31

RECOMBINANT ORGANISMS

Jessica Herzstein, M.D., M.P.H., Ed Fritsch, Ph.D., and John L. Ryan, Ph.D., M.D.

OCCUPATIONAL SETTING

Recombinant organisms are used routinely in the biotechnology industry and in academic laboratories. They are the source of many of the most innovative biopharmaceuticals that have contributed to medical science in the past 20 years. Because of the relative effectiveness of production techniques, certain processes may move from laboratory to pilot plant (often still a laboratory) to production with very little apparent change. Occasionally, relatively small facilities can produce large quantities of complex and previously rare or unattainable products. Increasingly, though, the scale of commercial manufacturing has grown significantly in order to support these valuable products.

The workforce at these facilities may be small but is very highly trained. Most biotechnology research and pilot production personnel have advanced degrees. Commercial facilities tend to be staffed with a mixture of individuals with high school or technical education and managers with advanced degrees. As a group, biotechnology workers are highly invested, both emotionally and economically, in the success of their new enterprises. Long work hours, secrecy concerning processes and product development, an emphasis on rapid progress toward production and very high economic stakes increase the potential for worker hazards.

EXPOSURE (ROUTE)

Exposure to three types of hazards must be considered in biotechnology research and production: recombinant organisms, biological (human- or animal-derived) reagents used for recombinant organism growth and non-biological hazards such as chemicals, radiation and recombinant products. Of these, biological

Physical and Biological Hazards of the Workplace, Second Edition, Edited by Peter H. Wald and Gregg M. Stave
ISBN 0-471-38647-2

reagents and chemicals/radiation pose the greatest concern. Exposure may theoretically occur through ingestion, inhalation, skin and mucous membrane contact, or skin penetration by a contaminated needle or other sharp object.

PATHOBIOLOGY

Recombinant biology is the ability to insert specific pieces of DNA into selected organisms for the purpose of creating desired products. Recombinant organisms are the resulting genetically modified bacteria, fungi, and cells. Humans have selectively bred animal and plant species for desired traits for thousands of years. Recombinant biology extends that activity to the molecular level and can produce very precise outcomes.

More than 80 biopharmaceutical products have now been approved in the USA, EU or both.[1] These fall into multiple broad categories including blood factors, hormones, hematopoietic growth factors, vaccines, monoclonal antibodies, enzymes and chimeric molecules (Table 31.1). Many more products are currently in development, and the human genome and bioinformatics efforts are likely to result in an explosion of new possibilities. In addition, new approaches to the production of biopharmaceutical products such as transgenic animals and gene therapy are aggressively being developed, extending the scope of technologies, facility design and environmental impact. Non-medical products include pesticide-resistant and pest-resistant plants,

Table 31.1 Selected products from recombinant organisms.

Biopharmaceutical class	Examples	Date first approved (EU or USA)
Blood factors	Factor VIII	1993
	Factor IX	1997
	Hirudin	1997
Hormones	Insulin	1982
	Growth hormone	1985
	Glucagon	1998
	Follicle stimulating hormone	1995
Hematopoieic growth factors	Erythropoietin	1989
	Granulocyte colony stimulating factor	1991
	Granulocyte–macrophage colony stimulating factor	1991 1995
	Alpha and beta interferon	1992
	Interleukin-2	
Vaccines	Hepatitis B surface antigen	1986
Monoclonal antibodies	Anti-CD-3	1986
	Anti-GII_bIII_a	1994
	Anti-HER 2	1998
	Anti-CD20	1997
Enzymes	DNase	1993
	β-Glucocerebrosidase	1994
Chimeric molecules	IL-2 Diptheria toxin	1999
	TNF Receptor-IgG	1998

Data are from the following sources: http://www.fda.gov, http://www.eudra.org/en_home.htm, http://www.phrma.org

industrial or food-processing enzymes, chemicals, fuels, and foods. In the future, specialty products may appear in clothing, construction, or transportation materials. Even before the public became aware of commercial recombinant technology in the mid-1980s, hundreds of start-up enterprises existed in the USA (and elsewhere). Continued progression from laboratory to pilot plant to commercial products is inevitable for a wide new array of products. As in every new industry, the potential also exists for health and safety hazards.

One of the fastest-growing areas in the biotechnology industry is gene therapy. Gene therapy employs viral and bacterial vectors to deliver specific human genes to patients with hereditary and acquired diseases. Recombinant vectors are often replication-deficient viruses, e.g. retrovirus, adenovirus, or herpes virus. These are used in gene therapy both for protein or enzyme replacement and for oncology. There are serious concerns that these viruses may become replication-competent in the host. Recognizing that large numbers (10^{10} or more) of viruses are often delivered emphasizes the need for caution, because there may be risks to the care-givers in preparation of the therapy or to the patient from the vector carrying the intended therapeutic gene.

Simple infection is the most significant biological hazard for workers. At the research stage, hazardous organisms such as drug-resistant microorganisms, hepatitis viruses and oncogenic viruses are frequently used. Potentially infectious organisms such as adenovirus have specialty uses in the field of gene therapy. Obviously, careful attention to biological containment and good microbiological practice is critical. Commonly, tissue culture work is carried out with non-pathogenic *E. coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Aspergillus niger* or mammalian cells. Exposure to the relatively non-pathogenic *E. coli* used for much recombinant technology work has not been associated with diarrheal or other gastrointestinal illness. Several studies have demonstrated the lack of colonization of workers with recombinant organisms.[2]

For production of recombinant proteins, the most common infection risk comes from exposure to reagents used to support growth and productivity of the recombinant organism itself. Mammalian cells typically used for production are not dangerous themselves, as non-pathogenic recombinant organisms are used exclusively and they have been extensively tested for a variety of known and possible infectious agents. However, material of animal or human origin, such as raw serum, partially purified blood components (human or bovine serum albumin) or even highly purified reagents from animals (bovine or porcine insulin) are frequently required. The risk of known or previously unknown infectious agents in the source animals or humans must be carefully assessed. The devastating impact of unknown HIV infection in the blood supply on the hemophilia population is ample demonstration of the concern becoming reality. More recently, the apparent emergence of a new variant of a human transmissible spongiform encephalopathy agent, new variant Creutzfeld–Jacob Disease (nvCJD), following from the epidemic of bovine spongiform encephalopathy in the UK, has again highlighted the possibility of infectious risk. Although the exposure of workers to such infectious agents is probably lower than for those receiving the products, the need for appropriate precautions is clear, especially when the volume of materials handled or stored may be significant.

Another potential biological hazard involves special applications of hybridoma technology. Typically, antibodies are produced from two fused murine cells to produce specific antibodies, creating an immortal cell line. Atypically, a theoretical hazard of this activity exists when one of the two cell lines is human, which carries the risk of latent or unapparent viral infection. In vitro human cells, such as lymphocytes, are susceptible to infection with tumorigenic virus. In addition, the murine fusion partner can carry mouse type C leukemia and sarcoma. Although these viruses are typically not infectious of human cells, it is not known whether residence in hybrid cell lines

could alter the host range. If so, this may create a new hazard whereby pathogenic animal viruses extend their host range to humans (and vice versa). Studies to date have shown no evidence of clusters of cancer or other diseases or elevated rates of disease in workers.

The pharmaceutical industry creates final recombinant products as well as intermediate biological products that are highly concentrated and physiologically active. Normal physiologic responses to inhaled or absorbed substances may include hormonal and allergic responses. This is the same problem encountered in the conventional pharmaceutical industry.[3] Workers exposed to estrogens, growth hormone or other physiologically active chemicals can be expected to have unintended but predictable responses.

Allergy to the concentrated product has been seen in biotechnologists. Products and intermediate products may elicit immune responses. In addition, product development involves laboratory animal handling, which has its own set of allergic issues. Normal engineering controls, with occasional use of personal protective devices, represent the best means of preventing sensitization or other unintended physiologic responses.

In addition to these concerns surrounding recombinant organisms and their products, other important non-biological hazards exist in the biotechnology industry. These may be of a substantially greater magnitude than in traditional research laboratories. For example, many laboratory research operations use radioisotopes, including tritium (^{3}H), carbon-14 (^{14}C), sulfur-35 (^{35}S), calcium-45 (^{45}Ca), phosphorus-32 (^{32}P), chromium-51 (^{51}Cr), and iodine-125 (^{125}I). Several of these are volatile and therefore quite dangerous. Safe handling and dose reduction techniques should be collaborative efforts between a laboratory radiation safety liaison and a consulting certified health physicist. Given the relatively young age of the biotechnology workforce and the reproductive implications of hazards such as ^{125}I, additional emphasis should be placed on workplace safety. Most monitoring is done by radiation badge, a process comparable to industrial hygiene monitoring. Health physicists should perform thyroid and whole-body scanning when needed.

A surprising number of physical hazards are associated with both research and product preparation in this highly technical industry. Biotechnologists must deal with compressed gas, cryogenics, humidified atmospheres (with associated building biological hazards), high-voltage electricity, as well as some flammable and corrosive materials. Heat and cryogenic burns are a common hazard of laboratory and start-up production facilities, especially if laboratory personnel transport laboratory-scale hoses for production use. Ultraviolet light and associated skin hazard may be encountered in manufacturing suites, research biological safety cabinets, and electrophoresis operations. Laboratory and even manufacturing operations can be hands on, with lots of lifting and frequent exposure to glassware. In industry operations, glassware handling (and cleaning) present opportunities for sharps exposure. For one biotechnology company, lacerations comprised 41% of injuries and back injuries accounted for 93% of lost time.

One largely unappreciated aspect of biotechnology is shiftwork. Relatively small labor forces are engaged in both research and production, yet the cell cycle is continuous in both settings. Round-the-clock care of cultures is dictated by biological realities. Shiftwork may be informal in the laboratory or formal in production facilities, but problems of shifting human sleep cycles are inherent in the production of cell cultures. Young research workers cheerfully accept poorly designed work cycles and do not think of their tasks in terms of shiftwork.

Biotechnology processes require significant chemical use, with the potential for exposure.[4] Classes of chemicals common in biotechnology include culture additives and antibiotics, solvents and extractants (including those used in chromatography and sequencing), and the corrosive additives needed to maintain a work-

ing chilled-water facility. Ethidium bromide is a genotoxic compound used for fluorescent staining in sequencing operations. Cell culture additives are frequently mutagenic. The most common acute chemical overexposures have been to acetonitrile, which is used as an extractant. Patients with mild cyanide-like toxicity of acetonitrile have been reported anecdotally in several emergency situations following spills and inappropriate handling during clean-up operations. One potentially hazardous repetitive process involves the use of acrylamide in gel preparations. The purchase of preformed gels can reduce the hazard of handling the neurotoxic bis-acrylamide powder. Finally, many production processes, especially those using *E. coli*, result in a fusion protein as the primary product. The fusion protein requires peptide bond cleavage with a strongly reactive and specific chemical in order to prepare the final product. This cleavage is carried out at large volume with highly reactive chemicals, including hydrofluoric acid. Precautions to prevent both exposure of workers and inadvertent release into the environment must be in place.

MEDICAL SURVEILLANCE

Reduction of health risks in biotechnology is attained through control of work hazard and exposure, which requires in-depth focus on work processes and the methods of containment. Medical surveillance is a strategy for disease prevention that is based on a risk assessment of the jobs performed by the worker.[5] Medical surveillance is usually targeted to specific chronic risks.

Medical surveillance is not routinely indicated for workers simply because they work with recombinant organisms. There is no prescribed medical surveillance related directly to work with recombinant organisms. Studies of workers in the biotechnology industry have concluded that there is no evidence of adverse health effects related to the unique aspects of recombinant DNA technology.[6,7] Medical surveillance data have documented neither the occurrence of clusters of disease nor an increased incidence of diseases in workers who are frequently exposed to recombinant organisms.[5] Medical surveillance for health effects related to exposure is best conceived when certain criteria are fulfilled:

1. The exposure can potentially cause an identifiable health effect.
2. It is reasonably likely that the disease or effect may occur (and it is related to work).
3. There is an acceptable and scientifically sound methodology for diagnosing the condition or disease.
4. Early diagnosis has the potential to reduce morbidity or mortality.

When data collection and understanding the prevalence of a work-related condition are the goals, surveillance may target specific conditions even if therapeutic approaches are not yet available.

Some common exposures that may warrant medical surveillance in biotechnology laboratories and industrial settings include: infectious agents, sensitizing chemicals, radio-isotopes, and animal handling (a potential for zoonotic infections and allergic responses). The goal is detection of early health effects and prevention of long-term morbidity related to agents such as animal dander, enzymes, and endotoxin.

Work with recombinant organisms that present no identifiable risk to human health (group I in the European Hazard Classification scheme) would not entail routine medical surveillance. For work with highly pathogenic organisms or biologically active substances such as enzymes that are expressed by genetically modified microorganisms, periodic medical evaluation may be desirable. Medical surveillance for infectious disease endpoints emphasizes the identification of workers at risk and the early diagnosis of an infectious process.[8]

The goals of periodic surveillance are primarily to (1) detect early signs and symptoms of disease and (2) detect changes in the health of employees indicating a need for changes in job functions and/or work process. The genetically modified organism does not typically have human health effects different from those associated with the unmodified organism. However, the genetically modified organism may cause allergenic responses or the expressed products may have toxic effects and these may warrant medical surveillance. The periodic evaluation usually includes a health questionnaire. Other components of the evaluation depend on the type of exposure and health effect and should be targeted to specific exposures and health risks.[9] For example, a lung function assessment is appropriate if there is potential exposure to asthmagen(s) or endotoxin.

In general, implementation of the hazard control plan at work protects the workers from a significant risk of health effects related to work. However, removal from work with certain pathogenic microorganisms or biologically active products may be necessary in the case of an allergic or a susceptible individual.

Is medical surveillance also indicated for a special subpopulation of workers who are more susceptible to health effects from recombinant DNA technology? Who is potentially susceptible to health effects related to work with genetically modified microorganisms? Similar to any work with pathogenic microorganisms, individuals with reduced immunocompetence (including steroid treatment) and individuals with less effective barriers to infection (usually related to disease of the respiratory tract or gastrointestinal tract or illness or injury of the skin) represent a potentially susceptible population. Preplacement evaluations are therefore recommended to identify persons with medical conditions that may increase risk of adverse health effects in work with infectious organisms, including recombinant microorganisms. The focus of the preplacement evaluation is the potential for altered host defenses.

PREVENTION

Guidelines for laboratory animal use were formulated in the 1960s, and with the expanding use of recombinant organisms in research laboratories, the NIH has developed guidelines for research involving recombinant DNA, which have been revised annually.[10,11] The CDC/NIH revised biosafety guidelines were issued in 1999.[12] There are four levels of biosafety containment (BSL).[12,13] Level 1 is for well-characterized agents not known to cause disease in healthy adults and of minimal hazard to personnel. Level 2 is for agents with moderate potential hazard and requires special training for personnel. Level 3 is for potentially lethal infectious agents and requires specific containment, protective clothing, and special safeguards, such that many laboratories are not able to handle this work. Level 4 is for exotic highly pathogenic, poorly understood pathogens and is extremely rare in either the academic or industrial setting. Very recently, a new European Council Directive on contained use of genetically modified agents was approved.[14] Member states have the option of requiring even more stringent safety measures.[15] The European Directive focuses on appropriate training and supervision in the workplace where genetically modified organisms are used. In the USA, the NIH Recombinant DNA Advisory Committee (RAC) has set up strict guidelines for most recombinant DNA technology including gene therapy. Thus it is clear that biological safety has become a paramount issue on a global scale and that recombinant organisms have provided the impetus for these developments.

REFERENCES

1. Walsh G. Biopharmaceutical benchmarks. *Nature Biotechnology* 2000; 18:831–3.
2. Cohen R, Hoerner CL. Recombinant DNA technology: a 20-year occupational health retrospective. *Rev Environ Health* 1996; 11(3):149–65.

3. Klees JE, Joines R. Occupational health issues in the pharmaceutical research and development process. *Occup Med: State of the Art Rev* 1997; 12(2):5–27.
4. Ducatman Alan M, Coumbis John J. Chemical hazards in the biotechnology industry. *Occup Med: State of the Art Rev*, 1991; 6(2):193–208.
5. Liberman DF, Israeli E, Fink R. Risk assessment of biological hazards in the biotechnology industry. *Occup Med: State of the Art Rev* 1991; 6:2.
6. Vidal DR, Paucod JC, Thibault F, Isoard P. Biological safety in the laboratory. Biological risk, standardization and practice. *Ann Pharmaceut Francaises* 1993; 51(3): 154–66.
7. Finn AM, Scott AJ, Stave GM. Genetic modification and biotechnology. In: Baxter PJ, Adams PH, Aw T-C, Cockcroft A, Harrington JM, eds. *Hunter's diseases of occupations,* 9th edn. London, 2000, 521–35.
8. Rosenberg J, Clever LH. Medical surveillance of infectious disease endpoints. *Occup Med: State of the Art Rev* 1990; 5(3):583–605.
9. Goldman RH., Medical surveillance in the biotechnology industry. *Occup Med: State of the Art Rev*, 1991; 6(2):209–225.
10. Department of Health and Human Services, National Institutes of Health. *Guidelines for research involving recombinant DNA molecules*; October 2000. 65 Federal Register 60328. Available at: http://www.nih.gov/od/orda/
11. McGarrity GJ, Hoerner C. Biological safety in the biotechnology industry. In: *Laboratory safety: principles and practices,* 2nd edn. 1995, 119–131.
12. Centers for Disease Control and Prevention, National Institutes of Health. *Biosafety in microbiological and biomedical laboratories*, 4th edn. HHS Publication no. (CDC) 93–8395 1999.
13. Gilpin Richard W. Research activities including pathogens, recombinant DNA, and animal handling. In: McCunney RJ, ed. *Medical Center Occupational Health and Safety* 1999; 115–136.
14. Vranch S. New directive on biosafety: the contained use of genetically modified micro-organisms. *Pharmaceut Technol Eur* 2000; 12(5):42.
15. Health and Safety Executive. *Compendium of guidance from the Health and Safety Commission's Advisory Committee on Genetic Modification*. London: HMSO, 1997.