short times (few minutes). More automated techniques which have the ability to estimate hourly or daily exposure levels are needed.

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GUIDELINES FOR EVALUATION OF AIRBORNE MICROBIAL CONTAMINATION OF BUILDINGS

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Abstract: *Although solution of building-related health problems requires accurate information on the nature and relative abundance of airborne microorganisms, many investigations have failed to provide the required data. There are no standard methods for obtaining this information, and assessment of only viable microorganisms may reveal as little as 1% of the total microbial airborne load. The sampling method strongly influences the results, so that comparison of counts obtained by different methods, either within or between investigations, is not valid. However, liquid impingers and filtration samplers allow viable and total counts to be made from the same sample. In sampling for viable organisms, the culture media selected are critical. For molds, both a general purpose medium and a medium to select for xerophilic fungi are essential. Because of rapid fluctuations in numbers in indoor air and other problems associated with sampling, there is a justified reluctance to set numerical guidelines. More reliance is placed on comparison of species composition and rank order of species in indoor and outdoor air. In the future, immunological, molecular, chemical and bioassay techniques are likely to play a significant role in investigations.*

Key words: Air sampling, contamination, airborne microbes, investigations

INTRODUCTION

As has been noted in general, for there to be no airborne microbiological hazard to the occupants of buildings, the microflora of the indoor air should be similar to that of outdoor air (ACGIH, 1989). We should therefore have objective data which are detailed and reliable, i.e. (a) precise identification of the bacteria, yeasts and molds and other filamentous fungi in the air, and (b) accurate quantification of the various categories of microorganism. For a variety of reasons which will be dealt with later, the second of these objectives is not

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attained, and in many investigations neither is the first. For example, as a result of either time constraints or lack of the high degree of skill required, two very important segments of the mold flora often appear in reports as "*Aspergillus*" and "*Penicillium*". In the first case, the members of the genus range from allergenic xerophiles such as *A. penicillioides* and species in the *A. glaucus* group (or more correctly *Eurotium* spp.) to toxigenic species such as *A. flavus* and *A. versicolor* and the respiratory pathogen *A. fumigatus*. Clearly, the potential significance for health of an atmosphere heavily contaminated with the first species is quite different from an atmosphere with a similar load of the last. As the first is xerophilic and the last hydrophilic, the abundance of one rather than the other can also tell us something about amplification sites within the building. There is a need for reliable species identification within the even larger genus *Penicillium* also. As in *Aspergillus*, the allergenic and toxigenic nature of different species vary considerably. In addition, detailed study has shown that there may be marked differences in the concentrations of different *Penicillium* spp. indoors relative to outdoor concentrations; some species are less abundant outdoors and others more abundant (Fradkin et al., 1987).

Air Sampling

Assessing numbers of viable microorganisms

The air sampling methods which are most often used in investigations are those in which the viable airborne bacteria and fungi in the air are collected on nutritive agar plates, which are then incubated to allow cells, spores and other potential colony forming units (CFU) to develop into colonies. These methods offer the best widely available means of identifying the organisms present. The range of current sampling methods has been reviewed by various authors (e.g. Flannigan, 1992b; Wanner et al., 1993). A crude assessment can be made by collecting microorganisms which settle out of the air on agar medium in open petri dishes. This method is, of course, non-volumetric and is biased in favor of large (heavy) spores or clumps of spores/cells which settle out more rapidly, and is also strongly affected by atmospheric turbulence. It is clearly much more appropriate to use a volumetric air sampler. There is available a range of volumetric samplers in which particles in air drawn in either through a slit or a perforated disc impact on an agar plate. Both slit-type impaction samplers (in which the agar plate rotates) such as Casella and Mattson-Garvin samplers, and sieve-type impaction samplers such as the Surface Air Systems (SAS) and Andersen N6 models are widely used. These single-stage sieve samplers are popular, but the original six-stage Andersen sampler is still preferred by many, because the particles are sorted out on different agar plates according to their aerodynamic size. Consequently, it can be deduced what size of particles the colonies on individual plates have developed from during incubation, and therefore where in the respiratory system the different organisms would most likely be deposited. The Reuter centrifugal sampler (RCS), in which particles impact at high velocity onto agar in a plastic strip as a result of cen-

trifugal acceleration of air drawn in by an impeller fan, is also a popular readily portable sampler.

A different method of obtaining a viable count is to filter microorganisms out of the air by drawing the air through a membrane filter in a filter cassette using a low-volume rechargeable pump. Such a system can be attached to a belt or harness and used to collect a "personal" sample. Aliquots of washings from the filter and dilutions of the washings can then be spread on a range of agar media. A liquid impinger can also be used in an essentially similar manner, although here the particles in air drawn in through capillary jets impinge on and collect in liquid. Portions of the collection fluid and dilutions prepared from it are then plated out as in filtration sampling.

Assessing total numbers of microorganisms

While these viable sampling methods enable many airborne microorganisms to be identified, they all have the very serious deficiency of assessing only those that are actually culturable. There can be a great disparity between the numbers of the organisms that are actually present and those that can be cultured. For example, when Palmgren et al. (1986a, b) used the CAMNEA filtration method to investigate a range of different work-related environments, they reported that the viable count could be as little as 1% of the corresponding total count. On the other hand, there were some instances when there was little disparity. Correlation between viable and total counts was much higher when the flora was dominated by fungi than when bacteria were dominant.

There are various ways of assessing the total numbers of airborne microorganisms. For example, total counts (viable + non-viable) can be made using impaction samplers in which particles are collected on the sticky surfaces of rotating rods (e.g. Rotorod), or on the sticky plastic tape of the 7-day or the glass microscope slide of the corresponding "personal" Burkard slit-type sampler. Counts are made under the microscope, but only those relatively few molds with distinctive spores can be identified in this way. We are then faced with using one type of sampler to assess the viable microflora present, and another type to obtain a total count, or to employ the same sampling method for both purposes. For the reasons given below, the second option should be taken. Currently, this means using a filtration sampler or a liquid impinger. Microorganisms in aliquots of the same washings that are plated out on agar medium can be stained with, for example, acridine orange and counted under a microscope (Palmgren et al., 1986a,b).

The sort of differences which can be found between the viable (culturable) fraction and the total count for a sample gathered over 4-hours on a Nuclepore membrane filter are shown in Table I, which also shows that the viable counts so obtained can be quite different from those for 3 (fungi) or 10 minute (bacteria) samples taken with a six-stage Andersen sampler at points within the 4-hour time frame.

In the absence of a standard method, the choice of sampler to be used is largely the outcome of the investigator's experience. However, the choice is

important for both quantitative and qualitative reasons. For example, dealing only with viable counts, the agar plates of a six-stage sampler are much less liable to "overloading" with spores than those of single-stage impaction samplers. The development of large numbers of colonies in close proximity to each other results in overgrowth of the plates and serious quantitative and qualitative errors in assessment of the microflora. The manner in which different samplers operate, their sampling times and efficiency in removing different sizes of particle from the air, extracting them from the airstream and collecting them on a surface or in liquid differ markedly. No valid comparison can be made of data obtained using different types of sampler, either in the same investigation or in different investigations.

Table I. Numbers of microorganisms in a restaurant as assessed by viable and total count methods (after Flannigan, 1992a).

	Number of microorganisms m^3 air						
	Spot samples (Andersen)*			Continuous samples (filter method)			
	Before open	Mid-day	Early evening	Plate count*		DEFT method†	
				a.m.	p.m.	a.m.	p.m.
Pantry							
Molds	212	141	129	3160	920	10200	7260
Bacteria	224	152	208	138	625	34910	5100
Kitchen							
Molds	812	334	506	n.s.	n.s.	n.s.	n.s.
Bacteria	212	127	527	n.s.	n.s.	n.s.	n.s.
Main restaurant							
Molds	35	565	24	521	348	2010	12360
Bacteria	106	244	88	417	1180	43250	13580

*Method based on culture on agar plates.
†Direct epifluorescence technique.
n.s., not sampled.

Another area which is not given the attention it requires is the choice of media used in conjunction with viable samplers. When these samplers are used, the interest is in isolating two major categories of microorganisms, bacteria and fungi, but not all investigators believe in using antibiotics to prevent interference with fungi and bacteria and *vice versa*. Other factors which influence what is isolated include the nutrient composition, pH and water activity (a_w) of media are also extremely important.

For primary isolation of bacteria, a general purpose medium (pH 7.0) is used, e.g. nutrient agar (Austwick et al.,1989) or tryptone soya agar (TSA). TSA is rich

in carbohydrate and therefore increases the chances of isolating nutritionally fastidious bacteria, although if rapidly growing types such as *Bacillus* are present they may swamp slower growing species. However, it is important to recognize that general purpose media are of no use for isolation of some bacteria. *Legionella pneumophila*, for example, is only one of a number which require highly selective media (Wanner et al., 1993).

Overgrowth on nutritionally rich media can be a greater problem in the case of fungi. The medium recommended by ACGIH (1989) for primary isolation of fungi is a malt extract agar (pH 4.5-5.0). This contains additional dextrose and peptone and is more usually used as one of a number of media in identifying species of *Aspergillus* and *Penicillium* (Samson et al., 1994). A malt agar containing only 2% malt extract (no dextrose or peptone) has been used by a number of investigators (e.g. Hunter et al., 1988; Austwick et al., 1989). An international group (Samson et al., 1994) has recently recommended its use because the isolation plates suffer less from rapidly growing molds such as *Fusarium* and members of the Mucorales (*Mucor, Rhizopus* and *Absidia*) and are satisfactory for isolating a wide range of other fungi.

Allergenically important fungi which do not grow well on general purpose media are slow growing xerophilic molds such as *Eurotium* spp., *Aspergillus penicillioides* and *Wallemia sebi*. They grow best at a lower a_w than other (hydrophilic) fungi, and therefore a low a_w medium has to be used in addition to general purpose media if they are to be detected and quantified. Low a_w media which have been used for this purpose have been malt extract agar incorporating 10% (w/v) sodium chloride (Flannigan et al., 1993) or 20% or more sucrose (Miller et al., 1988; Elixmann et al., 1990). Dichloran-glycerol agar (DG18), which is widely used in food mycology, contains 18% glycerol to reduce the a_w. It has been used successfully in air sampling by Verhoeff et al. (1990) and has been recommended for isolation of fungi from indoor environments (Samson et al., 1994). However, just as general purpose media are unsuitable for efficient isolation of xerophiles, low a_w media are unsuitable for isolation of some hydrophilic fungi, e.g. *Stachybotrys* and *Trichoderma*. It is absolutely clear that no thorough investigation of fungi in environments can be made using a single medium.

Table II. Levels of microbial contamination of air (Anderson sampler with malt extract agar as collection medium) and dust (1:50 dilution in peptone spread on DG18) in naturally ventilated homes and non-industrial work environments (after Wanner et al., 1993).

Category of contamination	CFU m^{-1} air		Fungi as CFU g^{-1} dust
	Bacteria	Fungi	
very low	<100	<50	<10,000
low	<500	<200	<20,000
intermediate	<2,500	<1,000	<50,000
high	<10,000	<10,000	<120,000
very high	>10,000	>10,000	>120,000

As Flannigan (1993) has pointed out, the temperature at which isolation plates are incubated is extremely important. If the interest is in isolating pathogenic bacteria, plates should be incubated at 37°C, but for a more general assessment of bacteria the incubation temperatures should be no higher than 25°C. However, for isolation of the allergenic thermophilic Actinomycetes, *Faenia rectivirgula* and *Thermactinomyces vulgaris*, 55°C is optimal. While 25°C or thereabouts is suitable for most fungi, incubation at 37°C (Austwick et al., 1989) will enhance counts of thermotolerant/thermophilic fungi such as the pathogen *Aspergillus fumigatus*.

Standards and Guidelines

Levels of microbial contamination of indoor air and dust have been categorized by some groups (Wanner et al., 1993) for naturally ventilated homes (Table II) and non-industrial work environments (Table III). However, there has been a reluctance to set numerical standards of guideline values because of the problems with air sampling (some of which have been mentioned previously). House dust may yield important information as its microflora can be said to mirror the air spora, at least in part, but here again there are also problems with sampling and assessment of numbers. ACGIH (1989) recommends that the rank order of fungal species in indoor and outdoor air be used in interpreting air sampling data. Comparison of the air spora of indoor air with that outdoors is extremely important. The presence or preponderance of some molds in indoor air, but not outdoors, can identify a problem inside a building. For example, as mentioned earlier in this volume, abundance in indoor air of such hydrophilic molds as *Aspergillus fumigatus, Exophiala, Fusarium, Phialophora, Stachybotrys, Trichoderma* or *Ulocladium* almost invariably indicates a very damp amplification site within a building. On account of the pathogenic and/or toxigenic nature of some of these species, e.g. *A. fumigatus* and *Stachybotrys*, it also indicates the need for urgent action. High levels of *Eurotium, A. penicillioides* and *Wallemia* can indicate a dusty atmosphere, and a potential cause of respiratory allergy.

Table III. Levels of microbial contamination of air and dust in non-industrial indoor work environments, methodology as in Table II (after Wanner et al., 1993).

Category of contamination	CFU m^{-1} air		Fungi as CFU g^{-1} dust
	Bacteria	Fungi	
very low	<50	<25	<10,000
low	<100	<100	<20,000
intermediate	<500	<500	<50,000
high	<2,000	<2,000	<120,000
very high	>2,000	>2,000	>120,000

Although bodies such as the ACGIH do not give numerical guidelines, a Canadian guide on office buildings based on five years of investigation of 50 air-conditioned federal government buildings (Nathanson, 1993) includes some guidance on numbers. The following are the main points:

(1) The "normal" air flora should be quantitatively lower than, but qualitatively similar to, that of outdoor air.
(2) The presence of one or more fungal species at significant levels in indoor but not outdoor samples is evidence of an indoor amplifier.
(3) Pathogenic fungi such as *Aspergillus fumigatus, Histoplasma* and *Cryptococcus* should not be present in significant numbers.
(4) The persistence of toxigenic molds such as *Stachybotrys atra* and *Aspergillus versicolor* in significant numbers requires investigation/action.
(5) More than 50 CFU m^{-3} may be of concern if there is only a single species present (other than certain common outdoor phylloplane fungi); up to 150 CFU m^{-3} is acceptable if the species present reflect the flora outdoors; up to 500 CFU m^{-3} is acceptable in summer if outdoor leaf-inhabiting fungi are the main components.

The numerical values above are based on 4-minute air samples collected with an RCS sampler and do not apply to other sampling procedures, other types of building or other climatic/geographical regions. What is the norm can only be based on extensive investigations (using well-defined procedures) of a range of buildings in a particular region. No threshold limit values can be set for exposure to molds in general or to particular species. Whether one subscribes to numerical guideline values or not, it is important to recognize that this document emphasizes two extremely important points made earlier. First, comparison with outdoor air is essential. Secondly, the species composition of the air spora is of primary significance—different species impact in different ways and to different degrees on health—and therefore attention should be focused on that rather than on crude counts.

Until relatively recently, the main emphasis in indoor air investigations has been on allergenic microorganisms, but recent epidemiological research indicates that some non-allergenic factors associated with fungi affect respiratory health. Mycotoxins produced by individual species of mold may have an important role, but it is also possible that some more general factor is involved. In the future, the overall approach to investigating the fungal burden in indoor air will have to take these factors into account. This will involve (a) assessing which allergenic and toxigenic species are present by sampling for viable fungi, and (b) obtaining a measure of the total amount of fungal material to which individuals are exposed in a work environment. In the next few years, immunological (Zwick et al., 1991) and/or PCR (Alvarez et al., 1994) and other molecular methods are likely to have an increasing role in the former, and assays such as those for fungal 1,3-ß-glucan (Rylander et al., 1991) or ergosterol (Miller, 1993) in the latter.

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UNDERSTANDING THE BIOLOGY OF FUNGI FOUND INDOORS

CHIN S. YANG, Ph.D.

Abstract: *Fungi are capable of surviving and flourishing in indoor environments and have caused significant problems related to indoor air quality. To be able to deal with this increasing environmental health problem, we need to know the basics of fungi and mechanisms of fungal growth and dispersal.*

Fungi are named according to the ICBN rules. Proper use of valid names and recognition of these fungi and related information in the literature will increase our ability to deal with them.

Fungi produce spores of different shapes and sizes, which affect collection efficiency of airborne spores. Consideration should be given when designing a new sampler or collecting samples.

The longevity and viability of fungal spores affect how we handle and control fungal contamination and its mitigation. Much more is to be learned regarding how fungi and their spores survive in indoor environments.

Some fungi have found a niche in buildings. To prevent and control their growth indoors, factors affecting their colonization and growth should be identified.

Key words: Fungi, ICBN, *Stachybotrys*, *Aspergillus*, ecology, longevity, viability

INTRODUCTION

Fungi have been involved in human suffering since time began. One of the earliest known fungal diseases is ergotism. Ergotism is caused by toxins produced by toxigenic fungi, primarily *Claviceps purpurea*. The ergot fungi infect rye, grains and other grasses. Ingestion of ergot-contaminated rye or other cereals causes ergotism. There are two types of ergotism recognized clinically: gangrenous and convulsive. Gangrenous ergotism affects the extremities as well as causes gastrointestinal symptoms. Convulsive ergotism affects the nervous

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system. Brain and spinal lesions lead to death or permanent mental impairment (Marasas and Nelson, 1987). Descriptions of ergotism can be traced back for five thousand years (Sorenson, 1993). Small outbreaks of ergotism were still reported as recently as the late 1970's (Marasas and Nelson, 1987).

Another fungus, *Phytophthora infestans*, caused the great potato famine in Europe in 1840's. The famine helped to speed up the migration from Europe to North America. This eventually resulted in shifting political power and wealth from Europe to North America. Fungi also cause diseases in plants and agricultural crops and in humans and animals. They also cause deterioration of foodstuffs and many useful materials, such as wood, leather, and paper products.

Medically, fungi are known to cause allergies, hypersensitivity pneumonitis and related diseases (such as humidifier fever), infections, mushroom poisoning, and mycotoxicoses. In addition, volatile organic compounds (moldy odors) released from actively growing fungi may also pose a health risk (Miller, 1990). Intuitively, moldy and musty odors often signal an unhealthy condition. Recent surveys and data suggest that fungal growth and amplification, such as in porous building materials (Morey and Williams, 1991), may be a significant contributor to as much as 34% of "sick building" cases (Ellringer and Yang, unpublished).

In order to advance our understanding of fungal colonization and contamination and to reduce and control fungal contamination in indoor environments, we need to better understand the biology of fungi, particularly those adaptable to indoor environments. This presentation focuses on a number of issues that have not been extensively discussed.

Taxonomy and Nomenclature of Fungi

Taxonomy is the key to all biological sciences. Fungal taxonomy is no exception. The critical importance of reliable fungal identifications was emphasized by Miller (1991). Misidentification of fungi is common in commercial laboratories. There is currently no professional or laboratory accreditation program available in the U.S. Registration as a medical technologist in mycology is often inadequate, because medical mycologists typically deal with very few fungi. Unfortunately, few mycologists are being trained in the U.S. In fact, very few mycology courses are currently taught in U.S. universities and colleges.

For many non-mycologists, name changes for fungi are common and confusing. Mycologists follow the International Code of Botanical Nomenclature (ICBN) to name and determine valid epithets so that we all talk and deal with the same fungus. The ICBN sets the rules and guidelines in naming fungi. Mycologists follow the ICBN to determine which epithet is a valid name when there is more than one epithet for the same fungus or the same name was applied to more than one fungus. Many fungi that we encounter in indoor environments have nomenclature problems. Three examples are provided to illustrate:

Two generic names, *Candida* and *Monilia*, have frequently been used incorrectly by medical professionals. Several species of *Candida* are known human

pathogens or opportunistic pathogens. They cause candidiasis. However, an older name, moniliasis, is occasionally used for candidiasis. The generic name *Candida* has been accepted to replace *Monilia*. The epithet *Monilia* is reserved for those fungi having teleomorphs in *Neurospora* and *Sclerotinia*. Another generic name often confused with *Candida* is *Oidium*, which is used for the anamorphs produced by species of *Erysiphe* (powdery mildews) and is not culturable.

Stachybotrys atra is the name commonly known to indoor air professionals. However, another name, *Stachybotrys chartarum*, was accepted to be the valid name and *S. atra* was reduced to a synonym of *S. chartarum* (Jong and Davis, 1976). In the same article, they also reaffirmed the segregation of *Stachybotrys* and *Memnoniella* as two distinct genera. Both groups of fungi have been isolated from indoor environments (Yang, unpublished). Species of *Stachybotrys* produce slimy spores in masses, while *Memnoniella* species produce dry conidia in chains.

Another group of common indoor fungal contaminants is *Aspergillus*. Many names in the genus require further research by mycologists to determine what are the valid names. *Aspergillus niger* is a fungus commonly encountered indoors and has been used for commercial production of a number of chemicals, such as citric acid. However, there are earlier names, such as *A. phoenicis* (Corda) (Thom 1840) and *A. ficuum* (Reichardt) (Hennings 1867), for the same fungus. Because the name *A. niger* is so widely used and because of its economic importance, the epithet *A. niger* has been proposed to be protected according to rules in the ICBN.

Sampling and characterization

Much discussion of sampling for fungi has focused on whether or not to sample, or air sampling techniques, and the selection of media. To sample or not to sample is often a professional judgment on a case-by-case basis. It is my opinion that we are not likely to see "perfect" air sampling equipment for fungal spore collection soon. Fungal spores vary in size, in morphology and in shape. Spores of *Penicillium* and *Aspergillus* may be as small as 2-3 µm diameter (Pitt, 1991; Klich and Pitt, 1988). Large spores, such as *Alternaria alternata* and *Epicoccum purpurascens*, may be as large as 20-63 x 9-18 µm for *A. alternata* and 15-25 µm (up to 50 µm diam.) for *E. purpurascens* (Ellis, 1971). Spores of *Alternaria tenuissima* can be as long as 95 µm. Conidia of *Cladosporium herbarum*, the cosmopolitan fungus, are mostly 8-15 X 4-6 µm but cover a range of 5-23 X 3-8 µm (Ellis, 1971). *Stachybotrys chartarum* has been reported to have spore sizes from 7-12 X 4-6 µm (Jong and Davis, 1976; Domsch, Gams & Anderson, 1980 & 1993) to 8-11 X 5-10 µm (Ellis, 1971).

Furthermore, conidial shapes of Hyphomycetes can be as simple as globose to ovoid in many species of *Penicillium* and *Aspergillus*. Some *Aspergillus* conidia may be smooth, finely roughened to spinose (Klich and Pitt, 1988). *Stachybotrys chartarum* spores were described as broadly ellipsoidal to subspherical with verrucose ornamentation by Ellis (1971), as ellipsoidal with bands and ridges by Jong and Davis (1976), or as smooth-walled to coarsely roughened with warts

and ridges (Domsch, Gams & Anderson 1993). Conidia of *Alternaria alternata* are often described as "ovoid, obclavate, obpyriform, or more rarely ellipsoidal, with a conspicuous basal pore, with or without a short conical or cylindrical apical beak not exceeding one third of the conidial length, medium brown, smooth-walled or warted, slightly constructed at the three to eight transverse septa, in the lower part each portion has one or two longitudinal septa" (Domsch, Gams & Anderson 1993). This long description of *Alternaria alternata* spores underscores the unusual shapes that we may encounter when sampling for fungi. Furthermore, conidia of *Cladosporium*, in chains or in clusters, have been encountered in air. All these different shapes, ornamentation, and spore aggregates will affect buoyancy of spores and the aerodynamics of air sampling.

Mycologists and plant pathologists have used a number of different nutrient media to isolate and grow fungi. Most common airborne fungi are saprophytic and are likely to grow on general media, such as malt extract agar (MEA) or potato dextrose agar. Xerophilic fungi require media of low water activity. Sucrose, at 20% or 40% concentration, added to MEA medium has been used to isolate and grow these fastidious fungi. On the other hand, mycologists have used a limited number of media in growing and describing fungi. To properly identify a fungus in culture, specific media are often necessary to grow the fungus. For example, Czapek yeast extract agar (CYA), CYA with 20% sucrose, and 2% malt extract agar (MEA) have been used to study and describe *Aspergillus* (Klich and Pitt, 1988). Growth rates, colony and conidia color on these media are measured and described. Therefore, the use of these media for *Aspergillus* identification is critically important. Most conidia-producing *Aspergillus* species can be readily identified on MEA and CYA (Samson, 1994).

To specifically isolate a fungus, selective media may be used. Czapek cellulose agar has been used to isolate *Stachybotrys chartarum*, although MEA and cornmeal agar were the media used to study and describe *Stachybotrys* and *Memnoniella* (Jong and Davis, 1976). Czapek cellulose medium, however, was used to test whether a strain of *Stachybotrys* and *Memnoniella* was cellulolytic or not. In our laboratory, we have been successful in using cornmeal agar for the recovery of *Stachybotrys chartarum* in air or in bulk and swab samples.

Longevity and Viability of Fungal Spores

Fungi produce spores for dissemination and survival. These spores may survive for a period of time until a suitable environment allows them to germinate, grow and produce more spores. In general, thick-walled spores, such as ascospores, are considered to be able to survive longer than thin-walled spores, such as basidiospores. Ascospores, belonging to *Wilcoxina* and *Tricharina* from herbarium specimens of more than ten years old, were revived and grown into cultures (Yang and Korf, 1985a & 1985b). Ascospores of morels (*Morchella* spp.) are known to survive in herbariums for as long as 20 years. Conidia of Hyphomycetes from one-year old dry cultures of *Epicoccum, Alternaria, Trichothecium, Stachybotrys, Memnoniella,* and species of *Aspergillus* were revived into cultures (Yang, unpublished). Conidia of *Penicillium* and *Aspergillus* have been

reported to be viable for over twelve years (Miller, 1990). The ability for fungal spores to survive an extended period of time makes controlling and mitigating fungal contamination in buildings much more difficult.

Not all airborne fungal spores are viable, although they are all allergenic and may be toxin-containing. The viability of airborne fungal spores depends on many factors, such as age of spores, radiation intensity, humidity, and desication. Some common airborne conidia, such as *Alternaria, Cladosporium, Cercospora*, have viability ranging from 20-96% as summarized by Lacey (1981). In addition, some fungal spores are viable but not culturable. Many basidiospores of mycorrhizal fungi, ascospores, and conidia of obligate parasitic fungi (such as *Erysiphe*) are difficult or impossible to culture on agar medium. They are either dormant, or require a stimulant or a host. Although these spores are most likely to be detected outdoors, they can get indoors through infiltration of buildings via cracks, doors and windows, or HVAC systems.

Ecology of Fungi In Indoor Environments

In nature, fungi occupy a wide range of niches, from animal guts to exposed rock in high mountains (such as lichens). In indoor environments, the prevalent airborne fungi found are similar to those detected outdoors (Yang et al., 1993). However, a number of fungi have frequently been encountered growing in building materials, such as carpeting, plaster, air duct liners, wall paper, water damaged ceiling tiles and sheetrock wallboard. These fungi include species of *Acremonium, Alternaria, Aspergillus, Chaetomium, Cladosporium, Paecilomyces, Penicillium, Stachybotrys* and *Ulocladium* (Morgan-Jones and Jacobson, 1988; Yang, unpublished). Their ability to grow on cellulose-containing products make them adaptable to indoor environments. In addition to the aforementioned fungi, there are many more fungi capable of growing on materials found indoors. Unfortunately, we know very little of fungal ecology in indoor environments even though we are the occupants of this environment. Recently, two new species of *Cladosporium* were described from building materials from Florida (Morgan-Jones and Jacobson, 1988). This discovery shows how much we know and still need to know about indoor fungal ecology.

Periodicity of Fungal Spores In Air

It is well established that certain airborne fungal spores peak during certain hours of the day or night. This periodicity is related to spore discharge mechanisms and environmental factors (Lacey, 1991). Fungal spores are released by two basic mechanisms: 1) active spore release and 2) passive spore release. For details on these two mechanisms, please refer to Lacey (1991).

Fungi with active spore release include such common airborne fungi as *Sporobolomyces, Epicoccum, Nigrospora*, and some smut-like yeasts. Dry spore fungi such as *Aspergillus, Penicillium*, and *Cladosporium* become airborne by passive force, such as air movement. *Sporobolomyces* is usually most abundant at night. Its spore release requires the absorption of moisture to build up release pressure. *Cladosporium* usually dominates airborne spore population during the day. Its

spores stay airborne owing to the upward movement of warmer air. It is, however, not clear whether the periodicity of spore release exists indoors.

Fungal spores are often released in a short time period and form a "spore cloud." The spore cloud may persist for a period of time until it is dispersed by air mixing. Results of air sampling can be greatly affected by whether there is a direct "hit" of the spore cloud or not. The magnitude of difference of "hit or miss" can be 100 times or higher. For more details on the spore cloud and its dispersal, see the excellent review by Lacey (1981).

Some fungi produce spores in slimy mass. These fungi include such indoor contaminants as *Stachybotrys, Fusarium, Trichoderma,* and *Acremonium.* Slimy spores may become airborne only when they become dry and disturbed or when they are attached to other particles. Their dissemination may be assisted by insects, animals, or water. Because slimy spores do not become airborne easily, its detection indoors should be considered significant. Any detection of *Stachybotrys* in air samples taken indoors should trigger further investigation of and search for the fungus. In a large indoor air monitoring study (USEPA, 1990), the detection of *Stachybotrys* sp. at low concentrations in three different locations of the same building failed to arouse any suspicion of possible contamination by this fungus because of low concentrations. This suggests that most investigators fail to recognize that there are different ways of spore dissemination.

CONCLUSION

Fungi have played a historic role in human affairs. They have become a major health problem in buildings where moisture control is poor or water intrusion is common. To prevent and control fungal contamination and amplification, better understanding of the basics of fungi in indoor environments is necessary.

Identification and characterization of fungal contaminants require the understanding of fungal taxonomy and nomenclature principles. The use of fungal names should be current and consistent.

Fungal ecology and factors affecting their growth, dispersal, survival and longevity in the indoor environment should be studied and understood.

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CYTOTOXICITY TESTING OF SAMPLES ORIGINATING FROM PROBLEM BUILDINGS

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Abstract: *The use of a modified colorimetric bioassay (MTT-cell culture assay) for the evaluation of the biological effects of moldy building material is reported. The MTT-cell culture test was carried out with a swine kidney cell line, sensitive for a wide range of mycotoxins. Four samples of crude extracts of paper-sheeted gypsum boards, contaminated with fungi of different genera* (Aspergillus, Alternaria, Penicillium, Fusarium, Stachybotrys) *were found to be highly cytotoxic in this assay as compared to a non-molded control. The presence of mycotoxins such as macrocyclic trichothecenes produced by* Stachybotrys *sp. but also cytotoxic metabolites produced by the other fungal genera could therefore be considered.*

Key words: Mycotoxins, cytotoxicity, bioassay

INTRODUCTION

Up to now the group of mycotoxins comprises about 400 compounds, all of which have in common that they are produced by different fungi. About 350 species are known to be able to form mycotoxins, and when considering the variations within the species, it is estimated that there are about 10,000 mycotoxin producers. Among them, the fungal genera *Aspergillus, Penicillium, Fusarium, Alternaria* and *Stachybotrys* have been recognized as major toxin producers. However, only a minority of the group of mycotoxins are known to occur under natural conditions, that is mainly as contaminants of food and feed. Eating of mold-contaminated food and feed is the most common route of entry of mycotoxins to man and animals, respectively (Betina, 1989). Besides, there is a considerable inhalation exposure risk when high count of airborne fungal spores are present (Smith et al., 1993). The biological effects of mycotoxins are manifold because of the quite different chemical structures of these low molecular weight compounds. Detection of mycotoxins could be carried out by

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physico-chemical methods, immuno-chemical methods and bioassays. Every technique has its particular advantage and is used depending on the analytical problem and the nature of the mycotoxin in question. Methods such as TLC, GLC, MS and EIA have in common that they are capable of detecting mycotoxins in samples far below those concentrations considered to be harmful to animals and humans. Nevertheless, there is no universal method for detection and quantification of the whole range of mycotoxins.

In practice, especially when different toxinogenic fungi are found on sample materials, the presence of more than one mycotoxin must be taken into consideration. The detection of many mycotoxins by chemical methods is not practical (Robb et al., 1983) or connected with an enormous analytical expenditure. In these cases bioassays, although not selective to identify individual compounds, can be used for screening purposes. They are able to alert the presence of a wide range of toxins which may be then subjected to chemical analysis.

Among the bioassays, the cell culture techniques have been proven to be very helpful for screening toxic activities of quite different sample materials. In previous studies we reported on the suitability of the MTT-assay for the cytotoxical evaluation of not only a series of mycotoxins standards but also on its use as a sensitive bioassay in screening of mycotoxin—contaminated sample materials such as cereals and feed (Gareis, 1994; Hanelt et al., 1995).

The aim of this study was to examine the cytotoxicity of moldy water-damaged building materials by the use of this assay.

MATERIALS AND METHODS

Samples

Samples of paper-sheeted gypsum board originated from residential homes with occupants complaining about serious health effects after water damage of the buildings.

Mycological Examination

For determination of fungi, the samples were grown on malt extract agar as well directly observed by electron microscopy.

Extraction

Five to 10 grams each of the sample material were cut in small pieces and given into 250 ml flasks. Samples were soaked with 100 ml of methanol overnight. The flasks were then treated with ultrasonification and the methanol layer decanted into separate flasks. Extraction of the samples was repeated with 50 ml each of chloroform and methanol on a rotary shaker for 30 minutes. The organic layers were combined and filtered through a paper filter. The filtrate was concentrated by rotary evaporation and the concentrate redissolved in 5 ml of aceton:methanol (2:1,v/v). As a control, non-moldy spots of the gypsum boards were selected, combined and extracted in the same way.

Cells

Swine kidney cells (SK), derived from an adherent cell line with an epitheloid morphology, were used as target cells and grown in an atmosphere of 5% CO_2 at 37°C in MEM (Minimum Essential Medium) with Earl's Salts supplemented with 200 IU penicillin/ml, 200 µg streptomycin/ml and 5% fetal calf serum) in microtiter plates until monolayers were dense (about 24 hours). Eight wells of the 96-well tissue culture place (row 1) remained empty and served as blanks.

MTT-Cell Culture Test

The principle of this bioassay is based on the clearage of the yellow tetrazolium salt MTT by viable, living cells to purple formazans (Fig. 1) and was carried out as described earlier (Hanelt et al., 1995).

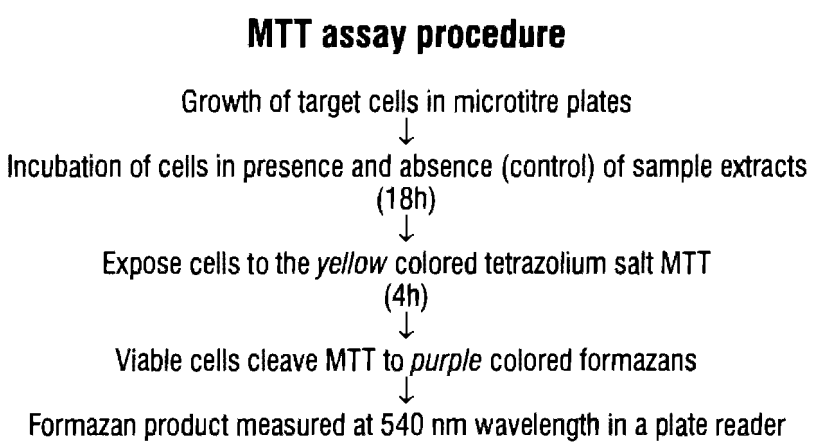

Fig. 1.

After decanting the cell culture medium from the microtiter plates, 100 µl of the complete medium containing 1.7% ethanol and 0.3% DMSO (v/v) were added to each well. Aliquots of the sample solutions were evaporated and dissolved in MEM containing 1.7% ethanol and 0.3% DMSO (v/v). Solubilization of the compounds was supported by ultrasonification. On separate plates serial log 2 dilutions of the sample solutions were prepared and 100 µl of each dilution were transferred in duplicates to the cell culture plate. Final concentrations of the samples tested ranged from 3.8 to 500 mg/ml of cell culture medium. Eight wells (row 2) served as cell control and received 100 µl of medium only. MEM containing 1.7% ethanol and 0.3% DMSO (v/v) was used throughout for serial dilutions and cell controls. All plates were incubated for 20 hours at 37°C in a humidified atmosphere with 5% CO_2. A volume of 20 µl each of the MTT (3-[4,5-dimethylthiazol-2-y1]-2,5-diphenyltetrazolium bromide) stock solution was then added to the wells and plates incubated for another 4 hours. Supernatants were then removed using a multichannel

micropipette and 100 µl DMSO were added to each well in order to dissolve the dark formazan crystals. The microtiter plates were thoroughly mixed on an agitation table for 5 min., and the optical density of each well was measured spectrophotometrically with an ELISA-Reader (Titertek Multiscan MC) at a wavelength of 510 nm using row 1 as the blank. Mean extinction values and standard deviations of each sample concentration were compared with those of the corresponding control and expressed as % cleavage activity in comparison to cell controls (100 %). The statistical significance of the test results was calculated by the students' t-test at p=0.01. The minimum concentrations of the test reagents to cause toxic effects were determined on the basis of the statistically determined values of 80% cleavage activity.

RESULTS

The mycological examination proved the presence of various fungi, with *Stachybotrys atra* and *Aspergillus* spp. predominating (Table I). Besides, *Fusarium* sp. and *Scopulariopsis* sp. have been recognized as contaminants.

Table I. Results of the cytotoxicity testing of samples originating from problem buildings.

Sample	No.	Origin	Mycoflora	Cytotoxicity
Gypsum board	2105-2	Oakland	*Stachybotrys atra*, *Scopulariopsis* sp.	+++
Gypsum board	2105-3	Oakland, Room 19 Crawlspace	*Fusarium* sp.	+++
Gypsum board	2105-4/5	Oakland, Rooms 4, 15, 16 Crawlspace	*Alternaria* sp. *Aspergillus nidulans*, *Stachybotrys atra*	+++
Gypsum board	Control from 2105-2/3/4/5		negative	–
Gypsum board	2204-1/2	Chicago Home Washer Room, Basewall	*Aspergillus wentii* *Aspergillus fumigatus* *Scopulariopsis* sp. *Stachybotrys atra*	++
Gypsum board	2204-3/4	Chicago Home Storage	*Aspergillus* sp. *Penicillium verrucosum*	+

Compared to the crude extract of the control, the extracts of all other samples reduced the MTT-cleavage activity of the SK-cells significantly, indicating the presence of cytotoxic mycotoxins in the sample material. As cytotoxic activities were also noted in samples not invaded by *Stachybotrys atra*, the occurrence of mycotoxins other than macrocyclic trichothecenes in these samples have to be taken into consideration.

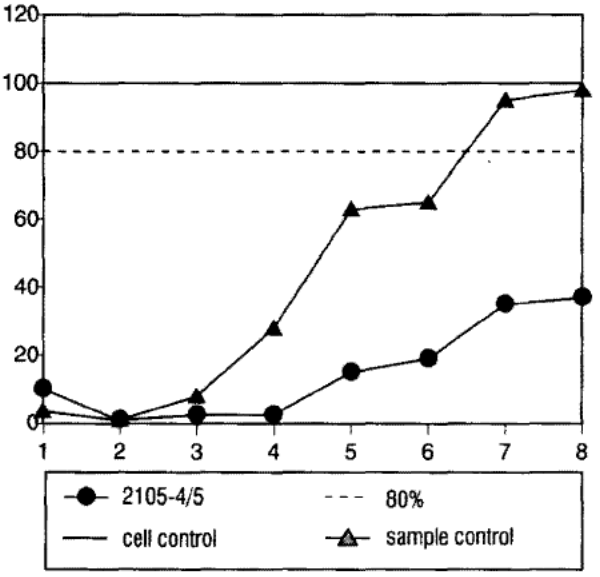

Fig. 2. Dilution step (log 2)

The graphs in Fig. 2 directly compare the control with the sample no. 2105-4/5. Cleavage activity of the cells was reduced till dilution step 6 of the control, while sample no. 2105-4/5 reacted cytotoxic in the assay till dilution steps below 8.

DISCUSSION

In 1983, Mosman described an alternative colorimetric cell culture assay using the tetrazolium salt MTT to measure cell proliferation and survival. The principal of this reaction is the reduction of the yellow colored MTT by mitochondrial enzymes to purple formazan crystal (Altmann, 1976). Based on this principle, MTT-assays have been used for various medical, microbiological and toxicological approaches. For mycotoxinogenic questions we have modified this assay and adapted the system (Gareis, 1994, Hanelt et al., 1995) for screening of crude extracts of samples of quite different origin. Swine kidney cells were routinely used as target cell line because its sensitivity toward a wide range of cytotoxic mycotoxins (Hanelt et al., 1995). The aim of this study was to examine the cytotoxic potency of building materials associated with human mycotoxicoses and to prove the suitability of the MTT-cell culture assay for such particular specimens. The results show that crude extracts of four of the samples were cytotoxic to the target cells as compared to the control. Different fungi (*Aspergillus* spp., *Scopulariopsis*, *Penicillium* spp., *Fusarium* sp. and *Stachybotrys*) were found as con-

taminants of these samples, that is why the presence of not only the macrocyclic trichothecenes of *Stachybotrys atra* must be taken into consideration.

Based on these results, the general cytotoxic potency of samples has been proven, which strengthen the exposure risk when inhaling dust and particular of this origin. However, further analytical work has to be carried out to identify the particular mycotoxins in question.

CONCLUSION

The etiology of mycotoxicoses must not be considered to be mono-causal. Toxigenic fungi produce not only one or two but several toxic compounds. Although improved analytical methods are available, it remains difficult to examine the relationship between exposure to mycotoxins and human health and to diagnose mycotoxicoses. Because of the wide toxigenic properties of the fungal species found to grow on different materials, analysis of particular mycotoxins could fail and are limited to known mycotoxins. The suitability of bioassays such as the MTT-cell culture assay as a rapid screening method has been proven in this study. Results allow to state if cytotoxic compounds are present and let identify the particular material as a risk factor to human health.

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ASSESSING IMMUNOTOXIC EFFECTS IN HUMAN POPULATIONS: LOGISTIC AND TECHNICAL ISSUES

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Abstract: *In performing field studies of workers exposed to xenobiotics, exposures may occur thousands of miles away from a facility that has the equipment or personnel to perform a detailed immune system evaluation. Because of this, numerous sampling and logistical problems have to be addressed to perform a quality study. Population selection and characterization as to confounding potentially immunotoxic exposures has to be factored, to the extent feasible, in the study design and interpretation. Selection of immunological endpoints has to be directed from both a rigorous review of the literature and the potential predictability of measurable endpoints to detect immunomodulation. Minimization of logistical problems which could affect results (time from sample acquisition to analyses, sample environmental control during transportation and before analyses, etc.) is paramount in the study design to minimize systematic effects. Validation of the methodology used with respect to reproducibility and accuracy and general quality assurance of all acquired data is also desirable.*

Key words: Epidemiologic pitfalls, selection bias, case-control, occupational hazards, immune system, xenobiotics, immunodulation

INTRODUCTION

Field studies in humans designed to detect immunomodulation from exposure to xenobiotics present some of the most challenging problems to epidemiologists and immunotoxicologists. Investigators must choose exposed populations with adequate exposure levels to detect potential effects, quantitate exposure on an individual basis if possible, and rule out concurrent exposure of the population to other potential immunomodulatory factors. A control group

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must be identified which is similar to the exposed group in all characteristics except for exposure to the xenobiotic under study.

Many exposures and circumstances can affect immune function in an individual, including sunlight, stress, medication use and illness; some of these factors may produce immune alterations as great or greater than those predicted from occupational or environmental exposure to xenobiotics (NRC, 1992; Plotnikoff, et al., 1991; Clement-Lacroix, et al., (1992); Hersey, et al, 1993; Baadsgaard, et al, 1990).

In vivo ultraviolet-exposed human epidermal cells activate T suppressor cell pathways that involve CD4+CD45RA+ suppressor-inducer T cells. Such exposures can best be evaluated by administering questionnaires to subjects, which must necessarily include sensitive topics such as recreational drug use and HIV infection. Sample acquisition (usually of peripheral blood and/or saliva) is performed at sites geographically and temporally distant from the controlled environment of an investigator's laboratory, yielding an assortment of new problems that would not occur in a clinical or hospital situation.

Some assays, such as lymphocyte transformation tests, which require almost immediate processing of blood samples, are difficult in field studies where blood samples must be transported to the laboratory over large distances or from remote locales. Some immunological and clinical tests which might yield important data in some studies (e.g., vaccination of study subjects to measure primary antibody response or bronchial provocation testing with workplace antigens) are rarely if ever used in immunotoxicity studies because of concerns about the risk/benefit ratio to study subjects.

Subjects involved in immunotoxicity field studies must be briefed about the nature and purpose of the study, provide informed consent and be notified of their individual test results and their possible clinical significance as soon as feasible after testing is complete. Since the application of immunotoxicologic techniques to populations exposed to xenobiotics is relatively new, there are difficulties in the interpretation of statistically positive results and their potential health significance.

Clinical Assessment

Clinical assessment of individuals usually starts with evidence of an immunological deficit or dysfunction. This immunological deficit or dysfunction is then investigated in order to associate the effect to exposure to a particular drug, toxic agent or exposure.

This section will focus on studies of groups of individuals with occupational or environmental exposure to a potentially toxic agent to detect (usually) subclinical immune changes. General issues in epidemiological study design and analysis are discussed in several texts (Rothman, et al, 1988; Kelsey, et al, 1986). The most common epidemiologic study design used in immunotoxicity research is the cross sectional study.

In such a study, exposure status and immunologic function are measured at one point in time or over a short period of time in study subjects. The immune

function of "exposed" subjects is compared to the immune function of a comparable group of "non-exposed" individuals.

The first challenge in conducting an immune assessment study is to identify the "exposed" group. In studies designed to evaluate the immunotoxicity of a chemical (as opposed to studies where immune function evaluation is prompted by a public health concern), the study should include populations at the upper end of human exposure unless previous studies have already established an immunotoxic effect in that range. Where possible, the study should incorporate individual estimates or measurement of dose, and utilize biological monitoring to estimate internal dose.

Once the exposed group has been identified, a clear definition is needed of who is eligible to participate in the study. For example, in an occupational study, all exposed individuals who have worked in a particular department might be considered eligible, while in an environmental study, eligible persons might include all residents of a community or a sample of households in a community.

It is important to enumerate the number of potentially eligible subjects, as well as the number who eventually participated, in order to assess if selection bias may influence the study findings. Selection bias may occur when an individual's willingness to participate varies with characteristics related to exposure status or health status of the individual. Although it is difficult to avoid or detect selection bias in a voluntary study, a high degree of participation makes it less likely that selection bias has influenced the results.

In many field situations, the potential immune effects of other chemicals present in the industrial or residential environment needs to be considered. The investigator should test for the presence of chemicals in the "exposed" and "control" environments and whether any other chemicals have either known or suspected effects on the immune system.

Exposures of individual study subjects to chemicals outside the study environment should also be evaluated. For example in an occupationally-based study, subjects could be questioned about chemical exposures in hobbies or second jobs. In a study of community residents exposed as a result of environmental contamination from a nearby factory, an assessment should be made of other contaminants in the local environment, as well as potential occupational chemical exposures of study subjects.

Statistical Concerns and Risk Factors

Known risk factors which might influence the outcome of immune function tests (such as age, gender, cigarette smoking, sunlight exposure, stress, use of certain medications and recreational drugs) should be matched in the design of the study or controlled in the analysis. However, there is limited quantitative data on the influence of these factors on immune function in the general population, and it is impossible for an epidemiologic study to match or analyze all potential factors. For example, differences in dietary habits, exercise levels, or community specific exposures to particular viruses might conceivably influence

comparisons between an "exposed" and "non-exposed" population. Yet it may be impossible to collect information on all such factors.

It is therefore desirable to select the control group to be as similar as possible to the exposed group in order to help match factors which cannot be measured. For example, in an occupationally-based study, the comparison group should be selected so that the community of residence, socioeconomic status and ethnicity is similar to the exposed group. In an environmental study, the comparison group should be selected from a geographical area whose residents have a similar ethnic distribution, socioeconomic status and employment pattern as residents in the exposed area.

The study should account for medical factors that might have a major impact on immune function. For example, individuals who are immunosuppressed as a result of chemotherapy or steroid treatment should be excluded from the study. Other medications and medical exposures (immunizations, medications, radiation) in the recent time period should be inquired about and evaluated in the statistical analyses.

Because many of the immune function tests have potential variability between laboratories or within a laboratory over time, it is desirable to have each test run by the same laboratory for all "exposed" and "unexposed" subjects in a particular study (or if that is not possible, to at least ensure that equal proportions of "exposed" and "unexposed" subjects are tested by each laboratory). The laboratory conducting the tests should validate each test procedure to assess measurement variability and variability within subjects before the analysis of study samples begins. It is desirable to recruit exposed and unexposed subjects into the study during the same time period, so that samples analyzed on a particular day include both types of subjects. This will minimize the effect on the study findings of any undetected changes in laboratory conditions that shift test results upward or downward on a particular day. The number and type of immunological endpoints which can conceivably be measured in a human clinical study is enormous.

Because of sample concerns for some tests, most have to be chosen *a priori*. Rigorous review of the available animal and human literature on the effects of exposure to a particular xenobiotic as well as the predictability of immunological endpoints measured in animal models to detect immunotoxicity (Luster, et al., 1992) have to be considered.

Interpretation

In addition to the characteristics that should be considered in evaluating the methodology of study, there are some issues that are particularly important in the interpretation of positive studies, and others that are particularly important in the interpretation of negative studies. In interpreting studies which show significant differences between the exposed and unexposed populations, it is important to recognize that large cross-sectional studies (i.e., studies with 100-200 exposed and control subjects, will have adequate statistical power to detect

relatively small differences (on the order of 10%) in immunological endpoints such as proportion of CD4+ and CD8+ cells).

Allthough such studies might be interpreted as positive, particularly if there is evidence of a dose-response relationship, there will be considerable overlap between the values of unexposed and exposed individuals, and it is possible that none of the individual values will fall outside the clinically normal range. Interpretation of such findings is complicated because there is little quantitative data on the degree to which such parameters need to be modified in a population before the population experiences an increased risk of disease (Trizio, et al., 1988).

In addition, because statistically significant differences measured between the two populations may be small in magnitude and of unknown clinical significance, the possibility that they reflect methodologic errors rather than a true biological effect is of concern. Methodologic problems that might spuriously produce such a finding may include selection bias, laboratory variability or lack of control for confounding variables. Careful design and analysis of studies examining changes in immune function tests as the primary outcome is therefore of critical importantce.

Evidence of a dose-response relationship is usually an important criterion in the assessment of a toxic exposure. However, both biological and methodological factors complicate the assessment of dose-response in human immunotoxicity studies. Traditional dose-response relationships may not always be present for immunoloogically-mediated effects. For example, experimental models suggest that high doses may be inhibitory while low doses might be stimulatory (Biagini, et al., 1993). For hypersensitivity phenomenon, the question of individual susceptibility or atopy may complicate the assessment of "dose-response" in the population.

In evaluating a "positive" study which does not demonstrate a dose-response relationship, a general issue is whether the dose estimate employed takes into account data on the absorption, metabolism and distribution of the chemical. Frequently, epidemiologic studies assume that the quantitative dose measurements available (i.e. concentration of the chemical in air, blood or urine) are proportional to potential dose at the target organ of concern (i.e. bone marrow, primary or secondary lymphatic organs). This is not always the case.

For example, air concentration may be a poor surrogate for internal dose if the compound can be absorbed through the skin as well as inhaled, or if respiratory protection has been used by some workers and not others. For chemicals with a long half-life, such as lead, an identical urinary lead concentration may reflect substantially different tissue-specific concentrations in long-versus short-term workers.

In addition to inferring dose level estimates for individual subjects from quantitative measurements taken at the time of the study (or from using available historical measurements) the dose-estimate used in the model should be restricted to what is considered the most biologically relevant time period. For

example, in a cross-sectional immunotoxicity study, the relevant time period may be the previous six months rather than the cumulative lifetime exposure.

Because the biologically relevant time interval is not known, and may differ for different immunologic outcomes, the assessment of dose reponse is even more complex than in epidemiologic studies of other outcomes. The healthy worker effect (HWE, apparent decreased mortality and morbidity in workers when compared to the general population) is also a potential confounder in the interpretation of the immunotoxic outcomes from exposure to xenobiotics, especially when exposed workers are compared to "normal reference values" for a particular outcome (Choi, 1992).

In evaluating a negative study, one important issue is whether the study size was adequate to detect a difference of a specified size in the immune function parameters of interest (statistical power). The statistical power of a study to detect a difference between two populations in the mean of a continuous variable (such as serum IgG level, proportion of CD4+ lymphocytes) depends on the size of the study groups, the mean and variance of the outcome in the study groups, the specified type I difference, and the size of the difference to be detected (Colton, 1974).

Power calculations are usually done *a priori* in planning an epidemiological study. However, there are occasions when they may be useful in interpreting a negative study result. In comparing the results of contradictory studies, one issue that should be considered is the precision of the differences in point estimate (i.e. the confidence interval for the estimated difference between the two groups). Of equal importance in evaluating a negative study is whether there is evidence that the "exposed population" actually had substantial (well documented biomonitoring or environmental sampling indicating exposure) to the xenobiotic of interest.

Other types of data on the immunotoxic effects of chemicals in humans are potentially useful. Case reports may arise from clinical identification of individuals with a particular exposure who have immune function changes or a disease of the immune system. Such reports are particularly valuable in generating hypotheses for well-designed epidemiologic studies, and may provide support for other toxicologic or epidemiologic data.

Other Epidemiologic Designs

Aside from the cross-sectional study design, two alternative epidemiologic study designs may be utilized in immunotoxicity studies. In longitudinal studies, one or more groups of people that are free of disease and that differ according to extent of exposure to a potential cause of the disease are compared with respect to incidence of the disease in each of the groups. A variant of this design that might be utilized in immunotoxicity studies would compare immune function test results within individuals before and after a defined exposure. Another variant would define "exposed" and "unexposed" groups cross-sectionally, administer immune function tests and follow the subjects prospec-

tively to assess relationships between immune function test results and development of clinical disease.

In case-control studies, persons with a given disease (the cases) and persons without a given disease (the controls) are selected: the proportion of cases and controls who have certain background characteristics or have been exposed to possible risk factors are then determined and compared. It should be considered that case definition may include individuals with a continuum of immunologic changes from exposure ranging from homeostatic immunologic responses to frank immunologically mediated disease. Case-control studies of the etiology of immunologically mediated diseases might identify increased risk of previous exposure to particular chemicals among the cases; such a finding would be particularly relevant if supported by toxicologic studies or evidence of immune function changes among humans exposed to the chemical.

CONCLUSION

The potential problems associated with designing and performing studies to detect immunomodulation from exposure to xenobiotics in humans have in common potential shortcomings with human epidemiologic studies in general, as well as some problems specific to studies concerning the immune system. From a population selection standpoint, control and exposed individuals have to be well characterized with as much information as possible concerning the body burden of the xenobiotic in exposed individuals, preferably with quantifiable measures such as biological monitoring.

Potential confounding factors (e.g., lifestyles, pre-existing disease, etc.) in both the exposed and control populations have to be factored, to the extent feasible, in the study design and interpretation. The selection of which immunological endpoints to be studied has to be directed from both a rigorous review of the literature concerning the immunotoxic potential of the exposure in question and the potential predictability of measurable endpoints to detect immunomodulation.

Minimization of logistical problems which could effect results (time from sample acquisition to analyses, sample environmental control during transportation and before analyses, etc.) is paramount in the design of the study to minimize systematic effects. Validation of the methodology used with respect to reproducibility and accuracy and general quality assurance of all acquired data is also desirable.

While clinical epidemiologic studies designed to detect immunomodulation are inherently difficult to perform and the acquired results sometimes inherently confusing due to wide variabilities in "normal ranges", they can be successfully performed with attention to pre-study planning and constant vigilance for potential systematic (or non-exposure related) effects. For a more in-depth review of the potential problems associated with designing and performing studies to detect immunomodulation the reader is referred to Biagini, et al., 1994.

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PANEL DISCUSSION 10/7/94

This discussion was transcribed and edited from the videotape of the conference. We apologize in advance for any inaccuracies due to technical difficulties. —The Editors.

Panelists: Auger, Flannigan, Morey, Rylander, Yang

Eckardt Johanning. I have a written question here. Can air samples from rooms with automatic ventilation be collected from air outlets? There is a graph on the sheet. Who wants to answer this?

A. ***Phil Morey:*** It looks like a diagram of air going in a room and then air going out through a return air grill. Looks like filters to collect spores on a filter put over a return air grill. I expect that it is a way to get a historical sample of what has been in the room. I don't know that anyone has done that, but it is reasonable. It looks like a filter on the return air grill and that would be a good way to get a historical air sample, although I would probably vacuum the carpet as another good way to get a historical air sample.

A. ***Ed Olmsted:*** In terms of trying to sample the air through a return, in order to collect spores you would need a very efficient filter and I suspect that the fan system would not be able to pull much air through the return, you would not be able to control the flow rates. It is kind of a hard one, possible but really it would take a lot of work.

Q. *Just as a follow-up to that question, would the spores that you collected, for example on a HEPA filter, to try to get a qualitative idea of what kind of molds were in that area, would those spores still be viable? If you sent the filter to the lab, could the lab identify the species?*

Ed Olmsted: You mean if you had a HEPA filter in a pan or tray?

Q. *For example, if you had a filter in an apartment and then you send that whole HEPA box to the laboratory, would the spores still be viable?*

A. ***Chin Yang:*** I guess some would still be viable, but some may not be.

A. ***Phil Morey:*** Just a brief comment. In the aspergillosis literature, which is a lung infection in hospital areas where there are immune compromised patients, there are a couple papers describing how HEPA filters were actually cultured, showing the organism was the same as that which caused the infection. Organisms may be viable on HEPA filters, the question would be, did the filter ever get wet? That would be another issue.

Q. *A question on sampling, particularly the denominator that you use for mold sampling per gram of settled dust or per square centimeter. Are there any guidelines or thresholds? It seems to me it is hard to use those numbers in any quantitative way, do you have any recommendations on that? Do you try to scrape it off or do you just grind up the whole filter? It would seem to me that the denominator is going to raise or lower your numbers dramatically and it would be very hard to compare a sample even from one part of a building to another.*

A. ***Brian Flannigan:*** I think the problem with settled dust is that the count you get will depend very much on the methods you use. In Europe, it seems as though there are two ways to get some sort of viable count. All some people do is to take 30 mg. of dust and spread it on a plate. Other people take 100 mg. of dust, put it into a diluent, shake it up, make dilutions and plate those out. They get far higher counts than you would with a 30 mg. sample of dust.

Q. *Also, if you use detergent or not that will make a difference?*

A. ***Brian Flannigan:*** Yes, they possibly might use trenated or peptone, but some people just use peptone, which will break up the spore clumps.

Q. *How do you compare that to the other bulk samples? That is even more difficult.*

A. ***Brian Flannigan:*** Yes, yes, I mean I don't think you can compare the numbers, but the point is, that it is the species that you are interested in. The species that are in the air ought to be reflected in the dust. As with any other bulk samples that you have, if you take a bulk sample from the wall, then you presume that that is having an influence on the air spora and if you box up the wall, that should be reflected in the air spora. So the range of species that you get should correspond, except you expect that there are some organisms that you will not find in the dust but you will find them in the air.

A. ***Phil Morey:*** Just one other brief comment that controls are always important. If you were doing say, vinyl wall covering, a case building and a control building, maybe some brand new wall coverings, say with filters, you could do it per gram, per square centimeter, or whatever, but new filters, filters from an air handling unit serving an area without a problem, and then your case area.

Q. *This might be addressed to a panel with more physicians on it, but I am starting to get more questions from the public in cases where there are known or suspected mold contamination problems where these patients are being treated by their physicians for either hyperactive or hypoactive thyroids and I haven't heard that specifically referred to today. I am wondering if this is a mere coincidence or if others are seeing this and what it might mean?*

A. ***Pierre Auger:*** That is a question I asked myself once in school. I had a case of thyroid disease that did appear. I did not find any literature on this, right now I can not say more on that.

A. ***Ragnar Rylander:*** It is difficult to give a reason why, but I think that by tradition, when allergists see someone suffering from lung problems, they think it is an allergy then build on these lines and allergies can cause these conditions and they then treat for allergies. I keep hoping that the number of people allergic to the whole environment that you see is very very small; and a lot of people suffer because they are worked up for allergic disease and then diagnosed as negative for allergies. Then they get the message "go home, you are hysterical." I am sorry to say this but it may be lack of knowledge of what is wrong by the physicians.

Questioner: In one instance, the caller said her thyroid was the size of a hen's egg, so there was probably something going on.

A. ***Ragnar Rylander:*** We can never draw any conclusions from one case. I would say that there is very little that suggests that there is a direct relation between these two.

(Unidentified) I just had a quick comment about the thyroid question. Thyroid problems are fairly common in the population and it is a standard part of a medical work up if the patient comes in complaining of fatigue, and other ill sorted symptoms. A physician is likely to order a thyroid test in children, a thyroid test and you can find a lot of disease. And so it may be just a coincidental thing that a lot of these patients are being found to have hyper or hypoactive thyroids. Something or nothing to do with symptoms.

Q. *I would like to elicit a little more information on some of the clinical immunotoxicities. In particular I am interested in the talks concerning people that have been poisoned, or affected by mycotoxins that develop these immunological symptoms, neurologicial symptoms and some of those people then go on to recover in a fairly short period of time. It is new to me to hear that a lot of those people do not go on to recover in a fairly short period of time. In fact, I think some of the data suggests that some people are sick ten years later if I interpreted what I heard correctly. I was wondering if anybody, besides following patients up clinically, did follow them up immunologically and found the same sorts of abnormalities in T cell subsets and things like that, after they recover, in people that do recover, or four or five years later in people who don't recover.*

A. ***Eckardt Johanning:*** In the New Yorker case we have not done the follow-up studies. The variations may be very great within each individual, depending on other factors that also can affect the immune system; it is very difficult to do it on an individual case.

A. ***Pierre Auger:*** I have not done much in the way of immunological study. We did it on the worker in the hospital but I was not sure of the data I got from the test laboratory, so I did not carry on afterwards. In the last cases I presented I did the immunological studies but these people had been away from exposure for at least four months.

Q. *I have a question regarding the ecology of microbes within the micro environments of the building. For example, within a contaminated ventilation system, has anyone seen or can they explain what sort of dynamics and variation are taking place in this environment on a seasonal basis. What affects the blooming of microbes under these sorts of conditions?*

A. ***Chin Yang:*** Moisture and water are the key. You have to have moisture and water, which is usually not a problem. As long as you have enough moisture and water around, they will keep growing and producing more spores. In the drier winter season they may stop growing until the next cycle.

A. ***Brian Flannigan:*** This is not really connected with growth in the system, but what we will find is that outdoor air will have very obvious seasonal variation. In summer, then, what you get are the phylloplane fungi in relatively large numbers, whereas in winter those organisms that you will find on leaves and the like will be in relatively low numbers. In fact, the total air spora will be much lower in winter. Therefore what you might expect to find in indoor air in summer is a higher proportion of phylloplane fungi than you might find in winter.

A. ***Phil Morey:*** I don't know that anyone has really studied this in detail. I can remember a couple of studies on induction units during the summer. Induction units are perimeter units under the windows of large buildings and the coils get moist but not terribly wet. You will find yeast, rhodotorula, sporobolomyces, if you sample above those units. But as the units dry out, and they are not used in the winter, you will no longer pick up those organisms. But no one has really studied month by month, that I am aware of.

Q. *I have seen a pattern, in schools for example, where once the heating season begins, there seems to be a higher rate of respiratory complaints. When there is clear microbial contamination in the ventilation system, how do the microbes respond to heat stress, or could hyphae be dried out and liberated?*

A. ***Phil Morey:*** As things such as a biofilm dry out, they become friable and break up. It is conceivable that those particulates will be disseminated. Thinking about something like *Stachybotrys*, if it is growing it will be wet, slimy; if it dries out the particulates could be disseminated.

Miriam Lonon, NIOSH microbiologist: Excuse me, can I mention something that we just did that might have some bearing on this? I would like a response to it, because we have not finished analyzing the data. At NIOSH over one period of the summer we conducted a number of investigations. We collected and examined bits of insulation from inside ducts, then looked at all of these to see what bacterial and fungal genera we isolated. The most prevalent fungus was yeast, which is not surprising I suppose, and the two most prevalent bacteria isolated from these bulk samples were *bacillus insolitis* and *thermophilic actinomycetes. Bacillus insolitis* is known to grow and sporulate at 0 degrees C., so it is cryotolerant, and *thermophilics* of course are heat tolerant, and they are both spore formers, and so we postulated that perhaps there is a seasonal switchoff as we

go from heating to cooling. Comments? Does that sound reasonable? That would account for the increased symptoms as you go to the heating mode.

A. ***Brian Flannigan:*** Well, certainly I would think heating would have a profound effect on the growth of those organisms. Whilst you've got heating on, if you've got sufficient moisture I could expect both of those things to grow, but also you might expect some other things to be growing. Did you find any other...?

Lonon: Oh certainly. These were just the commonly isolated bacteria from the bacteria containing ones.

Brian Flannigan: What about things like pseudomonas and acinetobacter?

Lonon: It varied from one sample to another. There were some pseudomonads, certainly, and others that were isolated as well as a lot of human commensals such as staph, but we were struck by how often *bacillus insolitis* was isolated. Then we went back and looked at our reports from previous investigations where we found that it was very commonly isolated. We are still wading through all these numbers.

A. ***Brian Flannigan:*** Yes, so what you are getting there are organisms that are survivors, in a way, because they are gram positives. Your pseudomonas will not survive too well under drying conditions. And bacilli and spore formers and also I think the spores of *thermophilic actinomycetes* are relatively resistant to drying out vis a vis pseudomonas and things like that.

Q. *I think several people might be able to respond to this question. The numbers that Dr. Morey put up as levels of concern or perhaps to use Dr. Rylander's term of "beware number," as I recall, are about 50 CFU per cubic meter of air, and the presence of even a few or even single spores of something like* Aspergillus. *It occurs to me that that kind of number and that kind of situation might occur fairly commonly outdoors. Is it that we would find indoor environments to have other properties that would make this kind of situation a problem for people, or am I wrong that this is not a common occurrence for total spores to be in excess of 50 CFU per cubic meter and the presence of an* Aspergillus?

A. ***Phil Morey:*** I did not mean to imply a guideline or anything like that. That was just raw data, 50 or 100 CFU per cubic meter of *Aspergillus versicolor* which are 90% of your isolates. In those studies in Florida, there maybe have been one or two colonies of that species outdoors, but no more than that, so its a marker of an unusual environment. If I remember those studies you might have some *Aspergillus niger* and *Aspergillus ochraceus* in small numbers outside, but here we have a totally different taxa indoors and it is telling you that there is a sense of which the numbers are defined.

A. ***Brian Flannigan:*** Yes I would say it is not so much the number, it is the fact that the organism is there. I have never got *Stachybotrys atra* from outdoor

air. If you find these inside the building you know or can be 99% certain that there is a dampness problem inside that building. You may not see it, you may have to strip down the walls, but it is there.

Q. *Yes, I was speaking of* Aspergillus niger *or* fumigatus, *though, which can both be found indoors as well as outdoors and probably are treated as a problem if found indoors.*

A. ***Brian Flannigan:*** I will say that you will not find *Aspergillus fumigatus* in any quantity at all outdoors unless you were next to a composting facility or something fairly unique like that. *Aspergillus niger*, in the U.K., at any rate, you very seldom find in outdoor air. There have been surveys done throughout the year; in the U.K. in outdoor air you might find *Aspergillus fumigatus* in larger quantities during the winter, which may seem strange, but this is tied up with feeding moldy materials to cattle and the like. This is where *Aspergillus fumigatus* would grow. If you've got it in a building, it also is a problem.

Q. *Gentlemen, you gave stories or case histories about where you went in and did sampling of evidently moldy wallpaper and wall boards and things of that nature, when you did that, was the objective to try and quantify what the products were, the molds, the bacteria that you were finding, or did you then use that information to help set up your remediation work, the type of isolation you would be utilizing? That would seem to me in a practical aspect the more useful results of that testing. I understand you have to be involved in the science behind it, but there are many people who unfortunately have clients that aren't going to be willing to pay for that. They come and say, "gee, we've got mold in here." I need a method of justifying to them that I should go in and do some extensive testing to identify the type of mold, or currently we make the assumption that if you've got mold it is a potential pathogen and err on the side of conservatism. Is that the approved method or is that overkill so to speak?*

A. ***Phil Morey:*** Some of that referred to my talk earlier this morning. In one or two of the buildings there is definitely building related illness or hypersensitivity pneumonitis. The main reason for the extensive sampling of the wall board and the vinyl wall covering was to try to get some sort of scientific basis on what should be removed. In other words don't remove wall board that is relatively uncontaminated. So the latter part of the question was the reason for much of the sampling. Then based on what was in the settled dust in interior areas of the building, non-moldy areas in the building, what type of clean-up needs to be done, short of physical demolition. So it was basically to guide the demolition aspect or the removal of the contaminated materials.

Q. *So in areas that may have shown some surface contamination, or of the wall board, or something of that nature, or insulation behind the wall, if you did not feel that the concentration was high enough, you would have recommended that they dry out and leave the material in place, versus, as one of the slides we saw yesterday showed, a multistory office building completely wrapped in plastic with the entire exterior being ripped off? Without knowing what the contamination level was throughout that building*

and what the building products are, it is going to be kind of hard to justify it when we've got an exterior block wall or something of that nature.

A. ***Phil Morey:*** I don't think you can generalize, and I tend to be driven by what the medical situation is in the building. If the building is to be reoccupied by say sensitized individuals, a couple of times I have seen that the physicians that I have been involved with in these studies want to have all the carpet, all the ceiling tiles, all the porous materials out of the building and then the non-porous materials cleaned up. Those are pretty rare situations where you have x number of cases of hypersensitivity pneumonitis. However, if your building were without disease or likely be without disease your remediation measures there can be less stringent. Use common sense and good judgement.

Q. *My next question is about building materials. I understand cement or pipes and cinder blocks are not an ideal medium for fungal growth, but in the presence of moisture, have you seen molds growing on these surfaces? Second question, if the proper stripping away of organic based building materials is done, could these types of materials that I have referred to serve as a reservoir for large numbers of spores, which can just let the problem come back again?*

A. ***Brian Flannigan:*** As far as brick work is concerned, absolutely fresh brick work uncontaminated with any organic materials will not support growth of microorganisms. But you will get all sorts of materials deposited on the brick work, it will condense on the surface and so on. If there is sufficient moisture this dirt will allow some molds to grow. I'll be saying something this afternoon about the actual moisture levels that are necessary. The main thing to notice is that everything in the building will potentially support growth if there is enough nutrient material; that nutrient material can be supplied by dirt, skin scales and so on. Therefore, you would have to look at all of these materials. But the moisture contents may differ quite considerably.

Questioner: *Most of the buildings with serious mold trouble that we know about at CMHC have really bad basements, and in many of those it is bare concrete. It didn't take very long, maybe a couple of months after construction, that there was mold on the concrete if it was periodically wet, it was growing well.*

A. Chin Yang: Fungus can contaminate masonry.

Eckardt Johanning: Thank you very much.

MYCOTOXINS AND NEUROTOXICITY

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Abstract: *Mycotoxins have been called "agents in search of a disease" (Schiefer, 1990). Medical literature contains little information concerning airborne mycotoxins. We would like to make the point that mycotoxins are potent neurotoxics agents. Chronic fatigue syndrome and psycho-organic syndrome comprise an array of symptoms which overlap. We considered both of these diseases as consequences of possible central nervous system injuries and hypothetically related to mycotoxins exposure.*

Key words: Mycotoxins, neurotoxicity, *Stachybotrys atra*, indoor air pollution, chronic fatigue syndrome, psycho-organic syndrome

INTRODUCTION

Fungi are one of the agents present in dust causing harm to human health. The lung and the upper respiratory airways have been considered the historical targets for disease subsequent to mold exposure. Mycotoxins are metabolites produced by fungi in certain favorable environment. They are polysystemic poisons and many of them are neurotoxic and immunotoxic. Since 1984, we have been confronted with patients complaining of fatigue, cognitive difficulties, repetitive respiratory infectious diseases and a complex array of symptoms consistent with the diagnosis of psychoorganic syndrome (POS) and/or chronic fatigue syndrome with immunodysfunction (CFIDS). We think that neurotoxicity from mycotoxins are an important aspect of mycotoxicosis. This report is based on soft data, on circumstantial evidence. Epidemiological analysis is not yet possible.

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SUMMARY OF TOXIC EPISODE INVESTIGATIONS IN VARIOUS BUILDING SETTINGS 1984-1994

Hospital[a]

Medical assessment has been carried out according to standard criteria for clinical evaluation. Chronic fatigue syndrome was defined by recognized signs (NIH Publication, 1990). One hundred sixteen (116) case summaries were forwarded to us. The main complaints were extreme fatigue and severe cognitive symptoms.

Fifty (50) were more thoroughly evaluated. They were:

nurses	29
patient attendants	15
secretaries	3
physiotherapist	1
occupational therapist	1
record keeper	1

The symptoms were:

Extreme fatigue at one point in time	100%
Neurocognitive problems	90%
Chronic sore throat	78%
Muscle soreness and/or weakness	74%
Frequent upper airways infections	50%

Routine hematological and biochemical findings were not specific.

Multiple air and surface samplings yielded by order of importance:

Stachybotrys atra
Aspergillus niger
A. Versicolor
A. Clavatus
A. Ochraceus
Penicillium brevicompactum
P. Cyclopium
Paecilomyces varioti

Alleviation of symptoms after decontamination among 22 available for follow-up:

2 had not recovered;
2 felt slightly better;
13 felt 80% to 100% improved;
3 felt symptoms were stress-related;
2 now had another diagnosis.

[a](Mainville, 1988)

Various Dwellings/Offices; Symptoms Relieved Upon Removal Of Moldy Material Or From The Setting.

- Husband and wife: extreme fatigue, hacking cough and cognitive symptoms
- Mother and 2 children: multiple respiratory infections, asthma, headache, fatigue
- 11 civil servants: in one office:
  5 with sick building syndrome
  3 with chronic fatigue syndrome
- Wife, husband, child with chronic fatigue syndrome with *Penicillium Brevicompactum*[b]
- Similar cases published recently[c] with Trichoderma viridae, *Penicillium frequentens, P. cyclopium, Phoma* species.

Harbour Station[d]

Medical evaluation was conducted by three physicians, one of whom is a neurologist. Neuropsychological testings were supervised by a neuropsychologist. Measurements of formaldehyde and Stoddard solvent were conducted by an industrial hygienist as recommended in "Le guide d'échantillonage de l'air en milieu de travail du Québec 9ième édition, *août* 1990". Measurements and identification were done by a mycologist.

This 50-years-old, two-story building with opening windows was free of formaldehyde and Stoddard solvent. The crawl space had often been flooded.

There were three occupants working in this building complaining of headaches, fatigue, sinusitis, problems of memory and intellectual concentration. Two occupants underwent more complete medical work-up three months after removal from exposure. The findings are summarized in Table I.

Aspergillus fumigatus, A. niger were detected in the sub-basement and crawl space, and *Penicillium aurentiogriseum* in the dwelling of the male worker.

Table I.

Male Director, 42 Years Old

Complaints:

Fatigue, irritability, cognitive symptoms, worst in fall and winter.

Physical examination:

Negative

[b](Auger, 1990)
[c](Auger, 1994)
[d](Auger P.L., Beaudet R., Bouchard R., Doyon J., Pépin P., Miller D.J., in preparation)

Laboratory:

Immunology: ↑ CD^4/CD^8 RATIO

Neuropsychological testing: Visual spatial anomaly, Type2 b encephalopathy

Female Secretary, 34 Years Old

Complaints:

Fatigue, headaches, cognitive symptoms, muscle soreness, recent asthma, worst in fall and winter

Physical examination:

Carpal tunnel and thoracic outlet syndrome

Laboratory:

Immunology: ↑ CD^4/CD^8 RATIO, ↑ IgM.

Neuropsychological testing: decreased cognitive functions. Type 2 b Encephalopathy

NEUROLOGIC DISORDERS AND NEUROTOXIC AGENTS

The environment is replete with toxins capable of damaging the nervous system: heavy metals, solvents, and pesticides are the most well known (Longstreth, 1994). Clinical manifestation can be acute or chronic. Encephalopathy is a frequent expression of harm following long-term chronic exposure. The earliest problems are subtle and include changes in behavior, mood and cognitive functions. More specifically for solvents, several cross-sectional studies from the Scandinavian countries have documented impaired neuropsychological performance among workers with long-term exposure to these agents. While some critics still doubt in the existence of this disease, the concept has gained wider acceptance, and two international workshops agreed on the possible association and refined the classification of neurobehavioral disorders caused by solvents. "There is currently sufficient support for the association of high long-term solvent exposure with long-lasting psycho-organic symptoms to consider this a clinical reality" (Lundbert I, et al, 1994).

The agreed classification is summarized below:

- Type 1: Symptoms only.
  Fatigue, memory and concentration disturbance, affective changes, sleeping disorders. Reversible 6-12 months after discontinuation of exposure.

- Type 2 A: Personality and mood changes.
  There are marked and sustained changes with obvious affective problems; apathy or aggression, *fatigue*, without cognitive abnormalities, partially irreversible.

- Type 2 B: Impairment in intellectual function.
  With mood changes, there is a difficulty in concentration, decreased memory and learning capacity. Psychometric tests are affected.
  This disease is partially irreversible.

- Type 3: Dementia.
  Global irreversible deterioration in intellect and memory often accompanied by neurologic signs and neuroradiologic findings.

CHRONIC FATIGUE SYNDROME

Chronic Fatigue Syndrome is a term recently coined for a long-known condition characterized by extreme fatigue accompanied by a polysystemic symptomatology: sore throat, headache and neurocognitive complaint. In 1988, clinical criteria were devised to help conduct epidemiological studies (NIH Publication, 1990). See Table II.

Table II.[a] Chronic Fatigue Syndrome: A Working Case Definition.

Major Criteria

1.—Onset of persistent or relapsing fatigue severe enough to reduce average daily activity below 50%

2.—Absence of other conditions producing similar symptoms

Minor Criteria

Symptoms

1.—Mild fever
2.—Sore throat
3.—Painful lymph nodes
4.—Muscle weakness
5.—Myalgia
6.—Fatigue after mild exercise
7.—Headaches
8.—Arthalgia
9.—Neuropsychological complaints
10.—Sleep disturbances
11.—Abrupt appearance

Physical criteria

1.—Low fever
2.—Pharyngitis
3.—Tender small lymph nodes

Diagnosis:

2 major criteria, and 8 clinical symptoms or 6 clinical symptoms and 2 physical signs

It must be pointed out that intoxication to heavy metals, pesticides and solvents have to be ruled out before the diagnosis of chronic fatigue syndrome may be invoked. Therefore it is easy to realize that clinical pictures of toxic encephalopathy and chronic fatigue syndrome vastly overlap.

PERTINENT MEDICAL LITERATURE:

There exist a number of different mycotoxins for which we have no data about their toxicity. Trichothecenes have been the object of more thorough laboratory testing. We know that they exhibit their toxicity through DNA and RNA synthesis inhibition. The brain and immunological system are therefore sensitive organs to these poisons (Feuerstein G., et al, 1989).

A considerable number of other fungal metabolites exist from which we have gathered few data. Preliminary results seem to unravel general and neurological toxics effects in many of them (Watson D. H., 1982). In Table III, it is possible to compare the acute neurotoxics effects of well-known agents compared to the only trichothecene studied in human (DAS).

Table III. Comparative Scale of Solvents and Mycotoxins Associated With Human Neurotoxicity.

Known Human Neurotoxics[a]
- Toluene: 65 mg/kilo (Inh-8hrs-70 kilos man)
- Xylene: 74 mg/kilo (Inh-8hrs-70 kilos man)
- Styrene: 37 mg/kilo (Inh-8hrs-70 kilos man)

Trichothecene In Man[b]
- DAS: 0.09mg/kilo (i.v. -rapid-70 kilos man)
- 0.26mg/kilo

A few case reports are gleaned from the medical literature. They can let us suspect the importance of neurotoxic effects of molds. Croft W., et al (1986), Johanning, et al (1993), and Recco P., et al (1986) described patients exposed to *Stachybotrys atra*. These people all complained of neuropsychological problems accompanied by fatigue with concomitant irritative symptoms. Nexo, et al (1983) relate cases of extreme fatigue alleviated by the removal of dusty carpets possibly contaminated by known toxicogenic *Fusarium* fungi.

Two other publications seem to entertain a similar hypothesis (Leving P.H., et al, 1992, Chester A.C., et al, 1994). They described cases of chronic

[a]From: Holmes GP, et al, Ann. Intern. Med. 1988:108:387-9.
[a]From: Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices, ACGIH, 1993-94.
[b]From: Feuerstein G. et al in chapter 5, in Trichothecene Mycotoxicosis: Pathophysiologic Effects, CRC Press, 1989.

fatigue syndrome in buildings with indoor air problems. Also, a few epidemiological studies of other symptoms than respiratory have shown significant relative risks of exposure to molds and humidity giving rise to symptoms like depression, aching joints, nausea, tiredness (Waegermaekers M., et al, 1989, Platt, et al 1989). Finally Gordon et al (1993) reported a neurological syndrome in a young man consisting of dementia and tremor possibly related to the presence of different toxicogenic *Aspergillus* and *Penicillium* in a moldy silage.

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HEALTH PROBLEMS RELATED TO FUNGAL EXPOSURE—THE EXAMPLE OF TOXIGENIC *STACHYBOTRYS CHARTARUM (ATRA)*

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Abstract: *The health status of office workers after exposure to fungal bioaerosol, esp.* Stachybotrys chartarum (atra) *and its toxigenic metabolites (satratoxin) was evaluated. Fungal exposure was characterized and quantified using microscopical, culture and chemical techniques. In a case-control study design, a 174 item health survey questionnaire and laboratory test (Red and white blood cell system, multi-chemistry, immunology/antibodies, lymphocyte enumeration and function) were utilized. Widespread fungal contamination of water-damaged, primarily cellulose material with* Stachybotrys chartarum *(St. c.) was found. St. c. produced a macrocyclic trichothecene, satratoxin H, and spirocyclic lactones. Strong associations with exposure indicators and significant differences between employees and controls were found for important health outcomes and several laboratory tests, mainly of the white blood cell system. Laboratory abnormalities showed trends and significant differences for workers with indicators of greater and longer exposure. Specific St. c. antibody tests (IgE and IgG) showed minimal differences.*

Workers with a history of prolonged and intense exposure to toxigenic Stachybotrys chartarum *revealed abnormalities of primarily the respiratory and central nervous system as well as the cellular and humoral immune system, though the magnitude of some laboratory differences were small.*

Key words: Epidemiology, immunotoxicology, *Stachybotrys atra*, Satratoxin H, health effects

INTRODUCTION

Several employees of a metropolitan, below street level office presented to an occupational and environmental specialty clinic with multiple and unusual

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health complaints.[1] Subsequent hygiene investigations of the converted exhibition and basement-level office building in New York City revealed that after several recurrent floodings, extensive fungal contamination of building materials (sheet-rock and insulation material), stored paper products (books) and air ventilation system had developed over few years duration. Biological sampling revealed an unusual indoor fungi, *Stachybotrys chartarum* (formerly called *Stachybotrys atra*), besides predominantly *Penicillium* and *Aspergillus spp.* primarily found on the sub-basement level used as a library, auditorium, storage room of paper materials. The other floors were connected to this visibly contaminated area through the ventilation system. Chemical analyses of a bulk sample of *Stachybotrys chartarum* contaminated sheetrock from the sub-basement level revealed presence of macrocyclic trichothecene, satratoxin H,[2] which is a potent protein synthesis inhibitor. Chemical compounds of trichothecenes have been reported to cause immunosuppression or alterations. Cases of human and animal toxicosis after ingestion of *Stachybotrys chartarum* contaminated food stuff have been reported sporadically. Immune-toxicological laboratory data and clinical knowledge about human cases esp. related to inhalation risk are very sparse and incomplete. Bioaerosol exposure effects from toxigenic *Stachybotrys chartarum* have previously not been studied using modern immunochemistry and flow-cytometry now used in clinical practice.

MATERIALS AND METHODS

Environmental Exposure Evaluation:

a) *Microbial sampling and analyses.*

Bulk (source) samples and indoor aerosol fungal identification was conducted on two occasions before bioremediation efforts were done and just prior to this clinical investigation using sampling methods for viable and non-viable fungi. Quiescent and aggressive (disturbing settled dust by simulating high indoor activity) air-samples were collected using an Anderson sampler and Burkhard trap. Malt extract agar (MEA), MEA with 20% sucrose (xerophilic fungi) and cellulose Czapek medium (*Stachybotrys chartarum*) were utilized to allow for selective growth of fungal species. Indoor air sampling results were compared with outdoors. Bulk samples were analyzed by dilution culture and microscopically by a leading U.S. specialty laboratory.

Air sampling for fungi was performed in the autumn of 1991 prior to the first remediation, in 1993 prior to the second remediation, and in 1994 after the second remediation. (Table 1) In each evaluation air sampling was performed quiescently, that is, under normal operating conditions and aggressively when interior finishes are disturbed abnormally so as to aerosolize settled dust and determine if unusual kinds of fungi occur in dust reservoirs. In the 1991 evaluation bulk samples of moldy interior finishes were also collected and analyzed for the concentration and kinds of fungi present mostly from the sub-basement level, where the flooding had occurred.

Table I. Summary of Sampling for Fungi in 1991 and 1993.

Description Of Sampling	Analytical Results
1991 Sampling (Prior to First Remediation)	
Quiescent air samples, N=24, MEA medium	Concentration range 10 to 163 cfu/m^3; *Cladosporium* and non-sporulating fungi dominate 16 of 24 samples; *Penicillium* and *Aspergillus (A. versicolor, A. glaucus, A. syndowi)* dominate or co-dominate 11 of 24 samples; trace of *Stachybotrys* in one sample.
Aggressive air samples, N=2 MEA medium	190 cfu/m^3 in one sample codominated by *Penicillium* and *Cladosporium*; $>10^4$ cfu/m^3 *Penicillium* in second sample.
Outdoor air samples, N=3, MEA medium	Concentration range 120 to 250 cfu/m^3; *Cladosporium* dominates 2 samples, *Aspergillus niger* dominates one sample.
Bulk samples, MEA medium	Up to 10^6 cfu/cm^3 *Stachybotrys* on gypsum board and books.
1993 Sampling (After First Remediation)	
Quiescent air samples, N=11, MEA+S medium	Concentration range, <LOW (30) to 540 cfu/m^3; *Cladosporium* dominates 5 of 11 samples; *Penicillium* dominates 3 of 11 samples; *Aspergillus versicolor, A. fumigatus* and non-sporulating fungi each dominate one sample.
Aggressive air samples, N=7, MEA+S medium	Concentration range from 700 to $>10^4$ cfu/m^3 in 5 samples dominated by *Cladosporium*; concentration of $>3x10^5$ cfu/m^3 in 2 samples dominated by *Penicillium* with significant amounts of *Aspergillus* species and *Cladosporium.*
Quiescent air samples, N=14, cellulose—Czapek medium	Concentration range 20 to 160 cfu/m^3 *Cladosporium* dominates 10 of 14 samples; *Aspergillus* species dominates 3 of 14 samples; *Penicillium* dominates one sample; trace of *Stachybotrys* in 3 samples.
Aggressive air sampling, N=7, cellulose—Czapek medium	Concentration range 250 to 1000 cfu/m in 5 samples, 4 of which are dominated by *Cladosporium* and one dominated by Aspergillus; *Stachybotrys* present at $2x10^4$ cfu/m^3 in 2 samples.
Outdoor air samples, N=8, MEA +S or cellulose - Czapek medium	Concentration range 50 to 200 cfu/m^3 dominated by Cladosporium (N=7) or non-sporulating fungi (N=1)

Malt extract agar (MEA) was used exclusively for the evaluation in 1991. Concentrations of *Stachybotrys* on moldy gypsum board and moldy books approached 10^6 colony forming units (cfu) per square centimeters (cm^2). The concentration of fungi in the 24 samples collected quiescently in 1991 ranged from 10 to 163 cfu/m^3. *Cladosporium* or non-sporulating fungi dominated most (16 of 24) samples. *Penicillium* or *Aspergillus* species (*A. versicolor*, *A. glaucus*, and *A. sydowi*) dominated or co-dominated 11 of 24 samples. A trace (one colony) of *Stachybotrys* was found in only one of the 24 air samples. Air samples collected outdoors were dominated by *Cladosporium* or *A. niger*. Aspergillus species such as *A. versicolor*, *A. glaucus*, and *A. sydowi* were not found in outdoor air samples.

The 1991 sampling results showed that strong growth sites of *Stachybotrys* were present on moisture damaged cellulose materials. Quiescent air sampling however suggested that *Penicillium* and several *Aspergillus* species, (*A. versicolor*, *A. glaucus*, and *A. sydowi*) were predominant contaminates in indoor air. The absence of *Stachybotrys* in all but one air sample is likely due to the use of culture medium (MEA) that is not selective for the fungus and the sticky nature of *Stachybotrys* spores which reduces the likelihood of their dispersion as an aerosol.

In 1993 air samples were collected on MEA plus 20% sucrose medium (selective for xerophilic fungi) and cellulose Czapek medium (selective for *Stachybotrys*). Quiescent and aggressive air samples collected on MEA plus 20% sucrose medium often contained *Penicillium* and *Aspergillus* species (maximum concentration exceeding $3x10^5$ cfu/m^3 in 2 aggressive samples). *Cladosporium* dominated all air samples collected outdoors on MEA plus 20% sucrose medium. The 1993 sampling results using this culture medium showed that the indoor mycoflora were atypical of what can be expected indoors or that strong reservoir or growth sites for *Aspergillus* and *Penicillium* exist in the indoor environment.

Stachybotrys was found in 5 of the 21 air samples collected on cellulose Czapek medium in 1993. In 2 air samples the concentration of *Stachybotrys* was approximately $2x10^4$ cfu/m^3 which indicates that very strong reservoirs of this kind of spore existed at the time sampling in 1993.

b) *Chemical analysis of source materials:*

Two bulk samples of water-damaged, *Stachybotrys* contaminated sheetrock paper from the sub-basement office and storage area was chemically analyzed after microscopical confirmation of *Stachybotrys* presence. Black material was scrapped from the largest sample (60 mg) and extracted with 20% methanol in chloroform under sonication for 30 min. The extract was passed through a short column of silica gel, washing the column with 8% methanol in dichlormethane. The eluent was removed, taken up in ethanol and analyzed by reversed phase high performance liquid chromatography.

Epidemiology and Serological Tests

Health complaints, medical, occupational and environmental history were surveyed with a standardized questionnaire in a study population (employees with a minimum of 3 months seniority); and compared with a control group. The convenience control group was selected to be closely matched by important confounders from same metropolitan area assuming similar residential and outdoor mold exposure. The study population (n=53) consisted of 39 female and 14 male employees with a mean age of 34.8 years and a mean employment of 3.1 years; with 10 active smokers (control group (n=21): female=11; male=10; mean age 37.5 years; active smoker n=4). Different exposure indicators were tested, i.e., status and duration of employment, location of office (ground floor=no visible contamination, and air test considered non-atypical; basement level office area= airborne exposure mainly through ventilation system; sub-basement=source of fungal contamination, large areas of visible moldy), "moldy" stains noticed in office space and participation in the water flood cleanup in sub-basement. Based on these exposure indices an internal comparison group was included in the statistical analysis.

A symptom complex score by major target organs and constitutional factors was formed to estimate severity of health effects (upper respiratory problems=nasal irritation, burning, stuffiness and congestion; lower respiratory problems=shortness of breath, cough, chest-tightness, wheezing; moldy environment or dust related respiratory distress; CNS=severe headaches, concentration problems, irritability, dizzyness or lightheadedness, sleeping problems, mental fatigue; eye=burning, irritation, blurry vision; constitutional=unexplained, low grade fevers, tender and swollen lymphnodes, flu-like symptoms and myalgia; skin=burning, erythematous rash, hair loss).

Laboratory Analyses of Blood Samples

A comprehensive immunological test battery developed by the National Institute of Occupational Safety and Health (NIOSH) immunochemistry research section in Cincinnati, Ohio to detect immunomodulation from exposure to xenobiotics was used to study the red and white blood cell system, serum chemistry, immune function and immunoglobulin antibodies. Peripheral venous blood was collected and handled in a routine manner and immediately shipped at ambient temperature to NIOSH, Cincinnati. Tests performed on fresh blood or isolated cells were initiated by the laboratory within 14-16 hours. It included:

- Flow cytometry
- Lymphocyte enumeration
- Lymphocyte function tests
- Natural killer cell, Mold specific antibody tests.

Antibody tests were done by the IBT reference laboratory, Kansas. For fungal specific IgE, fungal extracts (*Penicillium notatum, Aspergillus versicolor* and *Stachybotrys chartarum*) were coupled to paper discs by the diazo method and

were used to test sera by the modified RAST protocol.[3] For fungal specific IgG measurements, a microtiter-based EIA was used with the same antigens as above. Standard Labortatory procedures and quality assurance measures were applied to assure accuracy. Complete description of laboratory methods are available from the author.

Statistical Analysis

All data was computed, tabulated and analyzed for association and significance using parametric (ANOVA, logistical and linear regression) and non-parametric tests (Kruskall-Wallis [K-W] with a statistical PC package (SPSS for Windows). The significance level was set at $p<0.05$. To reduce Type II error risk (false negatives), because of the small study size and low statistical power, results with a significance level up to $p\leq0.10$ and important trends are presented if judged to be of potential clinical relevance. Variables that were positively skewed were log-transformed for analysis.

Participation in the study was voluntary and confidentiality was assured. The study protocol was approved by the Institutional Review Board (IRB). Because several employees had been noted to have health problems related in time and place to work activity in the contaminated office building, two extensive bio-remediation attempts were made and administrative control measures were in place to limit exposure to *Stachybotrys chartarum* materials approximately 18 and three months before this investigation was conducted in the fall of 1993.[4]

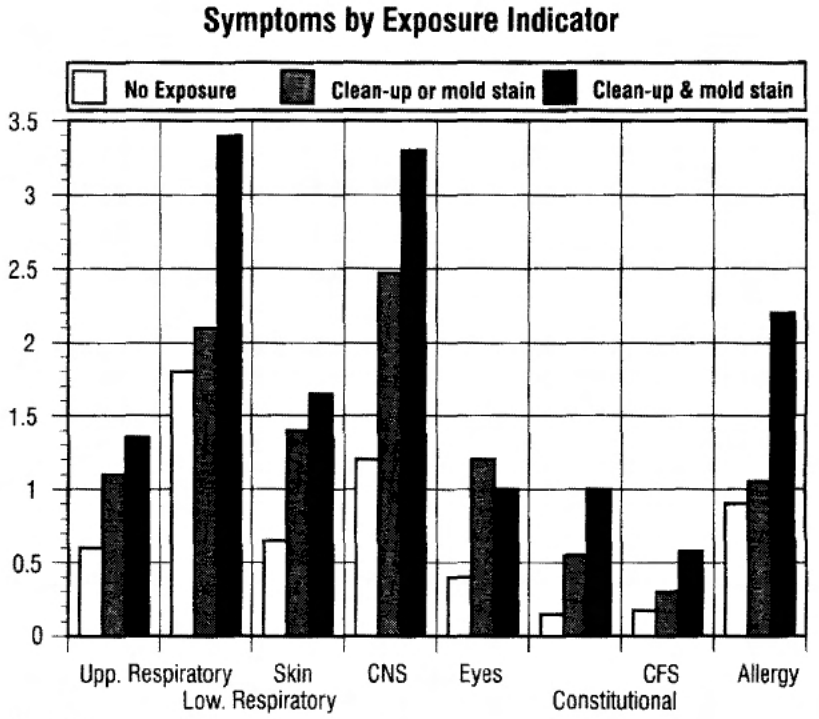
RESULTS

Health and Blood Study

Strong associations with exposure indicators and significant differences between employees and controls were found for important health outcomes and several laboratory tests. A greater proportion of employees in the contaminated office building had infections and symptoms in all major organ systems during the past year than controls. Differences between groups were statistically significant for lower respiratory system symptoms, dermatological, eye, constitutional symptoms, chronic fatigue (CFS) and allergy history. Indoor fungal exposure indicators such as *'sub-basement office location'* (highest contamination area), participation in the *'cleanup activity'* of the flood damage and visible *'moldy stains in office'* were associated with reports of disorders of several major organ systems, excessive chronic fatigue and allergy history (Graph 1). Duration of employment (mean 3.17 years, SD 2.5) was associated with upper respiratory tract ($r=0.27, p<.001$), skin ($r=0.24, p<.08$) and central nervous system (CNS) disorders ($r=0.27, p.054$). There was a trend (ns) for exposure indicators to be associated with frequent upper respiratory infections, fungal or yeast infections and urinary tract infection.

Mean white blood cell (WBC) count was slightly higher, but the proportion of lymphocytes (%) was slightly lower among employees working on either basement level. The eosinophil fraction (%) (p 0.06) was higher and total immunoglobulin E (IgE) count was more than twice as high (p=0.13) among all employees. Immunoglobulin A and G showed a clear trend, with slightly lower IgA and IgG levels in sub-basement workers, however, the mean IgM level was the highest among sub-basement and ground-floor workers. C-reactive protein (CRP) level among all employees was about half of the controls. Only two employees, who had been working on the basement and sub-basement level for more than one year—both with an atopic history—had a positive IgE test (RAST) for *Stachybotrys chartarum*. Subjects and controls had low mean IgG antibodies levels to *Stachybotrys chartarum* with minimal differences. Sub-basement office workers had the highest fungal specific IgG levels among subjects.

Lymphocyte enumeration analyses showed a trend, with few significant differences between subjects and control, although clinically these differences may appear minimal. Mature T lymphocytes (CD3), and subsets CD4 (Helper/Inducer), CD8 (Cytotoxic/Suppressor) and the CD4/CD8 ratio were lower in the exposed population, but CD 19 (earliest B cell) and CD 56 (NK, nat-

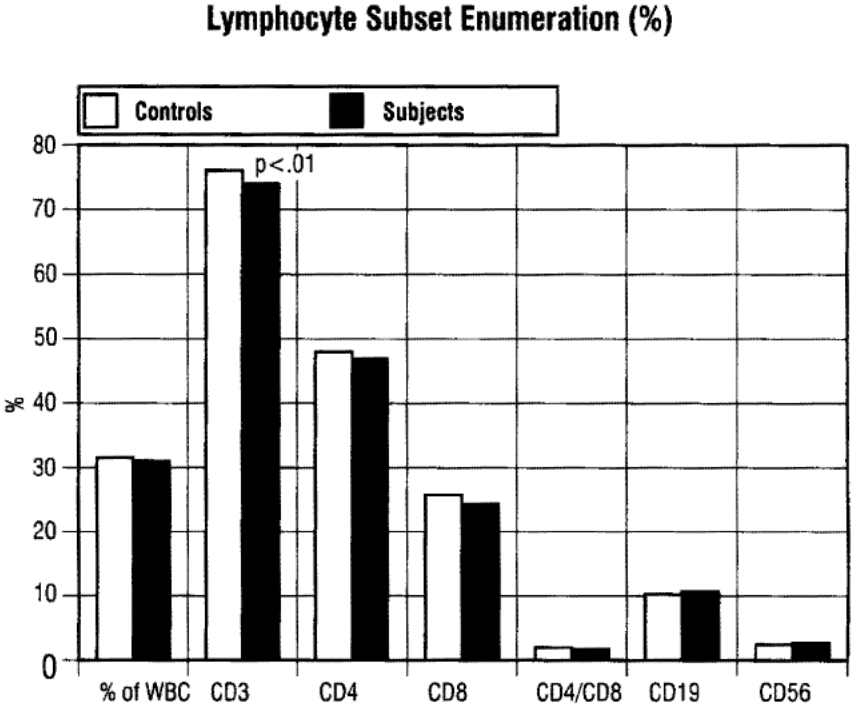


Graph 1

ural killer cells) were higher (Graph 2). Employees had a significantly (p=0.01 K-W) lower proportion of mature lymphocytes (CD3%) than controls.

Lymphocyte function tests (ConA and PHA Mitogen proliferation), but not PWM were consistently lower (ns) for sub-basememt vs. basement ground floor employees (Graph 3). Similarly, sub-basement and basement employees had consistently lower values than controls for all lymphocyte function tests (PWM, ConA and PHA). One of these differences (PHA-5) reached p<0.07. Finally basement and sub-basement employees with a history of recurrent infections tended to have even lower lymphocyte function tests levels.

Significant differences based on office location and comparison with controls were found for WBC count, CD3%, and natural killer cells (NK# and NK%). Laboratory WBC, neutrophil and eosinophil count and NK (CD56 # and %) dif-

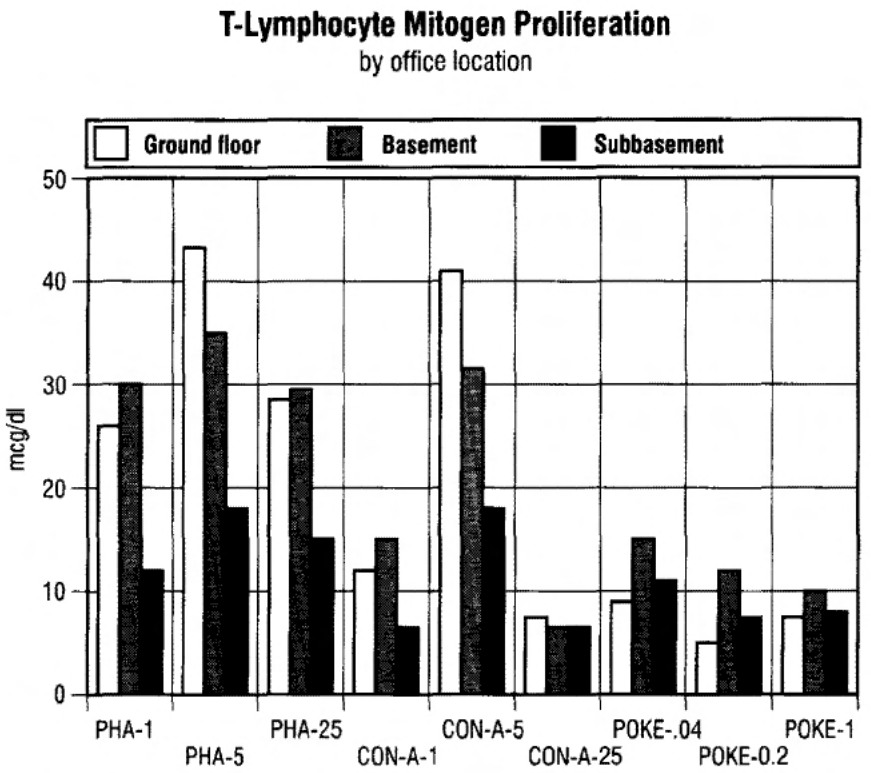


Graph 2

ferences were greater for basement and sub-basement employees, while CD3% did not differ greatly by office location, but remained lower for employees compared to controls. Separating out basement office workers with a history of 'yeast infections' did not substantially alter the previous results.

The regression analyses to associate health outcomes with selected laboratory tests, adjusting for possible confounders (gender, smoking, allergy history

and mold problems at home) showed a trend and borderline significant association of lower CD3% among those reporting health problems and changes since working in the contaminated building.



Graph 3

DISCUSSION

In this epidemiological and immunechemistry study of exposure to toxigenic *Stachybotrys chartarum* we found abnormalities of the cellular and humoral immune system, though the magnitude of some differences were small. Self-reported exposure indicators and documented contamination areas were associated with abnormal findings of components of the immune response system, primarily some nonspecific (Neutrophils, eosinophils, natural killer cells (NK) and C-reactive protein) and specific adaptive cells (T and B lymphocytes). There was a consistent trend of qualitative and quantitative measurements suggesting that higher and longer indoor exposure to *Stachybotrys chartarum* or its chemical products results in immune modulation or even minimal immune suppression. Marked leukopenia or acute "radiation-mimetic" effects on the blood cell system with subsequent sepsis like opportunistic infections were not

found as reported in earlier cases in Eastern Europe.[5,6,7] We did find a trend of increased frequency and unusual infections compared with a population of similar demographic profile, however it remained statistically non-significant.

T and B cells are unique in that they have antigen specificity and immunological memory. Repeated antigen contact, such as mycelia material typically results in an enhanced response. T cell defects or deficiency are clinically associated with a fungal (Candida), viral (CMV, Varicella, HSV), bacteria (Listeria) and protozoa infections and B cell deficiency with polysaccharide-encapsulated pyogenic organisms (*Streptococcus*, H. flu, *Staphylococcus aureus*, *Giardia* and *Campylobactor*).[8,9] Progressive and far greater T-cell deficiencies than in our investigation have been documented in AIDS and DeGeorge Syndrome. Secondary immune deficiency have been found in several medical conditions, pharmacological immunesuppression, infectious diseases, rheumatological diseases and metabolic disorders.

Lymphocytes have receptors for mitogen and can respond to these without prior sensitization. This can be used in nonspecific laboratory function tests. Phytohemagglutinin (PHA) and Concanavalin A (ConA) respond more with T-cells, and Pokeweed mitogen (PWM) is a T-cell-dependent stimulator of B-lymphocytes. Inability to proliferate and to produce cytokines or IgG upon stimulation is a possible sign of impaired immunity.[10]

Immune modulation and suppression of the humoral and cellular cell system after mycotoxin exposure have been studied in animal and laboratory experiments. Trichothecenes are considered the most potent small molecule inhibitor of protein synthesis through inhibition of the peptidyl transferase activity.[11,12] They have been investigated for use in cancer treatment[13] but alsoin chemical-biological warfare. Presence of fungal chemical metabolites has been reported in several cases of animal and human ingestion related to mycotoxicosis.[2,14,15] Mycotoxins, such as Satratoxin H of the trichothecene group, have been shown to cause depressed T or B lymphocyte activity, suppressed immunoglobulin and antibody production, reduced complement or interferon activity and impaired macrophage-effector cell function.[16] Impaired migration-chemotaxis and phagocytosis of human neutrophils have also been reported.[17] Weakened resistance to infections of salmonella, tuberculosis, listeria, herpes simplex, candida and cryptococcus has been demonstrated after trichothecene poisoning in various animal models. It may also suppress the tumor defense mechanism and weaken the host control of tumor cell growth.[16] Currently IARC classifies just aflatoxin (from *Aspergillus* spec.) with sufficient evidence for human and animal carcinogenicity, but trichothecene toxins (T-2 toxin, Fusarien toxins) with 'limited evidence' for animals and 'inadequate evidence' (no data avilable) for humans.[18] Satratoxin H has not been classified. Some epidemiological studies have shown a higher rate of upper respiratory tract and lung cancer in workers in the grain and food handling industry with high fungal product inhalation risk.[19]

There are currently no similar studies available for comparison of our in-vivo findings. The benefit of such investigations compared with some controlled

laboratory studies is, that immunological interactions of all contributing components of the body's defense system are taking place and impaired immune surveillance and suppression outcome can be studied in a more realistic setting. However, exposure conditions and dose of all office workers cannot be assumed to be similar and of equal range. After adverse health effects had been previously diagnosed in several office workers, environmental control measures and several remediation and administrative control attempts were made before our present investigation. This may have influenced our clinical and laboratory findings.

Presence of specific fungal antibodies have been used as possible mold spore exposure markers in the working environment,[20] we, however did not find in this study that higher immuneglobulin G and E antibodies to *Stachybotrys Chartarum* were statistically associated with studied health outcomes. Continued antigen exposure might have increased the formation of IgG_4 subclass complexes resulting in higher removal (turnover) and lack of serological elevation of IgG *Stachybotrys*.[21,22] Valid reference ranges for *Stachybotrys* IgG antibodies have currently not been firmly established.

Similar trends and changes of immunoglobulins (IgG, IgA, IgM and IgE) like ours were reported in a Russian study of workers handling mycotoxin contaminated foodstuff, primarily with desoxinivalenol (vomitoxin).[23] Increase of IgA production and IgA nephropathy and decrease of IgG and IgM after ingestion of vomitoxin was reported in a mice experiment.[24] Renal failure and IgG deposition in the glomeruli after inhalation of Mycotoxins (ochratoxin produced by *Aspergillus ochraceusl*) was found in a case of a farmer.[25]

The production of toxic chemical metabolites from *Stachybotrys chartarum* apparently depends on several environmental factors, such as temperature (2 to 40 C) and humidity (80-100%). High cellulose content material like wall paper, paper coated gypsum board (sheet rock), straw and books combined with very high water saturation has been shown to promote growth and presence of macrocyclic trichothecenes, mainly Satratoxin G and H with adverse biological activity.[26]

Few and sparce clinical reports of human cases of toxicosis from *Stachybotrys chartarum* are available. It was thought that mainly farmworkers and laboratory personnel (military and food safety research labs) belong to the high risk group of mycotoxin related diseases. However, one recently published case-report about an airborne outbreak of *Stachybotrys chartarum* toxins related illnesses in a family home to the effects of trichothecene chemicals.[27] In this case study no serological tests were reported. Indoor air contamination with *Stachybotrys chartarum* mycotoxins was thought to be responsible for extreme chronic fatigue syndrome in hospital workers.[28] Indoor air exposure may cause an exacerbation of asthma.[29,30]

Pathogenic effects, even fatal, of *Stachybotrys chartarum* or its chemical derivatives have been described in animals in the 1930s and 1940s by Russian and Hungarian veterinary researchers. In the USA the first detailed account of this in English and the experience of his own work came by Forgacs 1972.[31] Based on the German and English literature of case descriptions the following abnor-

mality have been reported after significant exposure: severe skin and mucous irritation (burning), bleeding disorders, conjunctivitis, upper and lower respiratory disorders, chronic fatigue syndrome, low grade fever and cardiac arrhythmia.

Conventionally, airborne fungal allergens have most often been associated with allergic diseases, such as allergic rhinitis, allergic asthma and hypersensitivity pneumonitis.[32] However, there is now growing evidence of direct cytotoxic and neurotoxic effects from microbial aerosols. Biochemical products, like endotoxin and ß-1,3-Glucan from bacteria, may be responsible for respiratory inflammatory disorders and organic dust toxic syndrome.[33]

Recently a German group presented to the German defense department, a treatment protocol for fulminant trichothecene poisoning based on animal experiments suggesting positive effects from use of steroids and activated charcoal combined with general supportive care.[34]

The role of fungi and mycotoxins in indoor air situations and possible adverse health effects have been reviewed.[35] Pulmonary exposure to toxic fungi in occupational settings such as food processing industry or laboratory may cause infections, allergy or hypersensitivity.[36] A task group of WHO concluded, that an association between trichothecene exposure and human disease episodes is possible, however only limited data is available.[37] This may have been related due to the lack of modern immunochemistry tests now available to clinicians and inadequate exposure assessment in the past. Toxicological health effects principally depend on the exposure dose and timing. As mentioned earlier, some immunological effects may only be transient and of short duration.

Flow cytometry analysis in clinical settings can aid in the diagnosis of immunopathology, although some alterations may not be specific or clinically important in the diagnosis of diseases. We found serological tests combined with clinical-epidemiological studies and exposure assessments helpful to correctly identify building occupants requiring medical and administrative intervention. Environmental control measures required removal of building material with dust restriction techniques similar to modern asbestos abatement.[38]

In conclusion, health status changes were associated with occupational exposure to *Stachybotrys chartarum*. Modern immune chemistry panels, which include lymphocyte enumeration and function tests, may aid in the clinical investigation of subjects with serious health problems after intense bio-aerosol exposure. Long-term clinical prognosis of *Stachybotrys* related illness and toxicosis in humans is unknown

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CLINICAL INVESTIGATIONS OF EMERGING DISEASES IN PATIENTS AND BUILDINGS: MANAGEMENT OF PATIENTS, EXPOSURE, AND REMEDIATION

MICHAEL J. HODGSON, M.D., MPH

Abstract: *Since 1987, sporadic outbreaks of a range of symptoms affecting the chest and central nervous system have been described after exposure to moldy environments without meeting criteria for the diagnosis of traditional respiratory tract diseases such as hypersensitivity pneumonitis or asthma. The outbreaks have generated much controversy. This manuscript summarizes a logical approach to emerging diseases. It reviews current practice guidelines in occupational and environmental medicine: "document disease," "document exposure," "establish linkages," "and intervene".*

Key words: Occupational and environmental health, clinical practice

INTRODUCTION

The practice of medicine often requires physicians to treat the unknown. Although the recognition of syndromes guides the use of diagnostic tools and the selection of therapies, the scientific basis of practice is often not as rigorously developed as physicians and scientists would like. Occupational and environmental medicine is, more often than not, the study and practice of emerging diseases. That is, the diseases are either not well characterized in thir clinical characteristics, the exposures leading to disease are unknown, or effective prevention strategies are unknown. This compounds problems in the use of diagnostic and therapeutic strategies.

When diseases have been studied for some time, exposure-effect relationships may be examined following well established causal thinking in a unified model of causation (Evans 1976). Often though, early on in the course of disease, only incomplete data are available. Physicians must still treat patients, decide

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on protection and prevention strategies, and explain their decisions to lay persons, such as employers, workers compensation referees, and the public. Over time, approaches to documentation have been derived that are less complete than Koch's postulates extended into environmental health. Models include the use of epidemiology (Bradford-Hill 1954), toxicology (Brennan 1987), and clinical criteria (Hodgson 1994) (Table I).

Table I. Causal models and criteria.

Epidemiology	
Consistency	Similar findings across multiple cohorts
Strength	High odds ratio
Temporality	Cause precedes the/effect
Specificity	Fewer other causes for syndrome
Coherence	Data from different kinds of studies converge
Dose-response relationships	More exposure make for more disease
Biological plausibility	Mechanistic considerations make the effect likely
Toxicology	
Structure-activity relationships	The chemical structure of a postulated agent suggests it might have those effects
Mechanistic testing	Laboratory testing, e.g., in vitro models, suggest an effect is likely
Animal studies	Similar effects have been documented in animals under controlled conditions
Clinical Approach	
Randomized controlled trials	Randomized therapeutic interventions suggest an effect
N-of-one clinical trials	Modification of exposure leads to a reproducible effect
Case reports and case series	Convincing clinical descriptions suggest causal relationships

In occupational health, physicians must not only treat the affected patient but incur a duty to a wider population. The fundamental basis of public health practice is the recognition that cohorts are at risk and must be protected. Therefore, public health practice must consider the importance of uncertainty, limited power and sample size, and type II errors much more strongly than traditional science. Outbreaks of putative mycotoxin-induced diseases since 1987, reviewed by Sorenson in this volume, and more recent reports of the chronic fatigue syndrome and immunological testing suggest that physicians are currently seeing a form of disease either poorly recognized in the past or that has emerged relatively recently. Although specific mycotoxin syndromes

such as ergotism, are well recognized, no generalized practice guidelines exist and physicians struggle with appropriate actions in the face of scientific uncertainty.

This paper will attempt to justify practice guidelines for management of patients, exposed cohorts, and remediation workers in the setting of scientific uncertainty. The approach has evolved through a series of investigations and discussions with colleagues.

CURRENT CLINICAL PRACTICE: INDIVIDUALS

Occupational medicine has evolved into a clinical discipline since the mid 1970s. Before then most occupational medicine specialists practiced in the corporate boardroom; surgeons ran plant dispensaries; and subspecialists tended to examine patients with end-stage disease in clinical settings for compensation purposes. Now almost half of practicing self-professed occupational physicians practice out of hospitals and out-patient clinics. In this transformation, de facto practice guidelines have evolved. These include 1) document disease, 2) document exposure, 3) prove or disprove linkages, 4) intervene, and 5) communicate.

Each of these steps must be scrutinized in the context of our knowledge on bioaerosols. In the presence of an as yet poorly characterized disorder, requires that the same steps be followed.

Document Disease

The process of differential diagnosis requires the sifting of patient complaints into symptoms and symptom categories, the definition of syndromes, and the postulation of pathophysiology. The latter then allows the selection of appropriate diagnostic tests. Without understanding the disease process, physicians are often unable to develop appropriate management strategies. Patients present with symptoms not diseases. Although diagnostic tests may exist to define specific characteristics, there is no broad recognition among physicians about mechanisms, clinical course, and prognosis. Nevertheless, linkage of emerging disease to exposures may follow epidemiologic, toxicologic, or clinical criteria as described in Table I.

Physicians often derive from patient symptoms encountered in other settings an understanding of the likely organ systems involved in a new setting. Documentation of disease may occur through the use of appropriate clinical tests in exposed and unexposed periods of time, while patients are symptomatic and asymptomatic, respectively. Therefore, using diagnostic tests, such as neuropsychological testing, lung function testing, or immunological tests, may indicate the presence of abnormalities. Similar guidelines here may allow the use of established laboratory methods to detect the presence of some abnormality associated with disease. In the absence of established clinical criteria, physicians must rely upon sound laboratory practices and basic empiric approaches to develop knowledge.

For example, any given laboratory test has a certain coefficient of variation. Although most laboratories do not routinely provide data on reliability, they should have those available. One may then use the intrinsic variability of a test to determine whether a quantifiable difference in a measurement system reflects changes in organ function at least potentially related to exposure and symptoms or whether differences between measurements are more likely to reflect random error. The use of 90% or 95% probability levels appears appropriate for most settings. If a patient presents with symptoms most likely related to an organ system, physicians may identify pertinent measures for that organ. If symptoms develop and resolve in parallel with exposure and laboratory testing simultaneously provides "abnormal" and "normal" results, it may be reasonable to consider the presence of a disease likely.

The use of mechanistic tests, such as of natural killer cell activity by Johanning (Johanning et al. 1993, described in this volume), in the context of clinical disease may serve at least two purposes: management of the patient and identification of screening and surveillance tests for remediation.

Document Exposure

The purpose of exposure assessment is, at least traditionally, usually to determine whether a dose adequate to cause disease is present. For syndromes in which dose-response relationships and dose interactions are not characterized, exposure assessment may serve a different purpose, namely to document the possibility that any pertinent exposure exists. For example, if a careful walk-through fails to reveal bioaerosol reservoirs, it may be unreasonable to attribute a disease to such a reservoir.

Other exposures may still exist, and, if disease appears convincingly related to a site, it may be reasonable to attribute that disease to whatever exposures are present and have been documented, if an association is plausible.

Documentation of exposure to bioaerosols relies primarily on visual inspection and sampling of reservoirs. Widespread agreement suggests that quantitative air sampling is not a reliable approach to the documentation of exposure for several reasons. It remains unclear whether the use of viable samplers or spore traps is superior. Mycotoxins may be analyzed in bulk samples, but airborne measurements have never been documented to be of use. Therefore, the presence of molds with the potential for mycotoxin production alone may suffice. Equally important, the absence of reservoirs should serve to document that a specific condition is unrelated to exposure.

A second purpose, described later, may rest in the attempt to document that bulk materials have the potential to cause certain adverse health effects.

Consider Linkage

An unusual disease may result from many other causes besides mold growth; not all mold growth must necessarily result in disease. Physicians must therefore consider whether a given disease results from a specific setting.

The framework of causal linkage has been discussed for many years. Individual components of the unified model include the epidemiology criteria defined by Bradford-Hill, toxicology considerations, and clinical causal models. In emerging diseases, each of these three may serve different purposes and alone suggest the presence of exposure-effect relationships to guide the management of patients.

Epidemiology

Most of the criteria (Table I—Epidemiology) cannot be used in a first cross-sectional investigation of an emerging syndrome. In fact, the tradition of outbreak investigations in public health practice follows a slightly different model. Index cases are reviewed, some working case definition is established, an instrument using that definition is developed and applied to cohort and controls, and subsequently some attempt at validation using a second, independent measure is undertaken. Surveillance documents the effectiveness of intervention.

In using this model to document an association between exposure and disease, traditional field studies with control groups and health outcomes measures, such as spirometry, immunological testing, or neuropsychological testing can be used.

Toxicology

Classic toxicology views mechanistic studies with controlled exposure and markers of dose (exposure and effect) as the essence of science. A hierarchy of considerations includes structure-activity relationships, in vitro testing, and controlled animal studies. Importantly, toxicology may be useful for viewing both markers of effect and for documenting the potential toxic effects of exposures.

Effect: For many of the recently described syndromes, no specific markers are available. On the other hand, effect measures of organ systems that are affected in animal studies may provide reasonable biologic outcomes. If, for example, T-2 toxin from *Fusarium* species is associated with immunologic depression in rabbits, the use of immunologic markers in humans may provide a reasonable parallel. If animal studies suggest the presence of central nervous system effects, neuropsychological testing is an appropriate measure.

In using this model to document an association between exposure and disease, researchers might characterize the effects of a specific toxin, present in the workplace, in animal studies, select a parallel measure in humans, and apply that measure to exposed and unexposed groups. The presence of differences would imply an effect of that toxin.

Exposure: The use of non-specfic toxicologic screening tests provides an interesting alternative to the problem of exposure assessment. Often, physicians are confronted with exposure in settings where agents have unknown or poorly defined toxicity. One possible use of such screening tests is to document the possibility that a human health effect convincingly associated with exposure tem-

porally may be causally related even if no prior studies have linked that kind of disease to the exposure at hand.

Clinical Causal Models

The paradigm of causal thinking in clinical medicine is the randomized controlled trial. An extension of that (Guyatt 1985, Hodgson 1993) is the n-of-one clinical trial in which a single subject serves as their own control with modification of exposure in a controlled fashion. This model can be used to associate rare diseases with reversible physiology to specific settings.

In using this model to document an association between exposure and disease, researchers may examine a patient while at and after removal from work for some weeks, repeatedly, to determine whether there is a consistent pattern to symptoms, physical signs, and laboratory findings. Of course, the more abnormalities can be identified and documented, the greater the likelihood that something "real" is going on.

Management

The major decision facing physicians is usually whether to remove an individual from the work place or the home that is considered contaminated. Discussions with numerous colleagues have suggested the following approach (Hodgson and Storey 1994).

- The presence of physiologic abnormalities usually warrants removal of that individual.
- The presence of differences in outcome measures between groups warrants timely intervention and often removal of all potentially affected individuals.
- The presence of symptoms alone requires further investigation. If no corresponding physiologic abnormalities can be identified, patients can often work. If large numbers of individuals are symptomatic and no useful clinical measures exist, e.g., nasal irritation and drainage, one may be forced to remove individuals anyway.

The management of groups of individuals with exposure obviously raises additional problems. The economic implications are much greater, and therefore some have argued that a greater degree of scientific certainty is necessary. In fact, public health practice has often been based on less certainty and greater probability of errors in a conscious attempt to protect the affected individuals.

SENTINEL EVENTS AND COHORTS

Patients with disease induced by bioaerosols do not usually work or live alone. Others exposed to the same environment are likely to be exposed to the same or similar agents. They are then at risk for developing similar disease. Physicians treating an index patient have no formally defined relationship to others from this cohort from a legal view. Because occupational and environmental medicine is a preventive discipline, many practitioners feel obligated to act

on the knowledge of risk. Although other members of a cohort are not patients, they do have the right to expect physicians to warn appropriately. Two separate questions arise: who pays? and what do physicians actually do?

When the exposure is occupational in nature and in jurisdictions where occupational disease is reportable, this is at least adequately resolved through notification of a health department. Even there, many health departments do not have adequate resources to follow up. When exposures occur at home, housing and health codes may be inadequate. Where a disease is as yet poorly defined, employers may be loath to embark on the investigation of a potential problem, although many are with or even without the support of their insurance carriers. Insurance carriers may be willing to pay for the examination of other exposed family members.

More interesting is the question of how to use clinical tests with inadequately defined predictive values. For many years, questionnaires and symptom checklists have been considered the most sensitive tools for the screening investigation of building-related diseases. Some tests may then subsequently be used to confirm or refute the presence of some pathology that is associated with the symptoms of concern. A negative test does not mean the absence of disease just as a positive test does not mean a disease is present. Only if the test has a well-defined relationship to organ function can physicians be certain that that organ is in fact dysfunctional. Therefore, the usefulness of pulmonary function testing, natural killer cell activity, and other screening tests will vary from outbreak to outbreak. On the other hand, it is easier to explain to patients that a disease is relatively mild or early in its course when all likely tests are negative.

Importantly, it is usually quite simple to identify a source of bioaerosols in a building and characterize it. It is often much more difficult to identify and characterize the nature of disease. Most physicians active in the field attempt to persuade all involved parties to focus on remediation rather than on disease documentation.

REMEDIATION WORK

Medical surveillance and protection guidelines for remediation workers in buildings with biological contamination has been described only once (Johanning et al 1993). Nevertheless, a logical approach suggests that those guidelines are appropriate. Any surveillance program must include education and training, initial examination, protection, and surveillance.

Education and Training

In addition to other usual training in hazardous waste, remediation workers should be informed about our knowledge and its limits in biological contamination. This includes fundamentals of dose-response relationships for sensitizers (for sensitized workers and for sensitization) and toxins, specific

effects of the agents at hand, and risk factors for sensitization. It should include the belief that exposure control strategies are likely to reduce the risk to a minimum (Morey P. 1993).

Initial Examination and Placement

The physical requirements for such remediation work are no different than for most general construction with the exception that this work must be done under respiratory protection. Depending on the nature of contaminants, either powered, air-purifying respirators or high-efficiency particulate air-cleaning respirators (negative pressure) must be used. Most workers should be eligible to perform such remediation work. Three considerations arise: respirator clearance, sensitization, and specific effects of the biologic agents.

Respiratory protection: Most individuals with lung function that represents only mild pulmonary obstruction or better are able to work under respiratory protection. Work under simulated conditions, i.e., having subjects don a respirator and perform acts similar to those required at work, should resolve any questions about ability to work.

Sensitization: Atopic individuals are widely considered to be at higher risk for developing sensitization after exposure to large molecular-weight sensitizing agents. The exact degree of risk is uncertain, but prospective studies of animal handlers provide some estimate of the risk even with relatively inadequate protection strategies. Clearly the vast majority of subjects do not become sensitized after short-term exposures. If protection strategies are in fact adequate, they should not be at increased risk for developing problems. On the other hand, they should know about the possibility and receive the opportunity to choose a different job.

Specific Effects: Various agents have been shown in animal studies to cause immune suppression, serve as carcinogen promoters, or be toxic to pulmonary cells. A medical surveillance program should incorporate some specific markers of end-organ outcome and, ideally, some mechanistic measure. For example, *Fusarium* T2 and *Stachybotrys Satratoxin* may be followed with tests of natural killer cell activity (Johanning 1993). Inclusion of a biological marker with some relationship to mechanisms may be guided from investigations of initially ill subjects. Conversely, if no markers were found among ill individuals, inclusion of such markers is not a useful approach.

Respiratory Protection

Few data exist on the level of respiratory protection needed. High-efficiency particulate air cleaners or powered, air-purifying respirators, are likely to have protection factors adequate to reduce exposure to the range commonly encountered without respirators.

Surveillance

No long-term studies exist on workers exposed to agents with mycotoxin exposure. On the other hand, Danish farmers thought to be exposed to myco-

toxins were shown to die at increased rates from all cancers. In addition, the literature on *Aspergillus* and aflatoxins suggests that at least long-term exposure is not benign. Surveillance programs should therefore address both sensitization and, long-term, attempt to identify a potential excess risk from carcinogen exposures or immune suppression. This will require both cohort follow-up studies and specific laboratory-based surveillance for outcomes of interest.

SUMMARY

Table II provides an overview of this approach in an investigation and management of emerging clinical syndromes. It follows current clinical practices and is generalizable to any new potential disease. Obviously, no single individual will be able to complete all portions of the evaluation. This approach follows a disease driven medical model and requires the participation of a physician with broad understanding of clinical syndromes and mechanisms. Exposure assessment in such settings generally requires an industrial hygienist or engineer with understanding of the other field. Use of innovative techniques such as described in this volume require the involvement of toxicologists who understand the use of appropriate screening techniques for specific agents. As toxicologic screening techniques become widely available, they may be incorporated very formally into exposure assessment and linkage strategies. Similarly, such screening techniques may guide the selection of appropriate biological endpoints, corresponding to the organ of interest.

Table II. Management of disease.

Document Disease	
Establish form	Identify pertinent physiologic outcomes from symptoms (markers of effect) characterize mechanisms
Establish work-relatedness	Determine abnormalities at and away from work (see also linkage)
Document Exposure	
Recognition	Walk-through Taxonomic identification Classification of possible health effects (mycotoxin producers, sensitizers, non-specific mechanisms)
Assessment	Quantify molds in reservoirs Determine presence of mycotoxins Perform screening tests for adverse health effects in isolated systems
Linkage	
	Epidemiology Toxicology Clinical

Management	
Patient	Remove from work if physiologic abnormalities are identified
Group	Screen for the presence of disease
	Intervene promptly
Intervention	
Workers	Respiratory protection
	Medical surveillance
Reoccupancy	
Site	Documentation of safety

Patients should undergo tests of organ function, such as neuropsychological measures or pulmonary function tests. Ideally they will serve as their own controls, ideally undergoing removal and re-exposure several times. In addition, measures indicating mechanisms, such as immunological testing, will be included. Exposure assessment will identify specific biological agents. Individual agents but more importantly building materials will undergo chemical analysis and toxicologic screening to document the presence of agents with the potential for causing adverse health effects. Workers conducting intervention should be adequately protected and undergo surveillance with the same measures that identified problems in subjects. After intervention, the same toxicologic approaches may be used to document safety for reoccupancy.

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A PROACTIVE APPROACH TOWARD THE RECOGNITION AND CONTROL OF FUNGAL CONTAMINATION IN SCHOOLS IN ATLANTIC CANADA

THOMAS G. RAND, Ph.D.

Abstract: *Microbial contamination of schools in Atlantic Canada has become a real and significant problem. The following article outlines a proposal I have made to various school boards in this area. The proposal involves training between 5 and 10 school maintenance staff members from each school board to conduct their own on-site inspections of school environments to recognize sources and signs of water infiltration and condensation, and to identify potential amplification sites. The intention of training is also to provide school maintenance workers with hands-on sampling practice (so they can do their own sampling for complaint and non-complaint building areas) and with information on contamination remediation procedures. It is hoped that training school staff to do this type of work will reduce the prevalence of microbial contamination in school environments and the costs associated with remediation.*

Key words: Indoor air quality investigations, biocontamination of schools, fungal contaminants

INTRODUCTION

Mycological investigations of Atlantic Canada buildings have been ongoing since mid-summer 1992. Since then, air and building material from 266 non-industrial buildings have been sampled for fungal contamination (Table 1). Findings indicated that of the 266 building sites sampled, some 166 (49%) had mold contamination problems. Schools were identified to be especially affected. Of the 144 school sites sampled, approximately 61% had contamination problems. These problems were manifest in having an indoor mycoflora qualitatively dissimilar to and quantitatively higher than that of outdoor air references, and/or comprising notoriously toxigenic species (eg. *Stachybotrys atra*).

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Table I. Summary of Mycological Investigations of Atlantic Canada Building Environments, from July 1992.

A) Types and Numbers of Building Environments*			
Federal government	4	(1)	
Provincial government	11	(6)	
Hospital suites			
	Operating	8	(3)
	Recovery	4	(1)
	Rehabilitation	18	(16)
	Administration	15	(4)
University	2	(2)	
Schools	142	(87)	
Office	35	(7)	
Homes	27	(4)	
Total number of building environments investigated	266		
Total number of building environments with confirmed fungal contamination problems			131 (49.2%)
B) Total Number of Samples Analyzed			
Air	1,658 (547 outside reference samples)		
Substrate	2,035		

*Numbers in parentheses indicate proportion of buildings with confirmed fungal amplification problems.

Microbial contamination of Atlantic Canada school environments is a real and significant problem. Building contamination by molds has not only affected air quality but has also been linked to health problems, especially among school children and teachers. Consequently, there has been a growing demand on government and private sector groups to investigate and remedy indoor biocontamination problems.

The recognition that relatively high numbers of schools in this area have been suffering from contamination problems has precipitated reaction among the school communities. School occupants and parents of school-age children have started to pressure school administrators to conduct mold and other microbial testing on a regular basis.

Upon discovery that there are unacceptable levels of biocontaminants in schools, school boards have come under intense pressure from building occupants and parent groups to expeditiously remedy problems found in their schools. Water- and mold-contaminated building materials have been removed and replaced, acres of contaminated school carpets have been discarded and, in a few instances, significant building reconstruction has taken place to ensure that biocontaminants and amplification sources have been removed. This has cost school boards a considerable amount of money. Some school boards in Nova Sco-

tia and in Prince Edward Island have spent from hundreds of thousands to several million dollars to remedy some of their school biocontamination problems.

One of my main concerns is the high cost to school boards of building inspections, and air and substrate testing for biocontaminants, since this may eventually prove to be a considerable disincentive to school boards wanting quality work done in this area. In my opinion, the excessive cost is due in part to the fact that building inspections and remedial work for microbial contaminants are usually conducted by environmental consultant companies, instead of by school board maintenance staffs. In only a few instances are maintenance staffs regularly doing their own work in this area.

One way I see costs being reduced is if maintenance staff within each or some of the various school board jurisdictions could be provided hands-on training and resource material. This would allow them to conduct their own inspections of school building spaces and the HVAC systems for fungi and other microbial contaminants. I believe that it is a wise strategy that investigations should be conducted by personnel familiar with building design, ventilation and maintenance. However, in speaking with school maintenance staff, it has become clear that staff members are uncertain about what they should do or be looking for. Possibly, this is because school maintenance staff usually have little familiarity with identifying potential building microbial amplifiers, knowledge of sampling methods for microbial contaminants in their school environment, remediation procedures, and recent literature dealing with these issues. They have received little if any training in this area, and lack sufficient resource material which they can consult prior to undertaking their own air quality investigations and inspections.

I have written to the superintendents of all the Nova Scotia and New Brunswick school boards since September (1994) and proposed training sessions for their maintenance personnel. This idea has been well received and I have received invitations from school board administrators to provide their staff with on-site training, before the end of December, 1994.

I have proposed a full-day session for 5-10 maintenance staff members from each of the school boards. The training will comprise two parts:

(1) The first will be presented as a seminar and highlight fungi and other microbials as building biocontaminants. The seminar objectives will be to provide attendants with an overview of fungi in school and other building environments, conditions which contribute to indoor microbial amplification, and signs of contamination. It will also present information on some of the health implications associated with exposure to airborne fungi in building environments. Literature will be circulated to training-session participants, which is referenced below.

(2) The second component of the training will involve an on-site inspection of a school with maintenance staff to recognize sources and signs of water infiltration and condensation in the building environment, and to identify potential microbial amplification sites. The objectives here will

also be to provide an overview of sampling strategies (non-complaint and complaint-based sampling, and air and bulk sampling), the necessity and significance of sampling, and remediation procedures. Staff members will also be provided with hands-on sampling practice with the RCS sampler, and swab and bulk sample collection methods. Workers will also be provided with directions on how to ship materials to microbiological laboratories for analyses.

It is hoped that this proactive approach will reduce the prevalence of microbial contamination in school environments and significantly reduce the costs associated with remediation.

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GUIDELINES ON ASSESSMENT AND REMEDIATION OF *STACHYBOTRYS ATRA* IN INDOOR ENVIRONMENTS

Sponsored by:

New York City Department of Health
New York City Human Resources Administration
Mount Sinai-Irving J. Selikoff
Occupational Health Clinical Center

Editorial note: *Because of frequent requests we present here the consensus guidelines from a recent expert panel meeting related to the topic of assessment and remediation. Participants were, among others, Dr. P. Morey, Dr. B. Sorenson, Prof. B. Jarvis, Dr. D. Miller, Dr. C. Yang, and Dr. E. Johanning.*

ENVIRONMENTAL ASSESSMENT

Criteria for Initial Inspection

Reports about potential *Stachybotrys atra* contamination in buildings should be followed up to ascertain whether a site inspection is warranted. The criteria for conducting an initial inspection include:

(1) presence of visible mold;
(2) evidence of water damage;
(3) symptoms which are consistent with an allergic or toxic response to *Stachybotrys atra* (e.g., respiratory illness, rashes and chronic fatigue) and are severe enough as judged by medical documentation to result in lost work days.

Inspection Procedures

If visible mold, water damage, and occupants exhibiting related symptoms serious enough to result in lost work days are present, a site inspection should be conducted. The results of all site inspections should be compiled into a written report, and made available to the building owner, employer and

Based on a panel discussion, May 7, 1993, District Council 37 AFSCME, 125 Barclay Place, New York, NY 10007.

employee representatives in the affected areas. Such an inspection should be conducted according to the following protocol:

Visual Inspection

Visual inspection is the most important initial step in identifying a possible contamination problem. Visual identification of black mold in chronically-wet areas is considered to be a possible indicator of *Stachybotrys atra*. Ceiling tiles, gypsum wall board, cardboard, paper, and other cellulosic surfaces should be given careful attention during visual inspection. The extent of any water damage and mold growth should be evaluated as this will be important in determining remedial strategies. Ventilation systems should also be visually checked, particularly for damp filters.

Bulk Sampling

a. If only a limited area is affected (i.e. all or part of an area that is approximately 30 square feet or less), the water damage is the result of a known occurrence, and no occupants are experiencing symptoms, then bulk (or other sampling) is not required. Remediation (as described under Remediation, Section A) should proceed without further evaluation, under the assumption that *Stachybotrys atra* or (other fungal contaminants) are present.

b. Bulk samples should only be used to document the presence and extent of *Stachybotrys atra* if extensive areas are affected, for example if visible mold occurs on areas larger than one wall board panel and water damage is a chronic problem, or if occupants are experiencing symptoms which may be related to *Stachybotrys atra* exposure.

c. When bulk sampling is required, designated personnel should collect bulk samples from appropriate areas (e.g. damp, moldy, cellulose-derived material) by scraping surface materials into a clean Ziploc plastic bag or by stripping the suspect surface with vinyl acetate tape.

Air Monitoring

a. Air sampling for *Stachybotrys atra* should not be part of a routine assessment. This is because air sampling methods for *Stachybotrys atra* are prone to false negative results and therefore cannot be used to rule out contamination. In addition, when the size of the affected area is small or moderate, decisions about appropriate remediation strategies can be made on the basis of visual inspection and bulk sampling.

b. Air monitoring may be required if there is evidence from visual inspection or bulk sampling that ventilation systems may be contaminated. The purpose of such air monitoring is to assess the extent of contamination throughout a building.

c. If air monitoring is conducted, personnel conducting the sampling must be trained in proper air sampling methods for microbial contaminants.

Evaluation of Environmental Data

Analysis

a. Documented quality control in the laboratories used for analysis of the bulk and air samples is necessary. *Stachybotrys atra* is easily missed if other species are present and microscopic identification of the spores requires considerable expertise. These services are not routinely available from commercial laboratories. The laboratory director should be familiar with the literature concerning *Stachybotrys atra*. A list of approved laboratories is available from the New York State Department of Health (518) 474-7413.

b. Samples should also be analyzed for the presence of other common indoor microbial contaminants.

c. Both indoor and outdoor air samples from nearby areas without signs of contamination should be collected and compared.

Evaluation criteria

a. Bulk sampling: Sampling results in excess of 1 colony forming unit per gram should be considered positive. Surfaces that were sampled and found to be contaminated with *Stachybotrys atra* need to be remediated, as described in Section III.

b. Air monitoring:

(1) Concentrations of mold in indoor air which exceed concentrations in outdoor air should be considered positive. Remediation of surfaces and general cleaning is required, as described in Section III.

(2) Airborne concentrations of 103-104 cfu/m3 or greater require immediate evacuation of all occupants.

REMEDIATION

Different levels of containment are necessary depending on the extent of the contamination problem. In all situations, the underlying cause of water accumulation must be rectified or the problem will recur. There must be a mechanism in place for ensuring an immediate response to these problems. Cleanup should be conducted when the affected area is unoccupied. In all remediations, a routine follow-up inspection at 6-12 months or sooner if visible mold contamination or water damage recurs should be conducted. Emphasis should be on ensuring proper repair of the building infrastructure, so that water damage and moisture buildup do not recur.

Four different levels of abatement, as described below, are identified, based on the extent of *Stachybotrys atra* contamination.

Level I: Small Isolated Areas (2 sq. ft. or less)

(1) Example: ceiling tiles

(2) Cleanup can be conducted by regular building maintenance staff. Such persons must receive training from a qualified individual on proper cleanup methods, protection, and potential health hazards, and should

be free from asthma, allergy and immune suppressive disorders. Gloves and a half face respirator should be worn. A full respiratory protection program, in accordance with 29 CFR 1910.134 is required.

(3) Contaminated absorbent material should be removed in a sealed plastic bag.
(4) Surrounding areas should be cleaned with household bleach.
(5) Special containment or evacuation measures are not necessary.

Level II: Larger Isolated Areas (2 - approximately 30 sq. ft.)

(1) Example: individual drywall panels.
(2) Cleanup can be conducted by regular building maintenance staff. Such persons must receive training from a qualified individual on proper cleanup methods, protection, and potential health hazards, and should be free from asthma, allergy and immune suppressive disorders. Gloves and a half face respirator should be worn. A full respiratory protection program, in accordance with 29 CFR 1910.134 is required.
(3) Surrounding material should be covered with plastic sheets and tape before removal.
(4) Contaminated absorbent material should be removed in a sealed plastic bag.
(5) Surrounding areas should be cleaned with household bleach.

Level III: Large Scale Remediations[1] (more than 30 square feet)

(1) Example: More than one wallboard panel in an area which cannot be isolated from personnel.
(2) Personnel trained in the handling of hazardous materials is necessary.
(3) Containment of the affected area is required.
    a. Complete isolation of work area from occupied spaces using plastic sheeting sealed with duct tape (including openings, fixtures and HVAC components) is required.
    b. A high efficiency particulate air (HEPA) exhausted negative air unit is required.
    c. Airlocks and decontamination room are needed for exit from work area.
(4) Contaminated material should be removed in double-sealed plastic bags.
(5) The work area must be HEPA vacuumed prior to the removal of isolation barriers.
(6) Cleanup workers should wear:
    a. Full-face respirators with HEPA cartridges or powered air purifying respirators.
    b. Disposable protective clothing, head gear, foot covering, gloves.

(7) Air monitoring:
   a. should be conducted during remediation to determine if spores are escaping during remediation and prior to removal of isolation barriers to assess the efficacy of the remediation.
   b. should be conducted after large scale remediation, to determine its effectiveness and whether an area is safe for symptomatic persons to reoccupy. If post-remediation air samples indicate the presence of SA, even in minor amounts, further investigation of possible sources is required.

Level IV: Remediation of HVAC Systems

(1) Personnel trained in the handling of hazardous materials are required for remediation of HVAC systems.
(2) Containment of the affected area is required.
   a. Complete isolation of work area from occupied spaces using plastic sheeting sealed with duct tape (including openings, fixtures and HVAC components) is required.
   b. A high efficiency particulate air (HEPA) exhausted negative air unit is required.
   c. Airlocks and decontamination room are needed for exit from work area.
(3) Contaminated material should be removed in double-sealed plastic bags.
(4) The work area must be HEPA vacuumed prior to the removal of isolation barriers.
(5) Cleanup workers should wear:
   a. full-face respirators with HEPA cartridges or powered air purifying respirators.
   b. disposable protective clothing, head gear, foot covering, gloves.
(6) If *Stachybotrys atra* is present in settled dust removal with a HEPA equipped vacuum and subsequent damp wiping is recommended.
(7) Air monitoring:
   a. should be conducted during remediation to determine is spores are escaping during remediation and prior to removal of isolation barriers to assess efficacy of the remediation.
   b. should be conducted after large scale remediation, to determine its effectiveness and whether an area is safe for symptomatic persons to reoccupy. If post-remediation air samples indicate the presence of *Stachybotrys atra*, even in minor amounts, further investigation of possible sources is required.
(8) Growth supporting material should be removed from ducts with a HEPA vacuum, where practical, if not removal of the affected component of the HVAC system is required.
(9) Contaminated material should be disinfected prior to removal. Decisions concerning the type of disinfection should be made by a qualified

individual, based on the extent of the growth supporting material. Decisions as to disinfection must be based on the extent of the growth substrate in the ducts. There are numerous "biocides" such as quaternary ammonium compounds (e.g. dimethylbenzyl ammonium chloride) that are employed routinely for disinfection and cleaning surfaces, particularly in hospitals and laboratories. Some of these biocides are recommended by manufacturers for use with cooling coils and condensation pans. In fact, the biocides are essential for maintaining the system. Household bleach is often recommended and can be used to clean coils. Chloorine dioxide or ozone are used for disinfecting inside of ducts.

Potentially toxic substances such as chlorine dioxide or ozone that are currently used for disinfecting duct work should not be used when the building is inhabited. Also, sufficient time should be allowed for the disinfectant to dissipate. These substances, however do have a short half life. As to whether they would be efficacious or not would depend upon the extent of the contamination and circumstances of application. As a safety factor it may be advisable to disinfect molded material within a duct system prior to cleaning.

(10) The causes of *Stachybotrys atra* accumulation and/or growth must be identified and corrective action taken.

Hazard Communication

When *Stachybotrys atra* is found, occupants in the affected area(s) should be notified of its presence by the building owner and the employer. Notification should include the description of the remedial measures to be taken and a timetable for completion. Group meetings held before and after remediation with full disclosure of plans and results can be an effective communication mechanism. Some individuals may require separate counseling. They should be encouraged to seek medical advice from a qualified occupational/environmental health practitioner if they are concerned about continuing health problems. Individuals seeking medical attention should be provided with a copy of all inspection results and interpretation to give to their medical practitioners.

CONCLUSION

In summary, prompt removal of contaminated material and infrastructural repair must be the primary response to *Stachybotrys atra* contamination in buildings. Emphasis should be placed on preventing contamination through proper building maintenance and prompt repair of water damaged areas.

Chronic exposure to airborne *Stachybotrys atra* poses a risk of debilitating health effects caused by irritative and allergic reactions. This risk is compounded by exposure to additional molds and other pollutants usually found in buildings contaminated by *Stachybotrys atra*. Laboratory tests for immune

markers associated with *Stachybotrys atra* exposure are not helpful at this time. Research should be pursued to refine such tests and characterize them more fully.

The simplest and most expedient remediation that properly and safely removes *Stachybotrys atra* from buildings should be used. This includes prompt removal, cleaning of contaminated sites and repair of the defects that led to water accumulation. Widespread contamination poses much larger problems that must be addressed on a case-by-case basis in accordance with published guidelines for remediation. Effective communication with building occupants is an essential component of all remedial efforts. Individuals with persistent health problems should be referred to physicians competent in evaluating health effects of microbial exposures.

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LEGAL ASPECTS OF INDOOR AIR QUALITY

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Abstract: *The design, construction, maintenance and use of a building creates a web of legal relationships based on rights and their correlative duties which involves everyone who has an interest in or uses the building. "Sick building syndrome" is caused by inadequate ventilation, and usually does not have long-term health effects. Building-related illness is caused by the presence of an environmental agent that causes illness. The problem may be compounded by inadequate ventilation. Biological contaminants such as fungi and bacteria can cause serious, long-term health effects. Indoor air quality (IAQ)/ building-related illness can also be an incident of the workplace in industrial and agricultural settings. Possible legal remedies for the victim of building-related illness include worker's compensation, disability claims, landlord/tenant, economic loss litigation, property damage/value litigation, and personal injury litigation. Personal injury litigation is the most important legal remedy for the individual victim, and has the greatest effect on all involved parties due to the deterrent effect of potentially large liability. Personal injury actions can be based on negligence, strict liability or breach of some other duty. Depending on the legal theory used, it may or may not be necessary to prove fault. IAQ problems arising from biological contamination usually result from negligence in that they are caused by recurrent or persistent water damage that has not been remediated, lack of proper maintenance, and, often, inadequate ventilation. It is necessary to prove exposure, causation, and the extent of damages. Expert testimony from a qualified physician is required. Owners are primarily liable because theyhave a nondelegable duty to provide a safe environment. Architects, developers/builders, contractors, building management, lessors, and even testng and remediation companies can be held liable if found to be responsible for the plaintiff's injury. Liability is best avoided by preventing the problem in the first place, or by promptly correcting a problem when it occurs.*

Key words: Building-related illness, worker's compensation, indoor air quality, disability claims, loss litigation, liability

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INTRODUCTION

A building creates a web of legal relationships between the architects, builders, contractors, owners, managers, lessors, tenants, occupants, visitors, trespassers, and just about anyone who has a property interest in, works on, or uses the building. These relationships are based on duties and their correlative rights which are either contractual or created by law. The most important legally imposed duty is the obligation of all parties to act with reasonable care, or as a reasonably prudent person would act under the circumstances, the failure of which can result in liability for negligence. Other duties include the duty of professionals, such as architects, to act in accordance with accepted standards of practice in their profession, and the nondelegable duty of an owner to provide a safe environment to building occupants.

Indoor air problems act as a catalyst to awaken these legal relationships when a party seeks to enforce its rights as a consequence of some other party's failure to fulfill a duty.

There are two basic types of indoor air problems: "sick building syndrome," and building-related illness. Sick building syndrome is caused by a combination of factors, including inadequate ventilation due to defective, improperly designed, operated or maintained heating/ventilation/air conditioning (HVAC) systems, and air contamination from toxic building materials or other products, such as volatile organic chemicals. Sick building syndrome is also called "tight building syndrome," and is usually seen in new or newly renovated "sealed" buildings which rely entirely on HVAC systems for ventilation. Sick building syndrome can cause serious health complaints in affected individuals, including respiratory and multiple chemical sensitivity ("MCS") type complaints. However, for most affected individuals, the consequences are relatively minor and transitory in nature.

Building-related illness is caused by the presence of some toxin or other environmental agent which causes illness. Inadequate ventilation usually compounds the problem, but is not necessarily a component. Building-related illness can have serious and long-term health effects. Biological contaminants, including fungi, toxins and bacteria, are a significant source of indoor air problems in that they are common, naturally occurring substances which, when conditions exist which allow them to proliferate, can cause severe health effects. Exposure to fungi (and fungal toxins) such as *Aspergillus, Penicillium,* or *Stachybotrys* can cause health effects including allergic reactions, asthma, hypersensitivity pneumonitis, immune dysfunction and neurological problems. Bacteria can cause a number of conditions, including Legionnaire's disease, which can be fatal, and Pontiac fever. These particular conditions are usually associated with water aerosols, such as cooling towers or humidifier systems.

Building-related illness can also be an incident of the workplace. Particulate contamination is most often encountered in industrial or agricultural settings, and can cause serious respiratory problems. Workers in these fields should be supplied with proper protective devices. Certain gasses, such as carbon dioxide,

carbon monoxide, and ozone, can cause health effects at high levels, and result from inadequate ventilation in the workplace. Volatile organic compounds are emitted by a number of materials and products, and can cause health problems. Pesticides, lead, radon, and asbestos are just some of the many other substances that may present health risks for building occupants.

The typical victim of building-related illness is a person who has been working (or living) in an older building that is poorly maintained, or in a new building or one that has recently undergone renovation. She has been vaguely ill for some time, with her condition worsening over time. Complaints of eye and throat irritation, respiratory problems, fatigue, neurological difficulties, and allergies or environmental sensitivities are most common. In many cases, the precise cause of her complaints is undetermined. Usually, other workers (or residents) in the same building or part of the building have had similar complaints to varying degrees. She has been to a number of doctors, with different diagnoses, and has eventually been diagnosed with environmentally caused illness.

The victim of building-related illness has a number of legal remedies available in different forums. These include:

Workers' Compensation: workers' compensation is a system designed to compensate workers who sustain injuries on the job. Employers are required by law to purchase compensation insurance, and claims are determined by an administrative judge. The claimant must only prove that he was injured in the course of employment, and the nature and extent of the injuries. Workers' compensation typically provides some percentage of lost earnings, compensation for permanent disability at scheduled rates, and payment for related medical treatment. Generally, workers who are eligible to receive workers' compensation are barred from directly suing their employer.

Disability claims: persons who become disabled as a result of building-related illness may file private insurance disability claims, or claims for Social Security disability. The claimant is required to provide medical evidence of their disability. The failure of insurance companies to pay disability claims is a fertile source of litigation.

Landlord/tenant: the law imposes a warranty of habitability on lessors, and tenants with leases also have contractual rights. Indoor air quality problems can create a "constructive eviction," allowing the tenant to vacate the premises and cancel the lease. A tenant can also sue for personal injuries, discussed further below, or other damages.

Economic loss litigation: businesses can suffer economic losses when their employees become ill, or they are forced to suspend operations or relocate because of air quality problems. Commercial tenants have rights under their leases, and can bring actions most commonly against the owner/lessor, or against other responsible parties, such as HVAC contractors, etc. If the company has business loss insurance, the insurance company is subrogated to the company's rights and will usually seek to recover from responsible parties.

Property damage/value litigation: biological contamination can be expensive and difficult to remediate, particularly when it stems from construction defects. The value of a property can be adversely affected and result in litigation by owners against responsible parties.

Personal injury litigation: is probably the most important legal remedy for the individual who is seriously affected by building-related illness, and has the broadest reaching consequences for all parties involved. A person who has suffered an injury as a result of another's negligence or other breach of duty (such as the owner's duty to provide a safe environment) can usually bring an action to recover fair and reasonable compensation for their injury and its consequences, including economic loss, disability and pain and suffering. Punitive damages may also be available to punish grossly wrongful conduct, and even if they are not, the size of jury verdicts often reflects what one might call the "degree of wrongfulness" of the offending conduct. The fear of potentially large liability is, unfortunately, a too often necessary economic motivation for people to do their jobs properly, and to attend to building occupant or worker safety.

IAQ-related personal injury litigation can be based on any of a number of legal theories: negligence, strict liability, or breach of an owner's or other's duty. It may or may not be necessary to prove fault. In any case, it is important to confirm the client's exposure, causation, and the extent of damages. Exposure is usually confirmed by a combination of properly performed microbiological sampling and testing, together with examination and evaluation by a qualified occupational physician. Antibody marker testing can be helpful in providing specific confirmatory evidence. Other possible causes should be excluded. The number of affected persons can also provide persuasive evidence: one sick person may be an anomaly and two sick people may be a coincidence, but three sick people are a toxic exposure. Causation and damages requires the expert testimony of a qualified physician. The doctor must testify, based on his review of records and/or examination of the plaintiff, that it is his opinion "to a reasonable degree of medical certainty" that the toxic exposure was the cause of the plaintiff's injuries, and as to the extent of the injuries.

IAQ problems arising from biological contamination are particularly amenable to personal inujury lawsuits based on negligence or other breach of duty in that their common denominator, which is conditions that allow fungi and bacteria to flourish, necessarily requires negligence. All these cases have in common: (1) recurrent or persistent water damage which has not been properly remediated; (2) lack of proper maintenance, and, often, (3) inadequate HVAC.

Who is liable to the victim of building-related illness? In most cases, the party primarily liable is the building owner. As discussed above, the owner has a nondelegable duty to provide a safe environment, and can be held liable for the failure to do so. The situation is complicated, however, where the owner of the building is also the plaintiff's employer, and the plaintiff is eligible for Workers' Compensation and therefore barred from suing his employer. In that case, it is

necessary to identify some other party whose wrongful act, breach of duty or defective or unsafe product caused or contributed to the potential plaintiff's injuries. These can include architects, who can be liable for improper design of the building, usually with regard to the HVAC or plumbing systems (creating conditions that allow biological contaminants to flourish), or moisture resistance. As a professional, an architect is liable for malpractice, which is a departure from accepted standards of practice in the locality.

Developers/builders can be liable for improper construction, including inadequate moisture resistance in materials or assembly, improper drainage, placement or assembly of plumbing, or improper HVAC. In some states, developers and builders are protected by "grandfather clauses" which protect them from suit after some time period has elapsed. General contractors have similar potential liability. Building management is the agent of the owner, and is often the party responsible for improper maintenance, or the failure to properly remediate potentially dangerous conditions. HVAC contractors, engineers or consultants can be liable for improperly designed, assembled or installed HVAC systems or their components. Lessors can be held liable for breach of their lease agreement or warranties implied by law. Even testing and remediation companies can be liable if they fail to properly perform testing or remediation, or fail to make proper recommendations.

There are a number of different types of statutes, standards and guidelines applicable to IAQ problems. Violation of a statute alone can create liability. For example, most states have labor laws that create a nondelegable duty on the part of an employer to provide a safe workplace. Violation of standards and guidelines, on the other hand, usually only provide some evidence of negligence. However, that evidence can be very persuasive. For example, violation of ASHRAE Standard 62-1989 for indoor ventilation indicates a failure to comply with accepted standards of practice and, for all practical purposes, is persuasive evidence of negligence or professional malpractice.

Potential liability for IAQ related injuries is best avoided by preventing the problem in the first place. Proper design, construction, maintenance, etc., together with attending to, rather than ignoring or dismissing worker or occupant complaints, is the best way to avoid liability. Promptly addressing and correcting conditions which cause IAQ problems will always result in less liability, because there will be less injury, than the too frequently taken approach of denying that a problem exists until it reaches a point where potential liability can be enormous.

Note: this article is a general discussion of legal issues and is not intended as legal advice for any particular situation.

THE AMERICANS WITH DISABILITIES ACT AND CONTAMINATED INDOOR AIR ENVIRONMENTS

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Abstract: *Indoor air contaminants, including the presence of bacterial or fungal agents, can cause disabling health effects among certain sensitive individuals. If such health effects significantly impair an individual's ability to function in a work environment or otherwise, that impairment may be considered a "disability" under the federal Americans with Disabilities Act. Where a disability is identified, certain legal requirements to accommodate the disability come into play. This article explores the conditions under which sensitivity to indoor air contaminants could be considered a disability. The article also analyzes the types of reasonable accommodations that an employer might be expected to provide under Title I of the ADA, so long as the accommodations do not cause the employer an "undue hardship." Possible accommodations might include removing the sources of indoor air contamination, improving ventilation, moving the affected individual away from the source, or providing work-at-home options.*

Key words: Americans with Disabilities Act, disabled or disability, employment, indoor air, legal remedies, reasonable accommodation, undue hardship

INTRODUCTION

The U.S. Environmental Protection Agency has characterized indoor air pollution as one of the greatest environmental risks facing Americans, second only to radon.[1] Indoor air pollution, including fungal or bacterial contamination,

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[1]*See* Cross, FB (1990) "Legal Responses to Indoor Air Pollution." Westport, Connecticut: Quorum Books, p. 3.

can increase the risk of cancer and birth defects, and cause eye and respiratory irritations, headaches, metabolic disorders, nausea, and other effects. Some, however, experience far more severe reactions to low levels of contaminants. For these individuals, exposure to indoor air pollution can cause more than annoying symptoms or an amorphous future risk. Like the proverbial canaries in the mine, they experience immediate and debilitating reactions to levels of contaminants that would not affect most people.

Many of those who are sensitive to indoor air contaminants could function at home and at work if their exposure to contamination were minimized or eliminated. With the enactment in 1990 of a new federal statute, the Americans with Disabilities Act[2] (ADA), such individuals may have a legal basis for minimizing their exposure to indoor air contamination. Title I of the ADA prohibits employers of more than 15 employees from discriminating against the disabled. Title II prohibits discrimination in the provision of public services. Title III prohibits discrimination in the provision of public services by private entities. This article will address the employment provisions of the ADA.

The Americans With Disabilities Act's Employment Provisions

In passing the ADA, Congress found that

> *The Nation's proper goals regarding individuals with disabilities are to assure equality of opportunity, full participation, independent living, and economic self-sufficiency for such individuals; and . . . [that] the continuing existence of unfair and unnecessary discrimination and prejudice denies people with disabilities the opportunity to compete on an equal basis and to pursue those opportunities for which our free society is justifiably famous, and costs the United States billions of dollars in unnecessary expenses resulting from dependency and nonproductivity.*

Many of those affected by indoor air contaminants could work or continue to work if they were able to avoid the contaminants barring their access. As Mary Lamielle, President of the National Center for Environmental Health Strategies, an educational and advocacy organization for MCS sufferers, has stated: "People who are sick from indoor air pollutants in public and private workplaces want accommodation and remediation—not litigation. . . . They want to be healthy and productive." The ADA may provide a mechanism to enable them to participate in the workplace and achieve economic self-sufficiency, while eliminating the expense that would otherwise result from their unnecessary alienation and dependency. These efforts might also improve the safety and quality of the environment for everyone in the workplace.

Under the ADA, employers are required not only to refrain from passive discrimination, but to undertake active efforts to provide access to the disabled. The ADA defines discrimination broadly to include the failure to make reasonable accommodations for qualified individuals with disabilities, so long as the

[2]42 U.S.C. § 12101 *et seq.*

accommodation would not impose an undue hardship on the employer. In the context of an individual reacting to indoor air contaminants, the law could be interpreted to require an employer to reduce the employee's exposure or risk facing liability under the ADA.

Because of the wide diversity of circumstances presented by employers confronted with disabled individuals, the law and its implementing regulations do not provide a blueprint of rights and responsibilities. Instead, they establish the basic parameters needed for case-by-case determinations. Key issues are: Who is "disabled"? Who is "qualified"? What is a "reasonable accommodation"? What constitutes "undue hardship"?

Who is Disabled?

The ADA states that "[t]he term 'disabled' means. . . . (A) a physical or mental impairment that substantially limits one or more of the major life activities of such individual. . . ."

That the definition includes both physical and mental impairments may help those with environmental sensitivities avoid the sometimes difficult burden of having to prove the physical nature of their disability in an area marked by great scientific uncertainty. The ADA is concerned with the effect of the disability on an employee, not with its precise diagnosis, nature or cause[3]. Thus, labeling the cause as "psychological" would not necessarily preclude relief.

Although the ADA does not require an individual to explain the precise nature of the disability, it does require that he or she prove that the disabling effects are "real." The Department of Justice's (DOJ's) statements regarding the ADA, as well as agency statements and case law interpreting similar statutes, indicate that the effects of sensitivities to indoor air contaminants can be recognized as legitimate disabilities.

In addressing the analagous issue of chemical sensitivities in the context of the provision of public accommodations, DOJ has stated that chemical sensitivities would be recognized as "disabilities" if the claimant could show that respiratory or neurological functioning were severely affected.[4] Interpretations of the regulations implementing the Rehabilitation Act of 1973,[5] the ADA-equivalent for federal agencies and programs receiving federal funding, are also enlightening due to the Congressional intent to model the ADA regulations after the Rehabilitation Act regulations. The definition of the term "handicapped" under the Rehabilitation Act regulations is essentially the same as the definition of the term "disability" under the ADA.[6] In interpreting the Rehabilitation Act regulations, the Supreme Court has stated that the agency "found that a broad

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[3]*See* Equal Employment Opportunity Commission and the U.S. Department of Justice, *Americans with Disabilities Act Handbook* I-1, I-28 (October 1991) [hereinafter *ADA Handbook*].

[4]56 Fed. Reg. 35549 (1991).

[5]42 U.S.C. § 701 *et seq.*

[6]*Compare* 41 C.F.R. § 60-741.2 (example of Rehabilitation Act regulations defining "handicapped individual") *with* 29 C.F.R. § 1630.2(g) (ADA regulations defining "disability").

definition, one not limited to so-called 'traditional handicaps,' is inherent in the statutory definition."[7] A number of courts have determined that those sensitive to indoor air contaminants are "handicapped" under the Rehabilitation Act.[8]

The same definition of "handicapped" is also included in the U.S. Department of Housing and Urban Development's (HUD's) regulations[9] implementing the Fair Housing Act's prohibition against discrimination against the handicapped in the housing context.[10] HUD has concluded as a matter of policy that individuals with environmental sensitivities could be considered "handicapped" under the regulations' definition,[11] and has so determined on several specific occasions.[12] Several state courts have found sensitivities to indoor air contaminants disabling under similar or narrower state laws,[13] as have the Social Security Administration and other federal agencies under narrower definitions of "disability."[14]

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[7]*School Board of Nassau County v. Arline*, 107 S.Ct. 1123, 1127 n.5 (1987); *see also Gilbert v. Frank*, 949 F.2d 637, 641 (2d Cir. 1991); *Doe v. New York University*, 666 F.2d 761, 775 (2d Cir. 1981).

[8]*Rosiak v. U.S. Department of the Army*, 679 F. Supp. 444 (M.D. Penn. 1987) (accepting without discussion that hypersensitivity to hydrocarbon fumes and dust, resulting from initial exposure to contact cement, is a handicap under the Rehabilitation Act); *Vickers v. Veterans Administration*, 549 F. Supp. 85, 86-87, (W.D. Wash. 1982) (hypersensitivity to cigarette smoke is a "handicap" under the Rehabilitation Act); *cf. Walders v. Garrett*, 765 F. Supp. 303, 350 n.4 (E.D. Va., 1991) (holding that Chronic Fatigue Syndrome "plainly fits within the [] definition of a handicap" under the Rehabilitation Act).

[9]24 C.F.R. § 100.201 (1992).

[10]*See* 42 U.S.C. § 3602(h)1

[11]*See* Memorandum from George L. Weidenfeller, Deputy General Counsel, HUD, to All Regional Counsel (April 14, 1992) [hereinafter HUD Memorandum].

[12]*See Corcelli v. Gilbane Properties, Inc.*, (Case Nos. 01-90-0255-1-5, 01-90-0512-1) (Dec. 11, 1990) (cited in HUD Memorandum, *supra* at 18); *cf.* HUD Concilation Agreement between HUD and Horner (complainants) and Wirtz Realty Corp., (respondent) (case no. 05-91-1198-1) (Feb. 27, 1992) (requiring landlord to reasonably accommodate complainants' chemical sensitivities); HUD Conciliation Agreement between HUD and LeRoy (complainants) and Michael Daniels/Cagan Realty (respondent) (case no. 05-92-1440-1 (Dec. 2, 1992) (requiring realtor's recognition that those with multiple chemical sensitivities may need special accommodations to reduce chemical exposures).

[13]See cases cited in HUD Memorandum, *supra*, at 13-14, including *Lincoln Realty Management co. v. Pennsylvania Human Relations Commission*, 598 A.2d 594 (Pa. Comm. 1991) (regrding housing discrimination); *County of Fresno v. Fair Employment and Housing Commission of the State of California*, 226 Cal. App.3d 1541, 1550, 277 Cal. Rptr. 557, 563 (5th Dist. 1991) (regarding employment discrimination); *Kallas Enterprises v. Ohio Civil Rights Commission*, 1990 Ohio App. LEXIS 1683 (Ohio Ct. App. May 2, 1990) (regarding employment discrimination); *Kent State University v. Ohio Civil Rights Commission*, 64 Ohio App. 3d 427, 581 N.E.2d 1135 (1989).

[14]See cases discussed in HUD Memorandum, *supra*, including *Kouril v. Bowen*, 912 F. 2d 971, 974 (8th Cir. 1990) (Social Security Act); *Kornock v. Harris*, 648 F .2d 525, 527 (9th Cir. 1980) (same); *San Diego (Cal.) Unified School District*, 1 National Disability Law Rptr., par. 61, p. 311 (May 24, 1990) (Department of Energy finding that a person with chemical sensitivities is "handicapped" under the Rehabilitation Act); *Montville (Conn.) Bd. of Education*, 1 National Disability Law Rptr., par. 123, p. 515 (July 6, 1990) (same).

Although the ADA and its implementing regulations, in contrast to workers' compensation and Social Security law, do not require particular types of evidence and proof, the chances of overcoming skepticism will be increased by garnering as much medical evidence as possible. A recent Supreme Court case, *Daubert v. Merrell-Dow Pharmaceuticals, Inc.*, has stated that, although evidence does not have to be "generally accepted," a judge is to serve as an evidentiary "gatekeeper" by evaluating the adequacy of the methodology used to reach the scientific conclusions. Thus, the scientific and medical communities will play a key role in providing the carefully documented scientific and medical information necessary to help those reacting to indoor air contamination assert their legal rights.

Even if an individual's physical or mental disability is recognized as legitimate, that disability will not be covered under the ADA unless it "substantially limits" a "major life activity," such as "working." An impairment is substantially limiting if it "significantly restricts the duration, manner or condition" in which an individual can perform the activity in comparison with the average person. Thus, individuals do not have to prove that they are completely dysfunctional before the law will deem them "disabled."

Because of the variation in the type and severity of symptoms associated with analagous chemical sensitivities, the DOJ, in promulgating the regulations implementing the ADA's public accommodations provisions, indicated that the determination of whether such sensitivities substantially impaired a major life activity must be made on a case-by-case basis, and "declined to state categorically" that chemical sensitivity is a disability.[15] The agency stated that it would be a disability if symptoms were severe, but would not be if they were minor "despite the sensitivity to environmental agents."[16]

"Working" is one of the "major life activities" that could be impaired by a disability. Determining whether an individual has a disability that substantially impairs his or her ability to work is somewhat more complex than determining whether there is an impairment of other major life activities, such as walking, seeing, hearing, or breathing. The analysis of whether someone is substantially impaired in the major life activity of working is necessary only if the person is not already substantially impaired in other major life activities. Thus, an individual whose sensitivity is triggered by contaminants not only at work, but in the environment at large, would be considered "disabled" without requiring the additional analysis required to determine if he or she were substantially impaired in the major life activity of working. However, if an individual's primary or most disabling sensitivity is to exposures in the workplace, then the Act's implementing regulations require the consideration of additional factors in determining whether he or she is disabled because of a substantial impairment in the ability to work.

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[15]56 Fed. Reg. 35549 (1991).

[16]*Id.*

These factors attempt to strike a balance between two extremes: requiring a person to demonstrate the inability to obtain any employment anywhere, and recognizing a disability based on an impairment in a single employment context. An individual must show that he or she is "significantly restricted in the ability to perform either a class of jobs or a broad range of jobs in various classes as compared to the average person having comparable training, skills and abilities."[17] The factors to be considered include the geographical area to which the individual has reasonable access, the type of job from which the individual would be disqualified due to the impairment, the number and types of jobs utilizing similar training and experience from which the individual would also be disqualified, and the number and types of jobs that do not use similar training or knowledge.[18]

The likelihood that those who react to indoor air contaminants will be deemed disabled thus turns on the severity of their reactions and the number or ubiquity of the contaminants to which they react. The reactions must be severe enough to substantially impair their functioning, and must be in response to contaminants that are common or diverse enough to render the search for alternative satisfactory employment difficult.

Otherwise Qualified Individuals

The ADA prevents employment discrimination against qualified individuals with disabilities. Whether an individual is qualified turns on whether that individual can meet the basic requirements of the job, with or without a reasonable accommodation.

The first step is evaluating whether the individual meets the educational, experience, certification, and other requirements established as prerequisites for the position in question. If an individual cannot meet one of the requirements and believes that the requirement itself is discriminatory, the employer must demonstrate that the requirement is "job-related. . . . and is consistent with business necessity. . . . "[19] The standard for a "business necessity," drawn from the Rehabilitation Act, states that

> *If a test or other selection criterion excludes an individual with a disability because of the disability and does not relate to the essential functions of a job, it is not consistent with business necessity.*[20]

That standard ties into the next step in analyzing whether an individual is qualified: determining whether the individual can perform the job's essential functions with or without reasonable accommodation. The regulations make clear that an employer cannot discriminate against an individual if that individual can perform the job's "fundamental" duties, even if that individual is

[17]29 CFR § 1630.2(j) (3) (i) (1992).
[18]29 CFR § 1630.2(j) (3) (ii) (1992).
[19]42 U.S.C. § 12112(6).
[20]*Technical Assistance Manual* at IV-3.

unable to perform its "marginal functions."[21] If an individual with environmental sensitivities could not perform marginal functions associated with his or her job but could perform the essential functions, he or she would nonetheless be qualified for the position.

Employees who react to indoor air contamination may require reasonable accommodations in order to be "qualified." The crucial issue, as revealed by the case law interpreting the Rehabilitation Act of 1973, is whether there are reasonable accommodations that will enable a disabled person to perform the essential functions of the position.[22]

Thus, the ADA can help only a portion of those who have environmental sensitivities. An individual who is not sensitive enough will not be "substantially impaired," will not be considered "disabled," and will therefore not have the benefit of the ADA's protection against discrimination. However, an individual who is too sensitive may not be able to find a reasonable accommodation that enables him or her to continue working, and thus may lose the benefit of the ADA's protection against discrimination because he or she is not "qualified." For those whose sensitivities are severe enough to be "disabled" but not so severe that they cannot be ameliorated by reasonable accommodations reducing exposure, however, the ADA may provide a mechanism for continued healthy and gainful employment and, depending upon the accommodation, for improved indoor air quality more generally.

Reasonable Accommodation

The ADA places a duty on employers to undertake affirmative actions to accommodate those with disabilities. The law specifically states that

> *The term 'discriminate' includes. . . . not making reasonable accommodations to the known physical or mental limitations of an otherwise qualified individual with a disability who is an applicant or employee, unless such covered entity can demonstrate that the accommodation would impose an undue hardship on the operation of the business. . . .* [23]

The law requires employers to modify the work environment or "the manner or circumstances under which the position held or desired is customarily performed" to enable disabled employees to perform a job's essential functions.[24] Since a "qualified individual" is one who can perform the essential functions of the job, the Act in effect requires an employer to make the reasonable accommodations necessary to render a disabled employee "qualified." The employer

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[21]*See* 29 CFR § 1630.2(n) (1) (1992). *See e.g., Ackerman v. Western Electric Co.*, 643 F. Supp. 836, 847-48 (ND Cal. 1986) (under California law, identical in this respect to Rehabilitation Act and ADA, 12% of total duties which employee could no longer perform due to asthma were not essential functions).

[22]*See Guice-Mills, supra*, 967 F .2d at 798; *Gilbert v. Frank*, 949 F. 2d 637, 642 (2d Cir. 1991); *Rosiak v. U.S. Dept. of Army*, 679 F. Supp. 444 (M.D. Penn. 1987).

[23]42 U.S.C. § 12112(b) (5) (A) (Supp. 1993); see 29 C.F.R. § 1630.9.

[24]29 C.F.R. § 1630.2(o) (ii).

is relieved of the obligation to provide a reasonable accommodation only if the employee would not be qualified despite the reasonable accommodation or if, as discussed below, the accommodation would result in an undue hardship.[25]

Reasonable accommodations are required not only to enable individuals with disabilities to perform the essential functions of the job, but also to ensure their ability to enjoy the same "benefits and privileges of employment as are enjoyed by. . . . other similarly situated employees without disabilities."[26] Such "benefits and privileges" include access to cafeterias, washrooms, meeting rooms, social events, and the like.[27]

After determining the job's essential functions, consulting with the disabled individual, and identifying and assessing potential accommodations, the employer must select "the accommodation that best serves the needs of the individual and the employer."[28] The employer is obligated to provide only an effective accommodation; the employer does not have to provide the best accommodation available.[29]

To be "effective," the working conditions provided need not be identical to the conditions provided others. If, for example, a worker could not enter the employer's lunchroom because of pollutants in that environment, the employer is not obligated to provide access to that lunchroom, so long as he or she provides another location where the employee can eat.

On the other hand, although the employer need not provide precisely the same working conditions as those available for other employees, the alternative conditions must provide the same sorts of benefits associated with other employees' working conditions. The Act prohibits discrimination by "limiting, segregating, or classifying a job applicant or employee in a way that adversely affects (that person's). . . . opportunities or status. . . ."[30]

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[25]*See School Bd. of Nassau County v. Arline*, 480 U.S. 273, 287 n.17 (1987) (interpreting similar provisions in Rehabilitation Act of 1973); *Guice-Mills v. Derwinski*, 967 F. 2d 794, 797 (2d Cir. 1992) (same); see also discussion of "qualified individual" above).

[26]29 C.F.R. § 1630.2(o) (iii). "Reasonable accommodation" also includes modifications to the job application process to enable a qualified applicant to be considered. 29 C.F.R. § 1630.2(o) (i). A chemical-free personnel office or alternative application procedures, such as permitting application submissions by mail, would be appropriate accommodations for the chemically sensitive.

[27]See EEOC, Technical Assistance Manual on the Employment Provisions (Title I) of the Americans With Disabilities Act III-3 (Jan. 1992) [hereinafter Technical Assistance Manual].

[28]Technical Assistance Manual at III-10.

[29]Id. at III-4. *See Harmer v. Virginia Electric and Power Co.*, 831 F. Supp. 1300 (E.D. Va. 1993) (company was not required to provide a smoke-free environment as a reasonable accommodation when employee could effectively perform essential functions without that accommodation); *Carter v. Bennett*, 840 F.2d 63, 68 (D.C. Cir. 1988) (under Rehabilitation Act, government must provide reasonable accommodation necessary to performance of essential functions, not every accommodation requested by plaintiff).

[30]42 U.S.C. § 12112(b) (1); see 29 C.F.R. § 1630.5.

There is some controversy regarding interpretation of the term "reasonable" in "reasonable accommodation." DOJ, in interpreting the public access section of the ADA (Title II), dismissed a complaint claiming that an individual was denied access to a public building due to a security guard's use of perfume. DOJ stated that it was not "reasonable" for a city agency to require security guards to refrain from wearing perfume. However, based on the clear terms of the law, the only basis for rejecting an effective accommodation would be that providing the accommodation caused the employer an undue hardship. An independent inquiry as to the accommodation's "reasonableness" does not appear appropriate.

Types of Accommodations

The Act anticipates a wide range of accommodations. It includes not only the physical alterations, such as wheelchair ramps, that are commonly associated with providing access for the disabled, but also changes in the nature and manner in which the jobs themselves are conducted. Although the ADA, its implementing regulations, and the Technical Assistance Manual developed by the enforcing agency, the Equal Employment Opportunity Commission (EEOC), suggest many possible accommodations, the list is far from exhaustive. The choice of an appropriate "reasonable accommodation" is ultimately a case-by-case decision made in light of the individual's and the employer's particular needs.

The appropriate accommodation for an individual reacting to indoor air contamination depends on the type and degree of sensitivity experienced by the individual, the contaminants triggering the reaction, and the demands of the workplace. There are several broad categories of appropriate accommodations: removing the offending contaminants from the workplace, removing the sensitive individual from areas necessitating exposure, or modifying the job to reduce exposure.

As one commentator on the applicability of the ADA to those with multiple chemical sensitivities (MCS) has noted:

> providing access to those with MCS boils down to providing good IAQ (indoor air quality). In IAQ terms, access. . . . means reducing the build-up and circulation of chemical emissions within the building.[31]

Because many of the "accommodations" that result in improving indoor air quality are simply "common sense worker protections," another commentator believes that "such actions will usually be difficult to attack as 'unreasonable'" "when required to enable a disabled employee to continue to work."[32] The

[31]Kosta, L. (1992): Access for the Disabled: Americans with Disabilities Act & Multiple Chemical Sensitivity, Indoor Air Review, Oct. 1992, at 6.

[32]Davis, ES (1990): Workers with Multiple Chemical Sensitivities: Legal Aspects (handout prepared for the American Public Health Association Annual Meeting, October 4, 1990).

same analysis may apply when the air contaminants are fungal or bacteriological in nature.

In some work environments, it may be relatively simple and inexpensive for employers to remove the contaminants triggering sensitivities. Removing conditions leading to bacterial or fungal contamination would be the first significant step. In others, an employer may not be able to remove the offending contaminants, but may be able to minimize their effect. For example, improved ventilation might mitigate the effect of the presence of some contaminants. As a reasonable accommodation, employers should be expected to meet at least the minimum air circulation standards recommended by the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE 62-1989). Air quality in particular areas could be improved by providing desktop air filters.

In many instances, the employer may be a tenant of the premises, and may therefore face certain additional obstacles to implementing accommodations. Although guidance documents and analogous case law do not address the issue, the flexible nature of the accommodation process would indicate that, where reasonable, the employer should enter into negotiations with the landlord to gain the landlord's cooperation and/or permission for the desired measures.

Alternatively, if the immediate working environment cannot be improved sufficiently, an employer may need to accommodate the individual by moving him or her away from the offending contaminants. Affected individuals may often be able to function with an accommodation as simple as placement next to a window that opens.

The ADA contemplates reasonable accommodations that involve not only changes in the physical environment, but changes in the nature and manner of the work itself. While such an accommodation may be less satisfying from the standpoint of improving indoor air quality, it may be of extreme importance to the affected individual.

If an individual with a disability can perform the essential, but not the marginal, functions of a job, a reasonable accommodation may involve reallocating or exchanging the "marginal" functions of the job with another individual.[33]

So long as the employee can still perform the essential functions of the job, an employer may be required to accommodate an employee's disability by allowing the work to be performed in a different time or manner than is customary. The ADA explicitly includes "job restructuring, part-time or modified

[33]ADA Handbook at I-42; see also 42 U.S.C. § 12111(9) (B) (including "job restructuring" within the definition of "reasonable accommodation"). In *Ackerman v. Western Elec. Co.*, 643 F. Supp. 836 (N.D. Cal. 1986), aff'd, 860 F. 2d 1514 (9th Cir. 1988), the court determined, under California law prohibiting discrimination against the handicapped, that an employer had failed to reasonably accommodate an employee suffering from asthma because the employer could have but did not reassign the 12% of her tasks that the employee was unable to perform.

work schedules" within its definition of potential "reasonable accommodations." Where the essential functions of the job can be performed at home, an employer could accommodate the employee by allowing him or her to work at home.[34]

In some cases, paid or unpaid leave may be a reasonable accommodation to allow an individual with a disability to cope with problems associated with the disability.[35] If inadvertently exposed to contaminants, an individual with chemical sensitivities may require a certain period of time away from the workplace to recover.[36] Leave may also be appropriate if the work environment becomes temporarily inhospitable.

If exposure to contaminants cannot be minimized by their removal or by adjustments in the way the job is performed, the reasonable accommodation of reassignment to a vacant position might be appropriate.[37] An employer "may not reassign people with disabilities only to certain undesirable positions, or only to certain [undesirable] offices or facilities,"[38] and may reassign an employee to a lower level status and pay only if no position of equivalent status is available.

Undue Hardship

An employer's obligations are not without limit: an employer will be liable for discrimination under the ADA for "not making reasonable accommodations to the known physical or mental limitations of an otherwise qualified individual with a disability. . . . unless. . . . [the employer] can demonstrate that the accommodation would impose an undue hardship on the operation of the

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[34]In *Langon v. Department of Health and Human Servs.*, 959 F. 2d 1053 (D.C. Cir. 1992), the employer had refused to accommodate an employee with multiple sclerosis by allowing her to work at home and then fired her based on her inability to come to the office. The D.C. Circuit, interpreting the Rehabilitation Act of 1973's similar requirements, reversed the trial court's grant of summary judgment for the employer, stating that whether the employee could have fulfilled the job's essential functions at home was question of fact.

[35]ADA Handbook at I-42; Technical Assistance Manual at III-6, II-23 to -24. See *Johnson v. Sullivan*, 764 F. Supp. 1053, 1066 (D. Md. 1991) ("there is a question as to whether the employer's handling of the requests [of the disabled individual, who suffered from heart disease, narcolepsy, and stress] for leave constituted 'reasonable accommodation'"); Sedor v. Frank, 756 F. Supp. 684 (D. Conn. 1991) (denying summary judgment for employer based on question of fact regarding whether the employer should have tolerated the absence of its employee as a reasonable accommodation to the employee's learning disabilities and emotional problems).

[36]During the period of recovery, the "employer cannot refuse to let an individual with a disability return to work because the worker is not fully recovered from injury" unless the employee cannot perform the essential functions of his or her job. Technical Assistance Manual at IX-4. One of the strategies the employer should consider in this context is reassignment to a vacant position. Id.

[37]42 U.S.C. § 12111(9) (B); 29 C.F.R. § 1630.2(o) (2) (ii). Several of the cases brought under the Rehabilitation Act noted employers' efforts to reassign disabled individuals. See *Guice-Mills v. Derwinski*, 967 F. 2d 794 (2d Cir. 1992); *Rosiak v. U.S. Dept of Army*, 679 F. Supp. 444 (M.D. Pa. 1987), aff'd without op., 845 F. 2d 1014 (3d Cir. 1988).

[38]Technical Assistance Manual at III-24; see ADA Handbook at I-43.

business. . . . "[39] The Act defines an undue hardship as "an action requiring significant difficulty or expense. . . . " Both financial and qualitative impacts are relevant.

The nature and cost of the accommodation are the first factors to consider. The cost to be considered is the actual cost of the accommodation to the employer, taking into account tax credits, tax deductions, or other sources of funding.[40] Then the financial resources of the facility or facilities making the accommodation are taken into account, as well as the number of people employed by the facility or facilities and the effect of providing the accommodation on its expenses and resources. In light of the complexity of modern business organizations, if the facility making the accommodation is part of a larger entity covered by the ADA, the financial resources and size of the larger entity responsible for the accommodation will be considered rather than the resources of and effect on the particular facility or facilities providing the accommodation. If the cost of the accommodation will cause the employer an undue hardship, then the disabled employee has the option of providing the accommodation or paying that portion of the expenses that constitutes an undue hardship.

Cost issues aside, the Act also requires consideration of the impact of the accommodation on the nature of the business operation and on other employees. Thus, if the individual's sensitivity were at odds with the very nature of the business, the employer could probably argue that it was unable to provide access for the individual without incurring an undue hardship. Similarly, if no other employees could undertake the tasks that the individual with a disability could not perform, the employer could probably demonstrate an undue hardship.[41]

Enforcement

Due to backlogs at the Equal Employment Opportunity Commission (EEOC), the agency responsible for enforcing the employment provisions of the ADA, and the slow nature of the judicial process, obtaining formal legal relief is likely to involve a long and expensive process. However, the informal assertion of the right to accommodation may be sufficient to prompt cooperation. If not, initiating the legal action may provide additional pressure.

To initiate legal action, a claim must be filed with the EEOC, which enforces the ADA in accordance with the procedures established for Title VII of the

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[39]42 U.S.C. § 12112(b) (5) (A) (emphasis added).

[40]Technical Assistance Manual at III-12 to -13. Tax credits for small businesses making accommodations required by the ADA are avilable under Section 44 of the Internal Revenue Code. Small businesses can obtain credit "for one-half the cost of 'eligible access expenditures' that are more than $250 but less than $10,250.

[41]*See, e.g., Dexler v. Tisch,* 660 F. Supp. 1418 (D. Conn. 1987) (Postal Service had not violated Rehabilitation Act in denying dwarf a Postal Service position because suggested accommodations (provision of assistant or distribution to others of tasks he could not perform) would unduly interfere with the nature of the operation, constituting an undue hardship).

Civil Rights Act of 1964.[42] The EEOC conducts independent investigations, attempts to settle disputes, and, if necessary, sues employers to remedy violations. From July 1992, the effective date of the ADA, until August 31, 1994, 93 charges based on the analagous disability of chemical sensitivity had been filed with the EEOC.

Employers with 15 or more employees are liable under the ADA for discriminatory acts occurring after July 26, 1992.[43] Normally, a charge must be filed with the EEOC within 180 days of the date of the alleged discriminatory act. However, in states with state laws that are similar to the ADA and that have met certain procedural requirements, the charge can be filed up to 300 days after the alleged discriminatory act.

An individual cannot sue his or her employer in court without first filing a charge with the EEOC. Beginning 180 days after the charge is filed, however, the charging party can request a "right to sue" letter from the EEOC, and will have 90 days after issuance of the letter to bring suit. Due to significant delays at the EEOC many claims are handled in this fashion. Once the charging party sues, the EEOC generally dismisses the charge filed with the EEOC.[44]

A wide variety of remedies is available. Where an employer is guilty of intentional discrimination, a court can order reinstatement, back pay, or other equitable remedies considered appropriate by the court, such as the provision of reasonable accommodation.[45] Attorneys' fees, expert witness fees, and court costs are also available.[46] The Civil Rights Act of 1991 gives those making claims under the ADA the right to recover compensatory damages as well, including actual and future monetary losses, mental anguish, and inconvenience.[47] The 1991 Civil Rights Act also gives complainants the right to recover punitive damages if the employer engaged in dicriminatory practices "with malice or with reckless indifference to the federally protected rights of an aggrieved individual."[48] Although available, compensatory and punitive damages are limited to anywhere from $50,000 to $300,000, depending on the size of the employer.[49] However, if the discriminatory practice complained of concerns the employer's provision of a reasonable accommodation, compensatory and punitive damages are not available "where the covered entity demonstrates good faith efforts, in consultation with the person with the disability. . . . to identify and make a reasonable accommodation. . . . "[50]

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[42]42 U.S.C. § 12117 (a); see 42 U.S.C. §§ 2000e-4, 2000e-5, 2000e-6, 2000e-8, and 2000e-9 (Supp. 1993) (enforcement provisions of Title VII of the Civil Rights Act).

[43]*See* 42 U.S.C. § 12111(5) (A).

[44]29 C.F.R. § 1601.28(a).

[45]See 42 U.S.C. § 2000e-5(g); Technical Assistance Manual at X-8.

[46]42 U.S.C. § 12205.

[47]See Civil Rights Act of 1991, § 102, Pub.L. No. 102-166, 105 Stat. 1072 (1991); Technical Assistance Manual at X-8.

[48]civil Rights Act of 1991, § 102, Pub.L. No. 102-166, 105 Stat. 1073 (1991).

[49]*Id.*

[50]*Id.*, 105 Stat. at 1072.

The ADA and Workers' Compensation

Because the reaction to contaminants is often caused, not just triggered, by exposures in the workplace, an employee must often consider workers' compensation in addition to the ADA. Although most workers' compensation laws bar any other civil remedies for injuries that are compensated by workers' compensation, an ADA claim would not be barred because the two laws seek to remedy different injuries. Workers' compensation addresses the fact that the injury was caused by the workplace, while the ADA addresses an employer's unwillingness to accommodate an individual with a disability, regardless of whether the disability was caused by the workplace.

Resources

Numerous sources of technical assistance are available to assist employees and employers in the search for reasonable accommodations. The National Center for Environmental Health Strategies is a clearinghouse for information and resources for the chemically sensitive.[51] The National Institute on Disability and Rehabilitation Research has also established, pursuant to congressional mandate, ADA Regional Business and Disability Technical Assistance Centers to provide a wide range of information and programs to all those potentially affected by the Act.[52] Additional information is also available through the Job Accommodation Network, a free consultation service funded by the President's Committee on Employment of People with Disabilities.[53]

CONCLUSION

The foregoing analysis of the ADA's employment provisions may be applicable to interpretation of the public accommodation sections of the ADA and prohibitions against discrimination under the Fair Housing Act, to the extent that the legal standards in these laws are analagous.

The ADA and other laws prohibiting discrimination against the disabled are not "the" answer to indoor air quality problems. However, in appropriate instances they may provide a powerful mechanism for improving indoor air or, at a minimum, for improving the well-being of those suffering from adverse health effects.

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[51]NCEHS, 1100 Rural Avenue, Voorhees, N.J. 08043, (609) 429-5358.

[52]The ADA Technical Assistance Center for the region including New York is the Northeast Disability and Business Technical Center, 354 South Broad St., Trenton, N.J. 08608, (609) 392-4004.

[53]Job Accommodation Network, P.O. Box 6123, 809 Allen Hall, Morgantown, West Virginia 26506-6123, (800) 526-7234.

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