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PRELIMINARY DRAFT
INFORMATION ONLY

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05/01/00

To: Mr. Charles Reaux

From: John H. Keene, Dr. P.H., CBSP

Re: Microbiological Sampling of Exploratory Studies Facility, Yucca Mountain, NV

At the request of TRW Health and Safety personnel, a protocol was developed to sample air for evaluation of airborne fungi in the Exploratory Studies Facility, Yucca Mountain, NV with specific emphasis on "cross drift" section in the area between the bulkheads prior to reaching the drilling equipment. This study was done to provide a basis for a health risk analysis to determine the relative safety of this space with regard to possible fungal contamination.

Background:

Fungal growth has been noted in the area between the two final bulkheads in the "cross drift" and in the area beyond the second bulkhead (drilling area). Concern has been noted regarding the potential health hazards associated with the mold growth in these areas. Previous studies were conducted which provided an evaluation of the types of fungi present in the area. However, these studies only involved surface sampling and did not quantify the numbers of organisms present on either surfaces or in the air. A health risk assessment of the potential health hazard associated with the observable fungal growth requires the quantitative comparison sampling of air in the area in question with air in "non-contaminated" areas, including outside of the facility.

Fungi, when present in the air, represent two potential health risks. These are:

1) Infection
2) Allergy.

Respiratory infections result from the presence of pathogenic fungi in the environment. Respiratory infection is relatively rare and may occur, when people are exposed to high levels of pathogenic fungal spores under, usually, dry conditions where the spores of the organisms have been released and are carried on respirable size particulates in the environment. Infection may also occur when persons are immunosuppressed due to some medical problem or treatment.

Allergic reactions can occur when personnel are sensitized to environmental fungi and there is a source of that particular type of fungus in the environment. Sources of fungi that can elicit allergic type reactions are, invariably, areas of high humidity or standing water containing some type of nutrient for the growth of the environmental fungi. Almost any organic material will serve as a nutrient for fungal growth.

Protocol:

The protocol involved taking comparative air samples for:

1) Viable fungal spores
2) Non-viable particulates
3) Respirable particulates

These samples were taken at specific points as noted on the accompanying diagram prior to reaching the suspect area and following sampling in the suspect area. Similar samples were taken in the suspect area with the exception of, as noted in Dr. Jurinski's report (See appendix A), the lack of respirable particulate sample between the bulkheads.

Since the air supplied to the suspect area for ventilation purposes comes from the surface (outside air), the testing was done to demonstrate whether or not, following ventilation, there was a difference in the quality of the air with regard to numbers and types of fungi present.

Air was sampled with an Anderson single stage air sampler to provide data on the numbers of viable fungal spores in the air of the areas sampled.

It is known that allergic reactions can be elicited by exposure to non-viable mold spores. A Bourkard non-viable particulate sampler was used to provide a comparison between viable and non-viable particulates.

Finally, since infection would require an actual deposition of fungal spores into the lungs of an exposed individual, sampling was performed to determine the amount of respirable size particles in the air of the various areas of the sampling site.

Results:

Relative humidity and temperature readings were made and while the RH in front of the 1st bulkhead was approximately 28%, the RH in the suspect area was in excess of 80%.

The results of the sampling are shown in Tables 1 and 2. No respirable samples demonstrated any respirable size particulates above the detectable limits of 50 micrograms.

The data indicate that:

1) Colony counts of molds in outside air and in the main tunnel range from 35 to 350 per cubic meter of air. These results were consistent in both the entry and exit samples obtained.
2) Colony counts of molds behind the first and second bulkheads in the area in question ranged from 3,000 to 37,000 per cubic meter of air, with the predominant organism being *Penicillium species*.
3) The non-viable spore sample results were consistent with the results of the viable samples.
4) Respirable particulate samples taken throughout the tunnel, including the area in question, did not demonstrate any respirable particulates above the detectable limit of the sampling process.
5) Low levels of *Stachybotrys* were found at the sampling site near the exhaust ventilation area prior to and following sampling within the tunnel. No *Stachybotrys* was found anywhere within the tunnel.

Discussion:

Mold spore counts in the lower range of those found in the suspect area are not significantly higher than the levels found in ambient outside air in many areas of the country. For example: The ambient mold spore level in Richmond, VA on April 28, 2000 was 4320 per cubic meter, or slightly above the lower limit of viable spores found in the suspect area.

However, the data indicate that the level of fungal spores, predominantly *Penicillium* species, is significantly increased in the area in question above the levels in either outside air or other areas of the tunnel. The organisms found (*Penicillium* species) are not generally considered to cause infection in exposed persons, nor do they produce significant levels of hazardous mycotoxins. In addition, the lack of respirable sized particulates would further diminish the potential for infection should the organisms found be pathogenic.

It should be noted that, exposure to the spores of *Penicillium* species could result in allergic type reactions, in individuals that are sensitive to these spores. While there are no standards or exposure limits for exposure to biological agents (non-pathogenic fungi), personnel who have a demonstrated allergy to molds should be considered at risk to potential allergic response when they are exposed to these agents. Persons with known allergy to *Penicillium* spores (or other fungal spores)can exhibit allergic responses when exposed to low levels of the allergens. Note that allergy to the antibiotic "Penicillin" does not necessarily mean that an individual would be allergic to the spores of *Penicillium* species. Respiratory protection capable of reducing the potential exposure for personnel with known mold allergies to a level comparable to existing outside air levels should be considered.

Penicillium is not generally considered to be a producer of large amounts of potent, volatile, hazardous mycotoxins and therefore, such volatile mycotoxins would not be expected to be present in the area in question in significant enough concentrations to cause health problems. Other agents such as certain species of Aspergillus could also produce mycotoxins under specific conditions of growth. If any volatile mycotoxins were to be produced in this area, it is most likely that they would be present in very small concentrations and the ventilation rate within the facility would make

it highly unlikely that any volatile, hazardous materials would be present in high enough concentrations to cause any health effects.

The presence of Stachybotrys near the exhaust port is not explained at this point. Stachybotrys is a common soil organism and is present in "rotting" organic material such as mulch. It has also been found associated with cellulosic building materials in buildings damaged by water. This organism was not found within the tunnel at any of the sampling sites and therefore is not considered to be a significant hazard for people entering the areas of concern.

Conclusions:

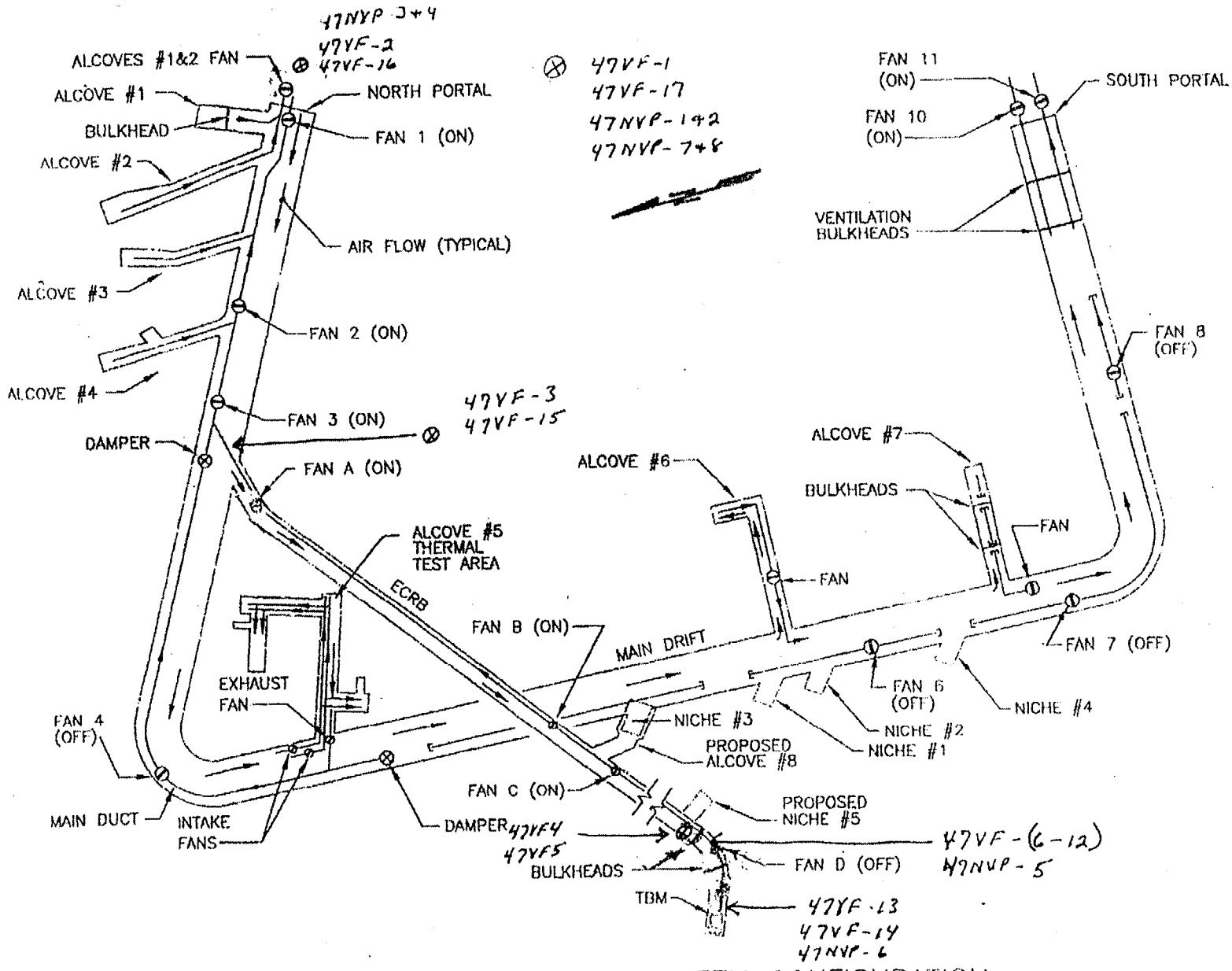
The area in question has an airborne fungal load, consisting of predominantly Penicillium species, which is significantly increased above the fungal concentrations in either outside air or other areas of the tunnel that were sampled.

Since Penicillium species is a type of environmental fungus which can cause sensitization and allergy, there is a potential for persons with mold allergy to exhibit allergic symptoms when entering the suspect area without some type of respiratory protection.

The health and safety concern should be for reducing particulate exposure recognizing that minimal respirable sized particles are present and therefore protection of personnel from exposure of nasal mucosa to the potential allergens should be the aim of the respiratory protection. Since there are no known negative pressure respirators that are 100% effective in removal of biological particulates, and no standards of acceptable levels of exposure to these agents, quantitative fit testing of respirators does not seem to be in order. Qualitative fit testing should be sufficient to demonstrate the efficiency of the respirators to be used.

Recommendations:

1) Personnel required to enter the area behind the first bulkhead in the "cross drift" should be questioned with regard to potential mold allergy.
2) Personnel with known mold allergy should either be restricted from entering the area, or provided with respiratory protection that will decrease the potential exposure to mold spores to: at or below the ambient levels of mold spores found in the outside air.
3) Based on the available information regarding the efficacy of negative pressure respirators for protection against biological particulates, qualitative fit testing should be appropriate, if respirators are necessary for entry. <u>Please note that this recommendation is made only for the protection against microbial particulates present and not any other hazardous materials that may be present</u>.



ECRB VENTILATION SYSTEM CONFIGURATION

Appendix A:

Communication of Dr. Joe Jurinski re: Sampling of ECRB

Yesterday I assisted Mr. Eric McKee of Bio-Tec, Inc. in the collection of airborne fungi samples from areas behind the bulkheads in the East-West Crossdrift. Samples of oil from the Tunnel Boring Machine were also collected for microbiological analysis. The sampling was conducted following a protocol developed by Dr. Keene of BIOHAZTEC. The protocol was followed with one exception; a second respirable dust sample from the space between the two bulkheads was not collected.

Three types of air samples were collected. These included standard respirable dust samples, Burkard (non-viable) samples and Anderson (viable) samples. The Burkard samples give information on the total number of spores (both "living" and "dead") while the Anderson samples give information on "living" spores. The Anderson samples also give more information on the types of fungi present compared to the Burkard samples.

Samples were collected from several locations at the site. Outside air samples were collected before and after entry to the tunnel. Samples were also collected at the entrance of the East-West cross drift before and after bulkhead entry. Air samples were collected from three locations in the area between the bulkheads and from a location behind the second bulkhead.

The sampling was started at the site around 9:00 a.m. and was completed by about 4:30 p.m. Mr. McKee and I left the site at 5:00 p.m.

Several observations were made during the survey.

Visible mold was observed in several locations between the bulkheads and in the vicinity of the tunnel boring machine. The mold was observed on the conveyor belt and on localized areas of rock and wooden cross ties. The mold appeared to be most prevalent on the conveyor belt at the second bulkhead. In this location, the mold was observed covering large areas of the belt surface, suggesting that it is spreading across the belt. The amount of visible mold appeared to increase toward the second bulkhead. Only localized areas of mold were observed on the opposite side of the second bulkhead. This was observed on the surface of a gauge panel. There was a noticeable musty odor in the areas where visible mold was observed. The 1/2 face respirators with combination (HEPA plus Organic Vapor) cartridges were effective in controlling the odor. The odor was noticed when the respirator was removed. It was reported to me that the temperature in the area was about 80 F and the relative humidity was about 80%. These conditions would be favorable for mold growth.

I took possession of the microbiologial samples from Eric McKee this morning to assist him with a timely delivery of the samples to the laboratory. The laboratory selected for use by Dr. Keene was Aerobiology Laboratories, Inc. of Reston, Virginia. I have worked with this laboratory for several years and was able to arrange a Saturday delivery of the samples.

Table 1: Results of Viable Microbial Sampling

Sample #	Description	Results (CFU's/m3)	Organisms
47VF1	Outside Air (Entry)	3.5E+01	Cladosporium species 100%
47VF2	Near Exhaust (Entry)	3.2E+02	Yeasts 78%; Alternaria 11%; Stachybotrys 11%
47VF3	Main Tunnel Y (Entry)	7.0E+01	Penicillium 50%; Pithomyces 50%
47VF4	1st Bulkhead (Entry)	No Growth	
47VF5	1st Bulkhead (Entry)	3.5E+01	Penicillium 100%
47FV10	2410 meters	4.4E+03	Penicillium 96%; Others 4%
47VF12	2410 meters	5.0E+03	Penicillium 96%; Others 4%
47VF8	2475 meters	5.0E+03	Penicillium 92%; Others 8%
47VF9	2475 meters	3.1E+03	Penicillium 88%: Aspergillus ochraceous 4%; Paecilomyces 3%
47VF6	2nd Bulkhead	3.7E+04	Penicillium 100%
47VF7	2nd Bulkhead	3.5E+04	Penicillium 98%; Others 2%
47VF13	Drilling area	9.2E+03	Penicillium 91%; Others 9%
47VF14	Drilling area	2.1E+04	Penicillium 96%; Others 4%
47VF15	Main Tunnel Y (Exit)	1.8E+02	Aspergillus versicolor 80%; Penicillium 20%
47VF16	Near Exhaust (Exit)	3.5E+01	Stachybotrys 100%
47VF17	Outside Air (Exit)	3.5E+01	Aspergillus versicolor 80%
47VF11	Control	7.0E+00	Mixed species

Table 2: Results of Non-viable Sampling

Sample #	Description	Pollen (#/cubic meter)	Mold Spores (# per cubic meter)	Type Spores
47NVP1	Outside (Entry)	4.4E+01	0.0E+00	
47NVP2	Outside (Entry)	2.2E+01	1.5E+02	Ascospores
47NVP3	Near Exhaust (Entry)	1.8E+02	4.4E+01	Pithomyces
47NVP4	Near Exhaust (Entry)	6.6E+01	4.4E+01	Ascospores
47NVP5	Suspect Area (2490)		8.4E+03	Penicillium/Aspergillus Group
47NVP6	Suspect Area (Belt area)		1.1E+05	Penicillium/Aspergillus Group
47NVP7	Outdoor Air (Exit)	1.3E+02	0.0E+00	
47NVP8	Outdoor Air (Exit)	8.8E+01	1.3E+02	Ascospores

HAZCOM AWARENESS FOR MOLD AND FUNGI BEHIND THE BULKHEADS

I, _____ , understand several types of mold and fungi are growing behind the bulkhead in ECRB and Alcove 7. The predominate mold is Penicillium. These molds may cause or precipitate allergic reactions and asthma usually after one or more exposure episodes. They may also cause illnesses ranging from flu like symptoms to respiratory distress.

Following several simple procedures to include the use of appropriate Personal Protective Equipment (PPE) will provide protection from any potential health effects from molds. Therefore; to protect you from these potential biohazards the M&O will require before entry into these areas:

1. Current medical clearance from the YMP Medical Director or other physician.
2. Current respirator fit-test.
3. Mandatory respirator wear during entry (can not remove for talking).
4. Mandatory use of disposable coveralls with hoods.
5. Mandatory use of nitrile or heavy latex gloves.
6. Disposal of all PPE into bags at exit point of bulkhead.
7. Mandatory hand washing upon exiting.
8. Showers are mandatory post exit for M&O employees and associates and recommended for short-term visitors.
9. Mandatory HAZCOM briefing prior to entry which will elaborate on these entry requirements.
10. If during the entry you begin to experience any medical symptoms such as shortness of breath, tight chest, allergic reactions report to your supervisor or leader and begin evacuation with assistance immediately.
11. If you develop flu like symptoms within 36 hours after entry, inform your supervisor or tour representative. They will contact the YMP Medical Director for or with you.
12. Any person who has asthma, allergies to molds, severe allergies, or immune compromising disease shall self-report and NOT enter the bulkhead areas (you do not have to indicate the specific medical condition that prohibits you from entering).
13. If you do not desire to enter for other reasons, contact your supervisor for instructions. Medical conditions may be discussed directly with the YMP Medical Director.

_____ Date: ___/___/___ _____

Employee or Visitor Signature Supervisor or Tour Representative

05/02/00