WALK-THROUGH SURVEY REPORT:

CONTROL TECHNOLOGY FOR FERMENTATION PROCESSES

AT

Wyeth Laboratories, Inc.
West Chester, Pennsylvania

REPORT WRITTEN BY:
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NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH
Division of Physical Sciences and Engineering
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PLANT SURVEYED:	Wyeth Laboratories, Inc. P.O. Box 565 611 East Nield Street West Chester, Pennsylvania 19380
SIC CODE:	2833
SURVEY DATE:	November 8, 1983
SURVEY CONDUCTED BY:	Kenneth F. Martinez James H. Jones
EMPLOYER REPRESENTATIVES CONTACTED:	T. Frank Gannon, Managing Director John A. Moffett, Manager, Fermentation Section Mary Lou Dodd, Industrial Hygienist
EMPLOYEE REPRESENTATIVES CONTACTED:	No Employee Representatives

I. INTRODUCTION

The National Institute for Occupational Safety and Health (NIOSH) is the primary Federal agency engaged in occupational safety and health research. Located in the Department of Health and Human Services (formerly DHEW), it was established by the Occupational Safety and Health Act of 1970. This legislation mandated NIOSH to conduct a number of research and education programs separate from the standard setting and enforcement functions carried out by the Occupational Safety and Health Administration (OSHA) in the Department of Labor. An important area of NIOSH research deals with methods for controlling occupational exposure to potential chemical and physical hazards. The Engineering Control Technology Branch (ECTB) of the Division of Physical Sciences and Engineering has been given the lead within NIOSH to study the engineering aspects of health hazard prevention and control.

Since 1976, ECTB has conducted a number of assessments of health hazard control technology on the basis of industry, common industrial process, or specific control techniques. Examples of these completed studies include the foundry industry; various chemical manufacturing or processing operations; spray painting; and the recirculation of exhaust air. The objective of each of these studies has been to document and evaluate effective control techniques for potential health hazards in the industry or process of interest, and to create a more general awareness of the need for or availability of an effective system of hazard control measures.

These studies involve a number of steps or phases. Initially, a series of walk-through surveys is conducted to select plants or processes with effective and potentially transferable control concepts or techniques. Next, in-depth surveys are conducted to determine both the control parameters and the effectiveness of these controls. The reports from these in-depth surveys are then used as a basis for preparing technical reports and journal articles on effective hazard control measures. Ultimately, the information from these research activities builds the data base of publicly available information on hazard control techniques for use by health professionals who are responsible for preventing occupational illness and injury.

The public debate over genetic engineering has focused on the possible hazards of genetically modified microorganisms, potential health hazards to workers involved with industrial applications of recombinant DNA (rDNA) techniques, and the potential uses of such technology. Several risk assessment experiments designed to investigate some of the characteristics of proposed host-vector systems which might affect hazard potential have been conducted. Likewise, the benefits of rDNA technology are being as vigorously promoted.

This particlular research effort was prompted by the anticipated surge of rDNA techniques in various industrial processes. Genetic engineering technology may be utilized in various manufacturing processes in the areas of agriculture, organic chemicals, energy, food processing, and pharmaceuticals. This potential growth and the possibility of uncharacterized occupational exposures indicate the necessity for careful evaluations of health risks. NIOSH is accustomed to examining new technologies for potential occupational hazards and developing recommendations for safeguarding the workers health.

Implementation of safeguards and protective engineering controls early in the growth of an industry can only minimize occupational health problems and avoid expensive retrofitting of production systems.

NIOSH is currently evaluating the potential occupational hazards involved with distinct applications of biotechnology and rDNA techniques. ECTB's involvement in this evaluation is the development of a study investigating the control technology being used to prevent occupational health hazards in the fermentation industry. This assessment will attempt to identify effective controls applicable to processes involving potentially hazardous microorganisms, innate as well as genetically modified, toxic processing chemicals, and biologically active products or intermediates. Documentation of effective controls and recommendations to minimize exposure in the fermentation industry will be accomplished through this assessment. The fermentation processes control technology assessment will impact imminent rDNA fermentation technology in addition to current fermentation technology.

This report contains results of this preliminary study, conclusions, and/or recommendations relevant to the operations at Wyeth Laboratories, Inc., a manufacturer of penicillin. This survey was conducted as one of a series of initial preliminary surveys of firms involved in fermentation processes. Based on the information obtained during these walk-through studies, potential candidates for in-depth survey sites will be selected. The in-depth surveys will involve more detailed evaluations of the engineering controls, personal protective equipment, employee work practices, and industrial hygiene and medical monitoring.

II. PLANT AND PROCESS DESCRIPTION

Plant Description:
Wyeth Laboratories, Inc., a Subsidiary Division of American Home Products, began producing penicillin in West Chester, Pennsylvania in 1943. At that time, Wyeth used a surface culture fermentation process. Since 1943, the surface culture equipment has been supplanted with large deep-tank reactor vessels (conventional submerged culture fermentor tanks) and in 1979 these vessels were replaced with larger capacity vessels. The fermentation process (not the recovery process) was purchased by Fermenta Products, Inc. in January 1984. The crude penicillin broth was extracted and processed into a penicillin salt by Wyeth until July 1984. Since that time Fermenta has recovered the penicillin.

Wyeth employs 16 hourly operators working in 4 shifts, 24 hours a day, 7 days per week. They also employ 4 production supervisors and 1 production manager.

Process Description:
The processes surveyed at Wyeth involve the production of penicillin V and penicillin G using the microbial strain Penicillium chrysogenum. This strain of Penicillium is non-pathogenic. The manufacture of penicillin is accomplished in four process steps: laboratory, inoculation, fermentation, and recovery. The inoculation and fermentation process steps are conducted in the same building, but the laboratory and recovery process steps are conducted in separate, adjacent buildings. The fermentation process is computer controlled and fully automated.

The laboratory process step includes the selection and maintenance of the microorganism cultures. The selection or screening process for microorganisms determines the culture to be used for the penicillin production process based on their ability to produce a desirably commercial quantity. Selected cultures must be identified and tested for pathogenicity and desired inability to co-produce harmful products or toxins. To help insure this, Wyeth must adhere to those regulations set forth by the U.S. Food and Drug Administration including the Good Manufacturing Practices guidelines. Maintenance of the selected microbial strain must ensure that this isolated culture is a pure, uncontaminated culture medium before being inoculated into the germination tank. This requires that the culture be regrown at intervals. The Penicillium spores (stored in a test tube of sand and rice) are cultured in a small bottle of medium for one week. The culture is then further propagated in corn steep liquor in an aspirator bottle. This bottle is placed in a controlled environment transfer room. The microbial culture is transferred manually and aseptically inoculated into the germination tank through a rubber tubing with nipples at either end.

The raw materials used to prepare the nutrient medium are composed of primarily liquids contained in holding tanks outside the building. They consist of a protein source (corn steep liquor), a sugar source (corn syrup), lard oil (used as an anti-foaming agent), sodium hydroxide, and potassium phenyl acetate. Solid components used as precursors include calcium carbonate and sodium carbonate. The raw materials are weighed and then combined in the Slurry Room. The raw materials, now the nutrient medium, is sterilized and

pumped to a heat exchanger -- the exchange of heat is used to sterilize the microbial culture.

The inoculation process step is segmented into two parts. In the first part, a 200 gallon germination tank, containing the sterile nutrient medium, is inoculated with the selected Penicillium culture prepared in the laboratory. The germination tank is designed to promote the initial logarithmic growth of the microbial biomass from the aspirator bottle. The batch mixture is aerated and mechanically agitated until the optimum volume of biomass is achieved. The final contents of the germination tank is aseptically transferred through an enclosed pipe network to the second part of the inoculation process step. In the second part, a 2000 gallon seed tank, containing the sterile nutrient medium, is inoculated with the microbial biomass mixture from the germination tank. Again, this mixture is mechanically agitated and aerated, continuing the logarithmic growth of the biomass, until the optimum level is achieved for proper fermentation in the fermentor tank. The contents of the seed tank is transferred through a pipe network to the fermentation process step.

The fermentation process step is where "fermentation" essentially occurs and the antibiotic of interest is biologically synthesized. A submerged, batch fermentation process is employed using a standard deep-tank reactor vessel with a top-mounted mechanical agitator (400 horsepower) and a bottom air sparger. Proper temperature conditions are maintained with cooling coils around the reactor vessel. Sterile air is passed through a 0.2 μm filter is used to aerate a producing microbial batch. Prior to inoculation, the fermentor tank is sterilized empty at 125°C for 40 minutes. The pre-sterilized nutrient medium is added to the fermentor tank during the cooling process. The fermentor tank is then inoculated with the biomass broth from the seed tank. This new broth mixture is aerated, mechanically agitated, and allowed to ferment for continued microbial growth and production of the desired penicillin base. Carbohydrates are fed continuously into the fermentor tank during fermentation. A continuous steam seal (20 pounds of pressure) is kept on all process lines. The composition of the biomass mixture is carefully controlled to promote maximum growth of the organism and/or penicillin production. Manual samples are extracted every 4 hours from a steam sealed port valve on the fermentor for analysis of batch culture morphology. Other process conditions are monitored and controlled by a central computer.

The recovery process step is segmented into two separate phases; separation and extraction. During separation, the penicillin/microbial broth is pumped to a rotary vacuum drum filter where a major portion of the suspended solids (mycellium and other solids) are separated from the penicillin liquid. The sludge is transported to a Parkson press to squeeze more liquid from the filter cake. The penicillin liquid is centrifuged for further concentration. The solid waste is taken in a dumpster to a landfill and any liquid waste is pumped to the plant treatment facility. During extraction, the pH of the penicillin liquid is reduced to 2. Amyl acetate is added to the broth in preparation for the counter-current extraction procedure to obtain a penicillin rich amyl acetate solution. Periodically, a carbon filter is used to remove impurities. The penicillin is crystallized with a continuous crystalizer and then basket centrifuged with an acetone wash. The penicillin

is dryed with a continuous (blanketed) tray dryer. The dryed crystals of penicillin are sent to a blender for homogenizing and then drummed. The finished penicillin is sold as a salt product.

The remaining liquid is pumped to another process for solvent recovery.

Potential Hazards:
The potential for exposure to hazards in the occupational environment within the fermentation industry is a three-fold problem. Exposure may involve potentially hazardous microorganisms (innate as-well-as genetically modified) toxic processing chemicals, and biologically active products or intermediates.

Presently, the microorganisms used by the penicillin industry, inclusive of the overall fermentation industry, for fermentation operations are non-pathogenic in nature. But future involvement with rDNA technology may produce microorganisms in need of more stringent containment requirements and equally stringent programs in occupational safety and health due to the increased health risks that they pose to the exposed worker. As indicated above, the microorganism utilized in the process surveyed at Wyeth for their penicillin operation (Penicillium chrysogenum) is a non-pathogen.

Acid and base compounds are used to adjust pH levels of biomass broth mixtures or concentrated penicillin liquids throughout the penicillin production process; acids are corrosive and irritating, whereas, base compounds are caustic and will cause burns. Dependent upon the compound being used and its degree of hazard potentiality, protective clothing should be worn and the appropriate control techniques implemented to prevent potential contact or exposure to these agents.

Amyl acetate is used as an extractant of penicillin from the fermentation broth in the recovery process step. Amyl acetate can have bodily effects if it is inhaled, contacts the eyes or the skin, or is swallowed. Overexposure may cause irritation of the eyes, nose, and throat with prolonged overexposure causing irritation of the skin. Severe overexposures may cause weakness, drowsiness, and unconsciousness. The current OSHA standard for amyl acetate is 100 ppm averaged over an eight hour workshift.

Acetone is used as a wash for the penicillin during basket centrifugation in the recovery process step. Repeated contact exposure (percutaneous absorption) to acetone may produce dry, scaly, and fissured dermititis. Inhalation of high concentrations of acetone vapors may irritate the conjunctiva and mucous membranes of the nose and throat. Systemic reactions to high concentrations include narcosis with symptoms of headaches, nausea, light headedness, vomiting, dizziness, incoordination, and unconsciousness. The current OSHA standard for acetone is 1000 ppm averaged over an eight hour workshift.

Penicillin can cause immunologic responses in susceptible persons due to inhalation of the penicillin molecule. It has been estimated that 8% of the population is allergic to Penicillin G. Reactions may vary from mild to severe depending on the particular individual exposed. There are currently no workplace standards for penicillin aerosols or dust.

III. CONTROLS

PRINCIPLES OF CONTROL

Occupational exposures can be controlled by the application of a number of well-known principles, including engineering measures, work practices, personal protection, and monitoring. These principles may be applied at or near the hazard source, to the general workplace environment, or at the point of occupational exposure to individuals. Controls applied at the source of the hazard, including engineering measures (material substitution, process/equipment modification, isolation or automation, local ventilation) and work practices, are generally the preferred and most effective means of control both in terms of occupational and environmental concerns. Controls which may be applied to hazards that have escaped into the workplace environment include dilution ventilation, dust suppression, and housekeeping. Control measures may also be applied near individual workers, including the use of remote control rooms, isolation booths, supplied-air cabs, work practices, and personal protective equipment.

In general, a system comprised of the above control measures is required to provide worker protection under normal operating conditions as well as under conditions of process upset, failure, and/or maintenance. Process and workplace monitoring devices, personal exposure monitoring, and medical monitoring are important mechanisms for providing feedback concerning effectiveness of the controls in use. Ongoing monitoring and maintenance of controls to ensure proper use and operating conditions, and the education and commitment of both workers and management to occupational health are also important ingredients of a complete, effective, and durable control system.

These principles of control apply to all situations, but their optimum application varies from case-to-case. The application of these principles at Wyeth is discussed below.

Engineering Controls:
Wyeth's penicillin production operation is a predominantly closed system once the process has graduated from the laboratory to the large-scale fermentation process steps. There appears to be limited potential for exposure to the microorganisms involved in the fermentation processes or the penicillin products of these microorganisms. All growth and holding tanks are closed during process operations. Batch broth mixtures or concentrated liquid penicillin are transferred between separate unit operations by a steam sterilized pipe network. Employee contact with the production process operation is minimal other than for equipment maintenance or manual broth sample extraction. There is minor potential for release of aerosols containing viable microorganisms and/or penicillin during manual sampling procedures and circumjacent to the agitator shafts, but the quantities involved pose minimal contact or exposure concerns. Potential aerosolization of liquid penicillin concentrates or microorganisms into the occupational environment is apparent around the rotary vacuum drum filter and possibly around the centrifuge (or any high any energy operation).

The majority of the large-scale process operations are either controlled or monitored by a central computer system This "automation" aids in limiting direct employee involvement, and therefore potential hazard exposure or contact, with the process operations.

Work Practices:
Wyeth utilizes a pre-employment training program to orient new employees with process equipment use, operation, and safety procedures. This training program is divided into three sub-programs; safety, fermentation, and extraction. The safety program is mainly concerned with product and raw materials contact. The fermentation sub-program is concerned with equipment operation and material hazards during the fermentation process step including acids and bases, relatively non-hazardous agents, and external skin (epidermal) agents. The extraction sub-program is concerned with equipment operation and materials hazard during the extraction process step involving solvent usage. Training is continued periodically to keep employees current on equipment use.

Monitoring:
The environmental health program in effect at Wyeth is monitored by the plant industrial hygienist. There is currently no routine viable sampling program but viable sampling has been conducted on limited basis in the past. Area and personal air samples (3-M passive dosimeters) are conducted annually for amyl acetate and acetone, where applicable.

Medical examinations are made available to fermentation and extraction process workers but are not required. These examinations include annual pulmonary function, audiometric, and chemical screening tests and bi-annual chest X-rays.

Personal Protection:
Wyeth production process workers are supplied with daily clothing changes. Safety shoes and glasses are supplied but are not required except where their use around certain process operations is explicitly stated. Disposable dust respirators are provided (not required) for raw material dumping stations. Gloves and head covering garments are also provided for those employees working at the penicillin bagging operation. Breathing air systems are located through-out the process building in case of fermentor coolant (ammonia) leaks or spills.

IV. CONCLUSIONS AND RECOMMENDATIONS

Wyeth has provided, for the employees, an occupational environment with an exhibited concern for worker safety and health. An outgrowth of this management attitude, in combination with current state-of-the-art technology, is a seemingly effective system of control measures. These control measures are, in part, responsible for the limited potential for exposure to the microorganisms, process chemical intermediates, and/or the biological products of the penicillin operation. Although occupational health and safety conditions appear to be reasonably-well controlled at Wyeth, an in-depth survey of this plant is not recommended for this Control Technology Assessment since it has been recently focused on the enzyme manufacturing industry alone. However, Wyeth should be recommended for future studies of controls in the fermentation industry.