"Suicide Genes" and Other Strategies:

Precautions for Our Environmental Future With Biotechnology

Introduction

During the past decade, biotechnology has become an established industry throughout the United States, Western Europe, and Japan. Although its influence was initially confined to the pharmaceutical and health care industries, the promise of biotechnology has the most immediate implications for agriculture. In some cases--particularly those involving agriculture and aquaculture, mining, and waste treatment--biotechnology applications could lead to many introductions of genetically engineered organisms into the open environment.

Conservationists hope that such applications will be done in ways that prove beneficial to the environment. In agriculture, for instance, by genetically engineering crop plants to resist pests biochemically, we may reduce our current dependence on chemical pesticides. Despite such tantalizing promises, however, caution must be exercised before large numbers of engineered organisms are introduced into the environment. Special attention must be paid to anticipating and evaluating side effects lest unintended consequences endanger public health, the environment, or wildlife.

Although the impact of introducing engineered organisms on such a large scale is currently difficult to predict, now is the time to begin considering how to deal with such releases. How can genetically engineered organisms be safely used in the environment? Can they be recalled or controlled once out there? If so, how?

Summary of a Workshop: "Safeguard Mechanisms for Post-Release Control of Genetically Engineered Organisms" convened by Audubon March 6, 1989

Such questions formed the backdrop for a workshop, "Safeguard Mechanisms for Post-Release Control of Genetically Engineered Organisms," convened on March 6, 1989, by the National Audubon Society [with support from The Joyce Foundation]. The participants at the workshop, gathered because of their diverse viewpoints, focused explicitly on the question: Are there technically feasible ways of engineering organisms so that they can do little or no harm once released into open environments?

In considering this technical issue, workshop participants also discussed whether or not it would be appropriate for federal legislation or regulations to encourage or require the use of post-release safeguards. Such an approach could offer a way of "designing protection ahead of time...by using new techniques to control and constrain genetically engineered organisms," noted Audubon's Senior Staff Scientist Jan Beyea.

The Audubon Society's interest in biotechnology issues stems from a specific concern about the threats posed by exotic species invading its national network of wildlife sanctuaries. The Audubon Society also has a more general interest in national and regional land-use policies affecting other restricted lands such as wildlife refuges and parks. These

national parklands and wilderness areas play an important role in saving patches of wild America. Biotechnology will bring new economic forces to bear on how these lands are used, and the need to protect them from development may intensify.

"In addition to a concern about exotic species and the possible need to protect refuges against their invasions, Audubon wants to know whether better crops and products made through biotechnology will change land use and damage wild lands," said Kathleen Keeler of the University of Nebraska and the U.S. Department of Agriculture facility in Albany, Calif., who recently studied these issues during a semester at Audubon as part of its sabbatical program. "If this technology can push back the limits on [agricultural] land use, we need to look at the implications."

Workshop Highlights

Deliberate release: a modest beginning.

Environmental tests of genetically engineered microbes so far have been modest in scope and cautious in design. Although no known damage has occurred or is predicted, biologists who think in ecological terms insist that, in planning for such tests and for any larger-scale uses that may eventually follow, scientists and regulatory officials give rigorous consideration to potential environmental consequences.

For instance, biologist Richard Condit of Princeton University in N.J., noted that current confidence in the safety of genetic engineering rests on "people saying nothing has happened in 10 years. But 'it' could be something very subtle; so we don't know [for certain] that nothing has happened." In terms of environmental questions, there is still little known about natural communities of microbes, a prerequisite for properly evaluating eventual impacts when genetically engineered microbes are released, he said.

However, biotechnology should be scrutinized not only because it might pose problems but also because it "might help in controlling conventional problems, such as weeds," Keeler pointed out. "Biotechnology might provide a handle on things that haven't work with conventional technologies."

A 1989 report of the Ecological Society of America strongly recommends long-term planning and oversight when considering the release of genetically altered organisms into the environment, said co-author and ecologist Robert Colwell of the University of Connecticut in Storrs, who participated in the Audubon workshop. In its report, "The Planned Introduction of Genetically Engineered Organisms: Ecological Considerations and Recommendations," the Ecological Society makes a strong case that ecological analysis will be an essential step in evaluating proposals for the deliberate release of genetically engineered organisms.

Colwell referred to other key points in the Ecological Society report, which sets out a systematic scheme for evaluating potential releases, recommending they be reviewed according to a "scientifically based regulatory policy that encourages innovation without compromising sound environmental management." The report also recommends that risk assessment "be sound and equal for both commercial and noncommercial research..." and that it involve "local, state,

national and international cooperation...." Moreover, "specific criteria for scaling regulatory oversight" are needed.

The current regulatory framework.

Because the biotechnology industry "is mammoth, with huge scope," many commercial products are being developed for applications in open environments, said Morris Levin of the biotechnology institute at the University of Maryland in Baltimore. Thus, plans for oversight are needed. However, although there is less confusion among federal regulatory agencies about overseeing deliberate release than existed a few years ago, there is "no real order yet," Levin said. "The relationships between federal agencies are complex...and, at the scientific level, the interagency communication is good. But at the policy level, it's a problem."

Although federal agencies are good at adhering to their mandated regulatory tasks, none of them directly addresses issues such as land use and societal impact of biotechnology, Levin added. Such questions "are just not part of their mandates."

Approaches for controlling engineered organisms.

One approach to these issues involves efforts to develop technological strategies for ensuring that genetically engineered organisms will do little or no harm after being released into the environment. Because microorganisms are both small and often hardy, special steps will sometimes be needed to monitor them and to prevent them from becoming established where they are not wanted. And, because genetically engineered organisms might "escape" from farm sites, mines, or other places where they are being used, it is important to determine ahead of time "what the options are" to minimize risks, Keeler said.

In small scale tests, the chief strategy is to impose a variety of physical constraints augmented by a few genetic limitations. Thus, isolated fields with limited access and surrounded by buffer zones are used for deliberate release tests, and provisions are made for monitoring any run-off waters, for taking air samples, and for sterilizing the soil, if deemed necessary.

At the genetic level, there are somewhat analogous strategies for making sure that genetic molecules remain where they are expected to be and that they do not go "roaming" into other organisms. For example, the use of genes for antibiotic resistance markers is restricted in organisms being released into the environment; while the resistance markers are very useful in laboratory experiments they may be transferred too freely in the open environment, thus undermining the medical usefulness of antibiotics. Safeguards can be imposed for both pragmatic and safety reasons.

Despite their appealing simplicity, physical means are not considered an entirely satisfactory restraint on microbes in the environment. Thus, such precautions can be burdensome, incomplete, and difficult if not impossible to use on a large scale. Hence, some microbiologists and environmentalists are contemplating a genetic approach--so-called "suicide genes"--as a means of restraining aberrant genetically engineered organisms outside the laboratory.

The notion that suicide, or conditional lethal, mutations may provide protection when

dealing with microorganisms can be traced back to early laboratory experiments involving crippled strains of genetically engineered Escherichia coli, the bacterium most commonly used in genetic engineering research. Although laboratory strains of E. coli are not in themselves particularly hardy, about a decade ago a concerted effort was directed toward disabling them still further, thus ensuring they could not survive outside the laboratory or colonize lab workers following accidental ingestion or other exposure. Genetic manipulations to disable microorganisms used in genetic engineering schemes became known as "biological containment." Techniques for accomplishing this goal were developed and made part of the federal National Institutes of Health (NIH) guidelines for conducting recombinant DNA research. In addition, a study by the National Academy of Sciences discloses that it considers suicide genes an appropriate containment measure for field-testing microorganisms.

Perhaps the key point to remember in evaluating the applicability of similar strategies for controlling environmental releases is that "complete containment cannot be achieved," said Ronald Atlas of the University of Louisville, Ken. "The aim is for...limiting distribution [of the engineered organisms] to minimize risk." Setting limits for microorganisms poses special problems, however, because "they are hard to track or see, and they can reproduce independently."

Another important component of earlier biological containment strategies "falls apart" when considering using engineered organisms outside the laboratory, Atlas continued. Microorganisms are now being designed and manipulated so they will survive rather than fail in particular open environments, such as in an open field. Survival--at least limited survival--is essential for the success of most biotechnology-based agricultural products now being developed, making the use of crippled lab strains out of the question. Moreover, "the early belief that [genetically engineered microbes] would have a 'burden' and not survive doesn't seem true from microcosm studies," he noted. And so, engineered organisms cannot be counted on to die off gradually of their own accord without doing damage.

Hence, the use of suicide genes appears attractive--the idea being that a microorganism can carry an inducible gene that, once triggered, will kill the organism and thereby halt its spread. The fundamental drawback now evident for this seemingly attractive approach is that its "scientific basis is not yet fully proven," Atlas said. Because funding to support research on developing strategies has been scarce, the promising potential of early work has not yet been realized.

Despite limited research, there are hundreds, perhaps thousands of genes of potential use in suicide schemes, said Stephen Cuskey of the Environmental Protection Agency (EPA) research laboratory in Gulf Breeze, Fla. Care must be exercised in choosing them and the gene-regulatory sequences used to control them. He recommended designing and testing a series of suicide "cassettes" (that is, packages of genetic material that are easily manipulated in the laboratory) that could be slipped into cells as needed. "There is a need for more than one cassette for redundancy, to ensure against loss of control by mutations," he said. In other words, if one suicide gene mechanism within an organism

happens to fail, other mechanisms must also be present as back-ups.

Suicide strategies under test.

Some kinds of E. Coli encode a lethal gene product known as "hok" (for host killer). The hok molecule contains 55 amino-acid building blocks. Somehow this molecule binds to and kills E. coli cells and many other similar types of bacteria that might be used in deliberate release experiments. Atlas and his collaborators recently engineered the hok gene, placing it on a so-called vector that makes it easy to move from one type of bacterial cell into another. They refer to this engineered entity as a "suicide vector."

When triggered with a molecular signal, cells carrying this vector commit suicide--more or less. Thus, many of the cells are killed but, after a lag, more cells begin to appear. Importantly, the new cells no longer carry the hok vector, Atlas found. The reason is that the cells had lost the vector and therefore survived. "That in itself is encouraging," he noted. "If the suicide vector were put together with other engineered traits, the cells would lose that information along with the host killing system." In other words, any threat posed by the engineered organism presumably would be nullified because the cells that survive lose the plasmid carrying those supposed threatening engineered traits.

If pushed to its limits, however, the same suicide vector system fails a critical performance test, Atlas said. In his experiments, a carbenicillin resistance gene was added to the suicide vector plasmid. The antibiotic carbenicillin kills bacterial cells that carry no resistance gene to it. Thus, adding the antibiotic to the growth media for such cells along with the hok inducer, faces them with a survival dilemma: loss of the antibiotic-resistance plasmid meant death, but death would also occur if the plasmid was retained while hok was turned on and made to function. After a growth lag in lab experiments, "the suicide gene failed, and a mutation occurred," Atlas said. This meant that a new strain of bacterial cell was created, one which contained an antibiotic resistance gene without a functioning hok gene. Although no such strains have arisen in soil microcosm studies, the failure of the suicide gene in lab tests points to the necessity for extreme caution in releasing genetically engineered organisms into the environment.

From these experiments, Atlas concluded that "a single suicide system won't do the job. Redundancy is needed, and must be built in to make suicide genes work."

Other strategies for controlling agriculturally useful microbes are being considered. For instance, David Sands and his colleagues at Montana State University in Bozeman are studying a native fungus, Sclerotinia sclerotiorum, as a means for controlling the Canada thistle weed. An important drawback is that the fungus also can kill several valuable crop plants. Hence, Sands is seeking ways of limiting its host range through mutagenesis.

One such way is through a fungal mutant that cannot make cytosine, a chemical required for growth. This mutant also cannot kill plants in greenhouse tests, making it like "a Doberman on a leash," Sands said. However, if cytosine is sprayed on the plants along with the fungus, it kills them. "So we might be able to add cytosine to weeds to enable the fungal mutant to grow [subsequently killing the weeds] and

then [the fungus would] die off when the cytosine supply is exhausted," he pointed out. Another fungal mutant cannot form spores, and thus does not survive through winter. Sands and his colleagues plan to develop yet another mutant in which a metabolic requirement is put under control of a promoter that responds to chemicals made by a specific plant--thus the survival and function of the microbe will be tied directly to that of the plant.

Comparable strategies may be applied to other engineered organisms, including fish being developed for aquaculture, according to Thomas Chen of the Center for Marine Biotechnology in Baltimore, Md. Several research groups, including his, are transplanting altered growth hormone genes to produce transgenic fish with the expectation that they will grow more efficiently than ordinary fish. Usually the transgenic fish are also polyploid, meaning they carry abnormal numbers of chromosomes, and thus are sterile. In this way, they have at least one built-in safeguard against reproduction if accidentally released. Other plans call for inserting diphtheria toxin genes into the fish under the control of a genetic promoter that could be selectively activated to kill them, if necessary.

The suicide strategy: an evaluation.

Suicide mutations provide some attractive schemes for helping protect the environment, but they are not altogether adequate for the task, according to several of the workshop participants. The suicide approach "will appeal to members of the biotechnology industry because it's their tools that are being used, and they might [see] it as sufficient, but it's not," noted Colwell. Moreover, it is worrisome that reliance on suicide genes when planning environmental introductions of engineered organisms might lead to molecular biologists "to learn no ecology," he added.

"We should not underestimate the potential of evolution to undo biological containment, no matter how good it is," Colwell cautioned. "I don't want to see any over-selling of a particular technical solution." Moreover, added Robert Wachbroit of the University of Maryland, College Park, a "technocratic solution to a problem that is not technical may not solve the real problem and can bring a false sense of security."

Cuskey has a different view. "I have no problem with genetic researchers having to 'learn ecology'," he said. But, "I feel that, to be fair, [we] should not make light of potential genetic solutions to biological containment based on the gut instincts of ecologists that such solutions are not tenable." It is clear that, for these important questions to be resolved, more research is needed.

On the policy level, Frank Grad of Columbia University Law School in New York City also saw some difficulties. To consider drafting a model law specifying use of biological containment schemes, there is a "need for a much clearer idea of what you're trying to protect," he said. For instance, "We don't want to have suicide cassettes frozen into regulations forever," he said. "There is a need for flexibility."

Nonetheless, biological containment "has a lot of attractive elements not only for scientists but also for regulatory agencies," pointed out Stan Abramson, an attorney formerly with EPA who now works with the firm of King & Spalding in Washington, D.C. Industry is

concerned with regulatory uncertainty. "If regulatory agencies were looking for particular biological controls, the companies at least would know what the handle will be," he said. The use of suicide genes to make genetically engineered organisms die off carries another distinct advantage for biotechnology companies--the greater certainty of repeat business, added Atlas.

Genetic safeguards such as suicide genes clearly represent a line of research that deserves more attention. Not only could such research provide precautions against environmental damage by genetically engineered organisms, it could also lead to a fuller understanding of the relationships between specific genetic mechanisms and the ecosystems in which those mechanisms function. However, genetic techniques of biological containment do not in themselves appear to present sufficient safeguards against all environmental risks posed by genetically engineered organisms. Furthermore, any assessment of how biological containment might work must take into account the ecological conditions in which suicide genes would operate. Thus, as Colwell and others pointed out, a full ecological analysis must remain an essential part of the process for evaluating deliberate release proposals.

This synopsis was written for National Audubon by Jeffrey L. Fox, a freelance writer living in Washington, D.C. With a PhD in biochemistry, he writes regularly about biotechnology and science policy related issues. He also is a contributing editor to Bio/Technology *and news and current topics editor for* ASM News.

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Audubon and Biotechnology

Audubon is focusing on the implications biotechnology will have on land-use and how it may affect biological diversity. The goal of our research is to identify natural systems and land areas of importance to wildlife that might be threatened by changes in the economics of farming and forestry because of advances in biotechnology, and the possibility of bioengineered species irreversibly altering the fabric of natural and managed systems. In the process, we examine regulatory strategies that will affect both national and international uses of this technology.

The workshop described herein is one component of Audubon's biotechnology program, which is coordinated through the Environmental Policy Analysis Department. EPAD is comprised of staff scientists, policy analysts, and other professionals from throughout the organization who come together to address specific scientific and policy-related issues of concern to Audubon and its members. The department regularly consults with and brings in outside expertise from industry and government, and sponsors a sabbatical program to advance interdisciplinary understanding of complex environmental problems.

National Audubon Society

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February 3, 1989

Channel Inn

Dr. Richard Condit
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Dear Dr. Condit:

Bruce Levin recommended you for a workshop the National Audubon Society is convening on March 6, 1989 in Washington, D.C. The purpose of the workshop is to bring scientists working on safeguard mechanisms for post-release control of genetically engineered organisms together with scientists knowledgeable about ecology and evolutionary biology. We hope to draft a set of recommendations to be used in developing model legislation or other policy documents. We have enclosed a description of the workshop and a list of participants.

Since you will not return from Panama until February 16th, I thought this might be one way of ensuring contact. I will call you on February 17 or 20 to see if you could possibly come to this one day workshop on March 6th. We would provide a $200 honorarium plus all expenses. As you may surmise from looking over the list, we very much need ecological expertise for this exercise.

Hoping you will be able to participate, I am

Sincerely yours,

Maureen K. Hinkle/mj

Maureen Kuwano Hinkle
Director
Agricultural Policy

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SAFEGUARD MECHANISMS FOR POST-RELEASE CONTROL OF GENETICALLY ENGINEERED ORGANISMS

Workshop Agenda

March 6, 1989
Channel Inn, 650 Water Street, SE, Washington, D.C.

9 a.m. Jan Beyea and Kathy Keeler: Purpose of workshop. What is Audubon trying to protect and why? Biotechnology sabbatical program for Audubon.

9:15 a.m. Introductions of participants.

9:30 a.m. Morris Levin: Overview of how agencies currently regulate environmental release of GEOs. What is required for mitigation, containment, spread and control. Maureen Hinkle, moderator.

10:00 a.m. Coffee Break

10:30 a.m. Ronald Atlas: Overview of safeguard mechanisms for post-release control of GEOs. State of the art, how long such mechanisms would remain effective, and known limitations of such mechanisms (microbes, bacteria and viruses).

11:00 a.m. David Sands: control mechanisms involving immunology, genetic locks.

11:15 a.m. Thomas Chen: macroorganisms.

11:30 a.m. Rob Colwell, discussion moderator.

12:00 noon - lunch in small groups of 6-7.

1:30 p.m. Panel discussion: Frank Grad, Morris Levin, & Stan Abramson. Possibilities for legal and regulatory authorities or risk assessment strategies to include control mechanisms as a requirement before marketing GEOs. Maureen Hinkle, moderator.

2:15 p.m. Robert Wachbroit: Advisability of technocratic solutions to environmental problems. Jan Beyea, moderator.

2:45 p.m. Coffee break

3:15 p.m. - 5:00 p.m. Conclusions and summary by Rob Colwell. Advice from participants. Discussion of appropriate biocontainment approach.

5:00 p.m. Reception

Safeguard Mechanisms for post-release control of Genetically Engineered Organisms

A Workshop Sponsored by the National Audubon Society
March 6, 1989 9 A.M. - 5 P.M.
Channel Inn
650 Water Street, S.W.
Washington, D.C.

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