Walk-Through Survey Report
of
New England Bio-Labs
Beverly, Massachusetts

Survey Date
December 8, 1981

Report Written By
Larry Elliott
Ken Wallingford

Survey Team:
Larry Elliott
Ken Wallingford
Mike Crandall
Wes Straub

Date of Report:
February 22, 1982

Report # 131.13

Industrial Hygiene Section
Industrywide Studies Branch
Division of Surveillance, Hazard Evaluations
and Field Studies
National Institute for Occupational Safety and Health
Centers for Disease Control
Cincinnati, Ohio

Place Visited:	New England Bio-Labs Beverly, Massachusetts
Date of Visit:	December 8, 1981
Persons Conducting Survey:	Larry Elliott Ken Wallingford Mike Crandall Wes Straub
Employer Representative:	Donald Combs Owner
Union:	No union
Standard Industrial Classification of Plant:	2831 Biological Products
Purpose of Survey:	To investigate and evaluate New England Bio-Labs methods of containment, environmental monitoring, and health surveillance for production/research processes utilizing or involving microorganisms and/or recombinant DNA techniques.

Abstract

On December 8, 1981 a walk-through industrial hygiene survey was conducted at the New England Bio-Labs, Beverly, Massachusetts. This company produces enzymes, by a fermentation process, which are used in genetic and biomedical research. This company is also conducting some limited research involving recombinant DNA. Assessments were made during the visit of fermentation process control technology, of laboratory and work practices, and of health and safety programs. This company had not developed an organized health and safety program, work practice guidelines, or any type of medical program. The report contains recommendations made to strengthen specific areas in order to minimize potential health and safety hazards.

Introduction

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 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 recombinant DNA technology are being as vigorously promoted.

The National Institute for Occupational Safety and Health (NIOSH) is responsible for conducting research, recommending occupational safety and health standards, providing technical assistance to workers and employers, and conducting training and education programs. NIOSH's responsibility extends to both existing and emerging technology which might impact on worker health and safety. Thus, NIOSH is evaluating the potential occupational hazards involved with distinct applications of biotechnology and recombinant DNA techniques. NIOSH's interest in biotechnology encompasses innate as well as genetically modified microorganisms, the biological products of these organisms, and the product extraction processes.

This particular research effort was prompted by the anticipated surge of recombinant DNA 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 human suffering and avoid expensive retrofitting of production systems.

Under the Occupational Safety and Health Act of 1970, Public Law 91-596, the National Institute for Occupational Safety and Health (NIOSH) was mandated and authorized to conduct research and health studies. Specifically, Section 20 (a) 7 states that NIOSH shall conduct and publish industrywide studies of the effect of chronic or low level exposure to industrial materials, processes, and stresses on the potential for illness, disease, or loss of functional capacity. Thus, this research is pursuant to the development of health standards applicable to a broad range of occupational environments. In compliance with this mandate, the Industrywide Studies Branch of the Division of Surveillance, Hazard Evaluations and Field Studies is conducting a study to assess the potential occupational hazards of biotechnology and specifically the area of research and commercial application of recombinant DNA technology.

The Engineering Control Technology Branch of the Division of Physical Sciences and Engineering is conducting a study to examine the engineering control techniques being utilized in fermentation processes. This study will attempt to identify effective controls applicable to processes involving potentially hazardous microorganisms, toxic processing chemicals, or biologically active products. Documentation of effective controls and recommendations to minimize exposure in the fermentation industry will be made after the study is completed.

In order to analyze the current technology for potential occupational hazards and to identify possible control technology assessment study sites, NIOSH has conducted walk-through surveys of six facilities utilizing recombinant DNA processes. Indentification of potential hazards, with recommendations to minimize worker exposure was derived from the information gained during the surveys. This report contains results of this walk-through survey and recommendations relevant to the operations at the New England Bio-Labs, a producer of enzymes for molocular biology. Recommendations applicable to the fermentation industry and industries employing recombinant DNA technology will be made after NIOSH completes the hazard assessments. In each facility, including New England Bio-Labs, an assessment of the following areas was made:

- Process operations with attendant potential for worker exposure
- Engineering controls (e.g. physical containment design, ventilation, exhaust gas filtration, waste product controls, etc.)
- Validation procedures (e.g., of sterilization, physical containment, and process termination)
- Work Practices
- Emergency and Accident Procedures
- Medical Surveillance
- Environmental Monitoring
- Numbers of Exposed Workers
- Employee Training and Education

This survey of New England Bio-Labs was conducted as one of a series of initial walk-through surveys of firms involved in biotechnology and/or the utilization of recombinant DNA techniques in research or production processes.

Description of The Company & Facility

The New England Bio-Labs are located in a newly constructed facility in Beverly, Massachusetts. New England Bio-Labs produce purified restriction endonucleases and other enzymes for use in genetic research. The company is independently owned and was started in 1974, producing oligonucleotides. The company has plans to expand the existing facility within the next year.

New England Bio-Labs produces their enzyme product line by fermentation processes. These fermentations are conducted in 10, 25, and 100 liter

fermentation vessels. The company is also involved in several research and development projects, one of which is recombinant DNA based. At the time of this survey no fermentation processes involved recombinant DNA techniques. The company was conducting the recombinant DNA research under voluntary compliance with the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA.

Description of Workforce

The New England Bio-Labs employ approximately 32 persons, of which 25 are research scientists or fermentation specialists. Seven personnel work at administrative or clerical duties. All of the research and fermentation specialists have advanced degrees with previous experience in research.

The employees work flexible hours on a daytime basis. The employees of New England Bio-Labs are not represented by a union.

Description of the Process

The various enzymes produced by New England Bio-Labs are made by batch fermentation processes. Depending upon the production quantity needed, a 10, 25, or 100 liter fermentation vessel may be innoculated with E. Coli from stock cultures. The fermentation vessels are innoculated by pouring the innoculation growth into the fermentor using aseptic techniques. The 25 liter fermenter is often used to innoculate the 100 liter vessel. After the growth phase of the fermentation process is completed the cells are harvested from the fermentation broth by centrifugation. The fermenter, centrifuge, and appurtenant piping and equipment are located in a room designated as the fermentation area.

After harvesting, the cell paste is frozen at -20°C to await the enzyme extraction and purification processes. The waste effluent from the centrifuge is inactivated with a chlorine solution and then drained to a septic tank.

The harvested bacterial cells are removed from the freezer and the cells are lysed by ultrasonication to release the intracellular fluid. The broken cells are centrifuged and the cell mass is flushed to the sewer. The supernatant of the centrifugation contains the enzymes which must be further extracted and purified via column chromatography and ion exchange. The enzyme as a final product is packed in a glycerol solution to prevent freezing when stored and shipped at -20°C.

Small quantities of various organic chemicals (e.g. ether, chloroform, piridines, etc.) and radionucleotides are used in the research and the product extraction phases at New England Bio-Labs. Potential for employee exposure exists to these agents in small quantities (micro to milligram equivilants) as well as stored quantities (liters).

The enzymes produced by fermentations at New England Bio-Labs represent milligram quantities per batch; one pound of enzyme would probably cost several thousands of dollars. Because the fermentation process is small scale with no pathogenic or recombinant organisms involved the management of New England Bio-Labs operates these processes at P-1 or less containment.[1]

Description of Industrial Hygiene, Safety and Medical Programs

Safety and Health

The company does not have an organized health and safety program. Because the production staff is small, there is no safety committee or scheduled safety meetings. The company does have on retainer a health physicist as a radiation consultant.

New England Bio-Labs has established an Institutional Bio-Safety Committee (IBC) in compliance with the NIH guidelines. The IBC is composed of 6 members; the owner of the company, the lab director, a bio-chemist, a physician with the local health department, a bacteriologist from a local hospital, and a college dean. This committee has been in existence for one year and meetings are held on an unscheduled basis. No formal chain of command has been designated by the company; the biosafety officer did not have a designated alternate.

Work practice guidelines have not been developed for specific procedures in the laboratory or production area. Employees are provided with laboratory coats, safety glasses, and a clothes change and shower area.

Industrial Hygiene

This company does not have an industrial hygienist on staff. Environmental monitoring has not been conducted in the research laboratory or production areas.

Medical Program

The New England Bio-Labs do not provide or require employees to take a pre-employment or periodic medical examination. No job related medical evaluations are performed on the employees. Serum banking is not conducted for employees working with recombinant DNA.

The company did not have any definite requirements for the reporting of accidental innoculation, ingestion, probable inhalation, lacerations or abrasions. There is no follow-up of employee illness to determine if it is job related. New England Bio-Labs does have an employee qualified in first aid and CPR for provision of emergency treatments.

Description of Past Exposures

Data on exposure of employees to organic chemicals and/or microorganisms does not exist since environmental or medical monitoring is not conducted at the facility.

Evaluation of the Plant Controls and Programs

Physical Containment Design and Engineering Controls

The 10, 25, and 100 liter fermentation vessels are contained in the fermentation laboratory and all three are used at various times for the production of small quantities of product enzymes. The 10 liter fermenter is a glass, bench-top model which is normally used for seed cultures. The 25 and 100 liter fermenters are pilot-size vessels and were manufactured by New Brunswick Scientific Co., Inc. The 100 liter fermenter was custom-modified for specific use at this facility and the 25 liter vessel is often used to inoculate the larger fermenter. Both pilot-sized fermenters are stainless steel jacketed pressure vessels with incorporated automatic and manual controls for the operation of the fermentation process. Both are equipped with top-mounted agitator (single seal design), air sparger, and steam sterilizable inoculation/sampling ports. There is no high fluid level alarm on either of these fermenters. Prior to each use, the fermenters are normally sterilized with steam. Additionally, both have packed glass wool filters for inlet and exhaust air. There is currently no program for either the routine cleaning or changing of these filters.

Two centrifuges are used to harvest the cells from the culture. They both are non-hermetically sealed units with self-discharging capability. The centrifuges are hard-pipe connected to the 25 and 100 liter fermentation vessels.

The fermentation laboratory is adjacent to the larger laboratory facility and is entered through a door which is normally open. At the present time, there are no provisions for controlled access to the fermentation lab. Within the fermentation lab is a smaller room which houses the two centrifuges. Both rooms involved in the fermentation process are ventilated with the recirculating general dilution conditioned air system which accommodates the entire facility. The fermentation laboratory is not under negative pressure during the normal operation of this ventilation system.

A floor drain in the center of the fermentation laboratory is used for the disposal of liquid wastes resulting from the fermentation process. There are currently no documented procedures for waste disposal. Additionally, the fermentation lab is not diked to contain accidental spills or leaks possible during the fermentation.

A room adjacent to the fermentation lab contains the autoclave which is used in the routine sterilization of the glassware and accessories used in the fermentation process.

The biochemical separation and purification processes such as ion exchange, affinity column, diffusion, and filtration are performed in upright glassdoor freezers kept at 0 to 4 ^{o}C. This operation takes place in the large laboratory area. The freezers are not ventilated.

Validation Procedures

Validation procedures are not used by the company to ensure the efficacy of the physical containment or engineering controls in place for the fermentation process. No pressure tests are performed on the fermenters to establish their integrity prior to use. No aerosol monitoring is performed routinely in the fermentation lab during fermentations to detect viable aerosols. No routine maintenance is performed on either of the centrifuges. No measurements are taken to ensure the efficiency of the ventilation system supplying air to the fermentation lab. Prior to discharge in the drain, the supernatant is inactivated with chlorine. Also, no validation procedures are performed for the autoclave.

Work Practices

Specific guidelines for safe work practices and laboratory techniques have not been developed by the company. Evidence of drinking, smoking in the laboratory area and the storing of beverages in laboratory refrigerators was noticed during the survey. Laboratory coats were not consistantly used by the employees as several employees were observed working without laboratory coats. Safety glasses and/or goggles were not observed in use at the time of the survey. Hearing protection, however, was required during the ultrasonication of the harvested cell mass.

Operators of the fermentation systems and centrifuge appeared to be well trained in the use of the equipment and the process methods. Distinct process protocols documenting process operations had not been developed. Cleaning of the laboratory and production areas was conducted by the employees in these areas.

Emergency and Accident Procedures

Safety precautions and plans for emergencies, such as laboratory accidents, have not been outlined and documented by the company.

Conclusions

New England Bio-Labs have provided, for the employees, an aesthetic environment with a relaxed and unconventional management approach. This company exhibits a relaxed attitude toward developing safety and health

programs. This attitude is based upon the contention that employees are working with characterized strains of viable organisms not known to cause disease in healthy humans. There are, however, laboratory practices and supervisory control aspects applicable to the New England Bio-Labs operations (1, 2, 3, 4). These guidelines not only provide sound recommendations, but also the objectives and reasoning behind incorporating bio-safety and health control measures into a research or production program. The recommendations provided by these references cover handling of non-pathogenic microorganisms, potentially hazardous chemicals and radioactive materials; concerns which New England Bio-Labs should recognize.

General experience has demonstrated that safe conduct of research or production processes involving hazardous entities is dependent on good work practices, availability and use of effective control technology, and effective management. Microbiological research requires containment features sufficient enough not only to exclude entry of contaminants into, but also prohibit escape of organisms from the system. Thus, attention to exposure control must receive equal emphasis as process integrity and production development. Safety and health, medical surveillance, and emergency programs should to be implemented and managed with the same degree of attention that the scientific production programs receive.

Recommendations

Safety and Health Programs

New England Bio-Labs needs to implement safety and health, medical surveillance and emergency programs and procedures. These should be documented, understood by all concerned, and specific for the company's operations. Emergency guidelines, laboratory practices, personal hygiene habits and practices, protective clothing and equipment requirements, maintenance procedures, and medical surveillance are some specific areas where documented guidelines are needed. Such guidelines will serve only as well as they are responsibly implemented. Without effective management and supervisory control these guidelines will only be ideals and not practices.

Medical Surveillance Programs

A medical program should consist of preemployment and annual physical examinations targeted with specific indicators in order that employees be monitored for abnormal health effects. Employees reporting to work from sick leave (after 3 or more days) should have a medical explanation of the illness. Employee records should be maintained and include notes on all illnesses, physical disorders, and accidental exposures. Uncertainty provides the strongest arguement for maintaining medical surveillance over workers engaged in recombinant DNA research[5]. Occupational exposures elsewhere in the biotechnology and pharmaceutical industries have produced a variety of illnesses and thus point to the need for medical surveillance[6].

Biological monitoring of worker body fluids for specific organisms carrying recombinant DNA molecules is costly and technically demanding. Monitoring of this nature is also not appropriate for the majority of employees at New England Bio-Labs. Quantitative and qualitative sampling of air and surfaces in the production and laboratory areas can be an effective measure of sanitation, environmental conditions, and efficacy of work practices. Environmental sampling programs for microorganisms, chemical agents and radioactivity have been utilized in hospitals, the food and chemical industries and containment laboratories for years. The principles of these programs are applicable to operations at New England Bio-Labs. The efficacy, sensitivity, and accuracy of environmental sampling and analytical techniques should, however, be considered and their validity must be established prior to utilization. The analysis procedure, sampling points and sampling schedules, thus established, should be formalized in writing.

Preventive Maintenance

A systematic scheduled maintenance program should be developed for the fermentation and centrifugation systems. The manufacturer of the fermenter should be asked to supply data on the failure rate or working life of the agitator seals. This data in consideration of the running time, operating pressure and temperature should be used to devise a preventive maintenance program for the fermenter. Since the harvesting of the bacterial cells occurs while they are still viable the centrifuge should be operated and controlled to prevent aerosolization of the organisms. Even though the organisms currently used are non-pathogenic, the handling of large volumes of microorganisms merit respect and precautionary measures. Implementation of such attitudes and precautions will smooth the transition to the use of more hazardous or virulent organisms.

Emergency and Accident Procedures

Plans should be developed for environmental emergencies, laboratory accidents, fire, and utilities failure. These should be documented and well understood by all employees.

Work Practices

Laboratory coats should be worn when conducting research experiments involving agents of no known or minimal potential hazard. This not only will aid in the protection of the researcher but also prevent contamination of the project.

Persons should wash their hands after handling viable materials, animals or when they leave the laboratory area.

Eating, drinking, smoking, the storing of food or beverage, and applying cosmetics should not be permitted in the work areas.

All procedures should be carefully performed to minimize the creation of aerosols.

REFERENCES

1. Laboratory Safety Monograph; A Supplement to the NIH Guidelines for Recombinant DNA Research. USDHEW, PHS, NIH. January, 1979.

2. Proposed Biosafety Guidleines for Microbiological and Biomedical Laboratories. UDHHS, PHS, CDC. Office of Bio Safety. 1980.

3. Green, M.E. and Turk, A. Safety in Working with Chemicals. The MacMillan Co., Inc., New York (1978).

4. The National Institutes of Health Radiation Safety Guide. DHEW, PHS, NIH. Publication No. 79-18 (1979).

5. National Cancer Institute, Office of Biohazard Safety; Medical Surveillance Programs - A Review with Recommendations. Bethesda: National Cancer Institute, July, 1980.

6. Medical Surveillance of Recombinant DNA Workers: Report of the CDC/NIOSH AD HOC Working Group of Medical Surveillance for Industrial Applications of Recombinant DNA. DHHS, PHS, CDC, NIOSH, Submitted to Director, Officer of Recombinant DNA Activities, NIH, January, 1982.