MANUAL FOR ASSESSING ECOLOGICAL AND HUMAN HEALTH EFFECTS OF GENETICALLY ENGINEERED ORGANISMS

Part One: Introductory Materials and Supporting Text for Flowcharts

by

Scientists' Working Group on Biosafety

a 1998 publication of

The Edmonds Institute

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Late in 1995, the member nations and regional groups of the Convention on Biological Diversity, called for development of an international protocol on biosafety which would "take into account the principles enshrined in the Rio Declaration on Environment and Development, and in particular, the precautionary approach . . .". A few months after that "call", the Edmonds Institute, a public interest, non-profit organization, invited a group of scientists from a broad range of disciplines to develop a biosafety handbook accessible to the public and reflective of maximum concern for ecological and human health. The scientists undertook to help both consumers and policy-makers evaluate likely impacts of genetically engineered organisms in a variety of settings and applications. The group met in a week-long workshop and their discussions led to a volume entitled *DRAFT Assessment of Genetically Engineered Organisms in the Environment: The Puget Sound Biosafety Handbook.* Subsequently, a second group of scientists, with a majority of the same members as the first, was asked to revise and expand the draft publication, to extend its scope to include a greater diversity of organisms. This manual is the result of their work.

In a very real sense, this manual is the gift of Mark Wheelis, Andrew Spielman, Philip Regal, Deborah Letourneau, Terrie Klinger, Anne Kapuscinski, Conrad Istock, Elaine Ingham, Norman Ellstrand, Pushpa Bhargava, and Sharon Akabas. The Edmonds Institute is indebted to them for the generosity with which they shared their time, their expertise, and their patience. We are similarly indebted to Michael Holmes, John Fagan, and Chris Mundt who were part of the group whose discussions and writings contributed to the draft that preceded this manual.

The Institute owes a large debt of gratitude to many, many others: to those anonymous reviewers who reviewed all or part of this manual, to Gordon Orians, Professor Emeritus of Zoology at the University of Washington, who managed a double-blind peer review for us, to Hugh Wilson and Kate Clancy whose candor benefitted various stages and details of this work, and to Ed Burrows, Karen Kovacevich, Elaine Knobbs, Kay King, Carolyn Carr, and Freida Morris whose logistical, clerical, and design support enabled two week-long meetings of the authors, two years of correspondence, uncounted phone calls and editing sessions, and five iterations of the manual.

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Beth Burrows
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Contents

Part One

Executive Summary

Modern molecular methods increasingly are used to produce organisms that express novel traits. Such methods commonly are referred to as "genetic engineering" (that is, the isolation of nucleic acid molecules from one organism and their subsequent introduction into another organism in such a way that makes them part of the permanent genetic make-up of the recipient and allows them to be inherited by offspring). Genetic engineering techniques currently are used for such diverse purposes as improvement of agricultural crops and crop yields, enhancement of farmed fish and shellfish broodstocks and their associated yields, production of microbes for bioremediation and other specific tasks, and changes in disease-transmission rates by insect vectors. Each of these purposes holds the promise of benefit to one or several groups. However, the potential benefits are accompanied by potential hazards to human health and the environment. These hazards arise from the inherently novel aspects of genetically engineered organisms (GEOs), and from our collective uncertainty about their short- and long-term health effects and behavior in the environment. We believe it is likely that at least some GEOs will pose substantial hazard to human health or the environment; while these may represent only a fraction of the GEOs released, the potential risks argue for careful scrutiny and cautious application of GEOs in the environment.

Examples of potential hazards include the following:

****Changes in ecological roles or functions.*** Engineered changes in growth rate, reproductive output or fecundity, longevity, tolerance of physical and chemical factors (e.g., temperature, salinity, water relations, pesticides, etc.) can all change the relative performance of GEOs with respect to naturally-occurring organisms. In some cases, the performance of GEOs will be enhanced sufficiently to negatively impact other organisms. An often-cited example of this is the potential for increased weediness among herbicide-tolerant crops. Increased weediness could have potential negative impacts on surrounding agricultural fields or on wild vegetation in nearby plant communities. Both impacts could have economic

consequences, either directly, through loss of valuable cropland, or indirectly, through loss of ecosystem services.

****Changes in genetic relationships.*** Many GEOs will retain the ability to interbreed with their non-engineered relatives. Interbreeding will allow the production of hybrid progeny expressing engineered traits. Such hybrids could be formed between GEOs and domesticated organisms, or between GEOs and wild organisms. In either case, hybridization will alter the distribution of phenotypes within domesticated or wild populations, and will serve to change the role of the organism(s) in the landscape. In the most extreme cases, introgressive hybridization could lead to genetic contamination of economically important crops (or stocks), or to extinction of native species or other species of local importance.

The potential for gene transfer is of special concern among prokaryotes (e.g., microbes), which differ from eukaryotes (e.g., crop plants) in their ability to transfer DNA between unrelated cells. Such lateral transfer of genetic material could allow engineered genes to move into populations other than the target population, with serious consequences for monitoring and containment. (Lateral transfer is also known to occur among eukaryotes, perhaps by the action of transposons, but its occurrence is thought to be less frequent than in prokaryotes.)

****Indirect effects.*** The indirect effects of releasing GEOs into the environment include changes in population mating structure, alteration of competitive hierarchies, disruption of trophic cascades, and modification of the physical and chemical environments upon which native species depend. Such alterations could lead to changes in community structure through changes in species number and population size. Indirect effects will be difficult to predict, detect, and monitor, but could have substantial impact on community and ecosystem function.

****Changes in allergenicity, toxicity, or nutritional composition of foods.*** The presence of foreign or novel proteins in "familiar" foods could prove hazardous to individuals who suffer specific allergies to those proteins. Further, the production of toxins, even at very low levels, could have adverse effects on human health over

the long term.

In addition, foods grown for human consumption cannot be entirely isolated from other organisms and exchanges of genes in the larger environment. Thus, food sources could become contaminated by novel genes introduced for purposes other than human consumption.

Biosafety Assessment and the Precautionary Principle

The goal of biosafety assessment is to discern, as far as possible, the potential for harm to the environment and/or human well-being stemming from GEOs and their products. While biosafety itself represents a goal that is not fully achievable, biosafety assessment can help to minimize the potential for harm. Our arguments in favor of biosafety assessment are predicated on the precautionary principle; that is, ". . . lack of full scientific certainty should not be used as a reason for postponing measures to avoid or minimize . . . a threat" (Convention on Biological Diversity 1994). A precautionary approach to the release of GEOs therefore requires shifting the burden of proof from those charged with post-release monitoring and management to those seeking approval for the release of new products. That is, the manufacturers and producers of GEOs intended for release must demonstrate that their products conform to the highest standards of human health and environmental safety. In a statistical context, the potential for harm is greater under conditions of type II error (that is, when an impact exists but is not detected statistically) than under conditions of type I error (when an impact is erroneously detected where none exists). Therefore, a precautionary approach should seek to minimize type II errors. (For more discussion of this, see Appendix E.)

Sources of Uncertainty

Our treatment of biosafety assumes that some amount of uncertainty is inherent in all biological systems, both natural and engineered. Because all uncertainty can never be accounted for, the behavior of organisms and biological systems cannot be predicted with total accuracy. We therefore expect that the

introduction of GEOs into the environment will be accompanied by "surprises", which may occur at low but significant frequencies, and which may pose threat or harm where none was anticipated.

Many factors will contribute to uncertainty in genetically engineered systems. The behavior of the GEO, the novelty of the trait(s) imposed, and the variability of the environment could all act to increase uncertainty. Uncertainty will further increase as the number of inserted genes increases. Early GEOs contained only one or a few novel genes; more recent work has focused on inserting multiple novel genes into a single recombinant genome, and some recent attempts have included the incorporation of tens of novel genes into a single GEO. Such gene stacking promises to confer additional uncertainty because of the potential for interactions between the inserted genes.

Uncertainty also will increase as GEOs released into the environment evolve. Living organisms are unique in their ability to evolve new traits or new combinations of traits. Such evolution is the product of a complex array of biotic and abiotic factors, and is often unpredictable in its effects. Our inability to predict the effects of evolution among GEOs increases the hazards associated with their release.

Examples from Non-Native Introductions

The intentional and unintentional introduction of non-native species and populations to local ecosystems provides many examples of the potential for unanticipated adverse effects of GEOs released into the environment. GEOs, because they bear novel genes and usually express novel traits, are novel organisms, and as such will constitute non-native introductions in every environment into which they are released. We know from our experience of (unengineered) non-natives that environmental, ecosystem, and economic damage can be wrought by species introductions; no less should be expected of some GEOs.

Past experience from the intentional introduction of non-native species has

shown that unforeseen effects often occur, sometimes long after the initial introduction, and that these can be quite damaging to the recipient ecosystems and, ultimately, to human welfare. For example, the introduction of mongoose to control populations of introduced rats on Pacific islands has led to the depletion or extinction of native bird species. In other cases, non-native insects introduced as agents of biological control have become significant pests themselves.

Unintentional introductions have caused similar harm. For example, the introduction and spread of non-native species of cord-grass (*Spartina* spp.) with the commercial cultivation of non-native oysters in Washington state (USA) has rendered large tracts of tidelands unsuitable for oyster cultivation, conferring substantial economic loss. In addition, the spread of introduced *Spartina* threatens the persistence of native plants and animals by altering local habitats and displacing native species.

Technical Precision versus Traditional Breeding

Some have argued that modern molecular methods allow more precision in manipulation of the genome than do traditional plant breeding or animal husbandry practices, and that this greater precision could decrease hazards associated with the release of GEOs. However, there is little or no evidence to suggest how (or whether) this precision in the genetic manipulation alone relates to environmental and human health effects of the entire (complex) organism. That is, we know little about the behavior of GEOs in the environment, and the technical precision with which they are produced may not necessarily reduce environmental impacts compared with traditionally-bred organisms.

It is certainly true that traditionally-bred organisms have caused human hardship and economic loss. For instance, the Irish potato famine and the ensuing displacement of human populations was the result of the failure of a traditionally-bred crop grown as a monoculture. The spread of weed-beets throughout European commercial beet operations is a further example of economic loss due to unanticipated problems with traditionally-bred crops (in this case owing to

hybridization between crop plants and weeds). Numerous other examples of hardship and failure, or of environmental degradation, can be traced to traditional breeding and agricultural practices. We suggest that the products of genetic engineering will be no less susceptible to serious, unforeseen problems, and will be no less capable of conferring hardship and loss.

Scope and Intent of the Manual

This manual offers a framework for systematically evaluating the safety of a planned release of a GEO or introduction of a genetically engineered food (GEF). A comprehensive array of taxa are considered, including microbes, crop plants, trees, aquatic plants, finfish, shellfish, arthropods used to improve agriculture and protect health, vertebrates, and foods. The assessment proceeds by means of sequential flowcharts, which ask multiple questions of the user, and which direct the user to various other flowcharts depending on the answer(s). The flowcharts are not exhaustive: while attempting to anticipate many of the situations or conditions encountered by the user, we have certainly not anticipated all such conditions, and therefore the flowcharts are necessarily incomplete. The flowcharts and their accompanying text should be interpreted as a way of thinking about the problem of biosafety of released GEOs, and should be used as an elementary guide to the sorts of information required of a biosafety assessment, and the sorts of problems that could arise following the intentional or unintentional release of a GEO. In this way, the manual might be used as a sort of field guide, with the expectation that individual users will need to alter and customize the assessment for particular GEOs of interest. We caution that this manual offers no assurance of complete safety. The manual is meant to guide consideration of the sorts of problems posed by the release of GEOs.

While the focus of this manual is primarily on planned releases of GEOs into the environment (e.g., the commercial application of genetically engineered crops), we recognize that unintentional releases will be unavoidable, will occur, and will likely increase in frequency as the scale of application increases. Users are therefore encouraged to consider the effects of both intentional and unintentional releases, and to attempt to minimize the effects of both.

We anticipate that users of this manual will include the designers and producers of GEOs, regulators and other decision-makers, professional biologists and other scientists, and other interested readers. The intention is to help inform decision-making at every level, from production through commercial application, in order to reduce the risks associated with the use of GEOs. Clearly, other sources of information will be required, and expert interpretation and analysis will be essential. Even so, we encourage users to seek the highest level of biosafety assessment possible before making decisions regarding the release of GEOs into the environment.

This manual evolved from the collaboration of scientists from a variety of disciplines, including microbiology, soil ecology, genetics, population biology, entomology, marine and freshwater fisheries, terrestrial and marine community ecology, evolutionary biology, and human health, nutrition, and disease, and further benefited from the comments of several anonymous reviewers. A biologist unaffiliated with the project that produced this manual managed a double-blind peer review of the work. Certain omissions may remain; this should not be taken as an indication of unimportance, but as the consequence of time and resource limitations when compiling this document.

Introduction

The diversity of life on earth derives from an exceedingly complex array of dynamic interactions. Human exploitation of the environment has altered and continues to alter the resulting balances, producing changes that cascade far beyond the directly affected sites and extend to biodiversity, human well-being, and even the global climate.

Among the environmental consequences of human actions have been immense changes in the species composition of most ecosystems, and the geographical ranges of many organisms, including those directly associated with humans, such as crop plants, domestic animals, and commensal animals (like the black rat). Many of these changes are generally considered to have been beneficial to humans, for instance the introduction of maize into the Old World, or rice to the New. Others have been unwelcome, such as the introduction of the gypsy moth into North America or the European rabbit into Australia (U.S. Congress 1993).

In addition to introducing organisms into new environments (deliberately or accidentally), humans have for 7,000 years or more been systematically altering the phenotype of certain plants and animals by selective breeding, both intra- and inter-specific. By this means most contemporary crop plants and domestic animals have been significantly altered from their wild forebears. Again, these changes are generally considered to have been beneficial to humankind, by increasing yield, improving nutritional quality or taste, or by enhancing preservability. But, as with introductions, these genetic changes have also resulted in significant problems. For example, hybridization between cultivated sugarbeets and wild beets has led to the evolution of weed beets that do not create a usable product and damage harvesting equipment, leading to loss of millions of dollars per year in Europe's sugarbeet industry (Boudry et al. 1993). Escape of an African subspecies of the honeybee in Brazil led to the evolution of Africanized honeybees in the New World that

disrupted the Latin American honey industry, caused human deaths, and killed livestock (Camazine and Morse 1988). Similarly, hybridization between wild rye and cultivated rye in northeastern California has led to the evolution of weedy rye that has rendered the region unsuitable for cultivation of rye for human consumption (National Research Council 1989).

Recently the human capacity for genetic manipulation has been expanded greatly by novel techniques of genetic engineering. As used in this Manual, a genetically engineered organism (GEO) is one that has been constructed by isolating nucleic acid molecules (the molecules that encode genetic information) from one organism, and introducing those molecules into another organism in a manner that makes them part of the permanent genetic make-up of the recipient, i.e., capable of being inherited by offspring. We also include in the definition those organisms constructed by the transfer of subcellular organelles from one cell to another, followed by the regeneration of an adult organism from the genetically altered cell, so long as the alteration can be transmitted to offspring.

Genetically engineered organisms have the potential to be significantly more novel than conventionally modified organisms, incorporating, as they sometimes do, genes from distant or unrelated organisms in combinations that are unlikely ever to have occurred naturally. These new constructs -- like the products of other new and powerful technologies before them -- offer a variety of plausible benefits. Unfortunately, along with potential improvement in human health and comfort come threats to human well-being and the ecological systems that sustain life on earth (Tiedje et al. 1989). The gene combinations produced by genetic engineering are in some (although by no means all) cases so different from those produced by more conventional manipulations that their effects on the dynamic interactions among organisms in nature remain largely unknown (Colwell 1989, Tiedje et al. 1989, Kapuscinski and Hallerman 1991, Regal 1994). Because of the very novelty for which they have been touted, GEOs and their products require careful scrutiny.

The need for care was apparent in 1995, when the (nation) parties to the Convention on Biological Diversity called for an international "action on biosafety",

recognized "the relatively short period of experience with releases" of genetically modified organisms, "the relatively small number of species and traits used, and the lack of experience in the range of environments, especially those in centres of origin and genetic diversity", and began creating a biosafety protocol (Convention on Biological Diversity 1996).

The goals of biosafety, as the term is used in this Manual, are to:

*assess in advance whether harm to human health and natural systems may result if any particular GEO is released into the environment;

*anticipate when a given GEO or any of its product(s) might be harmful if it becomes part of human foods;

*assess whether a GEO actually is likely to yield the benefit(s) it was designed to provide;

*anticipate possible hazards arising when GEOs are transported, intentionally or unintentionally, among different ecosystems and nations.

This manual offers procedures for identifying potential hazards associated with the release of GEOs created from viruses, bacteria, fungi, terrestrial plants including those providing food, marine and aquatic plants, finfish and shellfish, arthropods including those that are vectors of disease, and vertebrates. Where a specific hazard is identified, recommendations are made for minimizing the perceived risk (that is, minimizing the likelihood that a potential hazard will actually occur). The manual is intended to be accessible to the general reader and to be useful to scientists, decision makers, regulators, and those who develop and produce GEOs.

This work builds on the prior efforts of others. Some of the scientists who participated in the workshop that led to the first draft of this manual in 1996 were

not part of the workshop that wrote this version. And yet their work informed much of what is found here and in some cases is duplicated here with little change. Further, the flowcharts and the basic assessment design of this volume are modeled after those developed by a very large group of scientists for the Agricultural Biotechnology Research Advisory Committee (ABRAC)'s *Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish* (1995), (available on the World Wide Web at: www.nbiap.vt.edu/perfstands/psmain.html). In general, we have borrowed what we could from our colleagues and we wish to acknowledge that we have done so with great gratitude.

What is biosafety assessment?

In the context of this Manual, biosafety assessment is a step-by-step process that systematically examines the potential consequences of the deliberate or accidental release of a GEO and does so with sufficient thoroughness to enable a reasonably confident determination of whether the particular GEO can be used safely. Biosafety assessment includes elements of ***hazard*** and ***risk***. ***Hazard*** can be defined as a potentially adverse outcome of an event or activity. ***Risk*** is the probability of the hazard occurring (Smith 1992). The approach of this book is to focus on the *identification of hazards*, and then, in keeping with the precautionary principle,[1] to offer recommendations that *minimize specific risks*. We do not provide guidance on the estimation of specified risks; users interested in doing so should consult the extensive literature on risk estimation (see, e.g., Burgman et al. 1993 and Stern et al. 1996).

Broadly speaking, the goals of biosafety procedures are to minimize or avoid adverse human and environmental consequences by:

*anticipating detrimental effects that might follow the release of a GEO during experimentation or commercialization,

*designing monitoring systems for the early detection of adverse outcomes,

*planning intervention strategies to avert and, if necessary, remediate adverse environmental or health effects,

*defining regulatory authority to prevent the development and/or

[1] The precautionary principle as stated in the preamble to the Convention on Biological Diversity (UNEP/CBD/94/1) notes that, "...where there is a threat of significant reduction or loss of biological diversity, lack of scientific certainty should not be used as a reason for postponing measures to avoid or minimize such a threat".

importation of potentially dangerous GEOs,

*encouraging continued development of increasingly effective biosafety principles and procedures,

*providing public information about biosafety, and urging that such information become part of school curricula and teacher education.

The search for biosafety can benefit from ecological assessment, risk assessment, risk management for any identified hazards, monitoring, and, in some cases, regulation to prevent the creation and dispersal of undesirable organisms, for instance:

*a GEO that would proliferate and extinguish or greatly reduce a local species by predation, competition, genetic pollution, habitat disturbance, or infection; thus reducing biological diversity,

*a GEO that would do widespread damage to several species, to biotic community structure and its functioning, to soil fertility, to water purity, or air purity,

*a GEO that would harm human health through infection, toxicity, allergenicity, contamination, damaging nutritional or metabolic effects, spread of antibiotic resistance, or damage to agricultural systems,

*a GEO that exchanges its genetic material with one or more indigenous organisms creating a new ecological problem, or

*a GEO that proves to be genetically unstable and loses its intended beneficial purpose, or changes genetically by mutation or genetic exchange with surrounding organisms, and under natural selection evolves harmful properties. Some forms of genetic engineering may tend to confer future genetic instability; for example, GEOs constructed using transposons may

have a permanently elevated frequency of insertion mutations (Ribiero and Kidwell 1994).

The search for biosafety has international ramifications. GEOs will cross biological boundaries and geopolitical borders, whether intended or not, just as other types of organisms have done for a long time. This dispersal (or movement) may bring adverse consequences, particularly in countries and communities that are centers of origin and genetic diversity for crops and farmed animals (important as reserves of genetic sequences critical to the development of new crop strains and animal lines).

Hazards of GEOs are not just environmental; significant direct impact on human welfare is also of concern. For instance, excessive reliance on genetically engineered crop plants may displace traditional agricultural practices (Sharples 1987), with significant sociological effects.[2] Further, reliance on GEOs for control of disease vectors may lead to decreased application of more traditional methods, with consequent problems if the new strategy cannot be maintained indefinitely or is less effective than anticipated (Spielman 1994).

Although all countries are vulnerable to unanticipated hazards from the release of GEOs, due to their potential spread far from the point of introduction (Sharples 1983), countries most at risk are those with:

*insufficient scientific infrastructure to survey biological diversity and characterize the ecology of local natural and agricultural systems; or

*inadequate financial, political, administrative, scientific, or managerial capacities or will to establish and maintain a risk assessment program.

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[2] An example of the potential for such displacement can be seen in the response to large-scale plantings of transgenic crops containing genes from the bacterium *Bacillus thuringiensis* (Bt). Without careful management, such plantings can accelerate evolution of pest resistance to Bt and thus destroy the utility of a valuable agricultural tool (Gould et al 1992, Mellon and Rissler 1998). Many entomologists consider Bt an unusually benign pesticide that warrants extremely careful management, especially because there are no known acceptable alternatives (Snow and Palma 1997, Mellon and Rissler 1998).

In such cases more than even the usual caution is warranted in considering release of a GEO, since adequate assessment may not be possible.

A country in which a GEO is proposed for commercialization might best accomplish a biosafety assessment by using a case-by-case approach in which a "review-planning" team is formed of independent scientists (having a spectrum of expert knowledge consonant with the specific molecular biological, organismal, ecological, and applied dimensions of the proposed GEO), scientists from the body proposing commercial use of the GEO, and scientists and administrators from appropriate governmental agencies. The team would (a) define the kinds of procedures, experimental and observational data, and statistical analyses necessary to render a decision for or against commercialization using the procedures of this Manual and other sources of guidance, (b) produce a written plan with a timetable for completion of the data-gathering and statistical-analytical parts of the assessment, and (c) make its recommendation, based on analysis of the data gathered, for or against commercialization. A written report should be provided by the team, with minority reports if consensus cannot be reached. The whole process, to be well-executed, sustainable and widely accepted, should be open to public comment and review.

When a country contemplates importing a GEO for use, it might best evaluate the possible hazards and benefits using a team of its own similar to that suggested for the country developing the GEO. That team can review all documentation from the country of origin's risk assessment and plan and implement, where necessary, additional tests and risk assessments of its own, particularly where there is not sufficient data for the range of field conditions the GEO is likely to encounter in the importing country.

Why are GEOs potentially hazardous?

Because they reproduce, disperse, and evolve, GEOs pose safety problems considerably different from those posed by the products of technologies based solely on physics and chemistry. In contrast to most physical and chemical constructs, once a GEO is released, its multiplication, dispersal, and possible interbreeding with native organisms may make it impossible to retrieve or eliminate the GEO, or to alter or remediate its effects (Brown et al. 1984, Doyle 1985, Gould 1988, GAO 1988, Ginzburg 1991, Doyle et al. 1995, Dommelen 1996, Rissler and Mellon 1996, Klinger and Ellstrand 1994, 1998).

Although relatively little has been published on the capacities for dispersal by GEOs, many instructive examples can be found involving the introduction of non-engineered organisms (Elton 1958, Laycock 1966, Mooney and Drake 1986, U. S. Congress 1993, Drake et al. 1989, Simberloff 1991, Simberloff et al. 1997), including the explosive population growth of rabbits introduced to Australia, water hyacinth introduced to India and the USA, purple loosestrife and kudzu introduced to the USA, and the highly allergenic *Parthenium* (congress grass) appearing in India during the last half-century. These species and others all have caused immense damage since their arrival. By analogy, much harm could result from the environmental release of some GEOs.

Of course, numerous intentional introductions of non-engineered species (e.g., crop plants) have provided immense benefits to our species; so might some GEOs (Tiedje et al. 1989). The challenge is to determine in advance of release what will be of benefit and what will bring harm (Fincham and Ravetz 1990).

The assessment of risk for many GEOs may be dauntingly complex, combining as it does the micro-scale complexity of molecular biology, biochemistry and physiology with the macro-scale complexity of ecology, population genetics,

behavior, biogeography, and evolutionary biology. In this handbook we use knowledge from all these parts of biology to assess the potential hazards associated with GEOs and their products.

While we hope that the rush to exploit and release novel organisms will be tempered by careful scientific assessment and a universal commitment to biosafety,` we also recognize the urgent needs of many peoples of the earth, as well as the practical constraints faced by industries and governments. In this context, our scientific standards might appear too stringent, expensive, or unrealistic, and lacking sufficient concern for problems of hunger, disease, profit margins, politics, and administrative and legal imperatives. However, GEOs are not likely to provide a panacea for all human problems; many will not live up to excessively optimistic expectations, some will be ineffective, and a few are likely to be extremely dangerous. Prudence argues that we do all we can to ensure that a given GEO will be safe *and* effective before it is applied environmentally over wide areas and long time periods, or before it becomes part of human foods. Increasingly widespread and repeated application of GEOs will provide the opportunity for them to mutate, exchange genes, come under natural selection and evolve into something beyond the original engineered construct. This capacity to reproduce, disperse, interbreed, and evolve makes the effects of GEOs impossible to completely anticipate, and suggests caution until the benefits are well documented and the hazards thoroughly considered.

Like traditional methods of breeding, but to an even greater extent, genetic engineering can produce organisms with surprising new traits as well as surprising new effects on ecosystems. If we wish to realize the benefits of GEOs while reducing to the maximum extent feasible any adverse consequences (both to humans directly and to the surrounding environment), care must be exercised at every stage to perform as thorough an analysis as is possible, while recognizing that a complete analysis is not possible (Sharples 1983, Regal 1993, Regal 1994, Dommelen 1996, Kapuscinski et al. 1998).

All newly produced traits and their environmental effects and interactions --

whether expected or not -- need to be considered in any biosafety assessment. The relationship(s) between intended phenotypic changes, ecological effects, and effects on human health and welfare are diagrammed in Figure 1. To successfully design adequate and comprehensive tests for likely consequences of the release of a specific GEO, researchers and producers of a GEO should be familiar with the range of phenotypic traits expressed by the GEO and its parental organisms throughout their entire life cycles. In addition, researchers and manufacturers should consider the likelihood of local and long-distance GEO dispersal and the consequent ecosystems the GEO may be able to access. Table 1 provides examples of some of the many routes of organism dispersal.

Several mechanisms can lead to unexpected properties in a GEO, and to unanticipated problems caused by a GEO. These mechanisms need to be considered in any assessments. Among such mechanisms are the following:

* **Gene Flow.** Gene(s) from a GEO can be transferred unintentionally to populations of the same or different species; such gene transfer can cause unintended (and possibly adverse and difficult to discern) phenotypic change. Thus, for example, a plant might be genetically engineered to produce an industrially useful but toxic chemical. Through pollen dispersal, the (toxin-producing) gene might be transferred to other plant varieties of the same or related species. These species might be used as human food and if they were, the gene transfer might go unnoticed until enough adverse consequences manifested in the human population to stimulate a search for the cause of the problem. By that time, a great deal of the harm might have been done.

* **Second-site Change.** Unintended genomic changes can occur as a secondary consequence of genetic engineering. Such second-site changes can lead to production of new proteins that may be toxic or allergenic, or may disrupt or alter metabolic pathways that play a role in making the GEO useful, possibly even defeating the purpose for which the GEO was made in the first place.

* **Selectively Increased Transcription and Translation.** Genetic engineering may increase the production of an existing or new protein in a GEO. In turn, this might lead to alteration of a metabolic pathway, or the new protein might serve as a repressor or inducer of an enzyme system or compete for a limited supply of amino acids or other precursors of cellular biosynthesis. These or other unanticipated sequential changes, termed pleiotropic effects, could make the GEO of little use or possibly render it harmful.

* **Contamination of Desired Product.** A chemical product a GEO was designed to produce may carry with it small amounts of other molecules after processing. These other molecules, effectively contaminants, may reduce the value of the GEO product, particularly if they cause allergic or toxic responses.

Gathering and Interpreting Data for Risk Assessment

The hallmarks of high-quality scientific investigation are accuracy, thoroughness, systematic and rigorous analysis, experimentation whenever possible, quantification wherever possible, and common sense. These standards should be the goals of every stage in the assessment process, from laboratory investigations, small field trials, and larger field trials to computer simulation modelling (where useful) and commercialization (or widespread use by agencies in the case of public health measures, e.g., for disease-vector or pest control).

At the start of any risk assessment, Figure 1 (and Appendix A) and Table 1 should prove useful as reminders of the range of effects and dispersal mechanisms that need to be considered. At each of the following data-gathering stages it is important to compare the performance of a GEO with the unmodified organism(s) from which it was made.

*__Laboratory Investigations.__ These are of several kinds:
(1) basic molecular genetic analyses and analyses of physiological performance, done to characterize a GEO and indicate whether it expresses the intended phenotypic properties, and whether other properties are altered;
(2) microcosm (small scale) and mesocosm (medium scale, such as glasshouse) experiments, performed to allow somewhat more realistic study of the potential ecological impacts and the genetic stability of a GEO; and
(3) more complex experiments, progressing from initially "sterile" micro- or mesocosms to ones that include organisms found in the actual ecosystems the GEO might access (these latter experiments should include tests for genetic exchange with closely or distantly related wild or domesticated species). Recent examples of such experimental designs involving GEOs appear in

Holmes et al. (1998) and Muir et al. (1996).

***Small Field Trials.** These should be done only after laboratory investigations *suggest* that the GEO may be efficacious, genetically stable, and ecologically benign. Careful containment and monitoring remain important to prevent accidental release. Suitable experimental protocols are required, using well-understood designs, appropriate sample sizes, controls, and statistical analyses. Small trials should be done in (a) ecological settings where the GEO will be first used as well as in (b) other ecological settings to which the GEO is likely to gain access. Assays for genetic exchange to and from the GEO and for genetic stability of the GEO are very important at this stage (e.g., Mikkelsen et al. 1996). If efficacy cannot be demonstrated by this stage, there is no justification for proceeding with larger trials. If genetic exchange or instability appear likely at this point, the consequences of these effects will require additional exploration (e.g., Gliddon 1994, Darmency 1994, Rissler and Mellon 1996, Wang et al. 1997, Klinger and Ellstrand 1998). It is also important to conduct experiments to explicitly test for adverse ecological effects of the GEO brought about by one or more changes in its traits; for instance, enhanced competitive ability could allow the GEO to displace wild populations or species in the field setting. Although the spatial and temporal scale of field trials prevent testing for certain ecological changes, a thorough effort should be made to design direct or indirect tests for possible adverse ecological effects (Hallerman and Kapuscinski 1993, Regal 1994, Rissler and Mellon 1996).

***Computer Simulations**. Computer simulation models may aid in risk assessment, but should never be the sole basis for final decisions about the safety and efficacy of any GEO. When used in conjunction with laboratory and field data, such models may help to estimate such parameters as the probability of risk, the patterns and rates of GEO dispersal, or the spread of genetic material into a surrounding population after it has been transferred from the GEO (e.g., Muir et al. 1996). These models may also aid in identifying missing information, guiding additional experimental or

analytical steps in a risk assessment, or designing procedures for risk management and monitoring.

*__Larger Field Trials.__ If small field trials indicate both efficacy and safety, larger field trials can be done. The same requirements for good experimental design apply as with smaller field trials. Tests for genetic exchange and stability are again essential, as are studies of dispersal of the GEO and tests for adverse ecological effects. By integrating the results from small field trials and computer simulations, it should be possible to target experiments at the most likely types of adverse ecological effects.

*__Commercial Release or Widespread Application.__ Initially, commercialization or widespread environmental applications should take place in the areas where larger field trials have been completed and found to indicate a high probability of GEO safety and efficacy. When use in a different environment is contemplated, the field trials need to be repeated in the new environment before any general release is permitted there. Periodic monitoring after a GEO is released into a new environment is essential (Kapuscinski et al. 1998). This would entail statistically sound sampling to detect unexpected dispersal, gene flow, and ecological and human health effects. At the very least, DNA markers diagnostic for the GEO should be used to track the fate of released organisms and their descendants.

These tasks are neither easy nor inexpensive. Yet they are essential if GEOs are to be used with minimal harm. In the end, safety and effectiveness of GEOs are inseparable considerations, and those developing and producing GEOs should have as much of a stake in their safety as anyone.

How to Use this Manual

This Manual offers a framework for thinking systematically about the biosafety of GEOs. The document, which emphasizes maximum concern for environmental and human health, is not designed to recommend policies but rather to alert readers to the sorts of biological information required for safety assessment and to suggest means for proceeding with that assessment, questioning whether risk management is warranted, and discerning whether enough information is available to allow adequate assessment.

A major component of this design is a set of flow diagrams which utilize knowledge from many of the disciplines of biology to 1) allow the user to identify potential hazards and 2) guide the user to an informed decision regarding release of the GEO in question. Many kinds of organisms are covered, including viruses, bacteria, fungi, useful terrestrial plants including those providing food, marine and aquatic plants, finfish and shellfish, arthropods including those that are vectors of disease, and vertebrates. The user is cautioned that the text and flowcharts presented here were developed for single changes only. The effects of introducing multiple sequential alterations into a single GEO have not been explicitly accounted for here. Such multiple changes may render assessments more complex.

Accompanying the flowcharts is supporting text that provides scientific background for the questions and decisions of the flowcharts, presents more detailed risk management recommendations, and offers a glossary of scientific terms. Several appendices address specific issues related to the flowcharts.

So that readers and their colleagues may be able to trace their decision path through the assessment, a blank worksheet is provided. Once completed, the worksheet becomes a record and documentation of the decisions made and, where appropriate, of the rationale for any risk management measures taken.

In addition to the blank worksheet are several examples of completed worksheets for various projects that have been analyzed using the decision pathways of this assessment. The names and addresses of project assessors and consultants have been altered.

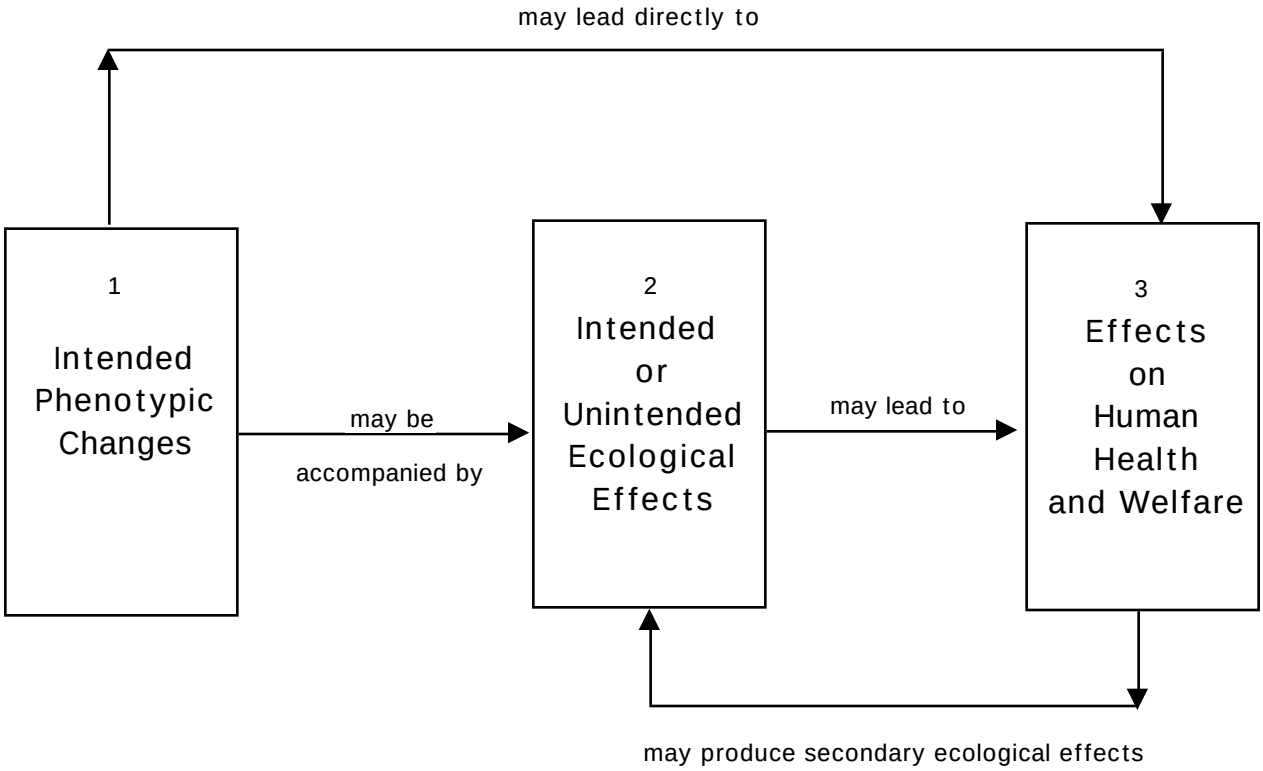
The Manual is published in two volumes to allow ease of use. In Part One are the introductory materials and the text, appendices, references, and definitions that support use of the flowcharts. Part Two contains the flowcharts and worksheets. It is the hope of the authors that the Manual eventually will be converted to an interactive computer program, available free to all users on the World Wide Web.

To use the printed Manual, the reader begins by looking (in Part Two) at the first flowchart (Overview of the Flowcharts) for orientation to the process, continues by reading (in Part One) the accompanying text for that flowchart, and then proceeds to the second chart (Flowchart I: Determination of Assessment Pathway). Answers to questions in Flowchart I will direct the user to other flowcharts relevant to his/her particular situation.

In proceeding through the flowcharts, users need read only those portions of the supporting text that correspond to the flowcharts and questions applicable to their project.

Figure 1. Relationships between Intended Phenotypic Changes, Ecological Effects, and Effects on Human Health and Welfare

A few general examples are given in each category.
For more numerous and detailed examples, refer to Appendix A.



1

Examples of Phenotypic Changes include changes in:
- Metabolism
- Physical Tolerance
- Behavior
- Morphology
- Life History
- Reproduction

2

Examples of Ecological Effects include changes in:
- Distribution of Organisms
- Abundance of Organisms
- Competitive Interactions
- Trophic Relationships

3

Examples of Effects on Human Health and Welfare include:
- Changes in Productivity
- Changes in Food Quality
- Loss of Cultural Diversity
- Loss of Biodiversity
- Changes in Social and Economic Relationships
- Increases in Human Population
- Changes in Patterns of Disease

Table 1. Possible routes of local and global dispersal that many GEOs, like their microbial, plant, or animal relatives, are likely to follow after intentional or unintentional release, and during transport of people and goods. These examples of organisms dispersing by the various routes are not exhaustive.

I. HITCHHIKING ON HUMAN-CREATED FORMS OF TRANSPORT

shipping at sea and on large lakes and rivers

via ballast water and sediments, e.g., marine larvae, shellfish, fish, arthropods, microbes, molluscs, algae

on all the surfaces and crevices of boats below water line, e.g., marine larvae, shellfish, fish, microbes, sedentary marine organisms, arthropods, molluscs, algae

on surfaces above water line, e.g., bacteria, bacterial and fungal spores, plant seeds

floating oil and gas drilling platforms, e.g., a variety of marine organisms

aircraft, e.g., microbes, seeds, insects and other terrestrial arthropods

ground transport (including agricultural equipment such as tractors), e.g., live plants, seeds, small mammals, microbes, insects and other terrestrial arthropods, pollen, many soil organisms and seeds when bulk soil, manure, and compost is transported

recreational boats, e.g., freshwater fish and invertebrates, aquatic plants, algae, microbes

containers used to transport live organisms, e.g., plants, fungi, seeds, fish, insects, bait buckets with fish or invertebrates

containers used to transport food, including live organisms traveling with frozen foods, seeds within fresh fruits and vegetables, grain crop seeds

transport of crop seeds, cuttings, and nursery stock, e.g., microbes, insects

on and in human bodies or clothing, especially bacteria, fungi, small seeds and viruses

trash/refuse/garbage, e.g., microbes, insects

navigation canals allowing active dispersal of mobile organisms, e.g., fish, aquatic invertebrates, and plants

transfers of water between municipalities and regions, for domestic and industrial use and irrigation, e.g., microbes, protozoa, viruses

II. NATURAL ROUTES OF DISPERSAL

flowing water, e.g., microbes, fish, algae, aquatic plants, aquatic insects, fish, arthropods, molluscs

subsurface flowing waters, e.g., soil microbes and invertebrates, cave organisms

on waterfowl and shorebirds, e.g., microbes, small invertebrates, seeds

terrestrial vertebrates, especially mammals (fur), e.g., seeds, pollen, small invertebrates

terrestrial and flying insects (flies, bees, ants), e.g., pollen, seeds, microbes, mites

rafting on logs and larger floating "islands" broken away from shorelines, on lakes, rivers, and seas, e.g., many kinds of terrestrial organisms

ocean and lake currents, e.g, multicellular and unicellular algae, larger aquatic plants, invertebrate larvae, fish, microbes

atmospheric circulation with subsequent deposition as rain, snow and dry fall, e.g., bacterial and fungal cells and spores, pollen, airborne seeds

autonomous locomotion, e.g., flying, walking, and swimming organisms

tornadoes, cyclones, hurricanes, floods, e.g., microbes, seeds, insects, birds, fish, shellfish, aquatic plants

Supporting Text for Flowcharts

How to Use the Flowcharts

The flowcharts are designed to help users evaluate whether a genetically engineered organism (GEO) or a genetically engineered food (GEF) poses specific genetic, ecological or human health hazards (adverse effects). Briefly, the charts allow users, on a case-by-case basis (one GEO or GEF at a time), to:

(a) determine if this Manual is appropriate for assessing the specific GEO or GEF in question;

(b) determine the potential for survival and reproduction of a GEO in whatever ecosystems it is able to access;

(c) identify potential genetic hazards posed by the introduction of a GEO into natural populations;

(d) identify potential non-genetic hazards posed by a GEOs in all ecosystems that are accessible to the GEO and suitable for its survival;

(e) identify potential adverse human health impacts from a GEF; and

(f) minimize the risk of hazards identified by this assessment.

The flowcharts use a variety of shapes to aid the reader. The meaning of most of the graphics is obvious. Circles indicate final decision points and questions always appear in diamonds. Most of the charts begin at the top with a question in a diamond labeled "1". Subsequent diamonds are labeled by number; the numbers are only labels and do NOT necessarily indicate the order in which questions are to be answered. Likewise, some rectangles that contain instructions are labeled by small letters.

Questions usually are answered with yes or no answers; sometimes there is

an option to answer "unsure" or "unknown" or "can't be estimated". Arrows point the pathway to be taken after each answer.

Explanatory material is not furnished for every statement and question but only where further clarification is necessary. To determine whether more detailed information is available for a particular flowchart, turn to the relevant discussion in the flowchart text. In that discussion, the explanations are identified (and marked) by flowchart (and flowchart number) and by question (diamond) number or instruction (rectangle) letter within each flowchart. For example, for more detail about estimating changes in species abundance, the subject of a question in diamond 3 of flowchart V. B., go to the V. B. <3> section of the flowchart text.

Please note that several paths in this framework lead to the Exit Routine which can then lead the user to the words " exit this assessment". This phrase was chosen to indicate that *this* assessment, although completed by a user, might not be a sufficient assessment for the organism or food in question. Other assessment schemes or further assessments may prove useful.

Further, in other places, the reader will encounter phrases like "consider disallowing release" or "consider allowing release" instead of more prescriptive language. In general, this reluctance to specify the final decision regarding release is in keeping with our intent to alert readers to the sorts of biological information required for safety assessment; the decision-making burden remains with the reader. Additionally, we recognize that decisions about whether or not to allow a release may be influenced by socio-economic considerations beyond the scope of this document.

In addressing the various questions on these flowcharts, it is imperative that the user consider *all ecosystems that are accessible to the GEO and that are suitable for survival of the GEO*. In many cases, this will include ecosystems in addition to those chosen for planned introduction. Guidance on how to identify suitable and accessible ecosystems issue is provided below under flowchart II. A. for questions <4> and <5>.

Overview of Flowcharts

The flowcharts begin with a schematic summary of the major decision-making pathways that indicates where each subsequent flowchart fits in the larger picture. Use of the flowcharts leads the user to one of several possible conclusions:

1. A specific hazard is identified and the user is led to consider minimizing the risk of this hazard.
2. Information is insufficient to answer an essential question in the assessment and so the user is directed to consider risk management.
3. A specific reason for exiting the assessment is identified and the user is directed to consult other relevant materials, such as national environmental regulations, biosafety guidelines, etc.
4. A specific highly adverse effect of the GEO/GEF is identified and the user is directed to consider disallowing release or use of the GEO/GEF.

Flowchart I. Determination of Assessment Pathway

All assessments begin with this flowchart which directs the user to the assessment pathway(s) most appropriate for the genetically engineered organism (GEO) or genetically engineered food (GEF) under consideration. The comments below follow a common format in which the Roman numeral (sometimes with a capital letter) specifies the particular flowchart, and the Arabic numeral refers to a specific diamond on the flowchart.

I.<1>

The importation of non-living GEFs that do not contain seeds or other propagules generally pose no genetic or ecological hazards. However, in cases where domestic or wild animals are able to access the GEF, there may be ecological hazards; in such cases, answer "no" to this question.

I. <2>

This assessment may be used for certain organisms that are the products of interspecific hybridization or chromosomal manipulation but are not technically GEOs under the definition set forth on page 2 (see discussion for I. A. <2>). Readers using this assessment for such an organism, should consider that subsequent directions and discussions about "GEO"s apply to their organism.

I. <3>

Gene flow between crops engineered for different traits can introduce harmful compounds into the human food chain. This is of special concern in the case of crops engineered to produce industrial or pharmaceutical compounds. Questions I. <3> and I. <4> are designed to identify situations in which such gene flow is possible.

I. <4>

Examples of potentially harmful biochemical compounds include known allergens, known toxins, novel proteins, and biochemical compounds known to be harmful in high dosage (e.g., fat-soluble vitamins).

Documentation from appropriate prior testing is required to answer "no" to this question. Consultation with toxicologists, immunologists, and other experts may be required. Readers may find it useful to peruse the food assessment in this manual (text and flowcharts for VIII. through IX.).

Flowchart I. A. Continuation of Assessment Pathway

This chart continues the process of identifying appropriate flowcharts or appendices.

I. A. <1>

The purpose of this question is to identify organisms created by traditional

(non-molecular) methods of breeding or husbandry.

I. A. <2>

Certain groups of organisms, e.g., crop plants, have been bred and improved through chromosomal modifications and interspecific hybridization for more than a century. These changes are not generally considered genetic engineering and this assessment should not apply to these organisms. This does not imply that all such changes for plants are without hazard nor should the decision to narrow the scope of this assessment be taken to imply any general endorsement of the Familiarity Principle. (For a discussion of the Familiarity Principle, see Dommelen 1998.)
In the case of some groups of organisms, e.g., finfish and shellfish, interspecific hybridization and/or chromosomal manipulations are so novel that the assessment in this manual may prove useful. For those working with such organisms and using this assessment, wherever the term "GEO" is encountered, the term should be taken to include their organism (although the organism is not technically a GEO under the definition set forth in this manual on page 2).

I. A. <4>

Clear documentation comes from appropriate empirical studies of the functioning of the accessible ecosystems. In the absence of such documentation, relevant advice may be available from ecologists and conservation biologists who are knowledgeable about the accessible ecosystems.

I. A. <5>

Some GEOs that exhibit alternate reproductive pathways (non-dioecy, parthenogenesis, apomixis, monoecy, selfing, neotony, etc.) may pose unusual hazards because a single individual or an apparently asexual form may establish a population without mating with another individual (see Appendix C: Assessment of GEOs with Alternate Reproductive Pathways). Examples of this might include cases where a single gravid female containing multiple offspring escapes, or where multiple individuals come from seeds within a single fruit, or where an insect larva produces offspring, or a single bacterium multiplies into millions of individual

offspring within a few days.

I. A. <6>

If information about efficacy is unavailable for a particular GEO, the user may insist that the supplier provide scientific evidence of its efficacy. If the potential benefits of using the GEO are great enough, despite the lack of evidence that the GEO is effective, a user nonetheless might wish to consider introduction of the GEO. In this case, use the flowcharts to identify possible hazards and determine what testing will be required. In most cases, a full range of testing in contained environments and in the field should be performed.

If information about effectiveness of the GEO is available from the supplier, carefully examine the conditions under which the tests were performed. Are they equivalent to the conditions at the planned application site? Are there ecosystems into which the GEO might spread from the new site that were not among the accessible environments previously tested? What tests has the supplier performed to show efficacy? If prior testing was done under environmental conditions that are very different from the ones in which the GEO will be released, those results cannot be extrapolated to the new conditions, and new tests are warranted. For example, there is evidence that some microbial GEOs do not perform similarly in different soils or in soils with different levels of organic matter (Holmes et al 1998). Each soil type therefore would require a separate test.

Flowcharts II. A., II. B., II. C., II. D., III. Survival and Reproductive Assessment

These flowcharts are meant to identify applications that can exit this assessment without proceeding to more difficult questions about the biology and ecology of the organism.

Flowchart II. A. Survival and Reproduction Assessment - Deliberate Gene Changes

II. A. <2>

Plants are specifically excluded at this point because deliberate chromosome manipulations and interspecific hybrids are already established and widely used techniques of crop development and improvement. This exclusion does not imply that all deliberate chromosomal manipulations and interspecific hybridizations of plants are without genetic and ecological hazards; however, these issues are beyond the scope of this document.

II. A. <3>

While the intent may not be to promote dispersal, once placed into the outdoor environment, most organisms, especially bacteria, fungi, protozoa, nematodes, plants, many insects and mites, cannot be prevented from spreading. Because these organisms or their propagules are microscopic, their dispersal is possible through many mechanisms. They can be carried on the surface of anything that is in the area of application, such as the feet of birds, the bodies of insects, the shoes and clothing of people, and the surfaces (and tires) of machinery. Many of these organisms are dispersed by the wind, either blown about individually or blown about in dust, on pollen, on seed surfaces, on plant debris, etc. Therefore, in cases where dispersal of microscopic stages could occur, answer "yes".

II. A. <4>

Determination of suitable ecosystems, where survival of the GEO is possible, is challenging. Familiarity with the parental organism can only provide partial guidance for assessing the range (broad vs. narrow) of environmental conditions under which the GEO might be able to survive, thus giving a sense of the potential survival range for the modified organism. For instance, in spite of assumptions that smolts and immature adult pink salmon could not survive in fresh water, the Laurentian Great Lakes experienced population explosions of this species two

decades after 21,000 juveniles were flushed down the drain of a Lake Superior hatchery (Kwain and Lawrie 1981, Emery 1981 - reviewed by Kapuscinski and Hallerman 1991). This demonstrates that pink salmon can survive, reproduce, and persist in a broader range of accessible ecosystems than had been expected from studies of their biology in their native range.

The zone of tolerance of the GEO to physical and chemical factors should be the primary consideration in evaluating the GEOs potential to become established in accessible ecosystems. A thorough review of the life history and environmental requirements of the parental organism is needed to determine the potential effects of the genetic modification on the GEOs tolerances for physical/chemical parameters (temperature, moisture, salinity, pH, dissolved oxygen, etc.). The tolerance of a species to combinations of physical factors is more difficult to assess than tolerances to individual parameters, but if such information is available, it should be evaluated.

An important source of information for determining the GEOs zone of tolerance is physiological data on lower and upper lethal limits for environmental factors (e.g., temperature, pH, oxygen availability, other inorganic or organic concentrations). These lethal limits set the lower and upper boundaries of the environmental conditions under which the organism can survive; that is, they define the organism's zone of tolerance. It is imperative, therefore, to assess whether or not: (a) the zone of tolerance of the GEO has expanded beyond one or both of the lethal limits of the parental organism; and (b) in cases where the zone is expanded, the GEO could survive in accessible ecosystems which are lethal to the parental organism.

II. A. <5>

If you haven't read the text for II. A. <4> above, do so before proceeding.

Methods of GEO dispersal are outlined in Table 1. Users should assess the potential to disperse to other suitable ecosystems in order to answer the questions about direct and indirect access to suitable environments. It is important to

recognize that indirect access can occur in several steps: an escaped GEO might pass through several ecosystems before reaching one in which it could survive and reproduce. Under certain conditions, intermediate ecosystems that would normally act as barriers to dispersal may not do so (D'Itri 1997).

The most common reason for going to the Exit Routine from one of these flowcharts is that the ecosystems accessible to the GEO clearly prevent the survival and/or reproduction of any escaped GEOs. It may be helpful to consider these ecosystems early on in the long-range plans for siting of releases (See the explanatory text under about project siting to avoid risk of certain hazards in supportive text for Flowcharts VI.A., VI.B. and VI.C.).

II. A. <6>

Very few applications will meet these criteria, because most ecosystems are not sufficiently isolated and of low enough concern to ensure that all escapees can be killed. The system must remain isolated under all conditions, including both fairly predictable events such as annual flooding and infrequent major disasters such as extraordinary flooding, wind damage, or forest fires (see further discussion of disaster preparation under several subheadings of Flowchart VI., "Risk Management Recommendations"). Appropriate but rare examples of isolation for the case of freshwater GEOs include artificial reservoirs, ponds, or abandoned quarries with no outlet.

The criterion of "sufficiently low concern" can be met only if: (1) the isolated system is not a live gene bank for any species of special concern; and (2) destruction of all GEOs (and perhaps all life) in the system is feasible and allowed by the appropriate management or government agency.

Flowchart II. A. 1. Impact of Deliberate Gene Changes

This flowchart is designed to assess organisms bearing a deliberate gene change and possibly bearing one or more additional genetic modifications.

II. A. 1. <1>

To answer "yes" to the first question on the flowchart, information is needed about the molecular characterization and stability of the deliberate gene modification, and the expression, functions, and effects of all the deliberate, induced genetic modifications. This assessment path can be bypassed if the only change is expression of a marker gene that has no demonstrated impact on traits such as those listed in Appendix A. In order to bypass this assessment path, however, users cannot simply *assume* that the marker gene has no effect on the physiology or fitness of the GEO but instead must test directly for effects of expression of the marker gene. For instance, the pesticidal property of a baculovirus against the cabbage looper, *Trichoplusia ni*, was reduced when a recombinant form of the virus bearing the bacterial lac Z gene and expressing the marker, β-galactosidase, was tested (Wood et al. 1993).

If the project involves a GEO for which the user cannot rule out expression of novel traits such as those listed in Appendix A, further assessment is needed in order to identify specific hazards. Phenotypic changes can pose ecological hazards, depending on other factors about the GEO and the accessible ecosystems. These other factors are addressed by subsequent questions in the flowcharts.

Typical marker genes include antibiotic resistance markers and ice-minus markers or marker genes conferring photoluminescence or altered pigment production. There is insufficient information to determine that these marker genes have "no significant effect" in real world situations. In the case of the antibiotic resistance markers, naturally-occurring antibiotic resistant microbes already exist, but there is little knowledge about the effects on particular ecosystems of greatly increasing the number of antibiotic-resistant bacteria. Until such time as a solid body of evidence is available, these marker genes must be considered within the assessment category of "insufficient information". Other marker genes clearly have effects on phenotypic characteristics, thereby leading the reader to answer "no" to the question.

II. A. 1. <3>

Presence of conspecifics in the accessible ecosystem(s) presents the possibility that GEOs could interbreed with the natural population unless the GEOs have been permanently sterilized. Some species, e.g., fish, plants, birds, and mammals, can also interbreed with closely related species. It is essential to assess whether or not GEOs can hybridize with populations of other species in accessible ecosystems because interspecific hybridization occurs commonly at low frequencies in nature for many groups, e.g., North American freshwater fishes (Hubbs 1955) and seed plants (Ellstrand et al. 1996). Hybridization among these species is relatively common because of external fertilization, weak reproductive isolation, and/or secondary contact of recently evolved species (Campton 1987; Grant 1981). Additionally, it is significant that interspecific hybrids are often fertile and able to transmit introduced genes to their progeny, allowing introgression to occur.

Gene exchange among broadly distant taxa: Transfer of functional genetic material can take place in nature between widely unrelated microorganisms due to transport by plasmids and viruses This transfer is not so rare that it can be assumed that transgenes will remain in the species into which they were engineered (Campbell 1981, Datta and Hughes 1983, Regal 1986). However, the transfer is not so common that it can be assumed that all species of microorganisms freely exchange genetic material. Much more research needs to be done on the natural history of genetic exchange in microbial communities before we can predict and understand this phenomenon with any great confidence.

There is also evidence that genetic material has passed between microorganisms and higher organisms (Doyle et al. 1995, Zhou et al. 1997). Among plants and animals, lateral transfer by pollination and sexual hybridization typically occurs only between species within the same genus or closely related genera.

II. A. 1. <4> and <5>

The efficacy of induced sterility varies greatly, depending on the species, sterilization methodologies (e.g., triploid induction, eyestalk ablation, removal of gonadal tissue, emasculation), specific protocols for a given methodology (e.g.,

specific level, timing, and duration of temperature or pressure shock in triploidy induction), and even technical skill of the applicator of the methodology. The literature on efforts to sterilize diploid freshwater and marine organisms by induction of triploidy illustrates this variability. Reported frequencies of triploids in treated groups ranged from 3-100%, with many reports in the 40-60% range; however, survival frequently is depressed by de novo triploidy induction (Ihssen et al. 1990). Usually, triploid organisms are sterile because their eggs or sperm contain chromosomes that would remain unpaired at fertilization and thus result in unviable embryos. However, triploids do vary among species in terms of development of reproductive structures, reproductive behaviors, and presence or absence of gamete production (Hallerman and Kapuscinski 1993). The presence of triploidy may not preclude alternate forms of non-sexual reproduction such as apomixis. In addition, the remote possibility, for large-scale, mass releases of sterile organisms to select for new parthenogenetic forms over time in the natural population should be considered in taxa that have parthenogenetic relatives.

The criterion for answering "yes" to these questions is that all individuals in the population of GEOs are permanently sterile. Evaluations at all appropriate life stages are required. For example, in a recent study of oysters in which triploidy had been induced to make them sterile, some cells reverted to the diploid state raising the possibility that fertility could be restored over time in these individuals (Blankenship 1994). Similarly, "male sterility" allelles are rarely 100% effective in plants when applied over a variety of environments. For microbes, there is no way known at this time to permanently prevent exchange of genetic material, and so for them the answer here will be "no".

II. A. 1. <7>

Populations might be of special concern for different reasons. Species may be designated natural treasures, assigned spiritual importance, or have scientific value based on local, regional, or cultural priorities; these will deserve special consideration and appropriate risk management.

Of additional concern is the undesirable loss of genetic diversity from natural

populations. To conserve this component of biodiversity, populations that constitute centers of diversity, or are declining, threatened, or endangered warrant special consideration. Human-induced species extinctions and declines of natural populations have increased dramatically over the past few decades. For example, significant declines have been documented for amphibians (Wake and Morowitz 1991), fish (Miller et al. 1989, Williams et al. 1989, Minckley and Deacon 1991) and other marine species (Norse 1994), freshwater mussels (Williams and Mulvey 1994), fungi, and other microfauna (Samways 1994).

Recovery and reintroduction of affected species are technically difficult and demand a long-term commitment to reach success. It is clearly prudent and cost-effective to prevent exposure of endangered, threatened, special, or protected populations to additional alterations via interbreeding with GEOs.

II. A. 1. <8>

Here users must consult relevant agencies that have oversight authority for introduction and use of non-indigenous organisms. This assessment is not applicable for evaluating the hazards specific to non-indigenous species. Depending on the non-indigenous species in question, government approval may be needed for the proposed project.

To gain background knowledge about the biological principles involved in assessing the environmental safety or risk of a non-indigenous species, users can consult a number of published papers and suggested protocols developed by various organizations. Examples include a discussion of the ecology of biological invasions (Mooney and Drake 1986), a review of introductions of marine species (DeVoe 1992), a review of harmful non-indigenous species (U. S. Congress 1993), a discussion of conceptual models (Kohler 1992), a discussion of genetic impacts of non-indigenous molluscs (Gaffney and Allen 1992), a discussion of unintentional effects of intentionally introduced biological control organisms (Simberloff and Stiling 1996), a suggested protocol for fish introductions in the United States (Kohler and Stanley 1984), an American Fisheries Society position statement on introduction of aquatic species (Kohler and Courtenay 1986), the "Revised Code of Practice to Reduce the

Risks for Adverse Effects Arising from Introduction of Marine Species" developed by the International Council for the Exploration of the Sea (Sindermann 1986, 1992), recommendations of European Inland Fisheries Advisory Commission (1988), and the protocol proposed in 1992 by the U.S. Aquatic Nuisance Species Task Force, reproduced in *Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish* (Agricultural Biotechnology Research Advisory Committee 1995).

II. A. 1. <9> and <10>

See explanation at II. A. <3>.

Flowchart II. B. Survival and Reproduction Assessment -Deliberate Chromosomal Manipulations

II. B. <1>

Generally, the intended utility of producing chromosomally manipulated organisms (e.g., triploid and tetraploid organisms) is to improve desirable product characteristics or to reduce environmental risk as a consequence of sterility. The risk of hazards posed by such organisms to natural ecosystems differ as a function of their degree of sterility or fertility and viability (see Flowchart II. B. 1.), involvement in mating behavior (see Flowcharts III. and IV. B.), and the nature and degree of phenotypic change (e.g., Appendix A and Flowchart V.). Further discussion of these factors for fish and shellfish appears in Hallerman and Kapuscinski (1993) and in Agricultural Biotechnology Research Advisory Committee (1995).

Although the sterility offered by inducing triploidy in some animals reduces environmental concerns about a modified organism, the issue of safety is complicated by three factors. First, the effectiveness of triploidy induction varies among species and the methods used. Second, in cases where triploids are functionally sterile, the males may still exhibit spawning behavior with fertile diploid females, leading to losses of entire broods and lowering of reproductive

success. Third, wherever large numbers of individuals are released, sufficient numbers of sterile triploids may survive and grow for an indeterminate number of years beyond the normal life span to pose heightened competition with diploid conspecifics or predation upon otherwise invulnerable prey. In some cases, such prey may be juvenile conspecifics; Kitchell and Hewitt (1987) report an example in which larger than normal triploid salmon can prey on juveniles of the same species. The assessment path through Flowcharts II. B. 1., III., IV. B., and V. is designed to address these three factors.

For species that are normally diploid, such as many vertebrates, tetraploid individuals in natural systems will pose a potential risk if they can mate with normal diploids and then yield triploid progeny that are functionally sterile (see Flowchart II. B. 1. <6>). For examples involving fish species, see Hallerman and Kapuscinski (1993). Large numbers of such matings, may produce large numbers of sterile individuals in the ecosystem that can compete with and reduce reproductive success of normal diploids, increasing the risk of extinction of the affected populations.

II. B. <3>

See discussion in text for II. A. <3>.

II. B. <4>

See discussion in text for II. A. <4>.

II. B. <5>

See discussion in text for II. A. <5>.

II. B. <6>

See discussion in text for II. A. <6>.

Flowchart II. B. 1. Impact of Deliberate Chromosomal Manipulations

This flowchart is designed to assess organisms modified solely by chromosome manipulations, such as induced tetraploidy and induced triploidy.

II. B. 1. <2> and <3>

See discussion at II. A. 1. <4> and <5>.

II. B. 1. <4>

See discussion at II. A. 1. <8>.

II. B. 1. <5>

To date, most tetraploid fish produced in the laboratory have demonstrated very low survival, so that few individuals reach sexual maturity. This factor mitigates against the capability of escaped tetraploids to interbreed with diploids and trigger possible declines in natural populations through the production of many sterile triploid progeny. Users are directed to the Flowchart VII. Exit Routine for research involving polyploids that exhibit extremely low survival with the caveat that the research project is small-scale. Users seeking guidance on how to identify an experimental scale appropriate for taking this exit should proceed to Flowchart III so that they can compare the factors that would lead to an *exit* versus those that lead to *risk management*.

II. B. 1. <7>

See discussion in text for II. A. <3>.

Flowchart II. C. Survival and Reproduction Assessment - Interspecific Hybridization

II. C. <2>

See discussion in text for II. A. <3>

II. C. <3>

See discussion in text for II. A. <4>.

II. C. <4>

See discussion in text for II. A. <5>.

II. C. <5>

See discussion in text for II. A. <6>.

Flowchart II. C. 1. Impact of Interspecific Hybridization

This flowchart is designed to assess the risk of losing natural populations of genetically distinct species.

II. C. 1. <1> and <2>

Questions in this flowchart address presence of both parental and other closely related species in the accessible ecosystem because the interspecific hybrid might hybridize with more species than just its parental species. See further explanation in text for II. A. 1. <3> .

II. C. 1. <3> and <4>

See text for II. A. 1. <4> and <5> for discussion of induced and permanent sterility.

II. C. 1.<5>

See discussion in text for II. A. <3>.

II. C. 1.<6>

See discussion in text for II. A. 1. <8>.

II. C. 1. <7>

See discussion in text for II. A. <3>.

II. C. 1. <8>

See discussion in text for II. A. 1. <7> .

II. C. 1. <9>

Some interspecific hybrids produced and reared in the laboratory have exhibited extremely poor survivorship, often at early stages of development (and those that do survive are frequently sterile). This reduces the risk that hybrids will interbreed with a parental or closely related species and thus reduces the risk of losing a natural population of a genetically distinct species through introgressive hybridization. Users are directed to Flowchart VII., the Exit Routine, for research involving interspecific hybrids that exhibit extremely low survival, with the caveat that the research project is small-scale. "Small scale" implies that the escape of all individuals would have no adverse effects on ecosystem structure or processes. Users considering the option to exit for small-scale research projects, should first proceed to Flowchart V. for consideration of possible ecosystem effects.

II. C. 1. <10>

See discussion in text for II. A. <3>.

Flowchart II. D. Transfer of Harmful Biochemical Compounds to Food Chain

This flowchart addresses situations in which hybridization with a GEO causes the transfer of a harmful biochemical compound to human or animal food chains. For consideration of what may be deemed "harmful", readers should consult toxicologists, immunologists, and other appropriate experts. Readers may also find it useful to peruse flowcharts and supportive text of the food safety section of this manual (VII. through IX.).

Flowchart II. E. Vectors Genetically Engineered to Reduce Disease

This chart applies to released GEOs that are intended to modify or reduce the abundance of a vector of a pathogen that causes disease. Vectors are agents that transmit organisms that are pests or causes of disease. Vectors are sometimes designed or engineered in the hope of disrupting pre-existing pest-vector relationships, as in the cases of replacing malaria-carrying mosquitoes with ones unable to carry malaria or replacing vectors of crop diseases with vectors unable to carry harmful disease-causing agents (Agrios 1988, Hoy et al. 1997, Sylvia et al. 1998).

Release of a genetically engineered vector intended to improve human health requires special consideration because success may depend upon the immune status of the affected people and on their willingness to accept the intervention. The ability to sustain the intervention is paramount.

II. E. <1>

Field trials should be small-scale in terms of the area of dispersal of the genetically engineered vector. In the case of an anti-malaria or anti-dengue intervention, such a field-trial could involve a single village or an isolated cluster of adjacent villages. No large-scale release should be attempted until the effectiveness

of the engineered vector has been demonstrated in prior field trials. Suitable comparison sites must be established. In the case of crop diseases, outdoor field trials carry a high risk of exposure to and escape of the genetically engineered vector, and tests therefore should be performed in contained spaces in the laboratory or greenhouse.

Flowchart II. E. 1. Vectors Genetically Engineered to Reduce Disease - Field Trials

II. E. 1. <1>

A body of laboratory observations and theoretical considerations should provide the basis for subjecting a community to any intervention involving engineered vectors. If the intent is to distribute an engineered construct that ultimately will reduce the competence of the native vector population, laboratory experiments must first convince the user that the construct actually and substantially reduces the ability of the vector to deliver an infectious dose.

II. E. 1. <2>

In the event that a drive mechanism is required to spread the competence-reducing construct through the target vector population, another body of laboratory experiments is required. A transposable element, or a sterility-inducing microbe such as *Wohlbachia pipientis*, might serve to insure that the construct is present in a disproportionate number of progeny.

II. E. 1.<3>

This question addresses proposed interventions designed to reduce vector-borne disease by diluting the native vector population through inundative releases of a vector-incompetent GEO. In this event, the overall density of vector organisms, including the released GEOs and unmodified vector organisms, will exceed their preexisting density. Such increased density could pose severe annoyance to people or animals (see text for II. E. 1. <8> and <9>.)

Adverse consequences of using engineered vectors are possible in other cases. For example, in order to replace a root-feeding nematode that carries a crop-disease virus, overwhelming numbers of engineered root-feeding nematodes might have to be introduced, thereby reducing or eliminating the viral disease while at the same time increasing crop loss due to root feeding by the increased number of nematodes.

II. E. 1. <4>, <5>, and <6>

If a useful drive mechanism is identified, users then need to provide theoretical or experimental assurance demonstrating that the construct cannot be disassociated from the driver. If the construct becomes inactivated by some mutation or separated from the driver by a crossover event, the drive mechanism could become distributed in the natural vector population. If such disassociation is possible, alternative drive mechanisms must be available; otherwise the proposed intervention may not be sustainable. This may lead to highly adverse effects. Lack of sustained intervention ultimately exposes people who have lost immunity during the preceding period of effective intervention to new episodes of infection. The most adverse outcome of such new episodes of infection would be a substantial increase in morbidity and/or mortality of people.

II. E. 1. <8> and <9>

Severe annoyance here refers to direct annoyance to people. Perhaps the most compelling example of severe annoyance to people occurs in the case of malaria vectors. The local human population may be subject to an increased level of annoyance by hematophagous arthropods. Further, these arthropods may continue to transmit other pathogens whose ability to cause infection is not reduced by the engineered construct. If such severe annoyance occurred during a field-trial, it is appropriate to consider disallowing large-scale releases of the GEO. If the intervention plan requires nurturing the GEOs (e.g. through continuous releases, provision of artificial breeding sites, intentionally increasing the opportunity of mosquitoes to bite people), the responsible agency assumes a more severe ethical burden. The residents of the release site may retain a "not in my back-yard" attitude that would preclude any such release. Indeed, a transposon-driven release may also require the residents to tolerate activities that reduce their personal comfort (due to

annoyance by hematophagous arthropods) or to increased incidence of some relatively mild disease.

In the case of viral disease vectors of crops, increases in the engineered vector population may induce burdens of another sort. Plant or crop losses due to disease could be replaced by losses due to increased feeding by engineered vectors.

Taken together, these considerations indicate that a small-scale field trial is justified if (1) the GEO reduces vector competence, (2) no drive mechanism is required or many alternative drive mechanisms are available, (3) the overall density of the disease-transmitting vector in the site does not exceed its preexisting density, (4) no similarly transmitted collateral infections are transmitted in the site and (5) no nurturing of the released population is required. A positive gain must be sustainable.

After performance of a single experimental field trial, there will be data concerning the effect of the release on the targeted population of humans or other species. These data should be used to evaluate whether or not additional releases are warranted.

Flowchart II. E. 2. Vectors Genetically Engineered to Reduce Disease - Large-scale Release

A large-scale program for distributing vector-incompetent GEOs should be considered only if results from at least one small-scale field trial suggest that sustainable improvement is attainable.

II. E. 2. <1> and <2>

The residents of the trial site must have accepted the prior release of GEOs (See text for II. E. 1. <8> and <9>.). They must have tolerated any annoyance and any collateral infections that were attendant on the release.

II. E. 2. <3> and <4>

Patterns of morbidity and mortality from the field trial site may demonstrate a health benefit even though the targeted infection remains as frequent in the trial site as in comparison sites. Such a demonstrable improvement in health would justify a large scale distribution of the GEO construct, provided that no adverse ecological effects seem likely.

A large-scale release of a genetically engineered vector would be justified if such an effort promised to reduce or eliminate risk of infection for a particular vector-borne pathogen. Such a release should result in a stable infestation of GEO organisms in which:

(1) The GEO population does not annoy people more than did the native population of wild-type organisms.

(2) The force of transmission of other pathogens is not increased.

(3) Even if the release results in an immediate increase in undesirable events, such as increased numbers of mosquito bites, no transient outbreak of disease results.

(4) The release does not compromise future disease prevention.

If these conditions cannot be achieved, the GEO should not be released.

II. E. 2. <5> and <6>

In the absence of evidence of parasitological or epidemiological benefit, additional field trials of the GEO might be warranted if observations from the first field trial suggested that the engineered construct had become established in the indigenous vector population or that the numbers of infectious vectors in the trial were fewer than in the comparison site.

Flowchart III. Potential Interference with Natural Reproduction

In some cases, such as releases of sterile male insects to reduce population sizes of pests or disease vectors, interference with natural reproduction is the intention of an introduction. (For such cases of biocontrol, refer to Flowcharts V. and V. C.) However, the questions in this flowchart are designed to cover at least two ways that **unintended** reproductive interference might occur:
(1) escaped GEOs are functionally sterile but still enter into mating behavior with fertile individuals in natural populations, yielding inviolable embryos; and (2) escaped GEOs are fertile tetraploids that breed with natural diploids, yielding sterile triploid progeny. An example of the first concern is evidence that presumably sterile, triploid male masu salmon and ayu exhibited normal courtship behavior toward mature conspecific females (Inada and Taniguchi 1991, Kitamura et al. 1991). Also, for example, one arthropod species may displace another because the mating activity of its males interferes with insemination of the females of related species (Ribeiro and Spielman 1986). Such interactions may produce unexpected effects on congenerics of the GEO.

III. <1>

This question applies to both fertile and functionally sterile GEOs. Regarding the latter, triploid males of some fish species exhibit testosterone levels comparable to those of diploid males (Lincoln and Scott 1984, Benfey et al. 1989). Despite abnormal gonad development, triploid rainbow trout exhibit normal sexual differentiation, and at least some triploid males produce sperm (Thorgaard and Gall 1979, Lincoln and Scott 1984). Should courtship and spawning behavior of triploid males sufficiently duplicate that of diploid males, the triploid males could successfully mate with diploid females. No viable progeny would result because the embryos would be aneuploids. However, were many triploids to secure matings, the loss of entire broods could reduce the reproductive success of the naturally existing population, increasing risks of loss of within-population genetic variation or of population extinction due to a demographic catastrophe (Boyce 1992, Shaffer

1987, Lacy et al. 1995).

It is feasible and cost-effective to assess courtship and mating capability in vertebrate animals from laboratory assays for steroidogenesis in individuals of a reproductive age. A negative result from properly controlled assays can clearly rule out the possibility that escaped GEOs will enter into reproductive behavior. In contrast, it is difficult to draw inferences from laboratory behavior experiments about reproductive behavior in natural ecosystems. Absence of a certain behavior in a laboratory environment does not mean that the behavior will be absent in the field.

III. <2>

See discussion in text for II. A. <3>.

III. <3>

See discussion in text for II. A. 1. <7>.

III. <4>

This question applies only to small-scale and contained research in project development. A case-specific approach to answering this question is strongly recommended, *because no generalizations can be made across taxa*. One way to approach this question is to give an affirmative answer only if the number of GEOs is at least two orders of magnitude less than the number of adults of reproductive age in each potentially affected population. An affirmative answer to this question leads to exiting the assessment; the user must base such an answer on an accurate count of the number of GEOs involved in the project and defensible estimates of critical demographic variables for the potentially interfered-with populations. Regarding the latter, necessary estimates of demographic variables include: the expected number of individuals of a reproductively mature age; the expected proportion of these individuals that will reproduce successfully (produce at least one viable offspring); and the expected reproductive success (number of viable offspring) per reproducing adult. Expected values involve estimating the mean and variance of a variable. Natural populations show temporal variability in these

demographic variables but will be most vulnerable to reproductive interference when they are at low ends of the range. Thus, an affirmative answer to this question must account for low values in the natural range of these variables.

Useful background information on how to estimate demographic variables in natural populations appears in texts on population dynamics for different taxa and in extensive literature in scientific journals (e.g., Rhodes et al. 1996). It is also particularly useful to consult local experts on population dynamics of local species. For example, for guidance on fish and shellfish, consult texts on fisheries population dynamics (e.g., Rothschild 1986) and experts on dynamics of local fish and shellfish populations.

Flowchart IV. A. Ecosystem Effects - Impacts of Introgression of Modified Genes

The Ecosystem Effects Assessment addresses this overarching question: if the GEO were to gain access to an ecosystem, either accidentally or by design, would adverse effects be possible or is there a specific reason to rule out such concern? Use of this section leads to one of the first four conclusions listed in the above discussion, "Overview of Flowcharts" and, in one case, specifically suggests disallowing release. Thus, for some GEOs/GEFs the assessment may be completed by concluding that the GEO/GEF is unlikely to present specific hazards. However, other assessments will direct the reader to consider disallowing release, or to proceed to risk management either because (a) specific hazards have been identified, or (b) there is insufficient information to complete the assessment and identify possible hazards.

IV. A. <1>

To answer this question, the user must have empirical data documenting whether or not the GEO expresses one or more phenotypic changes such as those listed in Appendix A.

To answer "no", the user must have supporting evidence about the organism's overall performance (see section below on familiarity). The response of "no" leads to the recommendation to consider disallowing release because the usefulness of the GEO is questionable if it has no phenotypic differences from the unmodified organism.

Phenotypic changes such as those listed in Appendix A mediate the organism's effects on ecosystem structure and processes. The potential for adverse effects depends on the numbers of the GEO accidentally or deliberately introduced into the accessible ecosystem, as well as on other factors addressed in subsequent flowcharts. Refer to Kapuscinski and Hallerman (1991, p. 101-103), Kapuscinski and Hallerman (1990, p. 6-7), Tiedje et al. (1989), and Snow and Palma (1997) for detailed discussions of how changes in traits may pose adverse ecosystem effects.

To determine if the genetic modification produces changes in specific traits such as those listed in Appendix A, the user must be familiar with the overall performance of the GEO throughout its life cycle. Familiarity is based on a combination of information sources, including: (a) knowledge and past experience with the parental (unmodified) organism grown in the same or similar environments, and (b) results of preliminary experiments specifically designed to test for *intended and unintended* phenotypic changes in the modified organism. Regarding empirical tests for phenotypic changes, two complementary approaches are suggested (Kapuscinski and Hallerman 1991, Hallerman and Kapuscinski 1993): a battery of laboratory experiments, in which selected environmental factors are varied while others are held constant; and studies in more ecologically realistic but securely confined mesocosms (Odum 1984, Voshell 1989, Rissler and Mellon 1993). As mentioned earlier, confinement may be extremely difficult for some GEOs, including microbes and vagile insects.

For some GEOs, information from current research, scientific literature or experts may be insufficient to assess the overall performance of the GEO and thus insufficient to give a clear affirmative or negative answer to this question. Following the precautionary principle, research projects involving such GEOs are

directed to risk management in order to develop appropriate confinement measures for the project. Lack of familiarity with the overall phenotype of the modified organism makes it difficult to reliably assess ecological effects posed by (1) expression of intended phenotypic changes in the modified organism that conform to one of the classes listed in Appendix A, (2) genetic modifications that are novel for the species as a whole (e.g., expression of antifreeze protein in tissues of transgenic Atlantic salmon or transcription of antisense DNA in tomato), or (3) unfamiliar effects of the genetic modification on other traits (e.g., the potential of antifreeze protein to expand the range of salmon or a temperate plant species into arctic ecosystems and thereby affect the structure of those biotic communities).

After substantial phenotypic testing, familiarity may increase to a point at which it becomes possible to give a clear affirmative or negative answer to questions about phenotypic changes. It is imperative that experiments involve proper measurements for these phenotypic changes, including variation across individuals (e.g., averages and standard deviations) and that inter-trait correlations and genotype-environment interactions be considered (Falconer 1989).

IV. A. [a]

Estimation of the frequency of introgression of the modified genes depends on the number of GEOs that could accidentally escape into the accessible ecosystem and the relative abundance of the potentially affected natural population. Possible values for the number of escaped individuals, from a minimum to a maximum number, can be developed by considering a range of scenarios that might trigger escapes from the proposed project. To develop appropriate scenarios, users may find it helpful to read text on "Project Siting" and "Design of Barriers" found below in supportive text for Flowchart VI. C. Risk Management : Containment Routines.

Gene flow depends primarily on the reproductive potential of escaped GEOs. Reproductive potential will be a function of: (1) survival rate and fertility or reproductive potential of the GEO and (2) environmental conditions affecting reproduction in the accessible ecosystem (e.g., availability of suitable habitats and length of the breeding season). One way to estimate the reproductive potential of a

group of escaped GEOs is to construct a life table, a traditional technique in population biology, taking into consideration impacts of environmental conditions in the accessible ecosystem (e.g. Emlen 1984, Chapter 3 or Price 1997, in press). This necessitates estimation of reproductive mechanisms (including asexual ones), different ages at reproduction, survival rates for each reproductive age, and fertility (or fecundity) at each reproductive age. Estimation of these variables requires substantial familiarity with the overall phenotype of the GEO, as derived from empirical measurements of GEO phenotypes and knowledge about the parental organism (see above discussion of familiarity in text for IV. A. <1>).

The next step in gene flow estimation is to estimate the frequency of introgressed modified gene(s) in the progeny generation of a natural population. This step may be difficult. Both the rate of spread of a modified gene and its rate of increase are strongly dependent on the ecological, geographical and/or social structure of the potentially affected populations (Gliddon and Goudet 1994). Additionally, users need to assess if the phenotypic changes exhibited by the GEO might alter directions or amounts of gene flow due to altered dispersal, mating behavior, or reproductive success (e.g., caused directly by expanded tolerance range for a physical factor in the accessible ecosystem, or indirectly, e.g., by the effect of this expanded range on the GEOs pollinators).

An experimental approach using innocuous genetic markers to measure rates of interbreeding between simulated managed populations and simulated wild populations can be informative. Experiments of this sort have been described for plants (Rissler and Mellon 1993 and specific experiments reported by Klinger et al. 1991 - radish; Arriola and Ellstrand 1996 - sorghum; Arias and Rieseberg 1994 - sunflower; Mikkelsen et al. 1996 - oilseed rape). It is critical to conduct such experiments in as many appropriate environments as possible using sufficient number of replicates and sample sizes to achieve statistical significance.

For certain organisms, direct experimental approaches may be impossible. Qualitative estimation of gene flow rates among natural populations is possible via a method described by Goudet (1993) and Goudet et al. (1994). This method involves

computer modelling and requires empirical estimation of the fixation index, F_{ST}, a measure of allele frequency heterogeneity among groups that is inversely proportional to gene flow among these groups (Wright 1943). By assaying the population genetic structure of markers detected by molecular genetic methods (allozymes, RAPDs, RFLPs, etc.), it is possible to estimate F_{ST} from data derived from natural populations. Gliddon and Goudet (1994) reviewed the application of this method to three actual populations including that of a marine mollusc (the dogwhelk *Nucella lapillus*), and outlined its potential application to predicting the flow of modified genes into wild populations of Atlantic salmon (*Salmo salar*). Other statistical methods involving estimation of gene flow from population genetic structure are reviewed and evaluated by Slatkin and Barton (1989).

Clear supporting evidence is needed for any prediction that escaped GEOs are grossly unfit and thus pose negligible gene flow (see discussion in text for IV. A. <2>). Whereas most traditional breeding of crops, for example, has been aimed at traits that are not likely to increase plant fitness, genetic engineering for pest resistance may increase crop survival and reproduction in the face of disease or pest attack. Additionally, if such genes are transferred through introgression to wild crop relatives, fitness advantages could accrue to non-target organisms (Snow and Palma 1998). An escape of genetic material through pollen transfer could have unknown effects.

IV. A. [b] and <2>

Introgressed individuals may have altered fitness relative to non-introgressed individuals. An evaluation of relative fitness must be supported by experimental evidence that compares the survival or reproduction of the introgressed GEO to the non-GEO parent under environmental conditions similar to those of the accessible ecosystem. Comparative fitness studies are few (Arriola and Ellstrand 1996; Klinger and Ellstrand 1994). For a thorough discussion of appropriate experimental designs see Rissler and Mellon (1993).

Some genetic modifications could yield a novel adaptive combination of traits so that near wild-type GEOs could survive, reproduce, and persist in natural

environments, thereby disrupting the existing organization of natural biological communities (Regal 1994). It is reasonable to consider many GEOs, such as genetically modified fish, microbes, or arthropods, as being near wild-type. Although numerous strains or stocks of fish and shellfish have been partially domesticated through consecutive generations of captive breeding (yielding increased fitness in captivity), no such strains have been shown to be so domesticated that their fitness in the wild is negligible (Kapuscinski and Hallerman 1991).

IV. A. [c]

If introgressed individuals exhibit lower fitness than non-introgressed conspecifics, it is necessary to assess potential demographic decline of a natural population by interbreeding with GEOs. Given gene flow rates (see discussion in text for IV. A. [a] above), the decline in fitness due to introgressed matings, and the size of the natural population, it may be possible to predict population decline through simulation modeling (Muir et al. 1996).

Flowchart IV. B. Ecosystem Effects on Reproduction

Users are directed to this flowchart if (a) the accessible ecosystem clearly lacks conspecifics or other species to which genetic material can be transferred (see Flowchart II. A. 1.) or (b) the GEOs cannot transmit genetic material to conspecifics or other species because they are functionally sterile (see Flowchart III.). Either case rules out the possibility of intra-specific or inter-specific introgression of engineered genes.

IV. B. <1>

Environmental conditions allowing survival of a GEO (as the user earlier determined in Flowchart II.A.), will not necessarily allow successful reproduction (e.g., pollen production, flower production, seed production, gonadal development, ovulation, sperm maturation, or spawning). Examples of abiotic factors that might

prevent reproduction of the GEO are temperature, salinity, daylength, photoperiod, nutrient concentration, oxygen concentration, and habitat or substrate availability. To invoke one or more of these abiotic factors as a reason to go to the Exit Routine in Flowchart VII., users need documentation that presence or absence of the factor clearly precludes reproduction. For instance, anadromous fish species typically spend their adult phase in salt water and reproduce in freshwater. Depending on the species, lack of freshwater does not necessarily preclude successful reproduction because some populations (e.g., some stocks of chum salmon and pink salmon) naturally demonstrate successful reproduction in saline waters of marine estuaries. Users should be aware that microorganisms and arthropods commonly enter dormant phases, so that lack of reproduction resulting from inappropriate conditions at one time does not preclude reproduction at a time when conditions may be more favorable.

To determine whether or not a given abiotic factor prevents reproduction of the GEO, familiarity with the reproductive biology of the GEO is essential. Knowledge of environmental requirements for reproduction of the parental, unmodified organism can provide some indication of requirements for reproduction of the GEO. Lack of knowledge about these environmental requirements requires answering "unknown" to question <1>. If there is sufficient familiarity with the parental organism's requirements for reproduction, the next step is to determine whether the genetic modification has altered any of these requirements in a way that would change the response to question <1>. Ideally, this determination should be based on empirical measurements of reproductive processes in the GEO in carefully controlled or contained environments. Scientific knowledge about interactions between the reproductive system and other aspects of the parental organism's physiology may also be helpful.

IV. B. <2>

See explanation of phenotypic changes listed in Appendix A in text for Flowchart IV.A.

IV. B. [a]

See supporting text for IV. A. [a] for a discussion of estimation of reproductive potential of GEOs**.**

IV. B. [b]

See discussion in text for IV. A. <2> for the rationale for estimating the fitness of descendants of escaped GEOs. For this question, however, fitness must be estimated for all descendants of the self-reproducing GEO population rather than for introgressed progeny produced by matings between engineered and unmodified adults.

Flowchart V. Effects on Ecosystem Structure and Processes

This and subsequent flowcharts require substantial information about complex and variable features of the ecosystem. User must have or gain sufficient knowledge of and experience with the accessible ecosystems to ensure that the assessments guided by these flowcharts are scientifically reliable and defensible. Users should be familiar with the following information about each accessible ecosystem: (1) structure (e.g., biological interactions among species as manifested by interdependence or by segregation in use of resources or space), (2) processes (i.e., patterns of nutrient and energy flow, such as is manifested by food webs), and (3) persistence (i.e., ability of an observed structure or species composition to persist within known limits through time). Detailed understanding of these attributes will allow development of a simulation model of the accessible ecosystem that can prove useful for informing assessments requested in this flowchart. Data on phenotypic changes exhibited by the GEO derived from laboratory or mesocosm experiments could be incorporated into the simulation model to assist with these assessments. See related discussion of experiments in the discussion of familiarity with overall performance of the GEO, located in text for IV.A. <1>.

If familiarity about ecosystem structure is lacking, users should conclude that assessment of the type and magnitude of species interactions is not possible and should proceed to Flowchart VI.B. for appropriate guidance on risk management. Assessment of the potential for adverse alteration of ecosystem structure or processes requires overall familiarity with structure, process, and persistence. Lack of sufficient familiarity in these areas prevents performance of a scientifically justifiable assessment and, thus, requires proceeding to Flowchart VI.B. for appropriate guidance on risk management.

V. <2>

If the GEO in question is designed to be a human or animal vaccine, this flowchart assumes that it will be regulated by an appropriate government agency. Whether or not this is really the case, such a GEO is beyond the scope of this assessment framework and so users are directed to go to the Exit Routine in Flowchart VII.

V. <3>

This question focuses on direct ecological interactions, such as parasitism, predation and competition, between escaped GEOs and populations of special concern. Previous questions in other flowcharts addressed only the potential for interbreeding between GEOs and populations of special concern.

Threatened, endangered, and declining populations are especially vulnerable to risk of extinction and therefore should be protected from novel interactions with GEOs. This protection is justifiable in light of the dramatic declines in biodiversity worldwide. Extinction can damage ecosystem structure or processes and indirectly threaten sustainability of other species in the ecosystem. To determine if populations of special concern occur in the ecosystem(s) accessible to the GEO, users should consult appropriate agencies. If the thorough inventories necessary to answer this question have not been performed, it should be assumed that such populations are present.

V. <4> to <7>

This flowchart distinguishes different categories of intended purposes of introducing the GEO. A particular GEO may sometimes to fall into more than one category; in this case, the user should focus on the primary intent of each gene modification, and consult all relevant flowcharts. Usually, a single pathway is dominant. For example, Bt corn is itself a harvestable resource; however, the purpose of the GEO, which now expresses insect pest resistance, is to kill herbivores that exploit the crop. Therefore, it is considered in the category of GEOs that reduces population density of other organisms.

In the exceptional case that the purpose of the GEO is unknown, the user will lack sufficient information to assess its biosafety effects, and should consider disallowing release. If, however, the purpose of the GEO is known, but not included among the options provided, the user may proceed to the generalized considerations of direct and indirect effects on ecosystem function in Flowchart V.E.

V. <4>

Examples of GEOs that may be introduced with the intention of reducing the density of conspecifics are the ice-minus strains of the bacterium *Pseudomonas syringae*. These non-virulent GEOs are released in large numbers to displace the wild type bacteria that act as nuclei for ice formation and initiate the freezing of crop plant tissue. Other examples would include sterilized male GEOs, used in an effort to reduce pest population size.

V. <5>

These GEOs are intended to provide a harvestable resource (e.g., food for humans or other animals, fiber, biochemicals, lubricants), and would include such GEOs as nitrogen-fixing cotton, herbicide-resistant crops, faster growing transgenic fish, or krill with enhanced lipid content.

V. <6>

GEOs that are introduced specifically to reduce populations of other species include pesticide-resistant predatory mites, incapacitated insect vectors of animal pathogens, pest-resistant plants, organisms that suppress plant pathogens, and other biological control agents.

V. <7>

GEOs used in bioremediation or processing of agricultural or industrial wastes include, for example, plants that can accumulate and process selenium from contaminated soils or microorganisms that mineralize spilled petroleum. The primary concerns about these GEOs involve their competitive abilities, and whether any toxic elements, compounds, or waste products could move into human or animal food webs (Roughgarden 1998). Secondary concerns involve indirect effects of these GEOs in the environment.

Flowchart V. A. Effects on Ecosystem Structure and Processes (Displacement)

This flowchart assesses GEOs designed to reduce the density of a population of conspecifics. Typically, this is accomplished when GEOs displace unmodified individuals in a pre-existing population. Note that GEOs designed to reduce the density of a different species are assessed in Flowchart V.C.

V. A. <1>

This question addresses *unintended* increases or decreases in the density of populations other than conspecifics. This might occur through predation or one of the many forms of competition. Answering "yes" directs the user to Flowchart V.E. to assess whether or not such changes in density pose adverse effects on ecosystem structure or processes.

V. A. <2>

To ensure that the GEO successfully displaces the unmodified conspecifics, some additional strategy or drive mechanism may be necessary (Ribeiro and Kidwell 1994) Examples of drive mechanisms include transposable elements and cytoplasmic incompatibility factors due to a microbe, such as *Wolbachia pipientis* . Note: the term "drive mechanism" is used in this context to denote an organism that effectively moves (disseminates) the GEO through the target population. This usage is somewhat different than that used in describing GEOs used to reduce disease.

V. A. <3>

A drive mechanism may constitute an exceptional asset that can only be used once and therefore should not be expended prematurely. Once released into the environment through one variety of the GEO, a drive mechanism may not be effective in any future release of other varieties of the GEO. The user has an ethical burden to consider whether *at this time* it is appropriate to expend such a unique asset.

V. A. <4>

For an explanation of the meaning and determination of "permanently sterile", see the discussion for II.A.1.<4>. A non-injurious GEO is one that does not cause severe annoyance, morbidity, or mortality to non-target species including humans. To answer "yes" to this question, users must have empirical data from experiments specifically designed to test for non-injurious status. Unexpected changes in this trait could occur through pleiotropic effects of the genes inserted or modified by the genetic engineering.

To assess whether a GEO is non-injurious, it should be tested in a laboratory environment that mimics as closely as possible the conditions existing in the ecosystem of proposed release, and its effects on non-target species examined. Transport mechanisms which might allow the GEO to access other ecosystems must also be considered (see potential dispersal mechanisms in Table 1). Each ecosystem the GEO is able to access should be examined for injurious effects on non-target

organisms.

V. A. <5> and <6>

To answer these questions, users need relevant empirical data for both the GEO and the target populations.

Flowchart V. B. Effects on Ecosystem Structure and Processes (Harvestable Product)

V. B. <1>

Transfer of introduced traits to wild relatives can occur through hybridization in plants and animals, or through conjugation in bacteria.

V. B. <3>

There are several methods of estimating changes in species abundance (see discussion of demographic variables for question III. <4>). Direct measures include counts of numbers per unit area or measurement of biomass per unit area. For some taxa, indirect methods include quantification of a product produced by the population, e.g. fecal matter, CO_2 , etc. The abundance of bacteria and fungi is especially difficult to estimate. For these and some other organisms, and wherever reliable measures are doubtful, the most likely answer is "no".

V. B. <4>

Substantial long-term population decline can be difficult to predict. In the absence of information or predictive capacity, users are directed to Flowchart VI. B. Risk Management - Insufficient Information.

V. B. <5>

This question requires substantial knowledge of the ecological success of the wild relative once the novel trait has been conferred in the wild species. Lack of

sufficient information leads the user to Flowchart VI. B. Risk Management - Insufficient Information.

Flowchart V. C. Effects on Ecosystem Structure and Processes (Biocontrol)

This flowchart guides assessment of GEOs designed to reduce the populations of organisms other than conspecifics; this constitutes a form of biocontrol. In answering questions in this flowchart, keep in mind that, due to pleiotropy, the GEO might exhibit traits that are unexpectedly different from those of the unmodified organism.

V. C. <1>

Respond "yes" if the GEO itself causes disease. Common examples of living agents of disease are viruses, bacteria, and protozoa.

V. C. <2>

This question refers to GEOs that carry a disease-causing microorganism.

V. C. <3>

It is important to assess if the GEO can cause disease in hosts other than the species targeted for reduction. Keep in mind that the genetic engineering itself might expand the host range compared to the host range of an unmodified parental organism (see Appendix A).

V. C. <4>

If the population targeted for reduction is resistant to the disease caused by the GEO, then releases of this GEO will be ineffective for the intended purpose. Thus, there may be no benefit to allowing a release.

V. C. <5>

See text for V. <3> for explanation of susceptible hosts.

V. C. <6>

Such change in disease-related traits may be an intentional result of the genetic engineering or an unintentional result due to pleiotropy. Thus, a negative response to this question should be supported by empirical data from experiments designed to test for *both intended and unintended* changes.

V. C. <7>

This question prompts the user to assess if the genetically engineered vector can transmit the disease it carries to hosts other than those affected by the unmodified organism. A negative responses must be supported by empirical data from experiments designed to test for expanded host range. Keep in mind that expansion of host range might be the intentional result of the genetic engineering or an unintentional result due to pleiotropy.

V. C. <8>

See relevant discussions in text for V. C. <3> and V. C. <7>.

V. C. <9>

Information from an unmodified parental organism is helpful for addressing this question, but pleiotropic effects might alter the potential of the GEO to carry other diseases compared to the parental organism.

V. C. <10>

In this context, the word "nuisance" applies solely to humans. A nuisance is "significant" if it results in the indigenous population rejecting use or presence of the GEO, as in the case of a community unwilling to suffer more abundant numbers of (biting) mosquitoes following the introduction of genetically engineered mosquitoes. (See discussion of genetically engineered malaria vectors in text for II. E.

1. <8> and <9>.)

The nuisance potential of the GEO may be assessed by interviewing residents of the affected area to determine whether or not they will tolerate the presence of the GEO.

V. C. <12> and <13>

It is important to note that many organisms switch their prey choice if their usual prey items decline in number. If a GEO has altered preferences or requirements for prey, it could produce significant changes in energy flow and nutrient cycling in the ecosystem (e.g., Connell 1975). To test for this effect, the GEO would have to be tested in a laboratory setting that mimics all the ecosystems it is likely to access. Test conditions would have to include all potential prey items the GEO might consume. If consumption patterns are found to be significantly different from those of the unmodified organism, the environmental (and economic) impacts of those changes would have to be considered.

It is essential to consider *all possibly accessible and suitable* ecosystems. Guidance on how to identify suitable and accessible ecosystems is provided above in text for II. A. <4> and <5>.

V. C. <14>

If the GEO (in comparison to the unmodified organism from which it was derived) has gained or lost characteristics that alter its ability to compete for food, space, or nutrients or that alter the metabolites it produces such that its ability to resist or inhibit other organisms in its environment is altered, the impact of those changes should be assessed. If its ability to inhibit or compete with other organisms is affected, the change may also affect related diseases in both plants and animals, as well as in human beings. To test for these kinds of consequences, the GEO should be tested in laboratory settings that mimic the environments into which the GEO is likely to be released and to which it will have access. All organisms that might be

affected by competition with the GEO, through all possible mechanisms of competition, should be assessed. (See any ecology textbook, such as Bush 1997, for a list of the mechanisms to consider.)

V. C. <15>

Biocontrol agents may exert their effect by producing a product toxic to the target organism; this product can take the form of a protein toxin, such as the insecticidal toxin *Bacillus thuringiensis*, or a metabolic waste product. Most of the earlier questions ruled out reasons other than toxicity for the GEOs intended effect on the target organism. Users who reach this point in the flowchart and also respond "no" to this question have failed to find evidence that the GEO could reduce the target population. Users therefore should consider disallowing release of the GEO and reconsider the effectiveness of this GEO for biocontrol.

If the answer to <15> is "unknown", it is likely that the GEO would not work for the purpose intended (since all other plausible mechanisms have been ruled out to reach this point).

V. C. <16>

If the GEO produces the effect for which it was designed, it may do because it contains a novel toxin (compared to the unmodified organism from which it was derived) or because it contains an altered level of a toxin already present in the unmodified organism. In either case, the toxin may have unexpected adverse impacts on other organisms. For example, when the beneficial insect *Chrysoperla carnea*, a natural predator of the European corn borer and other crop pests, was fed corn borers (*Ostrinia nubilalis*) reared on transgenic Bt corn, the beneficial pest predators exhibited reproductive problems and reduced longevity (Hilbeck et al. 1998). To identify unexpected adverse impacts on other organisms, it may be necessary to examine the effect of the GEO on other organisms in a laboratory setting which mimics the environment into which the GEO is likely to be released or to which it may have access. It is essential to consider *all possibly accessible and suitable* ecosystems. Guidance on how to identify suitable and accessible ecosystems is provided above in text for II. A. <4> and <5>.

To test their response, potentially sensitive organisms may be exposed to doses of the toxin present in the GEO in excess of those expected to be encountered after release. Note that unless the mechanism of action is well understood, a range of effects in addition to direct toxicity must be tested, including, for example, increases in mutation rate, changes in behavior, changes in carcinogenesis, changes in chromosomal abnormalities, etc.

V. D. Effects on Ecosystem Structure and Processes (Bioremediation)

V. D. <2>

Communities function through complex interactions along pathways connecting organisms with abiotic resources through transfers of energy, nutrients, information, or whole organisms. In most instances, changes in community structure (e.g., changes in relative abundance of species) do not trigger large or profound changes in major ecosystem processes (e.g., primary production) because compensatory dynamics of functionally similar species act to "buffer" the effects of a single species. However, certain changes can lead to substantial changes in important ecosystem processes (Connell 1975, Carpenter and Kitchell 1988, Wahl et al. 1995). Therefore, it is important to assess whether or not species interactions involving escaped GEOs could adversely affect ecosystem processes. For example, increased mouth gape (mouth size) due to increased body size of a GEO might enable the organism to prey on organisms not previously subject to predation. Such novel broadening of prey items could perturb the food web in ways that are difficult to predict.

Adverse effect on ecosystem processes can be illustrated by known examples from species introductions. Examples include: (a) common carp muddying clear lakes through their feeding activities; by increasing turbidity and affecting the balance between photosynthesis by phytoplankton and rooted macrophytes, carp affect habitat availability and food resources for a range of aquatic organisms Courtenay and Williams 1992); (b) predation by fish-eating fishes on planktivorous

fishes; by reducing predation upon large zooplankton, a decrease in planktivorous fish may increase grazing pressure upon phytoplankton, affecting the balance of photosynthesis by planktonic algae and rooted macrophytes (Carpenter at al. 1985, Kerfoot and Sih 1987); (c) an introduced clam in the San Francisco Bay estuary has, through its filtering action, caused the brackish parts of the system to switch from being dominated by planktonic organisms to being dominated by benthic organisms; (d) as fungal biomass is lost from the soil, trees in forest systems are lost (Ingham and Thies, 1996); and (e) as clerid beetles are introduced as fourth trophic level predators, ant prey are reduced, herbivory is increased, and plant species diversity in tropical forest understory is changed locally (Dyer and Letourneau 1998).

V. D. <3>

An example of detrimental waste products produced by GEOs is ethanol produced by *Klebsiella planticola* in concentrations large enough to kill plant roots (Holmes et al 1998).

Flowchart V. E. Effects on Ecosystem Structure and Processes - Other Biotic Interactions

V. E. [a]

When conducting this assessment, users need to consider how interactions will vary because the GEO and other organisms may perform different functional roles throughout their life cycles. Such progression is common among organisms that have several distinct stages in their life cycles such as nematodes, fish and insects (e.g., Stein et al. 1988). This assessment should also integrate information about the parental organism with an assessment of whether or not phenotypic changes expressed by the GEO (for examples, see Appendix A) may alter interactions between the GEO and other species in the ecosystem, including predation, parasitism, competition, mutualism, disease, and multitrophic level effects. In a review of ecological principles and ecological effects of intentionally stocked fishes, for example, Wahl et al. (1995) presented background information that is also

relevant for assessing ecological effects of accidentally escaped GEOs. In particular, they recommended that assessment be based on an ecological, community-based framework that integrates the relative importance of predation, competition, abiotic factors, and interactions among these factors across all life stages (see Figure 6 in Wahl et al. 1995).

Clearly, these considerations do not have well-defined standards by which to judge adverse alterations to the ecosystem(s) accessed. The reader is called upon to make a subjective judgement about whether assessment is possible and sufficient. *When in doubt, the precautionary principle should guide the reader to the conclusion that assessment is not possible.*

A number of species interactions are important to assess (Tiedje et al. 1989, Kapuscinski and Hallerman 1990, 1991). Assessment should focus on the following interactions:

(a) predator-prey interactions, particularly if the modified organism is a top-level predator, such as a fish-eating fish (Carpenter and Kitchell 1988, Mills and Forney 1988, reviewed in Kapuscinski and Hallerman 1990, p. 6-7), a predatory nematode (Ingham et al. 1985), insect (Letourneau and Dyer 1998), or a high-level predator such as *Pisaster* which, when removed, alters the structure of the community completely (Paine, 1980);

(b) competitive interactions that, for example, regulate densities of understory trees;

(c) symbiotic interactions, such as those that occur between mycorrhizal fungi and plants, nitrogen-fixing bacteria and legumes, or pollinators and plants, that are critically important for production in many crops (e.g., Ingham and Molina, 1991; Buchmann and Nabhan, 1996);

(d) parasitic interactions including wasps that regulate crop pests; and

(e) indirect interactions, in which the activities of the modified organism alter the density of another species or cause a change in abiotic factors, which in turn causes the density of other species to be changed.

Many important nutrient cycling processes are controlled by organisms

interacting with each other within the foodweb. Indeed, direct effects of GEOs on other organisms, as well as indirect effects that occur in trophic cascades can cause a reduction or increase in numbers of any of the species in the foodweb, alter the type of nitrogen present (Ingham et al 1989), and influence the plants that can survive and grow. Carpenter and Kitchell (1988) showed that toxic blooms of algae in Lake Mendota could be controlled by changing the top-level predator fish species in the lake in the following way: Sport fish had replaced native fish in the lake. The sport fish had eaten the native fish, which eat tiny crustaceans that compete with other organisms in water (zooplankton) that eat algae. Thus, lack of native fish allowed the competitors of zooplankton to prevail, so the zooplankton that eat algae were lost, and the algae bloomed. As stocking with sport fish was stopped, the native fish returned, and noxious algal blooms on Lake Mendota decreased significantly as a result.

Similar cascading trophic interactions have been described in soil (Ingham et al. 1985), freshwater and marine systems (Wooton and Power 1994, Steinberg et al. 1995), and forests (Marquis and Whelan 1994, Letourneau and Dyer 1998).

In some cases, GEOs may become pests to humans or to other species, either because the parental organism is a pest or because the phenotypic changes exhibited by the GEO are great enough to result in pestlike effects. For example, if the host range of an arthropod that acts as a vector for plant pathogens were to be expanded, the arthropod might produce a serious decline in the yield or the quality of economically important plants. (Note: Those plants currently might be food for a variety of animals, including but not limited to humans.) In another example, *Klebsiella planticola* engineered to produce ethanol, exhibited detrimental effects by causing the death of plants in certain soils where it was present. Such effects were never exhibited by its parent organism (Holmes et al. 1998). As a third example, the feeding activities of common carp greatly increase the turbidity of warm, shallow lakes, thereby eliminating freshwater plant beds and reducing populations of visually feeding predators (such as northern pike) and of waterfowl which depend on the aquatic plants (Courtenay and Williams 1992). Genetic modification that might increase the ability of carp to alter their environment (e.g., by inducing more

rapid growth or encouraging disease vectors that alter the range) therefore also have the potential to increase carp potential as a pest.

It is important to determine whether phenotypic changes exhibited by a GEO can increase its ability to adversely affect other organisms in the accessible ecosystem. For instance, if increased growth leads to larger size-at-age or ultimate size, the modified organism could have an advantage in competition for food, habitat resources, spawning sites, or mates. In short, an interaction is of concern if the activities of the GEO can affect the distribution or demography of another species. For example, albeit not an example of a GEO, the altered phenotype of honeybees as the suite of "Africanized" alleles spread through Latin America resulted in enhanced performance for this species. This in turn led to increased encounters of honeybees with livestock. Further, because of the aggressiveness associated with those alleles, the altered phenotype resulted in an increased number of livestock and human deaths from bee stings (Camazine and Morse 1988). (We emphasize that "Africanized" honeybees are *not* GEOs; they do, however, present an excellent example of a strongly negative effect stemming from an altered phenotype.)

Assessment of species interactions involving the GEO should specifically address displacement of populations of conspecifics or other species caused by alterations to the phenotype. There is growing evidence that oversized, hatchery-reared salmonids can socially dominate and sometimes displace smaller, wild conspecifics or closely related species through increased aggressive behavior or increased competition for food and space (e.g., Bachman 1984, Nickelson et al. 1986, Vincent 1987). This raises the concern that such displacement might be a more general phenomenon with GEOs exhibiting certain phenotypic changes that adversely influence their interaction with other organisms. Potential displacement of natural populations is a concern even if the GEO cannot interbreed with them because such displacement is the first step towards decline and extirpation of natural populations. Possible adverse ecological consequences include declines in genetic and species diversity, disruption of the ecosystem, and decreased sustainability of fisheries resources important to humans. This latter point is also relevant for the

discussion below on adverse ecosystem alterations.

Populations of exploited organisms are often both economically important to humans and ecologically important to long-term health and sustainability of freshwater and marine ecosystems (e.g., Christie et al. 1987). It is, therefore, important to assess whether or not interactions between escaped GEOs and populations of exploited species might adversely affect these populations, for instance through increased population fluctuations, through displacement due to increased competition or behavioral interactions, or through declines in abundance and genetic diversity (Wahl et al. 1995). This latter point is also relevant for the discussion (below) on adverse ecosystem alterations.

V. E. [b]

This step in ecosystem effects assessment (Flowchart V. E.) ultimately leads the user either to the Exit Routine or to Risk Management. Completing the assessment requires that the user have clear scientific evidence to support the conclusion that adverse ecosystem alterations are improbable or negligible.

Current understanding in ecology is that all ecosystems are in flux (Pickett et al. 1992). At best, systems have multiple, alternating "steady" states, with "steady" defined in relatively short time scales, no more than a few decades. However, as our ecological knowledge increases, the alternating states become more predictable, as does the direction of ecosystem change in response to regional or global factors. Addition of any new organism into a system, including GEOs exhibiting altered phenotypes, can change operation of the system and thereby can decrease the system's predictability to humans. At this point in the flowchart, therefore, users should assess whether or not the modified organism might have effects on the accessible ecosystem that could cause a shift to a state from which the ecosystem may not be able to return to a previous and more desirable state.

There is a growing literature on the concepts of ecosystem degradation and integrity. Ecosystem integrity is influenced by the diversity of ecosystem structure and processes, including some redundancy (Christie et al. 1987, Karr 1991).

Assessment of the potential to alter an ecosystem to a degraded state should address both environmental sustainability and human utilization (e.g., reduced water quality). Accessible ecosystems which have already been greatly perturbed from "healthy" states are particularly vulnerable to further degradation, and thus are more susceptible to adverse effects due to species interactions of escaped GEOs. Further, subtle changes in habitat quality can result in long-term, adverse effects on populations of non-target organisms that take years to detect (Doak 1995). A degraded natural ecosystem should not be treated as if it were an artificial system undeserving of protection of its natural structures and processes.

If the assessment concludes that adverse ecosystem alterations are improbable or negligible, Flowchart V. E. sends the user to the Flowchart VII. Exit Routine, whereupon if no food safety issues are presented by the GEO, no special confinement measures are advised and the assessment is considered complete. Before reaching this point, however, it is important to assess whether or not, through one or more of the assessed interactions, large-scale introductions of modified organisms could act as agents of natural selection on other organisms in the community, and if so, what might be the ecological consequences.

Flowchart VI. A., VI. B., VI. C.: Risk Management

Introduction

This section does NOT apply to microorganisms, because it is impossible to design barriers that can reliably confine microorganisms in an outdoor setting (and even in indoor settings with substantial direct or indirect connections to the outdoors).

This section applies only to introductions of GEOs determined to need risk management. It presents recommendations for the design and operations of a research project, field test, or large-scale field trial involving a genetically engineered

organism in order to minimize the risk of specific hazards identified through use of the appropriate assessment flowcharts. Planning and implementation of management measures should address all the factors discussed in this section, including project siting, determination of scale of the introduction, design and assessment of feasibility of barriers, and security. Operational requirements include a written operational plan, emergency response plan, training, and traffic control and monitoring , as well as a written review after the project is completed.

This section also does not address commercial-scale releases. It is expected that by the time commercial release is contemplated, the full range of laboratory, small field trials, and large field trials will have been completed, providing a reasonably comprehensive understanding of potential environmental or human health consequences. It is probable that in many cases significant hazards will be anticipated that cannot reasonably be eliminated by containment or other strategies. Nevertheless, political and economic factors may make release attractive despite those hazards. In that case, in contrast to small-scale field tests for which decision-making for release relies heavily on the feasibility of containment techniques, the ultimate decision to conduct large-scale commercial introductions of a GEO may focus on the feasibility of effective mitigation schemes for predicted environmental impacts.

Mitigation and Risk Management

Unfortunately, the issue of risk management and mitigation for large-scale releases is only in its infancy at this time. Most of the progress in this area has been in the area of transgenic crops, where gene introgression into wild populations is only a matter of time, and impacts on other organisms have already been demonstrated in small-scale tests. For example, despite recommendations that transgenic crops expressing the Bt endotoxins not be deployed on a large scale in agriculture due to evidence that the strong selection pressure of these crops would lead to pest resistance (e.g., Krimsky and Wrubel 1996), tens of millions of acres have been sown to Bt crops in the last several years. Serious efforts to mitigate the

introduction of millions of individuals expressing high levels of toxin against specific pest species are scientifically based resistance management schemes recommended in Mellon and Rissler (1998) and introduced by Alstad and Andow (1995). These schemes require that a certain percentage (approximately 15%-40%) of the crop be susceptible to the pest species controlled by the Bt endotoxin in the main planting, and that it be within a specified distance of the main crop so that it can act as an effective refuge for populations of particular pests. In addition, the "refuge/high dose" plans call for sufficiently high levels of endotoxin in the plants to kill the pests and minimize sublethal effects. They also include monitoring plans for regional pest levels (Bt crops should not be employed where pest pressure is low.) and for the level of resistance alleles in the population. The plans are based on simulation models with input from small-scale and greenhouse studies; no field data are available.

Although the detailed mitigation schemes designed to delay resistance to the Bt endotoxin expressed in transgenic crops are among the best examples of interventions that increase the safety of large-scale releases of GEOs with likely environmental impacts, they are still fraught with design uncertainties, biological knowledge gaps, and socio-economic feasibility problems for the short-term. Indeed, just to list some examples, commercial Bt cotton seemed to express low levels of Bt endotoxin under certain field conditions in Australia (Forrester and Pyke 1997), specific design effectiveness is limited by lack of knowledge about movement and mating behaviors in most pest lepidopterans, and monitoring growers for compliance is costly.

Several strategies of biological containment of novel traits expressed by GEOs have been proposed for commercial operations, but remain extremely controversial. For example, market pressures may work against the restriction of Bt crops to regions where no wild relatives occur. Given the increase in exposure in large-scale plantings, introgression of the Bt endotoxin gene into populations of wild relatives of Bt crops is likely over time. A recent suggestion for biological containment in plants proposed incorporating novel genetic material *only* into plant chloroplasts (Daniel et al. 1998). Unfortunately, this suggestion is flawed because it was based on

the assumption that there is no chloroplast gene flow through pollen to higher plants, an assumption that turns out to be incorrect (Stewart, Jr. and Prakash 1998, Cummins 1998).

In aquaculture, initial hopes of creating infertile triploid oysters for eventual large-scale dispersal of a non-native species were called into question as the oysters reverted naturally over time to diploidy (Blankenship 1994). Some aquaculturists are also proposing biological containment of transgenic fish by making them infertile through induction of triploidy. Across the small number of species where this issue has received attention, there is great variation in the effectiveness of triploidy induction, reproductive behaviors, and presence or absence of viable gametes (Hallerman and Kapuscinski 1993). The degree of sterility appears to be more complete in triploid female fish and shellfish than in triploid males (Thorgaard and Allen 1992). It appears that some adult triploid males still produce enough reproductive hormone in their bodies to allow them to enter into mating behaviors with fertile females. This means that male fish escaping from a fish farm still might reach sexual maturity in the wild and enter into mating behavior with females of the same or closely related specie, leading to infertile broods. This could reduce the abundance of the wild population, which would be especially serious for endangered species and other wild populations of special concern Further, triploidy will not lead to sterility in algae and in many fish and shellfish species (see Appendix C).

Finally, we must not forget that fertile GEOs or naturally occurring organisms that receive traits from GEOs through hybridization will undergo evolutionary adaptation in local environments. This will make it extremely difficult, if not impossible, to predict possible biosafety consequences over the course of many generations.

It is therefore our contention that large-scale deployment of GEOs should occur only under strong scrutiny and adequate, ongoing monitoring for possible, unanticipated adverse consequences. If adverse environmental effects have been identified for a particular type of GEO, then each proposed large-scale deployment

should involve careful case-by-case analysis.

Thus large-scale commercial deployment of GEOs should be subject to intense scrutiny and careful case-by-case analyses if environmental impacts have been identified. Mitigation plans should be thorough and explicit, and should specify alternative strategies in case the primary mitigation measures are less effective than anticipated. Experts familiar with the biology of the organism, as well as experts familiar with the ecology of the accessible ecosystems, should be among those consulted about mitigation plans. Consideration of mitigation plans, however, is beyond the scope of this manual, and will not be discussed further.

General comments on containment measures

Appropriate and effective containment should be expected or required of all GEOs determined to need risk management. The means of containment and its efficacy will vary with the GEO under consideration, the specific characteristics of the accessible environment, and the scale of introduction.

Containment generally increases in difficulty and decreases in efficacy with 1) decreasing physical size of the GEO or of it propagules; 2) increasing fitness or physical tolerance of the GEO; 3) the capacity of the GEO or its reproductive propagules to move about and disperse in accessible environments (vagility); 4) increasing duration, spatial scale, or absolute size of the project or introduction; and 5) proximity to environments that greatly facilitate dispersal (e.g., areas of high wind, rivers, oceans). These factors are briefly discussed below; we encourage users to minimize risk to the greatest extent possible by carefully designing and maintaining appropriate containment methods or devices. A list of some possible routes of dispersal of GEOs is given in Table 1. A more detailed discussion of containment methods for the specific cases of field tests of fish and shellfish is presented in the *Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish* (Agricultural Biotechnology Research Advisory Committee 1995); these methods may serve as useful examples for some

applications.

a. Effects of organism size

Small organisms, and especially microbes, are difficult to contain because they are: less easily seen or otherwise detected; typically grown or cultured at very high densities; and easily dispersed by wind, water, or other forces. Additional characteristics of small organisms and microbes can enhance their potential for spread beyond containment. Enhanced spreading due to effects of fitness, physical tolerance, or vagility (dispersal ability) is addressed in (b) below. For organisms that have alternative methods of reproduction beyond strictly dioecious reproduction, a viable population could be established in accessible environments by the escape of a **single** individual, seed, cyst, spore, or other propagule (as discussed in Appendix C, Section II).

b. Effects of fitness, physical tolerance, and dispersal ability

GEOs exhibiting high levels of fitness (that is, high potential for survival and reproduction), tolerance of adverse or severe physical factors, and/or high levels of dispersal ability will be difficult to contain. High levels of fitness will enhance the likelihood that larger numbers of individuals, gametes, or other reproductive propagules will be introduced into the accessible environment, and will further predispose the GEOs or their propagules to persist and spread once introduced or escaped.

Tolerance of adverse or severe physical factors increases the range of accessible environments in which a GEO could survive, and will make certain physical and chemical methods of containment ineffective. For example, a GEO able to tolerate a broad range of temperatures can thrive in a larger number of environments than a comparable GEO able to withstand only a narrow range of temperatures, and is more difficult to contain by physical barriers that rely on temperature treatment. Physical factors that could be important in this context include (but are not limited to) those listed in Appendix A under 'Tolerance of

Physical Factors'.

High dispersal ability will increase the likelihood that one or more individuals, reproductive bodies (e.g., sperm, small eggs, spores, cysts), or propagules will escape containment, and could significantly increase the effective distance over which the GEO could escape. For example, the pollen of wind-pollinated crops may be spread over a large area and the pollen of some insect-pollinated crops may also be widely dispersed; similarly, the seeds of wind-dispersed crops may be spread quite widely. Likewise, the gametes of fish and shellfish species that broadcast their eggs and sperm in the water column are spread over large areas of a marine or freshwater ecosystem. Dispersal ability generally increases with decreasing physical size of the GEO, as in (a) above.

c. Effects of the project's duration and spatial scale and number of GEOs

The likelihood of escape from containment increases in proportion to the duration and spatial scale of containment or release, and number of GEOs involved in the project. Increases in any of these factors increase the exposure of the GEO to the accessible environment. Firstly, the probability of escape is partly a function of time, so that the number of actual escapes is expected to increase as the duration of the project increases. Secondly, the probability of escape is partly a function of the size of the elements of the containment system that come into direct contact with the accessible ecosystem and/or dispersal routes to accessible ecosystems. Examples include the length of the perimeter of a confined crop field or the daily volume of effluent water from an aquaculture facility that discharges directly or indirectly into a natural waterbody. Therefore, the number of actual escapes is expected to increase as the spatial scale of the facility, containment site, or release site increases. Thirdly, the probability of escape is partly a function of number of organisms being contained; the actual number of escapes is expected to increase as the number of GEOs increases.

d. Proximity to environments that greatly facilitate dispersal

Certain environments exhibit physical properties that strongly tend to disperse escaped GEOs away from the containment site. Examples are environments characterized by high winds or by substantial flow of water (e.g., rivers, estuaries, marine environments). Marine and freshwater applications may prove especially difficult to isolate from accessible environments, and may require special consideration in the design of containment measures; detailed recommendations for such applications appear in the risk management section of the Performance *Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish* (Agricultural Biotechnology Research Advisory Committee, 1995). All containment methods or devices should be designed to withstand occasional events, such as storms, tornadoes, hurricanes, extreme tides, or vandalism, that will change the probability of dispersal away from the local area.

Case-specific approach complemented by review

Different research projects, field tests, and larger-scale introductions needing risk management will exhibit great variety in the biological features of the GEO, the specific risk(s) posed, and the features that affect the success of containment (e.g., scale, duration, project siting). This makes it *unfeasible for these guidelines to anticipate the best combination of containment measures for every possible case*. This section, therefore, presents general recommendations and relies on the user to develop the most appropriate combination of risk management measures that meet the objectives of either "containment indicated" or "no/negligible escapes", as specified in Flowcharts VI. A. or VI. B. Determination of what constitutes "containment" needs to be in reference to the specific hazard that has been identified for the proposed project, as reiterated on Flowchart VI. A. or VI. B.; the objective is to have negligible environmental consequences.

Users of this section should clearly recognize that the recommendations herein define the minimum requirements. Additional measures may be prudent in

certain cases. To assure that this case-specific approach results in adequate risk management, users of this section are expected to seek thorough review of their risk management measures, siting plans and scale of project prior to introducing the GEO into the field. It is essential that the reviewers include some experts familiar with the biology of the organism and some experts familiar with the ecology of the accessible ecosystems (see detailed discussion below in the subsections on peer reviews and site reviews).

Research projects versus commercial operations

It is likely that the range of containment options will be greater, and containment more feasible for GEO introductions in small-scale field tests than in large-scale commercial projects. Indeed, for some combinations of specific hazards, GEOs, and scales of desired release, no containment scheme will address all the issues posed by the identified hazards. However, in many cases, biological barriers or site selection can increase the feasibility of containment even for commercial-scale operations.

Project Siting

The ease or difficulty of managing a given project's specific hazards will depend to a great extent on the ecological attributes of the geographical location of the research project. For example, growing genetically engineered crop plants in regions that lack wild relatives for cross-pollination reduces the need to sterilize pollen or prevent the plants from flowering. It is especially important to assess if the physical and ecological attributes of the local site and the physical features of the project's facilities could still physically contain the GEOs during floods, storms, earthquakes, and other natural disasters known to occur periodically in the geographical area. For example, factors to consider for freshwater and marine GEOs are summarized in Table 2. Users need to develop a similar set of considerations for the GEO and geographical areas involved in their project. Physical barriers are

unlikely to contain mobile, small GEOs such as certain arthropods; careful design will be needed for physical containment of vertebrates, as well. Peer reviewers and site inspectors are expected to evaluate the adequacy of protection against accidental escape of GEOs under both common environmental conditions, as well as less frequent storms, and other natural disasters.

Some specific criteria for freshwater and marine sites

Although most freshwater research projects could meet the criterion of location above the 100 year flood level (designated in Table 2), many marine and estuarine research stations cannot meet this same criterion. Consequently, research projects sited at marine and estuarine locations should place greater emphasis on other management options. In many marine cases, the most feasible approach to protection against floods, hurricanes, or other natural disasters (e.g., wind and wave damage due to violent storms) is to keep the scale of the research project small enough so that all GEOs either can be moved safely to an alternative site or destroyed within a specified time. Movement or destruction should be completed between the time a disaster warning is received (for example, forecasts released by a local or national agency that issues weather reports) and before conditions become too dangerous to complete the action. The protocol for such emergency actions should be spelled out in the emergency response component of the project's written operational plan (see subsection below on operational plan).

Table 2. Minimum criteria for siting of a project when specific hazards of working with a freshwater or marine GEO have been identified.

Event	Freshwater	Marine
Flooding	Above the 100 year[3] level	Flood level and storm drain criteria not applicable - place greater emphasis on management of project's physical scale and other factors
	Storm drains designed for 100 year[3] rainfall event or storage provided	
	Surface runoff diverted around project site	Surface runoff diverted around project site
Wind Loading[1,2]	current requirements for laboratory facilities, where they exist	current requirements for laboratory facilities, where they exist
Snow Loadings[1,2]	current requirements for laboratory facilities, where they exist	current requirements for laboratory facilities, where they exist
Seismic Loadings[1,2]	current requirements for laboratory facilities, where they exist	current requirements for laboratory facilities, where they exist
Others[1,2]	current requirements for laboratory facilities, where they exist	current requirements for laboratory facilities, where they exist

1 These criteria apply to the project's rearing units and mechanical barriers located either indoors or outdoors. For indoor situations, the loading criteria will generally apply to buildings housing the rearing units and mechanical barriers. For outdoor situations, loading criteria will generally apply directly to the rearing units (e.g., fiberglass tanks and tank covers located outdoors) and mechanical barriers (e.g., structure of French drain, perimeter fencing located outdoors).

2 In cases where there are no current requirements for laboratory facilities, users should identify loading criteria that will prevent collapse of the physical facilities under both frequent events (e.g., the strongest seismic event expected to occur every 10 years) and exceptional events (e.g., the strongest seismic event expected to occur every 100 years). Users should seek the advice of structural engineers and other relevant experts to identify these criteria.

3. Refers to events expected (on the basis of historical records) to occur at mean intervals of 100 years.

Project siting to minimize certain risks

As summarized on Flowchart VI. A., the specific hazard posed by some projects involves adverse effects on populations of species that are threatened, endangered, or of special concern. Instead of implementing measures to minimize these risks, another option is to completely avoid this hazard by relocating the proposed project to a site where the accessible ecosystems do not contain such protected populations. If users are seriously considering such relocation, they should first utilize the assessment flowcharts (I. through V.) to evaluate the suitability of the relocation site. Specifically, it is important to determine whether or not the relocation site poses other specific adverse effects requiring management.

For example, for freshwater situations, siting of the research project in areas with interior drainage and no permanent waterbodies may be prudent until more experience with GEOs is available. In arid regions, there may be areas where all the runoff either percolates into the ground or evaporates. Any surface water bodies in these areas are temporary. In some cases, relocation of a project may reduce the numbers and types of barriers needed on the project site. The best reason for relocation is to allow effluents and drawdown water to be discharged to an environment known to be lethal to all life stages of the GEO. For instance, if it has been demonstrated that seawater is lethal for all life stages of a freshwater GEO, then projects involving such a GEO could be conducted at a site where it is feasible to discharge all effluents and drawdown water directly to the ocean (i.e., full strength seawater). In some cases, such a strategy might preclude the need for additional barriers in the project's effluent and drawdown water (see related discussions in subsections below).

Design of Barriers

This subsection discusses factors that should be considered in the design of different barriers used to confine GEOs within the project site. For each possible escape path in the system, the minimum expectation for each project requiring risk

management is to have sufficient numbers of barriers in series to achieve the risk management objective specified in Flowchart VI. A. or VI. B., for *all life stages* of the GEO occurring during the duration of the project. The risk management objective is either "no/negligible escapes", "release of the GEO is not indicated" (for microorganisms), or "containment indicated".

The entire set of barriers for the relevant system should *achieve the risk management objective for the hardest to contain life stage* encountered during the course of the project; usually this is the smallest life stage. Because no barrier type is 100% effective at all times, the overall reliability of confinement measures will depend heavily on the number of independent barriers present in series. Users are expected to determine the appropriate combination of types and total number of barriers needed to achieve the risk management objective. The number of independent barriers is site- and project-specific but a general starting point is to plan for three to five barriers. Where a constant feature of the surrounding accessible ecosystem has been demonstrated to be lethal to all life stages of the GEO (e.g. full strength seawater was shown to be lethal to all life stages of a freshwater GEO), additional barriers might not be necessary. Project reviewers and inspectors are expected to evaluate the adequacy of the chosen combination and total number of barriers.

At least four types of barriers to escape are available to the user:

Physical or chemical barriers

These are manipulations of physical or chemical factors to induce 100% mortality in one or more specified life stages of the GEO before such life stage(s) reach the accessible ecosystem(s). Physical and chemical barriers to terrestrial or airborne escape might include lethal temperatures or lethal doses of radiation, biocidal, or germicidal agents. However, some of these could cause grave harm to the wider environment and should only be used with extreme caution and in confined areas. For example, liquid effluents and air discharged from bioreactors containing microbial GEOs could be run through a chamber exposing them to lethal

levels of ultra-violet light, temperature, or a specific chemical. A broad literature exists on containment strategies for toxic industrial chemicals, infectious agents, and other hazardous materials, and the user is encouraged to consult this literature. (See, e.g., Buchholz 1998)

For freshwater or marine GEOs (e.g., fish, shellfish, algae, other aquatic plants), physical or chemical barriers might be feasible if the organisms are maintained in on-land rearing units. The water temperature, pH, or a chemical (e.g., chlorine, bromine, ozone) can be maintained at lethal levels in effluents from incubators or for the final effluent coming from all rearing units, followed by appropriate removal of the lethal factor prior to discharge of effluent water from the project site. Additional recommendations regarding freshwater and marine GEOs appear in the risk management section of the *Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish* (Agricultural Biotechnology Research Advisory Committee 1995).

Mechanical barriers

This category includes mechanical structures (either stationary or moving) that physically hold back one or more specific life stages of the GEO from escaping the project site. Mechanical barriers might be placed in series at critical locations in the facility or release site. Examples of mechanical barriers include enclosures (e.g., bioreactors, greenhouses or glasshouses, mesh cages, electric fences), particle filters, screens (e.g., floor drain screens, standpipe screens in aquaculture tanks), grinders with moving parts, and lids or covers for open tanks, raceways, etc. For examples of mechanical barriers used in aquaculture, see the illustrations reproduced in Appendix D (Agricultural Biotechnology Research Advisory Committee 1995).

Biological barriers

Biological features or alterations of the GEO can serve as barriers if they (1) prevent reproduction at the project site, thus avoiding risks of escape of small gametes, embryos, larval stages, spores, or cysts or (2) greatly reduce the possibility of reproduction or survival of the GEO in the accessible ecosystem. However, biological barriers that prevent reproduction may be extremely difficult or impossible to design for organisms that reproduce asexually (e.g., numerous species of plants and invertebrate animals exhibit budding in some or all generations of reproduction). As noted above at the beginning of the text for risk management, biological barriers are NOT feasible for microorganisms.

Biological barriers are usually not sufficient on their own, but they may be added to physical, chemical, and mechanical barriers to enhance the degree of containment. Examples of biological barriers include 1) lethal removal of GEOs before they reach a reproductive life stage; 2) use of GEOs of only one sex (applicable for strictly dioecious species ONLY); and 3) use of permanently sterile GEOs. For the specific case of commercial crop agriculture, barrier rows of alternate crops have sometimes been suggested as biological barriers. Although there is evidence that such barrier rows can reduce gene flow to outlying populations, they cannot completely prevent gene flow (reviewed in Klinger and Ellstrand 1998).

Scale of project as a barrier

This barrier is primarily applicable to research with GEOs, not to commercialization of GEOs. If the number of GEOs at the research stage can be kept so small that escape of all organisms would not impose the risk associated with the identified hazard, this barrier may be applicable. Nevertheless, it will be difficult to identify and justify a number that meets this criterion. If the GEO is a self-fertilizing hermaphrodite or a true parthenogen (see Appendix C), experimental scale cannot be used as a barrier because accidental escape of only one individual could establish an entire population of GEOs in accessible ecosystem. Although experiments with

such organisms should be kept as small as possible, multiple barriers of other types are required to achieve containment.

Example: barriers for escape paths of on-land aquaculture facilities

The following section applies to the special case of freshwater and marine organisms held in an on-land aquaculture facility, and is meant to provide a detailed example for extension to other systems.

Escape paths through the water-system

The accidental escape of GEOs from an on-land aquaculture facility might occur through any of the following components of the water system: influent water and makeup water (applicable in water reuse systems); effluent and drawdown water; waste slurries collected when filters are backwashed, screens scrubbed, or rearing units cleaned by siphoning; and aerosols from larval hatcheries of some shellfish. Therefore, each component of the water system should have a sufficient combination and number of mechanical or physical/chemical barriers to prevent escape.

Influent/makeup water. Surface waters require an appropriate set of barriers. Well water, other fully enclosed water sources, and municipal sources may not need barriers.

Effluent and drawdown water. All other factors being equal, the risk of accidental escape increases as the volume and frequency of water discharge increases. Static and closed water systems generally have no discharge except when draining the system. Water reuse systems and ponds may have a minor amount of discharge depending on operations and weather conditions. A flow-through system will have a continuous discharge. Although a sanitary sewer can serve as one barrier, discharge into sanitary sewers alone does not provide an adequate barrier to accidental escape in most cases because (1) many sewers bypass water to storm sewers

or surface waters during high-runoff events, or (2) some freshwater and marine animals can survive transit through the sewer and treatment plants. Prior to discharge to a sanitary sewer, effluent and drawdown water should pass through a sufficient set of barriers on the project site to minimize risk (that is, to achieve "no/negligible escapes" or "containment indicated", as specified in Flowchart VI. A. or VI. B.). For all types of water systems, the effluent drain capacity must be at least two times greater than the normal inflow capacity in order to handle simultaneous draining of a number of rearing units. Users are expected to identify the appropriate drain capacity and peer reviewers and site inspectors are expected to evaluate its adequacy.

For water systems that lack continuous flow-through, an alternative approach to preventing escapes via effluent and drawdown water is to locate the entire project in an indoor facility with no floor drains and the capacity to retain water from a specified number of experimental units. For instance, the facility could be designed to retain all the water if there was breakage of 5-20% of the experimental units. The user is expected to seek input from prospective peer reviewers and inspectors in order to select the appropriate water retention capacity. Additionally, any effluent from such an indoor facility should be treated as waste slurry (see below).

Waste slurries. These may hide small or dormant life stages of viable GEOs in the mixture of uneaten food, feces, shells from hatched eggs, and other particulate matter. Batch chemical or temperature treatment known to be lethal to smaller life stages of the GEO is recommended to kill any viable GEOs that might be present in waste slurries. For some species, on-site drying of waste slurries might be adequate. Final disposal of treated waste slurries should comply with all applicable environmental regulations; users are expected to obtain guidelines and regulations from their institution and, when applicable, from appropriate government units. In many countries, it is illegal to discharge such slurries into a freshwater or marine ecosystem. Examples of appropriate disposal of treated waste slurries might be: discharge to a sanitary sewer; discharge into a septic system, delivery to an institutional hazardous waste facility; or deposit in an approved land site.

Other escape paths.

The GEOs held in on-land aquaculture facilities might also escape through paths other than the water system. Users should determine if their project poses one or more of the escape paths described below and implement measures to protect against them.

Secure disposal of freshwater or marine animals or plants. Certain life stages of some species can survive long periods of time outside of water. For instance, adults of some bivalve molluscs (e.g.,clams, mussels, oysters) might survive three or more days outside of water as long as temperatures remain relatively cool and surroundings are slightly moist (e.g., a large number of adults packed closely together in a closed container). Therefore, users should anticipate and avoid situations in which animals might survive after disposal and be accidentally introduced into natural water bodies. The best way to avoid such problems is to initially place animals destined for disposal in secure, labeled disposal containers on-site and then deliver the containers to a designated, secure disposal facility, such as a hazardous waste facility or land disposal site.

Aerosols. Larvae of bivalve molluscs and of some crustaceans (e.g., shrimp, prawns, crabs) are much smaller than those of fish. Consequently, hatcheries for these organisms should be designed to prevent escape of larvae via aerosols into nearby freshwater or marine ecosystems. Hatchery exhaust fans, e.g., should be situated so that any aerosols that might be transported outdoors will not reach such ecosystems.

Equipment cleaning and storage. Certain life stages of some freshwater and marine GEOs could survive for a prolonged period after they are accidentally trapped in damp nets, small puddles in fish egg sorting machines, standing water in buckets, gloves or boots of workers attending to the GEOs, or other equipment. Therefore, all equipment that comes in contact with live GEOs should be properly cleaned and drained after each use. To ensure against accidental transport of live GEOs to another insecure site, such equipment either should be used and stored solely on the project site or should be disinfected using treatments lethal to all GEO life stages

and thoroughly drained prior to transport off-site. An inventory of project equipment is recommended.

Security

Users reaching this section have identified a specific adverse effect or hazard posed by the project's GEOs. Thus, security measures should be implemented both for research projects and commercial applications that need risk management. In the case of commercial applications, a number of socio-economic factors not addressed in this document may affect the user's decision regarding security measures. Pursuit of biosafety, however, ideally involves the implementation of security measures in commercial applications.

Security measures are needed to: (a) control normal movement of authorized personnel, (b) prevent unauthorized access to the site, and (c) for outdoor projects, eliminate access of predators that could potentially carry the GEOs off-site. Depending on the abundance and behavior of predator species present in the surrounding area, security measures might need to include electric fences, bird netting, and other exclusion measures. Users are expected to design an appropriate suite of security measures and peer reviewers and site inspectors are expected to evaluate their adequacy. Table 3 presents a suite of security measures.

Alarms

Installation of alarms at major escape routes from the project site should be carefully considered. Alarms provide valuable protection against not only possible failure of containment, but also guard against vandalism or terrorism that may lead to GEO release. Examples of alarms include: perimeter alarms on fences or greenhouses; intrusion alarms; and water level and flooding alarms if any life stage of the GEO can escape by water routes (e.g., freshwater or marine plants and animals, terrestrial plants that can survive water submersion, terrestrial animals that can

swim or float). Alarms need a battery or emergency power backup (Table 4). Alarms should alert designated personnel of breaches in physical, chemical, or mechanical barriers well before the breach can circumvent the project's entire suite of barriers. Adequacy of the alarm system should be justified in the worksheet and provided to peer reviewers and site inspectors. Ideally, all installed alarms should be connected to on-site visual or audible signals and a phone dialer that contacts personnel in a designated order. Automated alarm systems should not be the exclusive form of monitoring, but rather should provide a backup to human monitoring.

Stand-by Power

Stand-by power is needed to avoid possible failure of one or more of the project's barriers and to ensure functioning of alarms.

Operational Plan

All projects needing risk management should have an approved written operational plan. The plan should describe (a) how the project will be operated under normal conditions; (b) anticipated problems that may occur and how they will be addressed; and (c) an emergency response plan for disaster situations. The plan should address the major components of normal and emergency operations presented below. The entire written plan should undergo peer review prior to its implementation (see peer review and site inspection sections below)

Table 3. Measures for project security to minimize risk of identified hazards. Implementation of measures and choice of which to designate as "optional" or "essential" depends on features of the project and project siting.

Workers
background check[1]
access control
sign in and out
ID badge with photo
security training
Visitors
sign in and out
escorts required
Animals (outdoor facilities)
electric fences
bird netting
buried liners or suitable barriers against burrowing animals
Facility
Full perimeter control
Security alarms
Contracted responder or police response to security alarms
Signs and warnings
Written security plans
Peer and site review

1 Background checks should comply with relevant laws and institutional policies to protect rights of potential and hired employees.

Table 4. Types of alarms for project security in order to minimize risk of identified hazards.

Alarm Type	Recommendation	Comments
Water Level Alarms	required for GEOs that can escape by water routes	may require phone dialer, local signal, and power backup
Flooding Alarm	required if GEOs can escape by water routes and project site is below 100 year flood level; otherwise optional	effectiveness may depend on phone dialer, local signal, and power backup
Perimeter Alarm	required if GEOs are raised outdoors; otherwise optional	effectiveness may depend on phone dialer, local signal, and power backup
Intrusion Alarms	optional	effectiveness may depend on phone dialer, local signal, and power backup

Table 5. Training of project personnel to minimize risk of identified hazards.

Type	Recommended	Optional
Routine	Project Directors and other Project Staff	-
Emergency	All personnel designated to respond to emergencies	Refreshers plus drill for all personnel designated to respond to emergencies
Public	-	Recommended as an institutional responsibility

Training

Adequate training should be provided for all personnel who regularly work at the project site, including required reading of the operational plan. It is recommended that they sign a brief statement that they have read and understand how to implement the plan. Required and recommended types of training are presented in Table 5 above. Users are expected to design an appropriate training program; peer reviewers and site inspectors are expected to evaluate its adequacy for minimizing the risk of specific hazards identified for the project.

Traffic control

Control of traffic in and out of the confinement facility addresses intentional movement of personnel, equipment, wastes, and water and tissue samples. Such movements can facilitate escapes of GEOs. When drafting the traffic control portion of the operational plan, refer to the following previous sections for relevant recommendations: "Example: barriers for escape paths of on-land aquaculture facilities" (control of waste slurries, equipment and secure disposal of GEOs); and "Security" (control of personnel).

Record keeping

Adequate records should be kept to assess compliance with the operational plan (Table 6). This includes personnel and equipment logs as well as daily operations logs. Accounting for all genetically engineered individuals on the project site is an effective means of noting losses and discouraging theft. For groups of small organisms, numbers of individuals should be tracked on a frequent basis; one option is to estimate surviving animals based on daily counts of observed mortalities. Once organisms reach a larger size, exact counts of individuals should be maintained. Wherever feasible, individual tagging is strongly encouraged because it permits tracking of every modified individual.

Emergency response plan

Operations plans should include an emergency response plan that identifies the most common types of emergencies that a project could face and outlines what should be done to prevent dispersal of GEOs. The emergency response plan should address all types of natural disasters known to occur periodically at the project site (e.g., floods, hurricanes, typhoons, tornadoes, earthquakes). Ideally, projects situated in the path of frequently occurring natural disasters should be of a scale small enough to permit movement to a safe site or destruction of all GEOs before disaster conditions become too dangerous to complete the action.

Table 6. Recommendations for log books of projects needing to minimize risk of identified hazards.

Type of Log	Recommendation
Personnel logs (in and out of facility)	highly recommended
Equipment movement logs	highly recommended
Marking of individual animals	recommended when feasible
Operations logs	highly recommended
Animal inventory logs	larger animals - highly recommended
	smaller animals - recommended to the extent feasible

Responsible party. The project's on-site manager or a designated proxy should be available in person or by phone at all times to respond to emergency problems.

Notification of failure of confinement. In the event of failure of confinement, the

responsible party should notify responsible local agencies and, if one exists, any entity charged with biosafety oversight (e.g., Institutional Biosafety Committee). In most cases, the first local agency to contact is the local office of government agencies responsible for natural resource management and environmental protection (e.g., departments or ministries of forestry, fisheries, wildlife, park management, pollution control, environmental protection).

Recovery plan. The emergency response plan should include a plan for recovery of escaped GEOs in cases where the project site and biological features of the GEO allow recovery. The relevant government agencies for natural resource management and environmental protection should be involved in development of such a plan because one or more will probably have oversight authority over any recovery actions that occur in natural ecosystems.

Movement to safe site or destruction of GEOs. The responsible party should notify responsible local and regional government agencies, if they exist, that such an action will be taken. Oversight of the action by a member of the responsible agency is strongly encouraged. The emergency response plan should clearly define the event(s) which activate movement or destruction of GEOs.

Peer Review and Site Review

This section makes a distinction between review of projects prior to their start-up and periodic site review after start-up. In some cases, flexibility in this distinction is warranted. For instance, users or developers may be planning to conduct a new project, involving new types of GEOs, in a site used previously for another project with GEOs that already passed peer review and site review. If the new project clearly has the same specific hazards as the old project, less extensive peer review may be adequate but site review should continue. If the new project poses a different set of specific hazards, peer review prior to start-up is warranted. The review should address whether or not the existing configuration and components of the project site and barriers are adequate for the new project.

Peer review prior to start-up of project

Peer review of the project's siting, design of barriers, security, and operational plan is highly recommended. To ensure scientific validity of the review, reviewers should include scientists with expertise in the organismal and population biology of the project's GEOs and in the ecology of the accessible ecosystems. It may be beneficial to include a representative of a government agency charged with oversight of management of natural resources or environmental protection in the accessible ecosystems. It is important to remember that projects also need to comply with any local, regional, or national regulations governing the development and use of GEOs.

Site reviews after start-up of project

Site reviews are highly recommended and their scheduling should be the responsibility of the user's institution. The number of site reviews should be based on (a) the specific features of the project, such as the complexity of required risk management measures, and (b) findings during earlier site reviews. The purpose of site reviews is to determine whether or not the project is maintaining "no/negligible escapes" or "containment indicated", as specified in Flowcharts VI.A. or VI.B. Site reviews should determine whether: (1) physical facilities are performing and are maintained as expected; and (2) the operating and emergency response plans are being followed. Additionally, records might be checked to ascertain, for instance, if frequencies of routine barrier inspections and maintenance by project staff are adequate. Should problems in compliance with the operational plan be identified, additional unannounced site visits might be appropriate.

Documentation to submit to proposal and site reviewers

Users should be expected to provide the following documents to reviewers of both the project proposal and of the project site: a copy of this Manual, a completed

worksheet with attached documentation, and a written operational plan.

Project approval

Once the designated review team has decided that the risk management measures are adequate to address the hazards identified for a proposed project, it is advisable to obtain written documentation of this approval. The format of this approval is left to the discretion of the authorities involved.

Flowchart VIII. Overview of Food Safety

Introduction: Methodological Considerations

Several attempts have been made to assess the safety of genetically engineered foods (GEFs). The results of these attempts can be found, for example, in various national standards for food safety. For the most part, attempts at assessment have focused on the potential for allergenicity, toxicity or altered nutrient composition of the GEF (e.g., Clydesdale 1996). In addition to factors specific to allergenicity, toxicity or nutrient composition, several methodological issues underlie assessment of food safety in a more general way. These include, but are not limited to, factors affecting variability in effect(s) of the new food product, factors affecting assessment of exposure, and the ethics and practicality of testing novel foods on human subjects. What follows is a brief overview of some of these methodological issues.

Factors affecting variability of effect

The effects of genetically-engineered foods on human health could range from none to severe, and are likely to be highly variable in their expression and in our ability to detect them. Sources of variability include (1) the means and outcome of specific genetic manipulation(s) and (2) differential human response to the new construct or product. In short, genetic engineering of food may introduce new variables into a system already inadequately characterized. For example, the functions of most biochemicals in food remain poorly understood. Until the mid-20th century, fiber was believed irrelevant and possibly harmful in the human diet; and until the 1980s carotenoids were assumed to have little or no nutritional function independent of their vitamin A activity. We now know both of these to be incorrect. Moreover, in the body, food enters an internal environment composed of a collection of biochemicals that interact in complex ways to maintain life and health.

The variable *composition* of food is only one issue relevant to assessing its safety. Variability is further introduced by modifications resulting from food

storage, transport, and processing or preparation prior to consumption. Uncertainty in the estimation of food effects is increased by the fact that the diet of human beings is not composed of a single food, or even a single mixture of foods, but of an individually-chosen mixture of the foods available. And finally, the foods that have been selected may be beneficial, neutral, or detrimental to individual health, depending on characteristics of the individual consuming them. The above relationships can be illustrated by an example using the essential nutrient selenium. A plant's selenium content depends in part on levels of selenium in soil and water. The selenium content of bread therefore is determined by where the wheat was grown, and its impact depends on how much bread the consumer chooses to eat, the presence of other selenium-containing foods in the diet, and the consumer's individual requirement for selenium.

In the same way, a single GEF might have both beneficial and detrimental effects on human health, depending upon the individual consumer and the circumstances in which the GEF is consumed. If the consumer lives in region where selenium deficiency exists (and where there is no importation of food), a GEF with a higher selenium content could be beneficial. On the other hand, if the consumer lives in a region where selenium toxicity exists (and where there is no importation of food), a GEF with a higher selenium content could be harmful. The following paragraphs identify some sources of variability that contribute to uncertainty in the assessment of food safety.

A. Crop growth conditions

Assessment of the effects of production environment on the chemical composition of GEFs requires extensive data on the composition of unengineered foods (UEFs) and the range of variability produced by various growing environments. Such information is still grossly incomplete, and may not be available. Therefore, to make valid and meaningful comparisons between them, GEFs and UEFs would need to be grown under identical conditions in multiple environments. The most extensive studies examining the effects of various growing conditions on food composition have been performed by researchers

endeavoring to evaluate the impact of organic growing methods on the nutrient content of crops (Worthington 1998). Their studies show that food composition can vary widely, depending not only on the specific variety of the food plant being considered, but also on the season, temperature, water, mineral content of the soil, and sources of organic matter. Therefore, when comparing the nutrient content of a GEF to a UEF, even the establishment of a "normal range" for a given nutrient may be difficult or impossible.

Furthermore, the choice of which nutrients to measure can be highly debatable. One approach that has been used by some regulatory agencies is to recommend that if a given food is considered an important source of a particular nutrient, the content of that nutrient in the GEF should be compared with the content of that nutrient in the UEF. For instance, the vitamin C and beta carotene content of the Flavr Savr® tomato was compared with the vitamin C and beta carotene content of unengineered tomatoes, and it was judged that there were no important modifications in the content of these two nutrients. However, there is now considerable interest in the content of lycopene, a carotenoid found in tomatoes that may be an important anti-oxidant in the diet, and it therefore may have been advisable to measure measure lycopene content as well (Gerster 1997). The subtle but important biological significance of minute amounts of a nutrient in foods is illustrated by the extensive changes made to infant formulas, which have been continually modified since their development as nutrition scientists and public health professionals have increased their understanding of the significance of minute quantities of trace elements, nucleotides and various essential fatty acids found in human milk. Thus, the choice of which nutrients to screen for in the GEF and compare with the UEF should be informed by the most current knowledge in nutrition science.

A further complicating factor in safety assessment of a GEF is determination of the relevant standard to be used for comparison. For instance, some crops have been engineered to increase the endogenous (internally-produced) content of a pesticide, so that (presumably) less of the pesticide must applied to prevent crop loss by pests. In evaluating the relative safety of the GEF compared with the UEF,

several questions must be answered to determine the appropriate comparison. If the GEF contains a certain level of pesticidal compound, is that level to be compared to the level found in or on a UEF? If the active compound is sprayed on a UEF, and is therefore present but not systemic, can it be washed off or peeled off in food preparation? If the compound can be washed or peeled off, what assumptions should be made and what comparisons drawn to the pesticide levels in the GEF? Finally, are there alternative agricultural methods available to grow the UEF crop with lower levels of exogenous pesticide, and if so, should the pesticide levels in (alternatively grown) UEF crops be compared with the level of endogenous pesticide in the GEF? Clearly, the choice of the standard of comparison will substantially affect the outcome of the comparison and will impact the decision regarding whether or not to introduce a GEF.

B. Modifications produced by the handling of food

Most foods are modified in some way after they are harvested. In some cases they undergo only physical modifications (e.g. washing, cutting, cooking, etc.) but increasingly, they undergo much more extensive modifications, including combination with other ingredients. The effect of processing is to modify the composition of the foods being processed. Such processing may have either a beneficial effect (e.g., by destroying protease inhibitors) or a detrimental effect (e.g., destruction of vitamin C by high heat). What is relevant here, however, is that very few of the effects of processing on the chemical composition of foods have been characterized (Labuza et al. 1984). Adequate comparison of the biochemical equivalence of a UEF versus a GEF therefore requires that post-harvest alterations be standardized.

An additional complication is introduced by isolation and remixing of various food components in processed foods. Despite the presence of a seemingly large number of different food products, these products are increasingly derived from a relatively small set of raw ingredients -- proteins, fats and carbohydrates isolated from wheat, soy, corn and other crops. Variety in food products therefore does not necessarily imply diversity in raw ingredients. Dietary variability, which

was once counted on to minimize exposure to any single hazard, may no longer do so. The importance of this to the consumption of GEFs is that even a moderately diverse diet may expose the consumer to high levels of a particular compound or class of compounds from GEFs, thereby increasing risk.

It has already been stressed that food is composed of numerous chemicals whose biological significance is inadequately understood. Demonstrating the biological significance of a single nutrient is difficult enough; it is even more difficult to demonstrate the significance of a single food source of the nutrient. It would be nearly impossible to prove that a single modification in a cultivar had any impact on human health. The absence of such proof, however, does not imply an absence of harm. Furthermore, the accumulation of multiple, unregulated modifications over time may have a significant impact. For instance, if the fiber content of a single cultivar was modified to enhance the digestibility of wheat, it would be difficult to prove that this had adverse health consequences. However, if all cultivars of wheat were eventually modified, this would likely have a significant impact on the ingestion of indigestible fiber, which in turn could have an impact on the risk of colon cancer of the population (Kritchevsky 1997). Given the difficulty of obtaining accurate dietary intake data, and the fact that colon cancer is caused by many factors, it would be nearly impossible to document that a rise in colon cancer was due to the consumption of a modified food or group of foods. Nevertheless, in the absence of such scientific data, judgements about the impact of a given GEF need to be made.

C. Human food choice

Once a food is put into the marketplace, consumers determine its use. Where the number of available food products is extremely limited, the resulting diet can be assumed to be of limited diversity, and tracking the effects of a single food may be relatively easy. In an industrialized marketplace, however, new and complex food products continually enter the marketplace, in some cases at a rate of over 40 a day (Gallo 1995). In addition, a large proportion of meals in industrialized societies are consumed away from home and dietary patterns are highly individualized. These

consumption patterns affect the assessment of food safety of GEFs in several ways. As the number of foods increase, evaluating the impact of a single food becomes more difficult. As people around the world are eating more of their meals away from home, it makes labeling a less effective tool for tracking the consumption of any given food, and a less effective way of informing a consumer that a given food has been bioengineered.

D. Human biology

The effect of a food may not be uniform for all consumers The effect of the food depends on the physiological and/or pathophysiological characteristics of the consumer. Relevant physiological characteristics include gender, age, size, nutrition status, and genetic susceptibility.

The extent of variation in response to food is much greater than is generally acknowledged. Standards sometimes obscure this variation, as for example, in the establishment of nutrient standards (e.g. Recommended Daily Allowances or RDAs) or the determination of "safe" levels of exposure to chemicals. Human variability is important to the issue of allergenicity in GEFs because large proportions of the population are not susceptible to most allergens. Among the small proportion of people who are allergic, however, exposure may be fatal. Thus far, no available animal model can fully assess the human allergenicity of a substance. In many cases, it is technically possible to characterize the gene product, and compare the amino acid sequence to known allergens. Currently, it is assumed that about 90% of the known food allergens have been identified and sequenced (Clydesdale 1996). Therefore, one might assay a GEF to determine the presence or absence of known allergens. However, an estimated 10% of allergens have not been characterized, so if a protein is found in the GEF that is not found in the UEF, one might have to expose humans to testing to evaluate the allergenicity of that protein. Furthermore, the technology of genetic engineering may lead to the formation of new proteins of unknown allergenicity . The ethics and practicality of this aspect of genetic engineering is discussed in further detail in Section III.

Factors affecting only post-market assessment of exposure

The factors identified above complicate the measurement of post-market exposure to GEFs and make it difficult to assess the impact of a GEF on human health. In theory, labeling would allow post-market tracking of a food. In reality, however, due to a variety of factors including the technical difficulty of labeling produce, and the frequency with which people eat meals out of their homes, labeling can ***at best*** be expected to enable a consumer to control the consumption of a food. Factors that reduce the impact of labels include the practical difficulties of labeling unpackaged foods, the fact that some degree of literacy may be required for a label to be useful, and the fact that some prior education may be required to inform the consumer about the potential hazards of the GEF (e.g., allergenicity).

Even if labeling allows a consumer to minimize exposure, it is virtually impossible to determine who actually consumed the food and in what quantities. (In the United States, for example, 27% of all food is wasted at the retail, consumer and food service levels [Kantor et al. 1997]). Tracking consumption within households is difficult enough, but an increasing percentage of food in many countries is eaten away from home, and the ingredients are not necessarily known to the consumer. It is therefore so difficult to determine who has actually eaten a particular food or food component that it is often impossible to separate exposed and unexposed populations. From an assessment point of view, labeling is an inadequate and imprecise tool for tracking exposure.

Ethics and practicality

Genetic engineering may produce potentially hazardous substances whose impact cannot be accurately assessed in any but a human system. Determination of the safety of such a substance may require exposing large numbers of people to a hazard whose magnitude is unknown. As mentioned earlier, in vitro screening methods are available for about 90% of known allergens (see Hefle et al. 1996). However, for the remaining 10%, no current in vitro screening methods exist. Furthermore, the allergenicity of novel proteins is not fully assessable at this time.

For example, if a novel protein has a structure similar to known allergens, that may be sufficient reason to disallow introduction of the GEF. If the novel protein is different in structure from known allergens, the only way to fully assess its safety may be to expose susceptible individuals to skin tests, or to expose the population at large to the GEF and to conduct post-market monitoring. In such cases, one faces the ethical problems of allowing extensive exposure in humans for the purpose of safety assessment of a GEF. Furthermore, for the assessment to be valid, a statistician, an immunologist and an epidemiologist should be included in the design and performance of the test. If too few people are screened, or if the monitoring is inadequate, there is a risk of concluding that the GEF is safe when it is not.

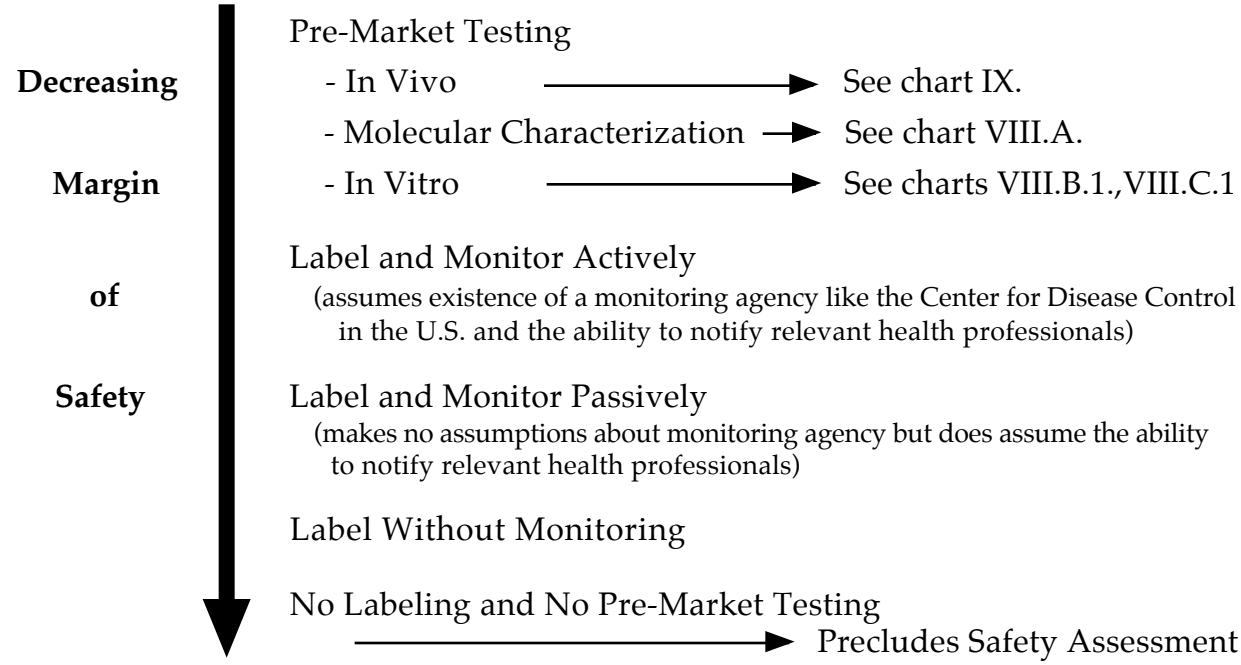
Many of the same issues arise with toxins as have been discussed for allergens. If the toxin is known, its level in the food can be measured, and in some cases there will be adequate in vitro tests available to assess safety. If in vitro tests are not available, human exposure may be necessary to assess the safety of the GEF.

Conclusion

The preceding discussion was intended to reflect the reality of and variability in present-day food systems. Factors outlined are those that would need to be assessed for the most thorough and rigorous evaluation of the safety of a GEF. However, complete assessment may be an unrealistic goal with respect to time, money, and feasibility. Clearly, the assessment strategy chosen will determine the margin of safety of the introduction of the GEF into the food supply (see Margin of Safety diagram below). Further, because our state of knowledge is not static, any assessment tool will itself need to be assessed over time in order to remain informed by current scientific knowledge about diet and health.

The graphic below shows how the margin of safety depends on the levels of testing, labeling, and monitoring performed. The greatest degree of safety is achieved by extensive pre-market testing, whereas the margin of safety approaches zero where there is no labeling and no pre-market testing. In such cases, assessment and monitoring are precluded.

Assessment Strategies for Genetically Engineered Food



The assessment scheme given in the following flowcharts presents an option for thorough and rigorous assessment of GEFs. It is recognized that in some cases users may not have adequate expertise, resources, or time to complete the full safety assessment of a GEF. Although the recognition of such circumstances in no way constitutes an endorsement of incomplete assessment, we recognize that decisions about assessment strategies are affected by many factors, some beyond the scope of this manual. We urge users to comply with the strictest assessment standards practicable under local, relevant conditions. Failure to adequately assess the human health impacts of a GEF could lead, in some cases, to serious detriment to public health. Further, it should be noted that in vivo testing of some GEFs could be dangerous, and all in vivo testing should be carefully considered before it is carried out. In no cases should in vivo testing be performed if other criteria (e.g., molecular characterization, in vitro testing) have not been satisfied.

The choice of a strategy for assessing impacts is a matter of complex decision-making, often dependent on socio-economic considerations. Nevertheless, assessment choices, however they are made, influence the margin of safety in introducing a new food into a population. At a minimum, labeling of a GEF is necessary (but not sufficient) for the detection and monitoring of any adverse consequences to consumers. However, labeling in and of itself cannot be considered an endorsement or a guarantee of the safety of a food.

Before a GEF is placed on the market, its potential toxicity, allergenicity, and nutritional content should be assessed. The following flowcharts (VIII-XI) provide examples for proceeding with food safety assessment. However, these flowcharts are neither complete nor sufficient on their own. Some of the tests called for may not yet be available, and additional tests may be required of some products. Hence, consultation with experts in the relevant area of food safety assessment is advisable at each stage. It might also be prudent to perform a cost/benefit analysis to evaluate at the outset whether or not a GEF provides any benefit to the consumer. If an unmodified food (UEF) with similar nutritional value is readily available, and if the UEF has a known (and acceptable) safety record with respect to toxicity and allergenicity, the introduction of a GEF might be deemed unnecessary.

VIII. <1>

The effect of some types of genetic engineering is to introduce new genes into the cells of a food-producing organism. Because a gene is typically the blueprint for a protein, that new genetic information can cause the organism to produce one or more new proteins. In turn, the food produced by that GEO will contain those new proteins. The effects of novel proteins on human health and nutrition in most cases have not been demonstrated.

Alternatively, other types of genetic engineering will introduce no novel gene products or proteins into the GEO or GEF. In such cases, the effects of novel proteins can be ignored, but it may nevertheless be prudent to assess levels of toxins or allergens known to be present in the unmodified (parent) organisms, or in comparable unmodified foods, because levels of toxins or allergens could have been

elevated to hazardous levels by the process of genetic engineering.

A few food products produced by genetic manipulations have been available to the public for some time and with no indication of adverse effects. (Examples include triploid oysters and hybrid striped bass). They may contain toxins or allergens at the same levels as those in the UEFs with which consumers are familiar and from which the GEFs were derived. There is little or no need for additional, extensive testing to demonstrate their safety, particularly where those allergic to the UEFS would be aware of the source of the GEFS. Although this flowchart routes such GEFs through questions about allergenicity and toxicity, it generally does not require of them extensive in vitro and in vivo testing for allergenicity or toxicity.

VIII. <2>

Molecular characterization of the novel protein (or gene product of interest) provides information about molecular structure that could help to identify potentially beneficial or potentially allergenic or toxic gene products. See text for VIII. A. <1> for a more detailed explanation of molecular characterization.

VIII. <3>

Compounds toxic to humans could be unintentionally introduced into GEFs, or the levels of toxins could be unintentionally elevated by genetic engineering. To test for such toxins, standard methods should be used in consultation with epidemiologists and other specialists. The most comprehensive safety assessment includes an in vivo testing regime, which should be carefully considered and carried out only according to strict safety protocols and under strict guidance from experts, and only AFTER in vitro tests have been performed. Carefully review the preceding discussion on methodological problems before interpreting the results of such testing.

In a subsequent flowchart (VIII. B.), users reach in vitro testing only after a series of questions allows the conclusions that (a) a novel toxin is present in the GEF or a toxin is present in the GEF at levels higher than that determined to be safe in the UEF and (b) the toxin(s) will not be destroyed by processing before being

consumed (VIII. <4> and <5>).

VIII. <4>

A toxin that is destroyed by processing poses little or no health hazard. ONLY IF the GEF is never and will never be consumed in an unprocessed state (that is, with the toxin still present). answer "Yes". In the absence of clear evidence that the food will be consumed ONLY after processing, answer "Unknown".

VIII. <5>

If the toxin is destroyed by processing of the food, it may pose little or no hazard to human health. In the absence of clear evidence that the toxin is destroyed by processing, answer "Unknown".

VIII. <6>

Genetic engineering could lead to the production of novel proteins of unknown allergenicity, lead to the transfer of an allergen from a food source of known allergenicity to one previously thought to be non-allergenic (see, e.g. Nordlee et al. 1996), or cause levels of existing allergens to be elevated. Consequently, levels of known allergens should be compared in the UEF and GEF, and screening for novel allergens should be performed if they are likely to exist (e.g., if novel proteins of potential allergenicity were produced by genetic engineering). Consultations with experts in clinical immunology would aid in designing assessments able to detect true differences between the UEF and the GEF, and ensure access to the most current tests and information available.

Molecular characterization should give information relevant to determine if a known or suspected allergen is present. If allergenicity is demonstrated or suspected, users of this flowchart can reach in vitro testing only after a series of questions allow users to conclude that (a) an allergen is present in the GEF that was not present in the UEF or an allergen is present in the GEF at levels higher than exist the UEF and (b) the allergen(s) of concern will not be destroyed by processing before being consumed(VIII. <7> and <9>)..

VIII. <7>

If an allergen is always destroyed by digestion, it poses little or no health hazard, and does not need further consideration here. In the absence of clear evidence that the allergen is destroyed by digestion, answer "Unknown".

VIII. <8>

If an allergen is destroyed by processing of the food, it may pose little or no hazard to human health. In the absence of clear evidence that the allergen is destroyed by processing, answer "Unknown".

VIII. <9>

An allergen that is destroyed by processing poses little or no health hazard ONLY IF the food is never and will never be consumed in an unprocessed state (that is, with the allergen still present and active). In the absence of clear evidence that the food will be consumed ONLY after processing, answer "Unknown".

VIII. <10>

Changes in nutrient content in GEFs can be benign (no effect), beneficial (nutrients are increased within safe levels), or detrimental (nutrients are increased or decreased beyond safe levels). Determination of which nutrients to test depends on the food product and on the nutritional status and food habits of the consumers. Such determinations should be made in consultation with a food scientist and a nutritional epidemiologist familiar with the methodological issues discussed earlier and with the nutritional needs and status of the population into which the GEF is to be introduced. Goals of such a consultation would include, but not be limited to: (1) determination of the important nutrients found in the UEF that should be measured in the GEF, (2) suitable databases for comparison of the GEF with the UEF, and (3) an analysis of the long-term impact of the GEF on public health. If there is insufficient information for completion of (1), (2), and (3), consider disallowing use of a GEF as food until such information becomes available.

VIII. <11>

GEFs exhibiting changes in nutrient content that are likely to be detrimental, either because of increased levels (potentially conferring toxicity) or decreased levels (potentially contributing to malnutrition), may be unsuitable for introduction to consumers. Consider disallowing release. Note too that decisions about desirability of nutrient level will depend on the nutrient and on the context. In some places where people have been ingesting higher-than-desirable levels of some nutrient (e.g., selenium), a lower amount of that nutrient may be deemed desirable.

Flowchart VIII.A. Food Safety Assessment: Molecular Characterization

The purpose of molecular characterization is to identify the molecular structure of the novel protein in order to predict its function (or probable mode of action) in the GEO. If the recombinant gene has a negligible effect on the metabolic or biosynthetic pathways of the GEO, then it is unlikely to significantly alter either the quality or safety of the food derived from it.

VIII.A. <1>

First, the nature of the transgene is considered. If it encodes a protein(s) that is known to be non-toxic and non-allergenic, and if it is unlikely to catalyze reactions that modify cellular metabolism in such a way as to generate toxins, then one class of risks is eliminated.

The possibility remains, however, that the transgene or its protein product might modify cellular gene expression, thereby generating new toxins or allergens. This possibility can be identified by answering the following questions:

1. Does the insertion site of the transgene interrupt one or more open reading frames within the DNA of the GEO? If an open reading frame is interrupted, then the expression of at least one gene is blocked. The question

then arises as to the identity of that gene and the function of the protein that it encodes, and the actual effect of the loss of that protein on the metabolism and regulation of the GEO.

2. If there are messenger RNA molecules (mRNAs) actively expressed from the sequences within the 20 kb domains flanking the insertion site of the transgene, are the levels and patterns of expression of those mRNAs unchanged in the GEO, compared to the unmodified organism? The genes whose expression is most likely to be disrupted by the inserted transgene are those nearby.

3. Is the transgene expressed in the parts of the GEO that are normally used for food?

If these questions all yield negative answers, then it is unlikely that the gene expression of the GEO is significantly different from that of the unmodified organism, and there is sufficient confidence that the genetically engineered food is safe to advance to monitored marketing.

VIII. A. <2>

If any one of the above-described questions (in VIII.<1>) yields a positive answer, then it is necessary to assess gene expression in the GEO more fully. For this purpose, we propose the use of the differential display technique (see Ausubel et al. 1994) or another method capable of exhaustively comparing the mRNA profile of the GEO to that of its unmodified parent(s). These techniques require significant technical expertise; however, in some cases, this approach will be preferred to in vivo testing.

Flowchart VIII. B. Toxins

There are many UEFs on the market that contain toxins at levels known to be safe for consumption. Some of these UEFs are products of unmodified organisms that also happen to be parental organisms of GEFs. There is little need to test toxicity of a GEF that contains only the same toxins as the UEF and only in amounts demonstrated safe in the UEF or lower. This flowchart quickly routes GEFs beyond testing for toxins IF they are **known** to have ONLY the same toxins as the UEF and ONLY at the same or lower levels than those of the UEF

Flowchart VIII. B. 1. Food Safety Assessment: In Vitro Testing for Anticipated Human Toxins

Two basic questions are asked in testing for anticipated human toxins:

(1) If food derived from the unmodified organism from which the transgenic organism was generated is known to contain toxins, are the levels of these toxins in the GEO within the norms expected for the unmodified organism?

(2) If the transgene is derived from an organism that expresses a toxin, was that toxin transferred to the GEO via the transgene, or were genes for enzymes critical to the synthesis of that toxin transferred?

These questions should be answered using specific analytical tests for particular toxins. If a toxin is detected, further analytical work should be performed to quantify the level and activity of the toxin in the GEF. In conjunction with clinical data from the literature, these analyses can serve as the basis for deciding if the GEF is appropriate for full scale marketing, and if so, to help formulate labeling and use instructions.

Flowchart VIII. C. Allergens

There are many UEFs already on the market that are allergenic. Some of those UEFs derive from parent organisms which later generated GEFs. Consideration of allergenicity of GEFs therefore involves two sets of questions:

(1) Is the GEF differently allergenic than the UEF, that is, are novel allergens present or are expected allergens present at levels that exceed the levels in the UEF?

(2) Will the consumer be aware of the potential allergenicity of the GEF?

The question of consumer awareness can be addressed, at least partially, by adequate labeling (although labeling is not a sufficient solution for those who eat in restaurants or other places where consumers are not likely to know the ingredients in their food).

The problem of GEF allergenicity different than that of the UEF is addressed by this flowchart. Where GEFs contain ONLY the same allergens and ONLY at the same level or lower compared to the UEF, users are routed beyond the time and expense of in vitro testing for allergens.

Flowchart VIII. C. 1. Food Safety Assessment: In Vitro Testing for Anticipated Human Allergens

In testing for **known** food allergens, three basic questions are asked:

1. If food derived from the unmodified organism from which the transgenic organism was generated is known to contain allergens, are the levels of these allergens in the GEO within the norms expected for the unmodified organism?

2. If the transgene is derived from an organism that expresses allergens, were those allergenic determinants transferred to the GEO via the transgene?

3. Are other common food allergens present in the food derived from the GEO?

These questions can be answered with the help of appropriate experts and, where available, standard laboratory tests for allergens, such as those which involve assessment of the reactivity of the test substance with immunoglobin E or sera active against the allergen of interest (Sanz et al. 1996). Existing tests for allergens, however, are limited by the fact that they cannot provide information on novel allergens.

Detection of allergens should lead to more detailed characterization of the levels and activity of antigenic material detected in the food. The results of the more detailed studies, along with clinical data regarding the (common) allergen(s) obtained from the literature, then may be used to decide whether the food is acceptable for human use, and if so, to formulate labeling and use instructions.

VIII. C. 1. <4>

Although this question allows the user to judge in the specific case what is a "negligible" impact on consumer health, clearly a level of allergen leading to near anaphylactic shock would not be considered "negligible" while a level of allergen that caused minor skin rashes in a few individuals might be considered "negligible".

Flowcharts IX. Food Safety Assessment: In Vivo Testing

In vivo testing can be used to establish that a given GEF is free of unanticipated allergens and toxins. Progression from animal studies, to small scale human studies, to larger scale trials minimizes the risk to human subjects and, ultimately, to consumers. In vivo testing should be performed only under carefully

designed and controlled conditions, under the guidance of experts, in compliance with relevant laws and professional standards, and only where the absolute hazard to health of the subjects is minimal.

Information from each step of this evaluation is best used in two ways: (1) to contribute to the decision whether or not to commercialize the particular GEF and (2) to provide information relevant to the labeling and use of the final product, if commercialization is permitted. At each stage of the study, expert agencies, statisticians, and epidemiologists should be consulted in order to estimate and refine for the specific situation the number of subjects and the appropriate dosage and timeframe needed to achieve the degree of certainty required of the study results.

IX. <1>

Short term animal tests are carried out to eliminate GEFs containing powerful toxins or allergens before humans subjects are exposed to them. Because of low cost and relative availability and uniformity, mice most frequently are the subjects of these tests. Mice can be tested for (a) acute physical effects observed from feeding at maximum feasible doses for short periods (e.g., 48 hours to 2 weeks) and (b) sub-acute effects resulting from feeding for somewhat longer periods (e.g. up to 90 days) at levels proportional to maximum dietary levels in humans.

IX. <2>

Because there are physical, chemical, and physiological limitations to the amount of a food that can be administered through feeding, it is not possible in short term animal experiments to detect toxins and allergens with the same sensitivity as can be done in toxicological experiments, in which extremely large doses can be administered. Thus it can be very useful to carry out longer term experiments with human subjects to be assured that a given food is free of significant toxins and allergens. In vivo testing generally proceeds in several steps designed to minimize hazard to human subjects, proceeding from short-term, low-dosage trials to longer-term, higher dosage trials only if earlier trials have indicated an acceptable level of safety. In vivo testing on humans should be performed only under the guidance of toxicologists *and* experts capable of assessing the safely of

drug testing in humans. Consultation with experts and conformity with relevant, acceptable testing standards and laws is essential. Further, *ethical* testing of human subjects requires the prior informed consent of all subjects.

If earlier human trials indicate the safety and desirability of a GEF, it may next be test-marketed in selected areas with careful monitoring to detect impacts on the health of consumers. Such a monitoring system would include implementation of: (1) a public health reporting plan whereby consumers can report to designated (and alerted) public health agencies any problems encountered with the GEF. and (2) a labeling plan whereby consumers are (a) made aware of the exact nature of the GEF and any dangers (e.g. allergenicity) it presents and (b) provided a means of reporting health impacts to a relevant and responsible agency without incurring cost (such as a toll-free telephone number, or local contact address).

Even after test-marketing is completed, labeling is desirable for continued safety monitoring and to provide consumers with sufficient information to make informed purchasing decisions. Ideally, labeling should: (1) specify that the product is genetically engineered; (2) indicate any unique characteristics of the GEF relative to UEF; (3) provide a mechanism for consumer feed-back to the developer or supplier and to any relevant regulatory agencies; and (4) provide information on special handling or preparation requirements.

APPENDIX A

This table lists six general classes of potential and intended phenotypic modifications, representative kinds of intended or unintended ecological effects and a few examples of affected human enterprises and matters of environmental protection. This table is not intended to be comprehensive; rather it should serve to remind the user of the great range of alterations and effects that need to be considered. Nor is it intended to imply that the effects of a single change are confined to one category. For example, a change in metabolism may affect growth rate, tolerance of abiotic factors, behavior, morphology, population structure, etc.

CLASSES	EXAMPLES OF INTENDED PHENOTYPIC CHANGE	EXAMPLES OF POTENTIAL INTENDED OR UNINTENDED ECOLOGICAL EFFECTS	EXAMPLES OF POTENTIAL EFFECTS ON HUMAN HEALTH AND WELFARE
METABOLISM	• Individual growth rates • Energy metabolism, pathways and rates • Photosynthetic and chemosynthetic pathway structures and rates • Rates of nutrient uptake and cycling • Amounts and types of nutrients used • Use of pollutants as nutrients, pollution degradation • Nitrogen fixation pathways and rates • Carbon dioxide (CO_2) consumption • Tolerance of elevated CO_2 • Expression of novel proteins or metabolites, increased metabolic wastes • Production of antibiotics, or biological toxins such as that from *Bacillus thuringiensis* (Bt toxin) • Antibiotic or pesticide resistance	• Altered feeding rates and efficiencies • Altered rates of nutrient cycling and biological energy transfers • Altered rates of photosynthesis and carbon fixation and plant productivity • Modified rates and patterns of nitrogen fixation • Shifts in competitive abilities among species • Changes in degree of pesticide and antibiotic resistance among target and naturally occurring species, and spread of antibiotic resistance genes by lateral transfer • Release of antibiotics, toxins, or increased concentration of novel metabolites • Decrease or increase of biological diversity	• Changes in agricultural productivity • Changes in forest production and timing of tree harvesting cycles • Changes in stock composition and productivity of fisheries • Increased dependence on aquaculture • Increased intensity and variety of food allergies due to novel proteins, hormones or other metabolites, or altered levels of normal proteins and hormones and other metabolites
TOLERANCE OF PHYSICAL FACTORS	• Temperature • Humidity/moisture • Soil chemical and physical properties, including nutrients and water potential • Light intensity • Salinity • pH (acid/base) • Water chemistry • Pressure • Oxygen, CO_2, and other gases such as those of anaerobic environments • Toxic chemicals/pesticides/antibiotics • Heavy metals (e.g., mercury)	• Geographical relocation, expansion or contraction of preferred habitats for species and ecological communities • Changed species/population phenology (seasonal timing of life cycles), including patterns of growth, development, and breeding • Altered geographical ranges of species • Altered patterns of dispersal and migration • Increase and change in routes and extent of biomagnification (concentration) of toxic substances, including heavy metals • Changed composition and diversity of ecological communities	• Change in geographical or local constraints on crop production • Changes in geographic or local constraints on disease vectors, pathogens, pests, or pollinators • New threats to persistence and abundance of terrestrial and aquatic wildlife • Increased invasiveness of noxious or weedy species • Loss of genetic diversity in natural populations

BEHAVIOR	• Reproduction • Territoriality • Migration, navigation, orientation • Chemosensory abilities, including pheromones and allochemicals • Motility/locomotion • Animal communication • New kinds and levels of plant secondary compounds • Colonization • Pathogenicity of bacteria, virus, and fungi • Mutualisms/coevolution • Pollination • Photoperiodism • Foraging patterns and feeding specializations and rates • Social behavior, communal and cooperative living, "altruism"	• Altered breeding patterns and cycles, and mate-recognition systems • Change in population abundance and species assemblages • Altered population dynamics and phenology • Changes in self-compatibility and incompatibility of plants • Changes in rates, plant species spectrum, and effectiveness of pollination • Increases and decreases in pathogenicities and patterns of disease transmission	• Changes in local and geographical patterns of abundance of wildlife, game, and commercially harvested species • Alterations in agricultural productivity • Increase or decrease in human, animal, and plant health as behaviors of pathogens, disease vectors, and pollinators change
MORPHOLOGY, ARCHITECTURE OF ORGANISMS	• Animal shape, size, color • Internal and surface geometry of unicellular algae and protozoa • Antigenicity of disease organisms and parasites • Skeletons and appendages • Leaf shape, pattern of plant nodal extension and branching, flower structure, branching and frond geometry of macrophytic algae • Spines,hairs, trichomes and other protective devices • Bacterial cell-wall characteristics • Mosaic segments of virus • Cell structure, organs, organ systems • Unicellularity, multicellularity	• Altered species interaction: predator/prey, herbivory, competition • Mate recognition • Changes in bacterial cell walls and some antibiotic resistances • Altered virus/host interaction • Changed crop plant architecture	• Increase or decrease in virulence of pathogens • Gains or losses in plant yields through changes of architecture (e.g., dwarf varieties of rice and wheat) • Increase or decrease in plant protection against pathogens and herbivores • New problems in conservation • New opportunities for horticultural innovation

CLASSES	EXAMPLES OF INTENDED PHENOTYPIC CHANGE	EXAMPLES OF POTENTIAL INTENDED OR UNINTENDED ECOLOGICAL EFFECTS	EXAMPLES OF POTENTIAL EFFECTS ON HUMAN HEALTH AND WELFARE
FACTORS CONTROLLING OR REGULATING NATURAL POPULATIONS	• Novel disease resistance • Reduced predation/parasitism • Habitat preferences, extensiveness of preferred and secondary habitats • Antibiotic or biocide sensitivity and resistance • Extinction, local and global • Increases or decreases in fitness (see next class below)	• Altered population and community dynamics • Release from preexisting ecological limits or establishment of new limits • Changed disease transmission • Lateral transfer of antibiotic and toxin resistances among bacteria • Changed trophic interactions	• Increase or decrease in pest and pathogen populations and the attendant problems • Decline or loss of therapeutic effectiveness of antibiotics • Origin of new pests, weeds, and pathogens (especially plant virus modification)
DEMOGRAPHY LIFE HISTORY, POPULATION GENETICS, AND EVOLUTION Phenomena required for a detailed understanding of populations, species, and more distantly related evolutionary lineages (Parameters providing comprehensive bases for risk assessment of GEOs of many kinds to be released to the environment)	• Population fitness (in the technical sense of population and evolutionary biology as a summary of ultimate population performance, based on the interaction of many of the life history and population genetic features below) • Average life cycle pattern, simple or complex • Mode of reproduction: sexual, asexual, or alternating between these • Frequency of reproduction • Average rates and patterns of embryonic and larval development • Patterns of metamorphosis • Age of reproductive maturity and age of last reproduction • Fertility and fecundity • Survival rates with age (survivorship), average longevity • Net and intrinsic rates of change in population size and density • Age-structure of population • Population distribution in space, time, and over habitat variations • Social organization, kin selection, inclusive fitness	• Altered population and community dynamics • Shifts in the composition of ecological communities and local biological diversity • Increased or decreased fitness of populations • Increased or decreased population sizes and densities • Increased or decreased population fluctuations, population stability • Altered age-structure in populations • Microevolutionary changes set in motion in the GEO population or surrounding natural populations • Changes in spatial and temporal distribution of population and species • Altered genetic structure of the GEO population and their parental populations, if the two are sympatric (conspecific introgression) • Increased interspecies hybridization • GEO evolution due to mutation, genetic exchange, and natural selection	• New problems in pest and pathogen control • Epidemiological problems • Commercially harvested and/or game species species yield change • Conservation and wildlife management practices require adjustment • Design of wildlife refuges and nature preserves require reconsideration and possibly change • Mitigation procedures become necessary to protect biological diversity and the genetic diversity of natural populations

	• Substratum affinities • Patterns of dormancy, diapause, aestivation, hibernation, and spore and seed banks • Sex, sex ratios, mating types • Population genetic structure, genetic recombination within populations • Genotype-environment interactions and correlations • Pathogen host ranges • Vector host range and competence • Geographical arrays of conspecific populations (metapopulations) • Specialized genetic exchange (sexual) mechanisms of bacteria (transduction, transformation, conjugation, retrotransposons, conjugative transposons, other mobile elements) • Gene flow among conspecific populations • Hybrid zones and geographical clines • Genetic exchange between species and phylogenetic lineages		

Notes: Extensive as the table is, it is not exhaustive. It is meant to convey a sense of the complexity to be considered when designing and implementing a risk assessment protocol for a specific GEO. Much of the complexity arises because one phenotypic change can elicit a cascade of changes in other interacting phenotypic traits that collectively determine the fitness and population performance of the GEO (pleiotropic effects). For decision points in the flowcharts of this manual, readers can use this table to list all relevant phenotypic changes appropriate for a specific GEO. Then, from the lists of examples of ecological and human related effects, or by analogy to them, the reader can create a list of potential effects to be considered in a risk assessment. Existing information may be extremely helpful in identifying hazards and evaluating efficacy of the GEO. At the present time, it is likely that existing information will be insufficient for a reliable risk assessment. New data will be necessary.

These new data must come from effective empirical tests for phenotypic and ecological changes, as well as for efficacy (of the GEO), and will generally include three complementary and sequential stages: (1) a battery of laboratory experiments where a few environmental variables are held constant, often in "test tube microcosms", (2) studies in more ecologically realistic, but securely confined "mesocosm" or glasshouses, and (3) field trials justified by favorable results from stages (1) and(2). During each of these experimental stages it is essential to test for microevolutionary changes that will alter the GEO(mutation and natural selection) in environmental settings where its use is anticipated.

The ecological and genetic parameters and considerations in this table are treated extensively in standard textbooks. Among the (English language) texts readers unfamiliar with the subject may wish to consult are: Atlas and Bartha (1993), Brock et al (1994), Falconer (1989), Ginzburg (1991), Griffiths et al (1993), Harper (1977), Hartl and Clark (1989), Killham (1994), Krebs (1985), Lewin (1994), Levin et al (1992), Lynch and Hobbie (1988), Stanier et al (1986), Schlief (1986), Stearns (1992), Sivertown and Doust (1993), Doust (1993), and Roff (1992).

Appendix B - Consideration of Other Assessment

(from 1.A)

The appropriateness of this biosafety assessment will vary depending on the method of transformation used to produce the organism and the intended application, i.e., the size and type of project involved. For example, this assessment may not be necessary for cases in which the specific genetic transformations or modifications were produced by traditional practices and methods, because many such cases have been subjected to prior scrutiny. Further, this assessment may be inappropriate when the scale of release is extremely small or restricted, or when unintended adverse effects are HIGHLY unlikely. And lastly, for some specialized cases, other types of safety assessment may prove more appropriate than this one.

Nevertheless, it is important for readers to recognize that novel traits produced by any method can create unintended and unforeseen effects, and can thereby confer unanticipated hazards. The prudent user therefore may choose to proceed with this biosafety assessment in order to evaluate such potential hazards. Alternatively, or in addition to this, the user may choose to evaluate potential hazards by the use of other standards or protocols more appropriate to the specific case.

If the user cannot find an assessment tool better tailored to the selectively bred or captively bred organism in question and chooses therefore to continue with this assessment, please recognize that this assessment was not intended or tested for selectively bred or captively bred organisms. If, with this understanding, the user still chooses to use this assessment, return to chart I. A. <2> and wherever reference is made to the "GEO", consider that to be a reference to your (unengineered) organism.

Appendix C: Assessment of GEOs with Alternate Reproductive Pathways

Non-standard reproductive strategies are the norm for prokaryotic organisms (section I) and viruses (section II), and are occasionally encountered among eukaryotic organisms (section III). After reading the appropriate section, the reader should proceed to section IV.

I. Prokaryotic Organisms

Many (perhaps most or all) prokaryotes have mechanisms of genetic exchange that allow the occasional transfer of DNA from one cell to another. Such genetic exchange is never an obligatory part of the life cycle, and most multiplication is asexual; genetic exchange is sporadic. The mechanisms share several features that distinguish them from eukaryotic genetic exchange mechanisms:

(a) they are unidirectional: one cell acts as a donor and the other cell acts as a recipient of the DNA (typically the donor contributes a fragment of DNA, and the recipient contributes a complete genome);

(b) they almost always result in a partially diploid zygote, with only some sequences diploid and the rest of the genome remaining haploid. There is a wide range, from as little as a few genes to, in the limiting case, the entire genome being diploid;

(c) the haploid state is regenerated by degradation of the exogenous DNA, rather than by a meiotic reduction division.

In almost all cases, the generation of recombinant cells requires homologous recombination in the brief interval between the introduction of exogenous sequences and their degradation. In certain specific cases, some genes may be integrated as an addition to the recipient genome rather than as a replacement. Since almost all prokaryotes have a single chromosome, molecular recombination (and not new chromosomal combinations) is required to generate recombinant cells.

Since the usual mechanism of recombination requires homology, most cases of interspecific DNA transfer do not result in recombination because of the lack of sufficient homology (similar species within the same genus may differ by 10-20% in DNA-DNA reannealing experiments). However, sometimes the presence of highly conserved genes, or of common transposons or similar sequences, will provide enough homology that recombination is possible between sequences from very different parents. Furthermore, any transposable elements on the exogenous DNA may transpose to the recipient chromosome via their own non-specific mechanisms of recombination. And of course plasmids, which are capable of replication

independent of the chromosome, do not require recombination in order to establish themselves as part of the genome.

Three basic mechanisms of DNA transfer are known:

(a) conjugation, in which specialized surface structures on the donor cell allow it to attach to recipient cells (not necessarily conspecific) and transfer DNA. This requires that the donor contain a plasmid encoding transfer functions (often termed a fertility plasmid). This process transfers the fertility plasmid, and occasionally some chromosomal DNA as well.

(b) transduction, in which a mistake during virus multiplication in the donor results in the accidental packaging of some host DNA into the virus, which will then inject it into the next host it encounters. This will usually be a conspecific because of the narrow host range of most bacterial viruses, but occasionally can cross species or generic boundaries;

(c) transformation, in which certain prokaryotic cells are able to take up DNA from solution. Sometimes there is a high degree of species specificity to this process, but in other cases it is nonspecific, and DNA from any source can be taken up. Given this process, obviously any cell has the theoretical potential to act as a donor to competent, non-specific recipient cells, since the death of a cell normally results in its eventual lysis and release of its DNA into the medium.

II. Viruses

Viruses are acellular entities that require entry into a host cell for their multiplication. Recombination between different viruses is possible if they simultaneously infect the same host cell. Often such recombination is via host mechanisms of homologous recombination; in such cases, recombination is normally between closely related viruses (but again, any transposons can move between unrelated viruses). Other viruses have special mechanism of nonhomologous recombination that allow them to recombine with the host chromosome, occasionally generating recombinant viruses containing some bacterial genes.

Most viruses have a single chromosome, and recombination requires molecular exchanges between the chromosomes of the two participating viruses. Others, however, have multiple chromosomes, and random assortment is an additional mechanism of recombination in such cases.

III. Eukaryotic Organisms

Eukaryotic organisms can exhibit a variety of reproductive modes or

pathways beyond those that are strictly dioecious. Alternate modes of reproduction include but are not restricted to monoecy, simultaneous and sequential hermaphroditism, self-fertilization, parthenogenesis, apogamy, apospory, encystment, fragmentation, and budding. Further, some life histories may contain more than one free-living phase, which may or may not be of alternate ploidy and morphology. The existence of multiple reproductive pathways will increase the likelihood of escape and establishment of GEOs beyond their intended containment or release site, and will therefore substantially increase the risk of introducing these organisms into the larger environment.

Of greatest concern is the fact that viable populations could be established by the escape of a **single** individual, cyst, spore, or propagule. Further concerns include the facts that 1) spontaneous expression of an alternate of life history phase could make containment difficult or impossible, especially if this alternate phase is cryptic; 2) once escaped, alternate life history phases could resist detection and eradication; 3) the spontaneous appearance of reproductive individuals where none were expected could render existing containment devices ineffective.

Because the risk of escape is substantially elevated, higher levels of containment will be necessary for organisms capable of alternate reproductive pathways. The user is encouraged to consider ALL possible reproductive and life history modes, and to take precautions sufficient to avoid release of ANY reproductive organism or propagule. A useful example for the special case of fish and shellfish appears in Appendix B of the Agricultural Biotechnology Research Advisory Committee's *Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish* (1995).

IV. Options

Once factors pertaining to alternative reproductive pathways have been considered, the user may choose to proceed with the project, and is directed to return to the assessment at I. A. <6>. If the user chooses at this point NOT to proceed with the project, the user can exit this assessment now.

Appendix D: Schematic Diagrams of Examples of Mechanical Barriers

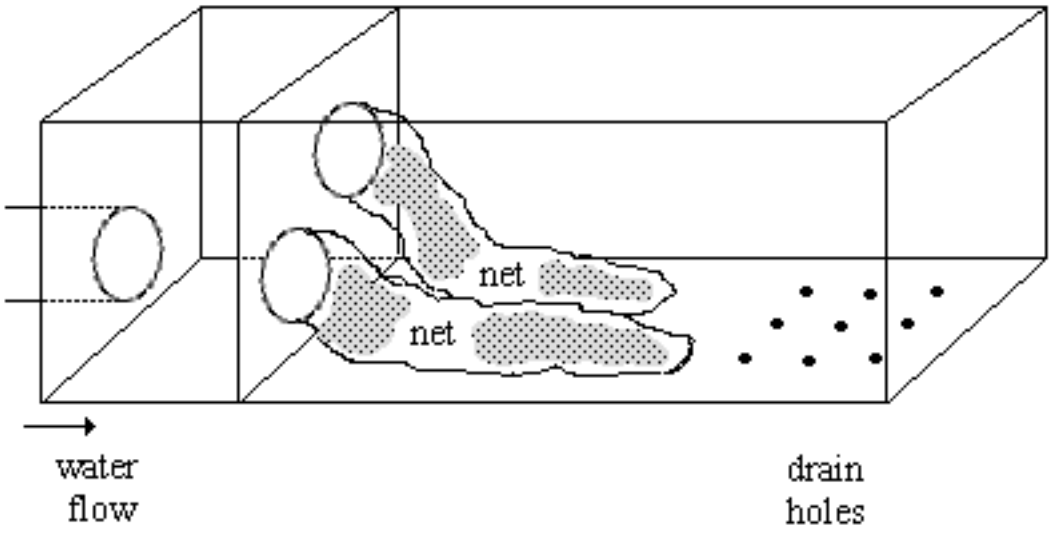


Figure D1. Sock filter trap. Effluent from any incubator or rearing tank holding fish embryos, larval fish, or small fish passes through such a trap. Any escaping organisms are trapped by a 0.3 mm mesh net. An overflow net will filter the effluent if the lower net should become occluded. Effluent discharged through the drain holes goes to the indoor laboratory's common effluent drain. Barriers for the common effluent drain are depicted in Figure D2.

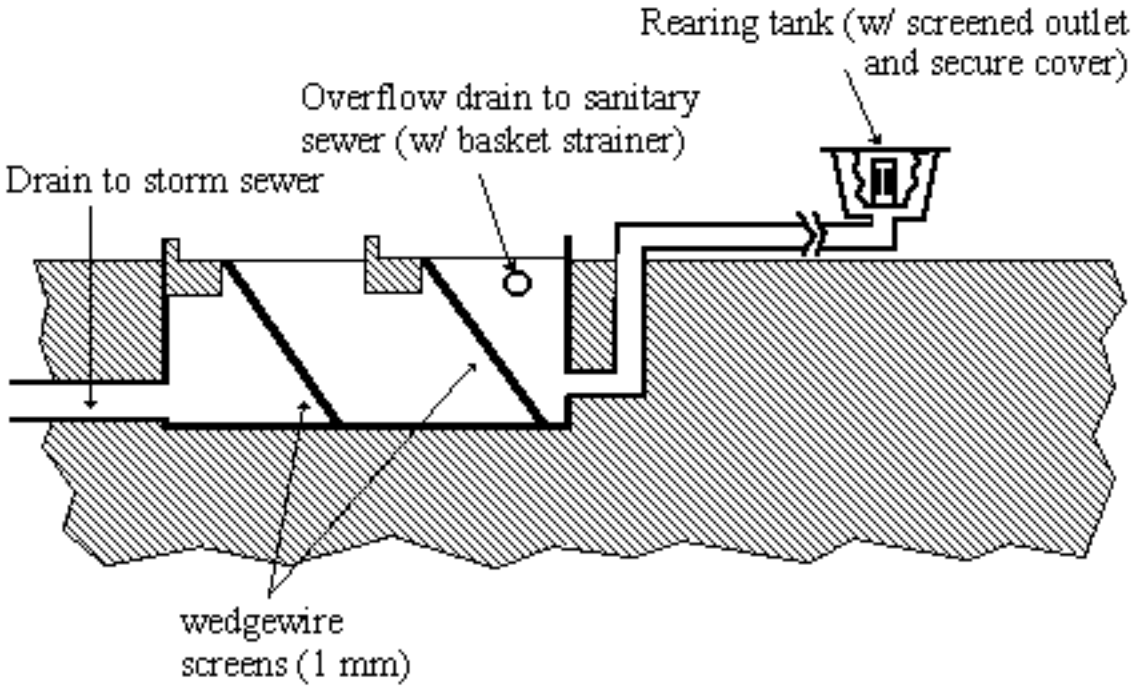


Figure D2. Two stainless steel wedgewire screens in series act as final barriers for the common effluent from flowthrough experimental units located in an indoor research facility for fish. There is a 1mm wide gap between the stainless steel wedgewires. Effluent from any experimental units holding fish smaller than a total length of 2 cm must first pass through a sock filter trap (Fig. D1). Effluent from units holding fish at or above a total length of 2 cm (equivalent to a head diameter of 2 mm) goes directly to these screens. Empirical tests showed that the screens will clearly retain fish at or above this size.

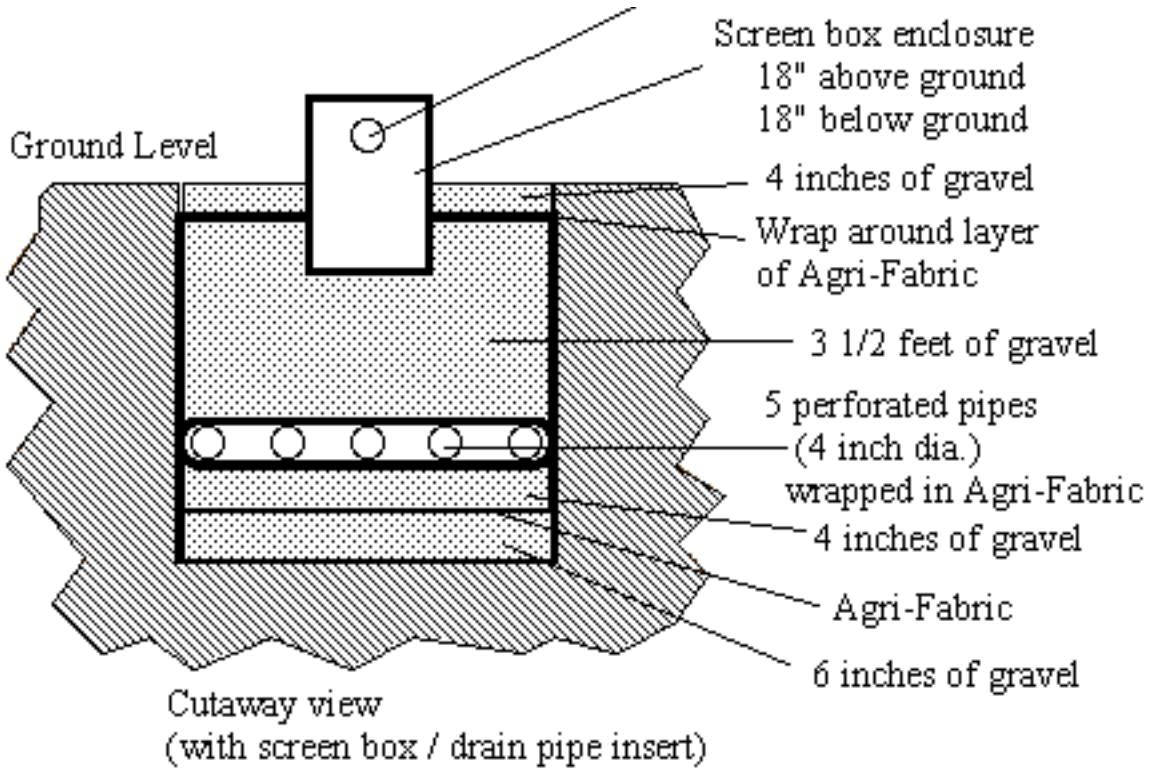


Figure D 3. Schematic drawing of a French drain for outdoor fish ponds. Normally run as static systems, such research ponds might be drained partially during sampling or entirely at the end of an experiment. The French drain is designed to retain the smallest possible size of fish reared in the pond. Water discharged from this drain eventually reaches surface waters. (Adapted from Cooperative State Research Service 1990, as cited in Cooperative State Research Service 1990a.)

Appendix E. Statistical Errors and Biosafety

Conclusions drawn from statistical analysis of a (biosafety) test might involve one of two types of error. A type I error occurs when the statistical analysis indicates that the GEO has an adverse effect when in fact no such adverse effect exists. A type II error occurs when the the analysis indicates that the GEO has no adverse effect when in fact it does have such an effect.

These two types of error have very different consequences. Consider, for example, a proposal to farm transgenic fish at a site where fish are likely to escape into a natural lake. Statistical analysis might be used to help discern whether the escaped fish could establish a population large enough to displace an important species already in the lake. Were a type I error made in the analysis, the finding would be that escaped fish are likely to displace an existing species in the lake although in fact, such an adverse effect would not happen. In this case, the proposal to farm the transgenic fish would be rejected in order to protect the lake ecosystem. On the other hand, if a type II error occurred, it would be concluded that escaped fish were unlikely to displace existing lake species when in fact they would displace an existing species. In that case (a type II error), the proposal to farm the fish would be accepted and some transgenic fish eventually would establish a reproducing population that outcompeted and displaced an existing species in the lake.

The potential for harm is greater when a type II error occurs than when a type I error occurs because most environmental and human health effects involve large time lags before recovery of the affected environment or of human well-being, and some environmental and human health effects are irreversible (Dayton 1998). Type I errors, in contrast, are usually limited to short-term economic costs (Dayton 1998).

A precautionary approach to biosafety would seek to minimize type II errors.

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Glossary of Terms

Abiotic characteristic limiting reproduction - physical factor in the environment (e.g., temperature, salinity) that limits or precludes reproduction by an organism.

Accessible ecosystem - the environment immediately accessible to an organism if it were to move or escape from a site, and more distant habitats in the contiguous environment into which the organism or its offspring can disperse.

Adverse decline - decline in population size that is undesirable or results in local extirpation or extinction of the population or species.

Agent of disease - the cause of a disease

Allergenic - having the ability to cause an allergic response.

Anadromous fishes - fish that spend the adult phase of their life cycles in salt water (or large bodies of fresh water, such as the Laurentian Great Lakes) but move up streams and rivers to spawn (e.g., Pacific salmon, Oncorhynchus spp.); anadromy is the opposite of catadromy (see below).

Aneuploid - bearing a number of chromosomes that is not an exact multiple of the haploid number typical for the species.

Antibiotic - a chemical produced by fungi or bacteria that kills or retards the growth of other microorganisms.

Apogamy - development of an embryo without the occurrence of fertilization.

Apospory - development of a gametophyte from a sporophyte without the formation of spores.

Arthropodicide - a pesticide whose action is against a specific arthropod or arthropod group.

Bearers - organisms (often fish or invertebrates) that carry their embryos (and sometimes their young) either internally or externally on or in their bodies.

Biological diversity - the number of species and their relative abundance within a given area, including also the phenotypic and genetic diversity maintained within the populations of these species.

Bioremediation - the use of organisms to remediate an environmental problem

Biotechnology - a term sometimes used to mean any biological manipulation conducted by humans to alter the phenotype or genotype of an organism; the term is also used in a more restrictive sense, as herein, to mean any form of biological manipulation using molecular biological or recombinant DNA technologies to alter the genotype and phenotype of an organism.

Budding - asexual reproduction by bud formation.

Captive breeding - controlled husbandry of an organism under conditions of confinement.

Cascading - progressing through a succession of stages, phases, or levels.

Catadromy - life history pattern of fishes which spend most of their life in fresh water but spawn in salt water (e.g., eels of the family Anguillidae). This pattern is opposite that exhibited by anadromous fishes (see above).

Chemosynthetic - ability of some bacteria and fungi to synthesize carbohydrate using energy derived from the oxidation of simple organic compounds such as iron or sulfur.

Chromosome manipulation - intentional change in the number of haploid sets of chromosomes, or intentional transfer of single chromosomes or chromosome fragments from one cell to another.

Cloning - to make multiple identical copies of part of a DNA molecule through molecular methods of biotechnology; or to make multiple identical copies of an entire organism through naturally-occurring asexual reproduction or through techniques of nuclear transplantation (see below).

Commensals - two species that interact in such a way that one is benefited while the other is not affected.

Competitive ability - ability of an organism to compete with other organisms for necessary resources (e.g., food, light).

Competitive exclusion - interaction between two species in which one species excludes the other from use of resources within a habitat; can lead to extirpation or extinction.

Competitive potential - the ability of an organism to compete with other organisms for necessary resources. See "competitive ability".

Congeneric - individuals belonging to the same genus.

Conjugation - a form of gene transfer from one bacterial or yeast cell to another. The transfer is accomplished by products of genes located on a small circular DNA molecule called a plasmid. The process of conjugation is found in nature and also used in genetic engineering.

Conspecific - individuals belonging to the same species.

Containment - the condition in which an organism or its genetic material is prevented from freely moving beyond a specific location.

Cryptic - difficult to discern, identify, or see.

Demography - study of the birth and death statistics of population and the calculation of the consequent growth or decline rates of the population.

De novo triploidy induction - spontaneous induction of triploidy from an alternate ploidy level.

Dioecious (Dioecy) - literally, two houses; having male and female reproductive organs on separate, unisexual individuals of the same species.

Diploid - bearing two haploid sets of chromosomes.

DNA (Deoxyribo Nucleic Acid) - genetic material that defines all heritable characteristics of an organism. DNA has two helical molecular backbones composed of sugar molecules (ribose) joined by phosphate molecules. Four bases (C A T G) join the two helical backbones across the middle of the molecule. Some viruses have Ribo Nucleic Acid (RNA) as their hereditary material.

Drive mechanism - a genetic element, such as a transposon, that maximizes transmission of the engineered construct from parents to progeny and causes a disproportionally large fraction of the progeny to inherit the engineered construct.

Ecosystem function - see processes of an ecosystem.

Ecosystem structure - see structure of an ecosystem.

Ecological competence - ability to survive, grow and reproduce in a specific habitat, and to maintain population numbers under ambient conditions of predation, competition, disease, and disturbance.

Environmental safety - the condition of being safe from environmental risk, detriment, or danger.

Encystment - enclosure within a resistant sac or cell; cyst formation.

Environmental effects - consequences to the environment caused by specific action(s). Examples include: (1) changes in the structure, function, or resiliency of an accessible ecosystem; (2) changes in the gene pool of populations resident in the accessible ecosystem; (3) decline in abundance of a population of threatened, endangered, or special concern species.

Epistasis - an interaction between genes in which one gene affects the expression of another.

Eukaryotic - containing a discrete, membrane-bound nucleus within the cell; includes most organisms with the exception of bacteria and cyanobacteria.

Extremely low survivorship - survival of very few individuals.

Fecundity - the number of female offspring produced per average female in the population, or the number of offspring produced per individual.

Field trial - an experiment or trial that is performed outdoors or in an uncontained environment.

Fitness - in population and evolutionary biology, the success in survival and reproduction of an individual organism, a population, or a species, relative to other individuals, populations or species; the number of offspring that survive to reproduce.

Fragmentation - asexual reproduction by detached parts, pieces, or structures of an organism.

FST - a measure of the reduction in heterozygosity of a subpopulation due to random genetic drift. It serves as a convenient and widely used measure of genetic differentiation between populations. In natural populations, observed values of FST include random drift, migration, natural selection, and mutation. In spite of the resulting complexity in interpretation, FST remains useful as an index of genetic differentiation (Hartl 1988).

Gene flow - the exchange and movement of genes within and between populations and species.

Gene introgression - incorporation of a gene into the gene pool of a population.

Genetic engineering - the deliberate production of genetically novel types of organisms through a variety of biotechnological techniques. These techniques can include: deliberate gene changes including changes in genes, transposable elements, non-coding DNA (including regulatory sequences), synthetic DNA sequences, and mitochondrial DNA; deliberate chromosomal manipulations including

manipulations of chromosome numbers and fragments; and deliberate interspecific hybridization referring to human-induced hybridization between taxonomically distinct species.

Genetic instability - any condition in which the original genetic expression of a genome undergoes spontaneous changes that alter the genome itself and (possibly) the traits expressed in its phenotype.

Genetic load - the proportion by which the population fitness is decreased by deleterious genes in comparison with an optimum genotype (Crow 1958).

Genetic swamping - inundation of a population by new or alternate genotypes of the same species or of hybrid derivatives descended form that species, with consequent change in genetic structure.

Genetically engineered food(s) - foods, food ingredients, and food additives produced through recombinant DNA techniques.

Genome - the genetic constitution of an organism; the complete set of chromosomes and all associated genes.

Genomic - of or pertaining to the genome.

Genotype - the genetic composition or make-up of an individual organism.

Growout - the growing of crops or animals to a harvestable life-stage.

Hazard - a potentially adverse outcome of an event or activity

Hematophagous - blood-feeding.

Hermaphrodite - an individual having both male and female reproductive organs. A simultaneous hermaphrodite has both types of gonads throughout its life. A sequential hermaphrodite may be protogynous (having an ovary first, then a testis) or protandrous (having a testis first, then an ovary).

Host - an organism harboring another organism (see parasitism)

Host range - all the possible organisms capable of harboring a specific organism

Indigenous - originating and growing or living in a particular geographic region or locale.

Indirect interactions - effects of one organism on (an)other organism(s) in the accessible ecosystem that occur through mechanisms involving abiotic factors or

additional species. Examples include, but are not limited to: (1) modification of the physical environment affecting its suitability as habitat for another species, and (2) cascading effects of altered trophic function in a biological community of multiple species.

Infectious material or agent - any living stage of an organism or any infectious substance(s) that can cause disease in other organisms or parts thereof.

Interspecific hybrid - an organism produced by mating between individuals of different species.

Interspecific hybridization - mating(s) between individuals of different species that result(s) in the production of viable offspring.

Interspecific reproduction - the production of progeny due to mating between individuals of different species. See interspecific hybridization.

Intervention - in pathology, an action intended to reduce the spread of disease.

Intraspecific selective breeding - the selective production of offspring for the purposes of agriculture or animal husbandry.

Introgression - the incorporation of genes of one species into the gene pool of another by backcrossing of fertile hybrids with one or both of the parent species.

Introgressive hybridization - introgression (see above) whereby the fertile hybrids backcross with the more abundant species, resulting in a population of individuals most of whom resemble the more abundant species but which also have some of the characteristics of the other parent species. A consequence of this process is loss of genetically distinct populations of one or both parent species.

Jumping genes - see transposons.

Lateral transfer - exchange of DNA and the genes it codes for directly from one organism to another rather that the vertical transfer from parent to offspring. Also called horizontal transfer, it is common among bacteria, but has also occurred in higher organisms. Plasmid-mediated conjugation, bacteriophage-mediated transduction, as well as transformation in bacteria are well-known forms of lateral transfer. Transposons (see below) are suspected of causing lateral transfer in higher organisms.

Life history - the developmental history of an organism from fertilization to death, including all changes in physiological, behavioral, and reproductive characteristics.

Marker sequence - a DNA sequence introduced into an organism for the purpose of unambiguously identifying the specified individuals or their progeny.

Metabolism - the physiological process that allows organisms to obtain the energy and materials necessary for development, growth, and reproduction.

Microcosms - small artificial systems used for performing small-scale testing in a confined environment.

Microscopic stage - any stage or phase in an organisms' life history that is microscopic in size.

Morphology - the physical appearance of an organism, including its form and structure.

Mosaic - in genetics, an individual in which component tissues bear different numbers of chromosomes.

Multitrophic interaction - measurable effects on organisms (e.g., in vigor, survivorship, reproduction, or abundance) due to feeding relationships with other organisms on one or more trophic levels.

Mutagenesis - natural or artificial procedures that cause mutations in organisms; used to create mutant organisms in research and biotechnology.

Mutation - a structural change in a gene or chromosome that alters the genotype and possibly the phenotype of an organism. Examples of mutations include base-pair changes, deletions, insertions, fusions, and chromosomal rearrangements.

Mutualism - an association between organisms of different species in which both benefit.

Mutualists - two species which both benefit from being associated.

Natural history - the typical life cycle, life stages, and behavior of an organism or species.

Negligible number of escapees - the number of escapees that is so small as to cause negligible biological or environmental consequences.

Net replacement rate - population growth rate.

Nitrogen fixation - the ability of some bacteria to remove elemental nitrogen from the atmosphere or water and convert it to nitrate, the form of nitrogen that is an essential nutrient for most forms of life.

Non-coding DNA sequences - Some of these are DNA sequences that serve as spacer regions (introns) between sequences that are parts (exons) of a complete protein coding sequence; they are spliced out of the message (mRNA) that provides a cell with complete instructions for assembling the protein. Other non-coding sequences come in a variety of longer and shorter repetitive forms; no cellular function is known for any of them.

Non-dioecious - without male and female reproductive organs on separate, unisexual individuals; monoecious.

Non-indigenous species - any species or viable biological material that enters ecosystems beyond its original range, including any such organism transferred from one region or country to another.

Non-reproductive interference - interference by one organism or species in the non-reproductive functions of another organism or species, e.g., through changes in competition, predation, parasitism, etc.

Non-target (non-target organism) - not intended to be affected by a process, technique, or event.

Novel trait - expression of a phenotypic (observable) trait not normally found in the species.

Nuclear transplantation - the process by which the haploid nucleus of an egg is removed and a genetically different diploid nucleus is implanted, causing the organism to develop the genetically determined characteristics of its new nucleus.

Nuisance - an organism or event that is undesired or bothersome.

Organelle - differentiated part of a eukaryotic cell that is responsible for a discrete function within the cell; examples of organelles are include the nucleus, mitochondria, and chloroplasts.

Overall phenotype - the sum of physical and biological characteristics of an organism.

Parasitism - relationship between two organisms in which one organism (the parasite) derives benefit by growing in or on another organism (the host), and in which the host organism either derives no benefit or is harmed.

Parental organism(s) - the organism(s) to be used as parents in cross-breeding, or the initial organism which is the recipient of introduced genetic material, or whose genome is to be altered by addition, removal, or rearrangement of genetic material.

Parthenogen - an organism that develops from an egg without fertilization.

Parthenogenesis - development of an individual from an egg without fertilization.

Pathogenicity - the ability to cause disease.

Permanently sterile - unable to gain or regain the ability to reproduce sexually.

Persistence - the ability to continue through time.

Phenology - study of phenomena, such as flowering, which occur periodically. The seasonal timing of the life cycle of any type of organism.

Phenotype - the observable physical or biochemical characteristics of an organism as determined by both its genetic make-up and environmental influences.

Photosynthetic - the ability of some bacteria, algae and green plants to use sunlight as a source of energy to create the carbon-based organic chemical compounds essential for life.

Plasmid - a small, circular molecule of DNA that may contain a variety of genes. Found in bacteria, although many artificial ones have been made. Some are capable of conducting their own transmission from one bacterial cell to another, and may also cause other plasmids that are not self-transmissable to also move between cells.

Pleiotropic effects - see pleiotropy.

Pleiotropy - the phenomenon whereby a single gene is responsible for a number of distinct and seemingly unrelated phenotypic effects.

Ploidy - a multiple of the basic number of full sets of homologous chromosomes in a cell. Haploidy indicates a single set of chromosomes. Diploidy indicates two full sets of homologous chromosomes. Higher ploidy levels are also known, e.g. triploidy, tetraploidy, hexaploidy, etc.

Polyploidy - having a number of chromosome sets that is greater than two.

Populations of special concern - populations that are centers of diversity, recognized national treasures, of scientific value, of spiritual importance, that have been identified as as threatened, endangered, or declining.

Predation success - success in feeding or in the capture of prey.

Processes of an ecosystem - the biological, chemical, or physical processes occurring in an ecosystem. Also called ecosystem function.

Prokaryote - characterized by the absence of a nucleus, nuclear membrane, and other membrane-bound organelles. Includes bacteria and cyanobacteria.

Propagule - asexual portions of an organism that are capable of dispersal and formation of a new individual.

Protein-encoding DNA sequences - These are either single stretches of DNA coding for a single protein, or parts of a complete protein-coding sequence that is spliced together by removing intervening (intron) sequences and joining the coding parts (exons) when the final RNA message is formed to provide instructions for the exact amino acid sequence of a given protein.

Recruitment - addition of new individuals to a population.

Regulatory DNA sequences - gene sequences that do not code for proteins that go into the structure or metabolism of an organism. They serve to turn other genes on or off, or they increase or decrease the activity of protein coding genes, yielding more or less production from these genes. It is often said that these kinds of genes control or regulate the expression of other genes.

Remediation - a process by which damage is fixed, repaired, or returned to an original condition.

Reproductive interference - disruption of the reproduction of a natural population, e.g., through changes in breeding behavior or by fertilization of eggs by aneuploid sperm.

Reproductive potential - capability for future reproduction.

Reproductively mature age - the age at which an individual first becomes reproductive; sexual maturity.

Resiliency - is the ability (of an ecosystem) to recover to a previous state or condition after a major change or disturbance.

Resistance / Resistant - the ability of either organisms or enzymes to counter the effects of toxic materials or disease or harmful environmental agent(s). Examples are resistance to malaria, or to antibiotics, insecticides, herbicides, or poisonous metals such as mercury, lead and cadmium. An organism or enzyme which exhibits resistance is said to be resistant.

Restriction enzymes - naturally occurring enzymes that cleave DNA molecules

at specific sites to produce short fragments.
Risk - the probability of a specific hazard (or set of hazards) occurring

Seed bank - the population of seeds in the soil that can potentially germinate and grow. Seeds can remain viable in the soil until conditions appropriate for germination occur.

Selection pressures - natural or artificial force that favors survival of one individual or group over another individual or group in the same environment or ecosystem.

Self-fertilizing hermaphrodites - organisms having both male and female reproductive organs that are capable of reproduction by fertilization of their own eggs.

Special concern - See populations of special concern.

Species - a group of organisms and populations that shares common genetic and phenotypic properties and are capable of interbreeding.

Sterile - unable to reproduce by sexual means.

Steroidogenesis - production of steroids by living organisms.

Stochastic variability - changes or differences resulting from chance or random events.

Structure of an ecosystem (ecosystem structure) - the relationship(s) between component parts of an ecosystem, primarily the biological relationship(s) among species in the use of food, space, and other resources.

Susceptible organism - an organism highly sensitive to or likely to be affected by a toxic material or element or a particular disease or vector

Symbionts - two or more individuals that interact closely, to the benefit of one or more of the participants.

Target (target organism) - intended to be affected by a process, technique, or event.

Tetraploid - individual bearing four haploid sets of chromosomes.

Toxin - a chemical substance poisonous to at least some organisms.

Transcription - the synthesis of messenger RNA from DNA.

Transduction - a form of gene transfer among bacteria (found in nature and also used in genetic engineering). The transfer is accomplished by a bacterial virus called a bacteriophage (or just phage). After the bacteriophage has replicated (copied itself) numerous times within its host bacterial cell, it forms protein wrapped viral particles containing its own DNA and often some parts of the host chromosomal DNA. After bursting the host, these particles can infect a new host and donate the chromosomal DNA sequences to the new host, often changing the genetic makeup of the new host.

Transformation - a form of gene transfer among, for example, bacteria; the process is found in nature and also used in genetic engineering. During transformation, one bacterial cell copies its DNA and releases the copy into the environment, or it dies and its DNA becomes free in the environment. Another cell takes in the free DNA and with some frequency exchanges it for the same region of DNA in its own chromosome. If the process brings in different (variant) forms of the genes, the receiving cell is said to be transformed. An example would be the substitution of a gene for antibiotic resistance for its susceptible counterpart.

Transgenic organism - An organism whose genetic composition has been altered to include selected genes from other organisms of the same or different species by methods other than those used in traditional breeding. (Adapted from The Language of Biotechnology: A Dictionary of Terms. John M. Walker and Michael Cox 1988. American Chemical Society Professional Reference Book: 238).

Translation - the process through which messenger RNA directs protein synthesis.

Transmissibility - the ability to be transmitted; in this case, the ability of an organism to transmit a disease to a range of organisms

Transposons - small DNA molecules that can move in and out of specific positions within the same chromosome or another chromosome of the same or different cell or plasmid. In moving, they may or may not leave a copy of their DNA base sequence behind. They are some times referred to as "jumping genes" or "selfish DNA". They are not well understood, but it is certain that they sometimes cause mutations.

Triploid - an individual bearing three haploid sets of chromosomes.

Trophic level - position in the food chain.

Trophic relationship - relationship in feeding habits between organisms of the same or different trophic level(s).

True parthenogen - an organism which reproduces exclusively through parthenogenesis; i.e., its reproduction never involves normal fertilization of an egg.

Unintentional trait changes (IV.A) - changes (to phenotypic traits) that are not deliberately made; can occur due to pleiotropy or epistasis.

Vagility - the tendency for an organism to disperse within or between environments.

Vector - a carrier; an organism that carries disease-causing microorganisms from one host to another, or a living organism that carries pollen or viruses from one plant to another.

Virulence - ability to cause infection, disease, or toxicity.

Zone of tolerance - for a given physical or environmental factor, the range of values over which an organism can survive (Fry 1971); organisms can acclimate to changing values of the factor within the zone of tolerance.

The Edmonds Institute

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Part Two: Flowcharts and Worksheets

by

Scientists' Working Group on Biosafety

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Late in 1995, the member nations and regional groups of the Convention on Biological Diversity, called for development of an international protocol on biosafety which would "take into account the principles enshrined in the Rio Declaration on Environment and Development, and in particular, the precautionary approach...". A few months after that "call", the Edmonds Institute, a public interest, non-profit organization, invited a group of scientists from a broad range of disciplines to develop a biosafety handbook accessible to the public and reflective of maximum concern for ecological and human health. The scientists undertook to help both consumers and policy-makers evaluate likely impacts of genetically engineered organisms in a variety of settings and applications. The group met in a week-long workshop and their discussions led to a volume entitled *DRAFT Assessment of Genetically Engineered Organisms in the Environment: The Puget Sound Biosafety Handbook.* Subsequently, a second group of scientists, with a majority of the same members as the first, was asked to revise and expand the draft publication, to extend its scope to include a greater diversity of organisms. This manual is the result of their work.

In a very real sense, this manual is the gift of Mark Wheelis, Andrew Spielman, Philip Regal, Deborah Letourn4eau, Terrie Klinger, Anne Kapuscinski, Conrad Istock, Elaine Ingham, Norman Ellstrand, Pushpa Bhargava, and Sharon Akabas. The Edmonds Institute is indebted to them for the generosity with which they shared their time, their expertise, and their patience. We are similarly indebted to Michael Holmes, John Fagan, and Chris Mundt who were part of the group whose discussions and writings contributed to the draft that preceded this manual.

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Beth Burrows
President/Director
The Edmonds Institute

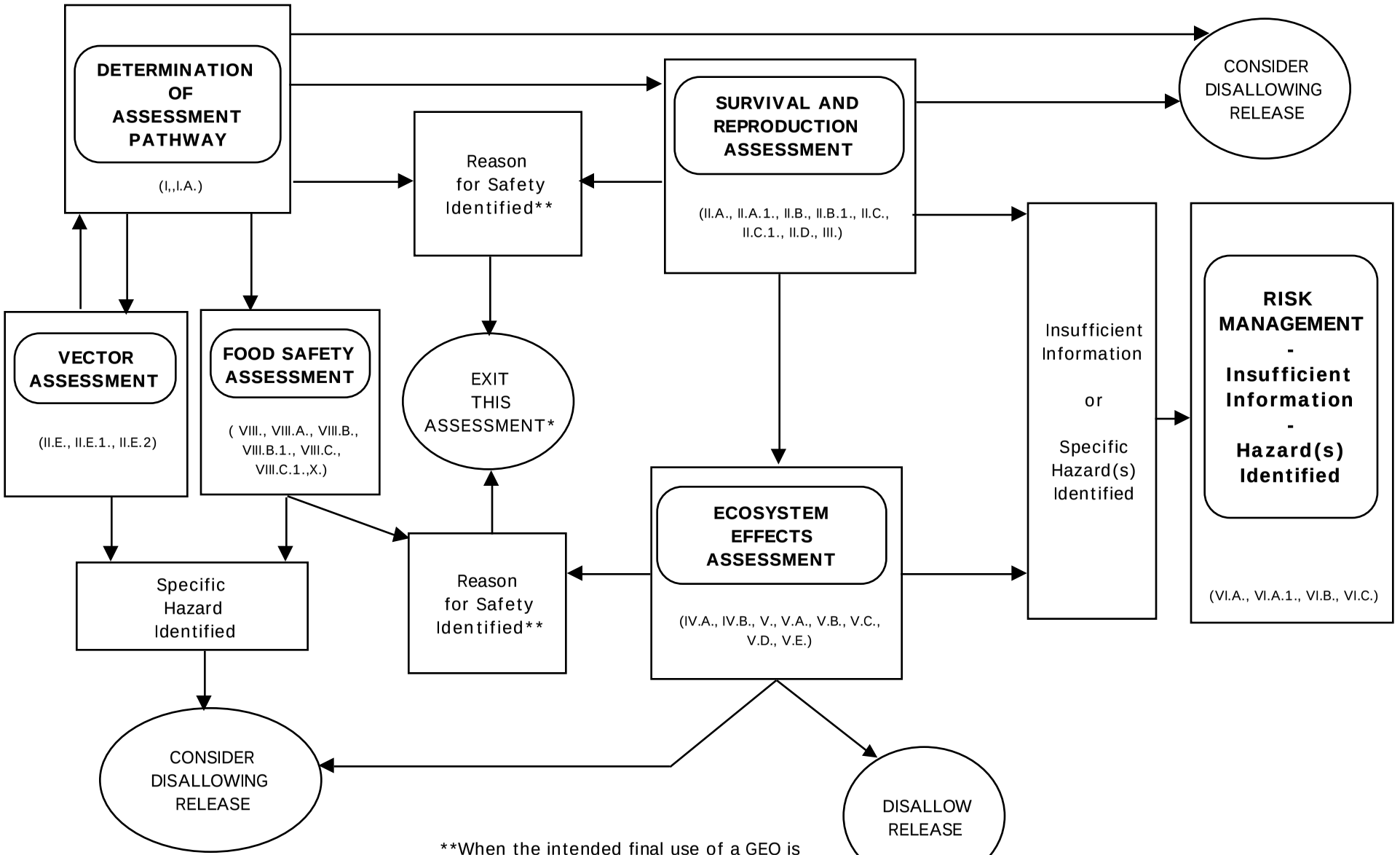
Contents

Part Two

FLOWCHART DIAGRAMS

OVERVIEW of FLOWCHARTS

(supporting text: page 23)



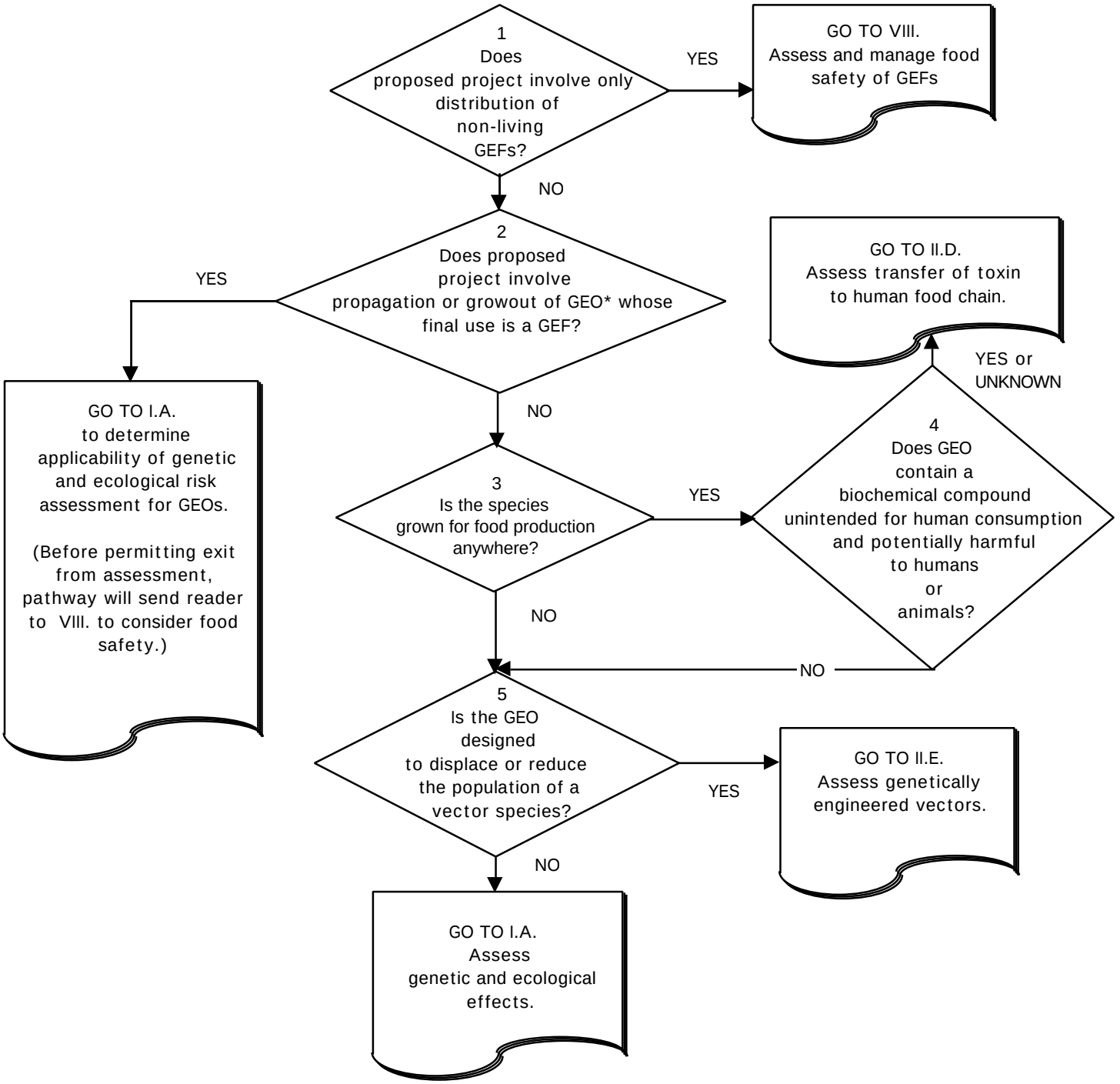
*In some cases, consult relevant government authorities for guidance on non-indigenous species.

**When the intended final use of a GEO is as a GEF, the assessment sends the reader to Food Safety Assessment before permitting exit.

I. Determination of Assessment Pathway

(supporting text: page 23)

The assessment is based on the precautionary principle. If answers to the questions in the assessment are unknown, the user is directed to further questions that will help determine appropriate risk management.

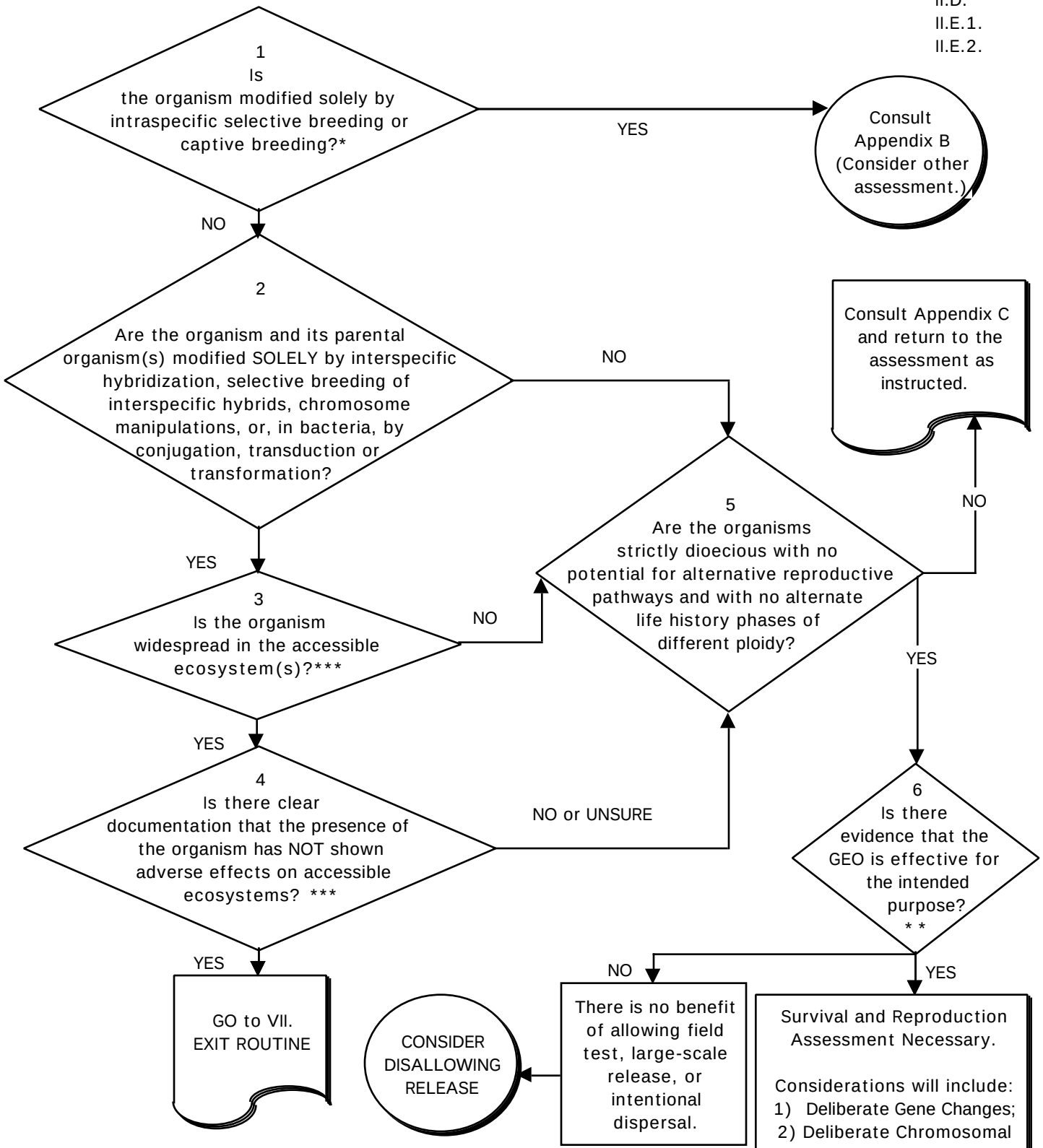


* As discussed in the text (see page 24), this assessment may be used for certain organisms that are the products of interspecific hybridization or chromosomal manipulation. For those working with such organisms and using this assessment, wherever the term "GEO" is encountered, the term should be taken to include their organism (although the organism is not included in the definition of a GEO used in this Manual (see page 2).

I.A. Continuation of Pathway Determination

(supporting text: page 24)

from I.
II.D.
II.E.1.
II.E.2.



* If the organism is a non-indigenous organism, consult relevant government agencies.

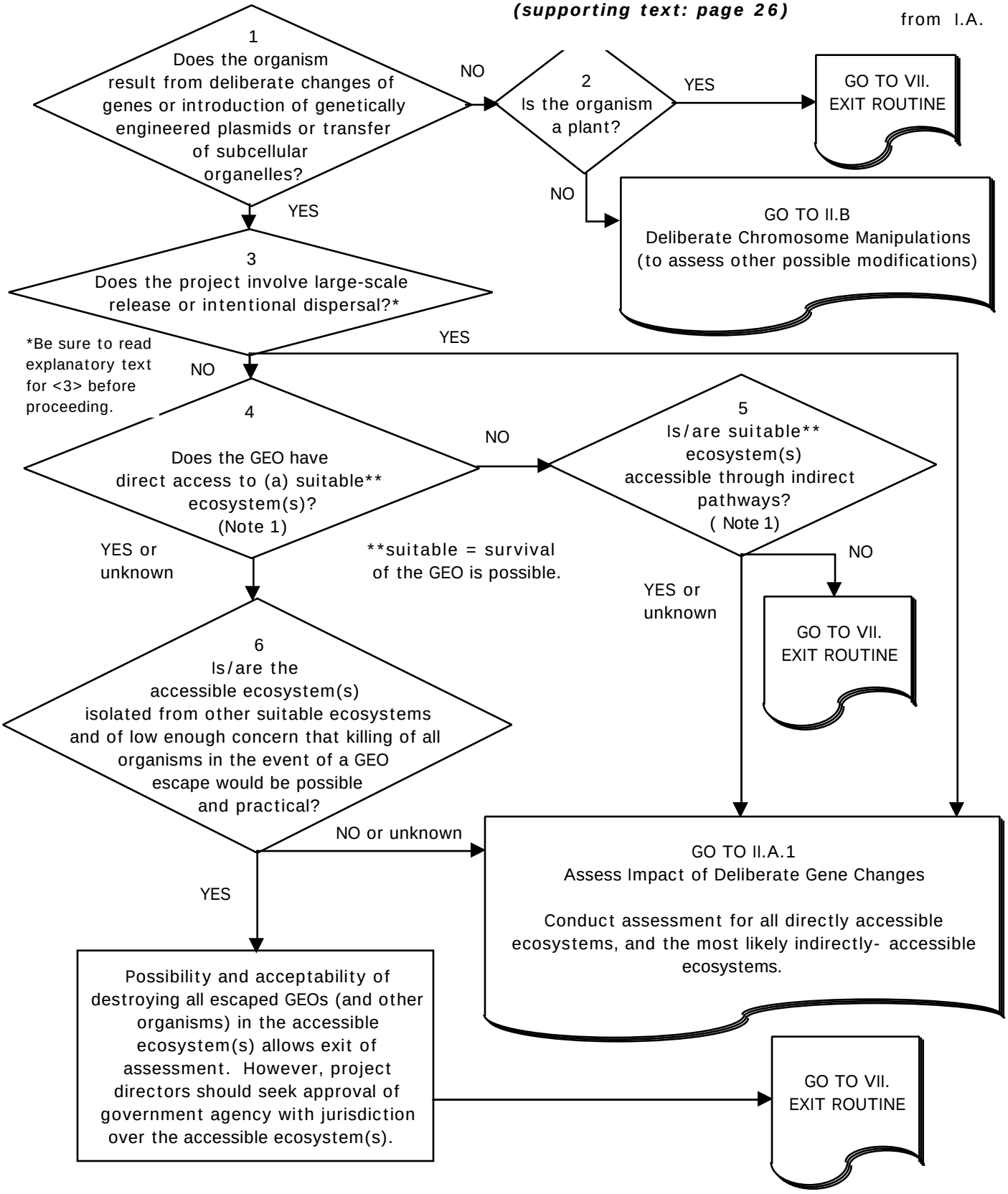
** For research and development projects, identify hazards if laboratory results indicate sufficient potential for effectiveness to warrant proceeding to field testing. For intentional dispersal or large scale release, identify hazards if lab and field test results prove efficacy.

*** For organisms or propagules capable of long-range dispersal (e.g. bacteria, fish, mobile insects, pollen) all accessible ecosystems must be considered.

II.A. Survival and Reproduction Assessment - Deliberate Gene Changes

(supporting text: page 26)

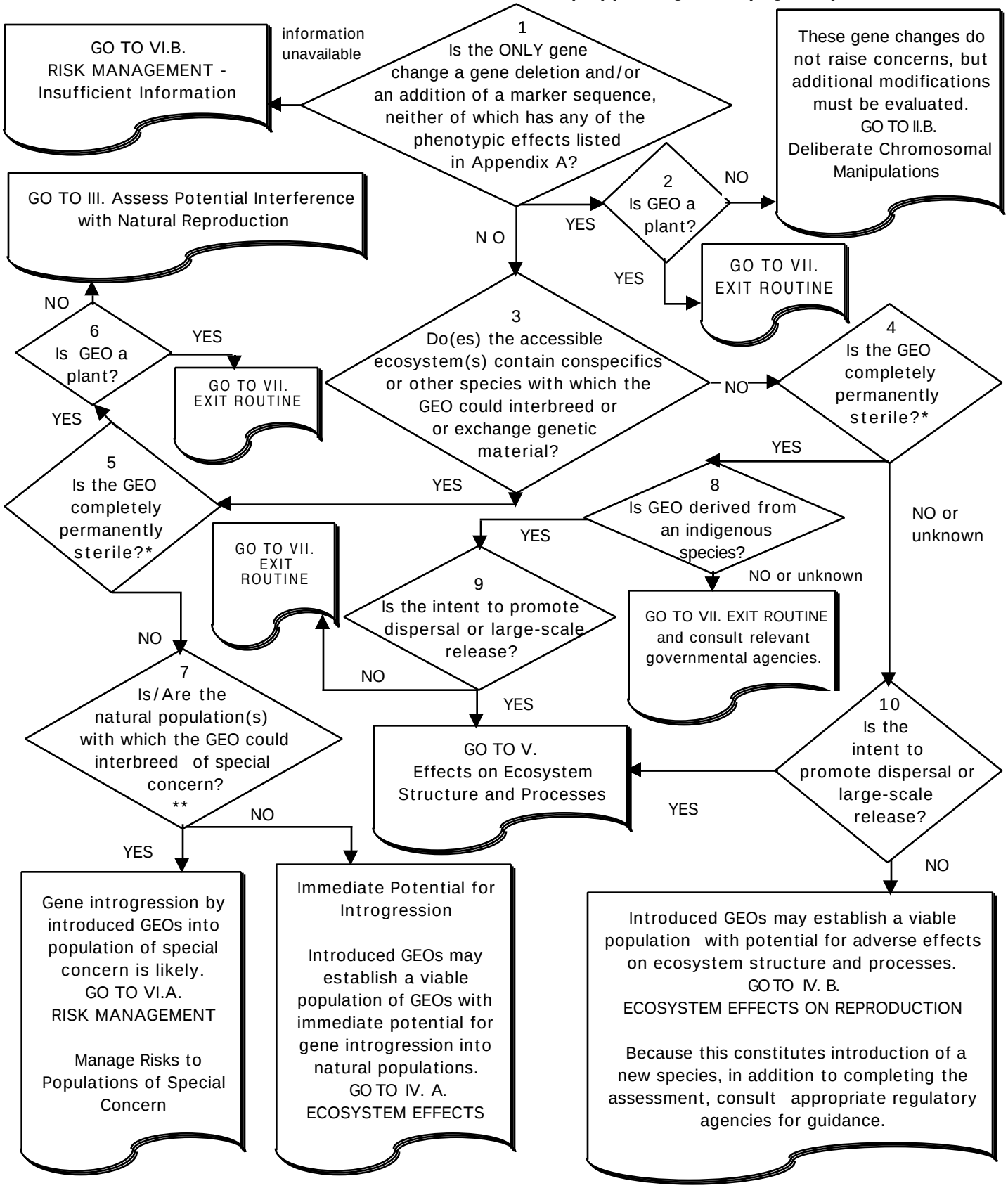
from I.A.



Note 1: Direct or indirect access is possible through numerous natural and human-created physical pathways. See Table 1 for routes of dispersal. For example, paths to consider for aquatic organisms include navigation canals, and interbasin water transfers (e.g., irrigation, municipal water supply, etc.).

II.A.1. Impact of Deliberate Gene Changes *(supporting text: page 29)*

from II.A.

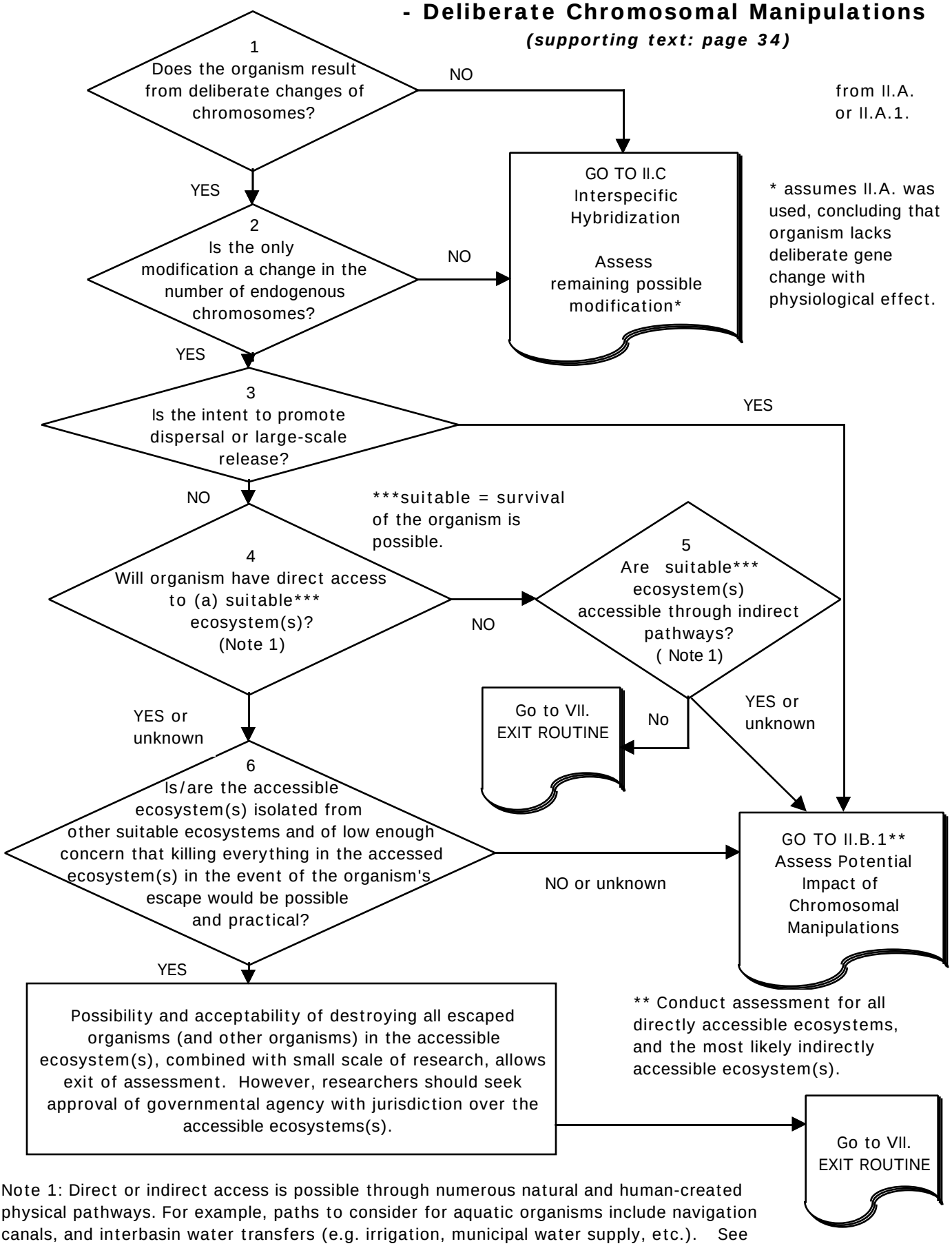


*Respond NO if organism is asexual or vegetatively reproducing or produces viable gametes (see Appendix C).

**Populations might be of special concern because they are: centers of diversity, national treasure, of scientific value or spiritual importance, threatened, endangered, or declining. If YES, one option is to move to a site where no species of concern are present. However, if this is considered, other topics in the assessment must be addressed. To explore the potential implications of site relocation, answer NO here and continue.

II.B. Survival and Reproduction Assessment
- Deliberate Chromosomal Manipulations

(supporting text: page 34)

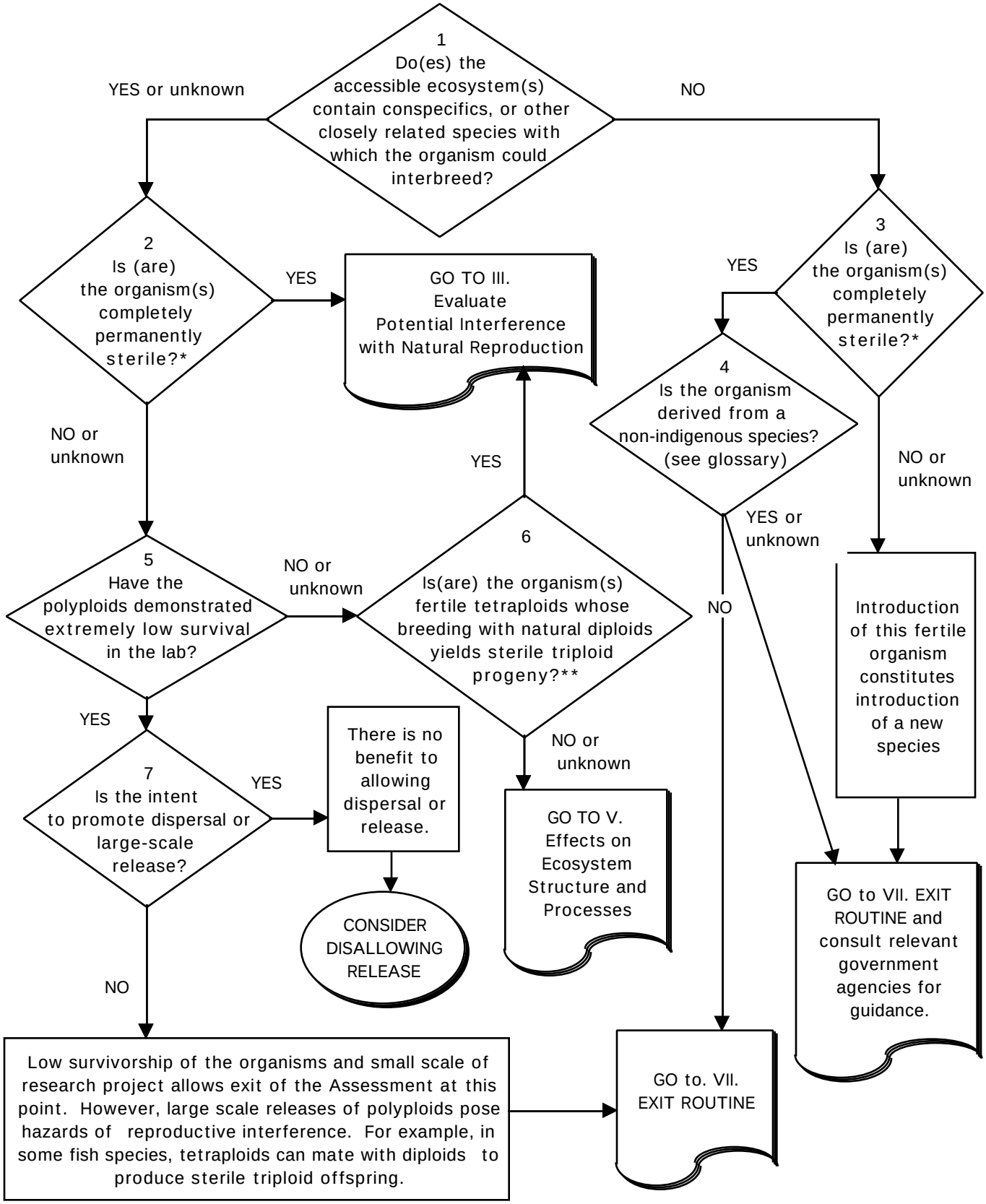


Note 1: Direct or indirect access is possible through numerous natural and human-created physical pathways. For example, paths to consider for aquatic organisms include navigation canals, and interbasin water transfers (e.g. irrigation, municipal water supply, etc.). See Table 1 in this Manual and Appendix A: Table 2 in the Performance Standards for Fish and Shellfish (ABRAC).

II.B.1. Impact of Deliberate Chromosomal Manipulations

(supporting text: page 36)

from II.B.



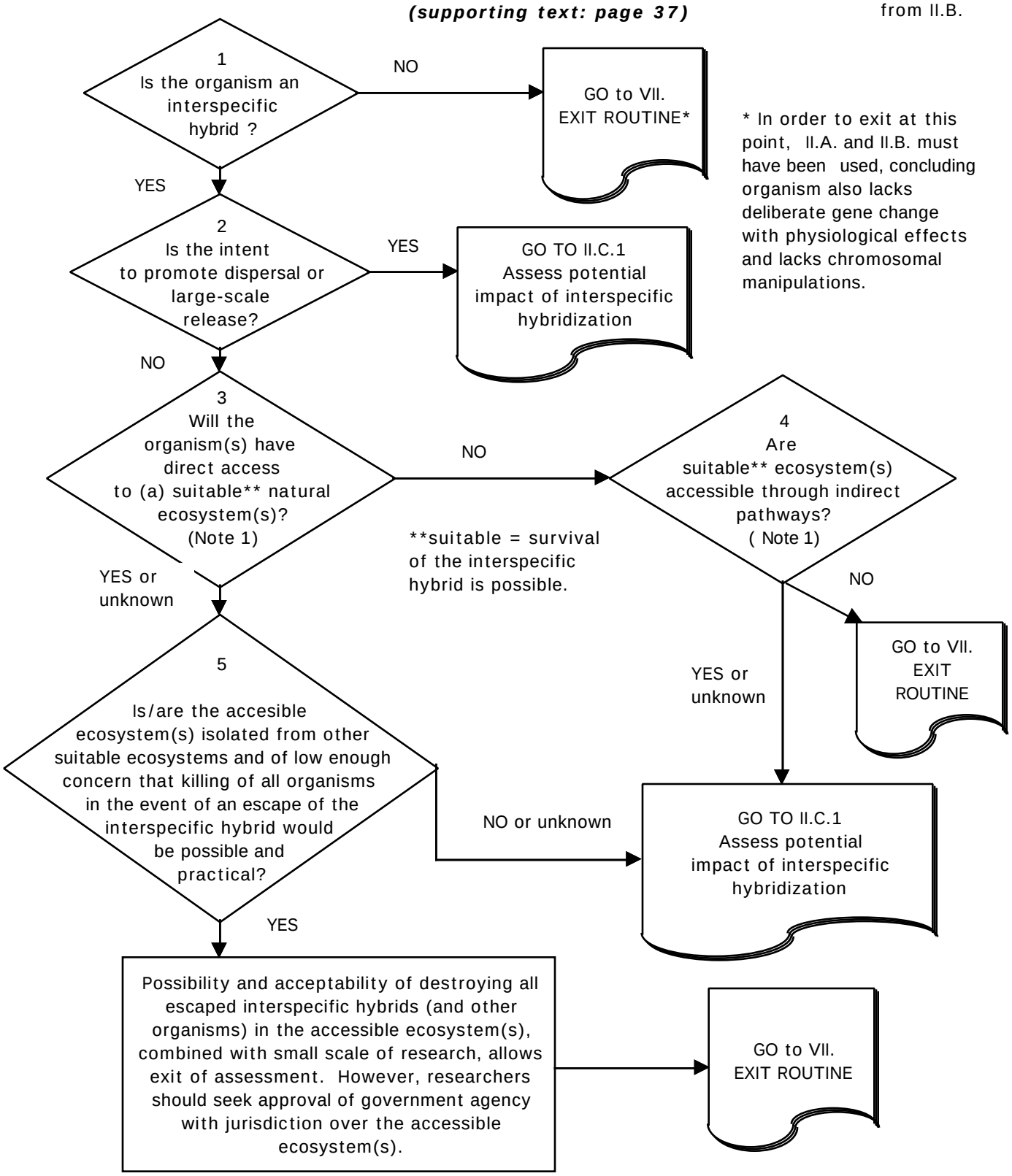
* Respond "NO" if the organism can reproduce from one individual (see Appendix C).

** This is known to occur in some species of fish and shellfish. Applicability of this to other species is under consideration.

II.C. Survival and Reproduction Assessment - Interspecific Hybridization

(supporting text: page 37)

from II.B.



* In order to exit at this point, II.A. and II.B. must have been used, concluding organism also lacks deliberate gene change with physiological effects and lacks chromosomal manipulations.

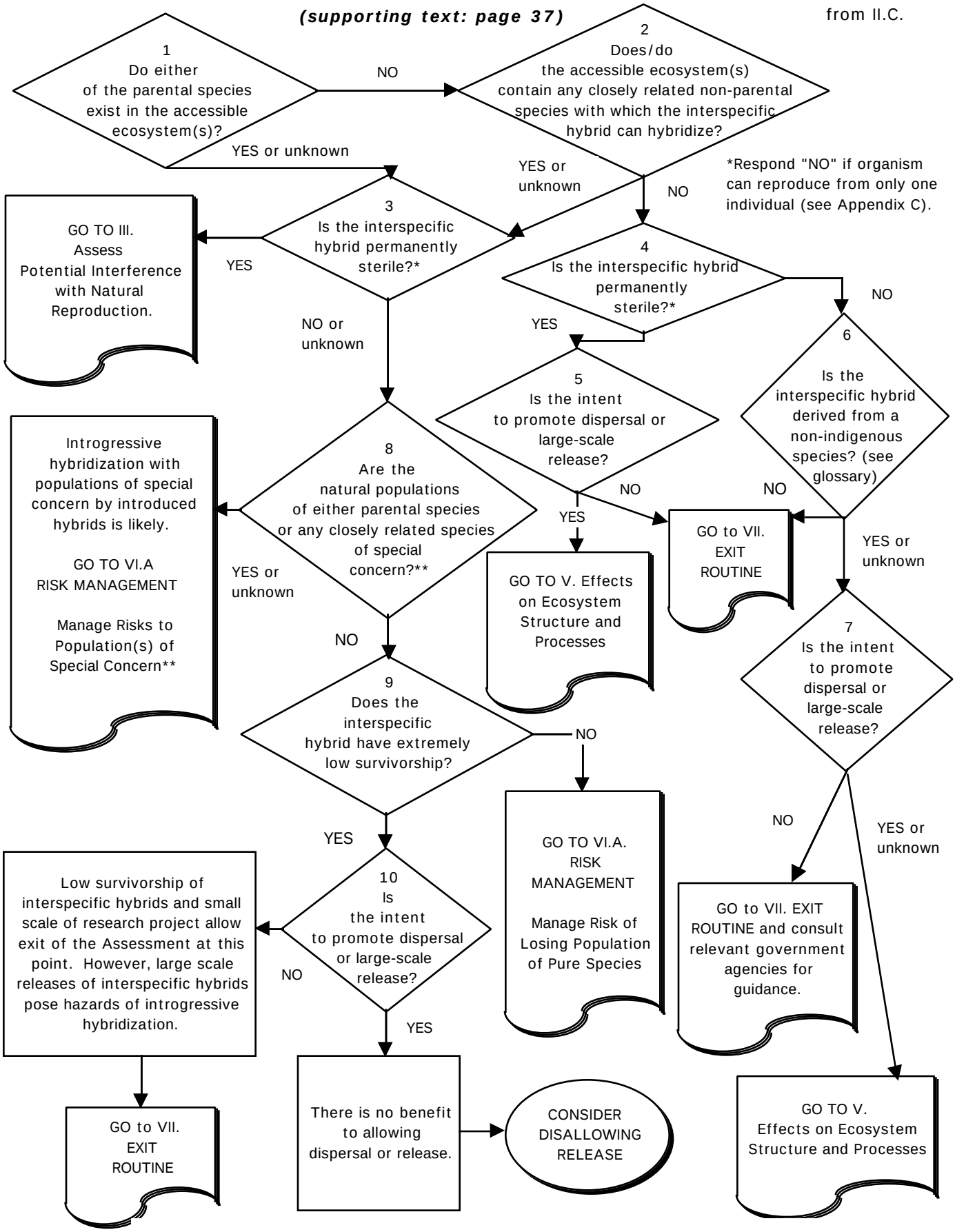
**suitable = survival of the interspecific hybrid is possible.

Note 1: Direct or indirect access is possible through numerous natural and human-created physical pathways. For example, paths to consider for aquatic organisms include navigation canals, and interbasin water transfers (e.g. irrigation, municipal water supply, etc.). See Table 1 in this Manual and also Appendix A: Table 2 in the Performance Standards for Fish and Shellfish (ABRAC).

II.C.1. Impact of Interspecific Hybridization

(supporting text: page 37)

from II.C.

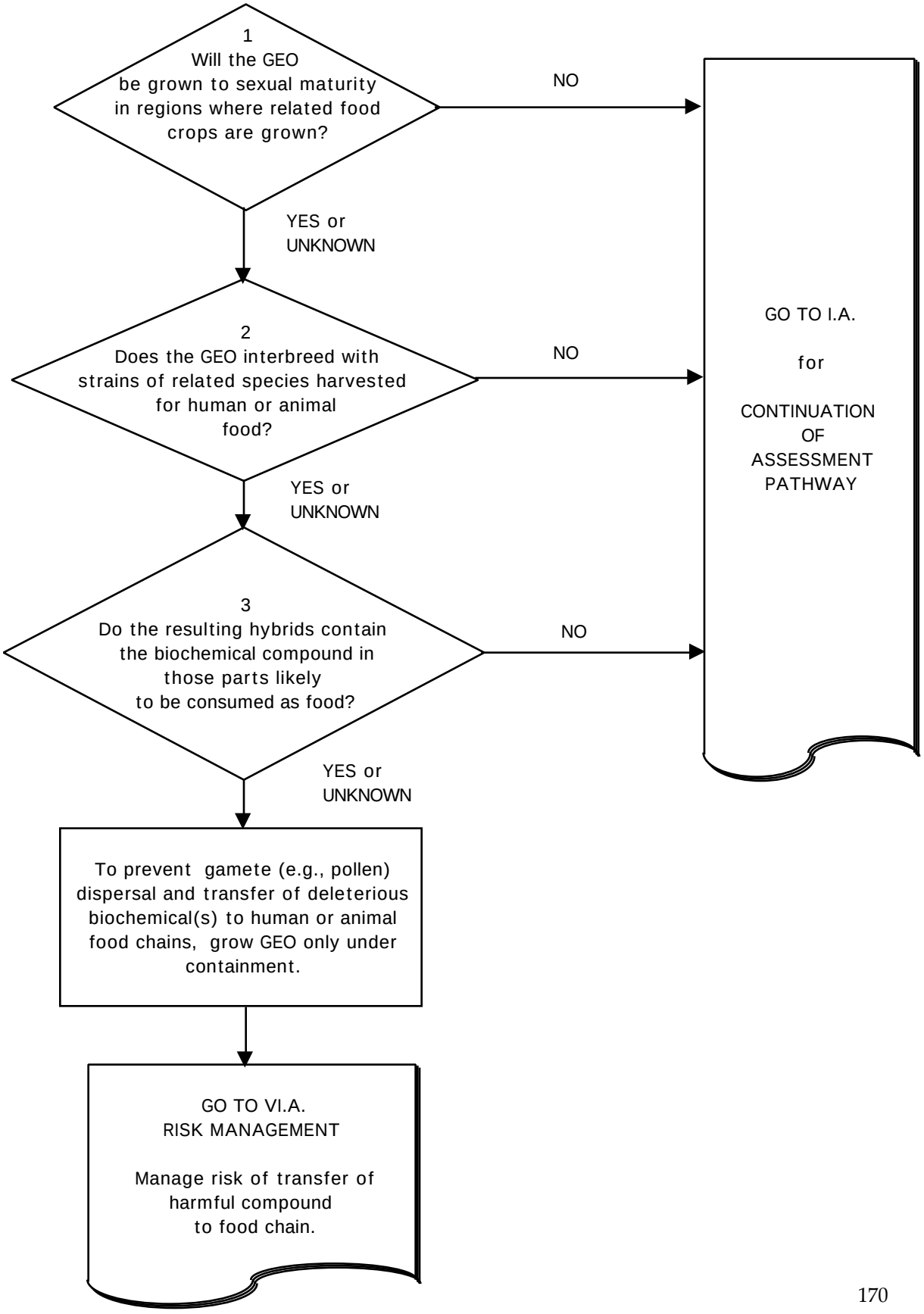


**Populations might be of special concern because they are: centers of diversity, national treasure, of spiritual importance, threatened, endangered, or declining.

II.D. Transfer of Harmful Biochemical Compounds to Food Chain

(supporting text: page 39)

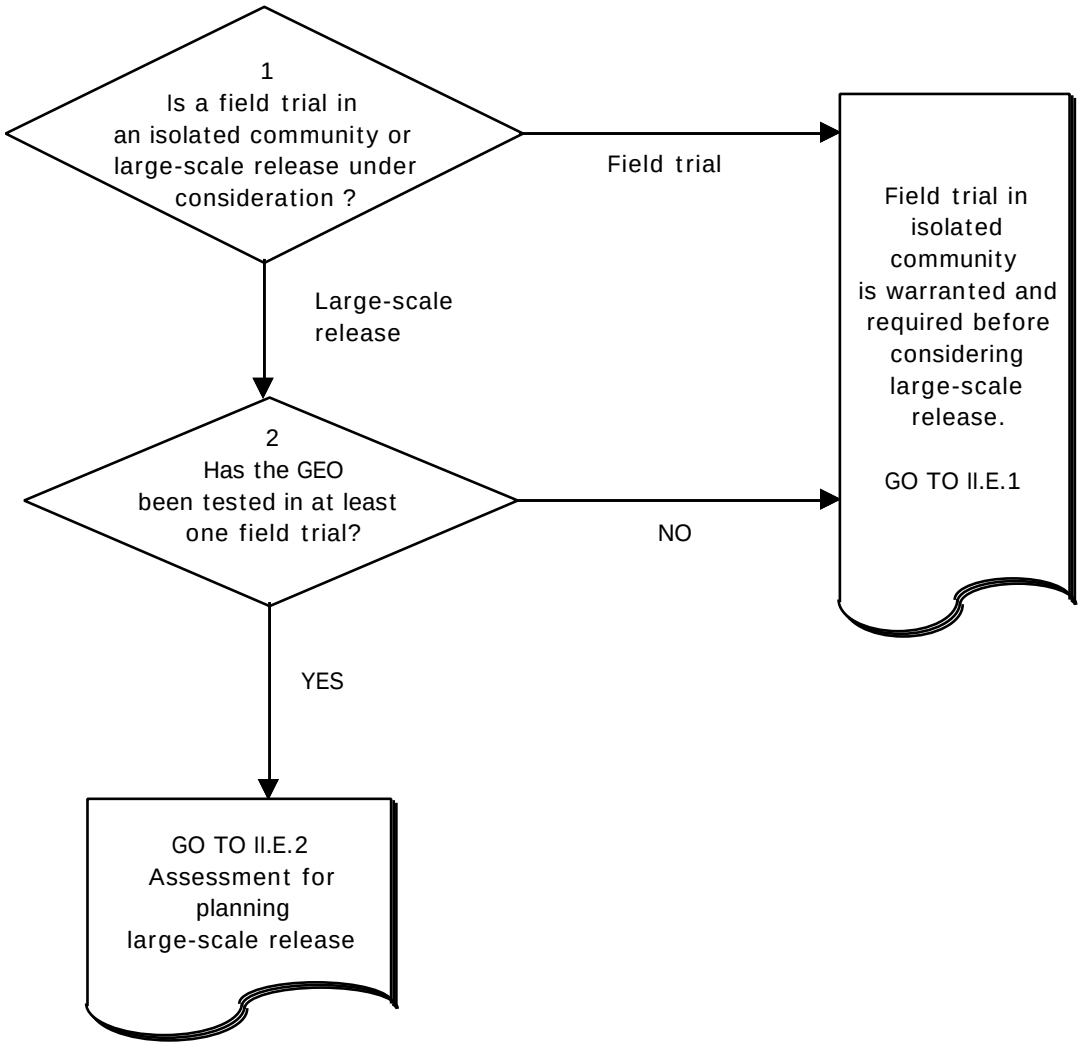
from I.



II.E. Vectors Genetically Engineered to Reduce Disease

from I.

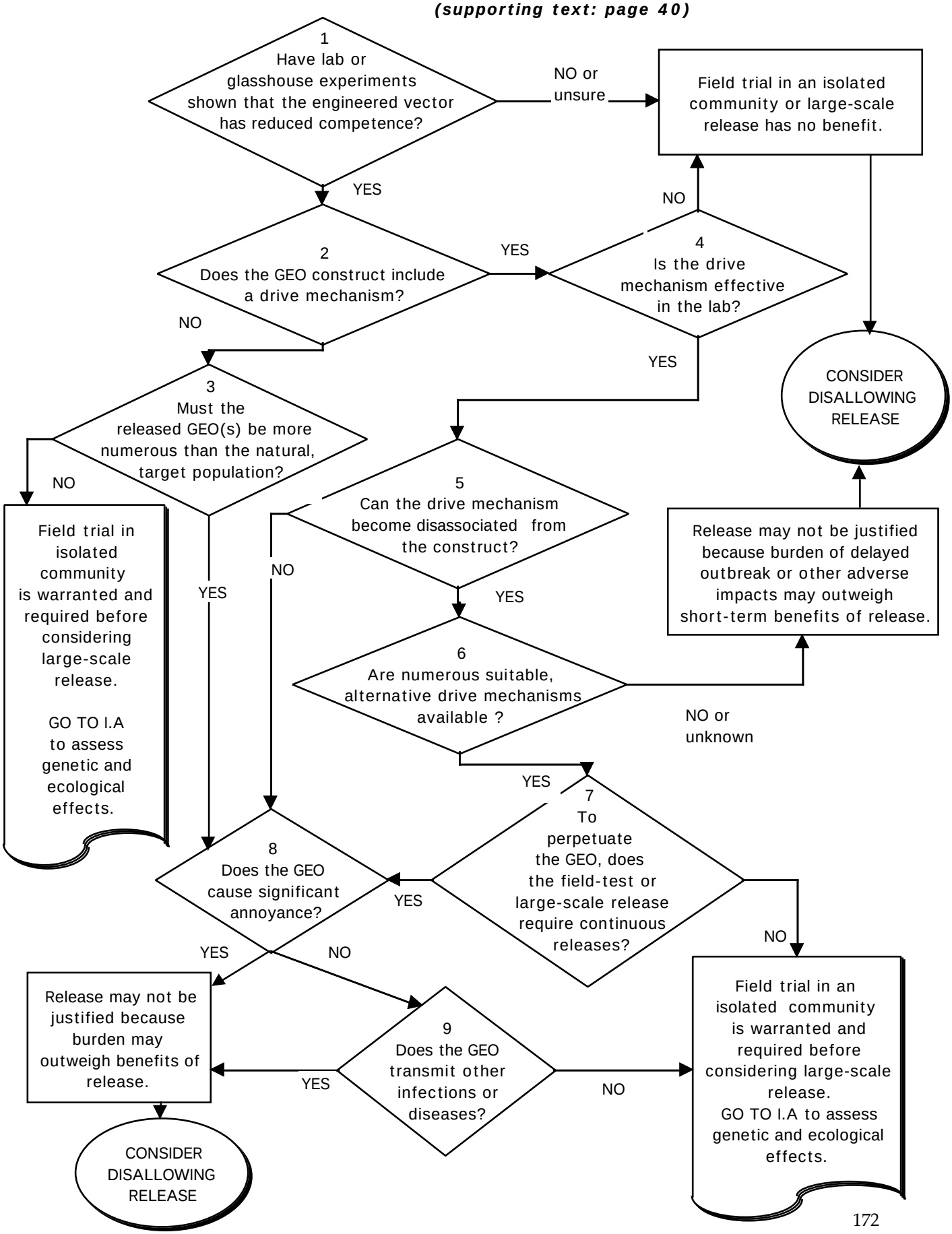
(supporting text: page 39)



II.E.1. Genetically Engineered Vectors - Field Trials

from II.E

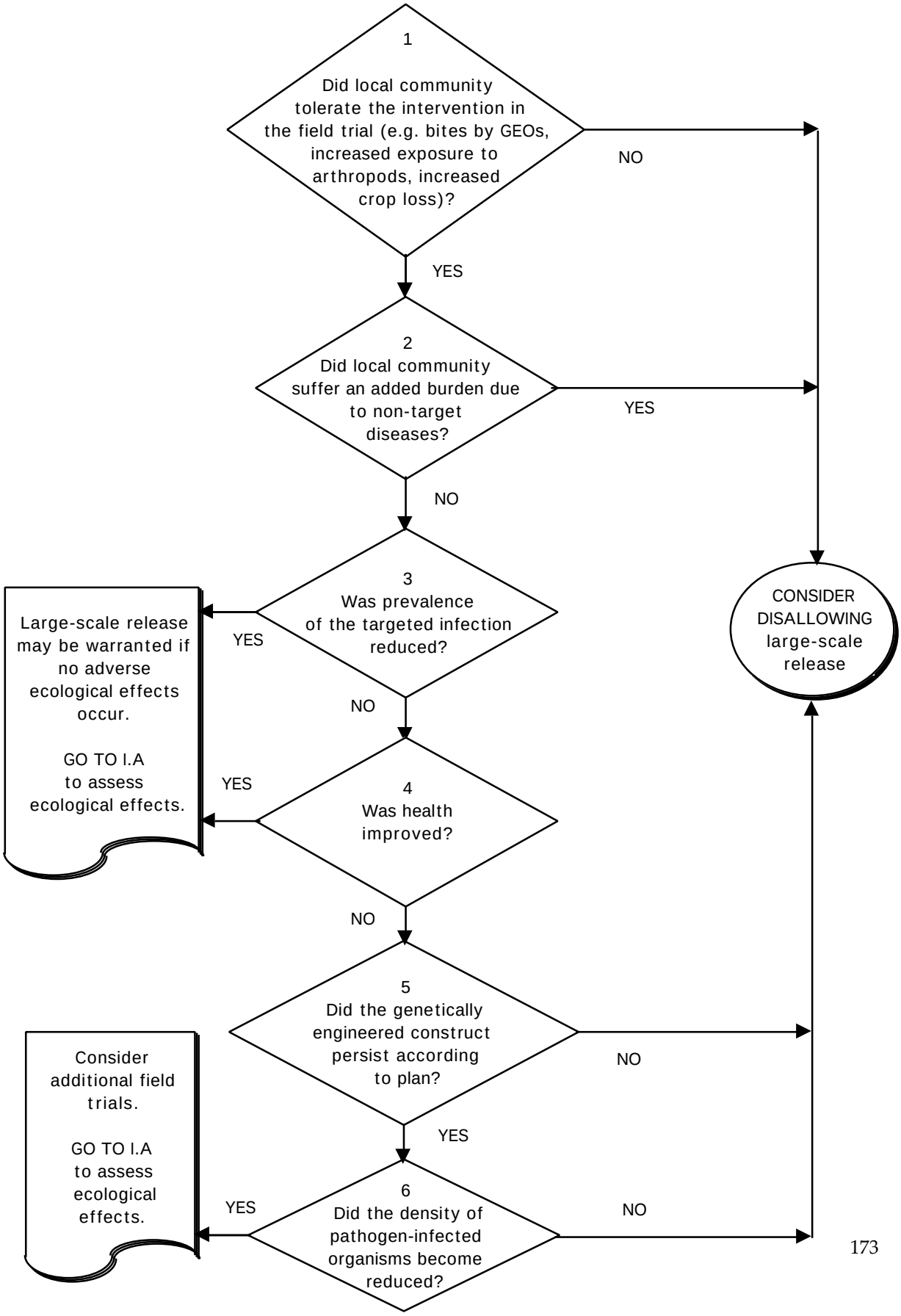
(supporting text: page 40)



II.E.2. Genetically Engineered Vectors - Large-scale Release

from II.E

(supporting text: page 42)



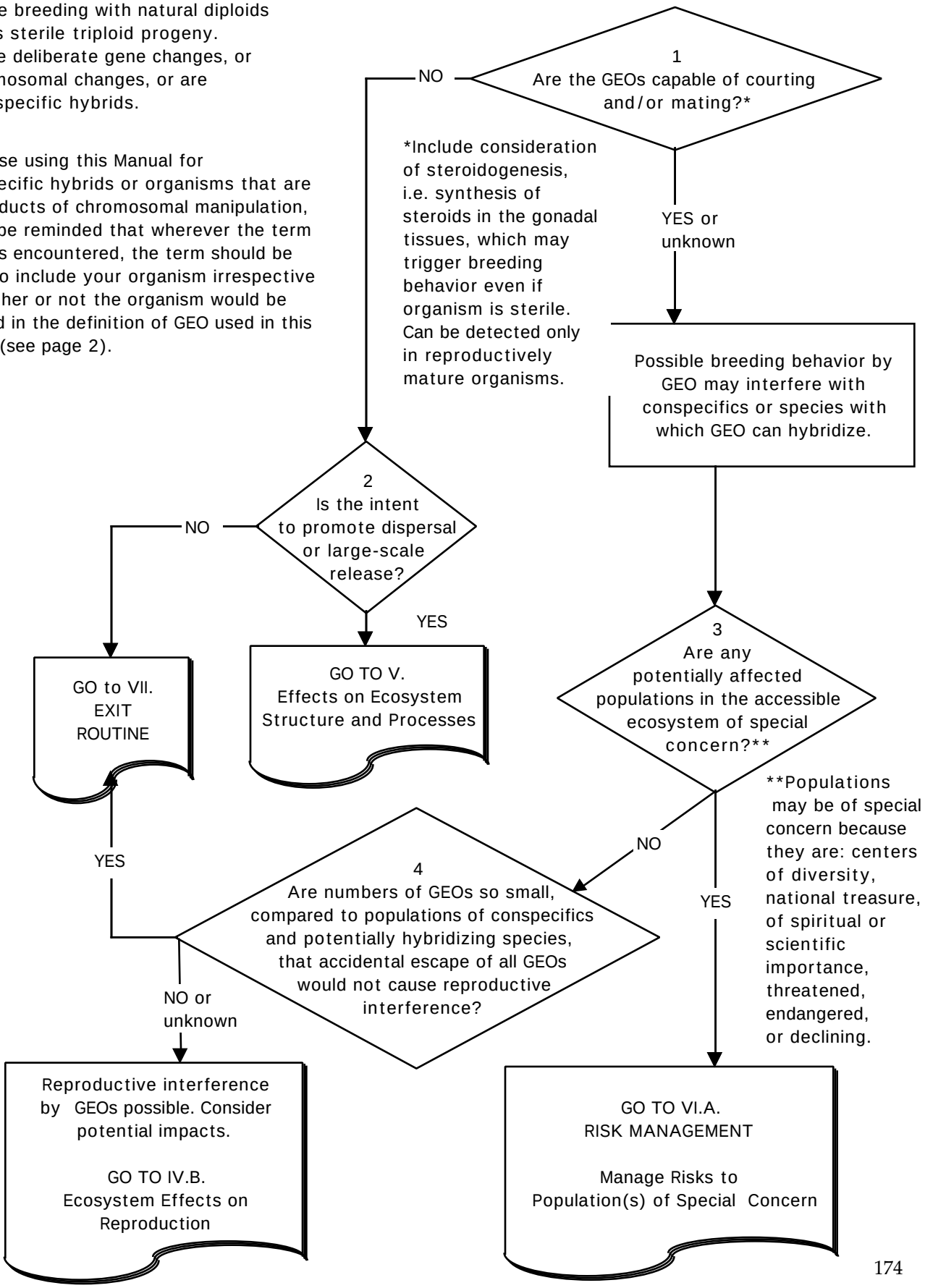
III. Potential Interference with Natural Reproduction

from II.B.1.
or II.C.1

(supporting text: page 44)

These organisms:
- ARE sterile or are fertile tetraploids whose breeding with natural diploids yields sterile triploid progeny.
- have deliberate gene changes, or chromosomal changes, or are interspecific hybrids.

For those using this Manual for interspecific hybrids or organisms that are the products of chromosomal manipulation, please be reminded that wherever the term "GEO" is encountered, the term should be taken to include your organism irrespective of whether or not the organism would be included in the definition of GEO used in this Manual (see page 2).

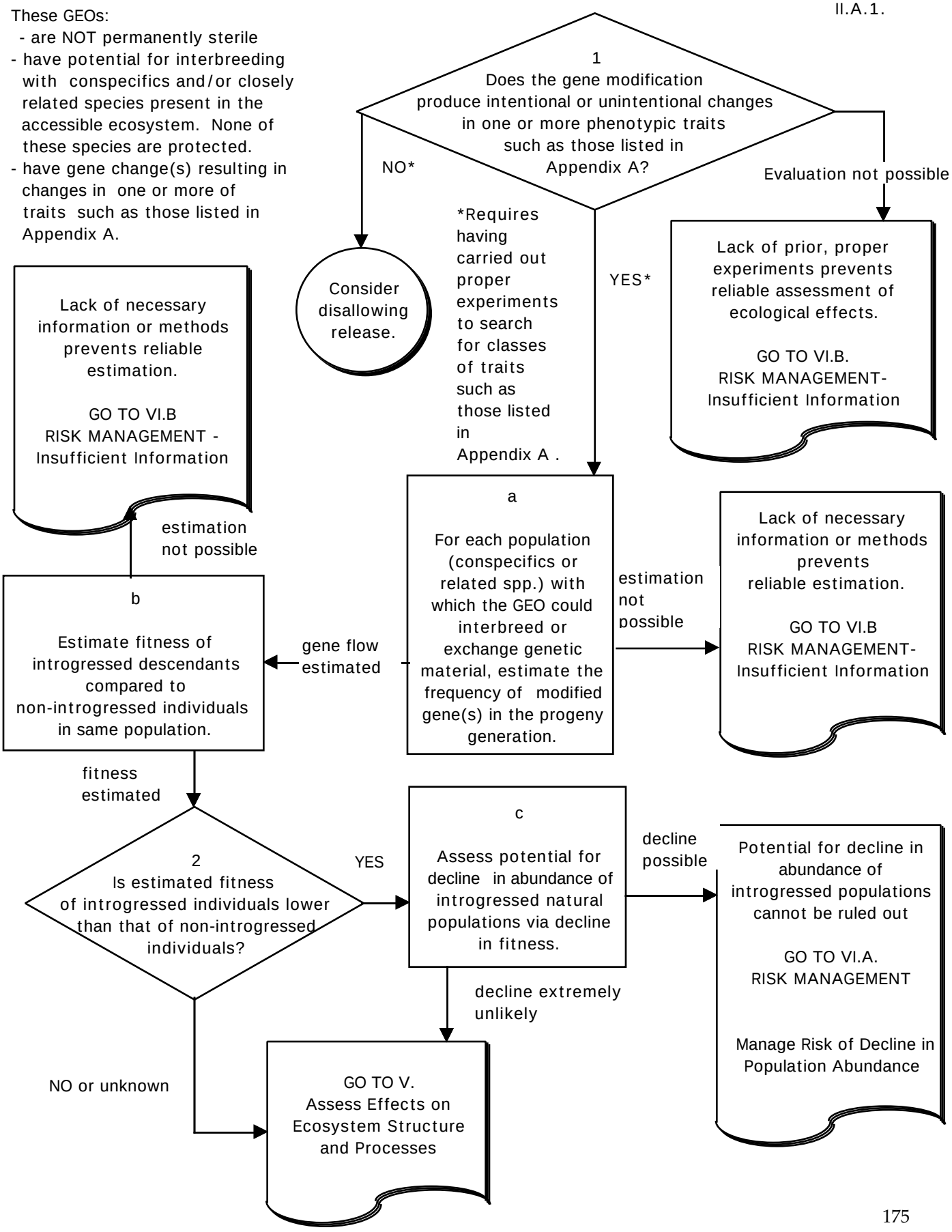


IV.A. Ecosystem Effects - Impacts of Introgression of Modified Gene(s)

(supporting text: page 46)

from II.A.1.

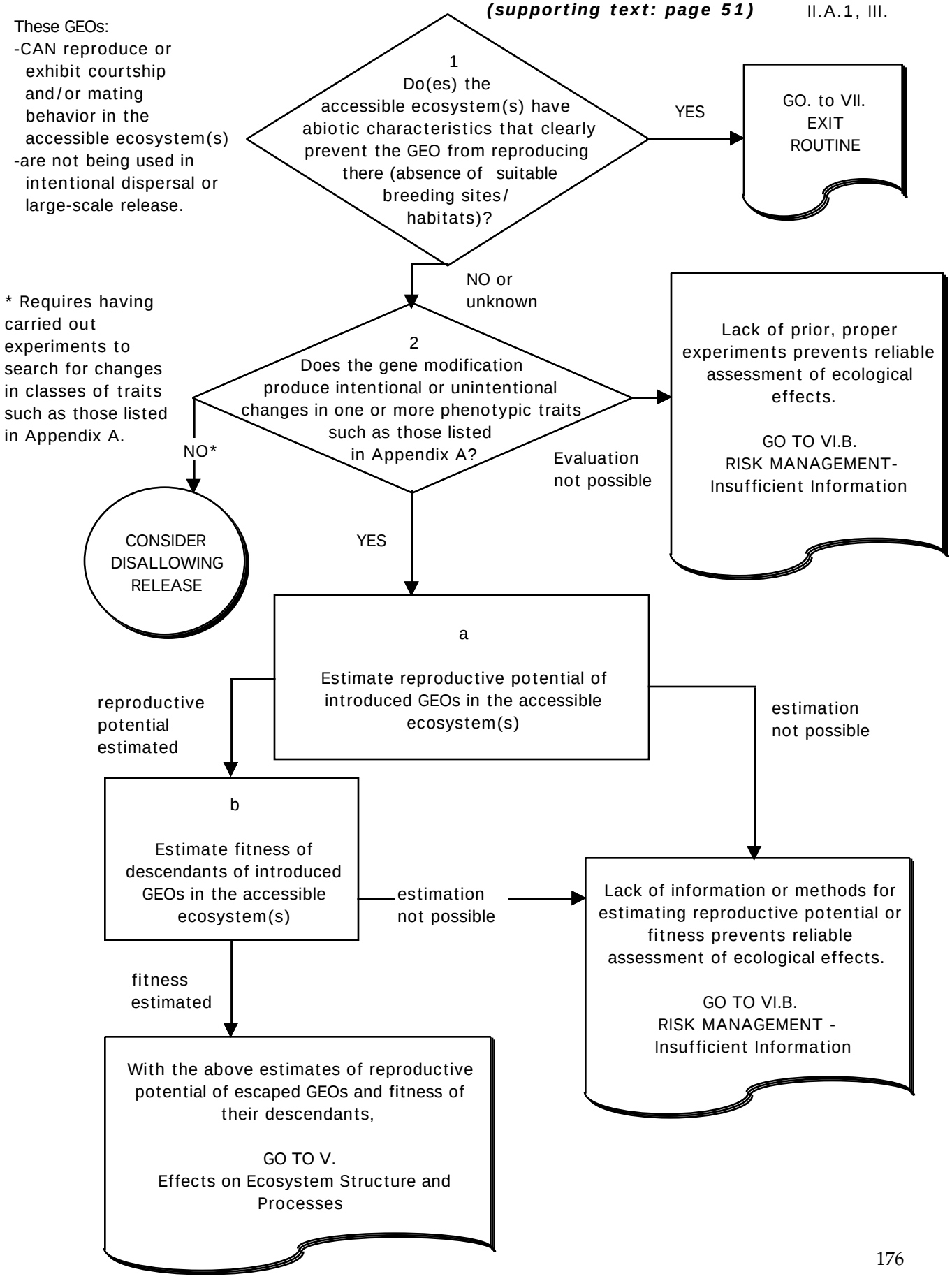
These GEOs:
- are NOT permanently sterile
- have potential for interbreeding with conspecifics and/or closely related species present in the accessible ecosystem. None of these species are protected.
- have gene change(s) resulting in changes in one or more of traits such as those listed in Appendix A.



IV.B. Ecosystem Effects on Reproduction

(supporting text: page 51)

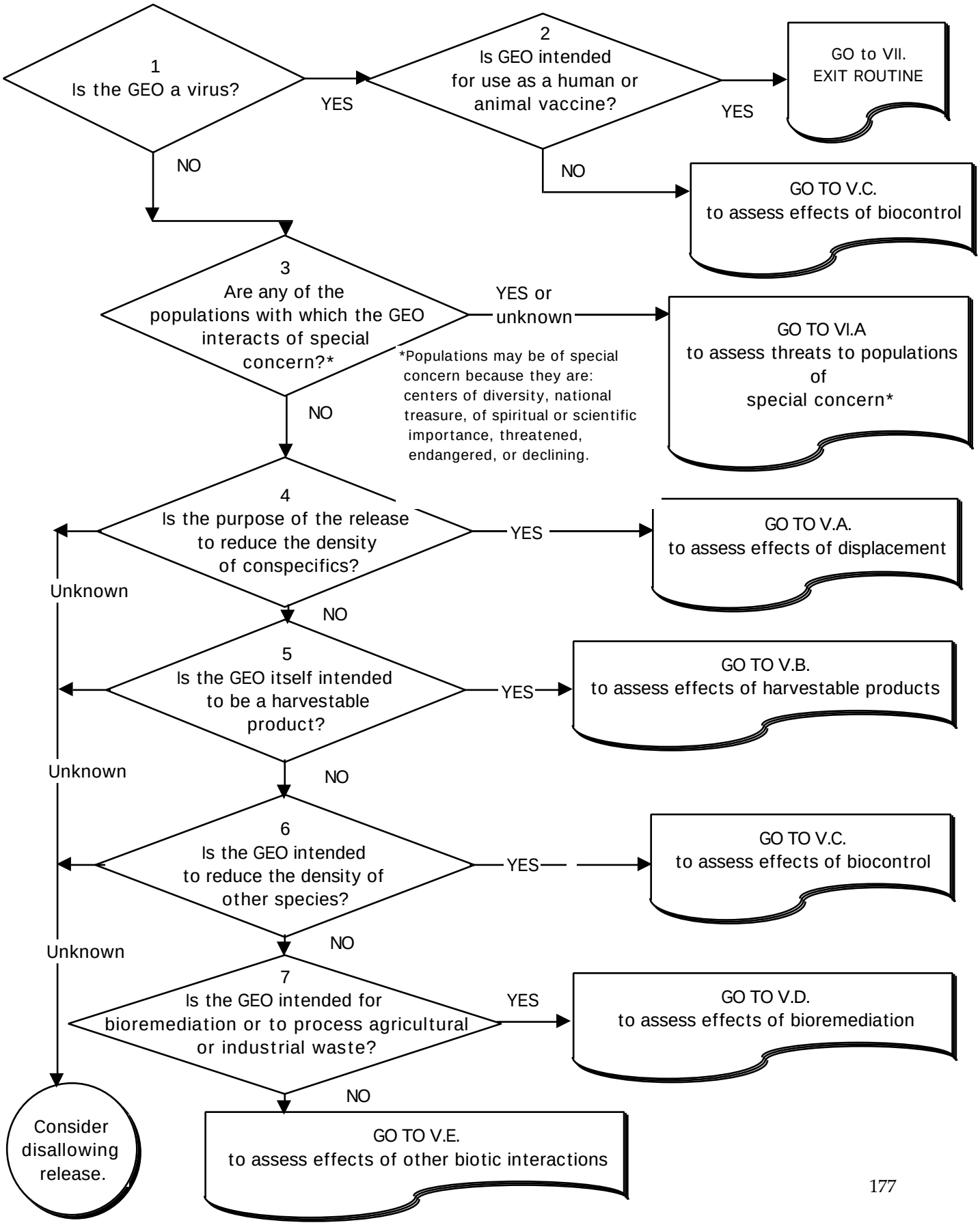
from II.A.1, III.



V. Effects on Ecosystem Structure and Processes

(supporting text: page 53)

from II.A.1., II.B.1., II.C.1., III., IV.A., or IV.B.

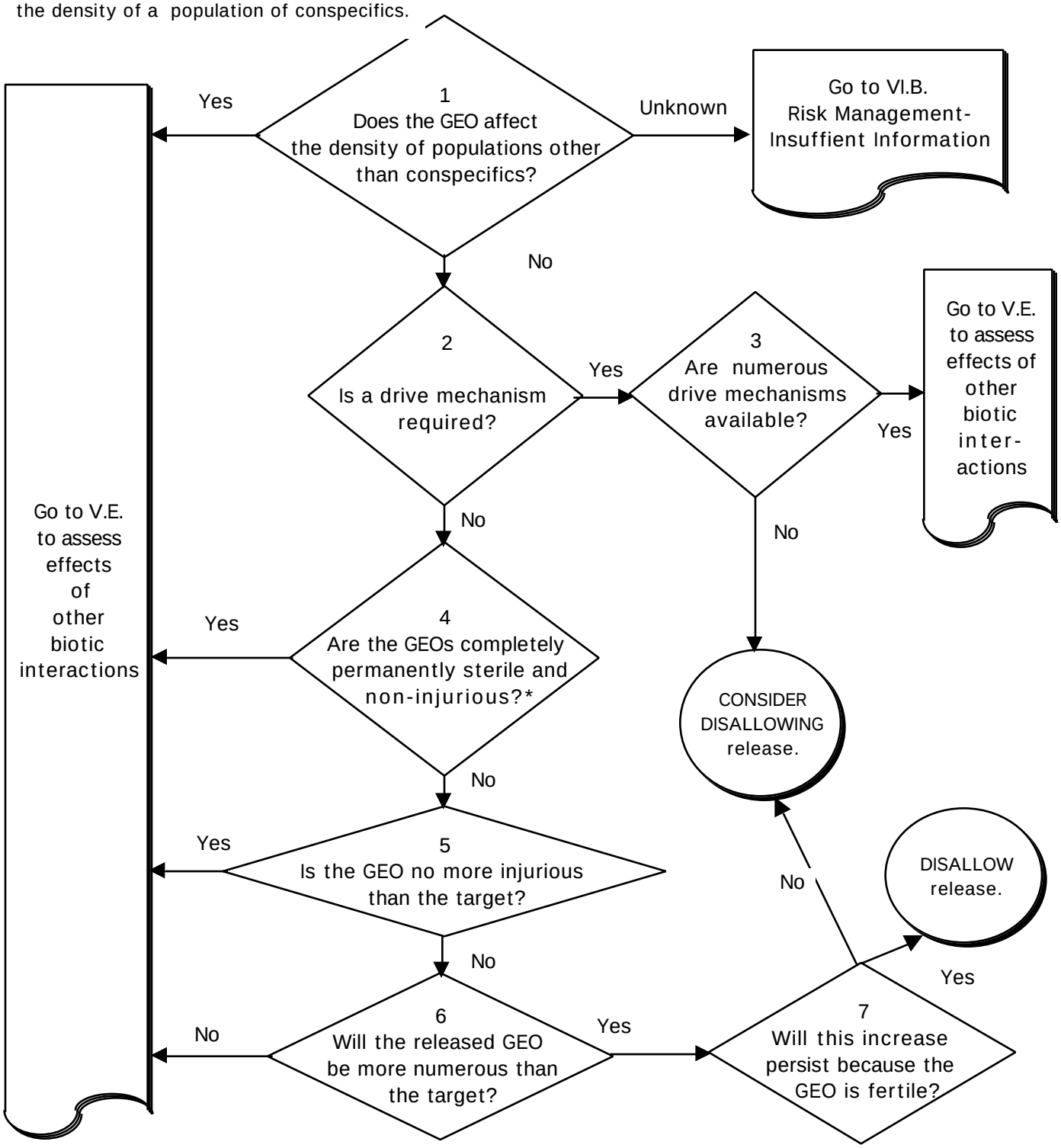


V.A. Effects on Ecosystem Structure and Processes (Displacement)

(supporting text: page 56)

from V.<4>

These GEOs are designed to reduce the density of a population of conspecifics.

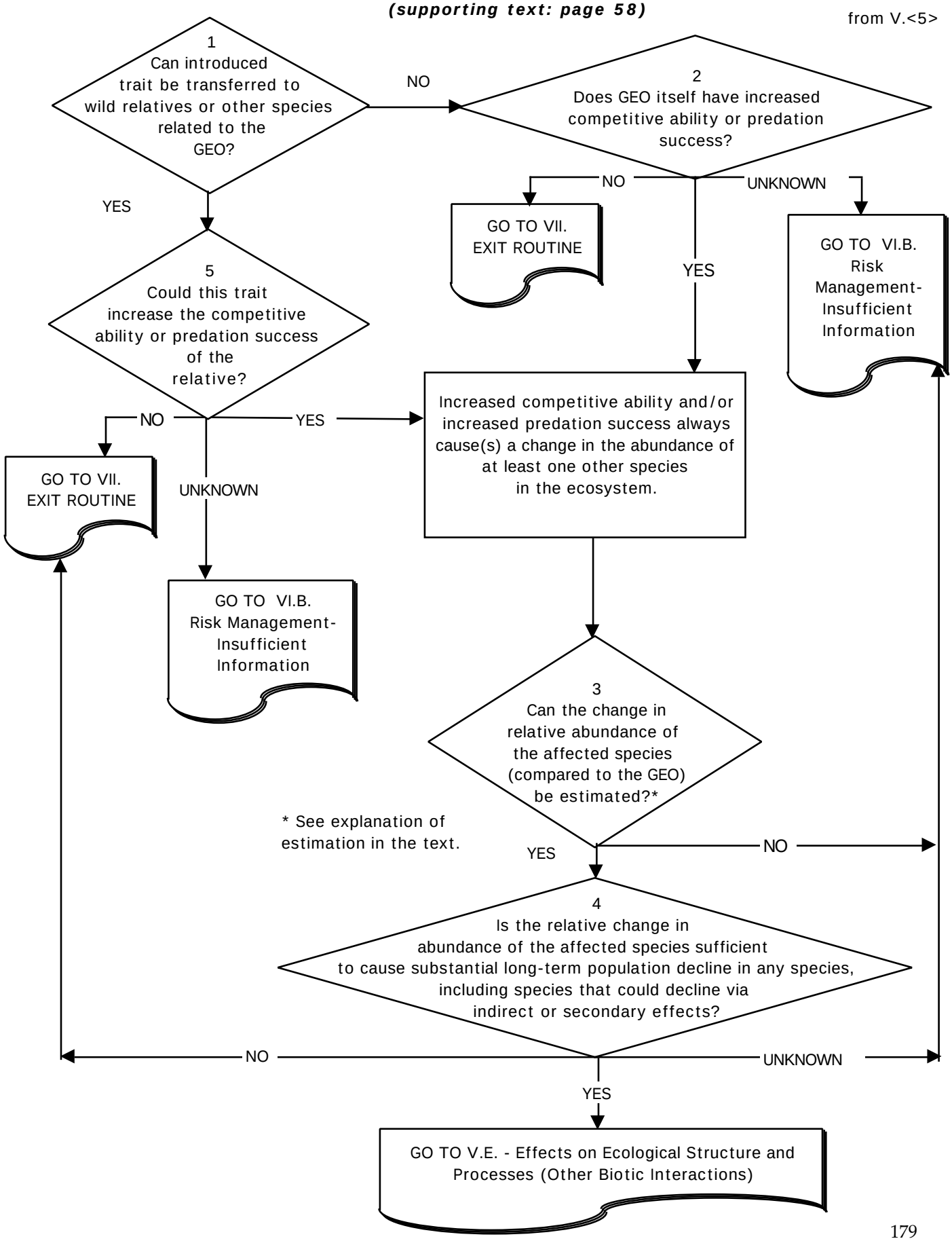


*Respond "NO" if the organism can reproduce from only one individual (see Appendix C).

V.B. Effects on Ecosystem Structure and Processes (Harvestable Product)

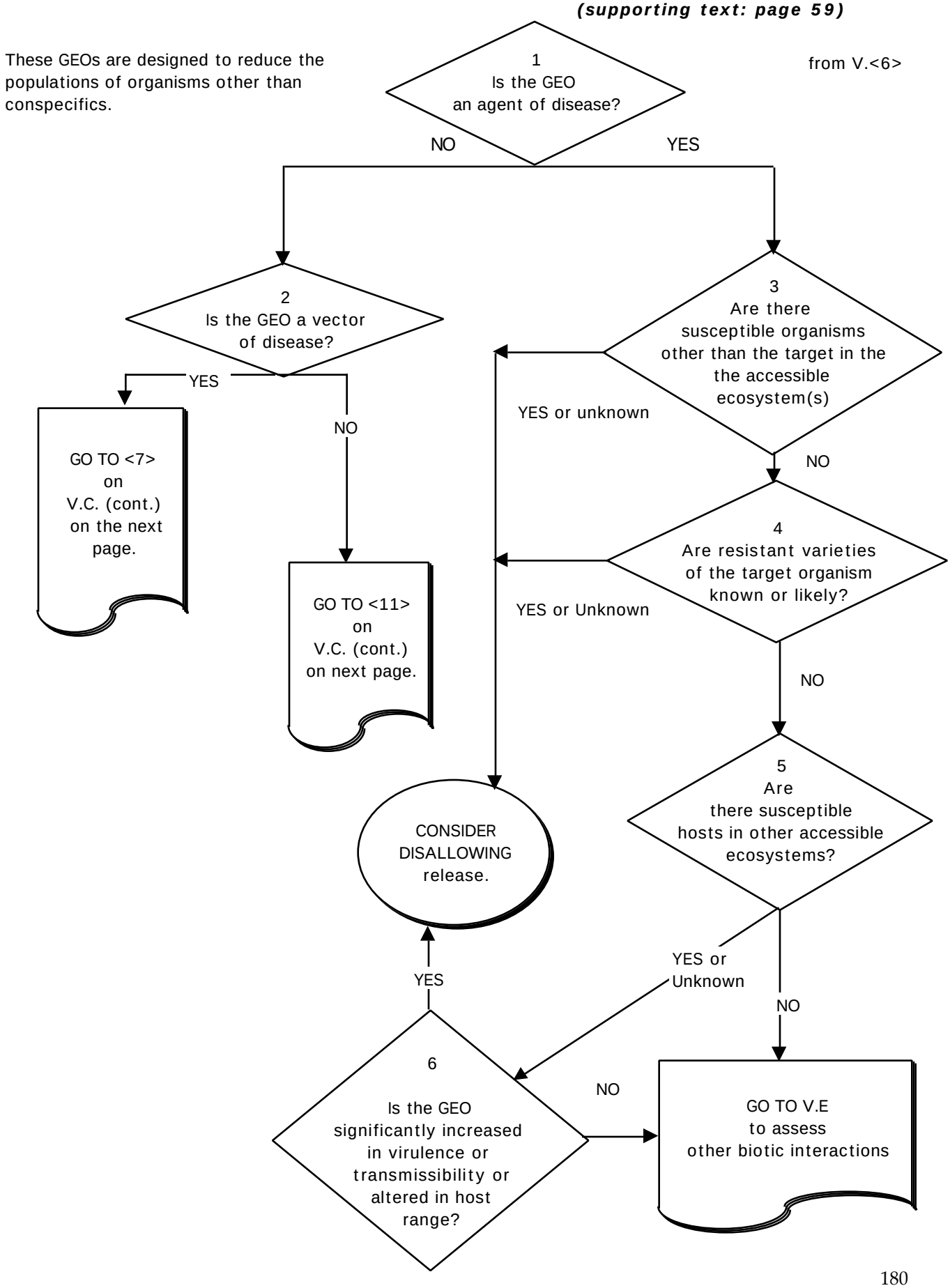
(supporting text: page 58)

from V.<5>



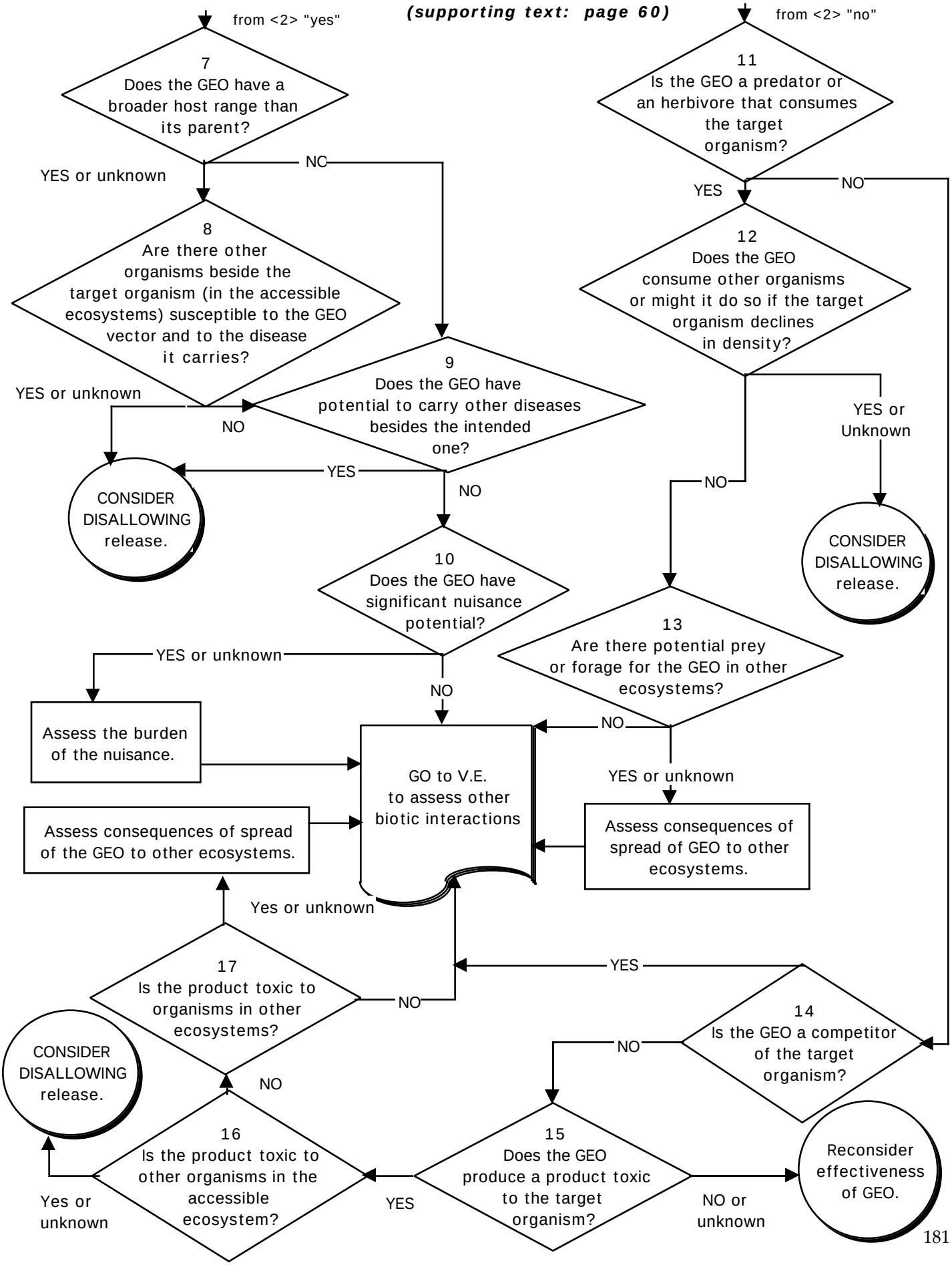
V.C. Effects on Ecosystem Structure and Processes (Biocontrol)

(supporting text: page 59)



V.C.(cont.) Effects on Ecosystem Structure and Processes (Biocontrol)

(supporting text: page 60)

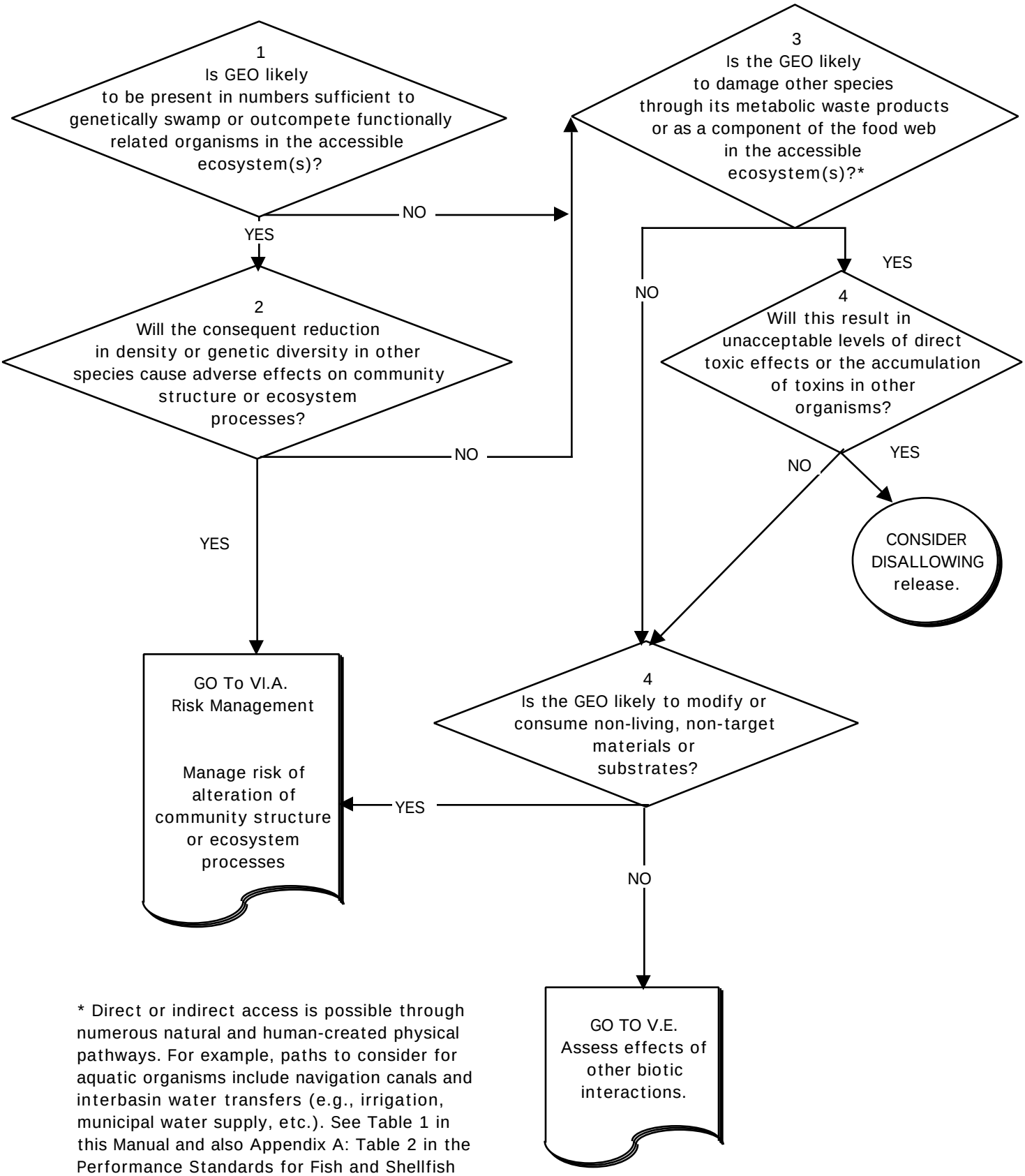


V.D. Effects on Ecosystem Structure and Process (Bioremediation)

(supporting text: page 63)

These GEOs are designed to consume undesirable material in the environment

from V.<7>



* Direct or indirect access is possible through numerous natural and human-created physical pathways. For example, paths to consider for aquatic organisms include navigation canals and interbasin water transfers (e.g., irrigation, municipal water supply, etc.). See Table 1 in this Manual and also Appendix A: Table 2 in the Performance Standards for Fish and Shellfish (ABRAC).

V.E. Effects on Ecosystem Structure and Processes
(Other Biotic Interactions)

(supporting text: page 64)

from V.A.
V.B.
V.C.
V.D.

a

* Assess type and magnitude of interactions between GEOs (both introduced GEOs and their descendants) and other organisms in the accessible ecosystem(s). Take into account the fitness of fertile GEOs and their descendants, as was assessed in IV.A.1 or IV.B.1.

Be sure to consider the following types of interactions:
- predation
- parasitism
- mutualism
- competition
- multitrophic
- indirect and cascading

Be sure to consider the following other organisms:
- conspecifics or widely distant taxa (including the probability of transgene movement by introgression)
- symbionts
- associated species on adjacent trophic levels
- associated species used by humans

* Consult explanatory text(s) for relevant type of organism. For fish and shellfish, see the Performance Standards Document (ABRAC 1995).

Assessment NOT possible

Lack of necessary information or methods prevents reliable assessment.

GO TO VI.B. RISK MANAGEMENT - Insufficient Information

Interactions assessed

b

* Assess potential for above interactions of GEOs to adversely alter structure or processes of the accessible ecosystem(s) in ways that would not have occurred if GEOs had not been introduced.

Be sure to consider alterations that are adverse because they:
- increase selection pressures on target populations that can then develop resistance;
- cause changes in species number, species abundance, species diversity or genetic diversity;
- increase density, transmission, dispersal, or virulence of pests or pathogens;
- alter the chemical composition of the environment
- decrease predictability of the state of the ecosystem;
- permanently alter the ecosystem to a degraded state for both long-term sustainability and human utilization;
- complicate management aimed at protection and utilization of resources such as fisheries, forestry, wildlife;
- permanently alter the ecosystem to a degraded state for both long-term sustainability and human utilization.

Assessment NOT possible

Adverse alterations ARE possible.

GO TO VI.A. RISK MANAGEMENT

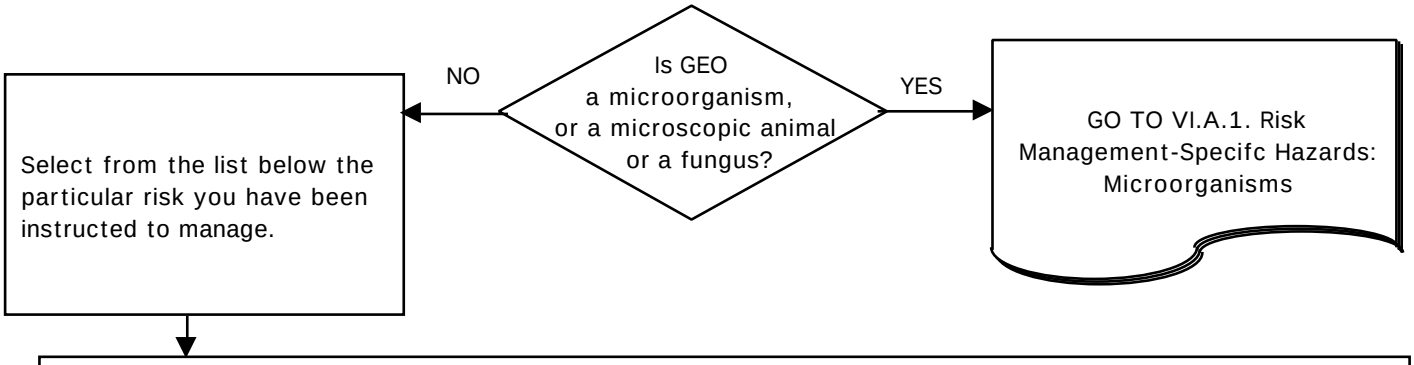
Manage Risk of Alteration of Ecosystem Processes

Adverse alterations are CLEARLY improbable or CLEARLY thought to be negligible.

GO TO VII. EXIT ROUTINE

VI.A. Risk Management - Specific Risks

(supporting text: page 69)



Manage Risks to Populations of Special Concern - from II.A.1., III., or V.

No/negligible escapes.*

Strict CONTAINMENT is indicated. Populations may be of special concern because they are: centers of diversity, national treasures, of spiritual importance, threatened, endangered, or declining. Concerns are gene flow, reproductive interference, introgressive hybridization, gene swamping, or displacement due to ecological interactions with GEO in these populations.

*No/negligible escapes = combined outcome of scale of project and effectiveness of barriers.

Manage Risk of Losing Populations of Pure Species - from II.C.1

No/negligible escapes.*

Strict CONTAINMENT is indicated. These GEOs are NOT sterile, and have parental/related species present, but none are protected species. Concern is that populations of parental or related species will become introgressed and swamped by interspecific hybridization, so that they no longer constitute a distinct species, thereby posing the risk of losing an evolutionarily important component of the affected species' genetic diversity.

Manage Risk of Transfer of Harmful Compound to Food Chain - from II.D.

No/negligible escapes.

Strict CONTAINMENT is indicated. These GEOs can reproduce in the accessible ecosystem(s), are NOT sterile, and have parental/related species present. Concern is that they could transfer harmful biochemical compounds to animal or human food chains.

Manage Risk of Decline in Population Abundance - from IV.A.

No/negligible escapes.

Strict CONTAINMENT is indicated. These GEOs are not sterile and have conspecifics and/or closely related species present but none are "species of special concern". Concern here is potential decline in the abundance of affected population(s) resulting from lowered fitness of introgressed descendants.

Manage Risk of Alteration of Ecosystem Processes - from V.D. or V.E.

No/negligible escapes* UNLESS specific plan in place to manage risks.

These GEOs CAN reproduce in the accessible ecosystem(s) and are NOT sterile. Risks of adverse alteration(s) in ecosystem processes exist. CONTAINMENT is indicated unless specific hazards are identified and management/mitigation of the risks is CLEARLY possible. This includes situations in which wild relatives or other species related to the GEO could gain competitive abilities from the GEO and become weeds or pests. (See text for examples.)

For each hazard (or set of hazards),
GO TO VI.C.
RISK MANAGEMENT - CONTAINMENT ROUTINES
to select a containment method that ensures there are either no escapes or the number of escapees is negligible.

VI.A.1. Risk Management - Specific Hazards: Microorganisms
(including microscopic animals and fungi):

Once released into a natural environment, microorganisms **normally cannot be contained** , may be transported over long distances, and may (in the case of procaryotes) exchange genes across very wide taxonomic gaps. In certain highly restricted situations a substantial degree of physical and genetic isolation may be feasible (e.g. greenhouse applications, high temperature thermal pools). Otherwise few strategies exist to manage identified hazards.

In certain situations the following may reduce risks to acceptable levels:
* Introduced or modified genes may be located on the chromosome rather than on a plasmid, reducing (but not eliminating) the likelihood of their transfer to other organisms.
* Re-engineer the GEO such that it is unable to survive outside the environment in which it will be released. This will require that the re-engineered GEO be re-assessed beginning with Flowchart I.
* If the GEO produces a product that is responsible for the adverse environmental effects, a second organism that consumes the product exists and may be introduced. This requires that the second organism be assessed beginning with Flowchart I.
* The GEO can be applied when all potentially affected organisms are senescent or dormant, if the GEO will die before the other organisms become active again.

RELEASE OF THE GEO IS NOT INDICATED IF the circumstances of release cannot ensure that:*
1) other organisms will not obtain the engineered genes,
2) non-target organisms are not killed or inhibited by the GEO,
3) the GEO does NOT have a competitive advantage over non-target organisms,
4) the GEO does not parasitize non-target organisms,
5) the GEO does not consume a non-target organism population reducing its size below recovery levels,
6) the GEO does not significantly alter nutrient cycling in the environment, and
7) the GEO does not cause other unacceptable environmental harm.

*In most cases, disallowing release of the GEO also indicates disallowing its use as a food. Those intending to use a GEO as a food after disallowing its release as a GEO have a responsibility to demonstrate that distribution of the GEO as a genetically engineered food will NOT result in any environmental release.

VI.B. Risk Management - Insufficient Information

(supporting text: page 69)

The precautionary approach of this assessment requires that in the absence of information to evaluate risk, the goal of risk management must be no/negligible escape* of GEOs.

Is GEO a microorganism, or a microscopic animal or a fungus?

NO

YES

GO TO VI.A. 1. Risk Management - Microorganisms

Select from the list below according to the flowchart that directed you to this page.

*Negligible escapes = combined outcome of scale of experiment and effectiveness of barriers.

Insufficient Information at II.A.1.

Containment indicated.

The phenotypic effect of the gene change(s) of these GEOs is unknown. Further risk assessment is not possible.

Insufficient Information at IV.A.

Containment indicated.

These GEOs are NOT sterile . Conspp. or closely related spp. ARE present in the accessible ecosystem(s), but none are protected spp. Because the GEOs have an unfamiliar overall phenotype, unknown reproductive potential or unknown fitness, no determination can be made of their impact on the structure or processes of the accessible ecosystem(s).

Insufficient Information at IV.B.

Containment indicated.

These GEOs are NOT sterile, and have NO conspecifics or closely related species present in the accessible ecosystem(s). No barriers to their reproduction in accessible ecosystem(s) are known to exist. Because the GEOs have an unfamiliar overall phenotype, unknown reproductive potential or unknown fitness, no determination can be made of their impact on the structure or processes of the accessible ecosystem(s).

Insufficient Information at V.A., V.C. and V.E.

Containment indicated.

Information is insufficient to assess the effects of the GEO on other organisms in accessible ecosystem(s) and/or on ecosystem structures and processes.

To ensure sufficient containment of GEOs for your project,
GO TO VI.C. RISK MANAGEMENT-
CONTAINMENT ROUTINES.

VI.C. Risk Management - Containment Routines

from VI.A.
VI.B.

(supporting text: page 69)

Select sufficient containment barriers from the categories listed below to assure that there are no/negligible escapes. Consult text of Risk Management Recommendations for siting and barrier details and explanations.

Ensure that GEO containment meets requirements for security, alarms, operational plan and inspection, as explained in the text of the Risk Management Recommendations.

Note: IF YOU CANNOT ENSURE SUFFICIENT CONTAINMENT, DISALLOW RELEASE OR USE OF GEO.

PHYSICAL OR CHEMICAL BARRIERS
Barriers that induce 100% mortality in any life stage of the GEO before reaching any accessible ecosystem (water temperature, pH).

BIOLOGICAL BARRIERS OF GEO
Barriers that prevent any possibility of GEO reproduction or survival.

MECHANICAL BARRIERS
Barrier devices that physically hold back any life stage of the GEO from leaving the project site (e.g., screens).

SCALE OF RELEASE
Maintain a population size small enough so that accidental escape of all organisms would not have adverse ecological effects.

WRITTEN OPERATIONAL PLAN REQUIRED
Develop and implement an appropriate written plan addressing all factors described in Operations subsection of Risk Management Recommendations.

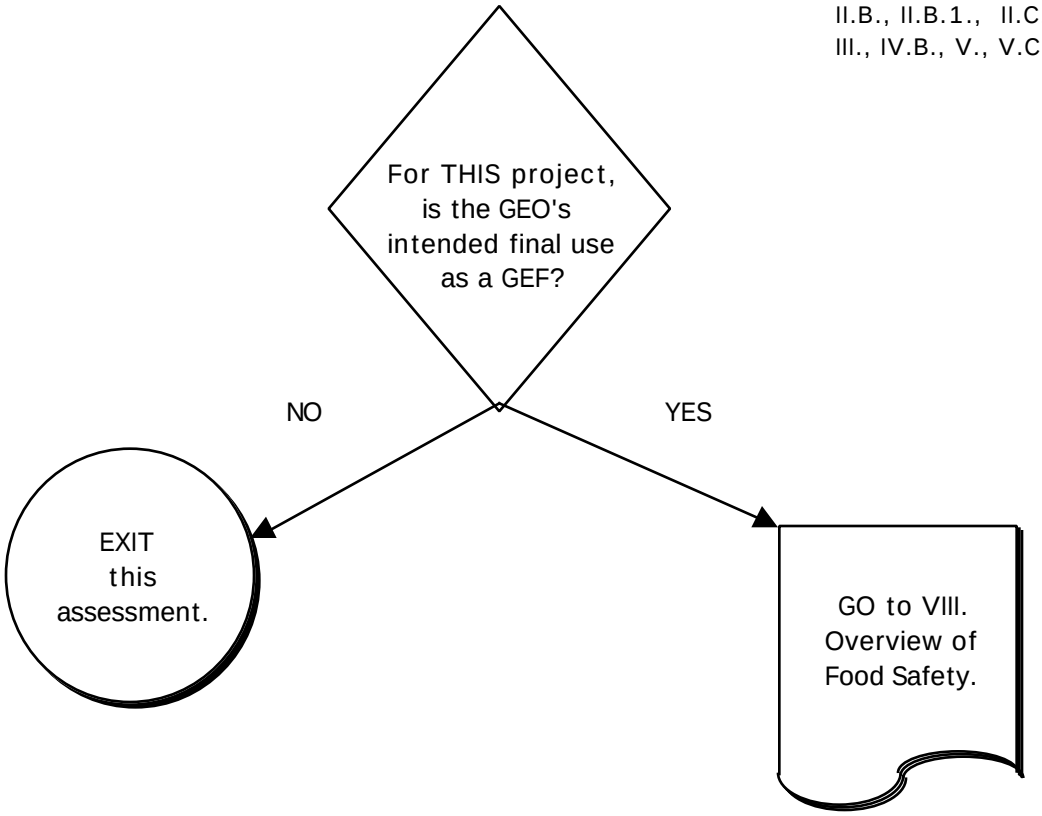
Did peer reviewers and site inspectors conclude that your project provides sufficient containment?

No → CONSIDER DISALLOWING release.

Yes → GO TO VII. EXIT ROUTINE

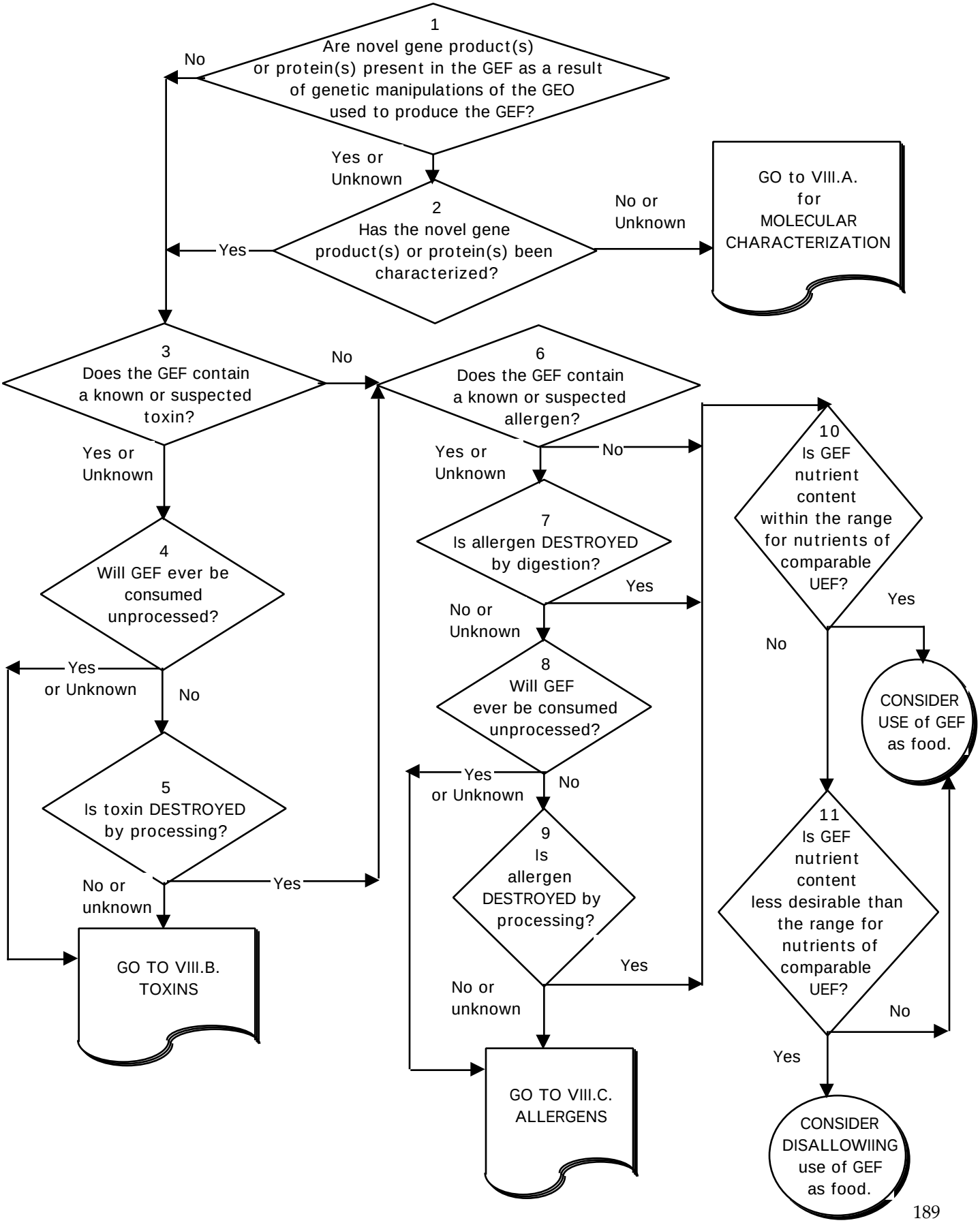
VII. Exit Routine

from I.A, II.A., II.A.1., II.B., II.B.1., II.C., II.C.1., III., IV.B., V., V.C., V.E.



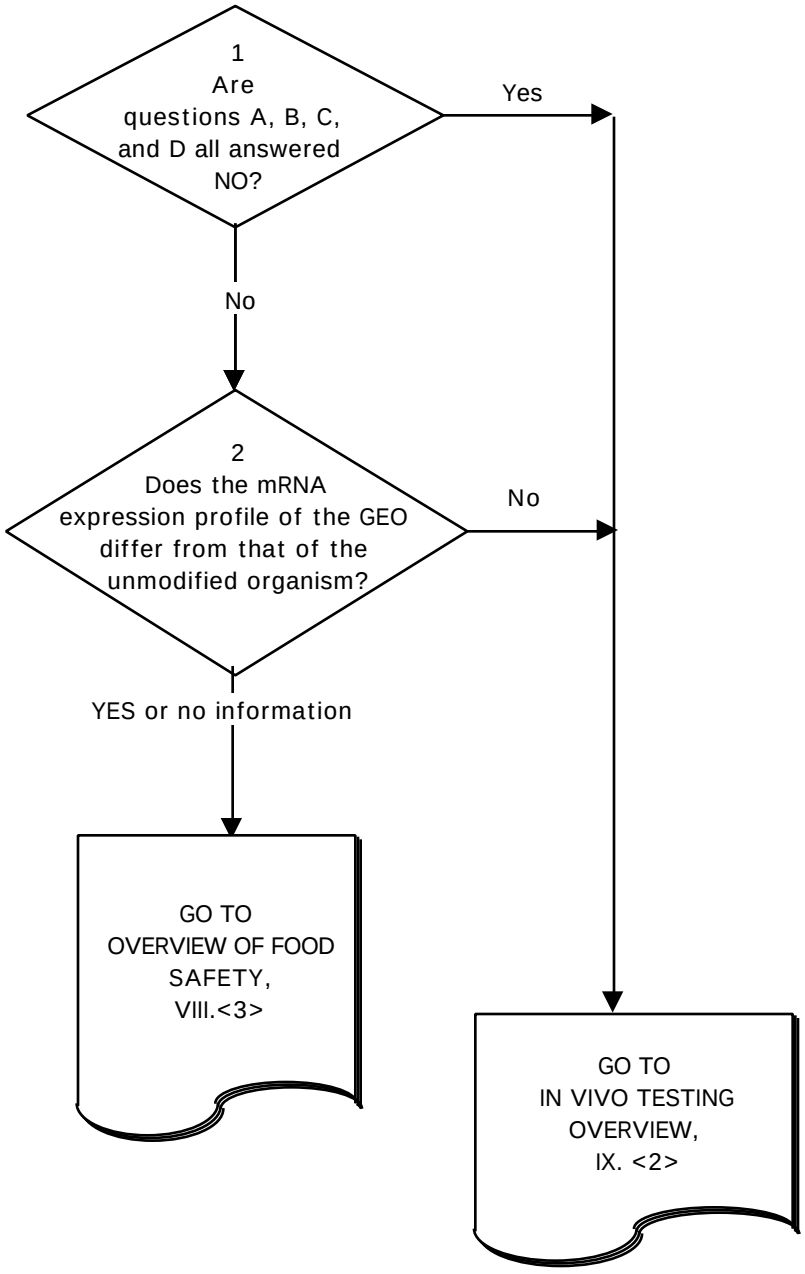
VIII. OVERVIEW OF FOOD SAFETY

(supporting text: page 96)



VIII.A. Food Safety Assessment: Molecular Characterization

(supporting text: page 109)



A. Does the transgene encode an enzyme or other protein that could interact with and alter known metabolic or biosynthetic pathways either catalytically or by modifying regulation?

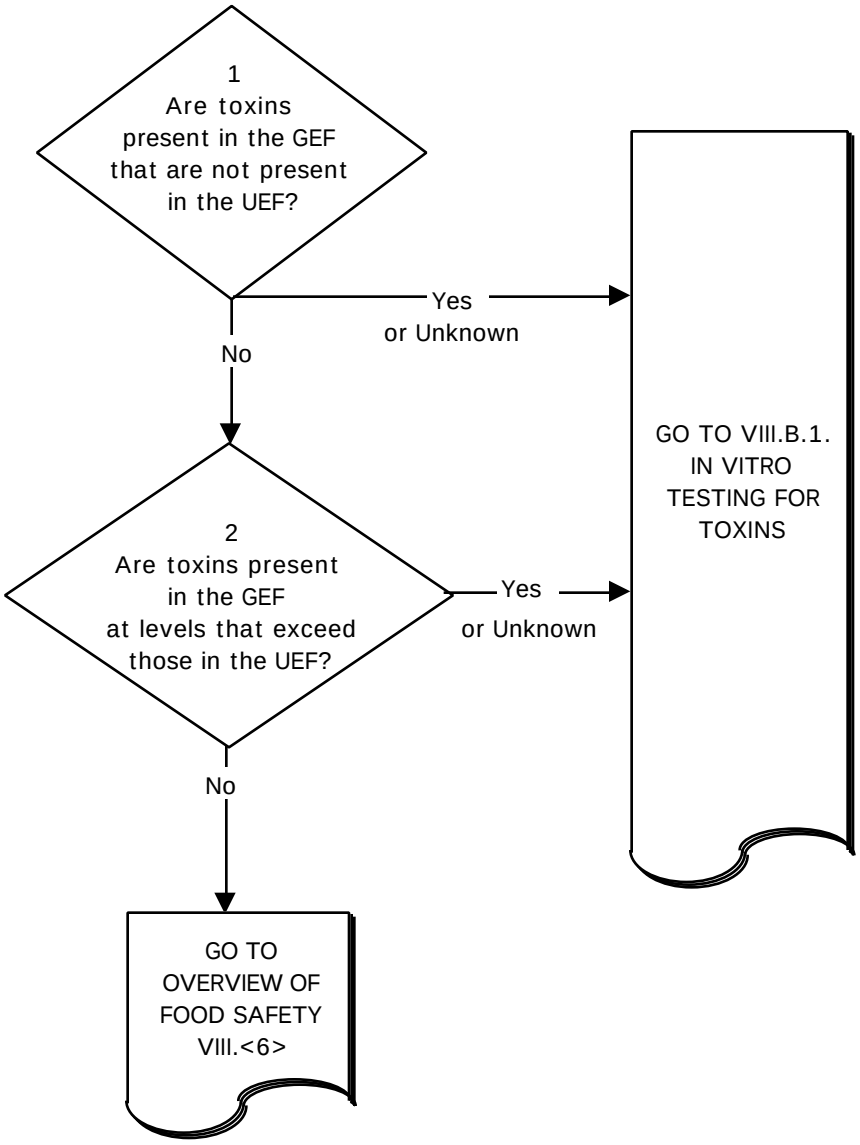
B. Does the insertion site(s) of the transgene interrupt one or more open reading frames within the genome of the organism?

C. If there are mRNAs expressed from sequences within the 20 kb domains of the genome that flank the insertion site(s) of the transgene, are the levels and patterns of expression of those mRNAs different in the GEO, compared to the unmodified organism?

D. Is the transgene expressed in those parts of the GEO that are normally used as food?

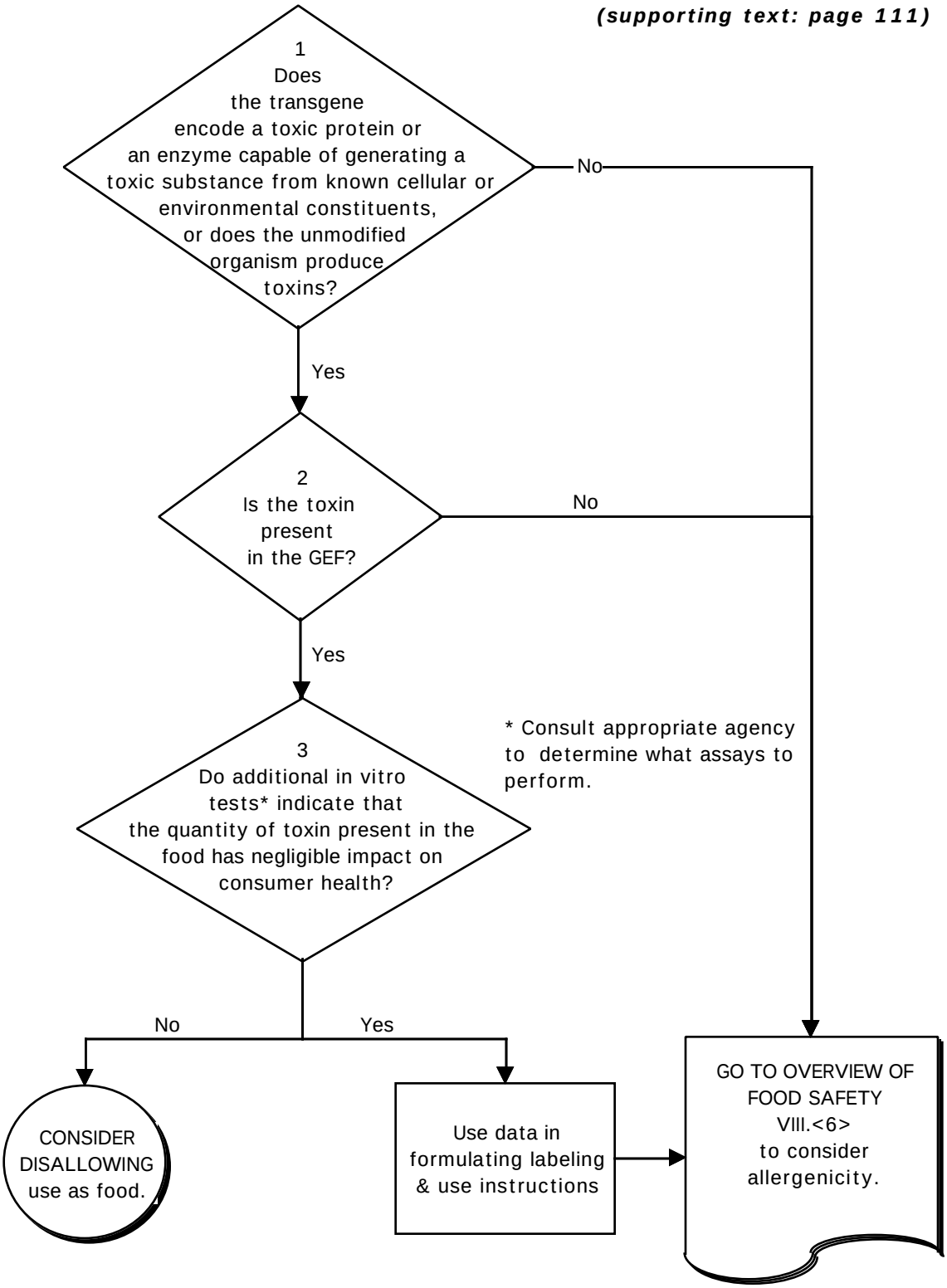
VIII. B. Toxins

(supporting text: page 111)



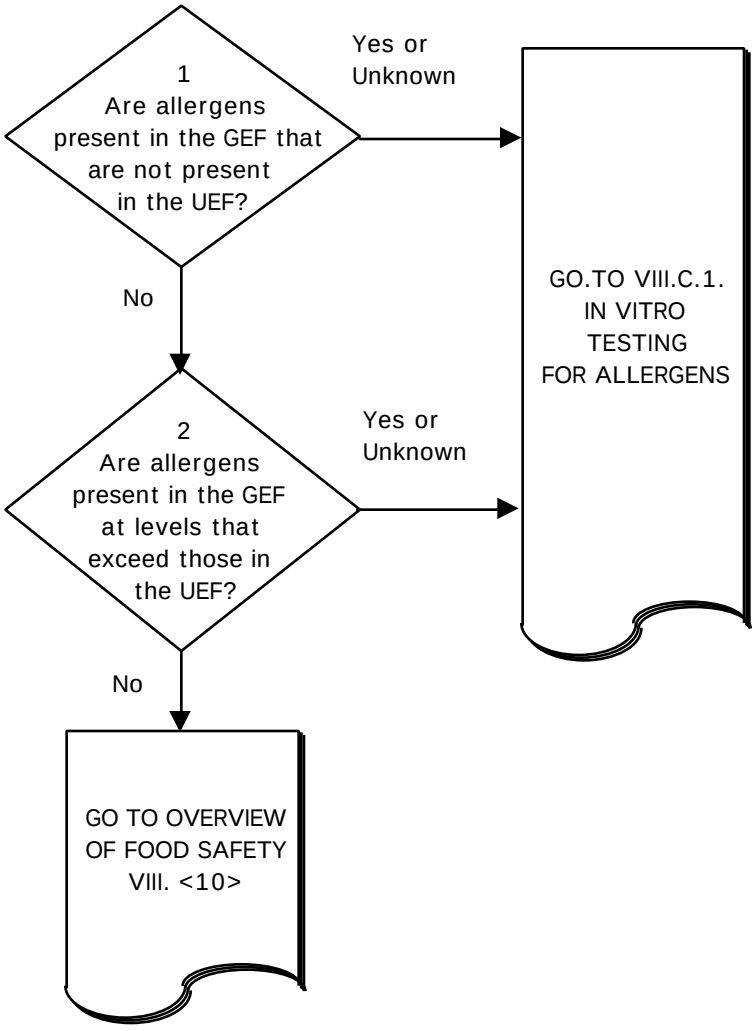
VIII.B.1. Food Safety Assessment: In Vitro Testing for Anticipated Toxins

(supporting text: page 111)



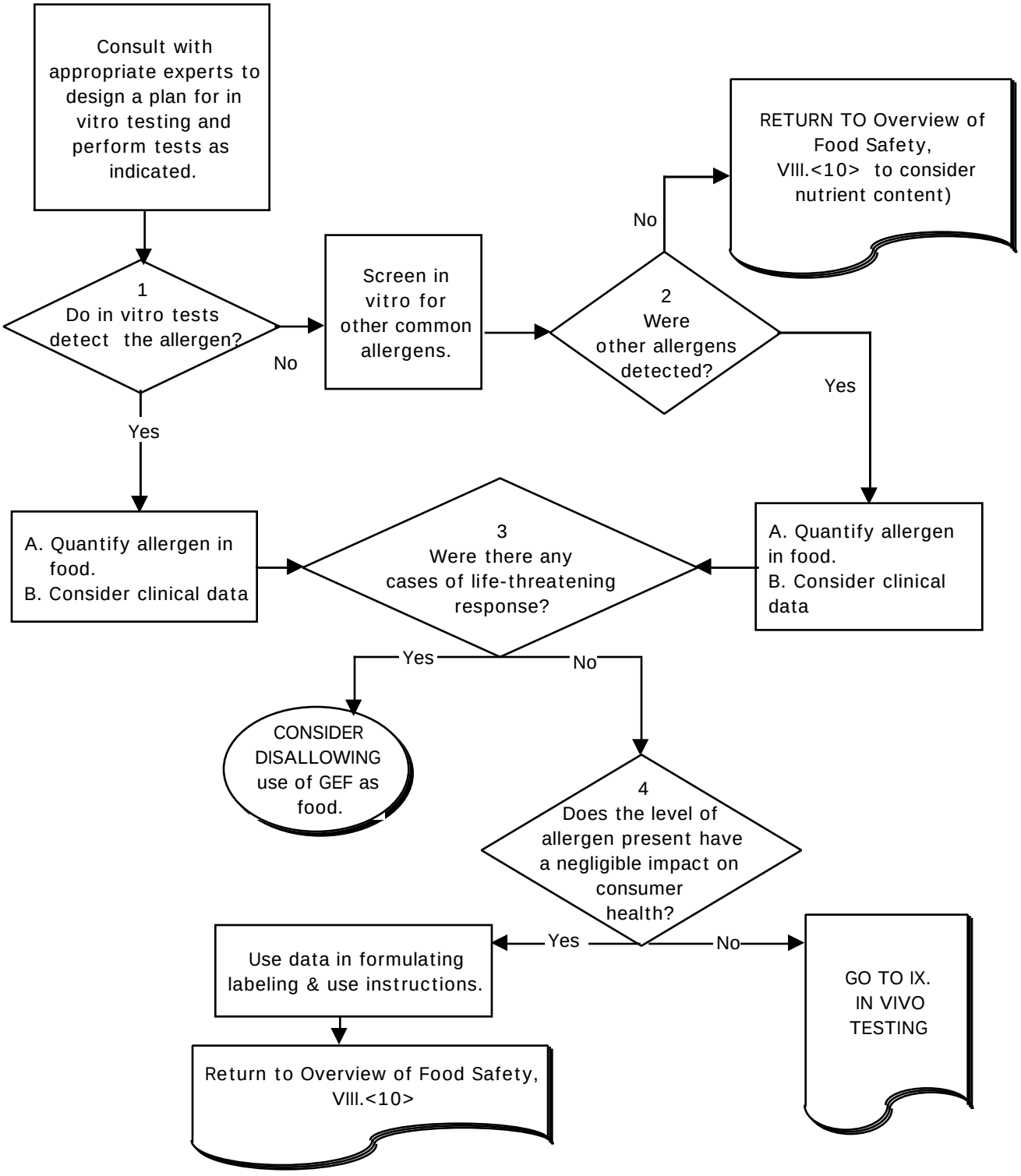
VIII. C. Allergens

(supporting text: page 112)



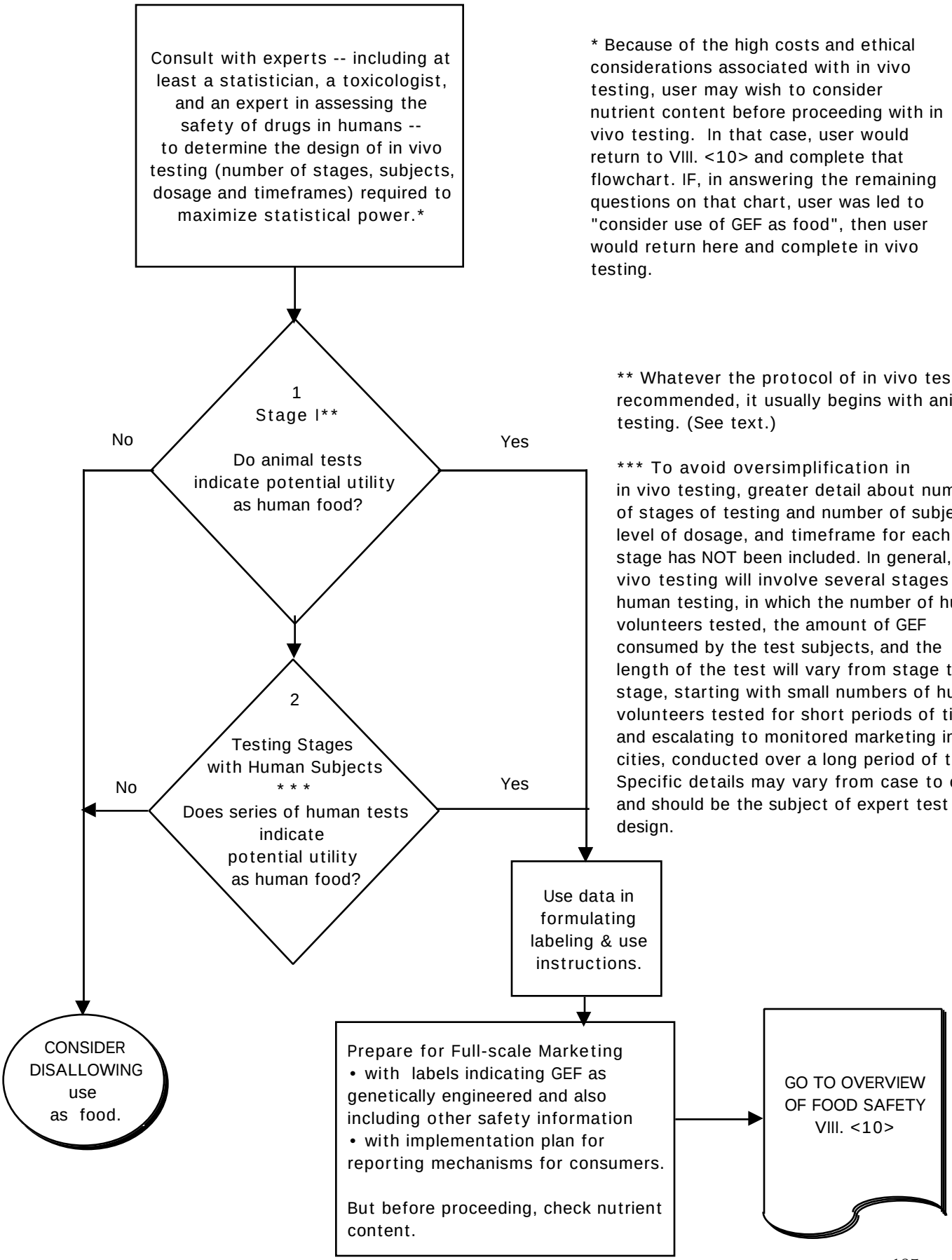
VIII.C.1. Food Safety Assessment: In Vitro Testing for Anticipated Allergens

(supporting text: page 112)



IX. Food Safety Assessment: In Vivo Testing

(supporting text: page 113)



* Because of the high costs and ethical considerations associated with in vivo testing, user may wish to consider nutrient content before proceeding with in vivo testing. In that case, user would return to VIII. <10> and complete that flowchart. IF, in answering the remaining questions on that chart, user was led to "consider use of GEF as food", then user would return here and complete in vivo testing.

** Whatever the protocol of in vivo testing recommended, it usually begins with animal testing. (See text.)

*** To avoid oversimplification in in vivo testing, greater detail about number of stages of testing and number of subjects, level of dosage, and timeframe for each stage has NOT been included. In general, in vivo testing will involve several stages of human testing, in which the number of human volunteers tested, the amount of GEF consumed by the test subjects, and the length of the test will vary from stage to stage, starting with small numbers of human volunteers tested for short periods of time and escalating to monitored marketing in test cities, conducted over a long period of time. Specific details may vary from case to case and should be the subject of expert test design.

WORKSHEETS

Worksheet
accompanying
The Biosafety Manual

Introduction

This manual is intended to aid users in assessing the genetic, ecological, and human health effects of genetically engineered organisms used in research, development, and small-scale or large-scale release. The flowcharts guide users in identifying specific hazards and managing the risk associated with each identified hazard, where possible. This worksheet is meant to accompany the flowcharts. The user should use the worksheet to document and track the decision path taken through the flowcharts and any plans for risk management.

Name of Assessor:

Identification Name of the GEO:

Purpose for which GEO was designed:

Describe proposed project:_____
_____
_____

On the worksheet below, record the pathway you took through the flowcharts. Please note that below, flowcharts are always listed in sequential order. *Attach written explanatory materials as directed.*

Flowchart Documentation

Please list the numbers of **all** flowcharts that you used:

_____
_____
_____
_____

Next to each flowchart used, indicate with an **X** where that chart led you and attach any explanations or rationales requested.

Flowchart
No.

I. Determination of Assessment Pathway led to:

____ I.A. to Continuation of Pathway Determination.
____ II.D. to assess Transfer of Harmful Biochemical Compounds to Food Chain.
____ II.E. to begin assessment of Vectors Genetically Engineered to Reduce Disease.
____ VIII. to begin Food Safety Assessment.

I.A. Continuation of Pathway Determination led to:

____ II.A. to consider Survival and Reproduction Assessment
____ VII. Exit Routine. *Attach rationale for arriving here from <4>.*
____ Appendix B to consider other assessment because organism is modified solely by selective or captive breeding. This then led to:
    ____ decision to pursue other assessment. *Indicate rationale for decision and name of other assessment protocol.*
    ____ return to I.A.<2> which led to a second consideration of this pathway (and a second "X")
____ Appendix C to consider Assessment of GEOs with Alternate Reproductive Pathways. This led to:
    ____ NOT proceeding further with the project and exiting this assessment.
    ____ returning to I.A.<6> which led to a further consideration of this pathway (and another "X").
____ Consider disallowing release because GEO is not effective for its intended purpose. *Attach rationale for your decision.*

II.A. Survival and Reproduction Assessment - Deliberate Gene Changes led to

____ II.A.1. to assess impact of Deliberate Gene Changes.
____ II.B. to assess impact of Deliberate Chromosomal Manipulations.
____ VII. Exit Routine. *Attach your rationale for seeking exit.*

II.A.1. Impact of Deliberate Gene Changes led to:

____ II.B. to assess impact of Deliberate Chromosomal Manipulations.
____ III. to assess Potential Interference with Natural Reproduction.
____ IV.A. to assess Ecosystem Effects from Impacts of Introgression of Modified Gene(s).
____ IV.B. to assess Ecosystem Effects on Reproduction.
____ V. to assess Effects on Ecosystem Structure and Processes.
____ VI.A. Risk management - to manage specific risks to

populations of special concern. *Attach written description of any identified hazard.*

____ VI.B. Risk management - to manage risk where there is insufficient information.

____ VII. Exit Routine. *Attach your rationale for seeking exit.*

____ VII. Exit Routine and consultation with relevant national, regional, and local government agencies regarding use of non-indigenous species. *Attach your rationale for seeking exit and indicate agency name(s) and recommendations.*

II.B. Survival and Reproduction Assessment - Deliberate Chromosomal Manipulation led to:

____ II.B.1. to assess Impact of Deliberate Chromosomal Manipulations.

____ II.C. to assess impact of Interspecfic Hybridization.

____ VII. Exit Routine. *Attach your rationale for seeking exit.*

II.B.1. Impact of Deliberate Chromosomal Manipulations led to:

____ III. to assess Potential Interference with Natural Reproduction.

____ V. to assess Effects on Ecosystem Structure and Processes.

____ VII. Exit Routine. *Attach your rationale for seeking exit.*

____ VII. Exit Routine and consultation with relevant government agencies regarding use of non-indigenous species. *Attach your rationale for seeking exit and indicate agency name(s) and recommendations.*

____ Consider disallowing release/dispersal because organism is not likely to produce desired effect. *Attach rationale for decision.*

II.C. Survival and Reproduction Assessment - Interspecific Hybridization led to:

____ II.C.1. to assess potential Impact of Interspecific Hybridization.

____ VII. Exit Routine. *Attach your rationale for seeking exit.*

II.C.1. Impact of Interspecific Hybridization led to:

____ III. to assess Potential Interference with Natural Reproduction.

____ V. to assess Effects on Ecosystem Structure and Processes.

____ VI.A. Risk Management - to manage risks to populations of special concern. *Attach a written description of any identified hazard.*

____ VI.A. Risk management - to manage risks of losing population of pure species. *Attach a written description of any identified hazard.*

____ VII. Exit Routine. *Attach your rationale for seeking exit.*

____ VII. Exit Routine and consultation with relevant government

agencies regarding use of non-indigenous species.
Attach your rationale for seeking exit and indicate agency name(s) and recommendations.

____ Consider disallowing release/dispersal because organism is not likely to produce desired effect.

II.D. Transfer of Harmful Biochemical Compound to Food Chain led to:

_____ I.A. for Continuation of Assessment Pathway.

_____ VI.A. Risk Management - to manage risk of transfer of harmful biochemical compound to food chains. *Attach a written description of any identified hazard.*

II.E. Vectors Genetically Engineered to Reduce Disease led to:

____ II.E.1. Genetically Engineered Arthropods-Field Trials

____ II.E.2. Genetically Engineered Arthropods-Large-scale Release.

II.E.1. Genetically Engineered Vectors - Field Trials led to:

____ I.A. Assess genetic and ecological effects by Continuation of Pathway Determination.

____ Consider disallowing release. *Attach rationale for decision.*

II.E.2. Genetically Engineered Vectors - Large-scale Release led to:

____ I.A. to assess genetic and ecological effects by Continuation of Pathway Determination.

____ Consider disallowing release. *Attach rationale for decision.*

III. Potential Interference with Natural Reproduction led to:

_____ IV.B. to assess Ecosystem Effects on Reproduction.

_____ V. to assess Effects on Ecosystem Structure and Processes.

_____ VI.A. Risk Management - to manage risks to population of special concern. *Attach a written description of any identified hazard.*

_____ VII. Exit Routine. *Attach rationale for seeking exit.*

IV.A. Ecosystem Effects - Impacts of Introgression of Modified Gene(s) led to:

____ V. to assess Effects on Ecosystem Structure and Processes.

____ VI.A. Risk Management - to manage risk of decline of population abundance. *Attach a written description of any identified hazard.*

____ VI.B. Risk Management - to manage risk where there is insufficient information.

____ Consider disallowing release. *Attach rationale for decision.*

IV.B. Ecosystem Effects on Reproduction led to:

____ V. to assess Effects on Ecosystem Structure and Processes.

____ VI.B. Risk Management - to manage risk where there is insufficient information.

____ VII. Exit Routine. *Attach rationale for seeking exit.*
____ Consider disallowing release. *Attach rationale for decision.*

V. Effects on Ecosystem Structure and Processes led to:

____ V.A. to assess the effects of displacement (by a GEO intended to reduce the density of conspecifics).
____ V.B. to assess the effects of a GEO intended to be or produce a harvestable product.
____ V.C. to assess the effects of a GEO intended as a biocontrol agent (to reduce the density of other species)
____ V.D. to assess the effects of a GEO intended for bioremediation or to process agricultural or industrial wastes.
____ V.E. to consider Effects on Ecosystem Structure and Processes of Other Biotic Interactions.
____ VI.A. Risk Management - to manage risk to population(s) of special concern. *Attach a written description of any identified hazard.*
____ VII. Exit Routine. *Attach rationale for seeking exit.*
____ Consider disallowing release. *Attach rationale for decision.*

V.A. Effects on Ecosystem Structure and Processes (Displacement) led to:

____ V.E. to consider Effects on Ecological Structure and Processes of Other Biotic Interactions.
____ VI.B. Risk Management - to manage risk where there is insufficient information.
____ Consider disallowing release. *Attach rationale for decision.*
____ Disallow release. *Attach rationale.*

V.B. Effects on Ecosystem Structure and Processes (Harvestable Product) led to:

____ V.E. to consider Effects on Ecosystem Structure and Processes of Other Biotic Interactions.
____ VI.B. Risk management to manage risk where there is insufficient information.
____ VII. Exit Routine. *Attach rationale for seeking exit.*

V.C. Effects on Ecosystem Structure and Processes (Biocontrol) led to:

_____ V.E. to consider Effects on Ecosystem Structure and Processes of Other Biotic Interactions.
_____ Consider disallowing. *Attach rationale for decision.*
_____ Reconsider effectiveness of GEO. *Attach rationale for decision.*

V.D. Effects on Ecosystem Structure and Processes (Bioremediation) led to:

_____ V.E. to assess effects of Other Biotic Interactions
_____ VI.A. Risk management - to manage risks of alteration of community structure or ecosystem processes. *Attach written description of any identified hazard.*

_____ Consider disallowing release. *Attach rationale for decision.*

V.E. Effects on Ecosystem Structure and Processes (Other Biotic Interactions) led to:

_____ VI.A. Risk management - to manage risk of alteration of ecosystem processes. *Attach written description of any identified hazard.*

_____ VI.B. Risk management - to manage risk where there is insufficient information.

_____ VII. Exit Routine. *Attach rationale for seeking exit.*

VI.A. Risk Management - Specific Risks led to:

____ VI.A.1. Risk Management-Specific Risks: Microorganisms.

____ VI.C. to choose appropriate methods of Containment.

VI.A.1. Risk Management-Specific Risks: Microorganisms led to:

____ Consideration of measures to manage potential risk(s). *Attach a written description of the risk management measures you plan to implement (and as suggested in VI.C. Risk Management-Containment Routines). Be certain to address the topics listed in the Risk Management Documentation section below. Attach your rationale for disallowing release in any case where containment cannot be ensured.*

VI.B. Risk Management-Insufficient Information led to:

____ VI.A.1. Risk Management-Specific Risks: Microorganisms.

____ VI.C. to choose appropriate method of Containment.

VI.C. Risk Management-Containment Routines led to:

____ VII. Exit Routine (due to peer reviewers' conclusion of sufficient containment). *Attach a written description of the risk management measures you plan to implement. Be certain to address the topics listed in the Risk Management Documentation section below.*

____ Consider disallowing release or use of GEO (due to peer reviewers' conclusion of insufficient containment). *Attach your rationale.*

VII. Exit Routine led to:

_____ VIII. Overview of Food Safety.

____ Exiting this assessment.

VIII. Overview of Food Safety TEXT asked that you consider what assessment strategy you deem appropriate to assess the safety of the GEF. *Attach rationale for choice of level of margin of safety. List tests that were indicated.*

VIII. Overview of Food Safety led to:

____ VIII.A. Molecular Characterization
____ VIII.B. Toxins
____ VIII.C. Allergens
____ Consider disallowing use of GEF as food.*Attach rationale for decision.*
____ Consider use of GEF as food. *Attach rationale for decision.*

VIII.A. Food Safety Assessment: Molecular Characterization led to:

_____ VIII. Overview of Food Safety **<3>** which led to:
_____ VIII.B. Toxins
_____ VIII.C. Allergens
_____ Consider disallowing use of GEF as food. *Attach rationale for decision.*
_____ Consider use of GEF as food. *Attach rationale for decision.*
_____ IX. In Vivo Testing, **<2>** which led to:
_____ Consider disallowing use of GEF as food. *Attach rationale for decision.*
_____ VIII. Overview of Food Safety **<10>** which led to:
___ Consider use of GEF as food. *Attach rationale for decision. Also attach labeling and use instructions, if any, and plans for implementation of consumers' reporting mechanism.*
___ Consider disallowing use of GEF as food. *Attach rationale for decision.*

VIII.B. Toxins led to:

____ VIII. Overview of Food Safety **<6>** which led to:
____ VIII.C. Allergens
____ Consider disallowing use of GEF as food. *Attach rationale for decision.*
____ Consider use of GEF as food. *Attach rationale for decision.*
____ VIII.B.1. In Vitro Testing for Toxins

VIII. B.1.Food Safety Assessment: In Vitro Testing for Toxins required consultation with experts.

Which agencies and/or personnel were consulted? *List names, areas of expertise and phone numbers.*

Which tests were performed? *List tests and results.*

The flowchart led to:

____ VIII. Overview of Food Safety **<6>** which led to:

____ VIII.C. Allergens
____ Consider disallowing use of GEF as food. *Attach rationale for decision.*
____ Consider use of GEF as food. *Attach rationale for decision. Also attach labeling and use instructions.*

____ Consider disallowing use of GEF as food. *Attach rationale for decision.*

VIII.C. Allergens led to:

____ VIII. Overview of Food Safety **<10>** which led to:
____ Consider disallowing use of GEF as food. *Attach rationale for decision.*
____ Consider use of GEF as food. *Attach rationale for decision. Also attach labeling and use instructions.*

____ VIII.C.1. Food Safety Assessment: In Vitro Testing for Allergens

VIII.C.1. Food Safety Assessment: In Vitro Testing for Allergens required consultation with experts.

Which agencies and personnel were consulted? *List names, areas of expertise and phone numbers.*

Which tests were performed? *List tests and results.*

The flowchart led to:

____ VIII. Overview of Food Safety **<10>** which led to:
____ Consider disallowing use of GEF as food. *Attach rationale for decision.*
____ Consider use of GEF as food. *Attach rationale for decision. Also attach labeling and use instructions.*

____ IX. Food Safety Assessment: In Vivo Testing Overview

____ Consider disallowing use of GEF as food. *Attach rationale for decision.*

IX. Food Safety Assessment: In Vivo Testing Overview required consultation with experts.

Which agencies and personnel were consulted? *List names, areas of expertise and phone numbers.*

Which tests were performed? *List tests and results performed at each stage of testing.*

The flowchart led to:

____ Consider disallowing use of GEF as human food. *Attach rationale for decision.*

____ VIII. Overview of Food Safety **<10>** which led to:

____ Consider use of GEF as food. *Attach rationale for decision, labeling and use instructions, plans for implementing a reporting mechanism for consumers.*

____ Consider disallowing use as food. *Attach rationale for decision.*

Additional Questions

1. Are you working with a non-indigenous species?
   ____ Yes.
   ____ No.

2. If yes, have you consulted any governmental and/or international agencies which oversee uses of non-indigenous species and complied with their procedures?
   ____ Yes
   ____ No

List names addresses, telephone numbers, and area of expertise of the experts you contacted for substantial advice in assessing effects of a proposed project and in designing adequate safety measures.

_____

_____

_____

_____ _____

Signature of assessor Date

Address, Phone Number, FAX Number, and Email Address

_____

_____

_____

_____

_____

_____

Risk Management Documentation

As part of good recordkeeping, the user should describe and provide the rationale for the risk management measures. Major points explained in the text on Risk Management Recommendations are listed below. Researchers and reviewers should read the text on Risk Management Recommendations before using this portion of the Worksheet. The risk management documentation should fully respond to these major points. For items which request a narrative response, attach your written responses and identify the numbered item being addressed.

Project Siting

1. Explain how the siting and structures of the project prevent accidental releases during flooding or other natural disasters.
   a. If project involves placement of GEOs in uncovered outside settings (e.g., fish tanks or ponds, garden beds), is there the potential for sudden high winds to wash organisms into a natural water body (accessible ecosystem) via water spray or waves?
      ____ Yes. Proceed to item 1. b.
      ____ No. Proceed to item 2.

   b. If there is potential for GEOs held in outside units to be washed via sudden high winds into a natural water body, what measures will be taken to adequately cover these outside units or otherwise protect against movement of GEOs by water spray or waves into nearby natural water bodies? (Explanatory diagrams may be useful).

Design of Barriers

The manual identifies four types of barriers: (1) physical or chemical; (2) mechanical; (3) biological; and (4) scale of endeavor.

2. Was the project site chosen because the surrounding accessible ecosystems are lethal to all life stages of the GEO?
   ____ Yes. Address items 2.a and 2.b.
   ____ No. Proceed to item 3.

   (a) Describe evidence that the accessible ecosystems are indeed lethal to the GEO.
   (b) Explain how the siting reduces the need for barriers on-site.

3. Could the project's GEOs potentially escape through any of the (water-borne, air-borne, or land-borne) paths listed below? Answer "Yes" if there is potential for escape or uncertainty about potential escape of GEOs via the listed path. Answer "No" only if escape is clearly precluded.

_____ a. Influent/makeup water?
_____ b. Effluent and drawdown water?
(Note: if discharge to sanitary sewer is used as one barrier against accidental escape of GEOs in effluent, at least one additional barrier is necessary.)
_____ c. Waste slurries or other waste material
_____ d. Disposal of GEOs?
_____ e. Aerosols?
_____ f. Equipment cleaning and storage?
_____ g. Windows and/or doors?
_____ h. Burrowing through walls, floors, and/or doors?

4. Have you identified additional, potential escape paths through air, water, or land? If yes, briefly describe each path.

5. For each escape path identified in items 3 and 4 above, describe the arrangement and types of barriers to escape; a diagram of layout of barriers at the site or facility may be useful. Describe: treatment and disposal of waste materials; secure disposal of GEOs; and cleaning and storage of equipment.

6. Describe how the types and numbers of barriers in series are sufficient to achieve the containment specified in Flowcharts VI.A. or VI.B.

Special Concerns

7. _____ If biological barriers are used for a given escape path, does the path have at least one other type of barrier? (Because of their variable efficacy, biological barriers cannot comprise the entire set of barriers.)

8. _____ If scale is used as a barrier, are you certain the GEO is not a self-fertilizing hermaphrodite or true parthenogen? Attach supporting evidence.

Security

9. Describe the security measures implemented to:
   a. control normal movement of authorized personnel,
   b. prevent unauthorized access to the site, and
   c. eliminate access for predators who could potentially carry GEOs off-site (applies only to outdoor projects).

Alarms

10. Describe and justify the adequacy of the entire set of installed alarms. Be sure to address the following:
   a. Have you installed an alarm at all major escape routes?
   b. Do all installed alarms have backup power?
   c. Describe the plan for notifying designated personnel.

Operational Plan

11. Attach the written operational plan. Required components are:
    a. Training.
    b. Traffic Control.
    c. Record Keeping.
    d. Emergency Response Plan.

Review and Inspection

12. Has your institutional biosafety committee, biosafety officer, or other appropriate expert reviewed and approved the proposed project and its risk management measures? If no, explain the status of review of your project.
    ____ Yes
    ____ No

Have you notified governmental agencies having jurisdiction over any aspects of your proposed project? If no, please explain.

____ Yes
____ No

Please list all required permits and authorizations and check appropriate line regarding status of your application:

	approved	pending	not yet submitted
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

COMPLETED WORKSHEETS
for
SAMPLE PROJECTS

The following are completed worksheets for sample projects examined using the assessment pathways of this Manual. The worksheets have been shortened to save space. Not all details of every "attachment" and discussion have been included, only enough to give the flavor of the biosafety decision-making.

Worksheet
accompanying
The Biosafety Manual

Introduction

This manual is intended to aid users in assessing the genetic, ecological, and human health effects of genetically engineered organisms used in research, development, and small-scale or large-scale release. The flowcharts guide users in identifying specific hazards and managing the risk associated with each identified hazard, where possible. This worksheet is meant to accompany the flowcharts. The user should use the worksheet to document and track the decision path taken through the flowcharts and any plans for risk management.

Name of Assessor: **C. Gigas**

Identification Name of the GEO: **triploid Pacific oyster**

Purpose for which GEO was designed: **to render sterile an exotic species so that it could be tested in non-native waters**

Describe proposed project: **investigation of resistance of triploid Pacific oysters to the disease MSX and dermo in Chesapeake Bay**

On the worksheet below, record the pathway you took through the flowcharts. Please note that below, flowcharts are always listed in sequential order. *Attach written explanatory materials as directed.*

Flowchart Documentation

Please list the numbers of **all** flowcharts that you used:
I., I.A., II.A., II.B., II.B.1., VII., VIII.

Next to each flowchart used, indicate with an **X** where that chart led you and attach any explanations or rationales requested.

Flowchart No.

I. Determination of Assessment Pathway led to:

- **X** I.A. to Continuation of Pathway Determination.
- ____ II.D. to assess Transfer of Harmful Biochemical Compounds to Food Chain.
- ____ II.E. to begin assessment of Vectors Genetically Engineered to Reduce Disease.
- ____ VIII. to begin Food Safety Assessment.

I.A. Continuation of Pathway Determination led to:

__X__ II.A. to consider Survival and Reproduction Assessment

____ VII. Exit Routine. *Attach rationale for arriving here from <4>.*

____ Appendix B to consider other assessment because organism is modified solely by selective or captive breeding. This then led to:

____ decision to pursue other assessment. *Indicate rationale for decision and name of other assessment protocol.*

____ return to I.A. <2> which led to a second consideration of this pathway (and a second "X")

____ Appendix C to consider Assessment of GEOs with Alternate Reproductive Pathways. This led to:

____ NOT proceeding further with the project and exiting this assessment.

____ returning to I.A.<6> which led to a further consideration of this pathway (and another "X").

____ Consider disallowing release because GEO is not effective for its intended purpose. *Attach rationale for your decision.*

II.A. Survival and Reproduction Assessment - Deliberate Gene Changes led to

_____ II.A.1. to assess impact of Deliberate Gene Changes.

__X__ II.B. to assess impact of Deliberate Chromosomal Manipulations.

_____ VII. Exit Routine. *Attach your rationale for seeking exit.*

II.B. Survival and Reproduction Assessment - Deliberate Chromosomal Manipulation led to:

__X__ II.B.1. to assess Impact of Deliberate Chromosomal Manipulations.

____ II.C. to assess impact of Interspecfic Hybridization.

____ VII. Exit Routine. *Attach your rationale for seeking exit.*

II.B.1. Impact of Deliberate Chromosomal Manipulations led to:

____ III. to assess Potential Interference with Natural Reproduction.

____ V. to assess Effects on Ecosystem Structure and Processes.

____ VII. Exit Routine. *Attach your rationale for seeking exit.*

__X__ VII. Exit Routine and consultation with relevant government agencies regarding use of non-indigenous species. *Attach your rationale for seeking exit and indicate agency name(s) and recommendations.*

____ Consider disallowing release/dispersal because organism is not likely to produce desired effect. *Attach rationale for decision.*

VII. Exit Routine led to:

__X__ VIII. Overview of Food Safety.

____ Exiting this assessment.

VIII. Overview of Food Safety TEXT asked that you consider what assessment strategy you deem appropriate to assess the safety of the GEF. *Attach rationale for choice of level of margin of safety. List tests that were indicated.*

(see attached)

VIII. Overview of Food Safety led to:

_____ VIII.A. Molecular Characterization
_____ VIII.B. Toxins
_____ VIII.C. Allergens
_____ Consider disallowing use of GEF as food. *Attach rationale for decision.*
__X__ Consider use of GEF as food. *Attach rationale for decision.*

(see attached)

Additional Questions

1. Are you working with a non-indigenous species?
   __X__ Yes.
   ____ No.

2. If yes, have you consulted any governmental and/or international agencies which oversee uses of non-indigenous species and complied with their procedures?
   __X__ Yes
   ____ No

List names addresses, telephone numbers, and area of expertise of the experts you contacted for substantial advice in assessing effects of a proposed project and in designing adequate safety measures.

Local official, Virginia Marine Resources Commission

Marine ecologist

Reproductive Physiologist

C.Gigas — Signature of assessor

August 1, 1995 — Date

Address, Phone Number, FAX Number, and Email Address

Department of Fisheries and Aquaculture
Virginia Institute of Marine Science
Gloucester Point, VA USA

Risk Management Documentation

As part of good recordkeeping, the user should describe and provide the rationale for the risk management measures. Major points explained in the text on Risk Management Recommendations are listed below. Researchers and reviewers should read the text on Risk Management Recommendations before using this portion of the Worksheet. The risk management documentation should fully respond to these major points. For items which request a narrative response, attach your written responses and identify the numbered item being addressed.

Project Siting

1. Explain how the siting and structures of the project prevent accidental releases during flooding or other natural disasters.
   a. If project involves placement of GEOs in uncovered outside settings (e.g., fish tanks or ponds, garden beds), is there the potential for sudden high winds to wash organisms into a natural water body (accessible ecosystem) via water spray or waves?

      ____ Yes. Proceed to item 1. b.
      ____ No. Proceed to item 2.

   b. If there is potential for GEOs held in outside units to be washed via sudden high winds into a natural water body, what measures will be taken to adequately cover these outside units or otherwise protect against movement of GEOs by water spray or waves into nearby natural water bodies? (Explanatory diagrams may be useful).

Design of Barriers

The manual identifies four types of barriers: (1) physical or chemical; (2) mechanical; (3) biological; and (4) scale of endeavor.

2. Was the project site chosen because the surrounding accessible ecosystems are lethal to all life stages of the GEO?
   ___ Yes. Address items 2.a and 2.b.
   _**X**_ No. Proceed to item 3.

   (a) Describe evidence that the accessible ecosystems are indeed lethal to the GEO.
   (b) Explain how the siting reduces the need for barriers on-site.

3. Could the project's GEOs potentially escape through any of the (water-borne, air-borne, or land-borne) paths listed below? Answer "Yes" if there is potential for escape or uncertainty about potential escape of GEOs via the listed path. Answer "No" only if escape is clearly precluded.
   **yes** a. Influent/makeup water?

**yes** b. Effluent and drawdown water?
(Note: if discharge to sanitary sewer is used as one barrier against accidental escape of GEOs in effluent, at least one additional barrier is necessary.)
_**no**_ c. Waste slurries or other waste material
**yes** d. Disposal of GEOs?
**yes** e. Aerosols?
**yes** f. Equipment cleaning and storage?
_**no**_ g. Windows and/or doors?
_**no**_ h. Burrowing through walls, floors, and/or doors?

4. Have you identified additional, potential escape paths through air, water, or land? If yes, briefly describe each path. **no**

5. For each escape path identified in items 3 and 4 above, describe the arrangement and types of barriers to escape; a diagram of layout of barriers at the site or facility may be useful. Describe: treatment and disposal of waste materials; secure disposal of GEOs; and cleaning and storage of equipment.
(see attached)

6. Describe how the types and numbers of barriers in series are sufficient to achieve the containment specified in Flowcharts VI.A. or VI.B. **(see attached)**

Review and Inspection

12. Has your institutional biosafety committee, biosafety officer, or other appropriate expert reviewed and approved the proposed project and its risk management measures? If no, explain the status of review of your project.
_**X**_ Yes
____ No

Have you notified governmental agencies having jurisdiction over any aspects of your proposed project? If no, please explain.
_**X**_ Yes
_**X**_ No

Attachment to Worksheet

Proposed Project: Investigation of resistance of triploid Pacific oysters to the diseases MSX and dermo in Chesapeake Bay

Risk identified (Agricultural Biotechnology Research Advisory Committee 1995):

The Pacific oyster, *Crassostrea gigas*, is not native to Chesapeake Bay, hence, triploidy will be used as a means of reproductive confinement. Triploid Pacific oysters, however, have shown a high frequency of hermaphroditism, as high as 29% (Allen and Downing, 1990. Performance of triploid Pacific oysters, *Crassostrea gigas*: gametogenesis. Canadian Journal of Fisheries and Aquatic Sciences 47: 1213-1222). An individual was observed which produced haploid eggs and sperm. The possibility of selfing has not been investigated. Further, recent observations suggest that a considerable proportion of apparently triploid individuals can progressively revert to the diploid condition. (Blankenship 1994. Experiment with Japanese oysters end abruptly. Bay Journal 4 (5): 1-4). Therefore, application of the precautionary principle would have me consult Appendix C and practice strict containment.

Should it be possible to rule out the possibility of selfing or conventional reproduction, a different pathway through the flowcharts would lead me to identify much the same set of risks. With exit from the flowcharts and consulting the Virginia Marine Resources Commission, however, specific requirements for risk management would depend on regulations of the state of Virginia or of other agencies (see recommendations). The only modification to the parental organism is a change in the number of chromosomes. It is proposed that the oysters will be stocked into a suitable natural ecosystem. Were the oysters to reproduce, it would not be possible to treat the ecosystem to eradicate the young. There are no native species with which Pacific oysters can interbreed; thus, risk is limited to that of introduction of a new species, due to reproduction of individuals for which triploidy turned out to be an ineffective means of sterilization.

Proposed risk management (Agricultural Biotechnology Research Advisory Committee 1995):

Although I was routed to consult relevant state and federal agencies, I voluntarily offer the following description of my experimental protocol. Siting of the experiment so as to minimize risk is not an option. Hence, oysters will be held in a tank into which unfiltered Bay water will be pumped - a vertical drop will preclude loss of gametes via influent water. Effluent water will pass through a UV sterilization unit and a filter removing particles smaller than oyster gametes. The tank will be held in a greenhouse - during the breeding season, aerosols from over the tank will be passed through a double screen to remove any larvae which may have become entrained. Equipment used in the facility will not be used elsewhere. Research animals will be killed and stored under freezing conditions for at least 24 hours before disposal. Access to the site will be limited. Personnel will be chosen carefully and thoroughly briefed about risks posed by introduction of the species to Chesapeake Bay.

Rationale for allowing use as food (See VIII.):

It is highly unlikely that these triploid oysters contain novel proteins or gene products because (1) the extra haploid set of chromosomes comes from an endogenous genome of the unmodified oyster species; and (2) the unmodified oyster species has been widely used as human food. Although some individuals in the human population may be allergic to shellfish, including oysters, it is not likely that these triploid oysters elicit a different response to their allergenic properties than do the unmodified oysters. Further, because the triploids look like oysters, those allergic to unmodified oysters have an effective warning “label”.

Worksheet
accompanying
The Biosafety Manual

Introduction

This manual is intended to aid users in assessing the genetic, ecological, and human health effects of genetically engineered organisms used in research, development, and small-scale or large-scale release. The flowcharts guide users in identifying specific hazards and managing the risk associated with each identified hazard, where possible. This worksheet is meant to accompany the flowcharts. The user should use the worksheet to document and track the decision path taken through the flowcharts and any plans for risk management.

Name of Assessor: **I. Punctatus**

Identification Name of the GEO: **transgenic catfish**

Purpose for which GEO was designed: **to produce faster growing catfish for aquatic applications**

Describe proposed project: **field testing of channel catfish expressing an introduced growth hormone gene, field testing to be in Alabama**

On the worksheet below, record the pathway you took through the flowcharts. Please note that below, flowcharts are always listed in sequential order. *Attach written explanatory materials as directed.*

Flowchart Documentation

Please list the numbers of **all** flowcharts that you used:
I., I.A., II.A., II.A.1., IV.A., VI.B., VI.C., VII.

Next to each flowchart used, indicate with an **X** where that chart led you and attach any explanations or rationales requested.

Flowchart No.

I. Determination of Assessment Pathway led to:

- **X** I.A. to Continuation of Pathway Determination.
- ____ II.D. to assess Transfer of Harmful Biochemical Compounds to Food Chain.
- ____ II.E. to begin assessment of Vectors Genetically Engineered to Reduce Disease.
- ____ VIII. to begin Food Safety Assessment.

I.A. Continuation of Pathway Determination led to:

_**X**__ II.A. to consider Survival and Reproduction Assessment

_____ VII. Exit Routine. *Attach rationale for arriving here from <4>.*

_____ Appendix B to consider other assessment because organism is modified solely by selective or captive breeding. This then led to:

____ decision to pursue other assessment. *Indicate rationale for decision and name of other assessment protocol.*

____ return to I.A.<2> which led to a second consideration of this pathway (and a second "X")

_____ Appendix C to consider Assessment of GEOs with Alternate Reproductive Pathways. This led to:

____ NOT proceeding further with the project and exiting this assessment.

____ returning to I.A.<6> which led to a further consideration of this pathway (and another "X").

_____ Consider disallowing release because GEO is not effective for its intended purpose. *Attach rationale for your decision.*

II.A. Survival and Reproduction Assessment - Deliberate Gene Changes led to

_**X**__ II.A.1. to assess impact of Deliberate Gene Changes.

_____ II.B. to assess impact of Deliberate Chromosomal Manipulations.

_____ VII. Exit Routine. *Attach your rationale for seeking exit.*

II.A.1. Impact of Deliberate Gene Changes led to:

____ II.B. to assess impact of Deliberate Chromosomal Manipulations.

____ III. to assess Potential Interference with Natural Reproduction.

_**X**__ IV.A. to assess Ecosystem Effects from Impacts of Introgression of Modified Gene(s).

____ IV.B. to assess Ecosystem Effects on Reproduction.

____ V. to assess Effects on Ecosystem Structure and Processes.

____ VI.A. Risk management - to manage specific risks to populations of special concern. *Attach written description of any identified hazard.*

____ VI.B. Risk management - to manage risk where there is insufficient information.

____VII. Exit Routine. *Attach your rationale for seeking exit.*

____ VII. Exit Routine and consultation with relevant national, regional, and local government agencies regarding use of non-indigenous species. *Attach your rationale for seeking exit and indicate agency name(s) and recommendations.*

IV.A. Ecosystem Effects - Impacts of Introgression of Modified Gene(s) led to:

_____ V. to assess Effects on Ecosystem Structure and Processes.

_____ VI.A. Risk Management - to manage risk of decline of population abundance. *Attach a written description of any identified hazard.*

__X__ VI.B. Risk Management - to manage risk where there is insufficient information.

____ Consider disallowing release. *Attach rationale for decision.*

VI.B. Risk Management-Insufficient Information led to:

_____ VI.A.1. Risk Management-Specific Risks: Microorganisms.

__X__ VI.C. to choose appropriate method of Containment.

VI.C. Risk Management-Containment Routines led to:

__X__ VII. Exit Routine (due to peer reviewers' conclusion of sufficient containment). *Attach a written description of the risk management measures you plan to implement. Be certain to address the topics listed in the Risk Management Documentation section below.*

____ Consider disallowing release or use of GEO (due to peer reviewers' conclusion of insufficient containment). *Attach your rationale.*

VII. Exit Routine led to:

____ VIII. Overview of Food Safety.

__X__ Exiting this assessment.

Additional Questions

1. Are you working with a non-indigenous species?

____ Yes.

__X__ No.

List names addresses, telephone numbers, and area of expertise of the experts you contacted for substantial advice in assessing effects of a proposed project and in designing adequate safety measures.

Local Fish and Game Department Official

Evolutionary biologist

Aquatic ecologist

I. Punctatus *August 1, 1995*

Signature of assessor Date

Address, Phone Number, FAX Number, and Email Address
Department of Fisheries and Aquaculture
Auburn University
Auburn, Alabama 36820

Risk Management Documentation

As part of good recordkeeping, the user should describe and provide the rationale for the risk management measures. Major points explained in the text on Risk Management Recommendations are listed below. Researchers and reviewers should read the text on Risk Management Recommendations before using this portion of the Worksheet. The risk management documentation should fully respond to these major points. For items which request a narrative response, attach your written responses and identify the numbered item being addressed.

Project Siting

1. Explain how the siting and structures of the project prevent accidental releases during flooding or other natural disasters.
   a. If project involves placement of GEOs in uncovered outside settings (e.g., fish tanks or ponds, garden beds), is there the potential for sudden high winds to wash organisms into a natural water body (accessible ecosystem) via water spray or waves?
      _____ Yes. Proceed to item 1. b.
      X No. Proceed to item 2.

Design of Barriers

The manual identifies four types of barriers: (1) physical or chemical; (2) mechanical; (3) biological; and (4) scale of endeavor.

2. Was the project site chosen because the surrounding accessible ecosystems are lethal to all life stages of the GEO?
   _____ Yes. Address items 2.a and 2.b.
   X No. Proceed to item 3.

3. Could the project's GEOs potentially escape through any of the (water-borne, air-borne, or land-borne) paths listed below? Answer "Yes" if there is potential for escape or uncertainty about potential escape of GEOs via the listed path. Answer "No" only if escape is clearly precluded.
   yes a. Influent/makeup water?
   yes b. Effluent and drawdown water?
   (Note: if discharge to sanitary sewer is used as one barrier against accidental escape of GEOs in effluent, at least one additional barrier is necessary.)

__**no**__ c. Waste slurries or other waste material
__**no**__ d. Disposal of GEOs?
__**no**__ e. Aerosols?
__**yes**__ f. Equipment cleaning and storage?
__**no**__ g. Windows and/or doors?
__**no**__ h. Burrowing through walls, floors, and/or doors?

4. Have you identified additional, potential escape paths through air, water, or land? If yes, briefly describe each path. **no**

5. For each escape path identified in items 3 and 4 above, describe the arrangement and types of barriers to escape; a diagram of layout of barriers at the site or facility may be useful. Describe: treatment and disposal of waste materials; secure disposal of GEOs; and cleaning and storage of equipment.
(see attached)

6. Describe how the types and numbers of barriers in series are sufficient to achieve the containment specified in Flowcharts VI.A. or VI.B.
(see attached)

Special Concerns

7. __**yes**__ If biological barriers are used for a given escape path, does the path have at least one other type of barrier? (Because of their variable efficacy, biological barriers cannot comprise the entire set of barriers.)

8. __**n.a.**__ If scale is used as a barrier, are you certain the GEO is not a self-fertilizing hermaphrodite or true parthenogen? Attach supporting evidence. **(not applicable)**

Security **(see attached)**

9. Describe the security measures implemented to:
   a. control normal movement of authorized personnel,
   b. prevent unauthorized access to the site, and
   c. eliminate access for predators who could potentially carry GEOs off-site (applies only to outdoor projects).

Alarms **(see attached)**

10. Describe and justify the adequacy of the entire set of installed alarms. Be sure to address the following:
   a. Have you installed an alarm at all major escape routes?
   b. Do all installed alarms have backup power?
   c. Describe the plan for notifying designated personnel.

Operational Plan **(see attached)**

11. Attach the written operational plan. Required components are:
    a. Training.
    b. Traffic Control.
    c. Record Keeping.
    d. Emergency Response Plan.

Review and Inspection

12. Has your institutional biosafety committee, biosafety officer, or other appropriate expert reviewed and approved the proposed project and its risk management measures? If no, explain the status of review of your project.
    __**X**__ Yes
    _____ No

Have you notified governmental agencies having jurisdiction over any aspects of your proposed project? If no, please explain.
    __**X**__ Yes
    _____ No

Please list all required permits and authorizations and check appropriate line regarding status of your application: **(see attached)**

Attachment to Worksheet

Proposed Project: Field testing of channel catfish expressing an introduced growth hormone gene in Alabama.

Risks identified(Agricultural Biotechnology Research Advisory Committee 1995): The accessible ecosystem contains conspecifics with which the transgenic catfish potentially could interbreed. The transgenic catfish are fertile; hence, there is a potential for reproduction of the transgenic fish, with possible introgression of the introduced growth hormone gene construct into the natural population.

In order to evaluate ecosystem effects of the deliberate gene change, I would need to have information regarding reproductive potential, gene flow, and fitness for a GMO population, as well as information about the structure and function of the accessible ecosystem. In particular, the current knowledge base makes it quite difficult for me to anticipate the fitness of transgenic catfish expressing an introduced growth hormone gene, or their descendants, in natural ecosystems. Hence, I conclude that lack of familiarity prevents reliable assessment of ecological effects, and I choose to practice risk management as appropriate in the face of insufficient information.

Risk management documentation (Agricultural Biotechnology Research Advisory Committee 1995): The project will be carried out in a secured outdoor pond facility near Auburn, Alabama. A portion of the facility was designed and built for purposes of confinement of genetically modified fish.

Project siting:

Question 1. The project site is over a mile from Sougahatchee Creek, the closest body of natural water. The top of the pond levees are approximately 36 feet above the estimated 100-year flood height for the creek.

1a. There is no potential for sudden high winds to wash organisms into the accessible ecosystem via water spray or waves.

Design of barriers.

Question 2. The project site is not inherently lethal to channel catfish; indeed, channel catfish populations occur naturally in the watershed.

Question 3. Transgenic catfish might potentially escape via influent/makeup waters, via effluent or drawdown waters, or via disposal of experimental animals. Procedures for minimizing associated hazards are described below under question 5. I find it untenable that catfish could escape from the facility in waste slurries, in aerosols, or via equipment cleaning or storage; details are presented below as responses to questions 9b and 9c, respectively.

Question 4. Human or animal encroachment. Procedures to minimize associated hazards are presented below as responses to question 9b and 9c, respectively.

Question 5. Barriers to escape of experimental animals via given paths are described below:

a. Influent/makeup water. The ponds' inlets will be double-screened, with a vertical drop of water into any pond or culture vessel. During drought conditions, water may have to be added to the ponds. This will be done only by personnel with authorized entry into the pond site. With a

maximum flow-rate of 9,500 gallons per hour through the overflow pipe, there is little chance of accidentally adding too much water through the ponds.

b. Effluent/drawdown water. The ponds' outlets will be double-screened. Screens will be hose-clamped to the end of the pipes. The mesh sizes used will be compatible with the confinement requirements of the life stage of the fish. Initially, a 250 micron saran screen will be used, and mesh size will be increased to 1/2 inch as the fish grow. Screens larger than 500 microns will be made of hard plastic securely clamped to the pipe.

Any water discharged from the ponds will pass into a catch basin emptied through a French drain (Figure A - page 225). The catch basin is a 0.3 acre pond into which all water from the experimental ponds drain. The bottom of the catch basin pond contains a French drain in a trench that is 70 feet long, 6 feet wide, and 5 feet deep. The French drain is designed to filter any water entering the catch basin through several layers of gravel and Agrifabric before entering perforated pipes located near the bottom of the drain. Filtered water then discharges off site into a open drain ditch, where it flows into a barrier pond about 1/2 mile away.

The barrier pond is an impounded reservoir containing fishes predacious on the various life stages of channel catfish. The water level of the pond will be maintained at nine inches below spillway elevation to fully contain any discharge from the experimental pond site.

c. Waste slurries. No waste slurries are at issue in this experiment.

d. Disposal of experimental animals. At termination of the experiment, the fish will be seined from the experimental ponds and humanely killed with MS-222. The ponds will be poisoned with rotenone to kill any fish which may remain. A group of bioassay carp in a cage will be placed in the ponds to confirm efficacy of the poison. The rotenone-treated water will be detoxified with potassium permanganate and the rotenone allowed to completely oxidize prior to the ponds being drained into the catch basin pond. Dead fish will be frozen for a period of not less than 24 hours before disposal by incineration at the Veterinary College.

e. Aerosols. Escape of animals via aerosols is not at issue for channel catfish.

f. Equipment cleaning and storage. Nets, boots, and small equipment will be washed down after use in water containing bleach, and allowed to dry thoroughly. Nets will be thoroughly dried. Equipment used on site will not be removed for use elsewhere.

Question 6. I believe that physical barriers render it impossible for fish to escape through either influent or effluent flows. Physical barriers should effectively preclude animal encroachment (see also 9c below). Chemical treatment of effluent provides an extra measure of fish confinement. Should fish escape, biological control, in the form of predation in the barrier pond, should provide yet another back-up system. Hence, I expect that no escapees will prove able to leave the experimental pond complex.

Special concerns.

Question 7. Not only a biological barrier (predators in barrier pond), but also physical and chemical barriers are involved in this risk management system.

Question 8. Although scale is not a barrier in this project, I offer the following information. Channel catfish are known to be gonochorists; i.e., there are two, genetically determined sexes, and reproduction occurs exclusively through union of sperm and egg gametes. The only selfing vertebrate is *Rivulus marmoratus*, an unrelated fish (Nelson, J.S. 1994. Fishes of the World, John Wiley and Sons, New York). Although there are some hybridogenetic fishes, e.g., *Poeciliopsis sp.*, a male's genetic contribution is necessary for reproduction to go forward (Vrijnhoek et al. 1977. Variation and heterozygosity in sexually vs. clonally reproducing populations of *Poeciliopsis*. Evolution 31:767-781).

Security.

Question 9.

a. Access to the experimental facility will be restricted to faculty, staff, and graduate students who have been instructed and tested on their knowledge of biosafety procedures for the experiment. Project personnel and authorized visitors to the experimental site will be required to log in and log out.

b. In order to preclude human encroachment, a ten-foot fence, topped with barbed wire, will encircle the compound, Gates will be locked when project personnel are not present. The experimental area will be posted and lighted. Staff will patrol the area intermittently during the day, seven days a week. University police will patrol the area at least twice during the night.

c. The ponds will be fully enclosed with 1/2 inch mesh, polyethylene bird netting placed from the ground up on the outside of the chain link fence and covering the top of the pond unit. A 1/16 inch wire screen perimeter fence, 18 inches high, also will be attached to the chain link fence. The double fencing and netting will restrict access by birds, waterfowl, and other predators such as snakes, rodents, and other animals. The levees will be mowed regularly, and any animals seen in the area that may cause damage to the outer perimeter of the dikes will be removed.

Netting, fences, levees, and water levels in the ponds will be formally inspected weekly. Filters of mesh size less than 1/4 inch will be inspected and cleaned daily. Those with mesh size equal to or greater than 1/4 inch will be inspected and cleaned weekly. A log of such inspections will be maintained. In addition, personnel working on the premises daily will promptly report any observed deficiency in the barriers.

Alarms.

Question 10

a. Alarms have been installed to announce overflow of any pond unit.

b. Alarms, and indeed all emergency equipment, are connected to back-up power.

c. Alarms will both produce a sound audible at the experimental site and set off beepers worn by one or more project personnel at all times. Any project personnel receiving an alarm will notify the principal investigator and any other appropriate designated personnel.

Operational plan.

Question 11

a. Prospective project personnel will be screened for sensitivity to the security issues involved, and will be trained regarding the importance of maintaining security.

b. Access to the experimental facility will be restricted as described above under item 9a.

c. A log will be maintained of: (a) numbers of fish in each experimental unit, (b) all movements of experimental fish, (c) all people entering or leaving the experimental site, and (d) all security checks.

d. An agricultural meteorologist will be designated to inform the principal investigator of the prospect of severe weather. Should project personnel on site determine that failure of confinement is likely, the ponds will be poisoned with a lethal dose of rotenone to kill the experimental animals. Appropriate state agencies will be notified promptly of any suspected or

known escapes of experimental animals. A sufficient supply of rotenone and potassium permanganate, which can be used to accelerate the decomposition of rotenone, and application equipment will be kept on the premises. In the event of suspected or known escapes, any actions undertaken would be carried out in accordance with the advice, and if practical, under the supervision of appropriate state authorities.

Review and inspection. Our institutional biosafety committee: (a) has reviewed and approved the proposed project and its risk management measures, and (b) will make both announced and unannounced inspections.

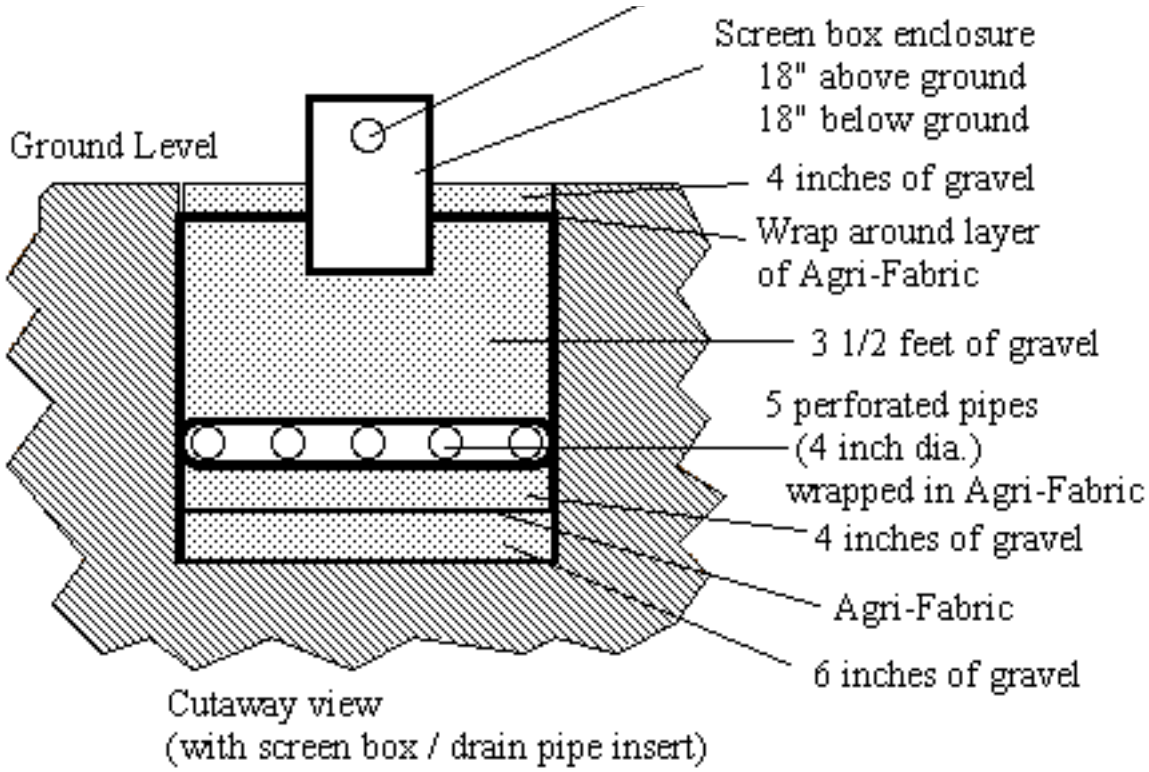


Figure A. Schematic drawing of a French drain installed for the effluent of each outdoor pond used in transgenic fish experiments. The French drain is designed to retain the smallest possible size of fish reared in the pond. Water discharged from this drain eventually reaches surface waters. (Adapted from Cooperative State Research Service 1990, as cited in Cooperative State Research Service 225.)

Worksheet
accompanying
The Biosafety Manual

Introduction

This manual is intended to aid users in assessing the genetic, ecological, and human health effects of genetically engineered organisms used in research, development, and small-scale or large-scale release. The flowcharts guide users in identifying specific hazards and managing the risk associated with each identified hazard, where possible. This worksheet is meant to accompany the flowcharts. The user should use the worksheet to document and track the decision path taken through the flowcharts and any plans for risk management.

Name of Assessor: **Soil Ecologist**

Identification Name of the GEO: ***Pseudomonas cepacia*** **containing an engineered plasmid with enzymes of 2,4-D metabolism and an antibiotic marker for tetracycline**

Purpose for which GEO was designed: **bioremediation**

Describe proposed project: ***Pseudomonas cepacia*** **engineered to detoxify 2,4-D, to be added to soil (10^{12} per cm^2 of soil surface) at toxic waste sites**

On the worksheet below, record the pathway you took through the flowcharts. Please note that below, flowcharts are always listed in sequential order. *Attach written explanatory materials as directed.*

Flowchart Documentation

Please list the numbers of **all** flowcharts that you used:
I., I.A., II.A.1., IV.A., VI.B., VI.A.1.

Next to each flowchart used, indicate with an **X** where that chart led you and attach any explanations or rationales requested.

Flowchart No.

I. Determination of Assessment Pathway led to:

_**X**_ I.A. to Continuation of Pathway Determination.
____ II.D. to assess Transfer of Harmful Biochemical Compounds

to Food Chain.

_____ II.E. to begin assessment of Vectors Genetically Engineered to Reduce Disease.

_____ VIII. to begin Food Safety Assessment.

I.A. Continuation of Pathway Determination led to:

2nd _X_ II.A. to consider Survival and Reproduction Assessment

_____ VII. Exit Routine. *Attach rationale for arriving here from <4>.*

_____ Appendix B to consider other assessment because organism is modified solely by selective or captive breeding. This then led to:

____ decision to pursue other assessment. *Indicate rationale for decision and name of other assessment protocol.*

____ return to I.A. <2> which led to a second consideration of this pathway (and a second "X")

1st _X_ Appendix C to consider Assessment of GEOs with Alternate Reproductive Pathways. This led to:

_____ NOT proceeding further with the project and exiting this assessment.

_X_ returning to I.A.<6> which led to a further consideration of this pathway (and another "X").

____ Consider disallowing release because GEO is not effective for its intended purpose. *Attach rationale for your decision.*

II.A. Survival and Reproduction Assessment - Deliberate Gene Changes led to

_X_ II.A.1. to assess impact of Deliberate Gene Changes.

_____ II.B. to assess impact of Deliberate Chromosomal Manipulations.

_____ VII. Exit Routine. *Attach your rationale for seeking exit.*

II.A.1. Impact of Deliberate Gene Changes led to:

____ II.B. to assess impact of Deliberate Chromosomal Manipulations.

____ III. to assess Potential Interference with Natural Reproduction.

_X_ IV.A. to assess Ecosystem Effects from Impacts of Introgression of Modified Gene(s).

____ IV.B. to assess Ecosystem Effects on Reproduction.

____ V. to assess Effects on Ecosystem Structure and Processes.

____ VI.A. Risk management - to manage specific risks to populations of special concern. *Attach written description of any identified hazard.*

____ VI.B. Risk management - to manage risk where there is insufficient information.

____ VII. Exit Routine. *Attach your rationale for seeking exit.*

____ VII. Exit Routine and consultation with relevant national, regional, and local government agencies regarding use

of non-indigenous species. *Attach your rationale for seeking exit and indicate agency name(s) and recommendations.*

IV.A. Ecosystem Effects - Impacts of Introgression of Modified Gene(s) led to:

_____ V. to assess Effects on Ecosystem Structure and Processes.

_____ VI.A. Risk Management - to manage risk of decline of population abundance. *Attach a written description of any identified hazard.*

__X__ VI.B. Risk Management - to manage risk where there is insufficient information.

_____ Consider disallowing release. *Attach rationale for decision.*

VI.A.1. Risk Management-Specific Risks: Microorganisms led to:

__X__ Consideration of measures to manage potential risk(s). *Attach a written description of the risk management measures you plan to implement (and as suggested in VI.C. Risk Management-Containment Routines). Be certain to address the topics listed in the Risk Management Documentation section below. Attach your rationale for disallowing release in any case where containment cannot be ensured.* **(see attached)**

VI.B. Risk Management-Insufficient Information led to:

__X__ VI.A.1. Risk Management-Specific Risks: Microorganisms.

_____ VI.C. to choose appropriate method of Containment.

Additional Questions

1. Are you working with a non-indigenous species?

____ Yes.

__X__ No.

List names addresses, telephone numbers, and area of expertise of the experts you contacted for substantial advice in assessing effects of a proposed project and in designing adequate safety measures.

Soil Microbiologist

Official from government soil conservation agency

Toxicologist

Soil Ecologist ***September 1,1997***

Signature of assessor Date

Address, Phone Number, FAX Number, and Email Address

Soil Ecologist
Oregon State University
Corvallis, Oregon USA

Risk Management Documentation -- see attached below

Pseudomonas cepacia engineered to detoxify 2, 4-D

Attachment to Worksheet

Risk Management:

Given the impossibility of containment, the possibility of the spread of engineered genes through a broad range of species (including organisms that could pose a direct hazard to human health), the known toxic effects of breakdown products of 2,4-D (Doyle et al. 1995) and the lack of organisms to utilize those breakdown products in the unhealthy soils to which 2,4-D is typically applied (Doyle et al 1995), and questions about the organism's efficacy when added to soils with other bacterial species which limit its growth, serious consideration must be given to questioning whether use of this organism should even be attempted.

Risk management would require strict quarantine of the system into which the organisms were placed (probably impossible) until such time as the engineered *Pseudomonas* died or were removed/killed (very difficult) and the system shown to be free of this organism. (Any methods for removing/killing these organisms would require careful monitoring to show that all have been eliminated, since bacterial populations can be reestablished from a single organism.)

Conclusion - the release is not warranted, because clear hazards cannot be adequately managed.

Worksheet
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Introduction

This manual is intended to aid users in assessing the genetic, ecological, and human health effects of genetically engineered organisms used in research, development, and small-scale or large-scale release. The flowcharts guide users in identifying specific hazards and managing the risk associated with each identified hazard, where possible. This worksheet is meant to accompany the flowcharts. The user should use the worksheet to document and track the decision path taken through the flowcharts and any plans for risk management.

Name of Assessor: **Soil Ecologist**

Identification Name of the GEO: ***Klebsiella planticola*** **engineered to produce ethanol from plant litter/debris**

Purpose for which GEO was designed: **bioremediation - to produce saleable products from plant residues**

Describe proposed project: **microcosm experiments to assess engineered organism's effect on soil biota and plant growth**

On the worksheet below, record the pathway you took through the flowcharts. Please note that below, flowcharts are always listed in sequential order. *Attach written explanatory materials as directed.*

Flowchart Documentation

Please list the numbers of **all** flowcharts that you used:
I., I.A., II.A., II.A.1., IV.A., V., V.D., VI.A., VI.A.1.

Next to each flowchart used, indicate with an **X** where that chart led you and attach any explanations or rationales requested.

Flowchart
No.

I. Determination of Assessment Pathway led to:

- **X** I.A. to Continuation of Pathway Determination.
- _____ II.D. to assess Transfer of Harmful Biochemical Compounds to Food Chain.
- _____ II.E. to begin assessment of Vectors Genetically Engineered to Reduce Disease.
- _____ VIII. to begin Food Safety Assessment.

I.A. Continuation of Pathway Determination led to:

_X_ II.A. to consider Survival and Reproduction Assessment

_____ VII. Exit Routine. *Attach rationale for arriving here from <4>.*

_____ Appendix B to consider other assessment because organism is modified solely by selective or captive breeding. This then led to:

____ decision to pursue other assessment. *Indicate rationale for decision and name of other assessment protocol.*

____ return to I.A.<2> which led to a second consideration of this pathway (and a second "X")

_____ Appendix C to consider Assessment of GEOs with Alternate Reproductive Pathways. This led to:

____ NOT proceeding further with the project and exiting this assessment.

____ returning to I.A.<6> which led to a further consideration of this pathway (and another "X").

_____ Consider disallowing release because GEO is not effective for its intended purpose. *Attach rationale for your decision.*

II.A. Survival and Reproduction Assessment - Deliberate Gene Changes led to

_X_ II.A.1. to assess impact of Deliberate Gene Changes.

_____ II.B. to assess impact of Deliberate Chromosomal Manipulations.

_____ VII. Exit Routine. *Attach your rationale for seeking exit.*

II.A.1. Impact of Deliberate Gene Changes led to:

_____ II.B. to assess impact of Deliberate Chromosomal Manipulations.

_____ III. to assess Potential Interference with Natural Reproduction.

_X_ IV.A. to assess Ecosystem Effects from Impacts of Introgression of Modified Gene(s).

_____ IV.B. to assess Ecosystem Effects on Reproduction.

_____ V. to assess Effects on Ecosystem Structure and Processes.

_____ VI.A. Risk management - to manage specific risks to populations of special concern. *Attach written description of any identified hazard.*

_____ VI.B. Risk management - to manage risk where there is insufficient information.

_____ VII. Exit Routine. *Attach your rationale for seeking exit.*

_____ VII. Exit Routine and consultation with relevant national, regional, and local government agencies regarding use of non-indigenous species. *Attach your rationale for seeking exit and indicate agency name(s) and recommendations.*

IV.A. Ecosystem Effects - Impacts of Introgression of Modified Gene(s) led to:

_X_ V. to assess Effects on Ecosystem Structure and Processes.

_____ VI.A. Risk Management - to manage risk of decline of population abundance. *Attach a written description of any identified hazard.*

_____ VI.B. Risk Management - to manage risk where there is insufficient information.

_____ Consider disallowing release. *Attach rationale for decision.*

V. Effects on Ecosystem Structure and Processes led to:

_____ V.A. to assess the effects of displacement (by a GEO intended to reduce the density of conspecifics).

_____ V.B. to assess the effects of a GEO intended to be or produce a harvestable product.

_____ V.C. to assess the effects of a GEO intended as a biocontrol agent (to reduce the density of other species)

_X_ V.D. to assess the effects of a GEO intended for bioremediation or to process agricultural or industrial wastes.

_____ V.E. to consider Effects on Ecosystem Structure and Processes of Other Biotic Interactions.

_____ VI.A. Risk Management - to manage risk to population(s) of special concern. *Attach a written description of any identified hazard.*

_____ VII. Exit Routine. *Attach rationale for seeking exit.*

_____ Consider disallowing release. *Attach rationale for decision.*

V.D. Effects on Ecosystem Structure and Processes (Bioremediation) led to:

_____ V.E. to assess effects of Other Biotic Interactions

_X_ VI.A. Risk management - to manage risks of alteration of community structure or ecosystem processes. *Attach written description of any identified hazard.*

_____ Consider disallowing release. *Attach rationale for decision.*

VI.A. Risk Management - Specific Risks led to:

_X_ VI.A.1. Risk Management-Specific Risks: Microorganisms.

_____ VI.C. to choose appropriate methods of Containment.

VI.A.1. Risk Management-Specific Risks: Microorganisms led to:

_X_ Consideration of measures to manage potential risk(s). *Attach a written description of the risk management measures you plan to implement (and as suggested in VI.C. Risk Management-Containment Routines). Be certain to address the topics listed in the Risk Management Documentation section below. Attach your rationale for disallowing release in any case where containment cannot be ensured.*

(see attached)

Additional Questions

1. Are you working with a non-indigenous species?
   ____ Yes.
   __X__ No.

List names, addresses, telephone numbers, and areas of expertise of the experts you contacted for substantial advice in assessing effects of a proposed project and in designing adequate safety measures.

Soil microbiologist
Agronomist
Forest ecologist
Wildlife biologist

Soil Ecologist **June 1, 1997**

Signature of assessor Date

Address, Phone Number, FAX Number, and Email Address

Soil Ecologist
Oregon State University
Corvallis, Oregon USA

Genetically engineered *Klebsiella planticola*
Attachment to Worksheet

Risk Management:

When plant residues are removed from the field, placed in air-tight containers with the genetically engineered bacterium and water, and allowed to ferment, ethanol (EtOH) can be removed within a matter of days. The remaining sludge material at the bottom of the container contains high levels of nitrogen and other plant nutrients and might seem a desirable fertilizer. However, the sludge material also contains high numbers of the live bacteria whose effects on soil and plants must also be considered before considering application to soil.

Klebsiella planticola is quite capable of surviving the ethanol levels its produces (Holmes et al. 1998). *Klebsiella p.* also lives in the root systems of all plants for which an effort has been made to look for the bacterium. *Klebsiella* uses the exudate material all plants produce, and is a particularly aggressive soil and rhizosphere bacterium. It does not reach extremely high numbers in soil - on the order of only 100 to 1000 individuals per gram of soil. Its normal soil function is to protect roots against pathogen attack, while using root exudates for energy. Alternatively, it decomposes plant litter, generating CO2, bacterial slime which the bacteria use to glue themselves to the surface of roots, leaves, and stems and other plant parts, and soil particles. The slime material appears also to have a role in soil pH maintenance. The *Klebsiella* engineered to produce ethanol does not make as much slime, since apparently the metabolic pathway for slime formation is disrupted by the insertion of the genes to generate ethanol. Under reduced partial pressures of oxygen often found in soil with any level of metabolic activity, succinate in the Kreb's cycle is diverted from slime formation. Ethanol is highly toxic to a wide variety of soil organisms, including roots of all terrestrial plants, beneficial myccorhizal fungi, disease-suppressive fungi, beneficial nematodes and protozoa (Jones 1989). Further, ethanol production coincides with an increase in the activity of many plant pathogens, such as *Pythium*, *Phytophtera*, and *Rhizoctonia*.

Engineered *Klebsiella planticola* will interfere with the naturally occurring parent organism because both utilize the same food resources. Thus it is likely that where the genetically engineered *Klebsiella* is present, the beneficial characteristics of the parent will be reduced or lost altogether. Plants will lose some of their protection against pathogens that the naturally-occurring bacterium provides. There will also be a loss of soil structure since the (declining) naturally occurring *Klebsiella* makes the "glue" that holds the soil microaggregates together.

Genetically engineered *Klebsiella planticola* is not intended to reduce the density of conspecifics but testing shows that it can (Holmes et al. 1998). Engineered *Klebsiella* is not intended to reduce the density of other species of organisms, but it does (Holmes et al. 1998).

Many species of plants, including endangered plants, could be affected if genetically engineered *Klebsiella* began to grow in their root systems. Plants cannot tolerate ethanol production in their roots systems, and in most cases, the rhizosphere is elevated with respect to the partial pressure of oxygen, such that this engineered bacterium would begin producing ethanol. Ethanol kills roots, in concentrations as low as 0.1% (Jones 1989). The genetically engineered *Klebsiella* produces 6 to 8% of its metabolites as EtOH. Thus, this organism cannot be allowed to be released, especially in areas with endangered species of plants. Ethanol in the concentrations that this organism can produce would also be detrimental to a wide diversity of wildlife, including earthworms, birds, mice, voles, shrews, raccoons, snakes, spiders, millipedes, centipedes, fox, etc. Any animal which burrows into the soil would be at risk, since their burrows are usually built within the root-zone of plants. The young of these species would

be especially at risk. Egg-laying and egg maturation of most ground-dwelling birds might also be affected, although data for these phenomena are lacking. Clearly, stringent risk management would be required.

The problem is how to prevent escape of the engineered organisms. Escape from airtight containers on farms would be extremely difficult to prevent, while escape from fertilizer material spread on fields would be a foregone conclusion. Once a bacterium is released in a field situation, it is impossible to prevent its spread. Bacteria are carried on the feet (or surface) of anything that touches the soil in the field -- birds, rats, mice, insects, etc. Further, bacteria can be moved in water, by erosion, by run-off, and by wind. Assessment of the spread and survival of the genetically engineered *Klebsiella* only in the surface layers of the soil would be inadequate to describe the extent of possible spread. Even to detect presence and spread in the soil, detection limits (number of individuals per gram of soil) would have to be set at numbers where bacterial reproduction can still occur (and from which a global spread of bacteria could occur). This necessitates the ability to detect the survival of only one bacterium in a large volume of soil. It takes only one bacterium to initiate an epidemic. In other words, once a bacterium is released into an environment, it cannot be recalled.

The possibility is small that genetically engineered *Klebsiella planticola* could be filtered completely from the products in the fermentation tank; use of those products might well mean (further) widespread dispersal of the engineered organism.

Clearly, release of this organism should not be allowed. The implications are too devastating. Genetically engineered *Klebsiella planticola* kills plants and beneficial soil organisms and enhances pathogens, is highly competitive, persists in soil, and causes changes in ecosystem structure and function, as well as substantial long-term effects. Its use for the purpose it was designed would require its addition to the soil in numbers that could genetically swamp or outcompete functionally related organisms. The possibility of its effective containment in a farm-like situation is very, very small.

Conclusion -- its release is not warranted because the hazards it presents cannot be adequately managed.

Worksheet
accompanying
The Biosafety Manual

Introduction

This manual is intended to aid users in assessing the genetic, ecological, and human health effects of genetically engineered organisms used in research, development, and small-scale or large-scale release. The flowcharts guide users in identifying specific hazards and managing the risk associated with each identified hazard, where possible. This worksheet is meant to accompany the flowcharts. The user should use the worksheet to document and track the decision path taken through the flowcharts and any plans for risk management.

Name of Assessor: **N. Spud**

Identification Name of the GEO: **Transgenic Sunflower**

Purpose for which GEO was designed: **to produce seeds that bear a high concentration of a chemical to be used as the primary active ingredient in human birth control pills**

Describe proposed project: **field testing in Kansas of Transgenic Sunflower, to examine performance under field conditions**

On the worksheet below, record the pathway you took through the flowcharts. Please note that below, flowcharts are always listed in sequential order. *Attach written explanatory materials as directed.*

Flowchart Documentation

Please list the numbers of **all** flowcharts that you used:
I., II.D., VI.A., VI.C.

Next to each flowchart used, indicate with an **X** where that chart led you and attach any explanations or rationales requested.

Flowchart
No.

I. Determination of Assessment Pathway led to:

____ I.A. to Continuation of Pathway Determination.
__X__ II.D. to assess Transfer of Harmful Biochemical Compounds to Food Chain.
____ II.E. to begin assessment of Vectors Genetically Engineered to Reduce Disease.
____ VIII. to begin Food Safety Assessment.

II.D. Transfer of Harmful Biochemical Compound to Food Chain led to:

_____ I.A. for Continuation of Assessment Pathway.

__X__ VI.A. Risk Management - to manage risk of transfer of harmful biochemical compound to food chains. *Attach a written description of any identified hazard.*

VI.A. Risk Management - Specific Risks led to:

_____ VI.A.1. Risk Management-Specific Risks: Microorganisms.

__X__ VI.C. to choose appropriate methods of Containment.

VI.C. Risk Management-Containment Routines led to:

_____ VII. Exit Routine (due to peer reviewers' conclusion of sufficient containment). *Attach a written description of the risk management measures you plan to implement. Be certain to address the topics listed in the Risk Management Documentation section below.*

__X__ Consider disallowing release or use of GEO (due to peer reviewers' conclusion of insufficient containment). *Attach your rationale.* **(see attached)**

List names addresses, telephone numbers, and area of expertise of the experts you contacted for substantial advice in assessing effects of a proposed project and in designing adequate safety measures.

Biochemical toxicologist

Pollination ecologist

Sunflower agronomist

N. Spud ***August 31, 1998***

Signature of assessor Date

Address, Phone Number, FAX Number, and Email Address

N. Spud
Department of Toxicology
University of California-Carmel
Carmel, California USA

Transgenic Sunflower
Attachment to Worksheet

Proposed Project: Field testing in Kansas - transgenic sunflowers engineered as a source of the primary ingredient in human birth control pills.

Risk identified:

The transgenic sunflowers create seeds rich in a biochemical compound to be processed as a pharmaceutical ingredient. Consultation with a biochemical toxicologist informed us that the compound would be produced at levels toxic to humans and other mammals. Consultation with a sunflower agronomist ecologist informed us that sunflower is an important Kansas crop grown for both human and animal consumption. Consultation with a pollination ecologist informed us that bees that typically pollinate sunflower often fly a kilometer or more (Arias and Rieseberg 1994). Therefore, without containment, it is likely that transgenic pollen will be moved to sunflowers in other fields. If that pollen fertilizes those plants, the resulting hybrid seeds may bear the compound in question at toxic levels.

Proposed risk management:

The flowchart suggests strict containment. To accomplish strict containment of pollen in Kansas would require measures that would interfere with the goal of the field testing, notably, to examine performance under field conditions. Therefore, we are disallowing release in Kansas (not because of peer review as suggested in the flowcharts but because of our own internal review). We are also seeking alternate sites for field testing where sunflower crops are not grown.

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Name of Assessor: **S. Tuberosum**

Identification Name of the GEO: **multicolored transgenic potato**

Purpose for which GEO was designed: **Potato engineered so that pigment expression varies from tuber to tuber, resulting in different colored tubers**

Describe proposed project: **Field test in Sweden - yield and color studies only. No human consumption. Plants to be grown in containment.**

On the worksheet below, record the pathway you took through the flowcharts. Please note that below, flowcharts are always listed in sequential order. *Attach written explanatory materials as directed.*

Flowchart Documentation

Please list the numbers of **all** flowcharts that you used:
I., I.A., II.A., VII.

Next to each flowchart used, indicate with an **X** where that chart led you and attach any explanations or rationales requested.

Flowchart No.

I. Determination of Assessment Pathway led to:

- **X** I.A. to Continuation of Pathway Determination.
- _____ II.D. to assess Transfer of Harmful Biochemical Compounds to Food Chain.
- _____ II.E. to begin assessment of Vectors Genetically Engineered to Reduce Disease.
- _____ VIII. to begin Food Safety Assessment.

I.A. Continuation of Pathway Determination led to:

2nd __X__ II.A. to consider Survival and Reproduction Assessment

_____ VII. Exit Routine. *Attach rationale for arriving here from <4>.*

_____ Appendix B to consider other assessment because organism is modified solely by selective or captive breeding. This then led to:

____ decision to pursue other assessment. *Indicate rationale for decision and name of other assessment protocol.*

____ return to I.A.<2> which led to a second consideration of this pathway (and a second "X")

1st __X__ Appendix C to consider Assessment of GEOs with Alternate Reproductive Pathways. This led to:

_____ NOT proceeding further with the project and exiting this assessment.

__X__ returning to I.A.<6> which led to a further consideration of this pathway (and another "X").

____ Consider disallowing release because GEO is not effective for its intended purpose. *Attach rationale for your decision.*

II.A. Survival and Reproduction Assessment - Deliberate Gene Changes led to

_____ II.A.1. to assess impact of Deliberate Gene Changes.

_____ II.B. to assess impact of Deliberate Chromosomal Manipulations.

__X__ VII. Exit Routine. *Attach your rationale for seeking exit.*

VII. Exit Routine led to:

_____ VIII. Overview of Food Safety.

__X__ Exiting this assessment.

(see attached below)

Additional Questions

List names addresses, telephone numbers, and area of expertise of the experts you contacted for substantial advice in assessing effects of a proposed project and in designing adequate safety measures.

Potato agronomist

S. Tuberosum ***25 November, 1997***

Signature of assessor Date

Address, Phone Number, FAX Number, and Email Address

S. Tuberosum
Department of Agronomy
Swedish National University
Karuna, Sweden

Multicolored Transgenic Potato

Attachment to Worksheet

Proposed project: Field testing in Sweden - Potato engineered so that pigment expression varies from tuber to tuber, resulting in different colored tubers. Yield and quality studies only. No human consumption. Plants to be grown in containment.

Proposed risk management:

Plants will not enter accessible ecosystem because they will be grown in containment. This cultivar rarely flowers, and never does so in Sweden. When it does flower in more temperate climates, the flowers are fully sterile, producing no pollen or seeds. Therefore the only vehicle for escape in this project are the tubers, which are units of potential vegetative reproduction.

The directly accessible ecosystem is too cold in winter for this particular cultivar which typically dies when the soil reaches a temperature of -1 degree C. for 24 hours.

The nearest "suitable" ecosystem is at least 100 kilometers from the study site.

Nonetheless, some containment measures will be taken to reduce the risk of escape of the transgenic plants. Specifically, the plants will be grown in raised beds that are plastic-lined and fenced (with fine metal mesh, one meter above the soil surface, one meter below the soil surface, etc.) to prevent mammals from entering the beds and digging up the tubers. All other animals in the area are too small to disperse the tubers. At the end of the experiment, all plants will be carefully removed to assess yield and color quality. The remaining soil will be sterilized by heating and the tubers will be incinerated.

Worksheet
accompanying
The Biosafety Manual

Introduction

This manual is intended to aid users in assessing the genetic, ecological, and human health effects of genetically engineered organisms used in research, development, and small-scale or large-scale release. The flowcharts guide users in identifying specific hazards and managing the risk associated with each identified hazard, where possible. This worksheet is meant to accompany the flowcharts. The user should use the worksheet to document and track the decision path taken through the flowcharts and any plans for risk management.

Name of Assessor: **Broccoli Grower**

Identification Name of the GEO: **Bt Broccoli**

Purpose for which GEO was designed: **to render susceptible broccoli plants toxic to Lepidopteran pests that are susceptible to *Bacillus thuringiensis* endotoxin, through expression of the *Bt* endotoxin in a commercial variety of broccoli**

Describe proposed project: **small-scale field test in California of a new transgenic construct for Bt broccoli**

On the worksheet below, record the pathway you took through the flowcharts. Please note that below, flowcharts are always listed in sequential order. *Attach written explanatory materials as directed.*

Flowchart Documentation

Please list the numbers of **all** flowcharts that you used:
I., I.A., II.A., II.A.1, IV.A., V., V.B., V.E., VI.A., VI.C.

Next to each flowchart used, indicate with an **X** where that chart led you and attach any explanations or rationales requested.

Flowchart
No.

I. Determination of Assessment Pathway led to:

- **X** I.A. to Continuation of Pathway Determination.
- _____ II.D. to assess Transfer of Harmful Biochemical Compounds to Food Chain.
- _____ II.E. to begin assessment of Vectors Genetically Engineered to Reduce Disease.
- _____ VIII. to begin Food Safety Assessment.

I.A. Continuation of Pathway Determination led to:

2nd ___X___ II.A. to consider Survival and Reproduction Assessment

_____ VII. Exit Routine. *Attach rationale for arriving here from <4>.*

_____ Appendix B to consider other assessment because organism is modified solely by selective or captive breeding. This then led to:

____ decision to pursue other assessment. *Indicate rationale for decision and name of other assessment protocol.*

____ return to I.A.<2> which led to a second consideration of this pathway (and a second "X")

1st ___X___ Appendix C to consider Assessment of GEOs with Alternate Reproductive Pathways. This led to:

_____ NOT proceeding further with the project and exiting this assessment.

___X___ returning to I.A.<6> which led to a further consideration of this pathway (and another "X").

_____ Consider disallowing release because GEO is not effective for its intended purpose. *Attach rationale for your decision.*

II.A. Survival and Reproduction Assessment - Deliberate Gene Changes led to

___X___ II.A.1. to assess impact of Deliberate Gene Changes.

_____ II.B. to assess impact of Deliberate Chromosomal Manipulations.

_____ VII. Exit Routine. *Attach your rationale for seeking exit.*

II.A.1. Impact of Deliberate Gene Changes led to:

_____ II.B. to assess impact of Deliberate Chromosomal Manipulations.

_____ III. to assess Potential Interference with Natural Reproduction.

___X___ IV.A. to assess Ecosystem Effects from Impacts of Introgression of Modified Gene(s).

_____ IV.B. to assess Ecosystem Effects on Reproduction.

_____ V. to assess Effects on Ecosystem Structure and Processes.

_____ VI.A. Risk management - to manage specific risks to populations of special concern. *Attach written description of any identified hazard.*

_____ VI.B. Risk management - to manage risk where there is insufficient information.

_____ VII. Exit Routine. *Attach your rationale for seeking exit.*

_____ VII. Exit Routine and consultation with relevant national, regional, and local government agencies regarding use of non-indigenous species. *Attach your rationale for seeking exit and indicate agency name(s) and recommendations.*

IV.A. Ecosystem Effects - Impacts of Introgression of Modified Gene(s) led to:

__X__ V. to assess Effects on Ecosystem Structure and Processes.

_____ VI.A. Risk Management - to manage risk of decline of population abundance. *Attach a written description of any identified hazard.*

_____ VI.B. Risk Management - to manage risk where there is insufficient information.

_____ Consider disallowing release. *Attach rationale for decision.*

V. Effects on Ecosystem Structure and Processes led to:

_____ V.A. to assess the effects of displacement (by a GEO intended to reduce the density of conspecifics).

__X__ V.B. to assess the effects of a GEO intended to be or produce a harvestable product.

_____ V.C. to assess the effects of a GEO intended as a biocontrol agent (to reduce the density of other species)

_____ V.D. to assess the effects of a GEO intended for bioremediation or to process agricultural or industrial wastes.

_____ V.E. to consider Effects on Ecosystem Structure and Processes of Other Biotic Interactions.

_____ VI.A. Risk Management - to manage risk to population(s) of special concern. *Attach a written description of any identified hazard.*

_____ VII. Exit Routine. *Attach rationale for seeking exit.*

_____ Consider disallowing release. *Attach rationale for decision.*

V.B. Effects on Ecosystem Structure and Processes (Harvestable Product) led to:

__X__ V.E. to consider Effects on Ecosystem Structure and Processes of Other Biotic Interactions.

_____ VI.B. Risk management to manage risk where there is insufficient information.

_____ VII. Exit Routine. *Attach rationale for seeking exit.*

V.E. Effects on Ecosystem Structure and Processes (Other Biotic Interactions) led to:

__X__ VI.A. Risk management - to manage risk of alteration of ecosystem processes. *Attach written description of any identified hazard.*

_____ VI.B. Risk management - to manage risk where there is insufficient information.

_____ VII. Exit Routine. *Attach rationale for seeking exit.*

VI.A. Risk Management - Specific Risks led to:

_____ VI.A.1. Risk Management-Specific Risks: Microorganisms.

__X__ VI.C. to choose appropriate methods of Containment.

VI.C. Risk Management-Containment Routines led to:

_X_ VII. Exit Routine (due to peer reviewers' conclusion of sufficient containment). *Attach a written description of the risk management measures you plan to implement. Be certain to address the topics listed in the Risk Management Documentation section below.* **(see attached)**

____ Consider disallowing release or use of GEO (due to peer reviewers' conclusion of insufficient containment). *Attach your rationale.*

VII. Exit Routine led to:

_____ VIII. Overview of Food Safety.

_X_ Exiting this assessment. **(see attached)**

Additional Questions

List names addresses, telephone numbers, and area of expertise of the experts you contacted for substantial advice in assessing effects of a proposed project and in designing adequate safety measures.

Broccoli agronomist

Broccoli Grower ***June 14, 1998***

Signature of assessor Date

Bt Broccoli
Worksheet Attachment

Risk Management:

Field test of Bt broccoli will be harvested for yield and insect damage estimates BEFORE the flowers emerge. Tests will be small-scale, carefully monitored (daily), and only one season in duration, thereby minimizing the potential for selection of resistant pest strains.

All plant material will be destroyed by incineration. None of the broccoli will be used as either food or feed.

Note: At the next stage of testing (large-scale field test), should yield and insect damage indicate that this Bt Broccoli warrants further consideration: (a) a refugia plan must be developed and implemented to minimize the potential for development of pest resistant strains of Lepidoptera, and (b) a food safety strategy must be developed and implemented.

The Edmonds Institute