DISCUSSION

How do you interpret your results (e.g., from the Andersen sampler) when you have 2 very different plate count results? What guidelines do you follow to do your interpretation?

CLOSURE

If one plate is blank, the other with many (>10 colonies), we use only the positive plate. If both plates have reasonable numbers of colonies, we use either an average or a range depending on the type of investigation.

DISCUSSION

In a recent American Industrial Hygiene Conference in St. Louis, a Canadian investigator stated that 50 or more Stachybotrys per cubic meter or 300 or more total fungi per meter cube is a concern level. Please comment.

CLOSURE

Fifty or more Stachybotrys/m^3 would prompt me to look for a source of Stachybotrys in the environment. In normally ventilated homes, 300 cfu total fungi/m^3 cannot be considered unusual. I do not feel an overall concern level can be set for total fungi. The environment, the population at risk, and the sampling modality must be a part of any guideline.

DISCUSSION

Are there problems in comparing outside sample fungi results vs indoor findings? (i.e.: varying normal flora etc)? How do you compare the results?

CLOSURE

Significant problems arise. 1) Sampler efficiency may change depending on wind speed. 2) The air spora may be differently (spatially) distributed indoors vs outdoors 3) Usually, generic identifications are used for such comparisons. Unless only one species occurs, such comparisons are not valid. We compare total spore counts indoors and out (assuming that total fungus exposure is meaningful with respect to disease). When looking at particular types of fungi (e.g., Penicillium) we identify each isolate to species.

DISCUSSION

Have you had any experience with the Biotest Centrifugal Air sampler?

Can the RCS centrifugal sampler be used successfully?

CLOSURE

Yes. It is a portable sampler that is useful especially for monitoring bacterial levels in places where larger samplers are inconvenient. However, 1) it cannot be field calibrated and the manufacturers' stated "effective volume" is apparently inaccurate; 2) the agar strips are not available with malt extract agar; 3) fungi rapidly fill the strips making enumeration difficult. Note that a new model that can be calibrated is currently being tested.

DISCUSSION

What about the "nut" in the building who is [not] really getting ill from molds; how do you determine the "nut" from the sick individual who is really ill because of the exposure?

CLOSURE

Sometimes you can't make the distinction. However, most illness that is clearly related to fungus exposure produces an immune response (either IgE or IgG) that can be detected serologically. Irritant or toxic symptoms should be clearly related to building occupancy. Often the unrelated cases will have a long history of similar problems in unrelated environments.

DISCUSSION

Do you recommend checking the calibration on cascade impactors for calculated versus actual size separation? Methods? Do you ever make mass measurements from impactor stages? If so, for what application?

CLOSURE

We do very little with size separation. Most fungus spores apparently travel as units and the known spore size tells us the aerosol size. Also, allergens are effective over a very large size range. We occasionally use the 6-stage Andersen, but trust the extensive characterization work done on this sampler by the Defense Department. We <u>always</u> calibrate the flow rate through the device. We have not made mass measurements from impactor stages.

DISCUSSION

Could you elaborate on the significance of non-viable proteinaceous fragments in onset of allergic response, especially in atopic and/or sensitized individuals? How do you identify these?

CLOSURE

Antigen fragments are an effective means of inducing symptoms in an appropriately sensitized subject. The only way to evaluate the concentrations of such particles is by immunoassay (e.g., RAST, ELISA, Immunoblotting).

DISCUSSION

Please elaborate on thermophilic actinomycetes sampling/analysis.

CLOSURE

Thermophilic actinomycetes can be assessed by culture plate sampling using a sampler (such as the Andersen) that efficiently collects 1um spores. TSA or nutrient agars are acceptable; they must be incubated at ~56°C. If appropriate antibodies are available, these antigenic units can be assessed by filtration sampling followed by immunoassay.

DISCUSSION

If rose bengal agar needs to be incubated in the dark, isn't this medium ineffective for identifying fungi that require light for spore production?

CLOSURE

Yes.

DISCUSSION

When you have enumerated fungal spores on sticky slides, do the numbers agree with colony forming units? Are there "non-culturable fungi"?

CLOSURE

There is usually a positive and significant correlation between spore trap and suction culture plate samples. However, culture plate sampling always estimates actual levels for culturable taxa because all airborne spores are never alive, and many are stressed and do not produce colonies in artificial culture. In addition, many spores do not germinate or grow in artificial culture and are not recovered by culture plate sampling. Most of the mushroom spores and all of the obligate plant pathogens fall into this category. Mushroom spores, by the way, are highly allergenic.

DISCUSSION

How much will speciation of all fungi (as you suggested should be done) increase the cost of analysis and time required for analysis of volumetric bioaerosol samples?

CLOSURE

Speciating all fungi would be ideal but impossible. Some fungi are relatively easy to speciate (e.g., Aspergillus). Others, including the common genus Penicillium are very difficult and expensive. I estimate that it costs us at least $150 per isolate to identify most Penicillium species, and it takes a minimum of 7 days to do the necessary cultures. With limited staff, it would probably double the time required for analysis.

DISCUSSION

What biocides are appropriate for use in HVAC systems while buildings are occupied?

CLOSURE

None can be used in an operating system so that occupants are at risk for exposure to the biocide.

DISCUSSION

What modifications (if any) can be made to the RCS/SAS samplers to improve the ~40% collection efficiency relative to the Andersen for viable organisms?

CLOSURE

The relative efficiency for the RCS is not known. For the SAS, decreasing the diameter of the sieve plate holes appears to increase efficiency, although it lowers sampling rate.

DISCUSSION

Is the reduced collection efficiency of the Burkard (indoor) for 5um particles due to the "relatively" low sample flow rate or some characteristic of the sampling head?

CLOSURE

It is probably due to the width of the slit.

DISCUSSION

Is there any concern for "sample shock" or "organism shock" when utilizing the high flow rates of the RCS/SAS for viable organisms?

CLOSURE

The actual speed with which the organisms strike the agar surface is not greater than that for the Andersen, especially if you use the sixth stage. The very small hole size causes the velocity of the air stream to be quite high. Probably some fragile organisms are killed or damaged by the impact.

DISCUSSION

Under what conditions is "aggressive" sampling, i.e., pounding on ductwork or fan unit filters, recommended, and is this practice not supporting artificial recoveries if the aggressive activity does [not] coincide with some similar workplace event, i.e., maintenance?

CLOSURE

Aggressive sampling constitute a "worst-case" situation, and can be very valuable in determining whether or not a particular reservoir/amplifier can (under any circumstances) contribute to the aerosol. Usually, aggressive sampling is done in ways that attempt to duplicate activities that might actually occur in the space.

DISCUSSION

Please comment on criteria utilized for establishing typical indoor/outdoor ratios and their applicability to data interpretation.

CLOSURE

Indoor/outdoor ratios are usually calculated on the basis of total particulate counts. It is usually assumed that if indoor levels are lower than those outdoors, then the indoor environment is not contaminated with respect to outdoors. Obviously, this is not a valid assumption. Unless the particles are of identical types indoors and out, and unless patterns and rates of penetration are known, one cannot assume an outdoor source for indoor aerosols.

DISCUSSION

We sometimes use allergen skin tests on building-responding patients. When the test material is marked for example "Cladosporium," is positive response useful in light of the difficulty in identifying species?

CLOSURE

A strong positive response to a fungal allergen indicates that the individual is probably atopic. How the atopy

relates to building occupancy is much more difficult to determine. Fungal allergen extracts available for skin testing are extremely variable, poorly characterized, and often mis-identified. The only way to clearly make a connection with building exposure, one would have to use for skin testing material made from a strain isolated in the building, and demonstrate that the reaction is unique to that strain. Especially with Cladosporium (which is always abundant outdoors), it is extremely unlikely that sensitization would occur only in connection with an indoor environment.

DISCUSSION

Is the agar to sieve plate distance critical for the Andersen sampler? If so, what should the distance be?

CLOSURE

Yes. The distance should be 2.5mm. For the original cast aluminum sampler using Andersen's original glass petri dishes, 27 ml of agar was necessary to achieve this distance. If plastic plates are used in this sampler, 47 ml of agar is necessary. Newer models currently available (the "Flow-sensor" samplers and the lathe-turned Andersens) have been modified for use of plastic petri dishes with 20 ml agar.

DISCUSSION

Using the N6 procedure, the average velocity per jet is 76.4 ft/sec (pretty high!). Has there been any research on whether this impaction velocity kills any important organisms?

CLOSURE

All the research has been with bacteria, and certainly some fragile organisms are killed. No studies have been done with fungi. Actually, the 5th stage would be just as efficient at collecting fungus spores (none of which are smaller than 1um) and the velocity would be only 42 ft/sec.

DISCUSSION

What types and concentrations of pathogenic fungi (Cryptococcus, Histoplasma, etc.) occur in abandoned buildings where large amounts of pigeon fecal material are being removed with and without prior formalin disinfection? Has anyone done this type of work? Assume volumetric sampling. What sampler was used?

CLOSURE

The fungus pathogen that is most likely to occur in indoor pigeon fecal material is Cryptococcus. Airborne levels have been measured (in a limited way) using the Andersen sampler by Ruiz et al. [42]. However, they did not use formalin disinfection. Reports of the use of formalin disinfection have been related to Histoplasma in outdoor sites [58]. Histoplasma cannot be evaluated using air sampling, so the data you request are unavailable.

DISCUSSION

If use of animals in the laboratory is prohibited, are there any methodologies for direct culture available (even though less sensitive) (e.g., described in the literature or possibly used by e.g., Fort Detrick?

CLOSURE

A visual screening method is available for Histoplasma (see sampling discussion above). Blastomyces is rarely isolated in connection with epidemics, even using animal inoculation procedures. Cryptococcus and other opportunistic pathogens (e.g., Aspergillus) can be isolated culturally using selective culture media or incubation conditions.

DISCUSSION

When do you feel would be a situation that air sampling investigation/evaluation should be conducted for the presence of [pathogenic] yeasts in the indoor environment?

CLOSURE

I would not recommend air sampling for these organisms. If disease exists, and a reservoir containing the organism exists, then air sampling is probably not necessary. If disease is not present, or if an apparent reservoir is not present, air sampling is unlikely to be of use. Air sampling cannot be effectively done for Histoplasma or Blastomyces.

DISCUSSION

What is the significance, if any, of the presence of Cryptococcus neoformans in an office? Assume no disease.

CLOSURE

Cryptococcus neoformans is an opportunistic pathogen and only causes infection if inhaled in very high numbers, or in immunocompromised people. The presence of the organism in an office environment means that a reservoir exists somewhere in the environment, and that the potential for

disease exists if an immunocompromised person occupies the environment.

DISCUSSION

Can you suggest a good reference for decontamination of an area covered with pigeon and bat droppings?

CLOSURE

Reported decontamination methods range from treatment with formalin [58] to drying and waiting [42]. Other possibilities include the use of other biocides (such as dilute hypochlorite or dilute hydrogen peroxide) that do not carry the potential risks of formaldehyde.

DISCUSSION

Are there documented cases of disease from workers exposed to pigeon and bat droppings?

CLOSURE

Many. Reviews of the literature are included in specific chapters on histoplasmosis, blastomycosis, and cryptococcosis in Rippon's textbook of medical mycology [14].

DISCUSSION

With the tremendous capsule surrounding the yeast cell do you feel that in using an impaction device (SAS, Andersen) with quiescent and aggressive sampling techniques you could collect viable organisms?

CLOSURE

Cryptococcus usually does not have the large capsule in saprophytic environments. Viable organisms of Cryptococcus can be collected using impaction sampling.

DISCUSSION

What would be the best mode of transport from the field to the laboratory (e.g., room temperature, ice pack, etc) Presume Histoplasma, Cryptococcus).

CLOSURE

Room temperature would be acceptable for Cryptococcus samples. The organisms have been shown to survive for up to 9 months in a dry condition without input of new substrate. Histoplasma has been less well studied. For long periods of transit (more than 24 hours), I would suggest maintaining the sample at approximately the temperature at which it was collected.

DISCUSSION

Due to the potential liability problems in recommending a formalin decontamination, what are other methods of decontamination that do not involve formalin or other materials of similar health risks?

CLOSURE

Drying and prevention of new substrate additions has been suggested for Cryptococcus, although only 85% of spores were dead after 9 months. Other biocides could probably be used (such as dilute hypochlorite or dilute hydrogen peroxide).

DISCUSSION

Is there a hazard of concern when pigeons roost on air intakes of building HVAC systems? How should it be controlled?

CLOSURE

Organic material building up in air intakes is certainly a concern, though not established as a health risk. Downstream filtration, if properly installed and maintained, should capture the majority of biological contaminants that would occur in such material. Methods for excluding pigeons is beyond the purview of this conference. Pigeon droppings, once they have accumulated, can be decontaminated by soaking with dilute formalin (or probably, dilute hypochlorite or peroxide).