RISK ASSESSMENT OF DELIBERATE RELEASE OF GENETICALLY-ENGINEERED MICROORGANISMS

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ABSTRACT

Hazard identification and evaluation of dose response and exposure, the standard approach to risk assessment, take on a new meaning when one evaluates the risks associated with deliberate release of genetically-engineered microorganisms (GEMs). Hazard identification of GEMs may be quite complex since one must consider not only their potential toxic effects on humans and animals, but also disruptions that the introduction of novel GEMs may cause in the ecological equilibrium. Initially, these changes might not be as obvious as direct toxic effects. Unlike with chemicals, for which the risk assessment scheme was initially developed, exposure to GEMs does not necessarily decrease with time due to dilution and degradation. This paper will describe conceptual models of risk assessment applicable to deliberate release of GEMs.

KEY WORDS: Risk assessment, genetic engineering, microorganisms, deliberate release

INTRODUCTION

New genetically-engineered microorganisms (GEMs) promise a great benefit to humanity, yet their use may pose a risk to public health or cause ecological damage. Thus, the potential adverse effects need to be reviewed and evaluated.

Application of genetic engineering results in three categories of products:

(1) Macroscopic plants and animals with altered genes, presumably improved from the original species; for example, more pest- and drought resistant plants, "super" cows, and "super" sheep.

(2) Products made by GEMs, such as insulin, growth hormone, interferon, various polypeptides, and vitamins.

(3) A GEM is itself a product designed to perform a specific task, and may be deliberately released into the environment for the following purposes:

(a) Pollution control, such as oil-digesting bacteria; xenobiotics-digesting bacteria, yeasts and fungi.

(b) Pest control; e.g., Pseudomonas fluorescens with inserted genes from Bacillus thuringiensis.

(c) Other uses, such as frost prevention by ice (-) strain of Pseudomonas syringae, nitrogen fixing bacteria, metal and oil recovery bacteria.

The data needed to evaluate risks posed by the use of products of genetic engineering are quite different for each category. This paper will focus primarily on category 3 because of its potential for the most immediate impact (products are already waiting for field testing), and potentially widespread use in various environments. Since the purpose of these GEMs is to perform their function in the natural environment such as soil, sewage, plant leaves, and water, a parent organism is generally selected that can survive well in these media. This is in clear distinction to microorganisms in category 2, which were specifically selected to be less able than the wild type to survive outside the container.

In order to place potential risks posed by a deliberate release of GEMs in the proper perspective, one must first address the misconception that naturally occurring products are safe, and that man-made products are inherently hazardous and unsafe. An example of a dangerous natural product is aflatoxin a potent hepatotoxin and liver carcinogen. Aflatoxin is a by product of the metabolism of the mold Aspergillus Flavus which grows on grains and peanuts. It is, therefore, a natural, but potentially very hazardous contaminant of grain products and peanut outter. Another example of naturally occuring hazardous entities is the numerous types of microorganisms causing infectious diseases, which certainly present a human health hazard. Just as naturally occurring microorganisms are not necessarily safe, GEMs created using recombinant DNA techniques are not necessarily more dangerous.

Another important fact to consider in risk assessment of a deliberate release of GEMs is that the degree of change in a genome of an organism does not necessarily correlate with physiological properties that this change in genome confers (Sharples, 1983). Slight differences in the genome can be associated with large differences in the organism's properties. For example, chestnut-blight fungus (Endothia parasitica) in Asia co-exists with Asian chestnut and causes no harm. When Asian chestnut-blight fungus was imported into the United States, however, it destroyed American chestnut trees, although it is not very different from the fungus that normally lives on American chestnuts and causes no harm.

Therefore, in the risk assessment of a deliberate release of GEMs one should not differentiate between naturally-occurring microorganisms and genetically-engineered microorganisms, and one should not assess risks based on the degree of human intervention used in the modification of the microorganism. Whenever the release of a large number of microorganisms into the environment (both natural and GEMs) is contemplated, the risks should be determined based on a specific organism. A case-by-case approach has to be applied because causal relationships between genome structure and functional properties are unknown at the present time. Knowing the DNA sequence of a gene does not provide information on how this gene will function in a particular cell. Therefore, risk assessment should be based on functional properties of the microorganism, and should be performed for each microorganism that is going to be released in large quantities into the environment.

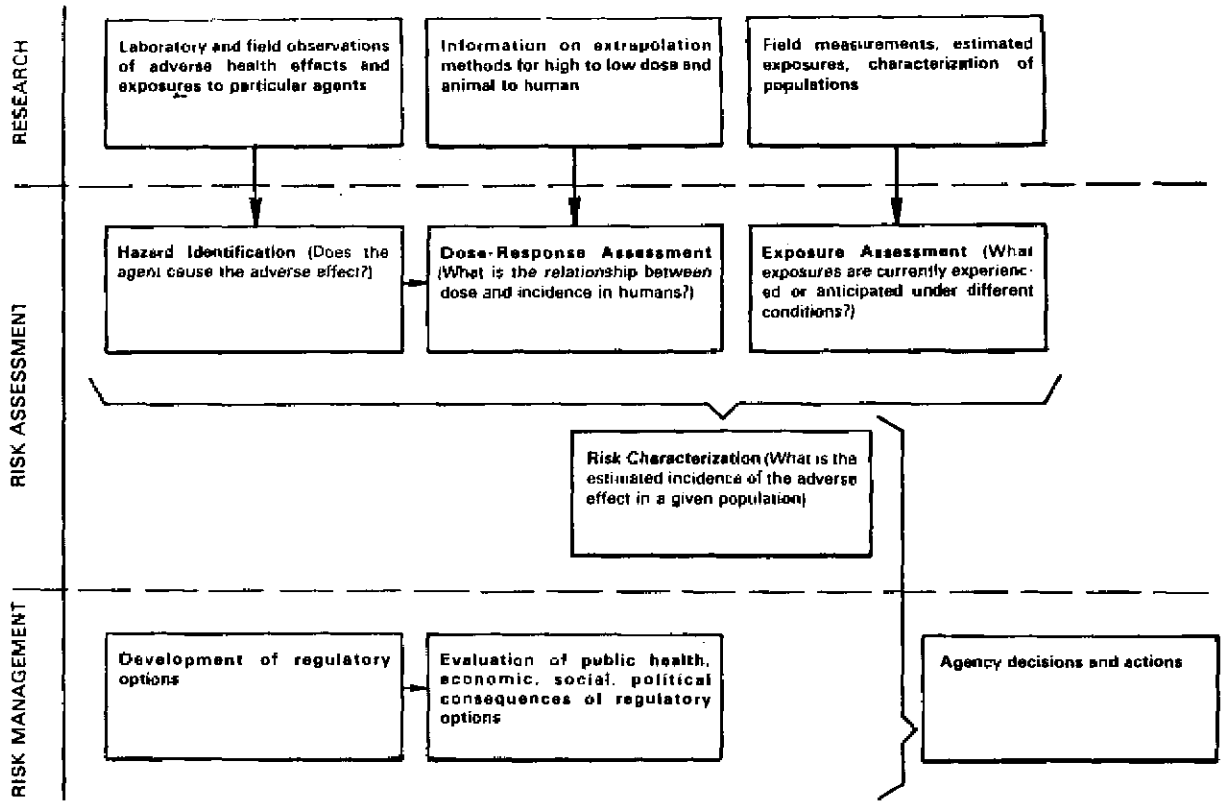

Figure 1. Elements of risk assessment and risk management

Hazard Identification

Figure 1 describes the risk assessment scheme for chemicals. In this scheme the main components of the assessment are hazard identification, dose-response determination and exposure assessment, leading to the risk characterization (Ruckelshaus, 1984; 1985). On the basis of risk characterization for a particular chemical, one can then perform qualitative or quantitative risk assessment. Can one use a similar scheme for microorganisms that are going to be released deliberately into the environment? In the case of chemicals, hazard identification is based on observed endpoints (presumably undesirable acute and chronic toxic effects or cancer); with microorganisms, the endpoints are not that clear. In general, microorganisms can be divided into two categories with respect to hazard identification, pathogens and non-pathogens.

In the case of pathogens, a scheme for hazard identification can be constructed somewhat similar to the scheme for chemicals. Minimal infectious dose (ID50), analogous to LD50 for chemicals, is the number of microorganisms that would cause infection (by ingestion, inhalation or dermal exposure) in 50% of the tested population. The ingestion ID50 is between 10^2 and 10^8 for most pathogenic bacteria; 1-10 for virusus and 1-100 for parasites (Batteile, 1985; Ward and Akin, 1984). Risk characterization of naturally-occurring pathogens can be performed similarly to risk characterization of chemicals by measuring the exposure levels (intake through food, air or water), and calculating the likelihood of infection.

Recently, an attempt has been made to perform a risk assessment for pathogens that may contaminate various environmental media as the result of sludge disposal (Battelle, 1985). The environmental fate and transport of various pathogens naturally found in sludge are being determined and combined with known infectivity dose response values. Various options of sludge disposal are considered (land application, distribution and marketing, landfill, ocean dumping), and an attempt is made to assess the risk of pathogens causing diseases resulting from each sludge disposal option. Such methodology could be applied for risk assessment of GEMs with known ID50. The transport and fate models have to be developed for each specific microorganism and the environment into which this microorganism is released.

Most of the newly constructed GEMs, however, are probably not pathogenic in human, animals, or plants. Therefore, for most GEMs an ID50 does not exist, nor does one know which endpoints to observe or even to expect. Since these GEMs must survive in the environment in order to perform their task, the question to ask in the case of a deliberate release of GEMs into the environmental media is not if they will survive (Alexander, 1985), but how long will these microorganisms survive in each particular environment, and what effects on the environment will they have during their survival. Obviously, the survival of each specific microorganism will depend on its interactions with the environment into which it is released.

Exposure

Figure 2 shows some of the environmental media into which GEMs could possibly be released. Microorganisms that might serve as pesticides, will be released into soil or onto plant surfaces. Microorganisms for pollution control will be released into sewage and water (Johnson and Robinson, 1984) or contaminated soil (Ghosal et al. 1985). Microorganisms used for oil recovery or metal recovery will be released into ore fields or oil drills (Moses and Springham, 1982; Curtis, 1983). In addition,

certain GEMs are planned to be released on plant surfaces to prevent frost (Lindow, 1983).

All the cited environmental media into which microorganisms might be released are already occupied by their natural biota. For example, to a depth of 15 centimeters in moderate temperatures, a hectare of average soil contains about 2×10^{16} bacteria, which weigh 2.6 metric tons. This soil also contains 8×10^{16} fungi weighing approximately 2 metric tons; 6×10^{17} actinomycetes, 7×10^{16} protozoa; and 3×10^{14} algae (Foth, 1984). In total, each hectare of soil into which GEMs would be released already contains about five tons of naturally occuring microorganisms. The interaction of microorganisms already present in the soil with the newly-introduced agents will determine their survivability and growth. It is possible that it would be safe to release a certain limited number of microorganisms, but unsafe to release a larger number (critical mass). Release of a critical mass of microorganisms into a specific environment could enable the introduced microorganisms to replace the natural populations. Therefore, quantitative relations between introduced and endogenous microorganism populations should be taken into account because ecological effects might depend, to a great extent, on the number of microorganisms that are introduced into a unit space.

Risk Characterization

From the above discussion it is clear that numerous factors must be considered before release of a large number of microorganisms into any environmental media can be contemplated (Figure 3). Such factors include the physiological properties of GEMs (nutritional requirements, growth, and metabolism); the quantity of GEMs released into a particular environment; and detailed characteristics of the environmental media. This includes physical characteristics such as soil porosity, structure of the soil particles, and water content; chemical characteristics, such as presence of mineral and organic materials; and biological characteristics which depend on the species already occupying each medium. Environmental interaction will be determined by a combination of these three factors. For example, survival and transport of GEMs will be determined by characteristics of GEMs and their interaction with physical and chemical factors in the environment. Intra- and interspecies gene transfer will depend on interaction of GEMs with biological factors. The interaction of GEMs with other species would again depend on biological characteristics of the particular environment. Potential exposure of humans, animals and plants would be also determined by these three factors.

1. soil
2. sewage
3. water
4. plant surfaces
5. ore fields
6. oil drills

Figure 2. Environments for deliberate release of GEMs

1. GEMs characteristics (nutritional requirements, growth, Survival)

2. Quantity of GEMs released into particular environmental unit

3. Characteristics of the environment:

   a. Physical

   b. Chemical

   c. Biological species occupying same space

4. Environmental interaction

   a Survival and transport of GEMs

   b Intra and interspecies gene transfer

   c. Interaction of GEMs with other species

5. Possible exposures of human, animals and plants

Figure 3. Points to consider

Examples of GEMs for Deliberate Release

Examples of GEMS intended for deliberate release are listed in Figure 4. Pseudomonas fluorescens is a bacterium that normally lives on roots of various plants (including corn). This species has been genetically altered by the introduction of a gene from Bacillus thuringiensis, which renders Pseudomonas fluorescens poisonous to soil parasites such as cutworms. Bacillus thuringiensis itself is used as a natural microbial pesticide against mosquito larvae, by spraying it over ponds which are breeding grounds for mosquitoes (Luthy et al., 1982). Therefore, plants that contain genetically-engineered Pseudomonas fluorescens on their roots are resistant to attacks from cutworms because their roots are poisonous to cutworms. In this example, the environment for a deliberate release is soil, and one must determine the fate and transport of this GEM in soil as well as its interaction with the soil's natural biota. Moreover, the toxic effects of this GEM on other species which might be exposed to soil should be established. If genetically-engineered Pseudomonas fluorescens has a prolonged survival time in soil, one would also need to consider its environmental fate and transport. Existing models for pathogen transport in soil may be useful to follow the environmental fate and transport of this GEM (BDM, 1980).

1. **Pseudomonas fluorescens with inserted gene from Bacillus thuringiensis**

2. **Pseudomonas syringae (ice (-) strain)**

3. **Pollution control microorganisms**

Figure 4. Examples of GEMs intended for deliberate release

The second example is genetically-engineered Pseudomonas syringae, which has been obtained from the wild type by deleting the ice nucleation gene. This change lowers the temperature at which this organism serves as an ice nucleation center. The bacteria that normally live on plant leaves are the sources of ice nucleation; therefore, the temperature at which these bacteria freeze determines the temperature at which leaves freeze (Lindow, 1983). In order to assess the risk of a deliberate release of Pseudomonas syringae, its survival on plant leaves should be considered, as well as its fate and transport in the environment where it is applied. Also, the possible pathogenic properties of this genetically-engineered species should be determined.

A third example of a possible deliberate release is pollution-control microorganisms. Many processes in decontamination of toxic wastes might involve the use of biological agents (Johnston and Robinson, 1984). Microorganisms are already used in sludge treatment and in wastewater treatment plants (Powledge, 1983). Presumably, with the help of genetic engineering, one can construct microorganisms that would have specific properties for desired pollution control. Such microorganisms will be

then released at various contamination sites, which might vary from sewage and sludge environment to water and contaminated soil (Chakrabarty, 1985). Again, to assess the risk posed by this type of a deliberate release, first it must be established whether these microorganisms are pathogens and what effect they have on the environment into which they are released.

CONCLUSION

What can be done with the information on microorganisms intended for deliberate release? The final goal should be to develop models for deliberate release of microorganisms that will accurately predict the fate and transport of these microorganisms and their public health and ecological effects. Ideally, a mathematical model that adequately describe all the environmental factors involved would be developed. The final output of the series of equations would describe the state of the system (environment) into which the new variable (GEM) is to be introduced. For example, given the number of GEMs introduced into a particular volume of soil, sludge, water or surface of plants, one should be able to predict the subsequent density of these GEMs at any chosen time and space. Provided that one knows the initial values (distribution and density of GEMs), and all relevant parameters for the model, this goal is achievable. The major difficulty lies in finding which equations adequately describe processes in nature (survival, competition, growth, transport, gene transfer), and in testing experimentally the relevant parameters in these equations. Because of the diversity of GEMs and the diversity of environments into which they will be released, it appears impossible to construct a generic model that would cover all possible instances of a deliberate release of GEMs. For example, mathematical equations describing the density of GEMs in aquatic environment will undoubtedly be different from the equations applicable to GEMs released into soil or onto plant leaves.

Since GEMs are not basically different from natural microorganisms (except for some special functions), models describing the fate and transport of natural microorganisms could be applied to a deliberate release of GEMs. However, only a few such models exist at present. Most of the models are designed for very specific purposes that do not take into account all the factors described in Figure 3. Therefore, new and more realistic models, requiring a great deal of interdisciplinary knowledge will have to be developed. These models should be designed taking into consideration knowledge from various disciplines such as ecology, microbiology, biochemistry, molecular genetics, soil science, agriculture and toxicology. Finally, the models should be tested in simulated real-life situations of deliberate release of microorganisms.

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