ABSTRACT

RICKY L. LANGLEY. Fungi as a Cause of Indoor Air Pollution : A Literature Review and Case Report. (Under The Direction of Dr. David Fraser)

Adverse health problems resulting from indoor air pollution have become more apparent over the last two decades. Because of the Legionella outbreaks in the 1970's, biologic agents have been recognized as one of the important causes of indoor air pollution. Biologic agents can cause either infections or allergies, and techniques for collection and handling of biologic samples are frequently unfamiliar to the industrial hygenist.

This paper is a review of indoor air pollution due to fungi and describes infections and allergies which may occur in building occupants. An approach to investigating sick building syndrome is also described. Also, an example of sick building syndrome at a local university due to fungal contamination is reported.

TABLE OF CONTENTS

INTRODUCTION

Ever since man learned to control fire, he has been faced with the potential health hazards associated with indoor air pollution. Soot has been found on the ceilings of prehistoric caves. It has been known for several years that burning of fossil fuels has been associated with outdoor air pollution and adverse health effects, but research on the health effects of indoor air pollution earnestly began in the late 1960's (1).

Indoor air quality, unlike outdoor air quality, is not directly regulated. Under the authority of the Clean Air Act, the Environmental Protection Agency regulates outdoor air quality. The EPA has constructed a framework for implementing ambient air quality standards (1). It also has a set of regulations for controlling air pollution sources.

Six criteria pollutants have been established which include total suspended particulates, sulfur dioxide, carbon monoxide, lead, nitrous oxides, and ozone. Recent improvements in the outdoor concentrations of these pollutants, except NO_2, have been noted (1).

However, as previously stated, no single agency has responsibility for regulating indoor air quality even

though the number of complaints, especially from office buildings, has been increasing (2). From 1980 to mid 1981, 13% of requests to the National Institute for Occupational Safety and Health (NIOSH) for health hazard evaluations were from workers in nonindustrial settings who felt their offices to be hazardous (3).

As the hazards from outdoor air pollution are being decreased from better air pollution control devices and the threat of legal suits to companies that exceed standards set by the government, more attention is being paid to the problems of indoor air pollution. Research has shown that individuals in more developed countries are spending less of their time outdoors. Thus indoor concentrations of air pollutants may be the main determinant of exposure for many of these pollutants (4). Table 1 shows the average hours spent per day in various locations by adults in 44 U.S. cities (1).

EXPOSURE TO INDOOR AIR POLLUTANTS

As can be seen from table 1, we spend a great deal of our time indoors. Although we consider a house or building a safe environment from the outside elements, potential health risks also exist in many of these dwellings. When evaluating health risks from exposure to air pollution, one must consider the dose that reaches the target tissue and not just the pollutant contamination in

the indoor or outdoor air. One can usually safely assume, however, that the higher the concentration of pollutant, the more likely it is to cause an adverse effect.

Personal exposures to pollutants represent time weighted average exposures from different locations (1). The determinants of indoor concentration depends upon many factors, including the following: outdoor level of the pollutant, indoor sources, the rate of exchange between indoor and outdoor air, and other characteristics of the structure and its furnishings (4).

Outdoor pollutants may enter a building through the ventilation system or through open windows, doors, and cracks in the structures. The indoor concentration of a pollutant from an outside source depends upon the concentration of the pollutant, the rate of air infiltration, the reactivity of the contaminant, the filter efficiency of the ventilation system, and upon particle size and shape (1).

Because of the oil embargo and increasing energy prices during the 1970's, individuals and institutions have been seeking ways to become more energy efficient. More than one third of the energy used in the U.S. is consumed by buildings (5). Common approaches to decrease the use of energy has led to adding insulation, reducing air exchange rates, and fuel switching. This increased efficiency has led to "tight" homes and buildings, which

has decreased the amount of energy consumed, but at the expense of the comfort and health of individuals (5,6).

SOURCES OF POLLUTANTS

The focus of this paper will be on microorganisms, fungi in particular, as a cause of indoor air pollution. However, many other agents may cause similar symptoms and will be briefly discussed. Since remedial action tends to be nonspecific for the majority of cases of buildings related illnesses, principles applicable in dealing with fungal related problems are also applicable in dealing with other viable and nonviable agents.

The 83 million housing units and hundreds of thousands of office buildings in the U.S. contain numerous sources of pollutants (1,4,5,6). Due to the energy crisis, the construction of homes and buildings has decreased the average air exchange rate into these facilities in order to save money. These "tight" and "super tight" homes have air exchange rates as low as 0.1 to 0.5 per hour, while more conventional homes have exchange rates between indoor and outdoor air around 1.0 per hour (1). Also, the American Society of Heating, Refrigeration, and Air Conditioning Engineers has lowered its recommendations for the amount of fresh air per person for a typical open office setting (7).

An often unrecognized source of indoor pollution is from transportation. It is estimated that about 5% of our time is spent in transit, while the remainder of our day is spent at home or in the office (1). In mass transit systems, the occupant to air volume ratios are much higher than in most indoor environments. Thus, substantial exposure to pollutants may occur (1).

Table 2 is a listing of many of the pollutants that have been found in indoor environments. Over 30 types of organic chemicals have been detected in buildings (6). These contaminants can arise from sources located outside the building, the building materials, building maintenance and cleaning materials, or the building inhabitant and the products they use (8).

TIGHT (SICK) BUILDING SYNDROME

The tight building syndrome, also often referred to as the sick building syndrome, is characterized by a significant number of building occupants expressing health complaints, in buildings not directly contaminated by industrial processes (2,4,6,8). These outbreaks have usually occurred in "new, sealed" offices, but this is not always the case. Likewise, the clinical picture may be fairly uniform, and a specific cause can be identified. However, others may complain of nonspecific symptoms

making it difficult to pinpoint a source of contamination (4,6,9).

The following is a partial list of symptoms reported in investigations of sick building syndrome: aching joints, muscle twitchings, back pain, hearing disturbance, dizziness, dry skin, discolored skin, skin irritation or itching, heartburn, nausea, detectable odors, sinus congestion, sneezing, chest tightness, wheezing, eye irritation, problems wearing contact lens, headache, fatigue, drowsiness, sensation of too hot or cold (2,6). The most common complaints include eye, nose, and throat irritation, headache, fatigue, sneezing, and difficulty wearing contacts.

Most individuals say that the severity of their symptoms increases during the day, and often over the course of a week, but symptoms improve when they leave the building or take a vacation (6). Even short breaks while at work to different areas inside or outside the building are often associated with noticeable improvement in symptoms.

FREQUENCY OF SICK BUILDING SYNDROME

As stated, sick building syndrome involves an excessive or a significant number of workers reporting symptoms. A certain background level of complaints is to be expected for any sampling of office workers (10). In

general, about 15-20% of workers in any office building will complain of nonspecific symptoms (9, 10). Hence, the syndrome is defined in the epidemiologic sense as the occurrence of symptoms above the background level.

Currently, more than one half of the workplaces in the United States are offices. It has been estimated that 30 percent of newly constructed and remodeled offices have signs of the tight building syndrome, and in those buildings, between 10-30% of the occupants are affected (11). These statistics do not take into account the new homes and mobile homes that are being built for energy efficiency (1).

No one knows for sure how common sick building syndrome is, but based on a telephone survey of 600 office workers in the United States, it is possible that 20% of office workers are exposed to environmental conditions described as sick building syndrome (9). A recent New England study demonstrates how serious the problem can be (9). State government workers in Maine and New Hampshire took a survey to identify the extent of sick building syndrome. Fifty-one percent of the workers were bothered by stuffy air most of the time, 16% at specific times and 27% occasionally. Thirty percent of the workers said that they had missed at least one and up to ten days of work during the previous year because of poor air quality. Sixty-eight percent stated that poor air quality was

causing a decrease in production and 24% sought medical attention for health problems related to poor air quality. If this survey is any indication of the problem in the total U.S., then millions of days of work and potentially billions of dollars are being unnecessarily wasted.

BIOLOGICAL AEROSOLS

There are numerous biological agents that can be transmitted by the aerosol route and that can cause discomfort or illness in man. For example, molds, dust, bacteria, viruses, fungi, danders, pollens, and insect parts can all be transmitted by the air.

Viruses and bacteria cause about 69,000 deaths per year due to respiratory illness in the U.S. They are also the most important cause of acute disabling illness with an average of 1.22 disabling colds per person per year in the United States (12).

Airborne allergenic agents are felt to be responsible for millions of disabling episodes of asthma and allergic rhinitis that occur each year (12). The aerosol route is also felt to be responsible for many of the hospital acquired infections that occur each year.

Pike has shown that many of the agents causing laboratory acquired infections can be transmitted by the aerosol route, which may not be the usual method of transmission (13). Even though the method of transmission

for 80% of lab acquired infections is unknown, it is felt that a majority of these are due to aerosol spread (13).

The most important single parameter useful in explaining the behavior of an aerosol is its size (14). Particle sizes may vary from 10^{-7} cm to 10^{-3} cm, and particles less than 5-10 microns are usually considered respirable (14).

Aerosols may be conveniently classified into two groups: Those >5 microns and those <5 microns. Particles greater than 5 microns tend to be removed in the nasopharynx, while particles less than 5 microns can reach the alveoli. The size of fungal species varies considerably, so they may be filtered out in the nose or they may reach the alveoli.

At least three mechanisms of deposition are known to occur (15). Impaction refers to a particle's failure to turn corners and thus impinge on the mucus surfaces of the nose and pharynx. This mechanism is effective for removal of most particles >3 microns in diameter. Sedimentation is the settling of particles due to gravity and is important for particles between 1-5 microns. Sedimentation occurs extensively in the small airways. Diffusion is the random movement of particles as a result of their continuous bombardment with molecules of gas. It occurs primarily with particles < 0.1 microns in diameter.

Deposition by diffusion takes place usually in the alveoli.

Many particles that are introduced do not settle at all but are exhaled with the next breath. These particles are often too small to impact or sediment to a major extent but are too large for significant diffusion to occur.

Some factors involved in determining the respiratory risk from aerosol exposure include: concentration of viable organisms, the influences of air volume and ventilation rate, environmental factors such as temperature and humidity, breathing rate, settling rate of particles, particle size, virulence of the organism, and susceptibility of the host.

FACTORS INVOLVED IN THE DEVELOPMENT OF DISEASE FROM MICROORGANISMS

It was known for a while that certain bacteria, viruses and fungi could be transmitted by the aerosol route. However, quantitative data on indoor air pollution and transmission of microbiologic agents is limited for most organisms (29). With the Legionella outbreak that occurred during a convention in Philadelphia in 1976 and the subsequent detection of the organism in the water of HVAC units, the importance of microorganisms as a cause of indoor air pollution came to the forefront (30).

After investigating many episodes of Sick Building Syndrome, it has been shown that fungi are one of the microorganisms responsible for illness in building occupants.

Characteristics of the host, agent, and environment all determine whether an individual will develop allergies or infection from microorganisms. Host factors that are important include age, sex, ethnic group, genetics, physiologic and immune status of the individual, nutritional status, human behavior, and preexisting disease. An individual has both specific and nonspecific resistance factors that protect him from disease. Included among the nonspecific resistance factors are the normal indigenous microflora, genetic factors, morphologic integrity of the skin and mucus membranes, nutrition, acute phase reactants, and hormones. Among the specific host resistance factors are immunoglobulins, complement, and cell mediated immunity.

Important aspects of the infectious agent that must be considered are its phylogenetic class, reservoir, life cycle, geographic distribution, latency, transmissibility, and pathogenicity. Pathogenicity or the ability to establish an infective process depends on the organism's invasiveness, evasiveness, and virulence.

Environmental factors that must be considered can be classified into three broad categories of physical,

biological, and socioeconomic. Physical factors such as climate and geology are important in determining whether an organism is likely to occur in an area. Biologic factors include local flora, fauna, and human population. Occupation, urbanization, and sanitation are important socioeconomic factors that must be considered.

TAXONOMY OF FUNGI

Taxonomists initially divided the living world into two kingdoms; the nonmotile, photosynthetic plants and the motile, nonphotosynthetic animals (16). Traditionally, fungi were classified as primitive plants. However, there are many characteristics of fungi that are not shared by plants. Because of the lack of agreement among taxonomists on how to classify fungi and certain bacteria, a third kingdom was proposed in 1866 by Haeckel. This kingdom, the Protista, is distinguished from the plant and animal kingdoms by their relatively simple organization (16). Controversy still exists in the classification of life forms, and Whittaker has proposed five kingdoms (17): Monera (bacteria, actinomyces and blue green algae), Protista (protozoa and other unicellular organisms), Fungi, Plantae, and Animalia. This last classification scheme will be used during this paper.

The classification of fungi is largely based on the characteristics of the sexual spores and fruiting bodies

present during the sexual stage of their life cycle (18, 19). However, the sexual spores and fruiting bodies (perfect life cycle) of many fungi are unknown. These imperfect fungi are classified on characteristics other than their sexual stage, such as the morphology of their asexual spores and thalli (the whole fungus including nonsexual portions and specialized structures (18).

The Fungal (myceteae) kingdom has three divisions: Gymnomycota, Mastigomycota, and Amastigomycota. The Amastigomycota is the division of primary concern to human health. Most forms included in the Amastigomycota class produce extensive, well developed mycelia, consisting either of septate or aseptate hyphae, although some single-celled organisms are placed here (19). Five classes, Zygomycetes, Trichomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes, are included in this division.

CHARACTERISTICS OF FUNGI (16, 17, 18, 19,)

General description

Fungus is a general term encompassing yeast, molds, and mushrooms. Yeast are oval, spherical (3-5 microns in diameter) or elongated cells which reproduce by budding. Molds are characterized by tubular branching cells which constitute a hypha. The hyphae are divided in most fungi by cross walls known as septa. The hypha is from 2-10

microns in diameter. As a thallus grows, its hyphae form a mass of intertwining strands called a mycelium. The mycelium forms the visible, usually dry colony of mold observed on natural substrate or on culture media.

The fungi have several distinguishing features from bacteria. Fungi possess rigid cell walls composed of certain polysaccharides such as chitin and mannan. The cytoplasmic membrane of fungi contain sterols. All fungi reproduce asexually and most can reproduce sexually. Fungi may be unicellular or multicellular. All fungi are heterotrophic, requiring organic foodstuffs, and most are obligate aerobes. See Table 3.

Reservoir and Airborne Distribution

Water and soil are two principal reservoirs for fungal populations. High humidity is required for fungal growth and spore germination.

Climactic conditions are important in the distribution of airborne fungal spores throughout the air, as well as in the return of the spores to soil, water, or ground. All atmospheric air contains certain varieties and amounts of fungal spores. The concentration of the spores differs according to location, altitude, time of day, season of year, condition of surrounding area, temperature, humidity, rain, snow, sunshine, windspeed, etc.

Most airborne fungi are found as spores or hyphal fragments. All fungi that cause allergies are airborne fungi. The most common airborne fungi belong to the genera Cladosporium, Alternaria, Aspergillus, Penicillium, Helminthosporium, Auerobasidium, Phoma, Nigrospora, Rhizopus, Mucor, Epicoccum, Stamphylium, Curvularia, Fusarium, Scopulariopsis, Cephalasporium, Chaetomium, Trichoderma, Streptomyces, Candida, Cryptococcus, and Rhodotorula (20).

Reproduction

In addition to growth by apical extension and branching, all fungi reproduce by asexual and most by sexual processes.

1 - Asexual reproduction

Three mechanisms of asexual (vegetative) reproduction are known: 1) sporulation, followed by germination of the spores; 2) budding; and 3) fragmentation of hyphae (16). Asexual reproduction involves the formation of a new clone without involvement of gametes and without nuclear fusion.

Asexual spores are sometimes referred to as conidia that form at the tips and sides of hyphae. Others develop within hyphae. The asexual spores aid in the identification of some species. These spores may be small, single cell microconidia or large single or multicelled macroconidia. The shape and arrangement of the conidia

are characteristic for a given spore (16, 21). There are several types of asexual spores (Table)

Budding is the primary asexual reproduction process in yeast, although some divide by fission. As the bud bulges out from the mother cell, the nucleus from the mother cell divides and passes into the bud. Cell wall material then is laid down between the mother and daughter cell, and the daughter cell eventually breaks away (16).

The third mechanism of vegetative reproduction is by fragmentation of hyphae. New colonies are capable of being formed from these fragments.

2 - Sexual Reproduction

For most species of fungi, except mushrooms, sexual spores are produced less frequently and less abundantly than asexual spores. They may be produced only under special conditions and not detected on usual culture (22,18).

Sexual reproduction can be accomplished in several different ways. The basic steps involved are the following: 1) The haploid nucleus of a male penetrates the cytoplasm of the female. 2) The nuclei of both fuse to form a diploid zygote nucleus, and 3) by meiosis - the diploid nucleus gives rise to four haploid nuclei (16).

One type of sexual spore is formed by fertilization of the contents of a female structure (oosphere) by male sperm. The resulting spore is known as an oospore.

Another type of sexual reproduction occurs when the tips of two hyphae come together and their contents fuse. A zygospore is formed from this process. When the sexual spores are formed in a sac called an ascus, the spores are called ascospores. When the sexual spores are formed in a structure known as a basidium, the spores are known as basidospores (18).

Characteristics and Identification of Fungi of Medical Importance

The identification of fungi depends on several factors. Among these are the shape, size, texture, color, number of cells, and thickness of the cell walls of the spores. Other features often examined are the fungal colony characteristics on growth media, the temperature, pH, light, nutritional requirements, presence of septae, and mycelial structure. More specialized antigenic tests can also be used to identify the fungi (22).

The major classes of fungi that are pathogenic to man are the Zygomycetes, Ascomycetes, Basidomycetes, and the Deuteromyctes (13, 17, 18). All except the Deuteromycetes have sexual and asexual spores. The Deuteromycetes, or Fungi Imperfecti, lack a sexual stage of reproduction.

The Zygomycetes are often referred to as bread molds. Most produce well developed hyphae that lack septae (17, 18, 22). Asexual spores include conidia, chlamydospores,

and sporangiospores (16, 17). Some are dimorphic, and sexual reproduction is by fusion of hyphal tips forming zygospores (18, 22). Examples of human pathogens include Rhizopus, Absidia, Mucor, and Basidiobolus (22).

The Ascomycetes are characterized by well developed mycelia that are septate (17, 18, 19). Sexual reproduction is by formation of a diploid ascus and after meiosis, eight haploid ascospores are formed. Asexual reproduction is by externally born spores (conidia) formed on normal hyphae (18, 22). Yeast forms reproduce by either budding or fission (18). Examples of human diseases caused by this class include histoplasmosis, blastomycosis, candidiasis, dermatophytosis, and some mycetomas (17).

The Basidomycetes include those fungi with which the layman is most familiar such as mushrooms, puffballs, stinkhorns, bracket and jelly fungi, rusts, and smuts. They are characterized by septate hyphae and the presence of sexual spores, basidiospores, formed on the surface of a specialized structure called the basidium (17, 18, 19, 22). Asexual reproduction may occur by various means, including the production of conidia (18, 22). An example of a human disease produced by these fungi is cryptococcosis.

The Deuteromycetes include fungi in which sexual reproduction has not been shown to occur. Asexual

reproduction is by conidia. A few yeast belong in this category, but most fungi have well developed septate hyphae (18, 22). If upon further study, a fungus in this group is found to have a sexual stage, then it is reclassified and placed in another class. Most of the human pathogens occur in this class. For example, Alternaria, Penicillium, Aspergillus, Cladosporium, Candida, Helminthosporium, Fusarium, Gliocladium, Stemphylium, Phoma, Scopulariopsis, Epicoccum, Trichoderma, Nigrospora, Trichospora, Pityrosporum, Microsporum, Trichophyton, and Coccidioides are included in this class (17, 18, 20).

INDOOR CONTAMINATION BY FUNGI

Fungi are ubiquitous organisms and can be found most anywhere they are sought. Given a carbon, nitrogen, and water source, fungi will grow under very extreme conditions and in very unusual places. The air in any "clean house" contains hundreds of types of biological and nonbiological particles (23).

Most indoor contamination results from outdoor sources and may build up to concentrations high enough to adversely affect the health of man (2, 23). Indoor buildup of bioaerosols results from material being shed and accumulating indoors and actual growth on interior structures (23).

The penetration of bioaerosols into a building seems to depend most on the extent of mass flow via windows and doors (2, 24). Additional ventilatory factors that are important include the incident wind speed and direction, negative pressurization either by fans and exhaust stacks, and airleak between structural cracks (2, 24). Windows and doors also contribute to bioaerosol entry into a building.

Fungi will grow indoors if given a carbon and water source. A relative humidity level greater than 70% is optimal for fungal growth. Numerous sources in a building will support the growth of fungi by producing standing water reservoirs. Humidifiers, evaporative coolers, self-defrosting refrigerators, and flush toilets all have a potential for becoming contaminated. Water disasters such as leaks in the roof or ruptured water pipes, often will allow abundant fungal

growth in carpet, furniture, or wood (2). Many of the appliances mentioned above are in contact with a moving stream of air which can pick up small particulates and spray them into a room or throughout a building depending on the ventilation system.

Another common source of molds in the indoor environment is house dust (24, 25, 26). House dust is a complex mixture of organic and inorganic compounds, including many biological agents (24). In a study by

Gravesen et. al., rooms with carpets had higher dust levels than rooms without carpets (25). It has been demonstrated that schools with carpets have an increased frequency of students with allergic symptoms than schools without carpets (27). Indoor surface contamination by biological organisms is dangerous usually only when the particles become airborne (24). However, practically any human or animal activity inside a building can elevate the background level.

Volumetric fungal spore studies in domestic interiors give counts that range from 1-6000 per cubic meter with maximum levels usually below 1600 per cubic meter (2). Indoor levels of fungi usually average about 40-50% of outdoor levels (2). Cladosporium species are the taxa most frequently recovered both outdoors and indoors in the United States during the summer. Levels are always more abundant outdoors (2). On the other hand, Penicillium isolates usually dominate in the winter and are often more frequent indoors than outdoors. Outdoor factors that appear to affect the indoor mold level include marked shade, marked levels of organic debris near the home, and natural or unkept property.

There are no set guidelines or levels that one can use to state whether a building has an excess amount of biological material in the air. As noted earlier, a wide range of fungi/m^3 can be detected in domestic interiors.

If the interior is used specifically for handling or processing biological material, then substantially higher counts can be found. For example, barns with moldy hay may have levels of millions of spores/m^3 (2).

A committee on bioaerosols has presumed a normal concentration of culturable spores to be 100-200 colony forming units (CFU) per m^3 and 200 CFU/m^3 of nonviable spores (28). They suggest that if levels greater than 800-1000 CFU/m^3 in affected areas, and if less than 800-1000 CFU/m^3 in nonaffected locations are found, then consider cleaning the office, inspecting the HVAC system and continue looking for the source. If elevated levels of nonviable spores (above outdoor levels) are found or elevated levels of any single spore type are found indoors but not outdoors, then attempt to identify the source (28).

ADVERSE EFFECTS OF FUNGI ON MAN

Fungi can adversely affect the well being of man in at least six different ways:(22)

1 - Infection - invasion of the body

- a - superficial - on the skin, hair or nails
- b - superficial - on the mucus membranes or genitalia
- c - localized, deep invasion of tissue
- d - widespread systemic invasion of the body

2 - Allergic fungal disease

3 - Poisoning by eating toxic fungi

4 - Poisoning by mycotoxins produced by fungi growing on stored food

5 - Starvation due to spoilage of stored food by fungi

6 - Starvation from crop failure due to fungal disease

This paper will first look at fungi as a cause of infection, then as a cause of allergic disease.

AN OVERVIEW OF FUNGAL INFECTIONS

Infections due to fungi can be divided into four groups, differing in the level of tissue invasion. These four groups include the following:(16)

1) Systemic or deep mycoses which involve the internal organs. They often disseminate widely and invade different tissues.

2) The subcutaneous mycoses involve the skin, subcutaneous tissue, fascia and bone.

3) The cutaneous mycoses involve hair, nails, and the epidermis. The responsible fungi are known as dermatophytes.

4) The superficial mycoses involve only the hair and the most superficial layers of the skin.

In most indoor occupations, fungi are rarely the cause of serious infections. However, conditions exist in certain indoor occupations that have the potential to

expose individuals to pathogenic fungi which possibly may lead to a serious infection.

Most microorganisms studied in the laboratory have caused accidental lab acquired infections. Dr. Pike has analyzed over 3900 cases of lab acquired infections (31). Of these cases 353 were due to fungi. Coccidioidomycosis, Histoplasmosis and Blastomycosis, fungal infections which can often be life threatening , accounted for almost 50% of these cases.

Because the deep mycoses have caused lab acquired infection and are potentially fatal diseases, these will be examined in some detail. In general, the deep mycoses are caused by saprophytic fungi (an organism that normally exists on dead organic material) in the soil, and inhalation of spores is usually the main route of infection. The earliest lesions are usually pulmonary, and the initial acute pneumonitis is often self limited. In their subsequent chronic stage, the diseases often begin insidiously and progress slowly and are characterized by granulomatous lesions (16). Fungi may spread via the blood stream and produce metastatic lesions in other parts of the body.

The subcutaneous mycoses are caused by saprophytic fungi in soil and on plants. Infection often occurs by direct implantation of spores or mycelial fragments into a wound (16, 21). The disease begins insidiously, slowly

progresses and is characterized by localized subcutaneous abscesses and granulomas (16). The infection may extend directly into adjacent tissue or may be spread by lymphatic channels. Rarely, it may spread systemically.

The cutaneous mycoses are obligate parasites of man and animals and only rarely soil saprophytes. The dermatophytes tend to produce inflammatory lesions of the skin. The hair and nails are also frequently involved. Part of the inflammatory lesion may be allergic in nature (16, 21).

The superficial mycoses are localized along hair shafts and in the nonviable layers of the skin. The infections are rarely of clinical significance (16).

INDOOR INFECTIONS DUE TO FUNGI

Fungi can cause infections by many routes, but this paper is mainly concerned with airborne spread of the agents and will focus on certain fungi that have caused "indoor infections" by this route. Even though the deep mycoses are rarely associated with indoor infections, they have been responsible for infections in diagnostic labs.

Infectious fungi, with the exception of the dermatophytes are usually saprophytic. Those fungi that cause infection, do so by becoming adapted to the host so that they are able to grow and produce disease (38).

Often, these infections occur in a host who has an immunologic abnormality.

Of the infectious mycoses that can be spread by the aerosol route, the ones of main concern are the following: Cryptococcus, Histoplasma, Blastomyces, Coccidioides, Aspergillus, and Sporothrix. Other fungi may cause infections, but primarily via contact or inoculation, therefore, they will not be included in this paper.

Cryptococcus is a member of the class Basidomycetes and is a spherical to oval budding yeast-like organism (4-20 microns in diameter) with a polysaccharide capsule (16, 21, 39). High concentrations of this organism are found in pigeon droppings and other avian excreta and in soil contaminated by this material. Inhalation of yeast cells is assumed to cause pulmonary infection with subsequent spread via the hematogenous system. Cryptococcus has a predilection for the central nervous system. It is assumed that many infections are subclinical and that most infections occur in immunocompromised hosts (16, 39). However, healthy individuals can become infected, and often these cases are associated with heavy exposure to dust (39).

Cryptococcus has occurred in certain occupations within fertilizer, flour or textile mills. Also, individuals that have worked in dusty barns, corn cribs and a dusty toy store have become infected.

Coccidiodes is a dimorphic member of the class Deuteromycetes (40). In infected tissue, it appears primarily as spherules that range from 5-60 microns in size. These spherules are filled with many endospores ranging in size from 2-5 microns (16). Coccidioidomycosis occurs usually in the South Western United States and is often called San Joaquin or Valley Fever.

Infection develops when arthrospores (2x5 microns) are inhaled and reach the lungs. Entry into the host leads to conversion of these spores into spherules (38). In 60% of infected persons, the disease remains asymptomatic (16, 21). About 40% of individuals develop an acute pneumonitis, often with pleurisy, and 3-5% of these may develop skin eruptions. The lesion is primarily granulomatous in nature and 1-5% of people develop a chronic pulmonary cavitary disease resembling tuberculosis (16, 21). Rarely, systemic spread may also occur.

Outbreaks have occurred in archaeologists and among farm workers exposed to the soil. However, laboratory personnel have often become infected from the arthrospores and many deaths have occurred (31).

Histoplasma is a dimorphic fungus occurring in vivo as a typical oval yeast (2-4 microns in length) within macrophages (21). Histoplasma is a member of the class Ascomycetes.

Because of epidemiologic studies on student nurses in the 1940's for TB, it was noted that individuals often had pulmonary calcifications on chest x-ray but negative TB skin tests. Subsequent skin testing with other antigens and soil studies concluded that *Histoplasma capsulatum* was responsible for many cases of pulmonary calcification (16).

Histoplasma capsulatum is a common soil organism, and inhalation of spores is responsible for infection. It is felt that bats may also serve as a carrier of this fungus in their intestinal tract. The infection may be inapparent, may appear as a primary acute pulmonary disease, may appear in a chronic cavitary form , or it may rarely dissiminate throughout the body (16, 21, 41). The inoculum or dose inhaled and the immunologic status of the host are factors that determine the severity of the disease.

Farmers, gardeners, nursery men, military personnel, and spelunkers are individuals likely to contract this disease. Many lab infections have been due to inhalation of cultured spores (31). Epidemics have been associated with bird roost, caves, cellars, chicken houses, cleaning or demolition of old buildings, farms, soil, and trees (41).

Blastomycosis is due to the dimorphic fungus *Blastomyces dermatitides*. It belongs to the class

Ascomycetes and grows as a budding yeast in human tissues. Infections occur by inhalation of spores 3-5 microns in size or by direct inoculation into the tissue.

The natural reservoir remains unresolved (42). The substrate for growth appears to be organic debris close to the soil. Infection apparently begins in the lung and spreads hematogenously. Destructive lesions are often noted in many organs. Skin lesions may result either from direct inoculation or hematogenous spread. The asymptomatic or mild self-limited form of pulmonary disease rarely occurs as with the other deep mycoses.

The disease occurs frequently in individuals that work outdoors, but no well defined association with occupation is known (42). There have been at least five reported outbreaks of Blastomycosis. All individuals involved had contact with the soil or organic debris. Also, several lab acquired infections have occurred (31).

Although not generally considered deep-seated mycoses, both Aspergillus and Sporothrix may rarely cause severe infections, especially in immunocompromised patients. Pulmonary sporotrichosis is a rare disease, while Aspergillus usually causes an allergic disease instead of an infection.

Sporotrichosis is caused by the dimorphic fungus *Sporothrix schenckii*. It occurs as 2x6 micron cigar shaped budding yeast forms in tissue. Sporothrix belongs

to the class Deuteromycetes. The organism's natural habitat is the soil, sphagnum moss and other plant material.

Most cases of sporotrichosis are due to direct inoculation, but cases of pulmonary sporotrichosis have occurred (38, 43). Pulmonary sporotrichosis usually presents as an upper lobe infiltrate and may cavitate. Inhalation of spores occurs in areas that allow large amounts of fungal growth or in areas where the fungus has grown, sporulated and then dried, allowing dispersal of the spores.

Persons at risk are primarily foresters, gardeners, construction workers, farmers, and children (43). Children often play with plant material and soil infected with Sporothrix.

Aspergillus is usually associated with allergic diseases but may cause infections, especially in immunocompromised hosts. Aspergillus either belongs to the class Ascomycetes or Deuteromycetes, depending on the species. Most aspergilli are not dimorphic, growing only in mycelial form (16). *A. fumigatus* accounts for over 90% of all infections due to Aspergillus. Spores are 2-5 microns in diameter, and the fungus is capable of growing over a wide temperature range (44). Inhalation of spores can lead to three types of pulmonary aspergillosis: 1)allergic bronchopulmonary aspergillosis with only

minimal tissue invasion, 2) active invasive granuloma giving rise to necrotizing pneumonitis and occasional dissemination (this form occurs primarily in immunocompromised patients), and 3) "fungus ball" growth in a preexisting cavity without invasion of lung parenchyma (32).

Agricultural workers, cane sugar processors, or cleaners exposed to spores in rye flour are often exposed to high concentrations of this organism (44). Immunosuppressed hospitalized patients often can develop infections from this organism, and often the source is external to the hospital (23, 38, 45).

There are a few other fungi that rarely can be transmitted by the aerosol route and cause serious infections, however the host usually has some type of underlying metabolic (diabetes mellitus) or immunologic disorder. With the AIDS epidemic, many microorganisms that were previously considered innocuous have been found to produce disease. All of the organisms previously mentioned can cause an infection in healthy individuals, thus they are covered in more detail.

Many fungi can produce superficial infections, especially by direct contact. However, these infections represent very unusual cases of indoor air pollution. Exceptions may include individuals handling animals and

contact with dressing room floors that are contaminated by fungi (athlete's foot).

DIAGNOSIS OF FUNGAL INFECTIONS

The diagnosis of infections from fungi can be roughly broken down into three broad categories: (1) history, physical exam, and chest x-ray, (2) culture and histopathology, and (3) serological and immunologic testing.

The patient's history often gives clues to the diagnosis. For example, in a patient with a cough, fever, chest pain and recent travel to the San Joaquin Valley, the possibility of coccidioidomycosis should be considered. Also the occupational history may give clues to potential exposures. If several employees working in the same area become ill and are found to have the same disease, then the physician should suspect the same agent in a coworker with similar symptoms. A good history will often point to the right diagnosis.

The physical exam is rarely diagnostic. However, certain lesions, in conjunction with the patient's history, may point to a fungal infection. For example, erythema nodosum or erythema multiforme, along with hepatosplenomegaly may be noted in histoplasmosis (46). Lesions that follow the lymphatic channels are often seen in sporotrichosis (16, 47). Cryptococcus is a cause of

chronic meningitis with signs and symptoms of fever, headache, disorientation, and papilledema (21, 39).

Lesions may occur throughout the body if the fungus disseminates, and these may be noted on physical exam or by x-ray examination. As mentioned previously, histoplasmosis can cause calcifications on chest x-ray that may appear similar to tuberculosis.

Histopathologic examination of sputum, pus, or tissue may identify a fungus as the cause of a patient's illness. Special stains may be needed to detect certain fungi. For example, sporotrichosis is difficult to detect by conventional histologic stains (43). Once stained, the shape of the hyphae, spores, or yeast-forms is used to identify the organism (48). The demonstration by histologic examination of fungi invading tissue is indicative of an infectious process.

The standard method of diagnosing an infection is by culturing urine, pus, blood, other body fluids, or tissue samples on different types of media. Sabouraud's glucose agar is frequently used to culture fungi (16, 21). Fungi tend to grow slower than bacteria, and by adding drugs, maintaining a low pH and low temperature, bacterial contaminants are prevented from growing (16). Fungal cultures are often incubated at different temperatures to get both yeast and mycelial forms. Colonial morphology is an important identifying characteristic and may vary

depending on the growth media (48). Once grown, microscopic evaluation of the colony is done to identify shape and size of the spores and hyphae. In certain conditions, the recovery of fungi by culture may not indicate an infection, but rather only fungal colonization or possibly contamination of the media.

If unable to classify the genus and species by morphology alone, then biochemical tests can be done to determine the identification of the organism (46). These biochemical tests often involve substrate utilization.

More recently serologic and immunologic testing has been developed to evaluate exposure to fungi (46, 48, 49, 50). Three serologic tests are frequently used to diagnose fungal infections. These include immunodiffusion, complement fixation, and agglutination tests (48).

Immunodiffusion techniques detect the reaction of antigen and antibody by the precipitation reaction (51). Immunodiffusion is one of the simplest and most direct means of demonstrating antigen-antibody reactions in the clinical laboratory. Reactions may be classified as single or double immunodiffusion depending upon the movement of the reactants. Basically, two circular wells are made in an agar dish. Within one well is placed the patient's serum, and in the other well is placed the fungal antigen. If the patient has specific antibodies to

that fungal antigen, the immune complexes will form and precipitate, and a line will be visible in the agar. However, this test is not positive in all cases of infection (41, 48, 49, 50).

In a complement fixation test, the fixation of complement occurs during the interaction of antigen and antibody (51, 52). The antigen-antibody complex uses up the complement. When antibody coated red cells are then added to the solution, these will be lysed by any remaining complement. The amount of remaining complement or red cell lysis is inversely proportional to the concentration of antigen-antibody complex. Rising titers indicate progressive infection, while decreasing titers indicate regression. As noted with immunodiffusion, this test may not be positive in all patients with infection.

Agglutination reactions involve clumping of antigenic particles by antibodies (51, 52). The reaction may be classified as direct or indirect. In the simple direct technique, a cell or insoluble particulate antigen is agglutinated directly by antibody. Indirect agglutination refers to agglutination of antigen-coated cells or inert particles which are passive carriers of soluble antigens. The agglutination technique detects the presence of specific antibody. The titer of agglutinating antibody is measured by taking a given concentration of target particle and mixing with progressively more dilute

solutions of antibody. The final dilution at which clumping occurs is referred to as the specific antibody titer.

Skin testing, using antigens derived from certain fungi has been used in the diagnosis of infection. Skin testing has been developed for histoplasmosis, coccidiodomycosis, and aspergillosis (41, 44, 46, 50). However, negative skin tests may occur in individuals with overwhelming infections, and cross reactivity between antigens may give false positive skin tests (16, 50).

TREATMENT OF FUNGAL INFECTIONS

Dissemination of fungi within the host is often fatal if untreated. Localized infections may occasionally reoccur without treatment. Whether or not to treat may be a difficult decision. Therapy is probably indicated if symptoms persist, if evidence suggests local progression of disease, if the patient is an infant, has a concurrent illness, an immune impairment, or belongs to a racial group predisposed to handling the infection poorly (46, 50).

Surgery as well as chemotherapy may be indicated in the patient. Surgical excision may be useful in some cases with residual pulmonary, cutaneous, or bony lesions. Chemotherapy is usually considered part of the therapy when surgery is contemplated.

It has only been since 1959 that effective antifungal agents were developed (16). Today, there are still only a few antifungal drugs available for treatment of infections. For the treatment of deep mycotic infections, Amphotericin B has been the mainstay. It is effective against all of the deep mycotic agents and most other fungi that may become invasive in the host. However it is not effective against the superficial mycoses (16). Amphotericin B, like some other antifungal agents, is derived from various species of Streptomyces. It works by binding to ergosterol in the plasma membrane, thus disrupting the fungal cell (16, 21).

Newer antifungal drugs such as Flucytosine, Ketoconazole, and Miconozole have been used alone or in conjunction with Amphotericin B to treat deep seated fungal infections (53). As with other drugs, adverse effects from these are multiple and potentially serious, so one must monitor the hematologic and metabolic systems carefully.

AN OVERVIEW OF ALLERGIC DISEASES

Numerous agents in the indoor environment can cause allergies. Included in this group are pollens, fungi, algae, actinomycetes, arthropod fragments, dust, and pumices (24). A large number of indoor particles reflects influx from the outside environment.

Numerous factors are important in the development of allergies, among which a history of atopy is very important. Atopy is a term that refers to the familial occurrence of common allergic disease and immediate allergies acquired by natural sensitization to common allergens. Some factors in the development of atopy include: season of the year, family history of allergies, geographic and racial factors, mucosal permeability, and immunologic disorders (54). Subjects are classified according to the degree of reaction to a battery of common allergens (55). Atopic subjects are more predisposed to occupational sensitization and allergic reactions to numerous agents than subjects that are not atopic.

Allergic reactions can occur in many organ systems such as the eyes, skin, nose, airways, and alveoli. Allergies affect 17% (35 million) of the population in the United States (56). The overall prevalence of asthma is 3%, but it is felt to be increasing in frequency. Atopic dermatitis occurs in 6.9 persons per 1000 (56). Twenty percent of the population experience some form of urticaria or angioedema at some time in their life, and animal allergy prevalence ranges from 11-32% (56). Allergic rhinitis occurs in 10-12% of the population (57). Other allergies to foods, insects, and drugs affect tens of thousands of individuals.

Asthma and allergic rhinitis are the third leading cause of limited activity in persons less than 45 years of age , and annual work loss due to these problems is reported at 5 million days (56). In the United States, it is felt that 2% of all cases of asthma may be occupationally related, while up to 20% of workers in various occupations in the United Kingdom have asthma (56, 58,).

Allergic reactions of the airways may be broadly classified according to their site of involvement and nature of reaction into four groups: allergic rhinitis involving the nose; the airways (allergic asthma); the airways and adjacent alveolar spaces (allergic asthma with pulmonary eosinophilia); and alveoli or peripheral bronchioles (hypersensitivity pneumonitis (24). A description of these as they relate to fungi will be discussed later.

Organic as well as inorganic agents can cause irritation or allergic sensitization of the skin. Allergic skin reactions may result from direct sensitization by an agent or from a secondary response to an infection within the body. Examples of the latter include the id reaction, erythema nodosum, erythema multiforme, urticaria, and annular erythema (22).

Other organ systems may be involved with allergic reactions. However, allergies due to fungi primarily

involve the lungs, skin, the eyes, and possibly the gastrointestinal tract (22).

MECHANISMS OF ALLERGIC REACTIONS

The amount of tissue damage has conventionally distinguished reactions due to normal protective immunity from allergic reactions. Four types of allergic reactions were classified by Gell et. al. in 1963 (51, 59). These reactions are the following: Type I (anaphylactic), type II (cytotoxic), type III (arthus), and type IV (delayed hypersensitivity).

Type I reactions are immediate and depend on IgE bound to mast cells and basophils. After antigen binds to the IgE antibodies, these cells release numerous pharmacologic substances. Smooth muscle contraction, increased vascular permeability, and bronchial spasms are examples of reactions that frequently occur. Hay fever, allergic rhinitis, asthma, and occasionally anaphylaxis are examples of clinical diseases due to type I reactions (51, 59).

Type II reactions occur when antigens present on cell membranes react to IgG or IgM antibodies. Cell damage often occurs from this type of reaction, and it is frequently known as antibody dependent cell mediated cytotoxicity. Autoimmune hemolytic anemia, leukopenia,

and thrombocytopenia may result from a Type II drug reaction due to quinidine.

Type III reactions, often known as arthus reactions, are due to the formation of complement binding antigen-IgG antibody complexes. Release of lysosomal products and activation of complement components leads to tissue damage. Allergic bronchopulmonary aspergillosis and hypersensitivity pneumonitis are two diseases whose mechanism of damage is mediated by type III reactions.

Type IV or cell mediated immune reactions result from the interactions between actively sensitized lymphocytes and specific antigens. These lymphocytes release lymphokines which are biologically active and mediate a local inflammatory reaction. Antibody and complement are not involved in these reactions. Examples of these reactions include tuberculosis and also hypersensitivity pneumonitis.

FUNGI AS A CAUSE OF ALLERGIC DISEASE

Fungi may cause allergies that, although rarely serious, are often debilitating. Most fungal allergies involve the respiratory tract, and these will be considered in more detail. Initial evidence establishing fungi as a cause of respiratory allergy was obtained by Prince in the 1920's (60). Numerous studies since this

time have contributed to our current understanding on fungi and allergic disease.

In the natural environment, man is potentially exposed to more than 100 different species of fungi, many of which are present in the indoor environment (61). There is no doubt that factors other than the mere existence and amount of fungi play an important role in fungal allergy. For example, the size of the spore is highly important. It is generally thought particles greater than five microns are filtered out in the upper respiratory tract and particles less than five microns can reach the alveoli. This is an oversimplification, because if the person is a mouth breather, then particles greater then twenty microns may penetrate into the bronchioles (62).

From air sampling of mold parts, Lowenstein states that spores comprise greater than 90% of the particles of relevant size (26). It has also been shown that spores and mycelia contain several distinct antigens. Attempts to define fungal allergens, however, are still preliminary, and standards for testing and reagent preparation are not fully developed.

Prevalence of type I allergy to molds ranges from 2-30% (62). This discrepancy may partly be explained by the overall quality of the extracts used for skin testing and

the ignorance of molds as a possible factor in respiratory allergy.(26).

The Deuteromycetes is the class of fungi most important as a cause of respiratory disease in man (20). The four genera Cladosporium, Alternaria, Penicillium, and Aspergillus represent the most common allergenic molds based on skin reactivity (20, 37). These genera are also associated with the highest percentage of fungal spore counts obtained by air sampling (23). About 85% of people with mold allergies will react with one of these allergens.

The respiratory allergic conditions which may be due to fungi include allergic rhinitis, asthma, allergic bronchopulmonary aspergillosis, hypersensitivity pneumonitis, and humidifier fever. Each will be briefly described. The main clinical problem is the extent to which a mold sensitive person's symptoms can be attributed to molds (37). This is because exposure to molds is a continuum without definite seasonal end points. Indoor spores are present throughout the year, and levels increase when human activity is present.

Allergic rhinitis consists of paroxysms of sneezing, nasal itching and congestion, clear rhinorrhea, palatal itching, and if severe, conjunctival irritation, redness and tearing, ear fullness, and pressure sensation in the cheeks and forehead (57). The symptoms may be seasonal or

perennial depending on the presence of environmental allergens. IgE mediated rhinitis may be caused by a wide variety of allergens including pollens, molds, dander, mites, excreta, etc. About 10-12% of the United States population suffer from allergic rhinitis, two thirds of which occurs before age 30 (57). Nasal symptoms from fungi are usually less intense than in pollen hayfever, persist for longer periods of time, and are more or less intermittent in nature, but showing acute exacerbation after heavy exposure (63).

As an example of rhinitis problems associated with molds, a study of nasal hypersensitivity in wood furniture workers by Wilhelmsson, et al, found that 3% of the workers had allergy to molds and 2% to wood dust (64). However, up to 16% of workers in the furniture factory had nasal perennial hypersensitivity, so for the majority of cases, the etiology of their symptoms was not determined.

Asthma is often defined as reversible obstruction of the airways. This airway narrowing may be due to contraction of airway smooth muscle, edema of the bronchial mucosa, or accumulation of bronchial secretions or any combination of these. An acute asthmatic reaction is believed to be an IgE mediated reaction, but a delayed IgG mediated reaction may cause a late asthmatic event. Serum studies in 102 adult patients presenting to an

emergency room by Dr. Platt Mills, showed that 90% had IgE antibody to one or more major indoor allergens (65).

For more than 60 years, molds have been considered a cause of asthma (66). It has been shown that fungal spores of Alternaria and Penicillium both can cause asthma, even though there is a large difference in the size of the spores (67). The shape of spores may be very different, their aerodynamic behavior is important in determining whether they can reach the lower respiratory tract.

Even though we know fungi can cause asthma, we don't know how often it happens. There have been reports of fungi growing within humidifiers and air conditioners leading to exacerbations of asthma (10, 24). Identification of mold aeroallergens is the main problem to progress. Detailed information on the kind and amount of antigen in the air and at different times and places, and reliable standard allergen extracts for diagnostic testing are required before we can fully correlate signs of asthma with fungal exposure (66).

Hypersensitivity pneumonitis is an allergic lung disease that results from sensitization and recurrent exposure to certain antigens. The disease is characterized by mononuclear inflammation of the terminal bronchioles and alveoli which tends to form granulomas, and may progress to fibrosis. The acute illness presents

with fever, chills, dry cough, malaise and dyspnea four to eight hours after allergen exposure. Infiltrates may be seen on chest x-ray, and physiologic changes can be found in pulmonary function that suggests a restrictive disorder. Acute attacks usually clear in one to four days. Repeat acute attacks or chronic exposure may result in pulmonary fibrosis.

Numerous microorganisms, including fungi, can cause hypersensitivity pneumonitis (68, 69, 70). Farmers are the occupational group most involved with up to 8-9% of farmers affected in some countries (70). Reports of fungal contaminated hot tubs or HVAC systems leading to hypersensitivity pneumonitis in office workers are becoming more frequent (32, 34, 71, 72).

Immunologically, this disease has been considered a classic Type 3 reaction, but recently, evidence of Type 4 involvement has been noted (73). Serum precipitating IgG antibodies to inhaled organic antigens can be found in over 90% of individuals with clinical disease.

Allergic bronchopulmonary aspergillosis (ABPA) is an allergic pulmonary reaction to inhaled *Aspergillus fumigatus*. The disease is characterized as definite, probable, or possible based on the following criteria: COPD, positive skin test with Aspergillus extract, high total serum IgE levels, typical roentgenographic findings with central bronchiectasis, general malaise with fever

and/or weight loss, productive cough with typical sputum plugs and/or hyphae and/or hemoptysis, high peripheral blood eosinophil counts (≥600 per m^3), positive precipitins against Aspergillus and/or positive specific anti-Aspergillus IgE (74). Disease is certain if criteria one through four and three of the last four are present. Disease is probable if criteria one through three and four of the last five are present, and disease is possible if criteria one through two and three of the last six are present.

The immunopathogenesis of ABPA is thought to involve type I and Type III reactions. During the acute illness, airway obstruction is predominant but over time, signs of interstitial fibrosis occur (73).

Although *Aspergillus fumigatus* is widely distributed, ABPA rarely occurs in the United States. It occurs usually in atopic people with a history of asthma. Individuals that work at sewage composting run a risk of heavy exposure to Aspergillus (44).

Humidifier fever is a recently described entity, probably allergic in nature, that has been reported in office workers exposed to contaminants in humidification systems (10, 24, 45). The disease presents with flu-like symptoms of headache, lethargy, myalgia, arthralgia, fever, shortness of breath, and occurring four to eight hours after exposure and resolving usually within 24

hours, regardless of continuing exposure. Symptoms recur on reexposure after an absence of several days from work.

Lung function studies during an acute attack show a restrictive pattern that improves during the working week. The diffusion coefficient is reduced during acute illness and arterial desaturation may occur. The chest x-ray is normal in the acute stage of disease and even after several years of symptoms, thus differentiating this disorder from hypersensitivity pneumonitis.

The cause is felt to be an antigen or antigens found in the humidifier (24). Precipitins to different microorganisms can be found in the serum of affected individuals, but antibodies may be seen in asymptomatic exposed individuals also. However, serologic testing is not felt to be a reliable method of determining the cause of the condition because inhalation challenge testing with extracts of organisms to which individuals have precipitins fail to produce systemic symptoms

DIAGNOSIS OF ALLERGIC DISEASES DUE TO FUNGI

As with the diagnosis of infectious diseases due to fungi, the diagnosis of allergic disease is multi-faceted. The requirements for good diagnostic tests are that they be standardized, accurate, reproducible, inexpensive, easy to perform, and present minimal risk to the patient.

Unfortunately, no test presently fulfills all these requirements.

The initial evaluation of any disease is taking a good history. Symptoms such as cough, wheezing, runny nose, nasal congestion, paroxysms of sneezing, itchy nose and palate, tightness of the chest, shortness of breath, onset of symptoms in relationship to work or environmental exposure, and rash are typical of allergic reactions.

Next, a physical examination should be performed. Typical signs often include pale, swollen nasal mucosa with watery secretions, eyelid edema, and red conjunctiva in allergic rhinitis. With asthma, tachypnea, wheezing, rapid pulse, pulsus paradoxus, hyperresonant lung fields, diminished breath sounds, wheezes and rhonchi may be noted. In hypersensitivity pneumonitis fever, chills, dry cough, tachypnea, cyanosis, and rales are often noted four to eight hours after exposure to environmental fungi that the patient has become sensitized to. In ABPA fever, bronchospasm, cough and expectoration of rusty brown mucus plugs are often noted.

Confirmation of clinical suspicions frequently relies upon laboratory testing. Tests frequently obtained include a complete blood count with differential, chest x-ray, pulmonary function testing, nasal swabs for eosinophils, and serum IgE levels.

Infiltrates are often seen on chest x-ray, especially with hypersensitivity pneumonitis and ABPA. Nasal swabs often show eosinophilia with allergic rhinitis. Complete blood counts frequently show elevation in the eosinophil count and often elevated serum IgE levels are present in the allergic patient.

Pulmonary function testing is frequently used to evaluate the respiratory status of the individual. In asthma and the early course of ABPA, evidence of obstruction is frequently noted. In hypersensitivity pneumonitis and late in ABPA, evidence of restriction is frequently noted.

More sensitive and specific "immunologic" tests have been developed to aid in the diagnosis of allergic disease.. Among these, both in vivo and in vitro procedures exist. The in vivo tests include skin tests and provocative challenge studies. Among the in vitro procedures, radioallergosorbent test (RAST), enzyme linked immunosorbent assay (ELISA), histamine release from basophils, and immunodiffusion tests are frequently used to diagnose fungal allergies (51, 75). A brief review of each follows.

Skin testing involves placing a suspected allergen onto the skin after a scratch into the epidermis is made (scratch test) or placing the allergen on the skin initially and then a point of a needle is passed through

the drop, and the skin is pricked (prick test). Occasionally, an allergen is injected intradermally under the skin similar to tuberculin testing. An interaction between the allergen and IgE fixed to mast cells in the skin with liberation of chemical mediators occurs (75, 78). Histamine released from the mast cells causes vasodilation (erythema or flare). Localized edema results from increased vascular permeability (wheal). Skin testing is usually positive in most individuals with type I allergy. A saline control, and a histamine control are usually included in the battery of allergens which are tested. The skin tests are graded on a scale from 0-4+ depending on the presence and size of the wheal and flare. When done correctly, the saline control is 0 and results greater than or equal to 2+ are usually considered positive. See Table

Both false negative and false positive reactions may occur, therefore, the skin tests must be correlated with the history. When discordance between the history and skin tests is noted, inhalation challenge or in vitro testing may be used to evaluate the importance of skin test reactivity.

Two types of challenge test are in use. The first involves nasal provocation testing, and the second involves pulmonary provocation testing.

Nasal provocation tests are considered research tools at the present. Uses include evaluating the effectiveness of immunotherapy, correlating results of other in vivo and in vitro tests, identifying new allergens, and substituting for bronchial challenge tests (75).

Bronchial provocation testing is used to evaluate the role of fungal allergens as the cause of an individual's respiratory symptoms. The results of post challenge pulmonary function testing are used to determine whether a patient has a positive reaction. A drop of $\geq$20% from baseline in the FEVI, FEF 25-75, or flow rate is considered a positive reaction. Also, an increase in airway resistance of >50% or a fall in specific airway conductance of $\geq$35% is considered a positive reaction. Many problems must be considered when evaluating provocation testing. Included are false positive reactions, difficulty standardizing the techniques, deciding what constitutes a positive reaction, time and expense involved, inability of patients to cooperate fully, and the frequent adverse reactions that may occur, some potentially life threatening. However, as technical aspects improve, provocation testing will be increasingly used to confirm suspected allergens as the cause of allergic symptomatology.

Numerous in vitro laboratory techniques to diagnose allergic or immunologic disorders exist. A brief

discussion on Radioallergosorbent test (RAST) and enzyme linked immunosorbent assay (ELISA) will follow. Immunodiffusion methods were discussed in the section on infections. Funderberg et. al. and Newman et. al. provide excellent reviews on the field of in vitro tests (51, 76).

Because immunodiffusion measures IgG, IgM, and IgA, other methods were developed to measure IgD and IgE, which are present in much lower concentrations in the blood. Of the newer methods, RAST and ELISA are frequently used to detect the presence of specific IgE to fungal allergens. However, as with most tests, each has its advantages and disadvantages.

For RAST testing, allergens are coupled to a matrix. The patient's serum is then added to the matrix-allergen complex and then incubated. After washing, radio-labeled iodine-125 anti-human IgE is added. After washing, the remaining bound I-125 is measured in a gamma counter and is a measure of the amount of specific serum IgE antibody to the allergen. This method can detect one nanogram of specific IgE antibody.

Problems with RAST testing for fungal allergies include: difficulty coupling the mold allergens to the matrix; competitive inhibition from other classes of immunoglobulins; and the failure of RAST to discriminate between monovalent and polyvalent allergens. Monovalent allergens do not cause histamine release and therefore

escape detection by skin tests, provocation tests, or histamine release tests, but are detected by RAST. Another problem with RAST testing is lack of standardization of the commercial tests.

ELISA allows for the measurement of either antigen or antibody. To measure antibody, usually the antigen is fixed onto a solid phase matrix. Then the patient's serum is added, and an anti-immunoglobulin tagged with enzyme is later added. If antibody is present in the patient's serum, then a color change will be noted after a substrate is added which reacts with the enzyme. The amount of color change can be quantitated by spectrophotometry, and is proportional to the amount of IgE antibody in the patient's serum.

ELISA requires less sophisticated equipment, less skilled personnel, no radiation exposure, and has the potential for automation as compared to RAST testing. However RAST is more sensitive and subject to less interference than the ELISA method (75). Other problems with ELISA include difficulty in defining the end point of the reaction, nonspecific background enzyme activity, and difficulty in labeling and purifying the enzyme conjugate.

TREATMENT OF ALLERGIC DISEASES

Millions of people suffer from allergic disease and treatment is necessary to relieve their symptoms. Even

though no single modality is completely effective in relieving symptoms, three methods exist which, when used singly or in combination, offer much relief. The three modes of treatment today include avoidance or environmental control, pharmacotherapy, and immunotherapy.

In the case of fungi, as with other allergens, avoidance is the most efficacious form of therapy. Because fungi are so prevalent in the environment, however, avoidance is often difficult. Mold sensitive individuals should stay away from dense foliage and decaying vegetation in wooded areas in the spring and fall and should not cut grass or rake leaves (77).

Indoor moisture, darkness, and accumulation of dust promote fungal growth. To control dust, removal of stuffed furniture and toys, thorough, frequent cleaning of carpets, and encasing mattresses in allergic proof covers is recommended. Filters on furnaces and air conditioners should be cleaned. Electrostatic air filters and Hepa filters effectively filter mold spores from the indoor air (78). Dark and damp areas such as basements and attics need careful cleaning and dehumidification to eliminate any fungal growth. Likewise, kitchens and bathrooms must be well aerated and cleaned. Basement walls and floors should be painted with moisture proof sealants. Dehumidification should be used continuously in damp areas.

Pharmacotherapy or drug treatment is frequently used to relieve allergic symptoms. Among the drugs, antihistamines, oral or topical decongestants, corticosteroids, theophylline, Beta-adrenergic agonists, and cromolyn sodium are the most frequently used. The mechanism of action differs among these agents.

Antihistamines work through competitive inhibition of histamine and are effective in reducing sneezing, itching, and rhinorrhea. Several classes of antihistamines exist, and if no benefit is noted from one class, another class of antihistamines can be tried. Side effects from antihistamines may include sedation, nervousness, dry mouth, blurred vision, decreased mental acuity, and insomnia.

Decongestants, whether oral or topical, are usually alpha-adrenergic agonists and act by stimulating constriction of the smooth muscles of the nasal mucosal vessels. Side effects may include elevation of blood pressure, nervousness, and restlessness. A rebound phenomenon known as rhinitis medicamentosa, which is characterized by a marked increase in nasal swelling and congestion, may occur after prolonged nasal topical use. Decongestants ore often given in combination with antihistamines.

Corticosteroids, whether oral or aerosolized, have been found very useful in severe cases of allergic

rhinitis or asthma. The mechanism of action, only partly understood, depends on their antiinflammatory activity and on their ability to potentiate the effect of adrenergic drugs on adenyl cyclase. However, severe side effects occur with prolonged use, and they should not be considered primary agents in the treatment of allergic symptoms.

Theophylline is a phosphodiesterase inhibitor and acts by increasing the cAMP and cGMP ratio, resulting in bronchial dilation and inhibition of mast cell release of mediators. This drug is currently used in the treatment of asthma as an oral agent. Serum levels should be monitored because toxicity may occur at levels above the therapeutic range of 10-20 micrograms per milliliter. Certain drugs and cigarette smoking can affect the metabolism of theophylline.

Beta-adrenergic drugs cause bronchodilation by activating the Beta receptors in bronchial smooth muscles and decreasing mediator release from mast cells. Drugs which are Beta-2 selective are preferred because of the side effects that may occur with nonselective Beta-agonist. These agents form the cornerstone of asthma treatment (77).

A recently introduced drug, cromolyn sodium is effective in the treatment of allergic symptoms. This drug works by prevention of mediator release from mast

cells, modulation of some types of reflex induced bronchoconstriction, and amelioration of nonspecific bronchial hyperactivity in some asthmatics (77). Cromolyn sodium is not effective in acute attacks of allergic symptoms, but it is useful as a prophylactic drug.

The third basic treatment of allergic disease is immunotherapy. After a complete history, physical examination, and appropriate skin testing has been performed, aeroallergens that may be an important cause of the patients symptoms can be identified. Extracts of these allergens can then be prepared for immunotherapy.

Immunotherapy involves the subcutaneous injection of dilutions of the extract usually into the patients upper arm. Over time, the strength of the extract is gradually increased until a maintainence dose is reached. Once this maintenance dose is reached, the frequency of injections is decreased until it is given monthly. Immunotherapy is continued for two to four years unless the patient has no relief of symptoms. In that case, reevaluation of the patient should be done.

Immunotherapy is felt to work by inducing the formation of blocking antibodies of the IgG class (79). This IgG blocking antibody thus is specific for the injected allergen and will bind circulating allergen without initiating a type I reaction upon environmental exposure to that allergen.

It is still difficult to evaluate the success of immunotherapy for fungal allergy for several reasons. One is that the potency of the extracts is unreliable. Also, spore specific fractions that may be important in mold sensitive patients are often not included in the extract. Third, it is often difficult for the average allergist to identify the clinically relevant fungi.

Immunotherapy is not without risk. Occasionally systemic symptoms and anaphylaxis have resulted when a large dose was given, or the injection was into a blood vessel. Local reactions are common such as swelling, erythema, and itching at the site of injection, and patients should be observed for at least twenty minutes after injection in case a systemic reaction may develop.

FUNGAL SAMPLING EQUIPMENT

One of the difficulties in evaluating sick building syndromes is determining when, where, what, and how one should sample for biological contaminants. Some feel that biological sampling should be done if local sources of nonmicrobial irritants have been ruled out and individuals have clinical symptoms consistent with exposure to biologic agents (80, 81). If sampling is to be done, then selection of the sampling device is the next step. Methods developed include both qualitative and quantitative analysis. Many of the devices used for

fungal sampling have been previously used to collect pollens. Particles must be deposited onto surfaces before their enumeration, either microscopically or in culture (82). This step poses problems reflecting the aerodynamic behavior of airborne particles, which depends upon the particle shape and size as well as atmospheric conditions.

Most methods for fungal sampling use one of the following approaches: Fallout, impaction on narrow surfaces, filtration, and acceleration with impingement of particles at flow channel bends (82).

Gravitational fallout methods have been used for numerous years to collect airborne organisms. Gravitational methods are simple, requiring no power source and easy to maintain. Particles are collected on a slide and are counted and expressed as a count per square centimeter (83). The recovery of airborne agents often parallels the clinical events. However, numerous limitations exist including the effects of wind speed, direction, and air turbulence on the deposition of particles. Recoveries tend to be biased towards the collection of large particles, leading to the near exclusion of small particles. Also, the inability to determine the volume of air that contributes particles for the sample is a limitation of this method (82).

A second technique involves the impaction of particles on narrow surfaces. The probability of particle

impaction varies with its terminal velocity and relative air speed, and inversely with the width of the target. Impaction efficiency increases with particle size and wind speed. Following impaction, particles are counted by a microscope.

Examples of such devices that work by this technique include flag samplers, rotorods, rotobars, and rotodisks (83). However, disadvantages with these devices include dependence on wind speed, frequent changing of the collection surface due to particle buildup , and limitation collecting agents smaller than 5-8 microns.

Filtration involves the collection of particles as they pass through a filter. Generally, air is drawn through the filter by a pump for a measured period of time. The particles can be either viewed directly by microscopic examination of cleared filters or the filter can be cultured. Problems noted with these devices include the necessity for a totalizing anemometer if data are to have volumetric implications, the effect of the filter digesting agent on the fungi, and the alignment of the intake with the air flow to avoid loss of sampling efficiency.

The fourth type of sampling involves impingement. This is a two stage process involving aspiration of measured amounts of air into a device and raising this input and its entrained particles to critical velocity

just before sharp bends in the flow channel are reached (82). The probability of capture varies with the particle size, acquired speed, acuteness of the turn and adhesive properties of the impingement point (82). Particles with stop distances exceeding the available clearance will impinge in the wells at points suitable for collection (82). An advantage to this method is that of high collection efficiency of small particles.

Impingement collection surfaces involve either liquid or solid media. Liquid impingers are used for short term, relatively low volume, calm air sampling in enclosed spaces. Because of shear stress in this process, damage to the organisms may occur. After filtration of the catch, particles may be viewed microscopically or cultured.

Solid surface collection occurs via the impingement of particles on semisolid or an adhesive coated solid surface. This method has been used to determine total number of cells, size distribution, total viable cells and variation of concentration per unit of time during a long sampling period (83). Examples of devices include spore traps, slit samplers, and sieve impingers.

The Andersen sieve impinger (impactor) is generally accepted as the standard instrument for collection of airborne viable particles in environments where the concentration of biologic aerosols is normally low (80,

81). The principle behind sieve impingers is that the interposition of perforated plates with different pore diameters in a flow channel promotes acceleration and impingement of viable particles so that the resulting colonies are optimally separated by size (82). Throughput of 1 ft^3 per minute of air is maintained. After incubation of petri dishes which have been used to collect the particles, counts of colony forming units are made, and the number of organisms per cubic meter in air can be calculated.

SAMPLING AIRBORNE FUNGI: IMPORTANT CONSIDERATIONS

Once the sampling device, usually an Andersen impactor, has been chosen, several other considerations are necessary before beginning to sample. An important factor is the proper calibration of the equipment. Samplers must be calibrated according to the manufacturers recommendations and should be checked daily when in use. Disinfection of the equipment is necessary to avoid contamination of the sample.

The proper size culture plates and media should be used when sampling for viable organisms. Standard media are available for the common organisms but special media preparation may be necessary if an unusual organism is suspected. Culture media should be checked for sterility

and ability to support growth of the suspected organism which is suspected (80, 81).

Once collected, the sample must be handled and processed carefully in order to avoid contamination. Incubation at different temperatures should be performed to check for the presence of fungi, bacteria, and thermophilic actinomycetes. The total number of colony forming units can then be counted and estimates of the concentration of organisms in the air can be made if the flow rate and duration of sampling are known.

A sampling strategy should be devised to determine the likely source of contamination so that corrective measures can be undertaken. Air samples should be collected near potential sources such as HVAC systems, office furnishings, and portable appliances. It is often helpful to measure outdoor levels of the suspected agent, since indoor particles may arise from outdoor sites.

Duplicate or triplicate samples should be obtained at suspected locations. Also, sampling of control sites is useful to compare the total number of organisms between the sites. One can then compare the prevalance of employee symptoms at control and suspected sites and correlate this with the microbial counts.

It is also important to obtain samples at the appropriate time. For example, sampling before and after HVAC systems are turned on may detect a large difference

in the concentration of organisms, and employee symptoms may be temporally related.

EXAMPLES OF INDOOR AIR POLLUTION DUE TO FUNGI

There has been an increasing number of cases of sick building syndrome attributed to biologic agents recently. Numerous state and federal agencies have investigated cases of biologically contaminated offices. Form 1971 to 1987, NIOSH has investigated around 450 complaints of poor indoor air quality in a wide variety of office settings (84). Five percent of their investigations have involved some type of microbiologic contamination. Several of those biological outbreaks involved fungi (85). Many of these buildings had a history of flooding episodes and mechanical systems plagued with pools of standing stagnant water (85)

The California Indoor Air Quality Program is an effort to determine the extent and nature of indoor air problems in California. Biological contamination was felt to be the cause of 16% of the complaints, and most cases were related to problems with internal moisture (86).

Arnow et al investigated symptoms of hypersensitivity pneumonitis in three office workers in a southwestern building complex (71). Numerous fungi were isolated from the air cooling system of the building. Numerous

symptomatic and asymptomatic subjects had positive precipitins to the fungi.

Wilhelmsson et al chose five furniture industries to investigate the incidence of workers with nasal allergies (64). Out of a total of 16% of workers with allergic symptoms, 3% of individuals had allergy to molds and 2% to wood dust as determined by skin tests and nasal provocation test. However, a high frequency of precipitating antibody against molds was found in the wood workers, in both symptomatic and asymptomatic workers.

Numerous other industries that work with organic materials have been associated with outbreaks of allergic diseases (69, 87, 88). Many diseases are often named for the organic material handled. For example, malt worker's lung and cheese worker's lung are due to fungal contamination of malt and cheese respectively.

It is often hard to control fungal growth in many industries that work with organic material, therefore elimination of allergic disease in these industries will be difficult. As an example, in the pork and poultry producing industries, pathogenic organisms are often found in swine confinement and poultry buildings. Aerosol exposure is highly likely to occur in these buildings (89). In other industries, such as antibiotic, bakery, cheese, and wine manufacturing, fungi are a necessary part of the process and thus exposure is practically assured.

Of concern to parents are reports of sick building syndrome due to biological agents in school houses (often dubbed sick school syndrome) (90). Studies of schools have shown numerous types of fungi, just as with most homes (25, 91, 92, 93). In certain schools, the number of mold spores is less than in local houses (91). Good housekeeping, the control of water damage, maintenance of ventilation systems, and the avoidance of carpets may decrease the microbial contamination in school houses (25).

INVESTIGATION OF SICK BUILDING SYNDROME

Sick building syndromes are becoming more prevalent, especially since the energy crisis in the early 1970's. However, determining the etiology of workers' complaints is often difficult and frequently unsuccessful. Several reasons, including the highly charged emotions of employees, the complexity of the buildings and often inconclusive epidemiologic and industrial hygiene sampling help explain this difficulty. Since 1971, NIOSH has investigated over 450 complaints of indoor air quality (84). Many of the following recommendations have been taken from their guidelines on investigating indoor air quality problems.

The major goals of any environmental health investigation are to determine whether the environment is

safe, to determine the cause of health complaints, to identify interventions to alleviate the existing problem, and to prevent future problems (94). Kreiss and Hodgson discuss three ways to investigate indoor air pollution (2). They break the approach into three areas: industrial hygiene, epidemiology, and ventilation engineering.

The industrial hygiene approach, which involves identification of potential hazardous processes and exposures followed by sampling for physical or chemical agents, frequently fails in determining the etiology. Numerous chemicals have been detected, but usually at low concentrations, far below occupational exposure standards (1, 4, 5, 6).

If the industrial hygienist cannot pinpoint a source for the complaints, epidemiology is often turned to for clues. Epidemiology, the study of disease in populations, can be useful to the investigation by identifying patterns which suggest new hypotheses and lead to appropriate environmental sampling strategies (94). Epidemiology can describe the complaints as functions of time, place, and persons, and can assess social factors and health outcomes which are not within the traditional industrial hygiene evaluation. Epidemiology and statistical analysis may be required to estimate the relative contribution of multiple

chemical, physical, and social factors in causing environmental problems.

Defined criteria, which depend upon the complaints, are used to establish a case definition. Then, cases can be compared by rooms, floors, or to other buildings to see if a difference exists. By this approach, certain clues may become apparent, and suggest either the likely source or the areas where additional sampling and investigation are needed.

Baker describes six steps in the epidemiologic investigation of a building (94).

1 - Establish existence and extent of the problem

2 - Look for patterns by time, place, person

3 - Formulate hypothesis to direct further study

4 = Systematically evaluate occupants

5 - Analyze multifactorial problems

6 - Present findings

Epidemiologic methods also have limitations. Frequently, too few people are in the building to reliably determine the association between specific exposure and health outcomes. Also, epidemiologic studies often cannot tell whether adverse environmental conditions led to increased complaints or whether health problems led to an increased awareness of the environment. Furthermore,

biases may be present and may affect the validity of the study.

If no source is located, then evaluation of the ventilation system guided by epidemiologic and industrial hygiene findings is performed. Frequently, contamination of the HVAC system is found. Limitations in fresh air intake are frequently noted, and adjustments can be made which will often solve the problem. Inadequate ventilation is by far the largest problem noted in NIOSH investigations of indoor air pollution (84). In many cases where no source is determined, increasing the fresh air intake has decreased the number of complaints.

NIOSH has suggested the following guidelines for investigating indoor air quality problems (84). While not specific for fungal investigations, these methods may help pinpoint areas that can contribute to fungal growth and that may need correction.

A - Initial Assessment

1 - Documentation of complaints (interview or questionnaire)

a) What health complaints have been experienced?

b) When was the first time they were noticed?

c) Was there any specific incident or event that was linked with the initial onset of the complaints?

d) How often do they occur?

e) How long do they last?

f) Are there any particular times during the day they occur?

g) Do they occur in particular areas?

h) Are there any specific activities, tasks, or unusual circumstances that accompany the problem?

i) When do the health complaints go away?

At this point, you can develop a case definition and determine how many people are involved. A graphic display of the floor plan and the cases can be made to see if any patterns emerge. It is often helpful to have a control building to compare with.

2 - Building Characterization

a) What is the building's age, basic construction, number of floors, number of square feet per floor, types of windows and do they open?

b) Who is responsible for the functioning of the building's systems?

c) Who is responsible for cleaning the interior of the building? How often is cleaning done?

d) Have there been any major renovations or operating changes? What were they? When did they occur?

e) Does the building have sprayed or foam

insulation? When was it applied?

f) What type of heating system is used?

g) What type of cooling system is used?

h) What type of humidification system is used?

i) How is the total ventilation system operated?

j) What floors and rooms are served by each system?

k) What type of filtration system is used? How often is it changed/maintenanced?

l) How much fresh air is being introduced into the ventilation system? Does this amount meet system specifications?

m) Where are the fresh air inlets and are they functioning properly?

n) Are there any possible sources of contamination located in the general vicinity of the air inlets?

o) How likely are contaminants to be drawn into the air inlets due to prevailing winds and inversions?

p) How does exhaust air leave the building?

q) Is the building being used for the same purpose(s) for which it was designed?

r) What type of activities are building occupants engaged in?

s) What processes or activities are present in

the building that may serve as contaminant sources? How are they vented?

The building composition and ventilation system will often provide important clues leading to a solution.

3 - A walkthrough survey is performed to ensure the accuracy of the interview and to assess the overall condition of the building and to determine that systems are functioning properly. Examples of some problem areas include:

a) specific equipment giving off fumes or heat
b) improper cleaning procedures leaving residues
c) filters and wet areas in the ventilation system may not be cleaned frequently enough or improperly draining
d) the air intake may be located next to the air exhaust
e) inadequate makeup air
f) the ventilating system may be out of balance or the temperature control may be inadequate

If at this point no specific problem can be found, then further investigation should done.

B - Intermediate Assessment

1 - Evaluate

a) CO_2 levels

b) temperature

c) humidity

d) provision of adequate amounts of outside air

2 - Additional evaluation of point source of contaminants may be necessary

3 - Eliminate or control all known and potential sources of chemical contaminants

4 - Eliminate or control all known and potential sources of microbial contaminants

C - Conclusions and Followup Assessments.

It is important to follow up to make sure your recommendations were followed and were successful.

The above steps are recommended to evaluate complaints of indoor air pollution. Early recognition of a problem with a timely and systematic evaluation are key factors to a quick, effective resolution and will reassure employees that action is being undertaken.

PREVENTION AND CONTROL OF FUNGI IN THE INDOOR ENVIRONMENT

One of the chief problems concerned with biological exposures in the indoor environment is the "threshold level." Individual sensitivity may vary and no levels

have been established for biological organisms, in contrast to industrial chemical exposures.

Because sick building syndrome is a public health concern, many levels of responsibility are involved. Individual workers, building managers, architects, contractors, manufacturers, and the government all have a responsibility to provide a safe working environment. Strategies for reducing risk to exposed populations must be based on defensible exposure-response relations, so that the cost effectiveness of control options can be evaluated on the basis of reduction in population exposures (5).

To prevent unnecessary financial burden on employees or employers by recommending unnecessary environmental changes, we must demand strict criteria for the diagnosis of allergy or infection (66). The minimal criteria for mold allergy should be that fungi are abundant in the indoor air, that the employees have IgE antibody to the same allergen, and that the employees have increased symptoms on exposure to the allergen. The presence of infection caused by the same fungus in two or more employees should suggest the work place as the common source.

There are three perspectives in dealing with sick building syndrome: contamination cleanup, preventive maintenance, and preventive design (96). The following

recommendations for microbial contamination prevention and control are compiled from numerous sources (23, 84, 85, 96, 97, 98).

1 - Repair all external and internal leaks promptly and permanently.

2 - Avoid stagnant water accumulation under cooling coils in air handler units. Proper inclination and continuous drainage of drain pans is necessary.

3 - Cooling coils should be run at a temperature low enough to permit adequate dehumidification so that spores and substrate impacted on coils may be washed away in condensate water.

4 - Maintain relative humidity at less than 60% in all occupied spaces and low air velocity plenums.

5 - Use only steam, not water, as the moisture source for humidifiers in the ventilations system. Humidifiers utilizing recirculated water are not recommended. The use of portable cold mist vaporizers is discouraged, since they are readily contaminated with microorganisms.

6 - Use filters in air handling units that have a moderate to high efficiency (80-90%). Prefilters should be used to clear the air prior to passage over the higher efficiency filters.

7 - Exercise caution in locating outdoor intake vents to avoid entry of microbial aerosols from cooling tower draft, sanitary, and other exhaust vents.

8 - Develop a preventive maintenance program to avoid slime buildup in cooling towers and drain pans.

9 - The building should be operated so that the inside pressure is slightly positive with respect to the outside.

10 - Discard carpet, furniture, ceiling tiles, and other porous furnishings that are contaminated with microbial agents.

11 - Air handling units should be constructed so that equipment maintenance personnel have easy and direct access to both heat exchange components and drain pans.

12 - Keep hot water temperatures above 120° F.

13 - Choose an HVAC system that fits the building's size and anticipated use.

14 - Fit the HVAC system with regulation generators that are flexible enough to adjust to the varying air pressures they will be subjected to by intake and outtake vents.

15 - Intake vents should be located where they will receive the largest supply of fresh air.

16 - At a minimum, outdoor air should be provided in conditioned air at a rate of 10 to 2.5 liters/second (20 and 5 CFM) per occupant for smoking and nonsmoking environments respectively.

17 - If the source of contaminant appears to be from the outside, then closing doors and windows is useful. The reduced fresh air must be supplemented by a carefully filtered air source.

18 - Maintain a dust free environment by good housekeeping, avoiding stuffed furniture, house plants, and pets.

19 - Antimicrobial agents may be necessary to fumigate moldy items that cannot be discarded.

20 - Animal or vegetable matter should be stored under dry conditions to prevent molding.

21 - In industries where dusty organic matter is processed, microbial spores are frequently released. In such situations, local exhaust ventilation and general dilution ventilation are often effective.

22 - If the source cannot be eradicated or if engineering controls are inadequate, use of personal protective equipment may become necessary.

CASE REPORT

Background

As mentioned in the literature, sick building syndrome is becoming more prevalent. Biological agents, including fungi, have been responsible for many cases. The opportunity arose to investigate worker complaints in a building at a local university. It appears that fungi may have been responsible for some of the workers' symptoms

The building was constructed in the early part of the twentieth century and has undergone numerous renovations. It has three floors and currently functions as both office space and class rooms for the liberal arts. There are no laboratories in this building. See Figures 1-3.

In 1984, a steam leak occurred in the ceiling of room 06. However, the leak was not detected until January, 1985. During 1984, room 106 had undergone renovation, and a change in its ventilation system. In November, 1984, black mold was noted growing on the floor, molding, and around two doors in room 106. A cleanup was undertaken later that month, and cracks were sealed. Fungal sampling was performed, and very heavy growth was found on the day of cleanup. Over the next several months, four people

quit working in room 106 due to an "unhealthy environment."

In November, 1985, a mold problem in the ceiling tiles of room 06 was brought to the attention of campus safety. It had developed approximately one year earlier. In December, 1985, aerosol sampling in the interstitial space between room 06 and 106 detected heavy fungal growth with Aspergillus species. Also Penicillium species and Aspergillus species were detected in heavy concentration in room 106.

New duct work was installed in this area in December, 1985, and the number of complaints decreased. In April, 1986, an odor and black mold growing on a wall behind a radiator in room 106 was detected.

In October, 1986, campus safety notified the Employee Health Service of workers in room 09 complaining of respiratory symptoms which the employees felt were work related. Four individuals were evaluated in the health clinic, and results of the history and physical examination were consistent with an allergic condition as the cause of their symptoms. As a result of this examination, questionnaires were sent to all employees in room 09 to determine if other individuals had similar complaints (see Appendix I questionnaire).

A few days after the questionnaires were distributed, a walk through survey of room 09 was performed. At this

MATERIALS AND METHODS

Subject questionnaire

The questionnaire (Appendix I) was distributed to the 135 full and part-time employees on all three floors of the affected building. A control building located several blocks distance from the affected building was conveniently chosen, and the questionnaire distributed to 80 individuals. The questionnaire was sent out once to the control building, but three times to the affected building.

Approximately one year later, a second questionnaire (Appendix 2) was distributed to 139 full and part-time individuals in the affected building. This number includes individuals who took the first questionnaire and individuals that were not working in the affected building at the time the first questionnaire was sent out. An additional 17 questionnaires were sent to the 18 faculty and staff that had left the affected building after the first questionnaire was distributed. One individual's address could not be found. The questionnaire was sent out twice to every individual.

During the interval between the first and second questionnaire, modification of the ventilation system to room 103 and 106 was performed. Also, a thorough cleaning of room 09 was undertaken.

Fungal Sampling

Numerous samples for fungi were taken both inside and outside the building. An Andersen two stage impactor with a flow rate of one cubic foot per minute was used. The first stage had an orifice of 1.5 millimeters, and the second stage had an orifice of 0.4 millimeters. The length of collection was usually either five or ten minutes. Fungi were collected on Sabouraud's agar plates. After incubation primarily at 35° C.,.the number of colony forming units was counted.

Dust sampling

Dust samples were collected from room 09 at two locations. Additional samples were collected from an adjacent building which was built at the same time as the affected building, and from the Environmental Safety building, which is located several blocks from the affected building.

Dust samples were collected with a high volume sample pump, using a preweighed five micron PVC filter. Flow rates ranged from 11.37 to 11.8 liters/minute, and sampling time ranged from 3 to 4.5 hours. Results are reported in milligrams per cubic meter.

Identification of yeasts

Initially, gross colony morphology on Sabourauds agar was used to identify the likely genus of fungi. Four different colony morphologies were identified from the sampling. The fungi were frozen and approximately one and a half years, later, speciation was attempted. Aspergillus versicolor and Cladosporium cladosporoides were identified. However, a Penicillium and another Cladosporium could not be speciated due to difficulty in growing the organisms.

Fungal extracts

Extracts from the fungi identified were prepared for skin testing by the Division of Mycology at the university. The antigens that were used for skin testing were derived from the spore fraction of each fungus and were filter sterilized. The carbohydrate content of the final extracts was then determined. A diluted concentration was used for skin testing.

Skin testing

Skin prick testing was offered to all employees in the affected building. After several announcements, 35 individuals volunteered for testing. The tests were performed by allergists from the university. Nine allergens and two controls were placed on the volar aspect

time, we were notified of employees in room 01 with similar symptoms. At this point, the decision was made to survey the entire building.

The ventilation system differs among the rooms in the building. The second floor was placed on a central system in the late 1960's. In the early 1980's, room 06, 106, and 010 were connected to central but separate HVAC units. Some of the other rooms, including 09, have window unit air conditioners, but many do not. Windows can still be opened in these rooms. No water circulates within the central systems.

Hypothesis and Statistical Analysis

Because fungal contamination had been detected earlier in one area of the building, and because fungi could be responsible for allergic symptoms, the working hypothesis was that fungal contamination was responsible for the workers' complaints. To evaluate this hypothesis, questionnaires were sent out, fungal sampling was done, and skin and immunologic testing was performed. Either chi-square or Fisher's exact method was used to evaluate the questionnaire responses depending on the cell sample size. A P-value of ≤ 0.05 was considered statistically significant. For skin test results, simple percentage comparisons were made among the floors in the affected building.

MATERIALS AND METHODS

Subject questionnaire

The questionnaire (Appendix I) was distributed to the 135 full and part-time employees on all three floors of the affected building. A control building located several blocks distance from the affected building was conveniently chosen, and the questionnaire distributed to 80 individuals. The questionnaire was sent out once to the control building, but three times to the affected building.

Approximately one year later, a second questionnaire (Appendix 2) was distributed to 139 full and part-time individuals in the affected building. This number includes individuals who took the first questionnaire and individuals that were not working in the affected building at the time the first questionnaire was sent out. An additional 17 questionnaires were sent to the 18 faculty and staff that had left the affected building after the first questionnaire was distributed. One individual's address could not be found. The questionnaire was sent out twice to every individual.

During the interval between the first and second questionnaire, modification of the ventilation system to room 103 and 106 was performed. Also, a thorough cleaning of room 09 was undertaken.

of the forearm at the site of a 2.5 millimeter prick, which was made with a four pronged skin prick instrument.

The allergens included the four fungi that were cultured from the affected building, a commercial Aspergillus mix preparation, dust mite antigen, oak, ragweed, and rye antigens. The controls were histamine and normal saline. The area of the wheal and flare was calculated and compared to the controls. A significant response was considered to be a wheal ≥9mm in area and a flare ≥16 mm in area after correcting for the saline reaction.

Immunodiffusion

Blood samples were obtained from each of the 35 individuals. The samples were centrifuged for ten minutes and the serum was removed. Double immunodiffusion in agar (Ouchterlony analysis) was performed on fourteen individuals identified as nonatopic by their history, but having positive prick tests. A sample of the patient's serum was placed in a central well. Surrounding this central well were five other wells in which a sample of the fungal extracts was placed. Lines of precipitation were searched for which would indicate the presence of IgG antibody to the fungi in the individual's blood.

RESULTS

Dust samples

Room 09 in the affected building was sampled for approximately four hours at a flow of 11.37 liters/minute for a total volume of 2701.7 liters. The dust concentration was 0.07 mg/m³. Another area was sampled in room 09 for 3.75 hours at a flow of 11.8 liters/minute. A total volume of 2664.8 liters was sampled, and the dust concentration was 0.08 mg/m³.

An area in the sister building adjacent to the affected building was sampled for approximately 3 hours at a flow rate of 11.8 liters/minute for a total volume of 2181 liters. The dust concentration was 0.05 mg/m³.

A third building was sampled at two sites. At the outside loading dock of this building, a total volume of 2730.8 liters was sampled, and the dust concentration was 0.1 mg/m³. At an inside office within the building, 2881.7 liters of air was sampled, and the dust concentration was 0.07 mg/m³.

Results of Fungal Sampling

Numerous fungal samples were obtained in the areas where people were complaining. Also, fungal sampling was performed in the sister building for comparison. See Table 5.

The number of colonies growing on the top and bottom plates was manually counted, but only the bottom plate

count was used in calculating the colony forming units per cubic meter.

The table shows a several fold higher number of colony forming units per cubic meter in the rooms with the employee complaints as compared to the adjacent building. In 1984 and 1985, Aspergillus was the predominant organism isolated from room 106. In later samples, Cladosporium was the dominant genus isolated throughout the building. However, Penicillium and Aspergillus were also collected later, but in lower concentrations. The identification of the colonies was by gross morphologic appearance.

From March 9 through March 13, 1987, a thorough cleaning was performed in room 09. The carpet was removed, all the books were removed and cleaned, and the walls were thoroughly washed. A sealant was placed on the floor, however one person complained strongly about the odor from the sealant. As can be seen in table 1, a marked fall in the fungal count could be noted, especially on April 2, 1987.

However, during sampling in the middle of April, marked increases in fungal counts were noted in room 06, 09, and 106. It can also be noted that the outdoor fungal counts were very high during the later days of April and may have contributed to the higher counts noted in the above rooms.

In July, 1987, the ventilation system to room 106 was markedly upgraded. High efficiency filters were installed, and bag filters were added. The number of CFU/m^3 dropped markedly after the changes were made. Likewise, the number of complaints reported decreased from this area. Individuals that occupied room 09 transferred to a different building and verbally reported improvement in their symptoms.

A followup questionnaire was sent out approximately one year after the initial survey and will be discussed below. See Appendix 2.

Results of Questionnaire

For the first survey, 128 people were sent questionnaires. Eighteen graduate students in room 01 did not receive the questionnaires due to secretarial misunderstanding in this area. One hundred six responses were received from the affected building for a response rate of 82.8%. Of 80 questionnaires mailed to the control building, 48 individuals responded for a rate of 60%.

Forty of 48 individuals on the ground floor (excluding graduate students in one area) returned questionnaires for a response rate of 87.5%. On the first floor, the response rate was 64%, and on the second floor, the response rate was 70%.

Of the respondents from the affected building 46 were male, 53 were female, and 7 were unknown. In the control building, 9 were male, 15 were female, and 19 were unknown. Of the combined responses from both buildings, 13% complained of shortness of breath, 42% complained of cough at work, 29% complained of eye symptoms, 11% complained of chronic cough or asthma, 15% complained of rash, 32% had a history of allergy, 27% had a family history of allergies, and 25% complained of symptoms due to their worksite. In the affected, building, 68.8% were nonatopic. In the control building, 66.7% were non atopic.

When the affected building was compared to the control building, there was no statistically significant difference noted in the following variables: asthma, prior personal history of allergy, family history of allergy, or overall complaints of work related problems. However, a statistically significant difference was noted in the location of the employees' complaints. The individuals in the affected building noted that symptoms occurred three times more frequently at work than at home.

Since the second floor is on a different ventilation system than the ground and first floors, the results of the questionnaire were compared between the ground and first floors combined to the second floor and control building combined. Again, no difference was noted in a

personal history of allergies or family history of allergies. However, individuals on the ground and first floors were almost four times as likely to complain of symptoms occurring at work and two times as likely to complain of work related problems, especially red or irritated eyes and sneezing.

When comparing individual floors and the control building, no statistically significant difference can be seen for history of asthma, prior history of allergies, or family history of allergies. However, the proportion of individuals with total number of work related symptoms is significantly different for the ground and first floor as compared to the other areas. See Table 6.

When the rooms with the most complaints (01, 06, 09, 106) were compared to all other rooms in the affected and control area, no difference was noted in the individuals' history of asthma, prior history of allergy, or family history of allergy. However, those with symptoms from the problem rooms were far more likely to have symptoms at work and to have symptoms they felt were work related.

Based on the questionnaire results and skin test results, modifications of the ventilation system were performed. About one year after the initial questionnaire, a repeat questionnaire (Appendix 2) was distributed to all individuals in the affected building and to seventeen of eighteen individuals that had left the

building. The purpose of the followup was to see if the ventilation changes had improved the indoor air quality.

A total of 51 questionnaires was returned. Results from the 51 responses are as follows: 13% complained of problems with shortness of breath, 35% with sneezing or coughing, 27% with eye symptoms, 16% with a family history of asthma, 18% with skin rash, 41% with allergies and 18% with work related problems, Eighteen percent of the respondents were smokers. There was no significant difference in the percentage of asthma or allergy by floor. Individuals on floor 0 and 1 were more likely to complain of work related problems then on floor 2 and more likely to have symptoms while at work than individuals on floor 2. There was no correlation in the length of time an individual worked in the affected building and the likelihood of having symptoms.

Only 20 individuals out of the 51 total responses participated in the first questionnaire. Of these 20 individuals, 6 no longer worked in the affected building. Only 3 individuals noted any change in their symptoms. Two noted an improvement in their symptoms and one individual noted a worsening in her symptoms. Both of the individuals that noted an improvement were no longer working in the affected building. The seventeen other individuals noted no change in their symptoms.

To exclude any bias that may be present due to differences in atopy, the 31 "new" workers in the affected building were compared to the 20 "old" workers. No difference was noted in their personal history of allergy. Also, there was no difference in overall number of work related complaints between the "old and new" workers. However, there was a statistically significant difference in the frequency of allergic type symptoms in the old workers as compared to the new. The "old" workers were twice as likely to have allergic symptoms as the "new" workers.

Skin Testing Results

Only individuals in the affected building participated in skin testing. Individuals included graduate students, faculty, and staff. Graduate students spent much less time in the affected building than staff or faculty.

Thirty-six individuals participated in this part of the study. Eleven were tested from the ground floor, fifteen from the first floor, and ten from the second floor. There were ten males and twenty-five females in the study.

A brief history was taken before skin testing (Appendix 3). Only five individuals were smokers at the time of the study. Eleven individuals reported a history

of allergy either to medicines or pollens. Twenty-four individuals reported no atopic history. Seventeen of these were female.

Wheal and flare responses were recorded on all except six individuals. In the first six individuals, the antigens were placed too close together on the forearm, and some of the flare reactions ran together. Thus, none of the flare reactions were recorded in this group.

If the individual's saline area is subtracted from the area of each of the other antigens, then we can exclude the underlying nonspecific reaction which may occur from administering the prick. Then, any area remaining that is ≥9 mm in wheal size is considered highly significant.

Results of the testing show that nine individuals had a significant reaction to dust mite antigen. Fifteen individuals had a positive reaction to Aspergillus. Six individuals had a positive reaction to Cladosporium I, while eight individuals reacted to Cladosporium II. Six individuals had a positive reaction to Penicillium.

Of the individuals with a history of atopy, six out of eleven (54%) had a positive reaction to one of the fungi. In the nonatopics, fourteen out of twenty-four (58%) tested positive to one of the fungi. For dust mites, six out of twenty-four (25%) nonatopics had a

positive reaction, while four of eleven atopic had a positive reaction (36%)

Results comparing skin test reactions by floor to atopic status are shown in Table 7. As noted, the first floor had the highest number of nonatopics with positive skin test. Most of the individuals were from room 106.

One individual on the second floor was classified as a nonatopic, even though his history was possibly suggestive of atopy. If he was classified as atopic, then 33% of the individuals with no history of allergy on the second floor would have positive skin test, and this is similar to the upper limits of fungal skin test reactivity reported by Gravesen (62).

Immunodiffusion

None of the fourteen individual nonatopics with positive skin tests had evidence of IgG antibody in their serum by this technique. Even after increasing the amount of antigen in the wells, no lines of precipitation were detected.

DISCUSSION

It is important to address the health complaints of workers, because they may point to a serious environmental problem. The conduction of an investigation should be well thought out and should focus on likely problems initially.

In this study, definite evidence of fungal growth which presumably was secondary to a break in a pipe was found in room 106. This was the area of the initial complaints. After the leak resolved and the walls in room 106 cleaned, the number of complaints markedly decreased, although the problem may not have been corrected.

When an allergic problem was suggested by the four individuals in room 09, further fungal sampling and comparison to levels in another building were performed. There was evidence of higher fungal counts in the affected building than in the control area. However, the source could not be determined. An outdoor source, as noted by the large increase in the fungal count starting in the spring, probably was a significant contributor.

The questionnaire results showed evidence of increased worker complaints on the ground and first floor as compared to the second floor and control building, suggesting that there truly was something occurring on these floors, adversely affecting employees. Also, the 20 "old" workers were more likely to have allergic symptoms than the 31 "new" workers, even though no difference was found in the rate of atopy. This suggests sensitization may have occurred in the "old" workers due to their worksite. Since no correlation between symptoms and length of work was noted, this also suggests that changes made in the ventilation system may have prevented

sensitization in the "new" workers by decreasing the fungal burden.

Knowing the design of the building's ventilation system may help pinpoint a source, since different rooms or floors may have different rates of symptoms, and may be on different ventilation systems. For example, in this study, individuals on the second floor were on a different ventilation system and had many fewer complaints than individuals in other areas. If changes are made in the ventilation system, then a followup questionnaire can determine if the changes were successful in improving employee complaints.

Skin testing and serologic testing may also be useful tools to see if individuals have become sensitized. This study shows a high rate of sensitization, especially among the first floor participants. This is in contrast to the 2-30% incidence of type I fungal allergy in the general population (62). Even though individuals can become sensitized to fungi in many different environments, it is unlikely that 80% of individuals with no predisposition to develop allergies would have positive skin test results to fungi isolated from the affected building if they did not have a common source of exposure. This source was most likely the affected building.

Some problems noted in performing this study were the misunderstanding or poor cooperation among secretarial

staff to distribute questionnaires to all the students. Also, the lack of interest in participating in a study when one is not bothered by symptoms was also a problem.

There was a fear among some individuals of participating in skin testing or giving blood, especially on a voluntary basis. Now, with the AIDS crisis, it may be difficult to explain to some individuals that one cannot contract diseases by donating blood.

Another problem encountered was explaining to maintenance workers with a minimal knowledge of biology the difference between sensitization and a simple dose-response effect. Many of the workers were unaware that fungi could cause allergic symptoms, and that once sensitized exposure to small numbers of organisms can lead to reoccurrence of symptoms.

It is difficult to draw any definite conclusions about the success of the ventilation changes because of the small number of individuals that answered both the initial and followup questionnaire. Even though lower fungal counts were noted after the ventilation change in room 106, no definite source was determined and possibly the problem may reoccur in other areas.

Room 09 was thoroughly cleaned, and the individuals located in this area were moved to another building. Verbally, many reported an improvement in their symptoms, but they declined to participate in the followup survey.

By fungal sampling, it was shown that the amount of contamination was decreased by thorough cleaning of the area. However, cleaning must be maintained on a regular basis to prevent future contamination.

If the personnel, money, technical resources, and time would have been available, a more thorough investigation could have been performed. A better approach to investigating the affected building would have included designating a single individual as the coordinator.

A better sampling strategy would have included total microbial counts and incubation of the culture plates at different temperatures to detect the presence of thermophilic organisms. Outside sampling should have been done concurrently with all indoor sampling. This may have led to the identification of the source. A regular schedule of sampling at certain locations and specific times should have been undertaken. Also, sampling immediately before and after changes in the ventilation system should have been performed.

The questionnaire should have been distributed the same number of times to each building, and a followup questionnaire should have been sent to the control building. Questions on smoking should have been included in the initial questionnaire.

Ideally, individuals in both buildings should have been skin tested and monetary compensation made available in order to increase the participation rate if necessary. Also, skin testing should have been properly performed so that both wheal and flare reactions could have been recorded.

The fungi isolated from the building should have been rapidly speciated, instead of frozen for one and a half years.

With the difficulties noted above in performing an investigation of sick building syndrome at a large university, what should a small plant or office building do when faced with a similar problem? A multifaceted approach involving occupational physicians, industrial hygienists, plant engineers, microbiologists and possibly an allergist would be ideal. However, this is unlikely to be the case. A simple approach would be to document an excess number of complaints. Then, one should evaluate the area for obvious sources of contamination. If none are found, then increasing the ventilation may alleviate the problem. If at this point symptoms are still present, then consultation with either the State OSHA office or private or university environmental consultants may be the most cost effective means of solving the problem.

Employees in the area should be kept informed of the progress of the investigation and remedial actions being

undertaken. In this way, potential problems between employees and management as well as potential law suits can be avoided.

APPENDIX 1

Building: Room Number:

Name:

Social Security Number:

1. Do you have problems with shortness of breath? If yes, explain.
2. Do you cough or sneeze at work?
3. Do your eyes water at work?
4. Do you have a history of asthma or chronic cough? If yes, explain.
5. Do you have a problem with skin rashes? If yes, explain.
6. Do you have a history of allergies? If yes, explain.
7. If you answered yes to the above symptoms, when are they worse? What makes them better?
8. Is there a family history of allergy or asthma?
9. Are there any problems you are having that you feel are work related? If yes, explain.

APPENDIX 2

QUESTIONNAIRE

BUILDING NAME

SS# ROOM# PHONE NUMBER

1) Do you have problems with shortness of breath? Yes No If yes, explain
2) Do you cough or sneeze at work? Yes No
3) Do your eyes water, itch, or burn at work? Yes No
4) Do you have a history of asthma or chronic cough? Yes No If yes, explain
5) Do you have a problem with skin rashes? Yes No If yes, explain
6) Do you have a history of allergies (including antibiotics)? Yes No If yes, explain
7) If you have answered yes to any of the above symptoms, when do they bother you the most? What makes them better?
8) Is there a family history of allergy or asthma? Yes No If yes, explain
9) Are there any medical problems you are having that you feel are work related? Yes No If yes, explain
10) Have you been a tobacco smoker within the last 2 years? Yes No
11) How long have you worked in the West Duke Building?

For individuals who completed the questionnaire in 1986, please answer the following additional questions:

12) Since the initial questionnaire in November, 1986 have you developed allergies or asthma? Yes No
If yes, please explain

13) For individuals with symptoms such as sneezing, cough, itching or burning eyes, have you noticed your symptoms to have improved, become worse, or not changed since November, 1986?
Please explain

14) What month did you note a change in your symptoms?

15) a) If you do not currently work in the West Duke Building, have your symptoms changed? Yes No

b) Did the symptoms change before or after leaving the building?

c) What month did you leave the building?

APPENDIX 3

Name:

SS#:

Date:

Please answer the following questions.

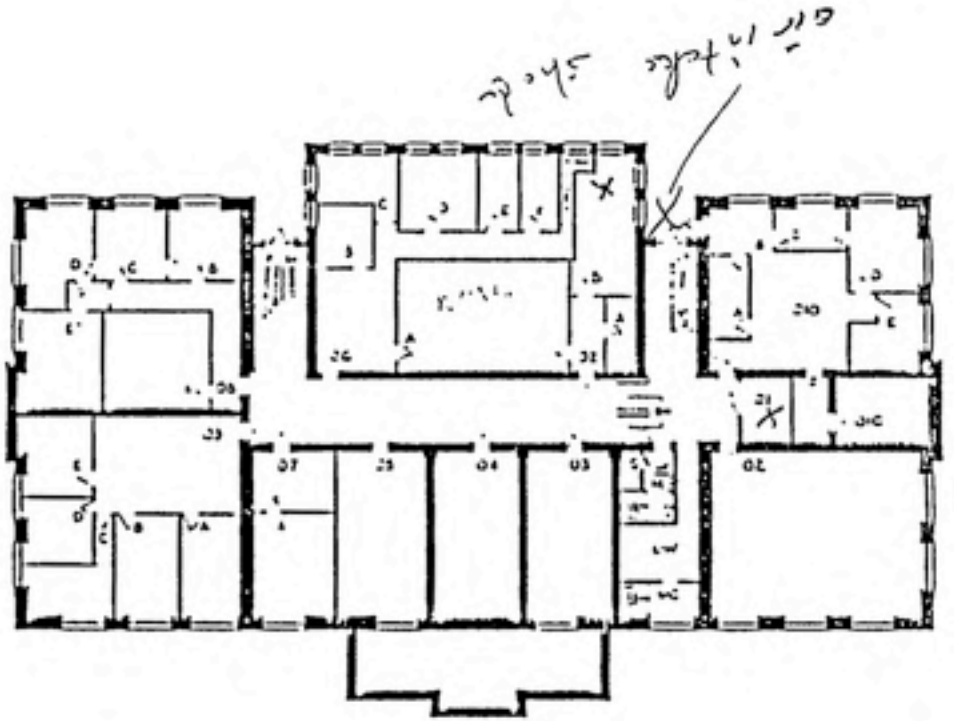
What medications are you currently taking?

Do you smoke? Y N If yes, for how long?

How many packs per day?

How long have you worked in the West Building?

Have you had any other problems that have occurred since the initial survey? If yes, please explain.



BASEMENT PLAN

SCALE: 0 5 10 15

WEST DUKE BLDG. 7202

DATE: 11-81

FIGURE 1

FIGURE 2

FIRST FLOOR PLAN

SCALE : 0 5 10 15

WEST DUKE BLDG. 7202

DATE : 11-01

SECOND FLOOR PLAN

SCALE : 0 5 10 15

WEST DUKE BLDG. 7202

DATE : 12-81

FIGURE 3

TABLE 1*

Average Hours Spent Per Day In Various Locations By Adults In 44 U.S. Cities

Location	Employed Men	Employed Women	Housewives
At home	13.4 (55.8)+	15.4 (64.2)	20.5 (85.4)
At work	6.7 (27.9)	5.2 (21.7)	- (0)
In transit	1.6 (6.7)	1.3 (5.4)	1.0 (4.2)
Outside	0.7 (2.9)	0.3 (1.3)	0.4 (1.7)
Inside other structures	1.6 (6.7)	1.8 (7.5)	2.1 (8.8)

*adapted from (1).

+Percentage of 24 h.

Table 2*

The Principal Indoor Pollutants

Respirable particles

NO, NO_2

CO

CO_2

Infectious, allergenic, irritating biological materials

Formaldehyde

Radon and radon daughters

Volatile organic compounds:

Benzene, styrene, tetrachloroethylene; dichlorobenzene; methylene chloride; chloroform

Semivolatile organics:

Chlorinated hydrocarbons, DDT, heptachlor, chlordane

Semivolatile organics: polycyclic compounds

benzo(a)pyrene, polychlorinated biphenols

Asbestos

*Adapted from (1).

TABLE 3*

Contrast Between Fungi and Bacteria

Property	Fungi	Bacteria
Cell volume (microns^3)	Yeast: 20-50 Molds: Not definable because of indefinite shape and size	1-5
Nucleus	Eukaryotic	Prokaryotic
Cytoplasmic membrane	Sterols present	Sterols absent (except for Mycoplasma grown on sterols)
Cell wall	Glucans; mannans; chitin, glucan- and mannan-protein complexes No muramic acid peptides, teichoic acids, or diaminopimelic acid	Muramic acid peptides; teichoic acids; some have diaminopimelic acid residues No chitin, glucans, or mannans
Metabolism	Heterotrophic, aerobic, facultative anaerobes; no known autotrophs or obligate anaerobes	Obligate and facultative aerobes and anaerobes; heterotrophic and autotrophic
Sensitivity to chemotherapeutic agents	Sensitive to polyenes and griseofulvin(dermatophytes) not sensitive to sulfonamides, penicillins, tetracyclines, chloramphenicol, streptomycin	Often sensitive to penicillins, tetracycline, chloramphenicol, streptomycin. Are not sensitive to griseofulvin or polyenes
Dimorphism	A distinguishing feature of many species	Absent

*Adapted from (16).

TABLE 4*

Asexual Spores Formed by Fungi

Conidia	This term is used sometimes generically for all asexual spores, or sometimes more specifically for spores borne singly or in clusters along sides or at tips of hyphae or of specialized hyphal branches (conidio -phores). Highly diversified in shapes, size, color, and septation. Large(usually multinuclear) and small (usually uninuclear) conidia are called macroconidia and microconidia, respectively.
Arthrospores	Cylindrical cells formed by double septation of hyphae. Individual spores are released by fragmentation of hyphae, i.e., by disjunction.
Blastospores	Buds that arise from yeast and yeast -like cells.
Chlamydospores	Thick-walled, round spores formed from terminal or intercalated hyphal cells.
Sporangiospores	Spores within sac-like structures (sporangia) at ends of hyphae or of special hyphal branches (sporangiophores). Characteristically formed by species of Phycomycetes.

*Adapted from (16).

TABLE 5^

Results of Fungal Sampling

Date	Room	Average CFU/m^3
1/7/86	09	*170
1/17/86	09	
1/21/86	010	194
3/27/87	09	109
	106	76
	112(s)+	42
4/2/87	09	20
	106	56
	112(s)	28
4/16/87	09	358*
	06	146
	106	•TNTC
4/23/87	outdoor	*353
	affected building	
	106	310
	06	141
5/21/87	outdoors	712
	106	130
5/22/87	outdoors	TNTC
	106	131
8/13/87	outdoors	510
	106	33

*Duplicate samples taken. If one plate had TNTC (too numerous to count) colonies, then only the other plate was reported.

+(s) = sister building.

•All plates had TNTC clolnies

^Average counts may represent sampling over different time periods.

Table 6

Questionnaire Results

Complaint	Floor[n] 0	1	2	control
S. O. B.*	12.8	12	13	8
Cough/sneeze	50	36	19	44
Eyes	31	30	17	25
Rash	22	6	9	17
Asthma	10	16	6	12.5
Allergy Hx	37.5	24	27	33
Family Hx	35	24	34	21
Work problem	39	39	13	18

[n]Results are expressed in percentages.

*S. O. B. = shortness of breath.

TABLE 7

Skin Test Results

Floor	Atopic	Nonatopic
Ground		
Total number tested	5	6
Total number with work related symptoms	5	5
Number with positive skin test to fungi	3 (60%)	3 (50%)
First		
Total number tested	5	9
Total number with work related symptoms	4	7
Number with positive skin test to fungi	2 (40%)	7 (78%)
Second		
Total number tested	1	9
Total number with work related symptoms	0	3
Number with positive skin test to fungi	1 (100%)	4 (44%)

References

1. Samet, J. M., Marbury, M. C., and Spengler, J. D. Health effects and sources of indoor air pollution. Part I[1-3]. Am Rev Respir Dis 1987; 136:1486-1508.

2. Kreiss, K., Hodgson, M. J. "Building-associated epidemics." Indoor Air Quality. P. J. Walsh, C. S. Dudney, and E. D. Copenhaver, eds. CRC Press Inc., Boca Raton, Florida. 1984:88.

3. Bensen, F. B., Henderson, J. J., and Caldwell, D. E. Indoor-Outdoor Air Pollution Relationships: A Literature Review, Publ. #AP-112, EPA, NARC, Research Triangle Park, N.C., 1972.

4. Samet, J. M., Marbury, M. C., and Spengler, J. D. Respiratory effects of indoor air pollution. J Allergy Clin Immunol 1987; 79:685-700.

5. Spengler, J. D., Sexton, K. Indoor Air pollution: A public health perspective. Science 1983; 221:9-16.

6. Hicks, J. B. Tight building syndrome: When work makes you sick. Occ Health Safety 1984; 53:551-57.

7. Mage, D. T., Gammage, R. B. "Evaluation of Changes in indoor air quality occurring over the past several decades." Indoor Air and Human Health. eds. R. B. Gammage and S. V. Kaye. Chelsea, MI, Lewis Publishers, Inc., 1985. pp. 5-36.

8. Holt, G. L. Sources of air contaminants in the office environment. Ann Am Conf Gov Ind Hyg 1984; 10:15-19.

9. Moretz, S. Are your workers safe from sick building syndrome? Occ Hazards 1988; 50: 58-62.

10. Morgan, M. S., Horstman, S. W. systematic investigation needed for tight building syndrome complaints. Occ Health Safety 1987; 56:79-88.

11. World Health Organization. Indoor air quality research. EURO Reports and Studies 103. WHO, Copenhagen, 1986.

12. Hinkle, L. E. Jr., Murray, S. H. The importance of the quality of indoor air. Bull NY Acad Med 1981; 57:827-844.

13. Pike, R. M. Laboratory-associated infections: Incidence, fatalities, causes, and prevention. Ann Rev Microbiol 1979; 33:41-66.

14. Fraser, D. A. "Sizing Methodology." The Industrial Environment: Its Evaluation and Control. Cincinnati, NIOSH. 1973. pp. 155-165.

15. West, J. B. Pulmonary Pathophysiology. Baltimore, Md., Williams and Wilkins, 1977.

16. Davis, B. D., et al. Microbiology. Hagerstown, Md., Harper and Row, 1973.

17. Emmons, C. W., Binford, C. H., and Utz, J. P. Medical Mycology. Philadelphia, Lee and Febiger, 1977.

18. Pelczar, M. J., Reid, R. D., and Chan, E. C. S. Microbiology. New York, McGraw-Hill, 1977.

19. Bold, H. C., Alexopoulos, C. J., and Delevoryas, T. Morphology of Plants and Fungi. New York, Harper and Row, 1980.

20. Al-Doory, Y. "Introduction'. Mould Allergy. Y Al-Doory and J. F. Domson (eds). Philadelphia, Lea and Febiger, 1984.

21. Myrvik, Q. N., Pearsall, N. N., and Weiser, R. S. Fundamentals of Medical Bacteriology and Mycology for Students of Medicine and Related Sciences. Philadelphia, Lea and Febiger, 1974.

22. Roberts, S. O., Hay, R. J., and MacKenzie, D. W. R. A Clinician's Guide to Fungal Disease. New York, Marcel Dekker, Inc., 1984.

23. Burge, H. A. "Indoor sources for airborne microbes." Indoor Air and Human Health. R. B. Gammage and S. W. Kaye, (eds). Chelsea, MI, Lewis, 1985.

24. National Research Council, Committee on Indoor Pollutants. Indoor Pollutants. Washington, D.C., National Academy Press, 1981. 9-393.

25. Gravesen, et al. Demonstration of microorganisms and dust in schools and offices. Allergy 1986; 41:520-525.

26. Lowenstein, H. Indoor allergens. J Allergy Clin Immunol 1986; 78:1035-1039.

27. Ibsen, K. K. et al. The indoor environment and symptoms of disease. Assessment of the conditions in

schools in Copenhagen. Ugeskr Laeger 1981; 143:1919-1923.

28. ACGIH Committee on Bioaerosols. Report of the committee on bioaerosols. Ann Am Conf Gov Ind Hyg 1985; 13:29-32.

29. Couch, R. B. Viruses and indoor air pollution. Bull NY Acad Med 1981; 57:907-921.

30. Fraser, D. W. et al. Legionnaires' disease. Description of an epidemic. N Engl J Med 1977; 297:1189-1197.

31. Pike, R. M. Laboratory-associated infections: summary and analysis of 3921 cases. Health Lab Sci 1976; 13:105-114.

32. Banaszak, E. F., Thiede, W. H., and Fink, J. N. Hypersensitivity pneumonitis due to contamination of an air conditioner. N Eng J Med 1970; 283:271-276.

33. Mc Sharry, C., Anderson, K., and Boyd, G. Serological and clinical investigation of humidifier fever. Clin Allergy 1987; 17:15-22.

34. Jacobs, R. L. Hypersensitivity pneumonitis caused by Cladosporium in and enclosed hot-tub area. Ann Int Med 1986; 105:204-206.

35. Mims, C. W. "Classification of fungi." Mould Allergy. Y. Al-Doory and J. F. Domson, (eds). Philadelphia, Lea and Feabiger, 1984.

36. Cole, G. T., Samson, R. A. "The conidia." Mould

Allergy. Y. Al-Doory and J. F. Domson, (eds). Philadelphia, Lea and Feabiger, 1984.

37. Patterson, R. (ed). Allergic Diseases Diagnosis and Management. Philadelphia, J. B. Lippincott, 1985.
38. Richard, J. L. Fungi as industrial hazards. Occ Health Safety 1979; 48:43-48.
39. Gordon, M. A. "Cryptococcosis." Occupational Mycoses. A. DiSalvo, (ed). Philadelphia, Lea and Febiger, 1983.
40. Pappagianis, D. "Coccidioidomycosis (San Joaquin or Valley Fever)." Occupational Mycoses. A. DiSalvo, (ed) Philadelphia, Lea and Febiger, 1983.
41. Larsh, H. W. "Histoplasmosis." Occupational Mycoses. A. DiSalvo, (ed). Philadelphia, Lea and Febiger, 1983.
42. DiSalvo, A. "Blastomycosis." Occupational Mycoses. Philadelphia, Lea and Febiger, 1983.
43. Goodman, N. L. "Sporotrichosis." Occupational Mycoses. A. DiSalvo, (ed). Philadelphia, Lea and Febiger, 1983.
44. Joseph, J. M. "Aspergillosis in Composting." Occupational Mycoses. A. DiSalvo, (ed). Philadelphia, Lea and Febiger, 1983.
45. Samet, J. M., Marbury, M. C., and Spengler, J. D. Health effects and sources of indoor air pollution. Part II[1-3]. Am Rev Respir Dis 1988; 137:221-242.

46. Goodwin, R A., Jr., Des Prez, R. M. "Histoplasma capsulatum." Principles and Practices of Infectious Diseases. G. Mandell, J. E. Bennett, and R. G. Douglas, (eds). New York, John Wiley and Sons, 1985. 1468-1476.

47. Bennett, J. E., "Sporothrix schenckii." Principles and Practices of Infectious Diseases. G. Mandell, J. E. Bennett, and R. G. Douglas, (eds). New York, John Wiley and Sons, 1985. pp. 1456-1459.

48. Rippin, J. W., "The pathogenic fungi and the Pathogenic Actionmycetes," Introduction to Medical Mycology. 3rd ed., W. B. Saunders Co, Philadelphia, 1988. pp. 2-11.

49. Chapman, S. W. "Blastomyces dermatitidis." Principles and Practices of Infectious Diseases. G. Mandell, J. E. Bennett, and R. G. Douglas, (eds). New York, John Wiley and Sons, 1985. pp. 1477-1484.

50. Stevens, D. A. "Coccidioides immitis." Principles and Practices of Infectious Diseases. G.Mandell, J. E. Bennett, and R. G. Douglas, (eds). New York, John Wiley and Sons, 1985. pp. 1485-1492.

51. Stites, D. P. "Clinical laboratory methods for detection of antigens and antibodies." Basic and Clinical Immunology. H. H. Fundenburg, et al (eds). Los Altos, CA, Lange Medical Publications, 1980. pp. 343-381.

52. Thaler, M. S., Klausner, R. D., and Cohen, H. J. Medical Immunology. Philadelphia, J. B. Lippincott Co., 1977.

53. Sanford, J. P. Guide to Antimicrobial Therapy 1986. San Antonio, Texas, Antimicrobial Therapy, Inc., 1986.

54. Pepys, J. Recognition of the allergic phenotype. Natural history of "atopy". J Allergy Clin Immunol 1986; 959-960.

55. Pepys, J. "Atopy." Gell PGH, Coombs RRA. P. Lachmann, et al (eds). Oxford, Blackwell Scientific Publications, 1985. p. 877.

56. Massicot, J. G., Cohen, S. G. Epidemiologic and socioeconomic aspects of allergic diseases. J Allergy Clin Immunol 1986; 954-955.

57. Druce, H. M., Kaliner, M. A. Allergic rhinitis. JAMA 1988; 259:260-263.

58. Pepys, J. Occupational allergic lung disease caused by organic agents. J Allergy Clin Immunol 1986; 1058-1062.

59. National Research Councel, Committee on Indoor Pollutants. Indoor Pollutants. Washington, D. C., National Academy Press, 1981. pp. 394-418.

60. Prince, H. E., Selle, W. A., Morrow, M. B. Molds in the etiology of asthma and hay fever. Texas St Med J 1934; 30:340.

61. Weeke, E., Bendixen, G. Micro-fungal allergy. Allergy 1979; 34:131-133.

62. Gravesen, S. Fungi as a cause of allergic disease. Allergy 1979; 34:135-154.

63. Howard, W. A. "Incidence and Clinical Characteristics of mould allergy." Mould Allergy. eds. Y. Al-Doory and J. F. Domson. Philadelphia, Lea and Febiger, 1984. 147.

64. Wilhelmsson, B., et al. Nasal hypersensitivity in wood furniture workers. Allergy 1984; 39:586-595.

65. Indoor allergens implicated in acute asthma. Allergy Observer March-April 1987:8.

66. Reed, C. E. What we do and do not know about mold allergy and asthma. J Allergy Clin Immunol December 1985; 76:773-775.

67. Aspergillus implicated in farmer's lung. Allergy Observer July-August 1987:3.

68. Licorish, K. et al. Role of Alternaria and Penicillium spores in the pathogenesis of asthma. J Allergy Clin Immunol December 1985:819-825.

69. Merchant, J. A. Agricultural exposures to organic dusts. Occ Med: State of the Art Rev 1987; 2:409-425.

70. Emanuel, E. A. "Mycotic hypersensitivity." Occupational Mycoses. A. F. DiSalvo (ed). Philadelphia, Lea and Febiger, 1983. 201.

71. Arnow, P. M. et al. Early detection of hypersensitivity pneumonitis in office workers. Am J Med February 1978; 64:236-242.

72. CDC. Outbreaks of Respiratory illness among employees in large office buildings - Tennessee, District of Columbia. MMWR 1984; 33:506-513.

73. Caldwell, J. L., Kaltreider, H. B. "Pulmonary and cardiac diseases." Basic and Clinical Immunology. H. H. Fundenberg et al (eds). Philadelphia, Lange, 1980. pp. 553-558.

74. Beaumont, F. et al. Environmental aerobiological studies in allergic bronchopulmonary aspergillosis. Allergy 1984; 39:183-193.

75. Kozak, P. P., Hoffman, D. R. "Critical review of diagnostic procedure for mould allergy." Mould Allergy. eds. Y. Al-Doory and J. F. Domson. Philadelphia, Lea and Febiger, 1984. p. 157.

76. Newman, L., Storey, E., and Kreiss, K. Immunologic evaluation of occupational lung disease. Occ Med: State of the Art Rev 1987; 2:345-372.

77. Domson. J. F. "Review of therapeutic procedure for mould allergy." Mould Allergy. eds. J. Al-Doory and J. F. Domson. Philadelphia, Lea and Febiger, 1984. p. 187.

78. Avoidance impossible? Dust filters can help. Allergy Observer June 1987:8.

79. Terr, A. I. "Allergic diseases." Basic and Clinical Immunology. H. H. Fundenburg et al (eds). Los Altos, CA, Lange, 1980. pp. 513-516.

80. ACGIH Committee on Bioaerosols. Bioaerosols. App Ind Hyg April 1986:R-19 - R-23.

81. AGGIH Committee on Bioaerosols. Bioaerosols: Guidelines for assessment and sampling of saprophytic bioaerosols in the indoor environment. App Ind Hyg September 1987:R-10-R-16.

82. Solomon, W. R. "Sampling techniques for airborne fungi." Mould Allergy. Eds. Y. Al-Doory and J. F. Domson. Philadelphia, Lea and Febiger, 1984. p. 41.

83. Finkelstein, H. Preliminary air pollution survey of aeroallergens. Lit. Rev. US Dept HEW, Public Health Service, Cons Protection Env Health Service, Nat Air Pollution Control Adm, Raleigh, NC, October 1969.

84. National Institute for Occupational Safety and Health. Guidance for Indoor Air Quality Investigations. Hazard Evaluations and Technical Assistance Branch, Division of Surveillance, Hazard Evaluations and Field Studies, Cincinnati, Ohio, January 1987.

85. Morey, P. R. Environmental studies in moldy office buildings: biological agents, sources and preventive measures. Ann Am Conf Ind Hyg 1984; 10:21-35.

86. Macher, J. M. Inquiries received by the California

Indoor Air Quality Program on biological contaminants in buildings. Advances on Aerobiology. Birkhauser Verlag Basel, 1987. 275-278.

87. Butcher, B. T., Salvaggio, J. E. Continuing medical education: occupational asthma. J Allergy Clin Immunol 1986; 78:547-555.

88. Proceedings of the Workshop Panel. Occupational allergic diseases. Clin Allergy 1986; 16:35-45.

89. Donham, K. J., Gustafson, K. E. Human occupational hazards from swine confinement. Ann Am Conf Gov Ind Hyg 1982; 2:137-142.

90. Seasonal allergic syndrome in school tied to recirculated, mold-laden air. Allergy Observer January-February 1988:1.

91. Dungy, C. I. et al. Aeroallergen exposure in the elementary school setting. Ann Allergy 1986; 56:218-221.

92. Rijckaert, G. Exposure to fungi in modern homes. Allergy 1981; 36:277.

93. Calvo, A., Dronda, A., and Castello, R. Fungal spores in house dust. Ann Allergy 1982; 49:213-219.

94. Baker, D. B. Epidemiologic investigation of office environmental problems. Ann Am Conf Gov Ind Hyg 1984; 10:37-44.

95. Melius, J. et al. Indoor air quality - the NIOSH experience. Ann Am Conf Gov Ind Hyg 1984; 10:3-7.

96. Bishop, V. L., Custer, D. E., and Vogel, R. H. The sick building syndrome: what it is, and how to prevent it. National Safety and Health News December 1985:31-40.

97. Burton, D. J. Indoor air pollution: solutions easier than determining causes. Occ Health Safety April 1985:68-69.

98. North Carolina Department of Human Resources. Indoor Air Pollution. Division of Health Services, Raleigh NC, August 1985.