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Genetically Modified Microorganisms

Biosafety and Ethical Issues

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1. Introduction

Over the last 30 years, the ability to modify specific genes in microorganisms has revolutionized numerous fields of the biosciences, including medicine, agriculture, and basic research into life processes. However, this capability raises concerns about the potential hazards posed by the technology. In response to these concerns, specific protocols have been developed to safely monitor the use of genetically modified microorganisms (GMMs). It is the scope of this chapter to review safety issues that have arisen and address bioethical issues that have become apparent through GMM use.

GMMs are defined as bacteria, fungi, or viruses in which the genetic material has been altered principally through recombinant DNA technology, in other words, by means that do not occur naturally. The first section of this chapter addresses GMM safety through risk assessment, identification of hazards, and the methods to use GMMs safely. Subtopics include safety issues of GMM foods and food products, environmental release of GMMs, and concerns arising from horizontal transfer of GMM deoxyribonucleic acid (DNA) to other organisms. Next, a brief review of protocols and recommendations developed by regulatory agencies for the safe use of GMMs is given. A final section on safety reviews strategies used to engineer suicide GMMs.

The second part of this work is devoted to looking at issues of bioethics and GMMs. Specific attention is devoted to issues of patents and GMMs, labeling of GMM foods, concerns over releasing *GMMs* perception of GMMs by the general public, biological warfare using GMMs, and the consequences of not using this technology.

2. Historical Developments in GMM Risk Analysis

The issues surrounding GMMs have been controversial from their inception. In 1972, shortly after Drs. Herbert Boyer and Stanley Cohen first published their breakthrough research in DNA recombination, a self-imposed moratorium on certain types of cloning deemed hazardous was enacted by many who pioneered the field. A year later, potential hazards from the release of genetically modified organisms (GMOs) were raised at a Gordon Conference on Nucleic Acids. In an open letter to the National Academy of Sciences, attendees of the conference agreed to halt progress in the area

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until an international panel could review the subject. Those initial concerns in led to the formation of the Recombinant DNA Advisory Committee (RAC) in the United States in 1974 and, internationally, to the formation of the Asilomar Conference in 1975. Both were charged with addressing these issues. The findings from the Asilomar Conference recommended replacing the moratorium with a set of guiding rules for some types of recombinant research that were identified as posing minimal risk and prohibiting other research deemed too hazardous, such as the cloning of DNA from "highly pathogenic organisms." These recommendations were used by the RAC in developing guidelines, in 1976, for recombinant work, the basis of which were adopted internationally by other government agencies *(1–3)*.

The strict guidelines laid out through these initial regulations were relaxed by RAC and international agencies after mounting evidence demonstrated that the technology itself was safe. In the United States, regulations of GMMs were moved from the RAC into the Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), and the US Department of Agriculture (USDA); whereas RAC regulated recombinant issues relevant to human therapeutic uses *(1)*. International safety regulations for GMMs have been developed through several national and international agencies, including the World Health Organization (WHO), the Food and Agriculture Organization (FAO) of the United Nations, the European Union (EU), and the Organization for Economic Cooperation and Development (OECD), as well as other national and international regional agencies. The specific regulatory roles of these agencies regarding GMM safety regulations are noted elsewhere (*see* Sections 5.1 and 5.2) in this chapter.

Although it has been up to key government agencies to enforce regulation, it has primarily been the responsibility of the scientific community and the developers of specific technologies to accurately identify and define specific GMM safety issues. For example, to address issues regarding the release of *GMMs* and their potential impact on the environment, an international symposium, sponsored by the American Society for Microbiology, was held in June 1985 *(4)*. The seminars at this conference addressed several ethical issues pertaining to GMMs, including an analysis on the impact of Frost Free Ice$^-$ *Pseudomonas* (the first intentionally released GMM) on leaf surfaces *(5)*, the potential use of recombinant vaccinia virus *(6)*, methods to monitor modified *Pseudomonas* released into the environment *(7)*, previously identifiable exchanges of DNA between different bacterial genera as a prelude to potential exchanges of DNA between GMMs and microbial communities *(2,8)*, and model systems to apply established chemical environmental risk analysis to assess GMM environmental impact *(9)*. Subsequently, numerous publications *(2,10–20)* and international symposia *(21–32)* have attempted to address these and other GMM safety and ethical issues.

As GMM technologies have become more refined and developed in increasing applications, society's questions about the technology have become more widespread and vocal. Increasingly, concerns have been expressed not only by researchers and regulators, but also by large segments of the public, who deem recombinant technologies unnatural, dangerous, or unnecessary. This vocal opposition has developed into political discussions that produced mandates that, in many cases, are no longer based purely on scientific arguments. Therefore, the future development and use of GMM technologies lies not only in their proven safety and success record, but also in how safe GMMs are perceived by the public *(33)*.

3. Biosafety and Risk Analysis

The major misconception in risk analysis is that the term *safety* does not imply a 0% chance that a given hazardous event will occur *(11)*. Rather, safety is about identification of risk factors and minimization of the likelihood that a given adverse event will occur. The problem would therefore seem to be to identify accurately the risk factors associated with GMMs and their probability of occurring. However, the potential risk factors are often difficult to define, especially considering the evolutionary nature of organisms *(34)*.

In some cases, risk factors are fairly obvious. For example, in a medical research study, it is conceivable that a cloned virulence gene in a host such as *Escherichia coli* could produce a novel GM pathogen. Production of such GMMs is not uncommon when fundamental questions of pathogens are under investigation. However, if the GMM is properly contained and the laboratory closely follows safety guidelines, the research can generally be carried out safely.

In other cases, risk factors are less obvious, such is for Ice$^-$ *Pseudomonas*. The strain is produced through a knockout of a surface protein that serves as nuclei for ice crystal formation. In principle, the GM pseudomonads will no longer serve as ice nuclei; therefore, fruits containing Ice$^-$ *Pseudomonas* will not be damaged by light frosts *(35)*. On initial analysis, it would seem difficult to imagine a reasonable scenario wherein an Ice$^-$ GM strain would pose a significant risk to humans or the environment. However, it was argued that such bacterial surface proteins may be needed to initiate water droplet formation in the atmosphere, and that bacteria normally blown up into the atmosphere might serve as the initial nuclei to produce rain. The concern that ice$^-$ bacteria would disrupt weather patterns has been widely reported *(36,37)*. However, because ice$^-$ bacteria are a natural part of plant microflora and classically induced ice$^-$ mutants failed to show an impact on weather patterns, it is generally accepted that the impact on rainfall by Ice$^-$ GMM would be extremely small or nonexistent *(35)*.

Herein characterizes the complexity of identifying GMM risk factors. Which risk factors raise reasonable concerns? There are some general principles involving GMMs and their genes that must be taken into account when assessing risk *(38)*. Consider, for example, GMMs released into the environment. GMMs or their recombinant genes have the potential to interfere with indigenous organisms by disrupting complex biological interactions *(39)*. That is not to infer that there is an inherent risk posed by these GMMs, but merely that the potential is there for such an outcome. If this risk potential is coupled to the realization that genes are transferred between different members of microbial communities and that the comprehension of the maintenance of genes in a population is still incomplete *(40,41)*, then there is some understanding of the difficulty identifying the true risks that GMMs pose to the environment.

The reality that science cannot provide absolute assurance about the safe use of GMMs has left policymakers looking for methods of regulation that address political realities. The 1992 United Nations Conference on Environmental and Development meeting in Rio de Janeiro, Brazil, formulated the *precautionary principle*. In essence, the precautionary principle states that politicians, when faced with uncertainty and potential risk from recombinant technologies, may act to prohibit the technology in the absence of scientific proof of the true nature of the hazards *(42)*. Therefore, based on

the precautionary principle, contamination and persistence of GMMs and their genes are regarded as a potential risk and are acceptable reasons to prohibit GMM releases into the environment or their presence in foods.

This interpretation of the precautionary principle has been used more extensively to restrict industrial use of GMMs in European nations than in the United States *(43)*. In the European Union, the precautionary principle has been interpreted as meaning that, despite current limited evidence of hazards from a given GMM, new risks may become evident in the future, and the prudent action is to ban a given practice. In the United States, the precautionary principle is interpreted more conservatively and is not based exclusively on a risk-free policy. Uncertainty in the US approach is addressed through regulatory agencies, which produce policy directives to maintain safe uses of GMMs *(44)*.

If there is a perceived risk with GMMs and their DNA, the question pondered is, why should GMMs be used in the first place? The obvious answer is the enormous potential that recombinant technologies bring to bioremediation, medicine production, food production, and a wide variety of other industrial processes. However, despite the promise of the technology, there has to be a point at which a given risk is unacceptable. At the Second International Symposium on the Biosafety Results of Genetically Modified Plants and Microorganisms, Hull proposed the following formula to assess risk *(29)*:

$$\text{Acceptable risk} = \frac{\text{Probability of hazard } A \times \text{Magnitude of the hazard } B}{\text{Benefit from this product } C}$$

Risk assessment is also, and perhaps more frequently, determined without the denominator benefit variable of this equation *(11,14,19,45)*.

Whereas the probability of hazard *A* has been estimated experimentally from the stability and transmittability of the DNA or product of the GMMs *(46–49)*, the magnitude of the hazard *B* seems the more difficult variable to quantify. Typically, biotechnology watch groups concentrate their efforts primarily on the magnitude variable and project grave consequences for GMM applications *(50,51)*, whereas industries using GMMs tend to concentrate their safety efforts on reducing the probability variable *(52)*.

One way that biotechnology firms accomplish their measure of safety is by testing and defining the probability variable as safe through the *substantial equivalence principle*. This principle defines the safe use of a GMM product as minimal risk if, for all practical purposes, it has the same impact on the environment as the non-GMM form. Finally, when risk is calculated using the Hull equation, the benefit of the product *C* is normally defined by direct comparisons of the result or value derived from the GMMs vs those technologies that do not use GMM technologies *(53)*. For example, if bioremediation is to be carried out using a GMM compared to a non-GMM, comparisons might include how completely the compound is mineralized in both systems and a cost–benefit analysis of both approaches.

4. GMMs and Safety Issues

4.1. Human Risk

Humans may encounter GMMs or their associated DNA in a variety of ways, including in food products, in GMM vaccines, or as interactions with released GMMs in the environment or laboratory. In the future, exposure to GMMs may also include

whole GMM foods and probiotics (viable organisms that have medical beneficial effects when ingested). Currently, human risks are generally placed in the following categories: increased exposure to antibiotic resistance genes; which may result in transfer of antibiotic resistance genes to indigenous flora, transfer of genes accidentally or intentionally that might produce human pathogens; production of GMM toxins; and activation of human immune allergies *(54,55)*.

4.1.1. Risk From Antibiotic Resistance Genes

Antibiotic resistance genes are the most widely used selectable markers for general cloning. However, there are credible concerns that antibiotic resistance genes might be transferred into other microorganisms, including known pathogens or opportunistic pathogens. In one obvious example, the most widely used microorganism in genetic research, *E. coli*, can readily exchange DNA with a host of known enteric pathogens via plasmids or transposons through methods such as conjugation, transduction, or transformation *(56,57)*. If a pathogen does successfully pick up an antibiotic resistance gene, it is in effect picking up a potentially novel virulence factor *(58)*. For this reason, regulatory agencies worldwide generally ban outright use of viable GMMs containing antibiotic resistance in foods and are attempting to minimize antibiotic genes in GMMs used to produce processed foods *(59)*. Certainly, widespread use and misuse of antibiotics is a major contributing factor to the worldwide epidemic of antibiotic resistance in a host of microbes; however, introducing antibiotic resistance genes may exacerbate the problem.

4.1.2. Risks of Human Toxicity

To determine the potential human toxicity of a specific GMM or GMM product, a series of defined toxicity tests must be completed *(20)*. To evaluate the toxicity levels of the product, analysis is used to determine the dosage at which no adverse effects are measured. This value is defined as the *no observed adverse effect level* (NOAEL) *(20)*. The NOAEL value is then used to develop a safe level for human consumption.

Toxic metabolites are typically analyzed through in vitro analysis, including Ames tests and cell line cytotoxicity assays and through in vivo animal testing analyses, including acute oral, subcutaneous, interperitoneal, and inhalation toxicity tests *(14,20)*. Typically, a large margin of safety is added to generate a safe level for human consumption. The NOAEL of whole food is calculated because whole foods are too complex to identify all effects the GMM products might have on the food. Foods are considered safe once it is established that the GM food is as safe as the traditional, non-GM food, thus complying with the concept of substantial equivalence. Hazard and risk analysis flow diagrams have been developed to use this paradigm *(20)*.

4.1.3. Risk of Allergies

Allergies are caused by the specific activation of an inflammatory process resulting from allergens interacting with immune effector mechanisms *(60)*. Specific allergies show geographic distribution primarily because of dietary considerations. Some of the best-documented examples of food allergies include peanuts, milk, hen's eggs, Brazil nuts, hazelnuts, walnuts, shellfish, celery, kiwi fruit, and rice *(54,61–64)*. Although typical symptoms of food allergens are not life threatening, severe anaphylactic reactions may be fatal *(60,61)*.

The concern derived from GMM foods is whether a novel protein expressed in a GMM will produce an allergic reaction. To test for the likelihood of an allergic response, the GMM protein can be tested for serological cross-reactivity with known allergens *(65)*, its amino acid sequence can be compared to that of known allergens *(66)*, or the GMM protein's stability in simulated gastric fluid can be determined *(67)*. Direct immunoglobulin E responses can be analyzed in animal models *(60,67–69)* or through skin prick tests *(70)*. To date, only one genetically modified (GM) food was ever found to have transferred an allergenic protein. The allergen was identified before the product was marketed, and its founder, Hybrid International, never marketed the GM soy *(64)*.

4.1.4. Risk From Unknown Pathogenicity

The lessons learned from the agricultural use of *Burkholderia cepacia* might serve as a model to identify the risks from released GMMs. Strains of this organism have been developed and used for their diverse metabolic properties, including their use as a biofungicide and their ability to biodegrade herbicides *(71–74)*. It is becoming increasingly evident that *B. cepacia* is also a pathogen in cystic fibrosis patients, causing serious pulmonary deterioration and associated fatal bacteremia *(74–79)*. These organisms were developed by classical selection methods; hence, they are not technically GMMs. However, even as the connection between the widespread use of *B. cepacia* and its frequency in cystic fibrosis patients is still under investigation, the connection is considered a warning by some against the widespread introduction of a novel microorganism into the environment *(80)*.

A second example perhaps better illustrates the potential harm that a specific GMM might inflict. During the course of a mouse sterility research program, a gene encoding interleukin-4 was inserted into mouse pox. Inadvertently, a GM virus that was highly lethal to mice was engineered *(81)*. Because the research group followed the proper safeguards, the GM virus was properly contained, and the experiment was terminated without further incident. However, the potential release of an unintended pathogen constructed through recombinant manipulation is an unacceptable consequence of this technology.

To assess the potential risk of a given ingested GMM as a human pathogen, animal models have been suggested and developed *(82)*. The first of these systems was developed using streptomycin-treated mice to develop complete human gut microflora. The findings from these model systems paralleled results between human and mouse responses to diet and the colonization by enteric flora. This suggests that these mouse gut models should be an excellent system to analyze the impact of GMMs in the human gut. Potentially, the flora of other parts of the human body could likewise be mimicked in such models *(82)*.

Another area in which unknown pathogenicity may arise is in the development of GM viruses used in gene therapy. This technology has shown remarkable promise in the treatment or therapy of many human diseases *(83–85)*. Unfortunately, there have been well-documented tragic failures with this technology, including the death of Jesse Gelsinger, who was under treatment for an ornithine transcarbamylase deficiency *(86)*. Also, the onset of leukemia in patients under treatment for the X-linked form of severe combined immune deficiency disease further raised concerns about the technology *(87)*.

Gene therapy, with its inherent risks, must be examined on a case-by-case basis by institutional and federal medical review boards before any trials are conducted *(86)*.

4.1.5. Risk From Known GMM Pathogens

There are relatively few scenarios by which a known GMM pathogen might infect a person. Interaction with known GM bacteria, fungi, or viruses containing pathogenic genes or pathogens genetically altered to analyze the organism could occur as a result of a laboratory accident. For example, a known pathogen with a gene knockout may inadvertently infect a laboratory researcher during the course of an experiment. The likelihood of such accidents is small if such experiments are conducted in the proper biosafety containment facility (*see* Sections 6.2 and 6.3). However, such accidents do happen.

In reviewing the cases of known incidences, many would have been prevented if proper safety measures were followed. In an extensive survey made in 1979, over 4070 cases of laboratory-acquired infections were identified *(88)*. These infections were collectively caused by 38 different species of bacteria, 84 different types of viruses, 16 species of parasites, 9 species of rickettsia, 9 species of fungi, and 3 species of chlamydia. Although it is not completely clear what percentage of these infections were actually obtained in the laboratory, there is an evident association with the type of work conducted in the laboratory and the acquired infection. In 20% of these cases, an identifiable incident resulted in the infection; in many of the other cases, workers contracted the infectious agent through an unknown incident *(89,90)*. Although these laboratory-acquired infections were presumably caused by non-GMMs, these incidents do indicate an area of concern regarding GMM pathogens.

Humans also might logically encounter pathogenic GMMs as the result of biological warfare or a terrorist action with GMMs used as the weapon. Obviously, the only way to prevent human exposure to these weaponized GMMs is to prevent their use. The 1972 international Biological Weapons Convention (BWC) with 150 signatories unfortunately has not been the last word on the development of weaponized GMMs *(91)*. The threat from GM weapons will need to be met by vigilance of the international community to prevent such weapons from development and deployment.

4.2. Environmental Impact

Although the number of GMMs released directly into the environment is currently relatively low, the large number of proposals to release organisms in the environment to remediate contaminated soils, improve soil fertility, manage pest control, and vaccinate livestock and wildlife has prompted active research and regulation into the safety of released GMMs *(92)*. Introduced here are some of the general concerns from different types of environmental releases, including determination of how to assess risk from released GMMs, methods to control the dissemination of GMMs, methods to monitor the impact of a GMM on microbial flora, and methods to monitor GMM activity.

4.2.1. Risk Analysis

As noted in Section 3, risk analysis of a GMM minimally involves the magnitude of a risk multiplied by the likelihood the risk will occur. To address these two variables, risk assessment for GMMs released into the environment needs to answer several questions *(93)*:

1. Are there potential hazards the GMM might impose on the environment?
2. How likely is it that the potential hazard will actually happen?
3. What are the consequences if the hazards are realized?
4. What management procedures, if any, are needed to control the risk?
5. What level of monitoring is necessary to confirm the risk assessment and determine whether control measures are efficacious?

A flowchart for events that might lead to an environmental catastrophe was developed by the US Office of Technology *(94)*. In this scenario, (1) a hazardous gene is inserted into a microorganism; (2) the GMM escapes into the environment; (3) the GMM multiplies and establishes a niche in the environment; (4) the GMM produces some "factor" that causes disease or damage; and (5) the hazardous effect is manifested in humans or other hosts. Each step can be assigned a probability for actually occurring.

In considering GMMs for release into the environment, it is imperative that the GMM demonstrate no adverse human health effects, be nonharmful to agricultural interests, and produce no irreversible damage to the ecosystem into which it is released *(95)*. Therefore, before the organism is released, an environmental impact study must be conducted in a controlled environment, such as in growth chambers or in greenhouses *(14)*. Data from these studies are used to identify the variables necessary to make a risk analysis of the release. In addition to any toxicological analysis of the GMM, a profile of the natural microbial population should be determined prior to the GMM release.

There are several issues that need to be addressed when considering how a given GMM will behave in a complex environment. For the industry using GMMs, an important issue is determining if the organism actually completes its task in its complex environment and how long the organism remains active *(96)*. Because of this need to maintain a stable form of the organism in the environment, safety concerns arise as to how that GMM will behave in an uncontrolled environment. First, an evaluation must be made to determine if conditions are favorable to permit cell growth beyond the release area. Once growth patterns are determined, an analysis must be undertaken to identify gene expression pattern changes when the GMM is released into the environment *(47)*. This might be the result of mutations that the GMM acquires as a result of stress factors from the environment. Such mutations could fundamentally change the niche of the organism *(97,98)*.

After the organism is evaluated, the impact the organism has on the local environment must also be evaluated. For example, chemical and physical impacts on the environment need to be determined *(99)*. Also, the impact generated by the GMM on indigenous microflora needs to be analyzed and evaluated *(28,93,96,100)*. The GMM itself needs to be monitored both inside and outside the release area to assess local impact. To aid in this analysis, evaluation trees have been devised to determine the potential hazards of GMM releases *(101)*.

In comparison to GM plants, there have been relatively few GMMs released directly into the environment that can be used directly as case studies for safety assessment. An analysis completed in late 1998 of the OECD databases identified that only about 1% of intentional GMOs released were bacterial, 0.3% were viral, and 0.2% were fungal.

Released GMMs included a variety of different organisms. *Sinorhizobium (102,103)* and *Bradyrhizobium* have been used to improve soil nutrition. *Pseudomonas (104)* has been used in a variety of plant or microbe model studies and to model GMMs released

into soils *(10,105)*. *Clavibacter xyli* modified with the δ-endotoxin has been used in environmental releases in pest control *(106,107)*. GMM release studies have also been carried out in animals, such as in the use of GM *Lactococcus lactis* in pigs *(108)*. Viral GM releases to date include the use of recombinant baculovirus for pest control *(109)*, of bacteriophages to trace and monitor pollution *(110)*, and in recombinant vaccines for rabies control *(19)*. Although not a comprehensive listing of all GMMs developed for environmental release, these examples give an overall indication of the types of organisms used for GMM releases.

To provide a framework for the safe use of GMMs to be released into the environment, the Safety in Biotechnology Working Party of the European Federation of Biotechnology developed a risk assessment classification system for the environmental release of GMMs *(19)*.

- Risk Class 1: GMM generally considered safe and highly unlikely to produce adverse effects.
- Risk Class 2: GMM that may produce local adverse effects, but are generally unlikely to produce a serious hazard. This includes localized transient displacement of indigenous species and temporary minor biological or chemical changes to soils. GMM can be treated or controlled. The organism is unlikely to disseminate beyond the treated site.
- Risk Class 3: GMM likely to produce serious adverse effects that act locally at the release site. GMM or application area can be treated or controlled. Impact includes a localized displacement of indigenous organisms and major changes in soil biochemistry that are restorable. The organism is unlikely to disseminate beyond the treated site.
- Risk Class 4: GMM will likely produce serious adverse effects likely to disseminate beyond the treated site. GMM and its DNA persist at the site and may result in serious damage to the soil fertility and vegetation. No treatment or control of the GMM is available.

When using GMMs *in situ*, care should be taken to ensure that the GMM does not become disseminated beyond its trial site. However, it may actually be impossible to restrict a GMM completely to its inoculation site *(38)*. Wind *(38)*, water *(38,111,112)*, and insects such as grasshoppers *(113)*, caterpillars *(114)*, Southern corn rootworm *(115)*, and earthworms *(116)* have all been shown to disseminate recombinant microorganisms from the initial inoculation site. It is reasonable to assume other physical processes that displace microorganisms will also actively transport GMMs to distal sites.

It is also important to have a fundamental understanding of the biology of the organism used to safely construct GMMs. Does the organism produce spores (i.e., *Bacillus, Clostridium*, etc.) or enter a dormant state (i.e., *Arthrobacter*)? What are the physical growth requirements for the organism? How does the organism behave physiologically once it is stressed? What is its affected host range? All of these are essential questions that require answers before the organism is released *(17,117)*.

4.2.2. Environmental Releases of Recombinant Viruses Used as Vaccines

In the United States, evaluations for the safe vaccination of organisms are reported through the "Summary Information Format for Environmental Release" and are carried out through the Center for Veterinary Biologists, which evaluates experimental vaccines. Because such vaccinations may include recombinant GMMs, their assessment is noted here. Several criteria are evaluated, including: (1) the location of the study; (2) the physical characteristics of the test site; (3) the qualifications of test per-

sonnel; (4) the design of the experiment; (5) the potential for dissemination of the organism; (6) the potential that the organism may become established in the environment, and (7) contingency plans if problems arise in the study *(58)*. Active programs have been developed to release GM vaccinia virus to eradicate rabies in fox, raccoon, and coyote populations in Europe and North America *(118)*. An episode in which a woman in Ohio apparently became infected with GM vaccinia virus after removing bait laced with the virus from her pet dog has raised concerns about this practice *(119)*.

4.2.3. Pest Control Measures Using Released GMMs

Several GMMs are under development for the use of pest management. These include mammal, insect, fungal, and plant pests *(120–124)*. It is essential in the development of a risk assessment for pest control that the GMM is proven safe for humans and nontarget organisms before the release is conducted *(124)*. Particular care should be taken to identify susceptible species indigenous to the release area. Microbes with especially broad host ranges should not be considered for pest control.

When a pathogen is released into the environment to control pests, there is always the possibility that infected animals will be intentionally moved to areas outside the approved release area. In Australia, non-GM myxoma virus was released to control feral nonindigenous rabbits in the early 1950s *(125)*. In 1953, a physician who wanted to control local native European rabbit populations released rabbits infected with the myxoma virus on his estate near Paris. The resulting infections originating from that release devastated wild rabbit populations in Europe, which in turn had an impact on local prey species and caused significant economic damage on rabbit farms in western Europe *(126)*. Although not a GMM pathogen, similar events could lead to the spread of a GMM pathogen beyond its intended control area.

4.2.4. Determining the Impact of Released GMMs on Microbial Ecology

A relevant and complicated component of risk assessment in environmental release of GMMs is profiling the indigenous prerelease microbial populations as a basis to detect changes in biodiversity resulting from the released GMM. Analysis of microbial ecology is a developing science, and the protocols necessary to analyze total microbial populations have yet to be standardized. However, several methods have been established to characterize microbial communities affected by a released GMM *(96)*. Examples include the use of protocols to monitor indigenous enzymatic activity *(127,128)*; and the use of culture techniques *(93,128–131)*, BIOLOG® GN microplates, and other metabolic analysis profiles *(132–135)*, fatty acid profiles *(136–139)* and a wide variety of DNA or ribonucleic acid (RNA) analysis methods *(129,140–153)*. Van Elsas and coworkers *(154)* extensively reviewed these and other methodologies designed to assess the effect of GMMs on diverse microbial ecosystems. Regardless of the method finally selected to analyze the impact of a released GMM on local microbial diversity, it is important to recognize that each protocol has its limitations. Therefore, it currently is not possible to derive a comprehensive picture of all the potential impacts from a released GMM.

Data from release studies have already shown significant differences in microbial communities when comparisons are made between GMM strains and the equivalent non-GMM strains. In one study, *Pseudomonas fluorescens* CHA0 significantly changed soil bacterial populations associated with cucumber roots *(129)*. Likewise, *Pseudomo-*

nas putida GMM has been shown to have an effect on natural fungal flora not targeted specifically by the GMM *(155)*. Impact studies have also been used successfully to analyze the effect of a GM baculovirus on a closed aquatic microbial community *(134)*.

4.2.5. Monitoring GMMs in Environmental Releases

Monitoring GMM cell growth may be accomplished using a variety of methods, including the use of selective and differential media and the use of reporter genes to follow cell growth, immunoblotting, cell profiling, or molecular biology techniques. Many of these methods have been extensively reviewed *(154,156,157)*. Identification of released GMMs using detectable proteins offers both inexpensive and relatively simple protocols to detect microorganisms harboring DNA modifications. β-Galactosidase *(111,115,158–160)*, luciferase *(96,105,161,162)*, *xylE* gene product *(156,163)*, and green fluorescent protein *(164,165)* have all been used effectively for detecting the presence of released GMMs.

Other enzyme markers have been proposed and used as methods to selectively isolate GMMs from the environment. Certainly, the use of antibiotic resistance markers is a feasible method to follow released microorganisms, and such methods have been successful *(41,166)*. However, both US and European agencies governing the use of released GMMs are making substantial attempts to limit, if not eliminate, the use of antibiotic resistance markers in released organisms *(14)*. One of the alternatives under investigation is the use of selective genes such as those that code for metal resistance *(158,167)* and catabolic genes *(168)*.

Immunoblotting techniques have also been developed to monitor released GMMs. One of the advantages of a serological approach is that it permits the detection of both nonviable and viable cells *(169)*. This approach is currently used to detect GM products in foods *(15)*. An alternative method of monitoring that offers similar advantages, although it is generally less sensitive, is lipid profiling of the environmentally released GMM *(159,170)*.

Increasingly, however, molecular DNA or RNA methods are the preferred protocols to monitor released GMMs. In addition to detecting the organisms, nucleic acid-based methods are able to detect the recombinant genes in other hosts, potentially identifying horizontal gene flow. There is a wide selection of methods available, including the use of Southern and Northern hybridization methods, polymerase chain reaction amplification, and gene-chip technologies *(171–174)*. The use of existing sequences, such as ribosomal RNA, highly conserved and unique genes, or specifically engineered unique sequences is considered the best approach for molecular monitoring *(39,156,170)*.

4.2.6. Survival of GMMs and Their DNA in the Environment

A critical consideration in determining safe release of a given GMM is determining whether the GMM is inherently more or less fit to survive in the environment *(46)*. With the advent of recombinant strains, it was generally believed that recombinant organisms would be inherently less fit than indigenous bacterial flora; thus, GMMs would not persist in the environment. Some studies have demonstrated this phenomenon *(175)*. However, there are also data that show GMMs either as stable as non-GM forms *(53,153,176,177)* or with enhanced stability *(160,178)*. Velkov *(47)* reasoned that persistence of GMMs or DNA from GMMs might result from a variety of different cellular processes, including adaptive processes associated with quorum sensing, acti-

vation of cellular responses that lead to resistance, and activation of hypermutagenic processes in the cell. Further, these processes might lead to persistence of the DNA itself via plasmid transfer to other species. Evidence has been found that some GMMs can be maintained in the environment for at least 6 years, even in the absence of their symbiotic hosts *(179)*. Studies with recombinant genes in chromosomes or plasmids showed persistence, but generally on the order of a month or less *(40,48,180)*.

Likewise, the genetic stability of the GMM construct is an important consideration when determining risk factors. GM fungi, for example, normally have stable chromosomal constructs *(181)*. However, GM fungal strains may become unstable after selective pressures are removed *(182,183)*. It has been noted that fungi altered as weed control agents may change their host range as a result of a change in the genetic structure of the GM fungi *(184)*. This increased host range may represent a potential hazard in environmental release of GM fungi.

4.3. Horizontal DNA Transfer

One of the major safety concerns surrounding widespread use of GMMs is their ability to exchange DNA with other organisms in an uncontrolled environment *(98)*. In the prerelease evaluation, studies should be undertaken to determine the stability of the construct as defined by its inability to transfer the construct horizontally to other organisms. If any instability is identified in this analysis, release of the organism should be reevaluated. Likewise, during the release, frequent monitoring should be conducted to determine if the construct is stable *(14)*.

It has been well established that bacteria are capable of exchanging DNA between very distant species, even between Gram-positive and Gram-negative organisms *(185–187)*. There is even evidence of horizontal transfer of bacterial genes to eukaryotic organisms *(188)*. Of concern is that, once released, a given GMM may transfer its modified DNA to indigenous species. A short list of bacteria identified as capable of exchanging genetic material under "natural conditions" has been generated *(189)*. These have been broken into four groups: Group I includes members of the genera *Escherichia, Shigella* (excluding *S. dysenteriae*), *Salmonella, Klebsiella, Enterobacter, Citrobacter*, and several *Pseudomonas* species; Group II includes several *Bacillus* species (*Bacillus subtilis, Bacillus licheniformis, Bacillus pumilus, Bacillus globigii, Bacillus niger, Bacillus natto, Bacillus amyloliquefaciens*, and *Bacillus aterrimus*); Group III includes members of the genus *Streptomyces*; and Group IV includes members of the genus *Nocardia*. These studies have shown DNA can be readily exchanged between members of the same group under natural conditions.

Therefore, novel GMMs should be evaluated to determine whether recombinant DNA present in the GMM could be transferred to other species. This analysis needs to be completed prior to an environmental release *(14,190)*. This precaution is necessary to minimize the chance that a transgene may be expressed and produce undesirable consequences from a novel combination of genes. Studies analyzing exchange of GM *Pseudomonas* DNA with other microorganisms in the rhizosphere and spermosphere showed that horizontal transfer could be greatly reduced if the genes are encoded in the chromosome rather than plasmids *(181,191)*. The frequency of transfer increases when the trait in question provides a selective advantage for the host, such as resistance to bacteriophages; acts as a virulence factor; confers additional substrate utilization; or

provides a bacterial antibiotic *(14,22)*. Fortunately, the frequency of transmission of DNA, even in "worse case scenarios," is low *(14,180)*. To minimize horizontal DNA transfer further, conjugal plasmids and transposons should be avoided in constructing GMMs used in environmental releases.

4.4. Safety Issues of Foods Derived From GMMs

A quarter of all food products are processed with the aid of microorganisms *(23)*. This includes foods composed of living microorganisms, foods produced through fermentation, and additives that use microbial components. Potentially, many of these foods could benefit from recombinant technology, including improved nutritional value, simplified downstream processing, or increased stability of the food products. However, above all else, foods produced through recombinant technologies must be proven safe.

Several factors need to be taken into account when considering the potential of GMM food safety, including (1) that the GMM is nonpathogenic; (2) whether it will colonize the human gut; (3) the possibility that the GMM will transfer its DNA to indigenous gut flora; (4) that the products produced from the GMM are safe; (5) that the vector components have an approved safe origin; (6) that genetic regulatory elements are safe to use; and (7) that specific foreign genes used in the GMM are safe *(16)*. A few food additives are produced by GMMs, including chymosin, pectinases, and aspartame. However, there are proposals to develop several other GMM foods or GMM-derived food products, notably in the production of cheese and buttermilk *(192)*.

Safety of foods produced via GMMs was studied extensively in the European Union through a joint commission of the FAO and WHO. Collectively, a joint report was generated to develop standards to assess the safety of GMM foods *(24)*. In their deliberations, foods containing viable or nonviable GMMs and those produced by fermentation were considered. This excluded highly purified food additives such as vitamins and GMMs used in the agricultural production of these foods. Additional regulations on novel food products have been published elsewhere *(193)*. As discussed in Section 5.1, GMM food safety in the United States is covered by the FDA, in Australia and New Zealand by the Australia New Zealand Food Authority, and in Canada by Health Canada *(194)*.

To identify potential hazards arising from the use of GMMs, a full accounting needs to be made of the host organism used, DNA donor organism used, specific biotechnology processes used to engineer the GMM, stability of the construct, and the specific genetic modifications used to make the GMM *(24)*. Comments and concerns arising from the initial listing of the components of the GMM should be addressed through examination of the GMM and food product. Once an initial analysis of the GMM is completed, information regarding the impact of the novel GMM and GMM-derived food product on human metabolism, both from direct ingestion and indirect exposure, needs to be reported properly. To make an accurate assessment of the potential risk from the GMM-derived food, a determination must be rendered as to the amount of product to be consumed *(24)*.

Specific attention has been brought to the use of antibiotic resistance genes in developing GMMs for food processing. Because such genes are widely used in processes to develop GMMs, there is a significant concern that the use of such genes could contami-

nate and transmit antibiotic resistance to normal host flora or even pathogenic organisms. Therefore, it is strongly recommended that the use of antibiotic genes be avoided in developing GMMs for food purposes *(24,59)*. Another concern is that the genetic modifications might activate the production of a toxin not found in the nonmodified strain. It is also conceivable that modifications of the organism can change its nutritional profile, thereby making it a less desirable strain than the nonmodified form. As noted in Section 4.1.3, arguments have also been made that GMMs need to be scrutinized particularly for their ability to cause human allergies, and that an assessment needs to include all populations, including those that individuals who are immune compromised *(24,64)*.

The method widely used to assess GM food safety follows the homologous concepts of substantial equivalence and substantially similar in the European Union and the United States. Through these principles, food safety involving GMOs is determined by directly comparing the GM and non-GM versions of the food product. This methodology has been adopted by the WHO, OECD, FAO, and FDA. The adoption of these concepts is not without its detractors. However, WHO and FAO insist that the substantial equivalence concept is intended to be developed as an initial analysis of the GMO food, not necessarily as a determinative evaluation of its safety *(23)*. This approach has been developed in large part because of the difficulty in applying conventional toxicology to determine the safety of any given GMM food. A typical investigation of a novel GMO food product is likely to include an in vitro analysis of the organism, a detailed analysis of the food product, and an analysis of the consumed product. It has therefore been proposed through FAO and WHO that both the GMO and the resulting food product be appraised separately using the principle of substantial equivalence to evaluate food safety *(24)*.

If living microorganisms themselves are used in the food product, such as is true of many dairy products, there is a reasonable concern regarding the impact of the GMM on the microbial flora of the gut. The organism could potentially transfer its recombinant DNA to indigenous flora of the gut or alternatively may interfere with complex interactions between different microbes. These concerns have been extensively analyzed and studied in lactic acid bacteria *(195)*. It is also possible that recombinant genes may be transferred into pathogenic organisms and convert opportunistic pathogens into pathogenic forms. Opportunistic microorganisms may also become pathogenic if the natural inhibitory effects of the normal microbial flora are altered *(24,26,195)*. Methodologies similar to those previously noted to evaluate complex ecosystems for environmentally released microorganisms may need to be investigated prior to producing viable GMM foods.

For novel GMMs and GMM products that cannot be determined using the substantially similar methodology, it will be necessary to use more extensive toxicological analysis called for through the precautionary principle *(95)*. These assays will normally include general chemical analysis, animal testing, cytotoxic evaluation *(196)*, antinutrition analysis *(26,44)*, and carcinogenic investigation *(14,20)*. For these foods, a case-by-case analysis will be necessary to evaluate human risk from exposure. Foods produced following either philosophy should be further analyzed by monitoring human populations to identify abnormal pathologies in susceptible individuals.

In contrast with the US regulations, since 1977 EU regulations have stipulated that viable GMOs must undergo regulatory approval requiring extensive documentation of the product's safety. Guidelines for safety using GMMs have been established for a variety of different GMMs for food processes; many of these have been published *(16,22,26,30,32,197–199)*. Following the recommendations put forth in the International Life Science Institute Consensus Guidelines, decision trees for assessing the safety of GMMs used in food have been developed. These decision trees are initially used to classify food GMOs into three risk groups termed the Safety Assessment of Food by Equivalence and Similarity Targeting (SAFEST). The different classes of SAFEST GMMs are noted next.

SAFEST Class 1 GMO foods are those in which no foreign DNA has been introduced, and the gene expression pattern is the same as for the unmodified organism. These organisms are considered substantially equivalent to the nonmodified safe microorganism.

SAFEST Class 2 GMO foods are those sufficiently similar to traditional foods. Such products are then assessed on their intended differences, with most of the analysis directed to evaluate the nature and consequences of the genetic differences.

SAFEST Class 3 GMO foods are for novel products for which there are no safe traditional foods to compare the GMM food product. Foods belonging to this category will require the most extensive testing to determine the safety of both the GMM foods and the GMM organism. Presumably, extensive toxicological investigation of the product will need to be undertaken, including the use of animal models. Because these foods will likely be extensively tested using conventional analysis protocols, it is less likely that the doctrine of substantial equivalence would be used in their assessment.

5. Regulations Addressing Safe Uses of GMMs

Listed in this section are general rules and philosophies covered by different international agencies to develop and maintain safe workplaces and safe use of GMMs. It is important that researchers using GMMs adhere closely to safety rules and recommendations when working with any organism that has a perceived risk. Indeed, there is a direct correlation between laboratory personnel who had fewer infections originating from laboratory strains and showed more awareness and concern about infectious agents used in their work, more readily identified hazards in their workplace, and generally maintained an enhanced respect of safety matters than those who generally reported more laboratory accidents *(17,200)*. Although GMM safety regulations vary in different nations, a representative list is noted here. Individuals working with GMMs need to determine the specific agencies and adhere to regulations covering their work.

5.1. United States and GMM Safety Regulations

In the United States, the use of GMMs is controlled by several government agencies, including the EPA, the FDA, the USDA, and the National Institutes of Health (NIH). The EPA generally regulates uses of GMMs that might have a potential impact on the environment; the FDA oversees GMMs used in food and pharmaceutical production;

the USDA regulates GMMs that have an impact on agriculture; and the NIH primarily is responsible for GMMs used in developing or studying issues related to human health. A brief look at each agency's contribution to GMM safety regulation follows.

5.1.1. The Environmental Protection Agency

In the United States, safe use of GMMs in industrial settings such as environmental releases, biofertilizers, and bioremediation is regulated by the EPA *(102)*. In a publication, "Biotechnological Program Under Toxic Substances Control Act," (www.epa.gov/opptintr/biotech/biorule.htm) GMMs such as those used commercially in biotechnology are defined as "intergeneric" and as defining "new" organisms. Before intergeneric organisms are used for commercial means, companies must first submit, 90 days prior to use, a document, "The Microbial Commercial Activity Notice" to the EPA. During this 90-days period, the EPA makes a determination on the document submitted. Likewise, the EPA also evaluates environmental releases of GMMs. At least 60 days prior to field tests, the experimental release application "Biotechnological Program Under Toxic Substances Control Act" (www.epa.gov/biotech_rule/pdf/t8669.pdf) must be properly submitted.

5.1.2. The National Institutes of Health

The NIH likewise has developed strict guidelines for the use of recombinant organisms and enforces its rules under the Office of Recombinant DNA Activities *(201)*. The approach taken through the NIH is to work directly with institutional biosafety committees by developing and institutionalizing standards of containment, both biological and physical. The office has identified four levels of GMM risk groups.

- Risk Group 1: Microbes not associated with disease in healthy adults.
- Risk Group 2: Microbes associated with human diseases that are rarely serious and are generally readily controllable through therapeutic or preventative measures.
- Risk Group 3: Microbes associated with serious human disease that may be controllable through therapeutic or preventive measures.
- Risk Group 4: Microbes associated with serious human diseases that generally lack effective therapeutic or preventative measures.

Using these criteria, the NIH has classified a wide variety of microorganisms into these risk groups. A few examples from each risk group are identified Table 1. The ranking of organisms through these guidelines is designed to maintain the appropriate safe handling of specific GMMs. Several general and specific species of bacteria have been designated as generally exempt from the NIH guidelines for inter- and intraspecies introduction of DNA, provided the appropriate biosafety level for the host is followed (Table 2).

5.1.3. US Department of Agriculture

The USDA's GMM safety regulations are primarily directed at recombinant microorganisms that are plant pathogens. Permits to use such an organism are handled through the Animal and Plant Health Inspection Service (APHIS) of the USDA. APHIS issues two types of permits pertinent to GMMs: those required for field testing of the potential plant pathogen and those required to bring a GMM plant pathogen into the United States or between US states. Permits for environmental testing must document

Table 1
Examples of Pathogenic Microorganisms Classified by Risk Group

Risk Group 1	*Escherichia* K-12, *Bacillus subtilis*, adeno-associated virus types 1 and 4
Risk Group 2	*Bacillus anthracis, Bordetella* spp, *Campylobacter* spp, *Escherichia coli* O157:H7, *Klebsiella* spp, *Listeria, Mycoplasma* spp, *Neisseria gonorrhoeae, Salmonella* spp, *Staphylococcus aureus, Treponema pallidum, Vibrio cholera, Blastomyces dermatitidis*, adenovirus, coronaviruses, hepatitis (A, B, C, D, and E), measles virus, mumps virus, rabies virus, rubella
Risk Group 3	*Brucella, Chlamydia* spp, *Coxiella burnetii, Mycobacterium tuberculosis, Rickettsia* spp, *Yersinia pestis, Coccidiodes immitis, Histoplasma capsulatium*, St. Louis encephalitis, Rift Valley fever virus, yellow fever virus, monkeypox virus, prions, human immunodeficiency virus (HIV), human T-lymphotrophic virus (HTLV), simian immunodeficiency virus (SIV)
Risk Group 4	Lassa virus, Machupo virus, Ebola virus, Marburg virus

Source: From ref. *201.*

Table 2
Examples of Bacteria Exempted From National Institutes of Health Regulatory Guidelines

Escherichia	*Bacillus* spp[a]
Shigella	*Streptomyces* sppa,[b]
Salmonella/Arizona	*Streptococcus* spp[a]
Enterobacter	*Serratia marcescens*
Citrobacter/Levina	*Yersina enterocolitica*
Klebsiella	Erwinia
Pseudomonas spp[a]	

Source: From ref. *201,* Appendix A.
[a]For complete list, see ref. *201.*
[b]Includes members with limited host ranges.

complete information on the organism, including sources and identification of all new genes used, reasons for the study, design of the study, and procedures to prevent dissemination of the organism from the test site. Permits for transportation of GMM plant pathogens require documentation on the organism, sources and identification of all new genes used, and how the organism will be used. APHIS, as part of its safety analysis, prepares an environmental assessment document for field tests. For movement permits, APHIS constructs a preliminary pest risk assessment, contacts the appropriate state department of agriculture, and conducts an on-site inspection of facilities along with state inspectors *(202).* To simplify the process of future biotechnology regula-

tions, risk assessment and permit issuing will be handled exclusively through the Biotechnology Regulatory Services unit of the APHIS.

5.1.4. US Food and Drug Administration

In 1972, the FDA published policy statements used to regulate foods derived from GMOs. In this document, the FDA determined that GMO foods that are not substantially different from their non-GM counterparts are determined as generally recognized as safe (GRAS). If a substance derived from a GMO is intentionally added to a food and is not determined as GRAS by the FDA, it is considered a food additive. Food additives, unlike GRAS products, are subject to review by FDA prior to use in foods *(59)*. The FDA suggests, but does not require, that comparative structural analysis of the GM protein be compared to known allergens. The FDA recommends that antibiotic marker genes used in the production of foods not contaminate food products. The FDA also urges care that antibiotic resistance enzymes present in the GM foods not reduce the efficacy of oral antibiotics *(59)*. The FDA noted that chymosin, the first GMM food product, was granted approval because the organism and antibiotic resistance gene were destroyed in the manufacturing process, and the products were nontoxic *(203)*.

For drugs produced in GMMs, regulation is not significantly different from those drugs produced in non-GM sources. The source organism and any resistance genes must be noted, and the final product should not show detectable levels of the antibiotic used in the fermentation process. The FDA also regulates GMMs to be used in gene therapy trials *(204)*.

5.2. International Regulations and Safety

Internationally, GMM safety is regulated by a host of national and international agencies. In many circumstances, non-US regulatory agencies have adopted regulations that parallel those in the United States. For example, the Japanese regulations on medically relevant GMMs were developed on principles delineated through the NIH *(201)*, but do differ in specifics of the organisms covered *(205)*. Likewise, the OECD, an international consortium with member states that include 17 European nations, Canada, the United States, Japan, Australia, and New Zealand, develops international safety guidelines for agricultural, industrial, and environmental release of GMMs that parallel regulations in the United States, yet differ in specifics *(206,207)*. Other multinational organizations involved in determining or advising on safety policies of GMMs include the WHO, FAO, and International Food Biotechnology Consortium.

To specifically address EU GMM users' regulations, several directives were issued that pertain to the safe use of GMMs. These directives include the commercialization of GMMs used as plant protection agents (Directive 91/414/CE), the manipulation of GMMs under contained environments (Directives 90/219/CE and amended portions in 94/51/CE and 98/81/CE), and the deliberate release of GMMs into the environment (90/220/CE) *(14)*.

6. General GMM Safety Considerations

Specific protocols and equipment are necessary to use GMMs safely in research and production facilities. Although reviewed in detail elsewhere *(17)*, a general outline is provided here. The emphasis here is on (1) developing safe practices in the GMM

facility; (2) developing control structures to prevent aerosols; (3) methods to contain GMMs; and (4) methods to protect personnel.

6.1. Containment Equipment

Fermenters, centrifuges, and centrifuge bottles are the primary containment barriers used to prevent the dispersal of microorganisms *(208)*. Biological safety cabinets of the various classes are used to provide varying degrees of protection. Class I cabinets are designed with open hoods with inward air flow, Class II cabinets are laminar flow hoods designed with inward flow with the supplied HEPA (high-efficiency particulate air) filter, and Class III cabinets or Glove boxes are designed to provide entirely enclosed systems *(209)*. Selection of equipment should be appropriate for the level of work done in the laboratory or facility.

6.2. Containment Facilities

Containment facilities provide the physical workplace for personnel using GMMs. The facilities should be designed to provide protection of those workers and prevent dissemination of the GMM beyond the immediate facilities into the environment. Included in this area are the physical barriers in the facility controlling air movement, differential air pressures used to contain GMMs, and equipment to treat GMM-contaminated wastes. To contain GMMs that represent different hazard levels, four classes of containment facilities have been developed. The following classes represent a combination of NIH and Japanese containment structures *(89,205)*:

- Biosafety Level 1 (BL-1); P1 (Japan): These containment systems are designed for use of GMM organisms that do not cause human disease and that work with organisms identified as NIH Risk Group 1. Biological safety cabinets are not required; work may be conducted in open laboratories using nonporous bench tops. Decontamination of work surfaces takes place daily and after spills. All contaminated liquid or solid materials are decontaminated before reuse or disposal. General laboratory practices include the use of mechanical pipetting devices and the wearing of protective coats, which should only be used in the laboratory. Eating, drinking, and smoking are prohibited, as is the storage of food in the laboratory.
- Biosafety Level 2 (BL-2); P2 (Japan): These containment systems are designed for GMMs or their DNA derived from NIH Risk Group 2 organisms. These are GMMs that pose some level of identifiable risk. Generally, work must be carried out in biological cabinets or chemical fume hoods, but work that does not generate aerosols may still be conducted on the open bench. Other regulations are similar to those of BL-1. Laboratory should be posted as BL-2.
- Biosafety Level 3 (BL-3); P3 (Japan): These are containment systems designed for GMMs and their DNA included in NIH Risk Group 3. Organisms at BL-3 are associated with significant risk to personnel. The facilities contain physical barriers, including sealed walls, floors, and ceilings. A biosafety laminator flow hood or glove box is used when manipulating viable cultures. There should be limited access to the facilities. Airflow is regulated to produce "negative pressure" within the facility and is appropriately discharged outside the facility. Lab coats or gowns should be autoclaved before laundering. A hazard sign needs to be posted identifying the class of organisms used. It is suggested that baseline serum samples be stored for persons at risk; periodic sampling may be collected to determine exposure. Although this principle was noted in Japanese protocols and not in NIH documents, it seems a logical precaution and so is noted here.

- Biosafety Level 4 (BL-4); P4 (Japan): These facilities represent the highest level of containment and are designed to protect personnel from extremely hazardous NIH Risk Group 4 organisms. These facilities are designed to be isolated completely from other parts of the facility, including physical barriers, ventilation, and waste treatment. Personnel change clothes and shower as they enter and exit the facility *(89)*.

Other general practices should be followed when using GMMs. Microbial cultures, of course, should be maintained and manipulated using aseptic technique to minimize the possibility of contamination and therefore cross contamination of the GMM DNA *(210)*. For maintenance, cultures should be properly stored frozen, cryogenically in or over liquid nitrogen or in a conventional freezer. Specimens can also be safely stored in a lyophilized form, but care must be taken to ensure the lyophilizer itself does not become contaminated with the GMM *(89)*.

Management of waste streams is of particular concern in the biotechnology industry. GMMs can be effectively controlled through conventional methods to eliminate microorganisms, such as proper autoclaving procedures. However, these techniques are typically ineffectual at breaking down DNA to the monomer level. Most DNA, notably chromosomal DNA, will typically be degraded rapidly by DNases in the environment *(21,211,212)*. However, some DNA is stable in fragmented form or as supercoiled forms, such as plasmids, for extended periods of time. The method suggested to minimize hazards from escaped GMM DNA is to use DNA exclusively from GMMs classified as nonhazardous and that do not contain mobile DNA. Finally, a full accounting of product production, recovery and processing, waste management, and accident reports should be completely documented. Methodologies for decontamination materials and accidents have been reviewed *(21,89)*.

6.3. Large-Scale Fermentation of GMMs

The OECD Council has laid out several principles to minimize general risk from GMMs used for large-scale industrial purposes. These principles, based on those developed for use of organisms in small-scale production, represent sound management of GMMs to minimize potential risks involved. Further, to be considered safe by the OECD, the microorganisms must have several traits that are deemed essential. The GMM (1) must be nonpathogenic; (2) must not harbor known viruses or co-contaminating bacteria; (3) must have been extensively used safely in a non-GM form for industrial purposes; and (4) must be unable to grow outside its industrial setting. The foreign DNA used in the host should be of limited size to minimize the inclusion of nonessential DNA; it should not provide additional stability to the construct unless such stability is essential for the construct's function; it should not permit increased mobility of the construct; and it should not confer resistance to other organisms that do not already possess the resistance. Finally, the GMMs themselves should not contain any deleterious properties.

To implement industrial GMMs safely, a series of principles termed the good industrial large-scale practice principles has been developed:

1. Exposure of GMMs and GMM products should be kept at levels appropriate for the organism used, the process developed, and the product produced.
2. Dissemination of the organism must be maintained through appropriate preventive engineering protocols and equipment. Use personal protective devices and clothing as needed.

3. Keep control equipment properly maintained through appropriate testing. Evaluate control protocols frequently to match the intrinsic nature of the GMM, the product produced, and the process used.
4. Monitor for the presence of viable organisms and their molecules outside its controlled environment.
5. Personnel involved in production and handling of GMMs need to receive proper training and have sufficient experience. This training and experience needs to be documented appropriately to ensure the safety of the production of the GMM.
6. A biological safety committee that consults with external regulatory committees should be established. This safety committee distributes its findings to worker representatives.
7. To ensure a philosophy of safety at the facility, a code of safe practice should be developed and implemented.

6.4. Small-Scale Field Release

The OECD has also developed safety practices, termed good developmental principles, for basic and applied research involving development of GMMs and for small-scale research field studies *(207)*. The good developmental principles are similar to the good industrial large-scale practice principles and are similar to previous practices described elsewhere *(207)*. The following is a summary and interpretation of the OECD principles:

1. Minimize levels of GMMs and GMM products at levels appropriate for specific field experiments.
2. Prevent the dissemination of the organism beyond the test area through appropriate protocols and equipment.
3. GMMs need to be monitored within the research site both during and after the experiment. Safe protocols should be developed to control the GMM at any step in the process to prevent harmful environmental effects.
4. Monitor for the presence of GMMs and their molecules outside the test area.
5. If GMMs are detected outside the test area, control methods must be implemented to prevent further contamination.
6. Appropriate protocols must be developed to terminate the experiment and properly dispose of waste generated in the experiment.
7. Personnel involved in production and handling of GMMs need to receive proper training and have sufficient experience to handle the GMMs safely.
8. Appropriate documentation needs to collected and maintained for all experimental trials.

6.5. Development of Suicide GMMs

One of the concerns regarding GMM releases is the possibility that they will linger in the environment long after their desired activity is completed. A further concern is that these organisms will continue to multiply and leave the release site. One method to prevent this outcome is to design a bactericidal mechanism into the GMM. However, the bactericidal trait introduced into the GMM needs to be sufficiently stable to minimize the possibility of revertants *(213)*.

One promising direction in preventing the controlled growth of released GMMs is the adaptation of the TOL benzoate mineralization pathway for suicide activation. By utilizing the *gef* family of genes, it has been proven possible to develop GMMs that will undergo controlled cell death *(213–217)*. Three members of this gene family, in-

cluding the *E. coli hok, relF*, and *gef* genes, effectively cause cell death by activating a cascade in the cell that leads to disruption in the cell's membrane potential, causing an influx of periplasmic RNase into the cytoplasm. The constitutively expressed *hok* (host killing) is normally blocked by the antisense RNA gene *sok* (suppression of killing). To use this system effectively in a GMM, *sok* is deleted or mutated to prevent its expression, and an inducible promoter replaces the *hok* constitutive promoter. Alternative suicide systems have been developed based on the *E. coli relF* gene *(218)* and streptavidin-based system *(219)*. Both systems were developed for use in *P. putida*, an organism more relevant than *E. coli* in environmental release. The *E. coli relF* system has been tested in both seawater and soil models *(218)*.

Additional suicide systems have been developed for this purpose *(220)*. However, all such induced-suicide systems remain highly ineffective. Even under laboratory conditions, there is still a significant survival rate (10^4) for the *hok* system. The *relF* and streptavidin-based suicide systems have reported cell resistance in the 10^6 to 10^8 range *(218)*. Although the *relF* and streptavidin-based systems have proven significantly more effective than the *hok* systems in suicide activation, their optimum efficiencies were obtained using isopropyl-β-D-thiogalactopyranoside induction, which would be impractical in an environmental release. Other induction methods may be developed to activate these killer genes in environmental systems. For example, linking these suicide systems to stress-induced control systems *(157)* or activation using the depletion of a substrate, such as a pollutant, *(221)* would be reasonable alternatives.

Alternative killing methods have also been developed. For example, the use of bacteriophages specific for a given GMM is potentially an effective way to control released organisms. Studies using the bacteriophage PhiR2f against GM *P. fluorescens* showed a 1000-fold reduction of cells in simulated soil environments *(39)*. In another approach, phage-resistant and rapid-ripening lactic cocci used in the production of cheese have been engineered with lysin, which autolyses the culture after the stationary phase *(29)*. This technology might be adapted in other organisms for environmental release. Finally, recombinant technologies have been used to engineer suicide fungal pathogens such as *Fusarium*, which is used as for biocontrol of parasitic broomrape weeds. Specifically, the fungus is engineered to be asporogenic *(222)*. These fungal GMMs are constructed through deletions in sporulation genes and are engineered as such to prevent the organism from spreading beyond the release site.

7. Ethical Issues and GMMs

7.1. Introduction to Ethical Issues

Ethical issues involving GMMs are both complex and contentious, involving parties with different attitudes and understanding of the issues *(223,224)*. Reiss *(225)* argued that not all ethical arguments are equal, and that ethical conclusions need to be based on reason, established ethical principles, and general consensus. The use of GMMs focuses in general terms on issues that look at the potential consequences of using GMMs (for example, damage to an ecosystem, introduction of antibiotic resistance genes) and that are intrinsically wrong ("polluting" the world with GMMs or their DNA). Researchers and end users of GMMs generally develop safety protocols to address the potential consequences of the genetic modifications present in GMMs and

usually do not identify GMMs themselves as intrinsically wrong. This in turn drives much of the conflict generated between the different sides in the GMM debate.

Some ethical issues primarily have an impact on personnel and institutions using the technology. For example, are GMMs truly patentable? At what point does the control of recombinant technologies through patents and litigation begin to impact seriously the very science from which they were designed? When GMMs are constructed in universities or other research institutions receiving public funds, who ultimately controls the profits and intellectual property derived from the GMMs? And, does a corporation have the right to withhold technologies based on intellectual property that could legitimately help developing nations?

Alternatively, there are ethical issues that are of more concern to the general population. Should people be eating GMM-derived foods? Just how "safe" are GMM foods? Should the environment be polluted with GMMs and GMM DNA? Could an environmental release be catastrophic? Should a person be compelled to accept GMM products even if they have strong personal convictions to the contrary? What will be the consequence of those who use non-GMMs and must now document that their products are GMM free? Who will have control and who will regulate safety issues? On balance, it also needs to be asked, if GMMs have this incredible potential and have demonstrated such little risk, is society overly cautious and missing out on the potential benefits afforded by GMMs? An accounting of many of these issues has been outlined elsewhere *(226)*. Because it is not possible to address all of these ethical issues, a subset of them is discussed.

How do ethical issues involving GMMs develop? The impact that GMMs have on the environment is an excellent example. The introduction of GMMs or their DNA into the environment will likely have some impact on the local microbial flora and, if not properly controlled or monitored, potentially may cause harm outside the control area. Such an event might introduce an undesirable gene, such as an antibiotic resistance gene, into the environment, which in turn might have an impact on human health through increased antibiotic resistance. It is then conceivable that the gene could be picked up by a pathogen, ultimately resulting in harm or death to individuals. It is easy to understand why an environmental release leading to increased antibiotic resistance of pathogens and human death is deemed immoral. How these concerns are addressed by GMM developers and regulators will ultimately determine how widely accepted GMMs will become *(223)*.

Other examples of GMM use less clearly demonstrate "harm." Which ethical issues result from actions that have no direct bearing on humans? For example, releasing a large number of GMMs might locally disrupt a natural ecosystem by interrupting a specific soil predator–prey relationship. If the action changes the soil, somehow making it less fertile, it can be said to damage the soil's "instrumental value." This phrase refers to the value of the nonhuman world in terms of its usefulness to humans *(12)*. Even if the disruption has no direct bearing on human health, human agriculture, or other human activity, it still can be acknowledged as causing a change in the intrinsic value of the site. In other words, inherent value can be derived from the nonhuman world itself *(12)*. Concerns for the intrinsic value of the earth in combination with the instrumental value drive much of the ethical debate regarding environmental issues.

In approaching issues of bioethics and GMOs, it is important to realize that this field of knowledge is unlikely to produce an ultimate resolution that will satisfy all parties. Reiss *(225)* established a framework that delineates a general standard for "ethical conclusions" by suggesting that they should be based on reason, use well-established ethical principles, and be derived from a general consensus. Although these may be difficult to formulate, a consensus on how to define these three principles should serve as a useful guideline to evaluate GMMs.

7.2. Public Concerns and Governmental Philosophies of GMMs

Pharmaceutical products made from GMMs are generally well received and do not receive much condemnation *(14)*. This is not generally true for GM foods *(224)*. Surveys conducted in 1999 to 2000 in Switzerland, the United Kingdom, and the United States showed a marked difference in US and European responses to GM food products. Whereas in the United States only 2% of the public felt that GM foods were a potential risk, 59% of Europeans shared this view *(227)*. To a great extent, in Europe food is not simply the fuel that drives bodies; it has a cultural value as well *(33)*. It has also been speculated that the difference in European and American views is based on wider support in the United States of agencies that regulate GMMs, such as the FDA. By contrast, the European experience includes a series of unrelated serious biotechnology incidents (noted in this section) that have led to deep public mistrust of EU regulatory agencies. In both the United States and the European Union, approaches to convince reluctant populations as to the true merit and safety of GMMs and GMM products need to be conducted via a well-meaning and thorough dialog with the public rather than the paternal approach frequently used in this debate *(33)*.

Another major problem currently facing the US vs European approaches to GM food safety issues are the conflicting philosophies of how safety should be determined. In the United States, GM foods are generally considered safe using the principle of substantial equivalence in that the GM food is essentially the same as the unmodified form. The FDA is far more supportive of GM foods than EU or Japanese granting agencies. This is indicative of the relative high percentage of GRAS status granted to the US GM food producers. The European model currently follows the precautionary principle, suggesting that a GM food or other GM food product must first be determined safe before it is released into the market *(33,34)*. Not surprisingly, in the United States, significantly fewer regulations detailing the specific use of GMMs have been generated than in Europe *(59)*. In 1996, the FDA produced a document, *Safety Assurance of Foods Derived by Modern Biotechnology in the United States*, that outlines this philosophy:

> "Based on our present knowledge of developments in agricultural research, we believe that most of the substances that are being introduced into food by genetic modification have been safely consumed as food or are substantially similar to such substances. Therefore, we do not anticipate that most newly added substances in bioengineered foods will require premarket approvals *(59,* p.3*)*."

This statement is not without substantial supportive data. The FDA rules are the result of intensive analysis of GM foods over the last 25 years. To date, despite extensive analysis, no GM food product brought to the market has shown adverse health effects. It is also clearly in the best interests of biotechnology companies to maintain

the safety of their products as documented that "no other foods in history have been tested and observed as diligently as the foods developed from modern biotechnology" *(52,* p.225*)*. Although the current FDA regulations do not require extensive testing, the FDA does specifically address concerns about limiting the use of antibiotic resistance genes in food products. The FDA noted that fermentation products, such as chymosin, which are made with GMMs containing antibiotic resistance genes, must demonstrate that they are free of "transforming DNA" *(59)*.

The cautious EU/Japanese approach arguably stifles research and production of GMM products that may be beneficial. The US model leaves itself open to criticism of a laissez-faire policy regarding GMM regulation, leaving too much control of safety in the hands of the industries that develop GMMs. However, an alternative safety model, similar to those developed for evaluating pharmaceuticals, lies between these two extremes *(33)*. In this approach, initial safety analysis requires physicochemical, biological, pharmacological, toxicological, and clinical testing of the product; this analysis is conducted and financed by the developing industry. This process is followed up through government epidemiological analysis, which acts as a population monitor to detect undesirable side effects. Finally, national agencies that specifically monitor the GMMs evaluate the product over the long term *(33)*.

In a similar approach, the OECD has developed the concept of *familiarity* in utilizing GMMs. Familiarity is based on overall knowledge acquired from (1) the host organism itself; (2) the environment in which the GMM is to be used; (3) the life cycle of the organism; and (4) criteria used in its construction *(15,42)*. Through familiarity, researchers and regulatory agencies can develop risk assessment based on previous knowledge and experience *(30)*. Using this approach, industrial GMMs can adopt the concept of familiarity as "an extended history of safe industrial use" *(206)* and agricultural GMMs can adopt the phrase of "an extended history of safe agricultural use" to develop safe GMMs *(42,* p.16*)*.

Other concerns and issues of bioethics also spring from recombinant technologies. In spite of almost 30 years of developing GMMs, there is still a significant level of distrust in the general population of this technology *(52)*. Although generally accepted in the production of medicines, such as recombinant vaccines and other therapeutic proteins, there is widespread fear of GMMs used as foods or in the production of food additives *(228)*. In part, this is a reflection of serious mistakes made in nonrecombinant biotechnology. Events such as mad cow disease and hoof and mouth disease outbreaks centered primarily in the United Kingdom, dioxin contamination of meats and poultry in Belgium, and the Starlink corn gene contamination in the United States and Mexico have convinced a large part of the population that biotechnology and, by association, recombinant technologies are inherently unsafe *(13,14,223)*. This connection of unrelated events may seem counterintuitive to those familiar with their actual causation. Nonetheless, these examples are frequently used in arguments directed against GM products.

Likewise, GM agricultural biotechnology has become engrained with the politics of globalization and the power of multinational agriculture industries *(229)*. Although a significantly larger issue in Europe, there are vocal advocates in North America and Latin America who likewise fear the development of GM food products *(229)*. If GMMs are to be developed to meet their full potential, there must be adherence to safe proto-

cols, appropriate analysis of potential risk factors must be identified, and concerns regarding their use must be transparently addressed. It is this final point that may be the hardest to fulfill; paternal assurances as to the safety of these technologies will not bring wider acceptance of their introduction *(34,230)*.

Despite the difficulties of gaining general acceptance by the public, examples exist of the general acceptance of GMM products *(223)*. These include the production of recombinant medicines through fermentation technologies and the use of GM viruses in gene therapy trials. The use of GMMs to produce pharmaceutical products has greatly reduced the cost of these drugs, making them more generally available. Further, because of the purification protocols used in producing drugs, GMM pharmaceuticals are inherently going to have the same risks associated with them as the non-GMM versions.

Novel GMM therapeutics that do not have complementary non-GMM versions, such as gene therapy, are another issue. The single human death and two documented leukemia cases resulting from gene therapy trials demonstrate the inherent risk of live GM viral vaccines. The FDA and European regulation agencies, as of September 2002, put a halt to the X-linked form of severe combined immune deficiency disease trials *(87)*. Obviously, clinical trials are inherently never risk free. Therefore, medical ethics committees at both the institutional and the national levels as well as the gene therapy patients themselves (or their guardians) must have sufficient understanding of the inherent risks of the therapy before such treatment is ever initiated *(86)*.

Another example of the general acceptance of GMMs is in the production of "vegetarian cheese." In the traditional process to make cheese, rennin containing the enzyme chymosin is extracted from the stomachs of calves. To make the enzyme more readily available, the chymosin gene was cloned into an *Aspergillus* expression vector, resulting in the production of recombinant chymosin *(192,231)*. Chymosin, which is a product of recombinant technology, is consumed. It is interesting to speculate why this product is so widely accepted and yet so many other GMM derived foods are rejected. Is it because it seems so much less humane to isolate the enzyme from a calf than from a fungus? Was it the marketing of the enzyme as vegetarian that brought it some level of consumer acceptance? Or, is it simply that consumers do not recognize it as the product of a GMM?

Another issue that relates patterns of concern about GMMs by the general public is the poor communication and misunderstanding that exists among the biotechnology industries, the public, and the media *(229,232)*. Whether a process or product is deemed safe is hardly important if the public does not have confidence that the scientific process used to evaluate the product is independent of industrial interests *(95)*. If the public perception is that multinational corporations, motivated by profit, deduce and report findings on the safety of their GMM product in a biased manner, it hardly seems likely that the process itself will provide assurances about the product's safety to a skeptical public. The public needs to be informed about the real risks, if any, of a given GMM product. An informed public should at least understand that the GMM product has been extensively analyzed, certainly for human harm, well before it is ever brought into the market, and that the process has been carried through without bias *(95,233)*. Communicating to the public true risk is perhaps best expressed briefly through the phrase "to produce the appropriate level of concern and action" *(234)*.

In identifying and communicating the risks of recombinant technologies, we are confronted with a truism: The scientific community evaluates safety and risk of these processes through interpretation of data, assigning probabilities and consequence based on data itself. Often, the scientific community evaluates public concern as not based on scientific data and therefore irrelevant to these issues. It is important to understand that, although public fears may have been devised using different methodologies, different standards of evidence, and different values, their concerns are every bit as rational and deserving of consideration as a scientific approach *(232,235)*. It is not possible or even necessary to try to convince everyone that a given procedure is entirely safe. Rather, it is critical that, through open dialog, it is possible to communicate that the assessment process, determined through a rational scientific approach, has been given a transparent account by all concerned, and the final evaluative outcome represents an honest conclusion of that process *(233)*.

7.3. Labeling of Foods and Food Products Derived From GMMs

In the United States, labeling is not required for any GM foods or GM food products. US citizens have indicated a preference for labeling that identifies products as GM foods *(228)*. If so, why does the FDA so earnestly resist the labeling process? FDA documents point out that labeling a product as being with or without GM DNA would become prohibitively complicated *(59)*. For example, to determine that a product is "GM free," documentation would need to be compiled and followed regarding the strains used, the food processing facilities used, and all transportation equipment used to ensure the noncontamination of the product *(59)*. It is imaginable that a process such as the production of acetic acid by GMM and non-GMM forms would require separate fermenters, separate transportation vehicles, and separate process equipment to be able to identify one product as a GMM product and the other as GM free *(52,59)*. Such a complicated and cumbersome process would undoubtedly increase the cost of the product. Finally, it is also believed that including labels on products warning the public about the presence of GMMs when there are no known hazards associated with the product may only serve to unnecessarily frighten consumers *(52)*.

The alternative, not labeling products as containing recombinant DNA, is unacceptable to many individuals, including those who profess a wish to maintain a GM-free diet. Many people practice forms of environmentalism that are arguably religious in nature *(236,237)*. Therefore, it might be further argued that these environmentalists' convictions against eating GMM foods are equally compelling as religious' prohibitions. This is true whether the GMM product is determined to be completely safe for both human consumption and environmental use *(232)*. However, the argument leads to the question, should individual consumers have the right to know and control their dietary intake even if a GMM product is deemed completely safe? This concern has led to mobilization of a large number of interest groups in the United States and Europe determined to require labeling on GM foods *(43,238,239)*. Already, governments in Europe and Asia have either recommended or enacted labeling requirements *(43)*. Recent government actions, including a resolution passed in the US Congress in defense of US GM products, *(240)* will likely only intensify what is already a divisive issue between the United States and the European Union.

A compelling reason to label GM foods is to help identify potential food allergens in rare susceptible individuals *(183)*. It seems unlikely that a GM food product capable of producing severe allergies would become established in a food product. This is largely because of the current level of allergen analysis conducted on GM foods. However, as pointed out by several watch groups concerned about GM issues, without proper labeling of products, if a problem were to exist with allergens in a small percentage of the population, no database will be available to make an epidemiological analysis of the food product *(43)*. In a reply to concerns raised by the Union of Concerned Scientists, the FDA indicated that the agency was reviewing this issue *(241)*.

The labeling issues are complex and divisive. Although dialog and consensus between concerned parties is the best approach to resolve some of these issues, it is most likely that resolution will be accomplished either through legislation or through national and international courts. Although not specifically involving GMM-derived foods, several lawsuits involving GM foods have been litigated against US regulatory agencies and biotechnology companies. Current class action suits are spearheaded by over 600 plaintiffs, including environmental groups such as the Union of Concerned Scientists, Greenpeace, and the Sierra Club *(242)*.

GM food labeling also has an impact on international trade. In a compromise designed to head off an international trade embargo between nations over GM labeling, the Cartagena Protocol on Biosafety Treaty was developed *(28)*. Signed in Montreal by more than 130 countries, this treaty bridges the extremes between not labeling GM products and the trade barriers likely to affect world GM producers. Specifically, the United States agreed to label foods derived from GMOs as "maybe" containing recombinant DNA products. Future requirements may be added to strengthen this reporting mechanism. In return, the protocol allows wider use of GM products in other nations without unilateral embargos threatened against exporters.

7.4. Ethical Issues of GMMs and Environmental Release

Two separate incidents are useful case studies regarding the ethics of released GMMs. In 1987, plant pathologist Gary Strobel of Montana State University conducted an unauthorized release of a mutated strain of *Pseudomonas syringe*, a GMM engineered to control the pathogenic fungi *Ophiostoma ulmi*, the causative agent of Dutch elm disease. He specifically violated a 1986 EPA rule delineating a 90-day notice prior to initiating field releases of GMMs *(243)*. In statements released by him to the committee, Dr. Strobel noted the *Pseudomonas* strain had not been genetically engineered in that it was a nonpathogenic transposon-marked organism. It was his contention that, by definition, the NIH guidelines regarding recombinant microorganisms did not apply *(244)*. In a separate incident, an accidental release of a GM virus containing genes from hepatitis C viruses and Dengue fever occurred at London's Imperial College. The accident was a result of inadequate containment *(245)*. Although no perceived harm occurred to human health or the environment from either of these incidents, public perception of these events helped galvanize concerns of legitimate and properly regulated releases of GMMs.

Potential uses of GMMs include examples by which the organisms will need to be released into an uncontrolled environment to be effective. The use of GMMs in pest control, bioremediation, wildlife and livestock vaccination, metal extraction, and crop

yield improvement all suggest that the use of released GMMs will represent a growing part of the biotechnology industry *(58)*. However, will such environmental GMM releases alter, in some fundamental way, the ecology of the systems into which they are exposed?

This question is certainly one of the most contested of all GMM issues, in large part because of the huge stakes involved. On one side of the issue are the biotechnology and agricultural interests, which see a potential panacea of benefits from controlled releases of GMMs *(179)*. On the other side of the issue are concerned environmental groups, which fear GMMs are inherently dangerous because of their very nature as organisms. The fear is that, once released, a GMM cannot be controlled if an undesirable event takes place *(50)*.

Both sides have compelling arguments for their views. Parties interested in release of GMMs can note an array of safety mechanisms developed that maintain the process as safe. Further, the benefits of GMM releases potentially help society in numerous ways. Parties opposed to such releases point out that the history of human colonization of the planet is a lesson in what can go wrong when nonindigenous species are placed in novel environments *(246)*.

It is easy to understand the source of these fears in evaluating the damage caused through the intentional or accidental releases of numerous nonindigenous organisms into the environment. For example, the establishment of human-released organisms such as kudzu plants and European starlings in the Eastern United States; mosquitoes, mongooses, and pigs in Hawaii; European rabbits, Cane toads, and domestic mice in Australia; and the brown snake on the island of Guam and the damage these organisms have inflicted in their respective environments underscore that concern. It is hard to imagine completely what the consequences might be of an uncontrolled GMM. Speculation of potential harm caused by a GMM release includes impacts on human health, crop damage, or damage to indigenous microbial communities *(10,18,89,190)*.

Regardless of the perspective on the issue, there must be one starting point in agreement: The ecology of vast areas of the world has been irreversibly changed through human activities. Ever-increasing demands on cropland and "pristine" environments to maintain substance for a growing world population and the needs to remediate lands already polluted beyond the capacities of natural systems require novel answers. Many of those potential answers will undoubtedly be devised from the ability to manipulate genes in microorganisms that are then released into the environment *(58)*.

It was generally believed, and some data support the fact, that most GMMs are effectively less fit to survive in complex ecosystems *(46)*. Even if true, like all organisms, GMMs do evolve, and some have been shown to persist for extended periods of time in harsh environments despite the best attempts to engineer biological and physical methods to constrain them. Further, because of the complex nature of these environments, it is virtually impossible to design experiments that will accurately assess all the parameters of whether a released GMM or its DNA will persist in the environment. Even if it were possible to design complex release studies for each GMM, the cost associated with such studies would be prohibitive.

Lenski argued *(46)*, however, that there is a reasonable approach to this conundrum. Lenski suggested that GMM applications appreciably similar to previous releases of GMMs or non-GMMs be used as a baseline to assess their risk. This approach would permit more intensive risk analysis of those applications evaluated as of greater risk.

Using this approach, a detailed assessment would be required for GMM applications that are novel and do not have comparative non-GMM releases.

Releasing GMMs into the environment also has definite social implications that invoke other ethical considerations *(95,224)*. Whereas research in a laboratory takes place in a confined, and therefore private, domain, releasing GMMs into the environment enters the realm of public involvement. It is inescapable that people do fear the technology. What responsibility do the GMM user and developer have to address those fears when analytical responses are ineffective? This is a difficult and important issue that is likely to elude simple answers.

7.5. GMMs and Patent Issues

Is it possible actually to patent a living GMM? That was the question put before the US Supreme Court in June 1980. Ananda Chakrabarty applied for a patent for a recombinant *Pseudomonas* that had been engineered to disperse oil slicks more effectively than the non-GM form. Several parties resisted the granting of this patent, including the US Patent Office, the USDA, and several individuals opposed to the process. By a 5-to-4 judgment, the US Supreme Court granted the patent to Chakrabarty on the grounds that the GMM was in fact a human-made invention and not a product of nature *(247,248)*. Despite this ruling, there are still moral considerations that need to be addressed in patent law, both in the United States and internationally. What moral code is used to address the granting of a given patent? What cultural norm should a court uphold to make such a determination? Court challenges based on the interpretation of the morality of a given patent are increasingly used as a method of choice by those opposed to the technology to block the granting of the patent *(226)*.

There are sound reasons for developing strong patent laws in nations. Patents provide protection of development costs and, certainly for companies, a way to receive a reasonable return on their investment *(248)*. Basically, all a patent provides is a way through the courts to find remedy of unauthorized use of the product of the patent *(235)*. Through the creation of a monopoly under the protection of the law, patents provide value for discoveries and thus incentives for private company investment.

A major concern that has developed from the patent process is the effect that patents have on science *(235)*. Notably, in the United States and in the United Kingdom, the undeniable monetary returns offered by the granting of patents have had an impact on the science conducted *(230)*. The process does provide funds for active research projects and is often used in the United States for general institutional needs. However, the patent process often imposes secrecy on the scientific process, including that of public institutions *(249)*.

Increasingly, the goal of research in many laboratories has become the creation of intellectual property. Should it not be the philosophical goal of publicly funded research that science is an enterprise that engages in free exchange of reason and thought? Further, issues of secrecy permeate the patenting process. In the United States, a patent must be filed within 1 year of the publication of the product; in the United Kingdom, the patent must be filed by the time the researcher publishes. Either way, the process stifles open scientific dialog of the findings, generally for fear of losing control of the patent. These and additional concerns stemming from patents were addressed recently by the Royal Society in the United Kingdom *(249)*.

Finally, once granted, a patent acts as a monopoly, entitling the inventor to exclusive rights in charging fees or exclusive use of that process or product *(230)*. That prohibition, through legal or economic pressures, may interfere with promising lines of research. Such arguments come full circle when it is realized that public funding through grants is often the catalyst that makes the research possible. However, because the patents are only granted as rights to prevent the unauthorized use of the product, it can be argued that the act of receiving a patent is not in itself immoral *(226)*.

7.6. Biological Warfare and GMMs

How does one conceive that biological weapons are more or less moral than conventional weapons? Since the September 11 attacks in New York City and Washington, DC, and the subsequent mailings of anthrax that resulted in several US deaths, this question has been part of active debate in the United States *(250)*. In fact, despite the US anthrax deaths, it is still believed by many that the effective use of bioengineered pathogens as weapons is likely quite low *(251)*. However, biological weapons pose many obvious advantages to their users, including their relative ease of production, relatively low cost, ease of concealment, and civilian vulnerability to bioweapons *(252,253)*. Although the development of GMM as bioweapons is more difficult to accomplish than development of non-GMM weapons, the fact that genomic databases provide details on the specific pathogenicity of genes may render methods to develop novel pathogens more efficient *(254)*. Many of the general issues regarding biological weapons have been reviewed elsewhere *(253,254)*.

The BWC treaty *(255)* banning the development and use of microorganisms as weaponized agents should have been the cornerstone in preventing the continued development of GMM-derived bioweapons. The threat of GMM-derived bioweapons was first addressed to the United Nations by Joshua Lederberg, discoverer of bacterial conjugation *(256)*. Unfortunately, it has become clear that microorganisms have been intentionally modified for weapons use, apparently first in the former Soviet Union *(254)* and later in other nations.

It has been suggested that enhancements provided by recombination technology could be used to deliver toxin genes (i.e., anthrax toxin, myelin toxin), antibiotic resistance genes (i.e., penicillin or tetracycline resistance), genes to change the mode of infection of a pathogen (such as addition of a respiratory mode), or genes to make microbes resistant to vaccination *(91)*. A short list of microbes implicated in GMM-derived bioweapons includes *Bacillus anthrax, Yersinia pestis, Francisella tularensis*, and smallpox *(91)*. Clearly, it is possible to ponder other destructive GMMs directed at humans as well, as demonstrated in the inadvertent creation of lethal GM mouse pox *(81,257)*.

Other forms of biological warfare are conceivable. Plant pathogen genes could be cloned into hosts normally associated with crops, or any number of pathogens could be engineered against livestock *(258–260)*. There are even suggestions that recombinant technologies currently used in biodegradation be adapted to consume metals, lubricants, or plastics that might be directed at opposing force weapons *(261)*. If true, such weapons would arguably not only be in violation of the BWC treaty by their very nature, but also a microbe developed and used in such a capacity could be isolated and turned against its developer, although presumably suicide genes would be engineered

into such strains. Regardless, such weapons directed at the fabric of industrialization could have far-reaching consequences in a modern world.

This misguided predilection to engineer microorganisms as weapons requires reasonable countermeasures. For example, it will likely be necessary in the foreseeable future to develop novel vaccines as a defense against GM viruses or GM bacteria. Although the extent of countermeasure development is unclear, in the Reagan administration active research into this question was conducted *(256)*. The United States has refused to support a stronger BWC, partly because of concerns about confidential business information and partly because of national security concerns *(245)*. It is reasonable to speculate that these national security concerns involve countermeasures developed against known bioweapons using much the same technologies that it took to develop them *(255)*.

7.7. The "Do Nothing" Principle

Many would argue, notably Jeremy Rifkin *(36)*, that the potential hazards of recombinant technologies are so vast that they should not be attempted, and the technology should not be approved. This argument, to ban a technology, has its consequences as well. There are serious problems, notably in pollution and food production or improvement, for which GMMs can provide obvious solutions.

For example, soils contaminated with xenobiotic compounds can be extremely recalcitrant. To remediate sites that are extensively contaminated, soils may have to be removed physically, treated with solvents or detergents first to remove the compound, and then be destroyed by physical methods, such as incineration or chemical neutralization. Such protocols potentially expose workers or local communities to aerosols and dusts containing the compound. A GMM that can effectively mineralize a toxic compound *in situ* would almost certainly be less expensive than conventional cleanup methods and expose cleanup crews to less of the pollutant.

The same principle is true in developing GMMs to make soil more fertile. It has been suggested that, over the next 40 years, food production will have to more than double to meet projected world populations *(52,230,262)*. Not a complete answer, but GMMs will likely compose part of the solution.

The concept of not using GMMs has other consequences as well. Species such as mice and rabbit populations periodically increase to produce significant plagues in Australia, resulting in major damage to regional farms *(124,263)*. The use of conventional methods to control these pests is simply inadequate to meet the staggering need both in realistically controlling their numbers and in limiting the harm conventional control techniques (poisoning, physical traps) might have on indigenous species *(263)*. The use of GM viruses has been proposed as a method to target and control individual pest species. However, as noted in Section 4.1.4, this approach comes with its own set of risks *(124)*. The ethical dilemma is which approach to take: allow destructive plagues to go unchecked, continue to use ineffective and environmentally damaging pest control methods, or look at targeted GMM control methods. Each comes with its own ethical consequences. If substantial data are gathered that the GMM control method is specific to its target organism and is effective in safely controlling pests, then in certain circumstances, GMMs may well become the preferred method to control pests.

7.8. Other GMM Ethical Issues

Finally, 3 billion years of selection and evolution have resulted in a biosphere full of microorganisms of incredible complexity and diversity *(12)*. Should the natural evolutionary processes that developed living organisms and therefore the integrity of individual species be respected? In other words, simply because there is the intellectual capacity to do so, is it appropriate to manipulate any organism of choice? These perhaps are ridiculous questions to ponder because humankind has been manipulating the genes of organisms for millennia using conventional genetic methods. Further, because there can be no return to a time when GMMs were not part of life on this planet, it is likely these questions represent moot concerns. However, not to appreciate and understand the relevance of this capability to manipulate life at the molecular level and the consequences of this ability are to deny what may be humanity's most profound impact on life on earth.

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