BIOLOGIC CONTAMINANTS

JAMES M. SELTZER, MD

Reprinted from **OCCUPATIONAL MEDICINE: State of the Art Reviews**
Published by HANLEY & BELFUS, Inc. © 1995
210 S. 13th St., Philadelphia, PA 19107 (215) 546-7293

JAMES M. SELTZER, MD

BIOLOGIC CONTAMINANTS

From the Pediatric Immunology and Allergy Division
Department of Pediatrics
University of California School of Medicine
San Diego, California

Reprint requests to:
James M. Seltzer, MD
Medical Director
Indoor Hygienic Technologies Corporation
9855 Erma Road, Suite 104
San Diego, CA 92131

Biologic contaminants have the potential to produce a wide spectrum of human discomfort and illness. Since most of us spend 90% or more of our time indoors, the nature and concentration of biologic contaminants are likely to have a greater influence on our health and well-being indoors than outdoors. In its 1989 Report to Congress on Indoor Air Quality, the Environmental Protection Agency stated that "biological contaminants are an important dimension of indoor air quality, can be the principal problem in some buildings, and can result in death, as in Legionnaire's disease, or serious infectious or allergic diseases."[12] Consequently, minimizing the risk of indoor environmentally induced illness should be as simple as identifying existing contaminants and removing them. Unfortunately, the task of detection, accurate identification, linking specific contaminants to human disease, effectively removing them, and then preventing their recurrence is anything but simple and straightforward.

Whether a biologic agent will survive, replicate, and disseminate all or parts of its structure depends to a large extent on its environment. *Ambient conditions* such as temperature, relative humidity (RH), and air movement affect its growth (amplification) and dissemination. *Nutrient sources* that can be used to feed vital biologic systems specific for a given organism must be present. Some organisms grow independently of other living organisms, and others require (obligate parasites) or make use of (synergy) other living organisms to further their own growth. Finally, a *source* of at least one *viable organism* must be available to contaminate and potentially colonize a given indoor environment.

Such sources may already exist in a contiguous indoor environment, or the organism or a portion of the organism may be imported by water, air, or fomites into the indoor environment.

Generally, biologic contaminants are indistinguishable from one another by the human eye and require specialized tools, such as a microscope, to confirm their presence. Exceptions, such as larger pollens and mold spores, usually cannot be characterized without the use of a microscope or culture media. Another exception, the gaseous byproducts of human, animal, and microbial metabolism, are invisible to the naked eye. Biologic contaminants that can cause human disease may exist as viable organisms or as portions of these organisms, e.g., cell walls, spores. As a result, we generally think of biologic contaminants as microbes or as microscopic derivatives of microbes or other living organisms, such as animals and insects. Generally, biologic contaminants derive from the following classes of plant and animal phyla: bacteria, viruses, fungi (molds and yeasts), protozoa, insects (e.g., cockroaches), and arachnids (e.g., dust mites).

Most animal- and insect-derived proteins produce human illness by inducing a type 1 (Gell and Coombs classification) immunologic response. This IgE-mediated reaction develops in 20–30% of people in the United States and results in an immediate release of mast cell inflammatory mediators. The onset of a clinical response is rapid, within seconds to an hour after exposure; hence the term immediate hypersensitivity (allergy). Microbes produce human illness through infection, induction of an immunologic response, or intoxication. The majority of illnesses are contracted through contact of the offending contaminant with the upper or lower respiratory tract epithelium. The remainder occur from skin contact and, occasionally, ingestion. Biologic contaminants become entrained in the indoor air in several ways: (1) when air movement sweeps over a source of living organisms, dead organisms, or portions of dead organisms not tightly bound to their substrate or reservoir, as occurs with natural or mechanical ventilation; (2) when the substrate or reservoir of the contaminant is physically disturbed, such as might occur during construction or renovation of a building or walking through a room or vacuuming; (3) solubilization of the solid contaminant in water particles in the air or reservoir, which may then become aerosolized; and (4) release of volatile gaseous compounds by the contaminant. The term *bioaerosols* refers to any airborne particles, large molecules, or volatile compounds that are living organisms, portions of organisms, or results of organism metabolism. Since there are many such organisms in our environment, some of which feed upon or inhabit others, bioaerosols frequently are complex and quite varied, always containing a number of biologic contaminants in various forms and concentrations. Table 1 lists the sources, characteristics, and potential adverse health effects of common bioaerosol components.

Evaluating indoor environments for the presence of potentially pathogenic biologic contaminants poses a number of challenges. First, investigators are often faced with several options for sampling of any given contaminant, and they must be able to select the most appropriate test(s) for any given situation. Secondly, they must know how, where, and what to sample. And finally, they must know how to interpret the test results correctly. An accurate analysis of the environment requires sufficient knowledge of the nature and extent of the health complaints, the characteristics of disease states that can be caused by biologic contaminants, locations where contaminants may reside, and an understanding of the limitations of the assays available.

TABLE 1. Characteristics and Sources of Common Bioaerosol Components

Living Source	Airborne Unit	Examples of Sources	Primary Human Effects	Lifestyle	Principal Indoor Sources
Bacteria	Organisms	Legionella	Pneumonia	Facultative parasites	Cooling towers
	Spores	Thermoactinomyces	Hypersensitivity pneumonitis (HP)	Saprophytes	Hot water sources, hot damp surfaces
	Products	Endotoxin	Fever, chills	—	Stagnant water reservoirs
		Proteases	Asthma	—	Industrial processes
Fungi	Organisms	Sporobolomyces	Hypersensitivity pneumonitis (HP)	Saprophytes	Damp environmental surfaces
	Spores	Alternaria	Asthma, rhinitis	Saprophytes	Outdoor air, damp surfaces
	Spores	Histoplasma	Systemic infection	Facultative parasites	Bird droppings
	Antigens	Glycoproteins	Asthma, rhinitis	—	Outdoor air
	Toxins	Aflatoxins	Cancer	—	Damp surfaces
	Volatiles	Aldehydes	Headaches, mucous membrane irritants	—	Damp surfaces
Protozoa	Organisms	Naegleria	Infection	Facultative parasites	Contaminated water reservoirs
	Antigens	Acanthamoeba	Hypersensitivity pneumonitis (HP)	—	Contaminated water reservoirs
Viruses	Organisms	Influenza	Respiratory infection	Obligate parasites	Human hosts
Algae	Organisms	Chlorococcus	Asthma, rhinitis	Autotrophic*	Outdoor air
Green plants	Pollen	Ambrosia (ragweed)	Asthma, rhinitis	Autotrophic*	Outdoor air
Arthropods	Feces	Dematophagoides (mites)	Asthma, rhinitis	Phagotrophic†	
Mammals	Skin scales	Horses	Asthma, rhinitis	Phagotrophic†	Horses
	Saliva	Cats	Asthma, rhinitis	Phagotrophic†	Cats

* Autotrophic = synthesizes carbohydrates.
† Phagotrophic = ingests food.
From American Conference of Governmental Industrial Hygienists: Guidelines for the assessment of bioaerosols in the indoor environment. Cincinnati, ACGIH, 1989; with permission.

One of the most difficult aspects of the evaluation process is relating the environmental sampling data to the discomfort or illnesses people have been complaining about in the target environment. Finding ubiquitous microbes or animal proteins, even in higher than expected concentrations, does not prove that they have caused human illness. The human illnesses associated with biologic contamination share many common features; in addition, they share features with illnesses unrelated to this etiology. Commonly, some people in a contaminated environment may be adversely affected by biologic contaminants while others sharing the same environment suffer from unrelated illnesses.

Once biologic contaminants have been identified in the indoor environment, their source must be identified and the contaminant removed or contained to minimize the health risk to the building's occupants. This may be difficult when the source is hidden or not even within the building. Also, multiple sources of contamination may exist, and each may need to be addressed. Lastly, whenever possible, steps must be taken to prevent a recurrence. The complexity of the task of (1) detecting and characterizing biologic contaminants, (2) determining which, if any, are responsible for human illness, (3) and how to deal effectively with the contaminants should be readily apparent. This chapter provides a basic understanding of these issues as they relate to bioaerosols.

CONDITIONS PROMOTING BIOLOGIC CONTAMINATION

Ambient Conditions

Ambient conditions help determine the environment in which organisms live, replicate, and disseminate. Temperature, relative humidity, outdoor and indoor wind currents, and light can greatly affect the development of biologic contamination.

Very low temperatures tend to inhibit the growth of many organisms that at room temperature readily replicate and thrive. However, the yeast *Sporobolomyces* and the mold *Aureobasidium pullulans (Pullularia)* grow and sporulate well in cool environments. Some microbes thrive at higher temperatures. While the fungal *Aspergillus* species can grow between 12–57° C (54–135° F), the optimal temperature range for its growth is 37–43° C (99–109° F), or body temperature. The bacterium *Legionella pneumophila*, Actinomyces species, *Micropolyspora faeni*, and *Thermoactinomyces vulgaris* grow best at temperatures in excess of 50° C (122° F). *L. pneumophila* can grow in water temperatures of up to 60° C (140° F) and even reside and survive within protozoa. Higher temperatures also increase the respiratory rate of organisms and thereby increase the load of contaminants from metabolism such as water vapor and CO_2.

One indirect effect of temperature on indoor air movement has been termed the stack effect. When the indoor temperature exceeds the outdoor temperature, hot air inside the building tends to rise in a column. This creates a positive pressure that forces the air through available openings in the top of the structure and reduces air pressure at the bottom of the building. This reduced pressure draws in colder air from outside to replace the exhausted air, thereby increasing the infiltration of outdoor air. If the air immediately outside the building is contaminated by biologic contaminants, the net effect is to increase the indoor concentration of these contaminants.

High relative humidity promotes the growth of many molds and bacteria by providing an abundance of the required nutrient water. The dust mite, a transparent microscopic arachnid and potent allergen for many people with allergies, grows best in high relative humidity, especially when RH exceeds 70%. In general, maintaining an indoor RH between 35–50% will minimize condensation and indoor dampness, reduce the growth of fungi, dust mites, and bacteria, and provide a reasonable comfort level for the building's occupants.

Wind currents from outside can transport contaminants from long distances and also stir up and aerosolize many biological contaminants that inhabit ground structures and soil. The wind can also increase the pressure gradient between the outdoor and indoor environment, driving the outdoor air's contaminants indoors. Kozak et al. found that Santa Ana winds, brought to Southern California from California's eastern desert, increased the outdoor viable mold spore count from a usual baseline of 1,000–1,500 spore/m^3 to 43,946 spores/m^3. Yardwork such as disturbing compost piles or mowing the lawn can increase outdoor mold counts greater than 1,000-fold and potentially increase the pollen concentration in the outdoor air of plants that are disturbed while pollinating.[18]

Because the outdoor air is a rich source of biologic contaminants, the availability of ports of entry into the building becomes important, especially if a building has little air leak. These tight buildings will protect the indoor air from outdoor contaminants better than those that leak more air or have open windows and doors. Most commercial buildings constructed in the past 20 years were built "tight" and depend on mechanical ventilation to supply fresh air and exhaust stale indoor air. This trend to build energy-conserving tight buildings continues even today. If the heating, ventilating, and air conditioning (HVAC) system does not provide adequate fresh air, air filtration, and exhausting of contaminants, it may actually concentrate outdoor and indoor contaminants inside the building. Also, larger and denser bioaerosols tend to settle out of the air more readily than smaller less dense particles, which tend to remain suspended longer. As a result, indoor environments with better ventilation generally clear the air of small respirable bioaerosols more effectively.

The degree and type of light can affect biologic contaminant growth. Ultraviolet (UV) light inhibits the growth of many bacteria and some molds. Kozak et al. found that marked shade around a house increased the indoor mold spore counts fivefold.[18] Yet, a total absence of light will inhibit the sporulation of some molds, such as *Alternaria* and *Drechslera* species.

Nutrient Sources

Living organisms require sources of nutrients for growth. Finding potential sources of nutrients in the indoor environment and outdoor surrounding areas may help locate the source of biologic contamination. Also, since different microorganisms have different growth requirements, the nature of the discovered nutrient source may suggest certain types of pathogens—for instance, food crumbs suggest the possibility of cockroaches. Virtually all living organisms require water, although some organisms, such as the mold *Penicillium*, require significantly less water than others for growth.

Generally, the most limiting and significant nutrient source is the presence of dead organic matter; however, some molds, many bacteria, and all viruses grow and replicate on or within living substrates. Molds and bacteria typically seed and grow on organic debris found in soil, compost and dung heaps, wood piles, hay, animal feed, and dead plants or leaves. They can also grow on building and finishing materials, especially if there has been wetting or water damage. Paint, wallpaper, carpeting (especially those backed with jute, which is a plant fiber), foam rubber carpet pads, drapery, upholstery fabric and filler, soap scum on bathroom tile and the bathtub, baseboards, hardwood floors, ceiling tiles, cement, exterior and interior wood beams, and framing and roofing material all can provide nutrients sufficient to support the growth of bacteria and fungi. Stagnant water from wells and hot water heaters, humidifiers, vaporizers, and condensate pans of HVAC systems and cooling units (water towers, air-conditioners, and evaporative coolers) can harbor bacteria, fungi, algae, and protozoa. Exposed sound and thermal insulation within walls and ventilation ducts provides an organic substrate for microorganism growth. Also, dust and dirt accumulation within insulation material or within ventilation ducts and other HVAC components add further substrate. Showers, water particle emitters such as vaporizers and humidifiers, and ventilation systems can aerosolize water particles adsorbed with live organisms or pathogenic portions of the organisms. These microbes may be living within the water source or on surfaces over which the water or air currents highly saturated with water vapor droplets move. Alternatively, the water or air currents may dislodge nearby biologic contaminants and sweep them up, disseminating them in the air or water stream (including potable water) a long distance from their nutrient source.

Microbial Sources

Table 2 lists some potential sources of biologic contaminants. Pets can produce moisture accumulation from urination and thereby promote microbial growth, especially if the substrate is of organic origin. Pet excreta provide nutrient sources for bacteria and fungi and contain proteins that can cause immunologic disease; for instance, pigeon and parakeet droppings can produce hypersensitivity pneumonitis, also known as pigeon breeder's disease. Animals excrete fungi that can be pathogenic, such as *Toxoplasma gandii* from cats and *Cryptococcus neoformans* and *Histoplasma capsulatum* from birds. Pets, via their saliva, hair, skin scales (dander), feathers, or urine, spread animal proteins that are allergenic to humans and that may persist for years after removal of the pet. Insects such as dust mites and cockroaches excrete potent allergens in their feces, and *Legionella* may grow within the cysts of amoebae. These cysts may protect this bacterium from the germicidal effects of chlorination of the water source.

Dust mites grow on desquamated human skin scales, found in greatest abundance in bedding materials and bedroom and bathroom carpeting. Mite growth is enhanced when the RH exceeds 55%, and it increases with increasing RH. Keeping potential dust mite sources dry and free of skin scales will minimize dust mite growth.

INDUCTION OF HUMAN ILLNESS BY BIOLOGIC CONTAMINANTS

The ability of airborne particles to reach different parts of a human respiratory tract depends on their size. Large particles, ranging 30–60 μm in diameter,

TABLE 2. Potential Sources of Indoor Air: Biologic Contaminants

Carpets	Bacteria, fungi, protozoa
Plants, animals, birds, humans	Standing water
Pillows, bedding, house dust	Humidifiers, evaporative coolers
Wet or damp materials	Hot water tank

From United States Environmental Protection Agency: Introduction to Indoor Air Quality: A Self-Paced Learning Module. Washington, DC, EPA Office of Air and Radiation, 1991, EPA publication 400/3-91/002.

usually consist of organic and inorganic dirt, fibers, and the larger pollens and mold spores. These are filtered out by the nasal vibrissae. Many pollens, mold spores, hyphal fragments, and smaller inert particles (some containing adsorbed biologic contaminants) ranging 5–20 μm will impact on the nasal mucosa or penetrate further down into the major lower airways—the primary and secondary bronchi. Bacteria, smaller fungal spores, and droplet nuclei generated by talking, coughing, and sneezing make up the group of truly respirable particles, i.e., those reaching the terminal airways, with size ranging 1–5 μm. Particles smaller than 1 μm generally are expelled from human lower airways with exhalation. Although viral particles fall into this last size category, they are usually adsorbed to larger droplet nuclei that remain in the respiratory tract, eventually penetrating the respiratory tract epithelial cells in cases of successful infection.

Biologic contaminants most often disseminate through air currents or water aerosols. Most illnesses of building occupants occur through the contact of these bioaerosols with mucosal surfaces, usually of the respiratory tract and sometimes the eye. The great majority of biologic contaminants cause human illness or discomfort via three mechanisms: (1) infection, (2) intoxication, and (3) immunologic responses. Bacteria, viruses, and some fungi are highly infectious. They enter the human host through the mucous membranes of the respiratory tract, through disrupted areas of skin (atypical mycobacteria), and sometimes through ingestion (*Salmonella*). Infection occasionally is iatrogenic, from the use of contaminated parenteral products such as blood. The microbial load (concentration) in the breathing zones of a building's occupants is an important determinant of the probability and eventual severity of infection, i.e., the greater the load, the greater the probability and severity of infection (see chapter 2). Some of these infectious agents are saprophytes, colonizing but not infecting the host, except when the normal body defense mechanisms are compromised or are overwhelmed by large numbers of infecting organisms. Mold spores that are small enough to enter the lower airways and grow well at 37° C (human body temperature), such as *Aspergillus, Candida, Geotrichium, Scedosporium, Paecilomyces,* and *Scopulariopsis* species, are normally saprophytes and do not cause infection. However, under certain conditions, they can become pathogenic, resulting in infection or hypersensitivity reactions. The desquamated skin of occupants in the building is the predominant source of the bacteria found indoors in normal buildings (*Micrococcus, Staphylococcus*), and our respiratory tract provides a second source of bacteria and viruses.[30]

Some microorganisms infect humans even though humans may not be the natural host, e.g., the anthrax bacillus contracted from the indoor processing of animal products from infected animals and Q-fever, a rickettsial organism usually contracted from infected laboratory animals or in buildings near animal housing.[30] Viruses may be spread by unconventional routes and cause infection: a measles epidemic within an elementary school spread by its HVAC system[25] and rabies contracted by inhalation of rabies virus in a bat cave harboring rabies-infected bats.[7] An unusual situation arose when *Acanthamoeba* infections of the eye occurring in contact lens wearers were linked to the use of tap water contaminated with this organism.[28]

Biologic contaminants rarely cause intoxication. A few molds synthesize mycotoxins as secondary metabolites. These are highly variable complex polypeptides that are generally not volatile and remain associated with fungal structures, including spores, or in the substrate on which the mold is growing. Many genera of fungi can produce mycotoxins, but this toxigenic potential and the likelihood of finding mycotoxins in the presence of these molds are species specific; *Aspergillus flavus, Penicillium viridicatum,* and *Stachybotrys atra* are frequent mycotoxin producers, whereas *A. fumigatus* and *P. chrysogenum* are not. Mycotoxins, when ingested, can produce central nervous system effects (anorexia, nausea, and fatigue), immunosuppression, gastrointestinal lesions, hematopoietic suppression, and suppression of reproductive function.[11] Some, such as the aflatoxin produced by *A. flavus,* are potent carcinogens.[1] Because mycotoxins are present in high concentrations in some mold spores, many health experts share the opinion of the Environmental Protection Agency that "it is reasonable to assume that these toxins have a systemic effect when inhaled, since inhalation more effectively allows entry for dissolved substances."[11] However, to date, despite several reports linking inhaled mycotoxins to human illness, there are few well-documented cases of inhalation-induced human mycotoxicosis. One such case involved a house heavily infested with *S. atra.*[8] In addition to toxins produced by fungi, many gram-negative bacteria produce endotoxin, a potent lipopolysaccharide moiety of the bacterial cell wall. This toxin produces fever, malaise, respiratory distress, peripheral blood leukocytosis with a shift to the left, and shock, which can be fatal. However, similar to mycotoxins, well-defined reports of human intoxication from inhaled endotoxin have been limited to experimentally induced air flow obstruction.[26,27]

Immunogenic substances (immunogens, antigens, allergens) that are integral parts of the structures of various plants, animals, and microorganisms or are released by them into the environment can cause indoor environmentally induced human illness. Immunogens stimulate the immune system to synthesize antibodies and induce lymphocyte and macrophage (cell-mediated) responses that are specific for the immunogen (and often for chemically and structurally related immunogens). Upon subsequent exposure, the body responds to these immunogens aggressively in an attempt to rid itself of the "foreign" substance. Antibodies bind or aggregate particles carrying the immunogen. Then, a cascade of various inflammatory pathways interact to try to kill (if viable) and clear the sensitizing agent from the body. Inflammatory mediators can be released from mast cells and basophils (immediate hypersensitivity or classical allergy). Macrophages and "memory" lymphocytes release cytokines that can activate other cells of the immune system, kill nearby cells, and attract more inflammatory cells (lymphocytes, eosinophils, basophils, macrophages, and natural killer cells) to the area, thereby increasing the inflammatory response. Once the inflammation becomes established, the initial immunogens may no longer be required to perpetuate the inflammatory response, which then becomes nonspecific and may persist for months to years. Infectious agents often cause tissue damage through their ability to act as immunogens, in addition to the direct cytopathic effects they can exert on the infected cells.

Biologic contaminants produce immunologically induced inflammation via all four Gell and Coombs pathologic mechanisms: (1) immediate (IgE-mediated) hypersensitivity or allergy, (2) cytotoxic antibodies against homologous tissue antigens (Group B hemolytic streptococcus causing rheumatic fever), (3) antibody-antigen complex disease (precipitins in hypersensitivity pneumonitis), and (4) delayed-type T cell-mediated immunity (*Mycobacterium tuberculosis*). The distinction is blurred somewhat because immediate hypersensitivity involves immunologically active cells (T and B lymphocytes) in addition to IgE and target cells (mast cells or basophils) that can enhance the inflammatory response; also, antibody responses, including IgE, can be detected against delayed-type hypersensitivity antigens (allergens). Reproductive progeny of plants (pollen) and fungi (spores), the excreta of insects and arachnids (dust mite, cockroach), structural components and byproducts of animals and insects (danders, saliva, urine, feathers, venom), and products produced from some plants (pyrethrins used in insecticides, cotton linters, kapok, and gums) generally induce immediate hypersensitivity responses only in previously sensitized individuals. Bacteria such as *Bacillus subtilis* can synthesize enzymes that stimulate specific IgE synthesis in a small minority of genetically predisposed people who become sensitized to these allergens. There have been a number of reports of occupational asthma in detergent manufacturing workers and in other occupations involving frequent large exposures to *B. subtilis* and other enzymes. The exact role of antiviral (e.g., respiratory syncytial virus) IgE in the pathogenesis of asthma remains unclear. Microbial antigens from fungi, bacteria, and protozoa can induce specific precipitating antibodies (IgG, IgM, and IgA) in susceptible individuals. A role for local immune complexes in the pulmonary parenchyma or airways has been suggested in the pathogenesis of hypersensitivity pneumonitis[3,23,29] and allergic bronchopulmonary *Aspergillosis*.[14] Cytotoxic antibodies may also contribute to the pulmonary parenchyma damage seen in hypersensitivity pneumonitis.[31] Plant resins from poison oak, ivy, and sumac can be spread in the indoor environment from contaminated clothing to the skin of family members. Individuals sensitive to these allergens can develop a contact dermatitis (delayed-type allergic response) to these resins and to other natural plant products, some of which may become airborne, such as ragweed pollen, which may mimic photosensitivity dermatitis or atopic eczema.[2,4]

Some molds[16] and indoor house plants[6] synthesize and release volatile organic compounds (VOCs) into the environment, which can result in a characteristic, sometimes irritating odor, such as the musty odor from mold overgrowth in rotting wood. Whether these VOCs of organic origin pose human health hazards other than the discomfort caused by their odor remains, as yet, undefined.

EVALUATION OF INDOOR ENVIRONMENTS

The evaluation of an indoor environment should begin with a history and description of the environment and the suspected illnesses it may be producing. This can often direct you toward the type or types of biologic contaminants for which to search. Next, a walk-through of the environment to look for potential sources of contamination is essential before undertaking formal testing or sampling, since the latter procedure is often expensive. The five senses are one of the most useful and cost-effective assets for evaluating indoor environments. We can often see, smell, or feel biologic contaminants. The third step, if necessary, that of formal testing or sampling, should be conducted by a qualified expert or team of experts if no obvious sources of contaminants that could account for the illnesses of the occupants have been identified by history and walk-through. Finally, a formal report of the findings *and appropriate conclusions* of the environmental evaluation should be provided to the individuals responsible for managing the environment of the building and the health of its occupants.

The Building's History

The history of the building should particularly focus on prior or current episodes of water damage. Kozak et al. found that recurring spills or leaks were the most common cause of mold growth in residences.[18] Inquire about one-time events that might have released a large amount of water into the indoor environment, such as leaky roofs and broken water pipes. Is the building owner or facilities manager aware of any water accumulation, such as slime accumulation in or inadequate draining of condensate pans beneath cooling coils in the ventilation system? Are there areas of condensation and moisture accumulation in the ventilation ducts, along cooling coils, or on or within walls, flooring, or dropped ceiling spaces? This condition may be caused by building deficiencies, such as inadequate conditioning of air moisture content and temperature, by inadequate thermal insulation of the ducts, or by misapplication of vapor barriers that are designed to keep moisture out of the indoor environment. Misapplying vapor barriers can result in condensation and moisture accumulation somewhere *within* the building envelope. Are there areas where moisture can seep through cement foundations on the ground floor or subterranean spaces and dampen structural elements, carpeting, floor and floor molding, and walls? While the occupants of the building may not identify actual water damage, they may notice "moldy" or other odors (e.g., fecal or sewer gas, hydrogen sulfide, odors) caused by volatile organic compounds released by contaminating mold and bacteria.[15,16]

Finally, review the maintenance of the building with the individuals responsible for this task, regardless of whether it is a residence or a commercial building. For commercial buildings, this review should be done by someone highly experienced with both the design and maintenance of HVAC systems, such as a mechanical engineer or some certified industrial hygienists. What is the design of the system *and* the ducts (review the blueprints)? Is it adequate for its current use, and does it provide sufficient fresh air, conditioning, and filtration of air? Are there attached or unattached water reservoirs, including condensate pans, cooling towers, water sprayers, and humidifiers, that could be contaminated and entrain bioaerosols into the supply air delivered to the breathing zones of the occupants? Has there been any renovation or construction in the past that might disrupt the function or integrity of the HVAC system? How often is the HVAC system inspected and balanced, the filters changed or washed, water reservoirs treated or changed? The types of chemicals used and how they are used to clean or treat the HVAC system, the carpets, the wall coverings and the furniture are all important potential sources of indoor air contamination. Germicidal agents containing formaldehyde, e.g., biocides and cleaning agents, can cause human tissue irritation, inflammation, and, in high concentrations, carcinogenesis.[13]

The Outdoor Environment

What are the characteristics of the outdoor environment in which the building is situated? Are there sources of biologic contaminants nearby, such as animals, grass, weeds, and trees; ongoing construction capable of disturbing the soil; or agricultural areas supporting plant and microbial growth? What is the seasonal likelihood of finding biologic contaminants in the air, including high levels of mold spores during the planting season in agricultural areas or during humid periods in late summer and early fall elsewhere? Consider regional pollen seasons. Are there sources of water, especially stagnant water, or exhaust air from buildings in the proximity?

Characteristics of the Illness

Find out the *who, when,* and *where* regarding people in the building who are ill, and determine *what* types of health complaints exist. Look for common themes or similarities among these health complaints that could point you toward certain classes of biologic contaminants. For instance, an illness complex of fever, myalgias, and headache in a large number of building occupants would suggest the possibility of an outbreak of humidifier fever, or possibly only a virulent strain of adenovirus (common cold). However, symptoms and signs of allergic rhinitis or asthma in only 10–30% of occupants would be quite consistent with the frequency of allergic hypersensitivity in the general population and would direct the search for common allergens. Because hypersensitivity pneumonitis usually affects only a small portion of the exposed population, even fewer individuals might be affected in a building contaminated with mold, bacteria, or protozoa that can cause this disorder. Find out if the people occupying certain portions of the building or sharing a common breathing zone are affected more frequently or severely. This shared breathing space characteristic was a red flag to investigators reporting outbreaks of tuberculosis in a shelter for homeless men[22] and on a U.S. Navy ship.[9] Also remember to consider alternative nonbiologic contaminants, such as chemical toxins or irritants, that can produce signs and symptoms of inflammation, toxicosis, or infection.

In the workplace, one of the major obstacles to obtaining a complete and accurate accounting of all workers who may be adversely affected can be the employer. If the employer or building owner was not the initiating force of your involvement in a potential building-related health problem, as often is the case in workers' compensation or litigation, the representative for the employer may not provide you with a complete list of employees who may be affected. The employer or building owner may be motivated to uncover an existing problem, but those reponsible for building maintenance or engineering may be less cooperative because you may be perceived as a threat to their competence. A list of potentially affected employees also may be incomplete because of lack of reporting by employees or employee health representatives due to fear, ignorance, or honestly not recognizing that the illness may be building-related. You must be sure to conduct your own unrestricted survey of employees in the building and of the building environment, including people who may have left for health reasons before your arrival. In addition, your ability to access the building and collect necessary data may be limited by an uncooperative building owner, employer, or representatives of the employer. Any lack of cooperation adds to the challenge of arriving at a valid conclusion based on factual information about the environmental conditions within the building.

Characteristics of the Health of Affected Individuals

Do any of the occupants have validly documented immediate (IgE) or cell-mediated hypersensitivity? Look for clues in the past and current medical history of affected individuals. What potential allergens have they been or are they currently exposed to, over what period of time, and in what concentrations? Have they had similar or related symptoms in the past and under what conditions? What is the health of family members; do others have allergy or evidence of some other related disorder? Is there a history of radiographically documented recurrent pneumonia, sinusitis, or other infections, especially if an organism has been identified that is normally a saprophyte? It is important to establish the presence or absence of potential biologic contamination in or around the home, even if the illness appears to be work-related.

The home environment may contribute to or be the cause of sensitization, with additional exposure and exacerbation of illness at the workplace; conversely, the current or a prior workplace may have initiated the sensitization process, and the home environment later can become the predominant trigger of the underlying immunologic mechanisms. This sensitization scenario may be particularly relevant for people working with organic grains, vegetable matter, and animals. In residences, people often find that allergic symptoms are most annoying after retiring and especially upon awakening. This is often due to the relatively high frequency of dust mite sensitization, the presence of pets in the house, and the ubiquitous caches for these allergens in the bedroom, including bedding, bedroom and bathroom carpets, upholstered furniture, and drapery. However, depending on the season, the windows may be open at night and permit entry of outdoor biologic contaminants, or the HVAC system may be on at night, disseminating contaminants present in the HVAC system or in the house. Residential heating systems do not usually introduce fresh air, but recirculate indoor air.

Inquiry also must define the current or past presence of pets or animals in or around the building, where they are housed, and if they have restricted access within the building. A past history of animals is important because the allergenic protein components shed by animals have a very long half-life (measured in months to years). Has there been a problem with insect infestation, particularly cockroaches? Are there upholstered furniture, bedding, draperies, or carpets that are more than 1 or 2 years old? How old is the carpeting, and how often and by what method is it cleaned?

The Walk-through

Once the history has been obtained, conduct an initial walk-through of the building. The purpose of this walk-through depends on the complexity of the problem and the building itself. Most disease-producing biologic contaminants in residences are associated with IgE-mediated disorders or with noxious odors. Commercial, industrial, and public buildings present a greater challenge because of the complexity of their air handling systems, the use of many chemical and/or biologic processes, the diversity of surrounding environments, and the large numbers of people often exposed.

Residential Environmental Evaluations

In residential evaluations, the history and illness profile often direct you toward a search for certain allergens in the environment. If symptoms are consistent with IgE-mediated disorders, and even if you have the advantage of knowing the sensitivity profile of the residents from prior testing, the visual search should include all potential biologic contaminants. The irrelevant allergens of today may become relevant tomorrow, since chronic exposure may trigger immunologic sensitization at some future date, especially in someone who has demonstrated the ability to make specific IgE to other allergens. The visual inspection should be thorough, with particular emphasis on contaminant sources suggested by talking to the owner and, if available, by immunologic skin or blood tests for IgE-mediated sensitivity. There is often more than one source of contamination in or around the home.

Be particularly alert for evidence of water accumulation or damage or for machinery or appliances that might contain a water reservoir both inside and outside the home. Table 3 provides a partial list of water or moisture reservoirs that may be found that can promote microbial growth. As described earlier, such reservoirs can both harbor bacteria, fungi, or protozoa as well as provide critical

TABLE 3. Water or Moisture Reservoirs in or around Buildings

Outdoors		
Cooling towers	Swamp coolers	Drainage ditches (e.g., drains)
Small lakes	Agricultural storage	Compost piles
Fountains	Air conditioners	Wooden structures
Poorly kept landscaping	Wood piles	Water softeners
Industrial HVAC exhaust	Under house crawlspaces	Sewer drains
Wells	Potable water storage containers	
Indoors		
Humidifiers	Air conditioners	Crawlspaces
Leaky appliances	Leaky plumbing	Refrigerator pans
Subterranean rooms	Ground level cement slabs and walls	Leaky roofs
Improperly placed vapor barriers	Water damaged carpets, ceiling tiles, walls, furniture	Bathroom showers, tubs, carpet, wallpaper and window coverings
Condensate on windows and cold water pipes	Condensate on ventilation ducts	Condensate on insulation

nutrients for these microorganisms to grow on other organic and inorganic substrates. Mold and bacterial growth may be obvious, appearing as colored fuzzy colonies growing on construction or finishing materials or as slime in accumulations of water, respectively. Evidence of water damage includes finding a musty odor from volatile byproducts of mold and bacterial metabolism, efflorescence (dried powdery deposit of minerals remaining after the water has evaporated) on walls or concrete, and softened wood or wallboard (when poked with a small sharp object). Moisture-damaged wood characteristically breaks out poorly and in short sections when pried up with a sharp implement, unlike the long thin slivers that are hard to chisel out, which are characteristic of dry healthy wood. Dirt and debris may be found in wall and ceiling insulation, within the furnace housing, or within ventilation ducts and on the duct (nonvisible) side of wall or floor registers. These accumulations can harbor mold and bacterial growth. Unfinished basements or crawlspaces beneath the house may contain damp earth or stagnant water. Water stains may be found on carpet, drapery, walls, baseboards, and ceilings, under sinks or refrigerators, or behind dishwashers and washing machines. Sagging doorway arches or floors may be evident. The potential for water damage may be suggested by poorly designed construction of the house: lots that slope toward the house, flat roofs or areas of the roof that represent a nondraining depression, skylights with flashing that does not seal tightly to the roof, cracks in the foundation. The moisture content of the walls, floors, and other building structures can be evaluated with a small sharp probe that can be pushed into the structure in question. Finally, sources of potential indoor and outdoor air disturbance, such as fans and even stand-alone air filtration devices, should be identified.

Nonresidential Building Environmental Evaluations

Nonresidential indoor environments are usually, although not always, of much greater size and scope than residential buildings. They rely on more complex HVAC systems, often have little natural ventilation, have tighter building shells, contain more industrial or office type processes, and are usually located in industrial or commercial parks surrounded by similar buildings. In addition, psychological factors from employee-employee and employee-employer relationships have the potential to play a much greater role in determining the type and extent of occupants' complaints. Consequently, these evaluations often require levels of expertise in areas such as medicine, industrial hygiene, mechanical engineering, and microbiology, which is significantly beyond what is required for the home evaluation.

Visual inspection of nonresidential buildings encompasses all the possibilities mentioned for the residential inspection plus evaluation of additional complexities created particularly by the ventilation system. Sources of water used by the HVAC system and by other industrial processes and moisture or water accumulating within and apart from the HVAC system must be evaluated for contamination. Because the building's occupants usually depend entirely on a combination of recirculated air and, to some degree, dilution with fresh air (often varies with outdoor temperature and time of day), the investigator must search for sources of biologic contamination of both fresh and recirculated air. This means that, outside, you need to determine the location of the fresh air intake grill, upwind potential contaminant sources, and the relationship of the building's exhaust grill to the fresh air intake grill, which could contribute to a reentrainment of exhausted building contaminants. Inside, the HVAC unit(s) needs to be entered, the efficiency and cleanliness of filters assessed, evidence of dirt or microbial growth noted, and the condition of the condensate pan and pan drainage evaluated. Unfortunately, the design of some HVAC systems makes entry into part or all of the HVAC unit difficult or impossible without dismantling parts of it. The inspection should note the hygiene of the duct interior spaces, any breaks in the integrity of the duct system, locations of breaks, and any contaminants or sites for potential contamination in those areas. The space between the ceiling and the floor above often serves as an exhaust and mixing plenum for circulating air. This space also contains many other parts of the building's structure, including insulated or uninsulated pipes and ducts, exposed fiberglass used for insulation, and deposits of dirt and debris from the initial or subsequent construction. Inspection should detect any areas of moisture damage (to ceiling tiles from pipe or roof leaks or condensation), dirt or mold accumulation (on insulation or insulation backing), and any evidence of insect, bird, or rodent infestation. Diffusers or diffuser grills in the ceilings or walls may collect dirt and grow mold and often must be removed for proper inspection.

Environmental Sampling

Assuming environmental conditions have been found that may predispose to biologic contamination or contamination has been identified, recommendations for clean-up and prevention of recurrence of contamination may be sufficient to resolve the health or odor problem. If no source of contamination has been found after a thorough inspection but the situation strongly suggests contamination, sampling for biologic contaminants involving the parts of the building in question may yield an occult source. Because testing can be the most expensive part of a building evaluation, easily exceeding several thousand dollars if evaluating for the presence of VOCs along with biologic agents, the investigator must be selective and sample for contaminants that are consistent with occupants' complaints. In addition, in the absence of overt signs of contamination, further investigation usually involves more aggressive inspection. It may be necessary to dismantle parts of the furnace or ductwork, remove flooring to get to subflooring, and remove portions of walls to evaluate insulation and inaccessible drywall. The decision concerning where to take a more aggressive approach to the inspection should be directed by the location of occupants' health or odor complaints, the design of the ventilation system, and the results of sampling. Also, the decision to undertake environmental testing and more invasive inspection must take into account the severity of the problem, the likelihood of other nonbuilding related causes of the complaints, and the appropriateness of further investigation in the absence of overt signs of contamination. Keep in mind that the indoor and outdoor environments are a dynamic system and can vary greatly depending on the weather, season, and activities occurring both indoors and outdoors.

Sampling generally should include both culture and particle collection methods. Many commonly used sampling methods are listed in Table 4. Some microbes are fastidious and grow poorly or not at all in standard culture media and conditions. Others are nonviable at the time of sampling or are damaged by the sampling process. Alternatively, the contaminant may be a nonviable product or portion of the microbe, such as part of a cell wall (endotoxin) or a metabolic product (mycotoxin). For these reasons, air sampling for culture always underestimates the true bioaerosol concentration. Generally, air samples should be collected outdoors in close proximity to the building's fresh air intakes. These outdoor samples serve as controls. Indoor air samples should be obtained simultaneously or in close temporal proximity to outdoor samples and near suspected contaminant sources, both before and after agitation of each source. Agitation of contaminated sources can produce a 1,000-fold increase in indoor airborne contaminants.[18,21] Ideally, control samples should also be obtained in a comparable building in the vicinity where there are no building complaints. Gravity or settling plates (petri dishes with culture media) have no place in the careful sampling of a building, since they significantly underestimate or fail to detect smaller biologic contaminants, such as mold spores and bacteria that can remain airborne for long times.

Culture plate impactors are most commonly used for bacterial sampling of airborne bacteria, using a general purpose media such as casein soy peptone agar or nutrient agar. These collection devices should not be employed where viable organisms may exceed 10,000 CFU/m^3, although the collection time may be reduced (smaller volumes of air sampled) to prevent overgrowth of the plate. Liquid impingers permit dilution of samples, thereby obviating the problem of overgrowth. Filtration methods frequently damage all but the most hardy bacteria and generally are only useful for bacteria that form spores, such as *Bacillus* and many filamentous bacteria. Specialized media or culture conditions can be used to select for certain types of bacteria. Human source bacteria and pathogenic bacteria grow best at about 35° C (95° F), thermophilic organisms at 50° C (122° F) or more, and many environmental organisms at 25–30° C (77–86° F). These factors dictate that cultures must be incubated as soon as possible, preferably within 24 hours, and in the appropriate nutrient and ambient conditions to isolate the type(s) of biologic contaminants suspected. Source sampling from contaminated water or washings of contaminated material, such as carpeting, are especially important for delicate organisms such as *Legionella*, which may not grow at all from air samples. Fluorescence microscopy also can be used to identify and speciate a number of pathogenic bacteria. Endotoxin can be measured from direct sampling of contaminated water or by air sampling onto solid or liquid media. Currently, the *Limulus* amoebocyte lysate (LAL) test, while not quantitative, provides an estimate of relative toxicity to mammals of the amount of endotoxin collected. This test is difficult to standardize, and comparisons or measurements between laboratories should be made with great caution. More analytic techniques,

TABLE 4. Commonly Used Samplers for Collecting Indoor Bioaerosols

Sampler	Principle of Operation	Sampling Rate, 1 pm	Recommended Sample Time	Minimum CFU Detected	Applications/Remarks
1. Slit to agar impactor	Impaction onto agar on rotating plate or stationary plate	30–700 continuous	Variable; 1–60 min or 7 day		Provides information on aerosol concentrations over time; bulky AC operation
2. Sieve-type impactors					
a. Single-stage portable	Impaction onto agar on "rodac" plate	90 or 185	0.5 or 0.3 min	22 or 16	About 40% as efficient as slit sampler; portable, useful as probe
b. Single-stage impactor	Impaction onto agar, 100-mm plates	28	1 min	35	Nearly as efficient as slit, bulky to handle, AC operation
c. Two-stage impactor	Impaction onto agar, two 100-mm plates	28	1–5 min	35	Same as 2b but divides samples into respirable and non-respirable fractions
3. Filter cassettes	Filtration	1–2	15–60 min or 8 hr	8–33	Some dessication loss; portable, inexpensive, useful as a probe
4. High-volume filtration	Electrostatic collection into liquid	Up to 1000	Variable		
5. All glass impingers	Impingement into liquid	12.5	30 min	3	Fungi require wetting agent; useful over wide range of particle concentrations
6. Centrifugal sampler	Impaction onto agar, plastic strips	40	0.5 min	50	Cannot be calibrated; small, portable, useful as a probe

From United States Environmental Protection Agency: Introduction to Indoor Air Quality: A Self-Paced Learning Module. Washington, DC, EPA, Office of Air and Radiation, 1991, EPA publication 400/3-91/002.

such as high-pressure liquid chromatography (HPLC), currently present problems with extraction efficiency that preclude their routine use for quantitating endotoxin.[1]

For fungal identification, liquid samples, washed bulk samples, and air samples can be plated on culture media such as potato dextrose, Sabouraud's dextrose, or malt extract agar. Malt extract agar has two additional advantages: bacteria do not grow well on it, and it is one of the diagnostic media for *Aspergillus* species.[5] Liquid samples and washings from bulk samples can be diluted before plating. Alternatively, timed volumetric sampling using sieve or slit impactors can be collected over a reduced time interval in areas of suspected high fungal spore concentrations. This will decrease the risk of fungal overgrowth that can prevent accurate evaluation of spore concentrations in such environments. Cultures for fungi generally are incubated at room temperature unless the only concern is *Aspergillus fumigatus*, which is incubated at 45° C. High-volume filtration samplers are used to evaluate airborne antigens or mycotoxins, since large amounts of toxin or antigen are usually required because of the relative lack of sensitivity of the assays for these nonviable biologic contaminants. Table 5 describes the sampling methods used to detect the presence of fungi associated with specific fungal diseases.

Air sampling for viruses is used infrequently, which may explain why viruses have only occasionally been implicated as causative agents in building-related illness. The viruses most commonly associated with airborne transmission are measles, influenza, adenovirus, and varicella. The same techniques used to collect bacteria and fungi for culture can be used to collect viruses. However, viruses tend to be more sensitive to environmental conditions and collection techniques. If delivery to the laboratory may be delayed even 1 hour, the transport media in which the sample has been placed should be refrigerated. Assays for the presence of virus involve either inoculating the material into a susceptible animal species or into tissue culture. The various effects of the virus on the host or host cells can be measured, virus particles in infected material enumerated, or other laboratory methods employed to detect portions of the virus.

Because of the large size of amoebic trophozoites and cysts, the two protozoa species that account for most human disease, *Acanthamoeba* and *Naegleria*, do not travel far on the water droplets that carry them. Sampling should be

TABLE 5. Sampling Modalities for Organisms Associated with Specific Fungal Diseases

Disease	Sampling Modality
Histoplasmosis	Source only; not culturable from air
Aspergillosis	Culture plate sieve or slit impactor with efficient collection to 1 μm
Hypersensitivity pneumonitis (HP)	1. Culture *and* particulate sieve or slit impactors with efficient collection to 1 μm 2. High-volume filtration (where antigen is known)
Allergic asthma and rhinitis	1. Culture *and* particulate impactors with known particle collection efficiency 2. High-volume filtration sampling (where antigen is known)
Toxicosis	1. Culture *and* particulate impactors with known particle collection efficiency 2. High-volume filtration sampler or liquid impinger
Sick building syndrome (SBS)	Air sampling usually not recommended; where necessary, use culture and particulate samplers of known particle collection efficiencies

From American Conference of Governmental Industrial Hygienists: Guidelines for the assessment of bioaerosols in the indoor environment. Cincinnati, ACGIH, 1989; with permission.

performed with high-volume samplers placed close to the suspected source. Samples are grown on nonnutrient agar plates seeded with a lawn of *Escherichia coli* and incubated at 43–45° C (109–113° F). Care must be taken not to contaminate the collecting devices with protozoa from contaminated rinse water. Pathogenic strains of these amoebae tend to be more thermophilic than nonpathogenic strains. If colonies of amoebae are observed, they are inoculated intranasally into weanling mice to test for pathogenicity. If available, fluorescence microscopy using fluorescein-bound monoclonal antibodies also can be used to identify pathogenic strains.

Sampling for nonviable biologic contaminants is frequently necessary, since they are relatively common causes of human illness in indoor environments. Because culture samplers are limited to detecting viable microbes and because nonviable or difficult to culture fungal spores are ubiquitous, air particle impaction sampling for mold spores and sometimes pollens (also nonviable) is essential for a thorough evaluation. Volumetric timed spore traps such as the Samplair or the Burkard (standard and personal) are excellent for sampling both larger and smaller spores and pollens. The rotorod, a rotating sampler, errs significantly in detecting the smaller-diameter mold spores and pollens. These detection devices may identify a number of airborne contaminants that did not grow on the culture plates. They also provide more accurate spore concentrations. Wipe samples of moldy areas or even better, scotch tape sampling (the sticky side of the tape is applied to the growth, then placed sticky side down on a glass microscope slide), are excellent techniques for identifying *growing* mold species. If mycotoxins are suspected, careful speciation is critical, since only certain species of mold are toxigenic. Mold cultured from indoor environmental samples (air or water) may produce measurable mycotoxin in culture. High-volume air samplers generally are required to collect enough airborne mycotoxin for detection by our currently relatively insensitive analytical methods. Complicating mycotoxin measurement, the laboratory techniques that can detect these toxins (gas chromatography/mass spectrophotometry, radioimmunoassay, and HPLC) are not readily available at most commercial laboratories.

Antigen levels in samples such as dust can be measured by in vivo and in vitro techniques: skin testing or inhalation challenges of affected individuals, radioallergosorbent tests (RAST), Ouchterlony double-diffusion, enzyme-linked immunosorbent assay (ELISA), and radioimmunoassay (RIA). For safety and practicality, in vitro tests are the predominant methods used for detecting antigens. In affected people, antibody responses to antigens, such as precipitins to fungal antigens that develop in hypersensitivity pneumonitis (HP), can be assayed by Ouchterlony testing. Even if sampling cannot detect viable or nonviable organisms, this technique can identify specific precipitins from serum of affected individuals against an extract from a suspected contaminated source. Some of these methods are currently in use and commercially available to quantitate levels of dust mite (both *D. farinae* and *D. pteronyssinus*), cockroach, and cat antigens. Such assays are not commercially available for the measurement of most fungal and bacterial antigens in *environmental* samples except to detect antigens associated with thermophilic actinomycetes. To confirm infection with a specific microorganism, most commercial laboratories can assay for *Legionella* antigens in urine, serum IgG (e.g., precipitin) responses to antigens of thermophilic actinomycetes and other causes of HP, and acute and convalescent titers of IgG against various viruses, *Legionella* bacteria, and amoebae.

INTERPRETATION OF RESULTS

The lack of established dose-response relationships between most biologic contaminants and human illnesses remains one of the major obstacles to the development of building standards for these potentially pathogenic substances. Even without such standards, the presence of levels of contaminants associated with valid disease states or the presence of measurable contaminants in excess of what would normally be expected is generally accepted as evidence of contamination. Consequently, to obtain interpretable information for most biologic contaminants, the levels of contaminants found indoors must be compared with the levels found outdoors and, ideally, with other "control" levels from areas within the building where the health of the occupants is not adversely affected. For some biologic contaminants that are generally not found indoors and that are known to cause disease, the presence of any detectable levels is significant. For other ubiquitous contaminants, such as *L. pneumophila* and *Staphylococcus* species, the finding of even elevated levels indoors may have no clinical significance.

Generally, measurable viable bacteria such as *Staphylococcus* and *Micrococcus*, in normal indoor environments originate predominantly from the building's human occupants. These bacteria derive from shed skin particles (dander) and respiratory secretions. Suggested maximum expected total levels of non-pathogenic indoor bacteria are 4,500 CFU/m^3.[1] Excessive quantities of human source bacteria might indicate overcrowding or poor effective ventilation. The presence of any pathogens, such as *Pseudomonas* or thermophilic actinomycetes, or of bacterial endotoxin would be abnormal and indicate contamination. *Legionella* must be present in high concentration in indoor air before it begins to infect occupants, and certain levels in contaminated reservoirs have been associated with outbreaks of *Legionella* pneumonia. A lack of at least a twofold higher level indoors makes the possibility of indoor amplification and dissemination of these bacteria highly unlikely. For endotoxin, levels 100–1,000 times background levels may be associated with disease.[1]

Indoor air fungal spore counts generally remain significantly below outdoor counts in the absence of indoor amplification. Kozak found indoor spore counts were five–ten times lower than outdoor levels in homes and schools in Southern California. But, disturbing a mold source outside could elevate spore counts to 100–1,000 times baseline values. Comparing homes with mold problems and those without, he found that homes without mold problems usually had fewer than 1,000 total mold spores/m^3.[18] In order of descending frequency, the most prevalent fungal spores were *Cladosporium* (all homes), *Penicillium* (91% of homes), nonsporulating mycelia (90%), and *Alternaria* (87%).[19] Generally, indoor mold spore counts should be no more than 50% of the outdoor counts in a structure free of mold contamination.[1]

Because mycotoxins are a part of the fungal cell wall, they can persist long after the cell is no longer viable. Therefore, the inability to grow toxigenic fungi in culture does not rule out the presence of mycotoxins. Some toxigenic species may only produce mycotoxins under certain nutrient and ambient conditions; consequently, the absence of measurable mycotoxins in cultured fungi does not eliminate the possibility of airborne mycotoxins. Conversely, the ability to isolate mycotoxins from mold cultures of sampled air or water does not guarantee that these fungi were producing mycotoxins in the indoor environment from which they were sampled. Also, some highly toxigenic fungi, such as *Stachybotrys atra*, cannot compete in culture with other common fungi like *Penicillium*. When the culture grows out and is read, few if any toxigenic fungal colonies may be found. Because of the complexity of measuring mycotoxins, an experienced mycologist should be consulted if mycotoxins are suspected.

Viruses typically cause well-defined illnesses characteristic of the pathogenicity of the infecting virus. Since most building-associated illnesses associated with the suspicion of a "sick" building are not characteristic of an obvious viral illness, sampling for viruses in indoor environments is rare. However, as little as one virion may be sufficient to cause infection. If a viral agent is suspected, sampling should be performed for that virus. Because viruses are obligate parasites and one would expect human occupants to be agents of amplification and dissemination, outdoor controls may not be necessary.

Finding protozoa indoors without finding them in control samples strongly suggests contamination. There are no normal background levels of pathogenic protozoa that one would expect in uncontaminated indoor environments.

Guidelines for nonviable antigen environmental loads have been established only for dust mites:[20,24]

< 2 μg *Der p I*/g or *Der f I*/g of dust = low,
2–10 μg/g dust = significant,
> 10 μg/g dust = high.

Suggested levels for the predominant allergen in cats, *Fel d I*,[10] are:

< 1 μg *Fel d I*/g dust = low
1–8 μg/g dust = moderate
> 8 μg/g dust = high.

Wood et al. found that levels of *Fel d I* as little as 2 μg/g of dust may be a risk factor for sensitization.[32] Because of the potential morbidity of hypersensitivity pneumonitis and because sensitized individuals react to extremely small amounts of the antigen, any levels of HP-associated antigens indoors would be unacceptable for people with this disorder. Although higher levels of antigen than for HP may be necessary to trigger symptoms in individuals with allergic asthma and rhinitis, very sensitive individuals with these common allergic disorders may become symptomatic upon exposure to low concentrations of biologic contaminants to which they are sensitive. Also, since allergens such as pollens and mold spores that cause typical IgE-mediated disorders are usually ubiquitous and established safe levels for these common biologic contaminants do not exist, we tend to accept background levels in uncontaminated buildings as safe. Given what we know about allergic sensitivity, "acceptable" background levels may be too high for a sensitive individual.

Relating Sampling Information to Human Illness

Some of the major questions that must be thoroughly asked, answered, and understood about the results of the environmental evaluation before beginning to relate them to human health issues are:

- Were the sampling assays selected appropriate for the environmental conditions and for the adverse health effects in question?
- Was the sampling done properly and with the proper controls?
- Did the technician and laboratory process the samples correctly?
- What is the sensitivity of the assay?
- What is the specificity of the assay?

Assuming all of the above issues were addressed adequately and the results of testing are considered valid, the next step, perhaps the most difficult one, is to relate the test results to the adverse health issues. The laboratory findings of biologic contamination must support the characteristics of the illness profile to have positive significance: for instance, the environmental presence of *Micropolyspora faeni* or *T. vulgaris* together with affected individuals with symptoms and signs of HP possessing serum precipitins to *M. faeni* or *T. vulgaris*. Similarly, findings of large amounts of dust mite antigen or mold spores to which one or more individuals affected with asthma or rhinitis symptoms show positive skin tests strongly suggests, but does not prove, that their indoor environment is contributing to their illness. Yet, finding small or even large amounts of *Legionella pneumophila* bacteria in a water reservoir or even in the air does not necessarily mean that individuals with pneumonia are infected with this bacterium, since large concentrations of this organism are only one criterion required to establish the diagnosis of *Legionella*-induced human disease. However, if a rising serum antibody response to *L. pneumophila* or any bacteria-specific antigen in the urine is found, causality can be established. Another common error is to attribute infectious illnesses in a building to high levels of human skin bacterial counts found on sampling. These findings may simply indicate poor effective ventilation or overcrowding, which may or may not contribute to occupants' complaints. Because of the complex interaction among (1) the nature and concentration of ubiquitous biologic contaminants, (2) the ability of the indoor environment to amplify, disseminate, and sometimes concentrate these contaminants, and (3) host (building occupant) susceptibility, exposure, and physiologic or pathologic responses to these contaminants, drawing valid conclusions about cause and effect can be problematic.

DECONTAMINATION AND PREVENTION

If contamination already exists, the causes of the contamination must be addressed. Building and mechanical systems design should minimize the infiltration of outdoor contaminants and provide adequate amounts of fresh air to dilute indoor air contaminants. Someone must assure that the settings and thermal controls of the HVAC system provide adequate ventilation with *fresh* air (standards now dictate a minimum of 15–20 cubic feet per minute per occupant in normal environments without excessive sources of contamination) *under all circumstances while the building is occupied.*[1a] Nutrient sources for biocontamiants should be minimized, and existing reservoirs must be removed. The HVAC system may require simple corrective action, such as replacing the current filters with more efficient ones during regular servicing of the unit(s) to maintain cleanliness, or more drastic measures may be necessary, such as replacing damaged or substandard ducts, moving air intake or exhaust grills, or even redesigning and replacing the entire HVAC system. Indoor and outdoor vegetation may need to be changed or maintained better to prevent accumulation of dead organic debris, an excellent nutrient source for biologic contaminants. Sources of moisture accumulation and leaks must be dealt with effectively, and *all* contaminated materials removed.

The relative humidity, if excessive, can be reduced with central or peripheral dehumidifiers; if too low, judicious use of humidifiers that do not use recirculated water may solve the problem. Office building humidifiers should be run through the central HVAC system, and heated "clean" steam can be added to the air. A source of potable water should be used with cold water humidifiers and removed once the water passes through the humidifier. Home humidifiers should use distilled or sterile water rather than tap water. The water should be changed daily and the device cleaned every 3 days with 5% chlorine bleach or hydrogen peroxide. The target range for relative humidity should be 35–50%. Water-damaged material, usually defined as wetting for longer than 24–48 hours without thorough drying, needs to be removed. If the water was contaminated, as occurs with sewage leaks, the material should be discarded regardless of how long it was wet.

The effectiveness of high-efficiency filters, such as high-efficiency particulate air (HEPA), electrostatic, or electronic (electrostatic precipitators) filters, in ameliorating clinical symptoms of allergy remains controversial. There is little doubt that these types of filters are able to remove 99.999% of airborne particulates that pass through them. However, their clinical effectiveness will be reduced in the face of continuing contaminating sources, such as an old carpet, mattress, or pillows. Carpeting should be vacuumed at least weekly to keep surface dirt to a minimum. Vacuum cleaners are available that minimize recontamination of the air by vacuumed dust leaking out of the system and collection bag, a common occurrence with most vacuum cleaners. If rugs are desired in the environment of someone sensitive to dust mite or mold, area rugs that can be sent out for periodic thorough cleaning should be used. Carpets, if present, should be cleaned at regular intervals with only modest amounts of steam (not chemicals, which leave a residue) and then completely dried for 24 hours with fans and heat. Successful execution of a regular comprehensive program designed to maintain a clean environment is foremost in minimizing biologic contamination.

The use of biocides to kill living biologic contaminants also is controversial. Well-defined human adverse effect profiles have been established for the active components of biocides, e.g., formaldehyde (formalin), hypochlorites, and phenols. Their ability to cause irritant, toxic, and even carcinogenic effects may be enhanced in susceptible individuals. Their use in ventilation or humidifier systems clearly results in dissemination of these chemicals throughout the indoor environment. Consequently, if biocides are used to clean or to minimize microbial growth in this manner, great care must be exercised to minimize contamination, and the risk must be weighted against the potential benefit. There is no established safe and effective biocide. The Environmental Protection Agency does register biocides and approves their use for certain indications, such as in cooling towers, where the anticipated levels of biocide indoors are small and the expected benefit great. Hydrogen peroxide in a 3–6% solution is one of the safest biocides available. However, one must keep in mind that the choice of biocide depends on what the user is trying to accomplish, because different biocides have different killing capacities and varying limitations.

IMPLICATIONS OF BIOLOGIC CONTAMINATION

Because biologic contaminants exist to varying degrees in every indoor environment, the potential for producing human illness is always present. The conversion from having the potential to induce illness to actually producing illness depends on a number of factors:

- The availability of a source of biologic contamination: a given, because all indoor environments contain a number of different kinds of viable organisms.
- The nature of the contaminants: their individual or collective abilities (considering synergy) to cause illness.

- The nature of the indoor environment: does it provide sufficient nutrients and ambient conditions to amplify pathogens; does it possess the mechanical or natural means to disseminate the organisms, their parts, or byproducts that can cause adverse health effects in the building occupants? How well does it clear various biocontaminants? Ultimately, these factors determine the concentration of biocontaminants that enter the breathing or body zone of a building's occupants.
- The genetic make-up, current health, and other relevant characteristics, such as age, of the building occupants. Certain groups, such as the very young, the elderly, and the infirm are often more susceptible to biologic contaminants.

Although we possess a solid understanding of many of the infectious and immunologic complications caused by biologic contaminants, much remains unclear about the ability of microbial products such as endotoxin or mycotoxin to induce human illness. We also lack essential knowledge of the dose-response relationships between many biologic contaminants and the human host that would permit the establishment of indoor environmental standards for these agents. This void exists for most toxic, carcinogenic, and many immunologic reactions to inhaled biologic contaminants.

Some of our ignorance stems from the complexity of the problem. Yet, some derives from our lack of attention to well understood nonmedical aspects of the problem that have great impact: the presence or absence of adequate *effective* ventilation; the hygienic aspects of the structural, ventilation, and design components of the building; the cleanliness of indoor and outdoor air; ambient indoor and outdoor conditions; and the degree of effective execution of building maintenance plans designed to minimize biologic contamination. These are only some examples of many building-related functions that affect the health of the occupants that we *do* have the knowledge to execute or correct but are often ignored. Finally, over the past 20 years funding for research to clarify these issues has paled in comparison to that for outdoor air pollution research.[17]

Do the fall-winter epidemics of respiratory viruses need to be as extensive as they always are? Couldn't we reduce their impact on human health by correcting deficiencies in effective ventilation and overcrowding in office buildings, schools, and hospitals? By reducing the indoor allergen load exposure to the 20–30% of the U.S. population that has atopic disease, couldn't we improve their health and reduce their susceptibility to respiratory tract infections? If builders, architects, and designers considered the health impact of their choice of mechanical systems, building and finishing materials, and the sequence and methods of construction or cleaning, wouldn't we stand a better chance of spending the 90% or more of our lives that we spend indoors in a healthier environment?

The problem of biologic contaminants can only be served well by a multidisciplinary team of health care professionals, engineers, building professionals, and, often, industrial hygienists and biologists who have broadened their scope of understanding about how the indoor environment affects the health of its occupants. Figure 1 can serve as a guide for conducting a building investigation for environmental contamination. Input from experts in several fields is often necessary at each junction of the evaluation process to define the problem accurately and correct any deficiencies. This strategy can be quite effective in the short-term. However, the major thrust of emphasis in construction, renovation, and building maintenance must shift from an almost exclusively reactive to a

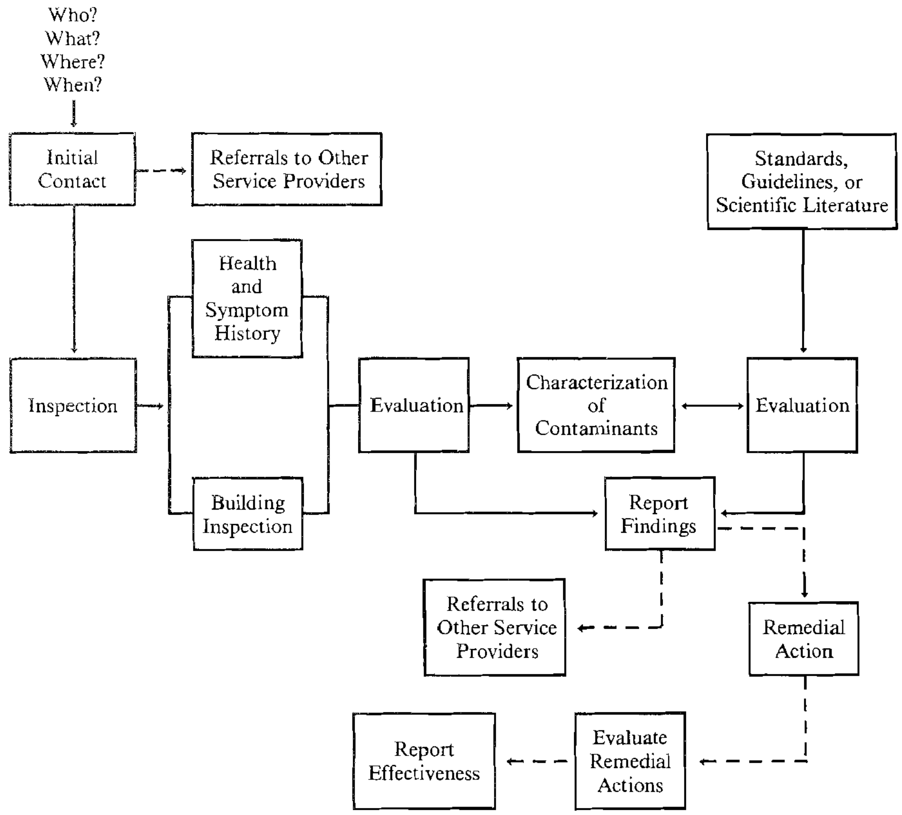

FIGURE 1. Flowchart of an investigation strategy for indoor air quality problems. (From United States Environmental Protection Agency: Introduction to Indoor Air Quality: A Self-Paced Learning Module. Washington, DC, EPA Office of Air and Radiation, 1991, EPA publication 400/3-91/002.)

proactive approach if we are to make a significant impact on the amount of indoor biologic contamination in both currently occupied and future buildings. To avoid the mistakes of the past and to create healthier and more productive environments for our employees, our patients, and our families, we need to build *all* homes and commercial buildings with an emphasis on providing a hygienic environment for the occupants at least equal to that placed on priorities such as energy, aesthetics, and structural integrity.

REFERENCES

1. American Conference of Governmental Industrial Hygienists: Guidelines for the assessment of bioaerosols in the indoor environment. Cincinnati, ACGIH, 1989.
1a. American Society of Heating, Refrigerating and Air Conditioning Engineers: Standard 62-89. Ventilation for Acceptable Indoor Air Quality. Atlanta, ASHRAE, 1989.
2. Arlette J, Mitchel JC: Compositae dermatitis. Contact Dermatitis 7:129–136, 1981.
3. Barrowcliff DF, Arbruster PG: Farmer's lung: A study of an early acute fatal case. Thorax 23:490–500, 1968.
4. Benezra C, Ducombs G, Sell Y, Fousserau J (eds): Plant Contact Dermatitis. New York, Dekker, 1985.

5. Burge HP, Solomon WR, Boise JR: Comparative merits of eight popular media in aerometric studies of fungi. J Allergy Clin Immunol 60:199–203, 1977.
6. Can house plants solve IAQ problems? Indoor Air Bull 2(2):1–5, 1992.
7. Constantine DG: Rabies transmission by air in bat caves [monograph]. Atlanta, Centers for Disease Control, 1967, PHS publication 1617.
8. Croft WA, Jarvis BB, Yatawara CS: Airborne outbreak of trichothecene toxicosis. Atmos Environ 20:549–552, 1986.
9. DiStasio AJ II, Trump DH: The investigation of a tuberculosis outbreak in the closed environment of a US Navy ship, 1987. Mil Med 155:347–351, 1990.
10. Dust Mite Task Force, International Association of Allergy and Immunology: Dust mite allergens and asthma—a worldwide problem. J Allergy Clin Immunol 83:416–427, 1989.
11. Environmental Protection Agency: Non-cancer health and discomfort effects of poor indoor air quality. In Report to Congress on Indoor Air Quality, Vol 2. Washington, DC, EPA, Office of Air and Radiation, 1989, EPA publication 400/1-89/001C.
12. Environmental Protection Agency: Report to Congress on indoor air quality; executive summary and recommendations. Washington, DC, EPA, Office of Air and Radiation, 1989, EPA publication 400/1-89/001A.
13. Environmental Protection Agency: Source and health effects of selected contaminants. In Introduction to Indoor Air Quality: A Reference Manual. Washington, DC, EPA, Office of Air and Radiation, 1991, pp 47–114, EPA publication 400/3-91/003.
14. Geha RS: Circulating immune complexes and activation of the complement sequence in acute allergic bronchopulmonary aspergillosis. J Allergy Clin Immunol 60:357–359, 1977.
15. Gerber NN: Odorous substances from Actinomycetes. Dev Ind Microbiol 20:225–238, 1979.
16. Kaminski E, Stawicki S, Wasowicz E: Volatile flavor compounds produced by molds of Aspergillus, Penicillium, and Fungi imperfecti. Appl Microbiol 27:1001 1004, 1974.
17. Kennedy JP: The Indoor Air Quality Act of 1991, extensions of remarks. Congressional Record E592, February 22, 1991.
18. Kozak PP Jr, Cummins LJ, Gillman SA: Endogenous mold exposure: Environmental risk to atopic and nonatopic patients. In Gammage RB, Kaye SV (eds): Indoor Air and Human Health. Chelsea, MI, Lewis Publishers, 1985, pp 149–170.
19. Kozak PP, Gallup J, Cummins LH, Gillman SA: Factors of importance in determining the prevalence of indoor molds. Ann Allergy 43:88–94, 1979.
20. Luczynska CM, Li Y, Chapman MD, Platts-Mills TAE: Measurements using cascade impactor, liquid impinger, and a two site monoclonal antibody assay for Fel d I. Am Rev Respir Dis 361–367, 1990.
21. Morey PR: Microorganisms in buildings and HVAC systems: A summary of 21 environmental studies. In Proceedings of the ASHRAE Conference IAQ 88. Atlanta, American Society of Heating, Refrigerating, and Air-Conditioning Engineers, 1988, pp 10–21.
22. Nolan CM, Elarth AM, Barr H, et al: An outbreak of tuberculosis in a shelter for homeless men: A description of its evolution and control. Am Rev Respir Dis 143:257–261, 1991.
23. Pepys J: Extrinsic allergic alveolitis. Monogr Allergy 4:69–131, 1969.
24. Platts-Mills TAE, Heymann PW, Chapman MD, et al: Mites of the genus Dermatophagoides in dust from the houses of asthmatic and other allergic patients in North America: Development of a radioimmunoassay for allergen produced by D. farinae and/or D. pteronyssinus. Int Arch Allergy Appl Immunol 77:163–165, 1977.
25. Riley EC, Murphy G, Riley RL: Airborne spread of measles in a suburban elementary school. Am J Epidemiol 107:421–432, 1978.
26. Rylander R, Haglind P: Airborne endotoxins and humidifier disease. Clin Allergy 14:109–112, 1984.
27. Rylander R, Haglind P, Lundholm M: Endotoxin in cotton dust and respiratory function decrement among cotton workers in an experimental cardroom. Am Rev Respir Dis 131:209–213, 1985.
28. Seal D, Stapleton F, Dart J: Possible environmental sources of Acanthamoeba spp in contact lens wearers. Br J Ophthalmol 76:424–427, 1992.
29. Spector WG, Heesom W: The production of granulomata by antigen antibody complexes. J Pathol 98:31–39, 1969.
30. Spendlove JC, Fannin KF: Source, significance, and control of indoor microbial aerosols: Human health aspects. Public Health Rep 98:229–244, 1983.
31. Wenzel FJ, Emanuel DA, Gary RL: Immunofluorescent studies in patients with farmer's lung. J Allergy Clin Immunol 48:224–229, 1971.
32. Wood RA, Eggleston PA, Lind L, et al: Antigenic analysis of household dust samples. Am Rev Respir Dis 137:358–363, 1988.