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Safety Precautions for Handling *Aspergillus flavus* Group Fungi and Aflatoxins

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SAFETY PRECAUTIONS FOR HANDLING *ASPERGILLUS FLAVUS* GROUP FUNGI AND AFLATOXINS

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Pathogenic or toxigenic fungi of the *Aspergillus flavus* group have been recognised all over the world, and the diseases they cause are widespread and of considerable medical importance. Aspergilli have been encountered most frequently as respiratory pathogens. The aflatoxins produced by strains of these fungi are highly poisonous and are among the most potent carcinogens known. The following guidelines indicate the basic precautions required when handling the toxigenic fungi and the mycotoxins they produce.

HAZARDS FROM *ASPERGILLUS FLAVUS* GROUP FUNGI AND AFLATOXINS

Working with the *Aspergillus flavus* group fungi and in particular with their spores (conidia) can result in several health problems:

1.1. The mould (fungus) spores may be inhaled and then set up irritation and infection in the lungs. This condition is called Aspergillosis. The general term Aspergillosis may embrace all infections caused by the Aspergilli but is usually restricted to the respiratory disease in man and animals.

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1.2 The spores are potentially allergenic and can affect certain individuals by causing severe sinus and respiratory allergenic responses.

1.3 Aspergilli can also cause Otitis (infection in the ear), Keratitis (corneal infection), Endocarditis (heart valve infection), and Maduromycosis (subcutaneous foot and hand infections) in man.

1.4 Aspergilli may cause chronic infections in which large, single or multiple, granulomatous lesions develop which resemble those caused by *Mycobacterium tuberculosis*.

1.5 The ill effects of such infections may be compounded if the fungi produce aflatoxins.

2.1 Toxigenic strains of *A. flavus* and *A. parasiticus* may produce aflatoxins in lesions on lungs etc. (see above) and these may cause damage *in situ* or may be translocated to other tissues and cause damage there. Also, the mycelium and conidia may contain appreciable quantities of aflatoxins and these could be harmful if inhaled, ingested or if they come in contact with the skin. Damage from aflatoxins may take the form of acute poisoning -- AFLATOXICOSIS or may result from CARCINOGENESIS.

2.2 Toxin production by this group of fungi has become of major importance in human and animal disease because of the direct toxicity and long term carcinogenic effects of aflatoxins. In

view of the existing knowledge of the health hazards due to Aspergilli and aflatoxins, safety of workers is a prime consideration and stringent safety measures need to be taken to protect those who handle cultures of Aspergilli, commodities contaminated by them, or aflatoxins.

SAFETY MEASURES FOR HANDLING MOULD CULTURES AND CONTAMINATED COMMODITIES

3.1 Workers should always wear a face mask or respirator and disposable surgical gloves when working with the mould cultures.

3.2 Inoculation of growth media/substrates and transferring of *A. flavus/A. parasiticus* spores should be done under a laboratory hood that is first sprayed inside with a disinfectant

3.3 Never handle the contaminated seeds with bare hands. Always use surgical gloves when examining seeds for external or internal fungal infection. Seeds should be examined for infection under an exhaust hood.

3.4 Seed for dry seed resistance testing should be prepared under an exhaust hood to draw the dust away from the worker.

3.5 When it is necessary to work with any contaminated material, workers should wear face masks to avoid breathing in spores or dust that may be associated with the contaminated commodity.

3.6 Samples of commodities for aflatoxin analysis should be prepared cautiously as grinding of dry samples may result in air-borne

dust. Even if no aflatoxin is present, there is potential harm from inhalation of mould spores or from allergic responses to inhaled dust. Use of a protective mask is essential.

3.7 Disposable laboratory coats are also helpful in protecting workers' clothing from accidental contamination with mould spores and aflatoxins.

SAFETY MEASURES FOR HANDLING AFLATOXINS

Aflatoxins are highly toxic and carcinogenic substances and strigent safety measures must be taken when it is necessary to work with pure aflatoxins or with extract preparations. The greatest risk of laboratory contamination comes from handling dry aflatoxins because of their electrostatic nature and resulting tendency to disperse in working areas.

4.1 When dry aflatoxins are handled, the worker should use a respirator or disposable face mask to prevent inhalation of the toxin. Disposable surgical gloves should be worn.

4.2 Whenever possible perform manipulations within a laboratory hood. Use a glove box if available.

4.3 Whenever possible the aflatoxins should be handled in solution.

4.4 Low-cost paper laboratory coats should be used for protecting workers' clothing from contamination with mycotoxins. These paper laboratory coats should be destroyed upon accidental contamination.

4.5 Workers must use a rubber bulb pipetting device not the mouth when pipetting toxin solutions to prevent any oral exposure to them.

4.6 Contamination of the mouth should be treated with a wash composed of 1% sodium perborate and 1% sodium bicarbonate in water. (a supply of this wash should be kept available in the laboratory).

4.7 If mycotoxins, toxin contaminated substances or cultures come in contact with the skin, the affected area should immediately be washed with full strength domestic bleach (e.g., 'Clorox' which contains 5.25% sodium hypochlorite). Any bleach solution which contains around 5% of sodium hypochlorite will suffice. Follow this treatment by washing the skin with germicidal soap or detergent and then rinse thoroughly in tap water. If the skin is too sensitive to wash with sodium hypochlorite solution, a solution (5%) of sodium perborate may be used instead. (supplies of the above solutions should be kept in a readily accessible position in the laboratory).

4.8 Protective goggles and face mask should be used when viewing chromatographic plates and mini-columns under ultraviolet light for the presence of aflatoxins. Similar protection is required when removing aflatoxin 'spots' from silica gel or when removing the gel from chromatographic plates. These processes should be carried out in a laboratory hood.

DECONTAMINATION PROCEDURES FOR LABORATORY GLASSWARE AND SPILLAGE

5.1 Mould cultures in flasks should be sterilized by autoclaving or by introducing a small amount of chloroform, replacing the cotton-wool plug, and heating in a steam bath until chloroform vapour can be seen condensing in the plug (ca. 10-15 minutes).

5.2 Mould cultures in petri plates should be placed in bleach solution (5% sodium hypochlorite). Allow at least 1 hour for effective decontamination.

5.3 Infected seeds in the petri plates should be kept in bleach solution (5% sodium hypochlorite) for 1 hour for decontamination.

5.4 Use surgical gloves (throw-away type) and face mask when it is necessary to handle mould cultures and contaminated seeds.

5.5 For general 'dish-washing' a solution of bleach diluted tenfold with water (0.5% sodium hypochlorite) should be used. Make sure that the entire surfaces of the glassware are wetted. All aflatoxins are destroyed by alkalis, strong acids, and oxidizing agents. Clean-up procedures involving the use of such reagents should be sufficient for decontamination. Sodium hypochlorite is a strong oxidizing agent, very effective in destroying aflatoxins and in detoxification. Ordinary liquid household bleaches contain 5 to 6% active sodium hypochlorite and are very effective in destroying aflatoxins in solution, in fungal spores and in mycelia.

5.6 Swab accidental spills of aflatoxin or of toxic substances with 5% sodium hypochlorite solution.

5.7 Contaminated waste materials such as filter papers, adsorbents etc., should be kept in tightly closed containers, should not be commingled with other waste materials, and should be incinerated. If burning is not practical such material may be decontaminated by soaking in 5% sodium hypochlorite bleach for an hour, care being taken to ensure thorough contact.

5.8 Prior to disposal, toxin-containing solutions should also be treated with bleach or strong acids.

5.9 Adsorbents on TLC plates should be soaked with bleach before they are removed from the plates.

5.10 Contaminated garments which are to be laundered should be soaked for an hour in 5% sodium carbonate solution or 1% bleach solution depending upon fabric and colouring.

GENERAL SAFETY PRECAUTIONS FOR HANDLING OF AFLATOXINS

6.1 Laboratory areas where work with *A. flavus* and aflatoxins takes place should be clearly demarcated. Such rooms should have warning signs - DANGER - AFLATOXIN - CARCINOGEN HANDLING AREA.

6.2 The work surfaces should be of glass or decolam sheet.

6.3 Laboratory floors, walls and ceilings should be cleaned using wet cloths and not by dry sweeping. A vacuum cleaner may be used to remove dusts, powder, etc. from inacessible areas.

6.4 Possible contamination of all working areas should be monitored frequently by means of a portable long-wave ultraviolet lamp, the presence of aflatoxins being indicated by their characteristic fluorescence.

6.5 The glass vessel containing aflatoxin should be kept within a metal container packed with adsorbent material. The container should be clearly labelled and bear a warning notice.

6.6 Eating, drinking, smoking, and chewing (beetle, gum or tobacco) should be strictly prohibited in all rooms in which work with *Aspergillus flavus* and aflatoxins is carried out.

6.7 Laboratory workers should receive regular medical examinations.