Recombinant DNA

Final Report

176.4

INDUSTRIAL HYGIENE CHARACTERIZATION OF COMMERCIAL APPLICATIONS OF GENETIC ENGINEERING AND BIOTECHNOLOGY

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Cincinnati, Ohio 45226
September, 1983

TITLE: Industrial Hygiene Characterization of Commercial Applications of Genetic Engineering & Biotechnology

SHORT TITLE: Genetic Engineering in Biotechnological Processes.

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ACKNOWLEDGEMENT

The authors thank Ms. Susan Kaelin, Mrs. Ruth McGrath, Mrs. Nancy Rosenberger, Mrs. Marianne Fleckinger, and Mrs. Nancy Beck for their help in preparing this manuscript.

ABSTRACT

NIOSH conducted walk-through industrial hygiene surveys at six companies involved in research and development of recombinant DNA techniques applicable to fermentation processes. Commercial application of recombinant DNA technology was evaluated by NIOSH because of its high growth potential, and to identify and prevent hazards during early stages of development. During each visit, the potential for worker exposure, engineering controls, work practices, and medical surveillance programs were assessed. Varying degrees of comprehensiveness, among the companies, were noted in all of the assessed areas. New companies in the field of fermentation technology, and industrial-type operations, were less sophisticated in approach to safety and health concerns than companies with years of experience in industrial operations. Recommendations were made in the areas of containment controls, environmental monitoring, medical surveillance, and preventive maintenance programs.

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INTRODUCTION

The public debate over genetic engineering has focused on the possible environmental hazards of genetically modified organisms, potential health hazards to workers involved with industrial applications of recombinant DNA techniques, and the potential uses and prospects of such technology. Various risk assessment programs designed to investigate some of the characteristics of proposed host-vector systems which might effect hazard potential have been conducted (1, 2, 3, 4). Likewise, the benefits from recombinant DNA technology are being vigorously promoted (5, 6, 7, 8).

During 1981, it became quite apparent that biotechnology will become a major factor in both the development of new products and the displacement of traditional methods of chemical synthesis. Biotechnology, the use of biological systems in industrial processes, has been used by several industry segments for many years. Recombinant DNA and genetic engineering as a whole are tools recently used in biotechnology to improve the speed, efficiency, and productivity of biological systems. It is these techniques that promote the vast potential for utilization of biotechnology in various manufacturing processes in the areas of argriculture, organic chemical synthesis, energy, food processing, and pharmaceuticals. Along with the potential utilization, emerge factors such as widespead process change, intense competition, extremely rapid transition from laboratory research to commercial applications, and the utilization of biotechnological processes by industries not accustomed to employing biological organisms. These new applications of biotechnology are in the preliminary stages of capitalization and development. Thus, industry, labor, and government are offered the unique opportunity to see industrial genetic engineering applications developed and conducted in a safe manner. Recommendations for safeguarding health can be most cost-effective if incorporated in the initial stages of process design. Certainly, implementation of safeguards and protective engineering controls early in the growth of an industry will minimize human suffering and avoid expensive retrofitting of production systems.

The National Institute for Occupational Safety and Health (NIOSH) is responsible for conducting research in occupational health, for recommending occupational safety and health standards, for providing technical assistance to workers and employers, and conducting training, educational, and surveillance programs. NIOSH's responsibility extends to both existing and emerging technologies. NIOSH evaluates new technologies to determine the existence or nonexistence of associated occupational health and safety hazards. Particular attention is paid to technologies which have high growth potential, or technologies for which exposures have not been characterized in order to identify and prevent hazards during early stages of technology development. Thus, NIOSH has evaluated the emerging commercial applications of biotechnology and recombinant DNA technology for potential occupational hazards.

NIOSH has been participating in the various Federal committees concerned with the safety of recombinant DNA research. NIOSH's involvement increased with the initiation of commercial applications and the potential for a greater number of workers to be exposed. Discussions of specific details of production or pilot-scale operations revealed the lack of information concerning potential risks to the workers in the field.

For example, the effectiveness of proposed and currently used containment devices and work practices (control technology) in safeguarding the worker from hazardous or protentially hazardous exposures was questioned. This question extends to the entire process, including processing chemicals, by-products of reactions, and end-products.

Another concern was what constitutes an appropriate medical surveillance program and when it should be applied. Those working with recombinant DNA processes will include a variety of job classifications such as scientists, technicians, equipment operators, and maintenance and custodial personnel. The varied nature of potential host-vector systems and the multitude of biologically active products therefore, make the establishment of an appropriate and effective medical surveillance programs a complex issue. The medical surveillance programs that do exist vary greatly, due to the lack of industry consensus on the aims and necessary components of such a program.

In order to address these questions and evaluate the current technology for potential occupational hazards, NIOSH conducted walk-through surveys of facilities utilizing recombinant DNA processes. Three of the six companies visited were participating in the National Institutes of Health (NIH) large scale voluntary compliance program and had submitted proposals on projects utilizing more than ten liters of cultured organisms. The other three companies were utilizing smaller amounts of cultured organisms, but were leaders in research applications, and/or suppliers of intermediate products to companies involved in large scale production. In each facility an assessment of the following areas were made:

- potential for worker exposure based on process design and operations.
- engineering controls (e.g., physical containment design, ventilation, exhaust gas filtration, waste product controls, etc.)
- validation procedures (e.g., of sterlization, physical containment, and process termination)
- work practices
- emergency and accident procedures
- medical surveillance and record keeping
- environmental monitoring

- number of exposed workers

- employee training and education

CURRENT UTILIZATION OF GENETIC ENGINEERING

Currently only two categories for the industrial utilization of genetically engineered organisms have been proposed. One involves distributing the organisms into selected environments to modify those environments.[6, 9, 10] The other involves the production of specific recoverable products from conventional fermentation processes with containment modifications voluntarily implemented to reflect the NIH Guidelines.[11] The NIH guilelines specify four levels of physical containment: P1-LS, P2-LS, P3-LS, and P4-LS (large scale). Each successive level of containment incorporates all requirements of the previous level(s). The level selected for a given procedure depends upon the level or degree of physical containment necessary for organisms or accessory product being handled, with P4 containment being the most stringent.

Examples of the first category include the "oil eating" organisms developed by Chakrabarty[10], organisms for mineral leaching and recovery, bacteria modified to consume and break down pollutants, and nitrogen-fixing organisms to improve crop yield.[7, 9, 10] These processes are currently far from commercial realization, therefore, this report will not discuss these applications. A full analysis of the ecological interactions and environmental impact of utilizing such organisms is required.

The second category, which includes large scale fermentations utilizing genetically altered organisms, was being conducted in the U.S. by three separate companies. One additional company is involved in application research and development, and two others are producing restriction enzymes. These were the only companies involved in Recombinant DNA research at the time of the NIOSH surveys, and all six companies were visited. A description of each company and production process may be found in Appendix A. In addition, a detailed description of the fermentation process may also be found in Appendix A.

GENERAL OBSERVATIONS

During the walk-through surveys, several observations were made that were not envisioned when reading the companies large-scale protocol submissions to the NIH. Recommendations relevant to the operations at each company surveyed were made and are contained in this report. It was found that years of experience in handling fermentation-type processes and active biological agents lead to more sophisticated approaches in dealing with containment. In companies less experienced in industrial-type operations, the approaches may be less sophisticated and their effectiveness less certain. This was noticeable in such areas as: inoculation of the fermenters, system validation design, training of equipment operators, and the development of process protocols and health and safety programs. It was observed that the need for rapid development of product lines can lead to insufficient emphasis on control and

detection of potentially adverse exposures. Specifically, improvised inoculation methods were used that could substantially increase exposure, when secure, e.g. closed systems, were available. It would appear that too much emphasis was placed on more secure aspects of the process, while an obvious weak link such as an inoculation procedure was in need of evaluation and redesign.

Another concern is the varying degrees of confidence in the validation procedures employed to determine culture inactivation and containment efficiency of the fermentation vessel. One company used thermocouples in remote parts of the fermenter to document through sterilization, while another company used a less thorough and less confident method employing indicator strips. Validation procedures for exhaust gas filtration systems, as well as the system designs, varied from company to company. One company continually checked and challenged the filtration systems while other companies relied on original validation data to insure a secure system. Two companies utilized incinerators after the high efficiency particulate air (HEPA) filters to assure complete exhaust gas sterilization.

No company has determined the efficiency, sensitivity, and accuracy of environmental sampling and analytical techniques that are used during production runs. The methods employed vary from company to company. Only one of the six companies had developed sampling protocols documenting procedures, sampling points, or sampling schedules. The prevailing practice, by the other companies, of placing a few settling plates around the fermenter did not supply meaningful quantitative data on the release of organisms from the physical containment equipment.

It was also observed that some facilites did not have a systematic preventive maintenance program for process equipment. Plans for process termination and equipment shutdown in response to power failure, emergencies, or natural disasters varied significantly between companies. Further descriptions of the facility designs and plant programs may be found in Appendix B.

It was observed that records of varying degress of comprehensiveness were being maintained on workers involved with recombinant DNA processes. The medical programs instituted by the companies also varied in objectives, essential elements, limitations, and applications. A complete description of Medical, Industrial Hygiene, and Safety programs employed by each company is contained in Appendix C.

Finally, diagrams of various plant processes are located in Appendix D.

CONCLUSIONS

The limitations of these walk-through surveys must be borne in mind. There was no industrial hygiene sampling conducted, only one production run at a facility was observed, and no observations were made of the extraction or purification procedures for the products of interest. As a result of these preliminary surveys, NIOSH believes further study will be required to

aderquately assess the need for and the effectiveness of control technology equipment and work practices. This will entail comprehensive engineering evaluations, industrial hygiene monitoring, and observation of production runs from inoculation through separation and purification to packaging of the product.

From these surveys, it became evident that some of the companies recognized the importance of safety and health programs and implemented these early in the initial development phase. Two of the six companies placed more emphasis on development of the production and scientific programs than safety and health, medical surveillance, and emergency programs. Although no tragic events occurred during, or as a result of, this inattention to safety and health programs, the opportunity did exist.

New developing companies need to recognize that health and safety programs should receive the same managerial attention and administrative support as the production and scientific programs. Guidelines and policies for laboratory procedures, work practices, protective clothing and equipment requirements, maintenance procedures, and medical surveillance 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. 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 designs sufficient not only to exclude entry of contaminents into, but also prohibit escape of organisms from the system. This is also applies to the use of chemicals and radiologically active agents. Thus, attention to exposure control must receive equal emphasis as process integrity and production development.

RECOMMENDATIONS

A medical surveillance program should consist of pre-employment and periodic follow-up physical examinations in order that employees may be monitored for abnormal health effects.[12] Follow-up examinations should not be comprehensive in nature but specifically designed around the host, vector or plasmid, and product process systems. A serological monitoring system should also be implemented and designed to meet the needs of the surveillance program. Immunizations should be considered for employees working with known pathogens. 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, operations, physical disorders, therapeutic medications, and accidental exposures.[12] Occupational exposures elsewhere in the biotechnology and pharmaceutical industries have produced a variety of illnesses, and thus point to the need for medical surveillance.[12]

Biological monitoring of worker body fluids for specific organisms carrying recombinant DNA molecules is costly and technically demanding. Monitoring of this nature may also not be appropriate in all cases. 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 control measures. Environmental sampling programs for microorganisms, chemical agents and radioactivity have been utilized in hospitals, food and chemical industries, and containment laboratories for years. The principles of these programs are applicable to the operations at each company examined. The efficacy, sensitivity and accuracy of environmental sampling and analytical techniques prior to utilization. The sampling procedures and analysis, sampling points, and sampling schedules, thus established, should be formalized in writing.

A registry of all workers employed with recombinant DNA processes should be established by each company and maintained for reference to determine any abnormal medical problems or unusual occurrences that someday may be associated with recombinant DNA processes. This registry should be linked to all other company records for an individual. NIOSH has had considerable experience in designing and maintaining such worker registries and can provide assistance in design, implementation, and logistical requirements. The comparability of individual company registries is desirable for optimum and successful surveillance on an industrywide basis.

A systematic scheduled preventive maintenance program for agitator seals, control valves, pressure relief valves, and equipment/facility safeguards should be implemented. Sterilization of fermenter, feed lines, feed tanks, inoculating device, exhaust ports, sampling ports, and extraction devices must be validated by a procedure that demonstrates effective sterilization.

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 more comprehensive validation and preventive maintenance program for the agitator seals. Such a system may prevent aerosolization or a spill due to failure of seal integrity. Even though the organisms currently used in recombinant DNA processes 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.

Containment controls could be enhanced by designing an unbreakable inoculating device for large fermenters. It should be capable of being sterilized after inoculating, but prior to detachment from the fermenter. The shake flask method of inoculating large fermenters is unwieldy and provides potential for exposure by accidental breakage, spillage, or aerosol production during detachment from the fermenter. Repositioning of the agitator drive and seals from the bottom of the fermenter could allow drainage of culture liquid resulting in a substantial exposure.

Physical containment of the fermentation process up to and including inactivation of the culture is mandatory. Physical containment of the biological material within a closed system should, preferably, also extend

through the cell lysis and product extraction phases. Capital investment for this would not only enhance production aspects but also would minimize potential for worker exposure to products and extraction solvents.

The P1-LS production area should be segregated from other production or research operations. This would maintain the integrity of the process and procedures outlined for recombinant DNA fermentation processes. Such system isolation will also enhance the containment of the entire operation to preclude accidental exposure to and from other employees and processes.

Specific procedures should be employed for handling hazardous chemical and radiological wastes. Only personnel trained in these procedures should handle the wastes. Protective clothing should be decontaminated or marked hazardous, so the laundry may handle these garments appropriately.

The use of formaldehyde as a disinfecting agent in laboratory areas merits certain precautions. Employees should wear rubber gloves while working with formaldehyde and when contact with the hands is likely. If the disinfection process involves occasional brief exposures to formaldehyde at concentrations in excess of 1.2 mg/cubic meter (1 ppm) appropriate ventilation or other engineering controls should be implemented, or if this is not feasible, an appropriate respirator should be used.[13] If respirator protection is used, a respiratory protection program would also be required, with emphasis on cleaning, maintenance and proper fit. A step by step procedure should be outlined and only personnel trained in the disinfection procedure and the respiratory protection program should be allowed to use formaldehyde. Access to the area during the disinfection procedure would be restricted. Detector tubes can be used to determine when it is safe to enter the disinfected area. Appropriate sampling methods should be used to assure that formaldehyde vapors are not escaping to other laboratories, offices, or public areas.

Maintenance of the fermentation systems, or maintenance in the laboratories, should take place only after lock out procedures have been implemented, a verification of clean systems has been made, and clearance from the operator and a supervisor has been obtained.

The use of a P-3 or P-4 level laboratory for P-1 or P-2 level work should require development of procedures for such use. If the P-3 or P-4 area is used consistently for work allowing relaxed procedures, as compared to P-3 or P-4 level requirements, the Safety and Operations Manual for the P-3 or P-4 facility should be revised. Additional practices, procedures, and safety precautions should be outlined for such use of the P-3 or P-4 facility. The revised policy and inherent concerns should then be discussed with all personnel involved, and then closely supervised to assure adherence to policy.

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APPENDIX A

DESCRIPTION OF COMPANIES AND PROCESSES SURVEYED

Large scale fermentation, utilizing recombinant DNA can be broken into two segments. The first segment involves process steps where viable cultured organisms are present. The second segment involves processing the culture to extract the desired product with no viable organisms present.

The batch fermentation process begins with a stock lypholized or freeze-dried culture of bacteria containing a plasmid, a small piece of DNA which is inserted into the host's genome and which codes for a new genetic product. This is used to inoculate an agar slant which is incubated until sufficient growth is obtained to inoculate small shake flasks. The inoculated shake flasks, after an appropriate incubation period, are used to inoculate a 5 to 50 liter seed fermention vessel. This seed culture is then incubated prior to being transferred to the production fermentation vessel containing nutrient media. Production vessels currently used for recombinant DNA work range in volume from 750 to 2000 liters. The production culture is incubated while pH, temperature, agitation, and supplied sterile air are automatically controlled.

After several hours of fermentation, the culture growth slows due to the decreased supply of nutrients. The organisms in the batch are then inactivated (i.e. cells are rendered nonviable) by a chemical killing agent. If the product is not sensitive to heat, then steam sparging or jacket heating may be used. Before the batch is removed from the fermenter for further processing, a sample may be taken and tested for completeness of inactivation.

The remaining process uses only inactivated or nonviable cells containing recombinant DNA. The first step in recovering the fermentation product involves separating the cell mass from the liquid media or broth. Currently, all products of recombinant DNA fermentations are expressed intracellularly. This intracellular product may fuse with cellular proteins (fused protein), or may exist free in the cytoplasm of the cell (free protein). The cells are separated from the broth by settling, coagulation, flocculation, filtration, or centrifugation. Centrifugation is currently the preferred method in recombinant DNA processes.

When the cell mass has been separated from the broth, the cells are lysed to achieve access to the cytoplasmic material. Lysing can be accomplished by ultrasonic disruption, grinding, pressure shearing, freezing and thawing or chemical agents. The lysed material is then centrifuged and the supernatant (liquid phase) or solid component retained, depending upon the mode of product expression, for further product separation.

The product may then be recovered from the cell in the case of fused proteins, or from the intracellular liquid in the free protein case by chemical separation. The fused or free protein product is further purified by chromatography, precipitation, or chemical derivation. Final product purification and isolation is accomplished by crystallization or spray drying.

DESCRIPTION OF THE COMPANIES

Company A

Company A started in 1971 as an independent research organization. This company conducts research in genetic engineering, biochemistry, and molecular biology. Results of their research may then be developed, on a pilot plant scale, as technology adaptable for commercial application. The company plans to market this technology rather than manufacture the products the technology will produce.

Company A was conducting research in eighteen project areas. Approximately one-third of these projects involved recombinant DNA research and the remaining projects have the protential for recombinant DNA applications. Pharmaceutical research efforts included the application of recombinant DNA technology for the production of insulin and interferon. The largest part of the company's research efforts were centered around organic chemicals and substitute fuel production. Resulting products of such research would be ethylene and propylene glycol and their oxides, ethanol, alkenes, and substitutes for lubricants and whale oil. Vaccines for animals and humans were also being developed.

Company A employed approximately 230 persons of which about 200 were involved with research and process development. Approximately 75% of this workforce had at least bachelors degrees and 50 individuals were Ph.D.'s. Because of the nature of the work, the typical employee hired by the company would be highly trained. The pilot scale setting would require very few, if any, line operators.

Process development for the production of insulin, interferon, ethanol, and ethylene glycol was underway. Development of fermentation, extraction, and purification techniques was also being conducted. Production processes utilizing recombinant DNA will vary depending on the nature of the product desired and the steps required to achieve the product. General process descriptions for specific products were not yet available partly due to the status of the projects.

Company A's fermentation processes were conducted in 10 liter or less capacities. Fermentation capacities larger than 10 liters would not likely be employed in process research or pilot scale development, and therefore, under the current voluntary system the company would not need to submit their project plans to the NIH Recombinant DNA Advisory Committee for review. Company A plans to stay at the lab or pilot scale level for their research projects.

Company B

Company B started in 1976 as an independently owned research organization. Research efforts by Company B, utilizing recombinant DNA, have resulted in somatostatin production (1977), human insulin (1978), and human growth hormone (1979). Company B is now in a transitional phase moving from strictly

research and development toward manufacturing and production. These three protein products are now in varying stages of clinical trials and all should be marketable by 1985.

Process development for the production of A&B insulin chains, pro-insulin, and somatostatin was underway. Development of fermentation and extraction techniques were also being conducted. Production processes utilizing recombinant DNA will vary depending on the nature of the product desired and the steps required to achieve the product. General process descriptions for fusion proteins and direct expression techniques are outlined in Diagrams 1 and 2 (Appendix D) respectively.

Company B's fermentation capacity conmsisted of 10 liter shake flask, and 600 liter fermentation systems. The 10 liter glass fermenters were located in the main production room with the 600 liter fermenter. The 10 liter fermenters were inoculated using a shake flask. Inoculation of the 600 liter fermenter was accomplished via shake flask, or from the 10 liter fermenter by transporting the inoculum in a glass flask with an attached needle and introducing the contents of the flask into the fermenter through a diaphragm port. The 10 liter and 600 liter fermenters were loaded with nutrients by continuous piping from feed tanks. The feed tanks and lines constituted a closed system that was sterilizable and maintained under positive pressure (about 10 psi). One feed tank was filled with a chemical killing agent for inactivation of the process or for emergency measures.

The fermentation process was monitored and terminated when optimum cell growth was attained. Termination was by chemical or physical means depending on the product or process. Harvest of the cells was accomplished by centrifugation followed by physical lysis of the cells. The crude product was then purified and extracted using processes such as chromatography. The extraction, cell lysing, and product purification processes were not observed during the survey. These processes should be observed and evaluated for potential hazards when the company has developed them to the state of continuous production.

The company employed approximatley 80 persons of which about 68 are involved directly with research and process development. Very few personnel were actually designated as operators of fermentation systems. Manpower requirements for monitoring fermentation processes and systems were anticipated to be no more than 10-15% of the total workforce.

Company C

Company C has Divisions of pharmaceuticals, agricultural products, cosmetics, electronic medical instruments, cardiac pacemakers, and research which make up the corporate structure.

Company C's vast experience in the fermentation industry recently has been applied to the research and development of recombinant DNA technology. This was exemplified by the sophisticated equipment and systems used in the

company's recombinant DNA processes. Company C was conducting its research and developing production under voluntary compliance with the NIH Guidelines for Recombinant DNA Research.

The company employed approximately 6,000 persons of whom about 4,200 were engaged in direct production of pharmaceutical products. Since recombinant DNA fermentation was in the research/production development stage, very few employees were involved. The anticipated number of workers involved with recombinant DNA fermentation processes was expected to be minimal due to the nature of the process and the manpower requirements dictated by the system technology.

Fermentation processes, including those involving recombinant DNA, start in a P-2 or P-3 lab setting. Ampoules of culture stored in liquid nitrogen are used to inoculate a shake flask containing the appropriate nutrients. After an incubation period the inoculum (the contents of a shake flask) is transferred, in a specially designed metal container, to the production floor. This container is designed to preclude escape or entry of viable organisms during inoculation of the fermentation vessel. Metal vessels of 10 liters, 150 liters, or 2000 liters were used for recombinant DNA fermentations, depending upon the process, stage of development, or stage of culture growth. Culture is introduced into 150 liter or the 2000 liter fermenters by a stainless steel vessel that is sterilizable after transfer of the contents and before the vessel is deteched from the fermenter. The fermenters are charged with nutrients, water, and inoculum using closed systems designed to maintain physical containment.

The fermentation process was monitored, and then terminated by thermal techniques. The batch was then removed from the fermenter by pressure. Subsequent extraction of the desired product was done by centrifugation or lysogenesis, depending upon the nature of the process and the desired product. Product extraction operations were not viewed during the visit.

The fermenter, inoculating device, shake flask, and all exposed equipment were sterilized with steam after use to prevent comtamination of succeeding operations and work environment.

Company D

Company D was a subsidiary of a large pharmaceutical corporation. Veterinary biologics have been produced at this facility for the past twenty years, and the facility has been under the control of Company D for the last seven years.

Company D was conducting fermentations for the parent company, using bacteria containing a recombinant molecule that codes for human leukocyte interferon. This production was conducted in a recent addition to the production building which houses a 130 liter fermenter and a 1000-liter fermenter. The parent company plans for Company D to conduct these fermentations until a fermentation facility is completed on the east coast. The production of

interferon will be used for clinical trials and toxicological studies. The interferon production was being conducted under voluntary compliance with the NIH Guidelines for large scale uses of organisms containing recombinant DNA molecules.

Company D employed approximately 175 persons, of which about 45 are located at the facility which houses the recombinant DNA project. Maintenance, administrative, and research personnel are included in this 45. Six personnel are currently assigned to the recombinant DNA project. It is unlikely that more than six individuals will be required to conduct the fermentations.

The batch fermentation process began by inoculating 4 liter flasks of media with stock cultures of bacteria containing the plasmid coding for interferon activity. These stock cultures were kept at -70°C until needed. The 4 liter flasks were incubated 6-10 hours at 37°C. After incubation the inoculum was gravity fed, through a plastic tube, into stainless steel pressurizable inoculation cannisters.

Meanwhile, the medium for the fermenter was prepared in a stainless steel jacketed tank in a room adjoining the fermenter room. The media was pumped from this tank through Tygon^{R} tubing which entered the fermenter through the vessel's upper viewing window. The tubing was detached from the tank and the fermenter after the transfer was complete. The wall port through which the tubing entered the room was then sealed. The viewing window of the fermenter was closed, and the fermenter and contents were then steam sterilized at 15-16 pounds of pressure for 45 minutes. The fermenter was than brought to atmospheric pressure.

The fermenter was inoculated by connecting the inoculation cannister via stainless steel flexible tubing and swage lock quick connectors to a steam sterilizable inoculation port. The transfer was made by applying slight positive pressure to the surface of the liquid in the cannister. The cannister, tubing, and inoculation port were steam sterilized in place. The production culture in the fermenter was incubated while pH, temperature, agitation, and dissolved oxygen were controlled automatically. When the fermention was completed, the contents of the fermenter were returned to atmospheric pressure, and a chemical inactivating agent was introduced.

The chemical inactivant was added using a pressurized stainless steel 40 liter cannister. The cannister was connected to the fermenter by flexible stainless steel tubing fitted with swage lock quick connectors at both ends. The chemical inactivant was transferred through the inoculation port by air pressure (2-4 psi). After the transfer, the inoculation port valve was closed, the tubing was disconnected, and the inoculation port cover was fastened in place and supplied with steam. The contents of the fermenter were agitated for one hour to assure total inactivation of the culture.

Company E

Company E produced purified restriction endonucleases and other enzymes for use in genetic research. The company was independently owned and started operation in 1974, producing oligonucleotides. This company had plans to expand its existing facility within the next year.

Company E produced their enzyme product line by fermentation processes. These fermentations were conducted in 10, 25, and 100 liter fermentation vessels. The company was also involved in several research and development projects, one of which was recombinant DNA based. At the time of the survey, this company was not conducting fermentations involving recombinant DNA techniques. Company E's research in recombinant DNA was conducted under voluntary compliance with the NIH Guidelines.

This company employed approximately 32 persons, of which 25 were research scientists or fermentation specialists. The remaining employees were performing administrative or clerical duties. All of the reseach and fermentation specialists had advanced degrees with previous experience in research.

The enzymes produced by batch fermentations at Company E represent milligram quantities per batch. Because the fermentation process was small scale with no pathogenic or recombinant organisms involved, the management reportedly operated these processes at P-1 or "less" containment.

Company F

Company F started operation in 1956, and at the time of the survey was the world's leading manufacturer of radioactive chemicals for research and radiopharmaceuticals. Other radioactive products from this company included chemicals for clinical diagnosis, liquid scintillation chemicals, and products used to calibrate diagnostic and research instrumentation. Nonradioactive products, such as monoclonal antibodies and enzymes for genetic research were also produced by this company.

The enzymes for genetic research were produced by batch fermentations in 14 and/or 150 liter fermenters. Product extraction procedures are carried out at the same facility. Company F was conducting limited research in recombinant DNA.

Company F employed approximately 1600 persons, of which about 140 were located at the facility surveyed. Of the 140 employees, only a very small group were assigned to conduct the fermentations. At this facility, more than half of the 140 employees had advanced degrees.

The various enzymes produced by Company F were made by batch fermentation in a 150 liter fermenter. The 150 liter fermenter was operated at a 100 liter working capacity, and was inoculated through hard piping from a 14 liter fermenter. E coli is the culture organism used in the fermentation process. Company F maintained a culture library of 250 strains of organisms, thirty of which were actively used cultures.

When the growth phase of the fermentation was complete the culture was inactivated by rapidly chilling with ethylene glycol. The chilled cells were then separated from the fermentation broth by centrifugation. The fermenter and centrifuge were connected by hard piping. The cells were stored and handled at -20°C until enzyme extraction from the cell mass occurred. The fermenter, centrifuge, and appurtenant piping and equipment were located in an area capable of qualifying as P3-LS containment.

The harvested cells were lysed to release the intracellular fluid. The lysed cells were centrifuged and the cell mass was flushed to a kill tank for sterilization prior to passage to the sewer. The supernatant from the centrifugation contained the enzymes which were further extracted and purified via column chromatography. The enzyme, as a product, was shipped to another plant for packaging and customer delivery.

APPENDIX B

EVALUATION OF THE PLANT CONTROLS & PROGRAMS

EVALUATION OF THE PLANT CONTROLS AND PROGRAMS

Physical Containment Design

Physical containment laboratories at the P-1, P-2, and P-3 levels in each company were toured. These laboratories were designed and presumably operated within the NIH Guidelines. The only observed infractions of these guidelines were eating and drinking in some of Company B's and E's laboratories.

Production areas at Companies B, C, D, E, and F were also evaluated during the site visits. Company A had no large scale production areas because it operated only at a research and development (laboratory scale) level. Production areas at the four plants B, C, D, and F were designed to meet the NIH requirements for P1-LS level of containment.

Company B's P1-LS production area was under-construction at the time of the survey. The production room incorporated additional features that could qualify it as a P-3 laboratory. The company had experienced rapid growth and expansion, and the result was a poorly chosen location for the production room and a poor arrangement of equipment within. The production room was immediately adjacent to high traffic areas and the public reception area of the facility. This problem was compounded by a "fire door" (opened into the room) that egressed into the hallway from the public reception area. The door was locked and only openable from inside the production room. Sloped floors had been installed in the room, yet none of the access doors had thresholds. A large spill could feasibly flow under the door. Ongoing construction and facility modifications will possibly correct some of these problems as the production process develops.

The production room housed a 600 liter (metal) and a 10 liter (glass) fermenter. Single pass ventilation, negative air pressure in the room, floors sloped to drains in order to contain large spills, and controlled entry were additional features found; these features are not required by NIH for P1-LS. Non-porous and readily cleanable walls, floor, and ceiling were also planned. The integrity of the containment design may have been compromised by the (breakable) glass 10 liter fermenters, the shake flask method of inoculating the large fermenter, and the poorly located (bottom mounted) agitator drive and seals of the 600 liter fermenter.

Company C's P1-LS area with a 2000 liter fermenter, modified to contain recombinant DNA organisms, will be used for development and production of clinical trial material. This fermentation system was still in the developmental phase, and undergoing sterilization validation procedures at the time of the survey. Fermentation with recombinant DNA work was not housed in a distinctly confined room, but was in a pilot production area in immediate proximity to conventional fermenters of the same size. Access to the tops of the fermenters was by a single stairway and catwalk. Descriptions of fermenter modifications may be found in Diagrams 3 & 4 of this report (Appendix D).

Company D's large scale fermentation laboratory (production room) contained a 1000 liter and 130 liter fermenter. This room surpassed the NIH requirements for a P1-LS level of containment. A diked area around the 1000 liter fermenter was designed to contain the entire contents of the fermenter plus extra volume for the addition of disinfectant. A HEPA inlet and exhaust filter system for room ventilation (85% of room air was recirculated), negative air pressure in the room, and controlled entry were additional features of the production room not required by NIH for P1-LS. Non-porous and readily cleanable walls, floor, and ceiling were another feature of the facility. All doors in the facility had a rubber seal on the frame and threshold.

The fermenter used for production of cells containing interferon was a stock design equipped with accessories and containment modifications for recombinant DNA fermentations. The fermenter was a stainless steel jacketed pressure vessel with incorporated automatic and manual controls for the operation of the fermentation process. The vessel was designed and equipped with a bottom-mounted agitator, air sparger, mechanical foam breaker, and steam sterilizable inoculation sampling ports. There was no high fluid level alarm on this particular model of fermenter. A silicon double mechanical seal, that was steam sterilizable, mounted the agitator into the vessel.

The centrifuge used to harvest the inactivated cells from the culture was a hermetically-sealed unit with self-discharging capability. The centrifuge was connected to the fermentation vessel and the supernatant holding tanks by quick-connect fittings and flexible tubing.

Company E's 10, 25, and 100 liter fermentation vessels were contained in the fermentation laboratory and all three were used at various times. The 10 liter fermenter was a glass bench top model normally used for seed cultures. The 25 and 100 liter fermenters were pilot-sized vessels manufactured by New Brunswick Scientific Co., Inc. The 100 liter vessel had been custom-modified for specific use with incorporated automatic and manual controls for the operation of the fermentation process. Both were equipped with top-mounted agitator (single seal design), air sparger, and steam sterilizable inoculation/sampling ports. There was no high fluid level alarm on either fermenter. Prior to each use, the fermenters were normally sterilized with steam. Both fermenters had packed glass wool filters for inlet and exhaust air. The company had no program for either the routine cleaning or changing of these filters.

Company E used two non-hermetically sealed centrifuges which had self-discharging capabilities. The centrifuges were connected directly to the 25 and 100 liter fermentations vessels.

Company E's fermentation laboratory was adjacent to the larger laboratory facility and was entered through a door which was normally open. There were no provisions for controlled access to the fermentation lab. Within the fermentation laboratory was a smaller room which housed the two centrifuges.

Both rooms were ventilated with the recirculating general dilution conditioned air system which accommodated the entire facility. Neither room was under negative pressure to the rest of the laboratory facility. Both rooms lacked dikes to contain accidental spills or leaks.

Company F had 14 and 150 liter fermentation vessels, used in the production of research enzymes, contained in a fermentation laboratory qualifying as P3-LS containment. These fermenters were pilot-sized vessels and were manufactured by New Brunswick Scientific Co., Inc. The 150 liter fermenter was custom-engineered for specific use at this facility and is inoculated during production runs from the 14 liter vessel in a "bump-tank" arrangement. Both fermenters were stainless steel-jacketed pressure vessels with automatic and manual controls for the operation of the fermentation process. Both were equipped with a top-mounted agitator (double mechanical seal design), and anticlogging air sparger, and steam sterilizable inoculation/sampling ports. There was also a high fluid level alarm on the 150 liter fermenter. Prior to each use, the fermenters and appropriate feed lines were sterilized in place with steam. The sterilization and process operation steps for the fermenters were outlined in checklists for the operator. Both fermenters had HEPA filters in place for the purification of inlet and exhaust air. A routine program for the cleaning and/or replacement of these filters was currently in effect.

A centrifuge was used to harvest the cells from the culture. The centrifuge was a hermetically sealed unit with self-discharging capability and was connected to the 150 liter fermenter with a hard-duct rapid chill system. Additionally, the centrifuge was modified with a custom-engineered plexiglass aerosol retainer to minimize worker exposure to viable aerosols created by centrifugation. This control had proven to be quite effective in significantly reducing the release of viable aerosols as determined by inhouse industrial hygiene sampling (Diagram 5, Appendix D). The process operation steps for the centrifuge were outlined in checklists for the operator.

The fermentation laboratory was a controlled access area requiring a key card for entry. A small room contained within the fermentation laboratory housed the centrifuge. The door to the centrifuge room was closed during production. The fermentation laboratory was ventilated with a single pass, general dilution, conditioned air system which was filtered by a HEPA bag-in/bag-out arrangement. A routine program for the cleaning and/or replacement of these filters was currently in effect. The fermentation laboratory was under negative pressure with respect to adjoining rooms during the normal operation of the ventilation system. The centrifuge room was under negative pressure with respect to the fermenter room since the potential for the generation of viable aerosols was greatest during centrifugation. The fermentation laboratory was diked to contain any possible spills and still had room for the addition of a chemical inactivating compound. Additionally, the laboratory floor, walls, and ceiling had been sealed with a non-porous material to prevent the escape of fugitive viable organisms and allow for more

efficient cleaning and disinfecting. The cleanup procedure was outlined in a checklist for those performing the cleanup. Ultraviolet (UV) lights were also used in the fermentation laboratory to further reduce the possibility of the escape of viable organisms, and to reduce the potential for contamination.

Engineering Controls

Attention to process integrity is critical with pharmaceutical fermentations. This concept of containment strives to not only prohibit escape of organisms from, but also to exclude entry into the fermentation system. Thus, the engineering design and measures implemented to achieve this dual aspect of containment, and to comply with the NIH Guidelines, can be quite sophisticated. Because of the design of the large scale operations observed, it is possible that more risk for worker exposure to recombinant DNA organisms (e.g. from accidental spillage or aerosol production) could occur in the shake flask laboratory than on the production floor.

The ventilation systems, fermenter exhaust gas filtration, waste products control, biological safety cabinets, access barriers, and all interior room surfaces in the P-1, P-2, and P-3 laboratories of each company met the NIH Guidelines requirements. Company A had installed an automated security computer system which monitored the ventilation systems, access areas, safety cabinets, centrifuges, autoclaves, and ambient environment of their laboratories.

The NIH Guidelines for the P1-LS containment level do not require specific ventilation measures, distinct room confinement, or locked access to the production room. Companies B, D and F had added these features to their production areas. Air supplied to the rooms and the fermenters was HEPA filtered. Fermenter exhaust gas is subject to HEPA filtration and incineration at these companies. Companies D and F also HEPA filtered the room exhaust air from the production area.

Characteristics of the fermenters used by the companies for organisms containing recombinant DNA molecules were as follows:

Company B

1. Inoculation, at the time of the survey, was accomplished by using a shake flask. The company has since employed the use of a stainless steel inoculating device similar to Company C's.

2. Agitator shafts penetrated the bottom of the fermenters through double mechanical seals. Sterile coolant was circulated between the seals at a pressure greater than that in the fermenter.

3. The 600 liter fermenter was operated at atmospheric pressure.

4. Sterilization of the fermenter exhaust was accomplished by HEPA filtration and incineration.

5. Thermocouples are reportedly now in use by the company to verify the sterilization process of the fermenter, but not its ancillary piping.

Company C

1. Inoculation, sample collection, nutrient addition, and transfer of culture fluids could be performed with closed system piping. All pipes and feeder tubes were connected to a fermenter at its top.

2. Each fermenter had its own separate system of feeder lines with the exception of electrical service, steam, and pH control (the latter two were double-check valved).

3. Agitator shafts which penetrate the fermenter shell had double mechanical seals and were top mounted on the fermenter. Sterile coolant was circulated between the seals at a pressure greater than that in the fermenter.

4. The 2000 liter fermenters were operated at a slightly positive pressure (approximately 5 psi).

5. Sterilization of fermenter exhaust was accomplished by double filtration.

6. Drainage pipes at the bottom of fermenters were sealed closed.

7. Before initial use, the ability to sterilize an entire fermenter and its ancillary piping was verified by a test protocol employing thermocouples.

Company D

1. Inoculation, sample collection, nutrient addition, and transfer of culture fluids was performed with closed system piping. All pipes and feeder tubes were connected to the top of the fermenter.

2. Preparation of the inoculation device, at the time of the survey, provided the opportunity for aerosol production and thus potential for exposure.

3. Agitator shafts penetrated the bottom of the fermenters through double mechanical seals. Sterile coolant was circulated between the seals at a pressure greater than that in the fermenter.

4. The 1000 liter fermenter was operated at a slightly positive pressure (approximately 5 psi).

5. Sterilization of the fermenter exhaust gas was accomplished by HEPA filtration and incineration.

6. A routine preventive maintenance program to change o-rings on the headplate and viewing port existed in accordance with the fermenter manufacturer's recommendations. The entire fermentation system was pressure tested during the sterilization process step.

Company E

1. Inoculation of the 100 liter fermenter was accomplished with closed system piping from the 25 liter vessel.

2. The agitator shafts were of the top mounted, single seal design.

3. The inoculation and sampling ports were steam sterilized.

4. Exhaust and inlet air for the vessel was filtered by packed glass wool filters.

5. No validation procedures of complete system sterilization had been conducted.

Company F

1. Inoculation, sample collection, nutrient addition and transfer of culture fluids was performed with closed system piping. All pipes and feeder tubes were connected to the top of the fermenter.

2. The fermentation vessels were pressure tested prior to each use to determine their integrity.

3. Both vessels were equipped with top-mounted agitators with double mechanical seals.

4. Both fermenters were equipped with HEPA filters to sterilize exhaust gases.

5. A quarterly preventive maintenance schedule is followed for the fermenters with special emphasis on valves and seals.

All liquid wastes from the fermentation systems and laboratories of each company were inactivated before dispensed to the public sewers. Company A further treated its liquid waste in a bio-waste system located at the facility site. Prior to disposal, none of the process materials were recycled or treated (other than sterilization by live steam or addition of a chemical killing agent) by any of the companies. Each company handled solvent and radiation wastes separately from the inactivated liquid wastes. These wastes were not disposed of in public sewers.

Validation Procedures and Practices

In all of the companies, except Company E, the biological safety cabinets and walk-in hoods received scheduled performance testing and certification. Autoclaves were monitored by integral recording charts to document performance in all companies except Company E. All centrifuge equipment received periodic maintenance checks by all companies except Company E. Validation of active sterilization was conducted on systems as required under the NIH Guidelines.

Steam sterilization effectiveness of the fermentation system was checked by Companies B and C using thermocouples. Originally Company B was using indicator crayons or strips which melt at a specific temperature. Such as indicator system does not identify areas of the system which may have not reached the desired temperature and may therefore not be thoroughly sterilized. The company has reportedly stopped using this method and has utilized the thermocouples since the survey visit. Company F pressure tests each fermenter, to determine containment integrity prior to each use. Company E employed no validation measures to ensure the efficacy of the physical containment or engineering controls in place for the fermentation process.

Chemical inactivation of the culture was checked and validated by all companies to document total cell inactivation in response to the NIH Guidelines.

Work Practices

Companies B and E were the only companies, at the time of the surveys, which did not have specific guidelines for safe work practices. All of the companies had instituted training programs for the operators of the fermentation systems. Companies B and E were the only companies of the six who did not have an established safety policy or procedure outlined for maintenance performed in laboratories or on fermentation systems. Company B has since reportedly developed guidelines for safe work practices and procedures for performing maintenance. In the other companies maintenance of systems, or in laboratories, was performed only after lock out procedures had been implemented and approval granted from the line operators and a supervisor. In all of the companies the cleaning of the P-2, P-3, and production areas was the responsibility of the employees working in those areas.

Emergency and Accident Procedures

Companies A, C, D, and F all had developed detailed plans for emergencies and laboratory accidents. These plans outlined areas of responsibility for individual response in the event of fire, utilities failure, environmental emergencies, laboratory accidents (i.e. biological and radiological spills and/or aerosols). Company B was drafting plans for emergencies and accident procedures at the time of the survey. Company E had no plans to develop safety precautions, plans for emergencies, or laboratory accidents.

APPENDIX C

DESCRIPTION OF INDUSTRIAL HYGIENE, SAFETY, & MEDICAL PROGRAMS

DESCRIPTION OF INDUSTRIAL HYGIENE, SAFETY, & AND MEDICAL PROGRAMS

Industrial Hygiene

Companies C, D, and F had industrial hygiene programs while Companies A, B, and E had no such support or programs.

Environmental monitoring for organisms consisted of RODAC (Replicate Organism Direct Agar Contact) plates, air impaction onto agar plates, settling plates filled with agar, and liquid impinger techniques. Company C utilized all of these methods while Companies B, D, and F used only settling plates and air to agar impaction methods. Companies A and E had not conducted any environmental monitoring while Company F had performed the most critical evaluations of appropriate and feasible methods for environmental monitoring.

Environmental monitoring for chemical and physical agents was reported by Companies A, C, and D to be conducted on an as needed basis in the case of an accident or suspected exposures. Company D actively conducts testing for formaldehyde concentrations during general disinfection procedures. Companies A and F employ a radiation protection officer who monitors the use of radioactive materials and supervises decontamination and clean up of radioactive spills. Company F had a very comprehensive industrial hygiene program involving monitoring for radiological, chemical, physical, and biological hazards.

Safety and Health

Companies B and E were the only companies of the six without an organized and well documented safety program. Companies A, C, D, and F had formal safety programs consisting of written procedures, scheduled meetings, and inspections. Company F maintained that all employees hired, regardless of experience or education level, must receive basic training sessions in safety and health, and are required to comply with the safe work practices defined by the company.

Each company had a functional and appropriately staffed Institutional Bio-Safety Committee (IBC) in compliance with the NIH guidelines. Only companies C and F had designated an alternate bio-safety officer and an orderly chain of command.

Medical Programs

The medical programs instituted by the companies varied in objectives, essential elements, limitations, and application. This variety was attributed to the individual company's perception of medical surveillance and the products and processes pertinent to each company.

Company A did not require pre-employment physicals or comprehensive annual medical examinations of employees. All injuries were required to be reported to the employee's immediate supervisor. Employees on sick leave were required to have clearance from a physician before returning to work. Employees with open wounds, or those on antibiotic therapy, were excluded from working in recombinant DNA areas.

Preassignment and annual blood serum samples were required and stored for employees working with recombinant DNA techniques. The IBC was investigating the need for specific medical tests of medical certification prior to working in recombinant DNA areas.

There was no exposure data available because environmental monitoring had not been conducted. Thus, no qualitative or quantitative results existed to document range or incidence of exposure to research material.

Other potential health hazards were radioisotopes and organic chemicals such as chloroform, formic acid, toluene, and formaldehyde. Proper usage, storage, and disposal requirements had been discussed by the company with the employees involved with these hazards.

Company B was in the process of developing a formal medical program for employee health surveillance. This program was being developed under the direction of the company's Director of Human Resources and a consulting physician, a specialist in industrial medicine. They were arranging emergency clinical support, pre-employment physicals, and blood serum sample aquisition and storage. Employees on antibiotic therapy were required to report to their supervisor and were excluded from working with recombinant DNA organisms. Production workers involved in the production of human growth hormone and interferon would be subject to fecal monitoring.

Data on exposure of employees to recombinant DNA organisms utilized in fermentation processes was limited to the environmental monitoring previously mentioned. No quantitative results were available to document range or incidence of exposure.

Other potential health hazards were radioisotopes and organic chemicals such as chloroform, pyridine, formic acid, and formaldehyde. Proper usage, storage, and disposal requirements had been discussed by the company with the employees involved with these hazards.

Company C's medical department was staffed by a director of industrial medicine, five full-time physicians, and eight nurses. Pre-employment physicals were given to newly hired employees. Comprehensive annual physicals were given to all employees, and annual physical examinations were mandatory for all laboratory and production personnel (including recombinant DNA workers). Company policy stated that all injuries must be reported to the plant physician, and any employee on sick leave for more than five days must report to the physician before returning to work. Employees undergoing antibiotic therapy were excluded from working in recombinant DNA areas. Employees involved with recombinant DNA processes who developed chronic or acute illness were required to see the physician. Preassignment blood serum samples were required and stored for individuals working with recombinant DNA processes. When appropriate, immunizations were given to those employees working with infectious organisms.

Data on exposure of employees during fermentation processes with organisms containing recombinant DNA was limited to the results of the environmental monitoring previously mentioned and the medical surveillance program implemented. No quantitative data was available to document range or incidence of exposure. The company voiced concern about the practicability of the methods currently used for environmental monitoring, yet recognized the need for such monitoring.

All Company D's employees receive pre-employment and scheduled annual follow-up medical evaluations. The pre-employment evaluation included an occupational history, medical history, medical examinations, chest x-ray, urinalysis, complete blood count, and evaluation of twenty-six serum parameters. The annual medical evaluation consisted of all the above parameters, plus a drug and medication history for the year that was obtained. Serum banking was conducted on all employees working with organisms containing recombinant DNA molecules; serum samples were taken each year and retained for seven to ten years for reference purposes only. The company had a physician on retainer to conduct the medical examinations.

Employees working with recombinant DNA were required to report all injuries to the Employee Health Services (EHS) and the Principal Investigator (PI) within 48 hours. Any employee illness that may have been considered job-related is reported to the PI and EHS. All illnesses were required to be reported within 48 hours. Employees were not allowed to return to work with recombinant DNA material until so approved by their physician and EHS. Accidental injuries found to result in exposure to recombiant organisms would necessitate a follow-up investigation by a safety team and an industrial hygiene evaluation if necessary. Employees would also be followed medically for any abnormal health patterns if exposed to recombinant organisms, e.g., urinary tract infection due to ingestion.

A supplemental medical history questionnaire was administered to recombinant DNA workers. This questionnaire was designed for work with E coli K-12 systems. Exclusion of employees with immunodeficiency disease from work with recombinant DNA systems was based on multiple parameters and evaluated on a case-by-case basis. Some of the parameters considered were: severity and type of disease, type of project, and likelyhood of exposure. Immune disorders were not absolute contraindications for working with recombinant DNA containing organisms.

Employees on antibiotic therapy could work on recombinant DNA projects unless serious or prolonged illness was a factor. Corporate policy stated that employees on antibiotic therapy were not necessarily at risk for work with E coli K-12 systems. Medical evaluations were planned for all employees prior to termination of employment.

Company E did not provide or require employees to take a pre-employment or periodic medical examination. No job related medical evaluations were performed on the employees. Serum banking was not conducted for employees working with recombinant DNA.

The company did not have any definite requirements for the reporting of accidental inoculation, ingestion, probable inhalation, lacerations or abrasions. There was no follow-up of employee illness to determine if it was job related. Company E did have an employee qualified in first aid and CPR for administration of emergency treatment.

Company E had no exposure data available because environmental monitoring had not been conducted. Potential for employee exposure to small quanitities of various organic chemicals and radionucleotides did exist.

All of Company F's employees received pre-employment and scheduled annual follow-up medical evaluations. Pre-employment evaluations included a medical history, occupational history, medical examinations, chest x-rays, and the collection of two intermittent baseline blood sera which were stored at -70°C for 5-10 years, at which time new samples were then obtained. Pre-employment, as well as annual medical evaluations included urinalysis, visual and hearing acuity tests, pulmonary function testing, blood serum analysis, and an electrocardiogram. A complete immunization history was maintained on all employees. Permanent employees received immuno-electrophoreses and allergenic response testing.

The company employed a full time medical director at corporate level, a physician (part-time basis) to conduct the medical evaluations, and a licensed nurse (part-time basis) to provide first aid and immediate health care. Medical surveillance procedures for all personnel had been devised. These procedures covered employee health monitoring requirements, the reporting of illnesses, and supervisor/employee responsibilities. Authorization to conduct microbiology and biotechnological operations and daily health survey forms were required of all employees working in the bio-restricted area.

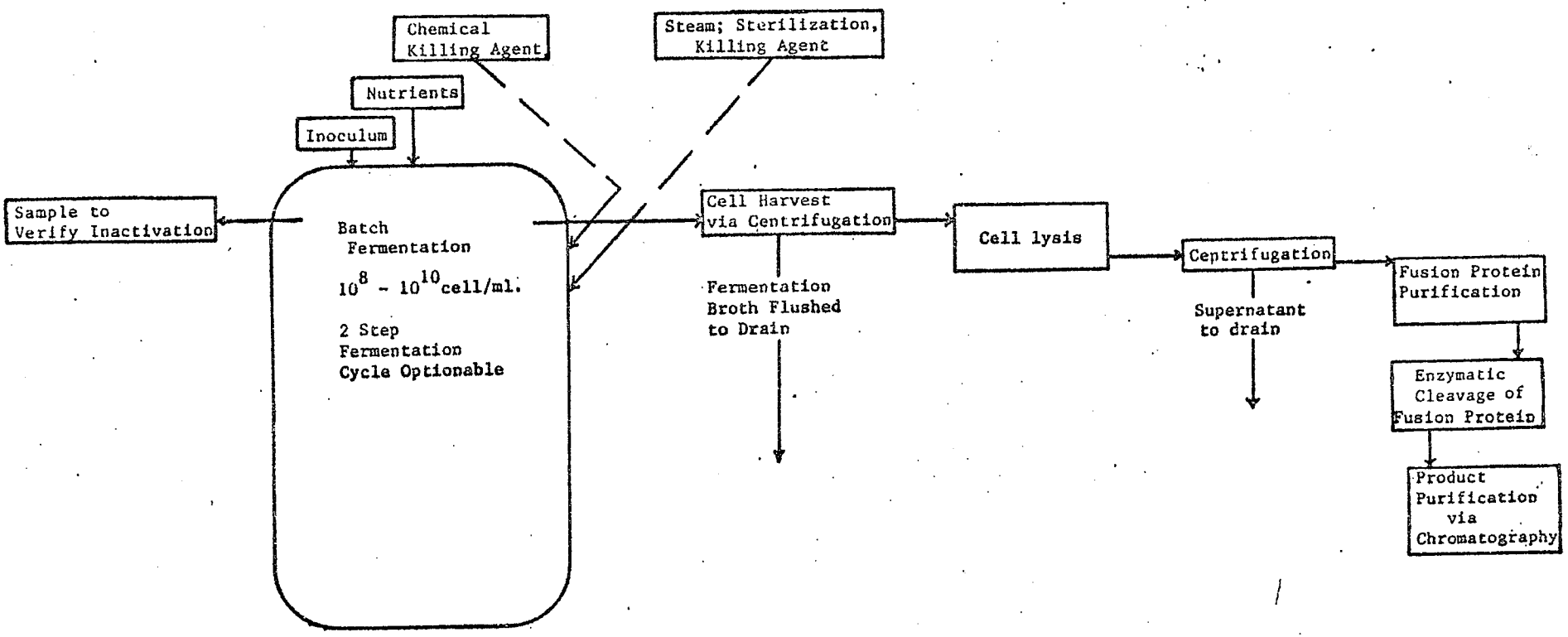
Potential for exposure to organic chemicals and radiation was controlled by the company's safety and health programs. Routine environmental monitoring included measurements for volatile organics and radioactive contamination. Settling plates and slit air to agar impactors were used for viable aerosol sampling. Data on exposure of employees to organic chemicals, microorganisms, and radiation was accumulated and maintained by the Environmental Control Office.

APPENDIX D

DIAGRAMS

DIAGRAM 1

FUSION PROTEIN PROCESS APRIL 1980



* Currently using 10 liter fermenter, preparing to step up to 600 liter fermenter

DIAGRAM 2

DIRECT EXPRESSION PROCESS APRIL 1980

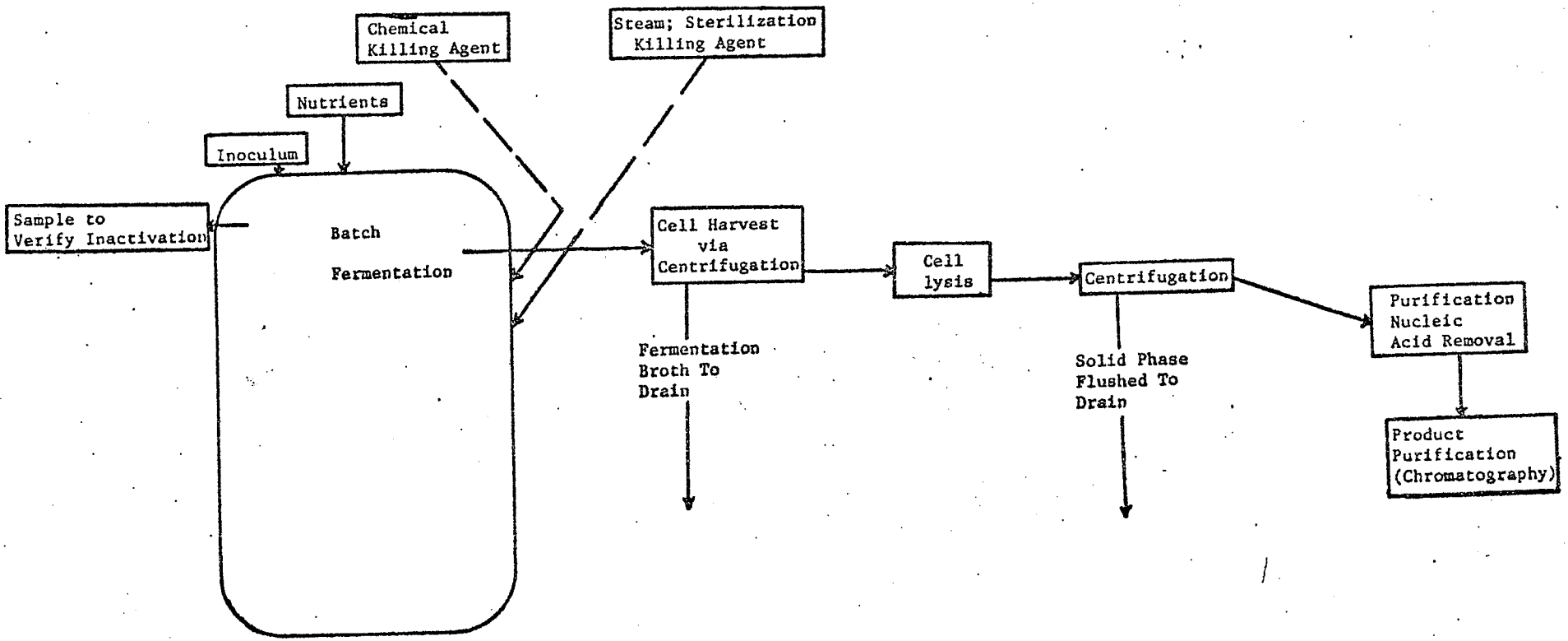
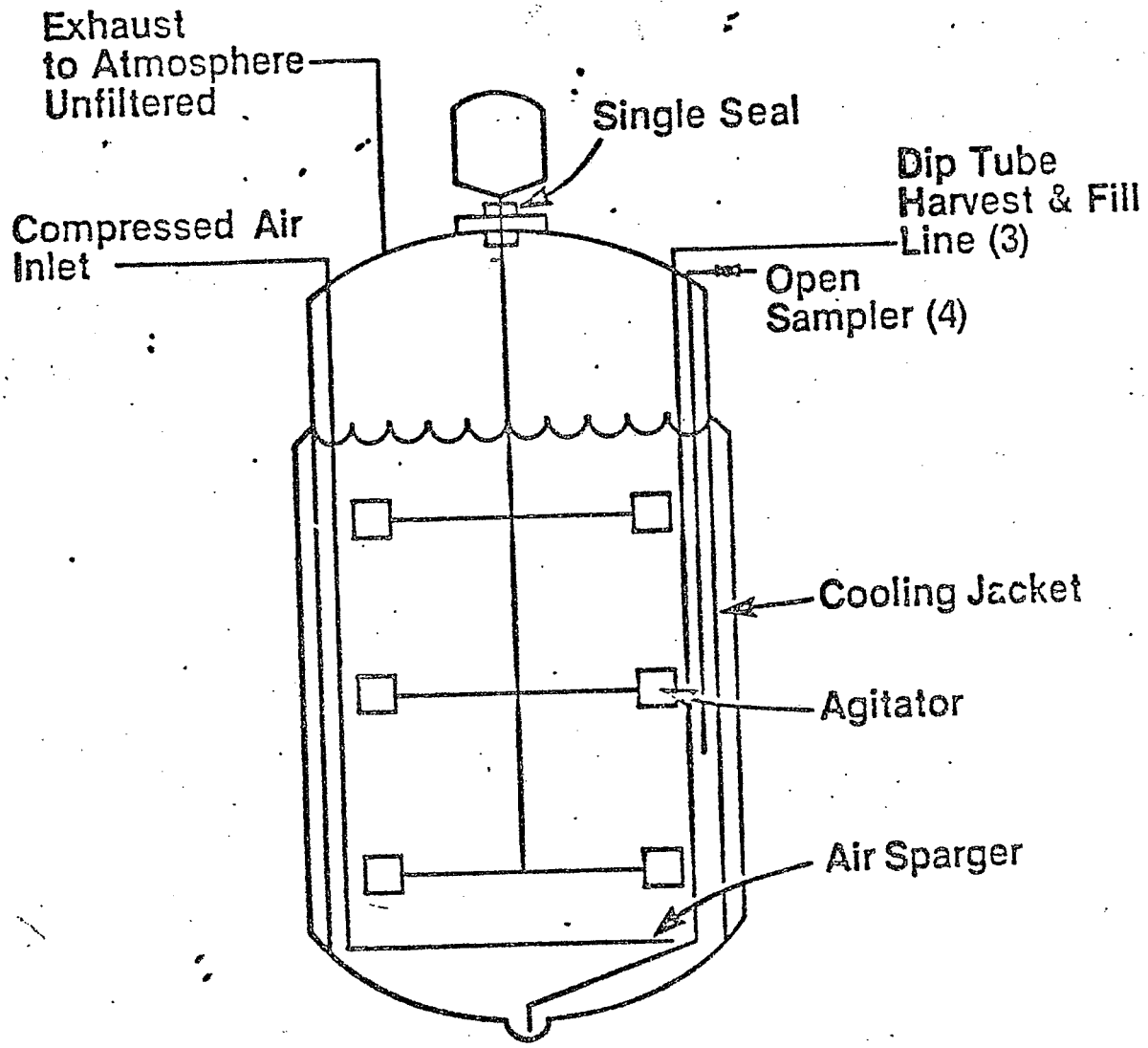
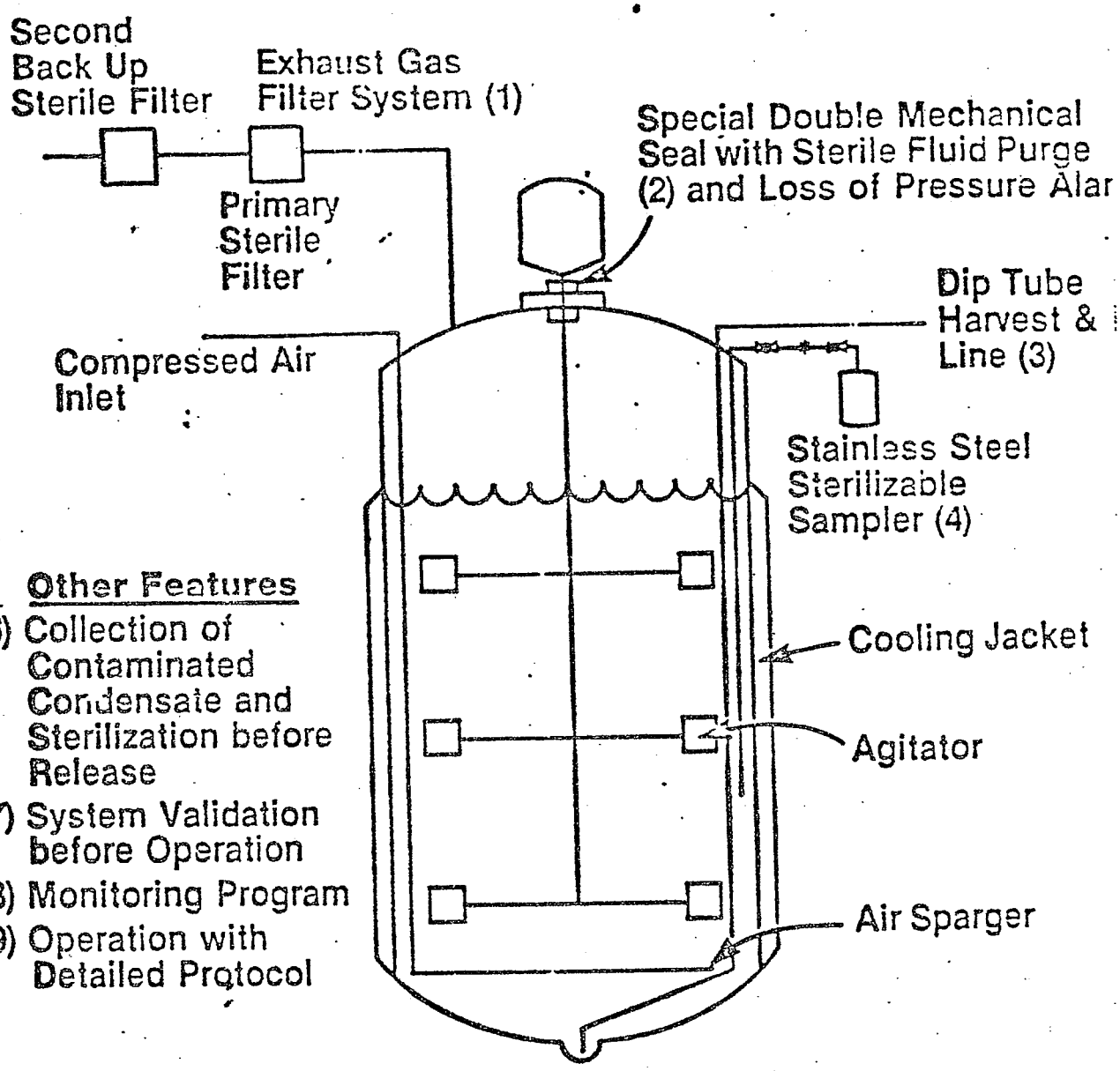


DIAGRAM 3

Features of Standard Fermentor



Features of Contained Fermentor



AEROSOL CONTROL IN CENTRIFUGE ROOM-COMPANY F