Sampling of the Environment for Biologic Materials

The "sick building syndrome" and indoor air quality is receiving a great deal of attention in the media. A desire to improve heating/cooling efficiency in high rise buildings has led to a reduction in the amount of outdoor air brought in for circulation. Complaints reported by workers in these structures may be attributed to actual conditions, but may also be related to other factors totally unrelated to the work environment. To establish a cause and effect relationship to conditions in a particular building, detailed studies are sometimes required to determine how the indoor environment differs from the outdoors and from other buildings where none of the workers experience unusual symptoms.

Generally, materials suspected or proven to be associated with the sick building syndrome fall into two general categories. One relates to the chemical composition of the work environment; the second has to do with the biological environment, represented mainly by mold spores and bacteria. A worker's response to the work environment may also reflect a preexisting condition the worker has had prior to employment in the "sick building." As an example, a worker having previously become sensitized (allergic) to a particular mold spore could be exposed to that same mold growing in the work environment and become ill. If the problem is recognized and corrected, the worker's symptoms should clear. Since allergies affect a relatively small population, only limited numbers of people would be affected.

Normally, volatile chemicals such as formaldehyde are not found in our environment unless they have been introduced into that environment as a consequence of a manufacturing process or were accidentally discharged into the area. Since these materials are not normally found in the environment, their presence in anything above trace quantities can be assumed to be associated with the work area, work materials or the result of off-gassing (emissions) from the materials used in the construction or furnishing of the building.

Biological materials are more difficult to evaluate. Biological materials, including fungal spores and bacteria, are a normal part of our environment, both indoors and out. Many misconceptions regarding techniques for performing scientific measurements of biological materials exist in the general public and even among many health care professionals. Association of these materials with disease is possible only when one can demonstrate the presence of a particular organism previously associated with that disease, in numbers sufficient to cause symptoms/infection. Influx of bacteria and mold spores from the outdoors occurs in all structures. The amount of influx will depend to some degree on the structure itself, the ventilation system (heating/cooling/humidification), and the presence of filtering systems.

In addition to the normal influx of outdoor organisms into a building, additional organisms can grow within the building. This occurs when there is just the right combination of factors, including the proper temperature, humidity and material on which the organisms can grow. Some of these conditions are inherent to the building structure while others are related to the furnishing of the building itself. As an example, the bacteria associated with Legionnaires' disease could be growing in the water in a high-rise cooling tower, while *Aspergillus* mold spores could be growing on a wicker basket on a worker's desk. The first problem would represent a maintenance problem with the building, while the second could be due to careless over watering of a plant with resultant mold growth occurring on the wicker material.

TECHNIQUES USED TO STUDY BIOLOGICAL MATERIALS

When the mere presence of an organism in a building is required to prove a causal relationship

with a disease, any number of simple sampling techniques can be used. These techniques could include obtaining swabs from suspected items or utilizing open culture plate (also called gravity plate) collection. Unfortunately, these techniques for studying the environment have many limitations and are of no scientific value when comparisons are made between different buildings, between the building and the workers' normal home environment or between the building and the outdoors.

The open culture or gravity plate collection is especially likely to provide misleading information. Many factors can influence which spores will be collected, including air currents, temperature, humidity, size and weight of the airborne spores. Larger spores may be routinely overestimated using this collection method while the smaller and lighter spores may not even be collected.

Scientific comparisons are possible only when measured volumes of air are sampled and when results of surveys are expressed in terms of such volumetric measurements. The number of isolates of a particular organism per cubic meter of air has meaning. Under some conditions, one could, theoretically, duplicate this exposure in a controlled laboratory and determine if the same complaints are reproduced. This type of challenge study is especially suitable for diseases that are due to allergic mechanisms such as asthma. Some bacteria and fungal spores can cause disease only when they are living (viable). Others are capable of producing illness even when no longer living. For example, mold spores are antigenic (can cause allergy) long after they have lost viability. Ideally, to adequately sample the biological materials in any environment one must use a combination of viable and non-viable sampling methods. In the past, the most commonly used volumetric instrument for viable organisms was the six stage Andersen sampler. Measured volumes of air were drawn through a series of plates, each containing a petri dish with growth media. Presently, most people use the Andersen N6, an instrument containing only the bottom stage of the six stage sampler. Viable spores that impact on this single plate are then allowed to incubate. Depending on the characteristics of the various organisms, growth can usually be observed within 3 to 6 days. When data from these studies is compared to a simultaneous sampling of the out-doors, one can determine if the indoor air flora represents normal influx of outdoor organisms or if there is a possible indoor problem. Under normal conditions the same relative distribution of spore types is found in the indoor and outdoor environments with the indoor concentrations averaging 10 to 80% of the outdoors at the time of sampling. Several non-viable sampling techniques can be used. Ideally, the instrument chosen should be capable of giving volumetric data. However, if no such instrument is available, the presence of reservoirs of spores which may be non-viable may be confirmed using various simple and inexpensive techniques described below. One volumetric non-viable sampler used commonly by allergists is the rotorod impaction sampler. The collection surface of this instrument is a Vaseline coated small plastic rod. These rods are placed in a special holder attached to a motor. When activated, the rods spin and particles in the air impact on the coated plastic rods. After a timed period of sampling, the rods are removed and examined microscopically. Many mold spores have unique morphology and are identifiable by direct microscopic observation. Others lack distinguishing characteristics so that identification is difficult. These latter spore types are counted into broader groups. The aerodynamics of the rotorod are poor and only larger particles are picked up by this instrument. The instrument increasingly used for non-viable spore collection is the Burkard spore trap, an impaction sampler with excellent aerodynamic characteristics. The collection surface of this instrument is a coated glass slide. As with the rotorod, counts and identifications are made by microscopic examination. The Allergenco MK-3 instrument is yet another spore trap which operates on the same principle as the Burkard. Now available are Zefon Air-O-Cell cassettes, which function like a Burkard/Allergenco slide but which are disposable, making volumetric sampling possible for those without expensive instruments.

Other simple techniques are available to supplement Andersen and spore trap sampling. One technique involves the use of clear tape, which may be pressed against suspected surfaces, and then

pressed onto glass slides. These slides are examined under the microscope to determine whether or not active or invasive mold growth is taking place, or if spores present are simply the result of normal fallout from outside sources. Bulk samples are handled much the same way, in that portions of samples are examined directly under the microscope. There are some situations in which culturing environmental samples is helpful. Generally, however, culturing of bulk samples is discouraged since cultures can provide misleading information. Often, if the problem is long-standing, the predominating spore types present may have lost viability and will not germinate. (These spores still remain antigenic to allergic persons.) And, culturing will reveal different spore types, present from normal trapping. Thus, direct examination under the microscope will provide the most accurate information. In the same regard, swabbing surfaces for culture may provide misleading information since spore types differ greatly in regard to growth on laboratory media, and because all surfaces may trap spores which are a part of normal air flora.

Ideally, to obtain the maximum amount of scientific information regarding the types and concentrations of mold spores and bacteria present and to determine their likely origin, one should have Andersen studies in areas of the indoors suspected of having a problem. An outdoor Andersen sample is necessary in order to interpret the indoor results. An additional Andersen study in an area considered free of problems is also helpful as an indoor control. Non-viable samples (Burkard/Allergenco/Zefon Air-O-Cell) conducted simultaneously with all Andersen samples is ideal, but not always possible, although the new Zefon Air-O-Cell cassettes are making the addition of non-viable sampling easier than ever. If a volumetric non-viable method is not available, other techniques such as tape lift sampling may be employed to provide non viable data regarding potential reservoirs of spores.

Each sampling method has limitations and drawbacks. However, information gathered with a combination of many of the described techniques can result in a valid scientific evaluation of the micro-biological flora in indoor locations.