GUIDELINES FOR

MANAGEMENT AND HANDLING

OF SPENT MYCELIUM

FROM BULK DRUG INDUSTRY

MAHARASHTRA POLLUTION CONTROL BOARD

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November, 2005

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PREAMBLE

The spent (waste) mycelium from the bulk drug industry can exhibit hazard characteristics if not handled properly. For disposal of spent mycelium, it is subjected to bio composting followed by its use as agricultural manure. This guideline follows and implements the Maharashtra Pollution Control Boards' Pollution Control Policy in this regard. It aims to protect the health and safety of the community and the environment when handling mycelium and its further treatment.

1. GENERAL

1.1 Objective

The objective of this guideline is to provide general guidance and set the framework for the management of potential risks to human health and / or the environment resulting from activities involving handling of mycelium.

1.2 Area of validity

This guideline is binding for the Pharmaceutical and Bulk Drug Industries handling mycelium and holding Authorisation under Hazardous Wastes Rules, Consent to Operate under Water Act and Air Act from Maharashtra Pollution Control Board.

1.3 Scope

This guideline applies to the handling, i.e. production, transport and use of all types of mycelium, including recombinant organisms, in and between laboratories, pilot plants, greenhouses, production areas, including the infrastructure, as well as to field trial areas. It includes the inactivation and safe disposal after composting of potentially infectious material.

Conventional biological waste treatment systems (e.g., biological wastewater treatment plants) are excluded from these guidelines.

1.4 Responsibility

The Occupier of the Pharmaceutical and Bulk Drug Industry is responsible for implementation of these guidelines.

2. BASIC PRINCIPLES

2.1 Risk Management

When handling mycelium, risk management is based on an initial identification of the potential hazards followed by an evaluation of the associated risks and institution of the appropriate technical and organisational control measures.

Classification

Mycelium is classified into the appropriate risk group either according to international or national lists of organisms or based on a risk assessment verified by the State or Central Government Authority.

Hazard Identification

Prior to the commencement of activities with mycelium previously not handled at the site and / or novel types of handling, hazards must be identified and risks evaluated. This includes the classification of the mycelium into risk

groups, considerations on the type and scale of handling of the mycelium, and the suitability of installations and equipment.

A *systematic* hazard analysis (e.g., Zurich Hazard Analysis) must be conducted prior to the handling of mycelium in World Health Organisation risk group 3 and 4.

2.2 Safety Measures

Containment measures

The containment measures required for laboratories, production and other facilities must correspond to the risk group of the mycelium used and the way they are handled (e.g. scale, potential for aerosol formation). Facilities are can be designated as BL 1 (biosafety level 1, basic biosafety level) through BL 4 (biosafety level 4, maximum containment).

Facilities, in which mycelium of different risk groups are handled, must be designed to the level appropriate for the safe handling of the organisms in the highest risk group.

Labeling of Biohazard Areas and Material during Storage and Transportation

Biological hazard zones i.e. BL2 and higher must be clearly labeled at the entrance e.g., using the international biohazard warning sign (Schedule III and Schedule IV under Rule 6 of The Bio-Medical Waste (Management and Handling) Rules, 1998) and indicating the type of containment (BL2, BL3 or BL4) and the potential special hazards. Emergency contact persons responsible for biosafety in the work area should also be signposted.

Work practices

PPE

Work practices and personal protective equipment (PPE) must be in line with the mycelium (risk group, exposure routes and means of transmission) and the processes used.

Operating Instructions

If hazardous mycelium (risk group 2 and above) are handled, operating instructions must be elaborated and made available in the work areas.
These operating instructions must include information on the potential effect of the mycelium and safety precautions in an emergency.

The operating instructions must be clear and written in a language understood by the workers. The instructions should be, wherever appropriate, complemented by oral instructions.

Maintenance / Cleaning

Safety equipment must be maintained in proper working order by carrying out routine maintenance according to an established program.
Maintenance and cleaning work in areas of category BL2 or higher may be carried out only after it has been ensured that the maintenance or cleaning staff are not exposed to biohazardous material.

The work may be carried out only under the supervision of a qualified person or by individuals who have undergone suitable safety training.

Inactivation / Waste disposal

Mycelium material which might pose a risk to the environment must be inactivated by disinfection, sterilization or incineration prior to final discharge into the environment.

The filtered Mycelium cake must be thoroughly washed with water or suitable reagent to remove any traces of active ingredient. The ability to differentiate between material before and after inactivation must be assured.

Disinfection

Methods used for disinfection must be periodically validated (in-house or contractor) to confirm their efficiency.

Emergencies

Written emergency procedures in case of spills or accidental release of mycelium must be established (first aid, prevention of spreading, decontamination and safe disposal of potentially contaminated material). Provisions for proper documentation and internal and external communication strategies must be included as part of the contingency plan.

2.3 Organisational Measures

The Industry generating mycelium shall obtain appropriate registration / approval to ensure evaluation biological risks in the work area.

The Principal Investigator (PI) (leader of project, head of laboratory, pilot plant, production plant or other person who directs biological activities) who

- is responsible for the safe handling of mycelium under his / her supervision
- establishes inventories of mycelium
- identifies those workers exposed to biohazardous material
- evaluates biohazards in the work area
- is the contact person to the local authorities in all biosafety matters
- initiates and supervises training programs
- performs routine performance checks

Glossary

Biohazardous agents See: Hazardous mycelium.

Mycelium Natural or genetically modified viable cells, cell clusters, spores, viruses or genomic elements capable of replication (pathogens but also nonpathogens).

BL Biosafety level (BL1 to BL4) according to known or expected risk category of organism or experiment. The levels are designated in ascending order by degree of protection provided to personnel, the environment, and the community.

Containment facilities Containment facilities are rooms and areas in which potentially hazardous mycelium are used.

Disinfection Selective verified treatment aiming at removing the infectious activity of specific pathogens. Pathogens are thus rendered non-virulent.

Handling of Mycelium

Their production or use, i.e. application, storage, processing and treatment, packaging, repackaging, mixing, destruction and transport on-site and off-site.

Hazardous Mycelium

Inactivation Destruction of the biological activity of organisms, viruses and cell components, such as infectious materials.

Pathogens Disease-causing organisms

Principal investigator (PI)

Leader of project, head of laboratory, pilot plant, production plant or other person who directs biological activities.

Recombinant organism See: Transgenic organism

Transgenic organism Organism (virus, microorganism, animal, plant etc.) whose genetic material has been altered by any method that overrules existing natural barriers of sexual recombination and/or propagation, and still has potential to transfer and/or to multiply this new genetic material.

Annexure 1

World Health Organization Risk Group Definitions

Pathogens should be handled and contained depending upon their biological characteristics. These characteristics or factors provide for an indication of the associated risk of exposure and the risk of disease of the host. The factors that are associated with the risk of exposure are the host's work activity, proficiency, age, sex, immune status and medications being used. The factors associated with the risk of disease to the pathogen include virulence, infectious dose, route of infection, toxigenicity, agent's host range, and the availability of effective preventive measures and treatment. Other factors that determine how susceptible one is to an infectious agent are the host's natural defense mechanisms and the chance for opportunistic infection.

The World Health Organization (WHO) has classified infective microorganisms by risk group. These are numbered 1-4 as follows:

- **Risk Group 1** no or low individual community risk

  A microorganism that is unlikely to cause human or animal disease, such as *E. coli* K-12 and *Saccharomyces cerevisiae*.

- **Risk Group 2 moderate individual risk, low community risk**

  A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited. Examples include Herpes viruses, HIV (clinical work), Varicella-Zoster virus, and polioviruses.

- **Risk Group 3 high individual risk, low community risk**

  A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available. Examples include HIV (non-clinical work), Mycobacterium tuberculosis, Coxiella burnetti, Brucella, and Hanta virus.

- **Risk Group 4 high individual and community risk**

A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available. Examples include: Ebola virus and Hemorrhagic fever.

Annexure 2

Biosafety Level Definitions

Biosafety Level 1 (BL1)

Biosafety Level 1 is suitable for work involving agents of minimal potential hazard to personnel and the environment. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Personnel have specific training in the procedures and are supervised by a scientist with general training in microbiology or a related science. Contaminated materials that are to be decontaminated at a site away from the work place are placed in a durable leak-proof container that is closed before being removed from the work place. Special containment equipment is generally not required for manipulations of agents assigned to BL1.

Biosafety Level 2 (BL2)

Biosafety Level 2 is similar to BL1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that: personnel have specific training in handling pathogenic agents and are directed by competent scientists; access to the workplace is limited when work is being conducted; and certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment. Contaminated materials that are to be decontaminated at a site away from the work place are placed in a durable leak-proof container that is closed before being removed from the work place.

Biosafety Level 3 (BL3)

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is conducted with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Work Place personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices. Contaminated materials that are to be decontaminated at a site

away from the work place are placed in a durable leak-proof container that is closed before being removed from the work place.

Biosafety Level 4 (BL4)

Requires maximum containment procedures.

Annexure 3

Case Study On Implementation Of Guidelines For Composting Of Mycelium

The prolonged use of chemical fertilizers, insecticides, herbicides for increasing crop productivity in our country has resulted in changes in microbiological population, nutrient imbalance, deterioration of physical property of the soil, drop in soil fertility.

Several methods have been developed by which organic residue or waste biomass can be converted safely into biofertilisers.

Mycelium is generated during the fermentation process of the manufacture of Rifamycin B. Rifamycin B is further chemically converted to Rifampcin, a bulk drug used in the treatment of tuberculosis and leprosy.

At one Bulk Drug unit in Maharashtra, the fermentation process is a submerged fermentation and the fermenters are harvested for 265 hrs. The broth is deactivated with Formaldehyde at the end of harvesting and filtered through a Rotary Vacuum Drying Filter using Perlite as a Filter Aid. The Filtrate containing the product is taken for chemical synthesis and the mycelium on the Filter medium is removed from the Vacuum Dryer as a mycelium cake.

The deactivated mycelium is completely biodegradable and is used as a raw material for making biofertiliser by the method of composting. With addition of different ingredients and other components, this composted mycelium has been proved as one of the best soil enricher, soil conditioner and agro manure.

Composting is a biochemical process in which diverse and mixed group of micro-organisms break down organic material into a black coloured humus substance.

In the early stage of composting mesophiles consume part of soluble carbohydrates resulting in the formation of more biomass and release of heat.

Due to this phenomenon, heap temperature is raised and this favours growth of thermophylic organisms.

Addition of water helps in maintaining moisture content in the range of 50-60% which helps in cooling the heap. A small addition of cowdung slurry helps as a seed for composting.

Breakdown of proteinaceous matter leads to liberation of ammonia and rise in pH.

Turning of heap at regular intervals helps in aeration and enhance the composting rate.

Composting is complete in about 35 to 45 days time.

NADEP method has been adopted for composting of our mycelium.

In this method heaps of 1m height by 1m breadth and suitable length are made.

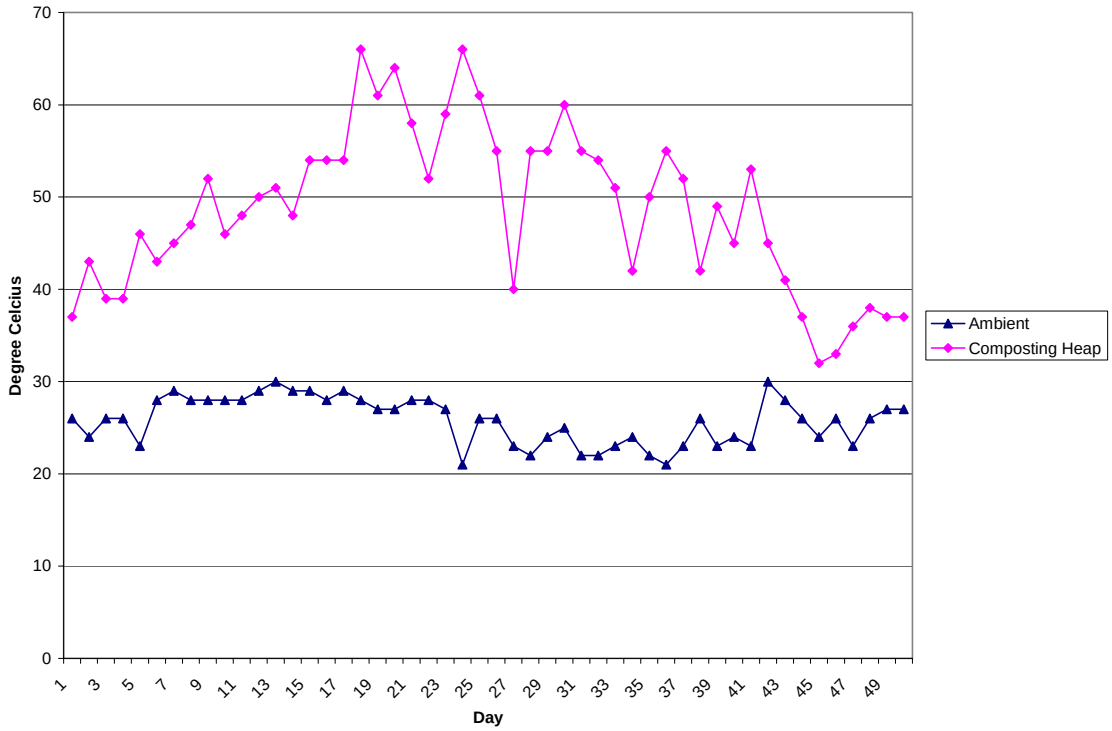
In this heap, alternate layers of grass and fresh mycelia are spread.

Cowdung slurry is added for starting the composting process.

Heaps are covered by jute bags and the moisture content is maintained between 50-60%.

Holes provided in the heap as well as turning of the heap help in aeration.

Figure 1: Temperature Profile



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