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Supplement to Minutes

Agricultural Biotechnology Research Advisory Committee

Performance Standards for Safely Conducting Research With Genetically Modified Fish and Shellfish

Part II. Flowcharts and Accompanying Worksheets

July 31, 1995

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Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish

Flowcharts

and

Accompanying Worksheets

Prepared by

U. S. Department of Agriculture
Agricultural Biotechnology Research Advisory Committee
Working Group on Aquatic Biotechnology
and Environmental Safety

July 31, 1995

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ACKNOWLEDGMENTS

The Performance Standards and associated documents are the outcome of a broad interdisciplinary collaboration involving numerous individuals. The Working Group on Aquatic Biotechnology and Environmental Safety (Appendix E) prepared the first draft of the Standards, convened an international workshop to seek interdisciplinary input into development of the Standards, and guided revisions of the text, flowcharts, and worksheets. The Agricultural Biotechnology Research Advisory Committee, including members appointed in 1992 and 1994, provided important guidance at key steps in this project. Two students at the University of Minnesota made significant contributions: Craig Acomb initiated development of the Flowcharts; and Carolyn Carr contributed significantly to completion of the Flowcharts and to Appendix B. Approximately 100 participants at the 1993 workshop (Appendix F) contributed the majority of the comments upon which this final draft and accompanying documents are based. A number of other individuals from the United States and other countries submitted additional comments. Wendilea LeMay and Julie Karels (University of Minnesota, Dept. of Fisheries and Wildlife), and Eva Russnack (USDA, Office of Agricultural Biotechnology) did the majority of the crucial clerical tasks.

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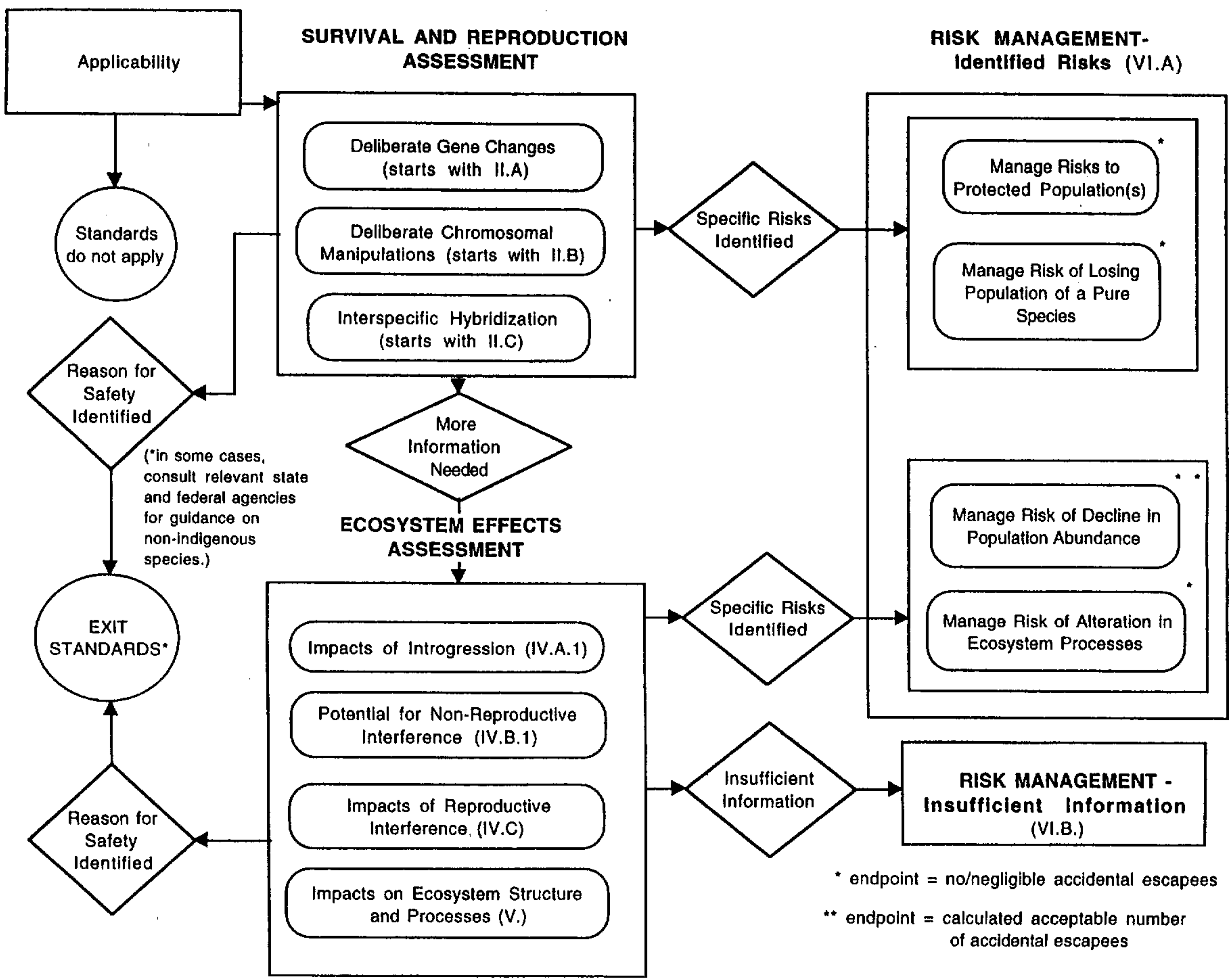
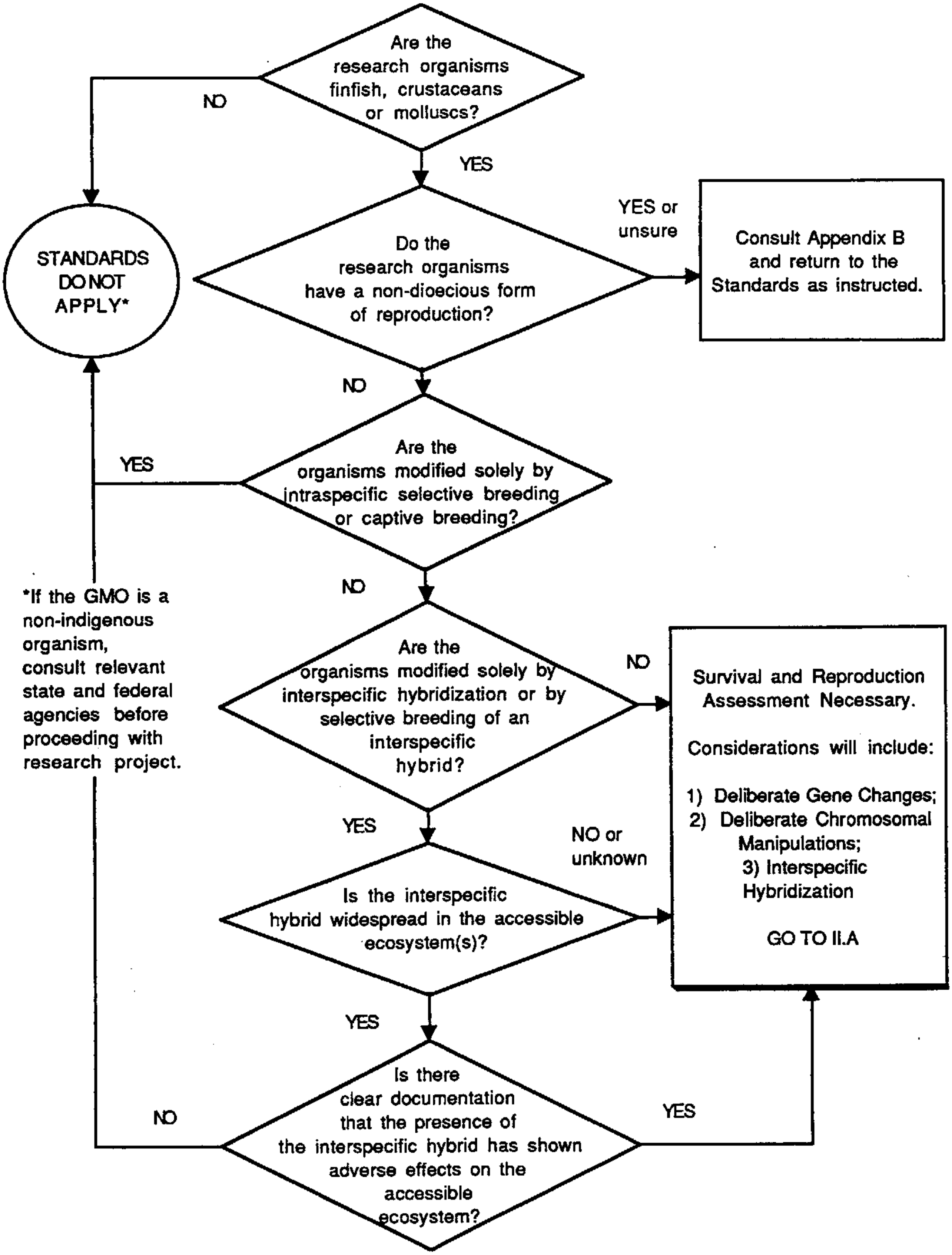
FLOWCHARTS

OVERVIEW of Performance Standards Flowcharts



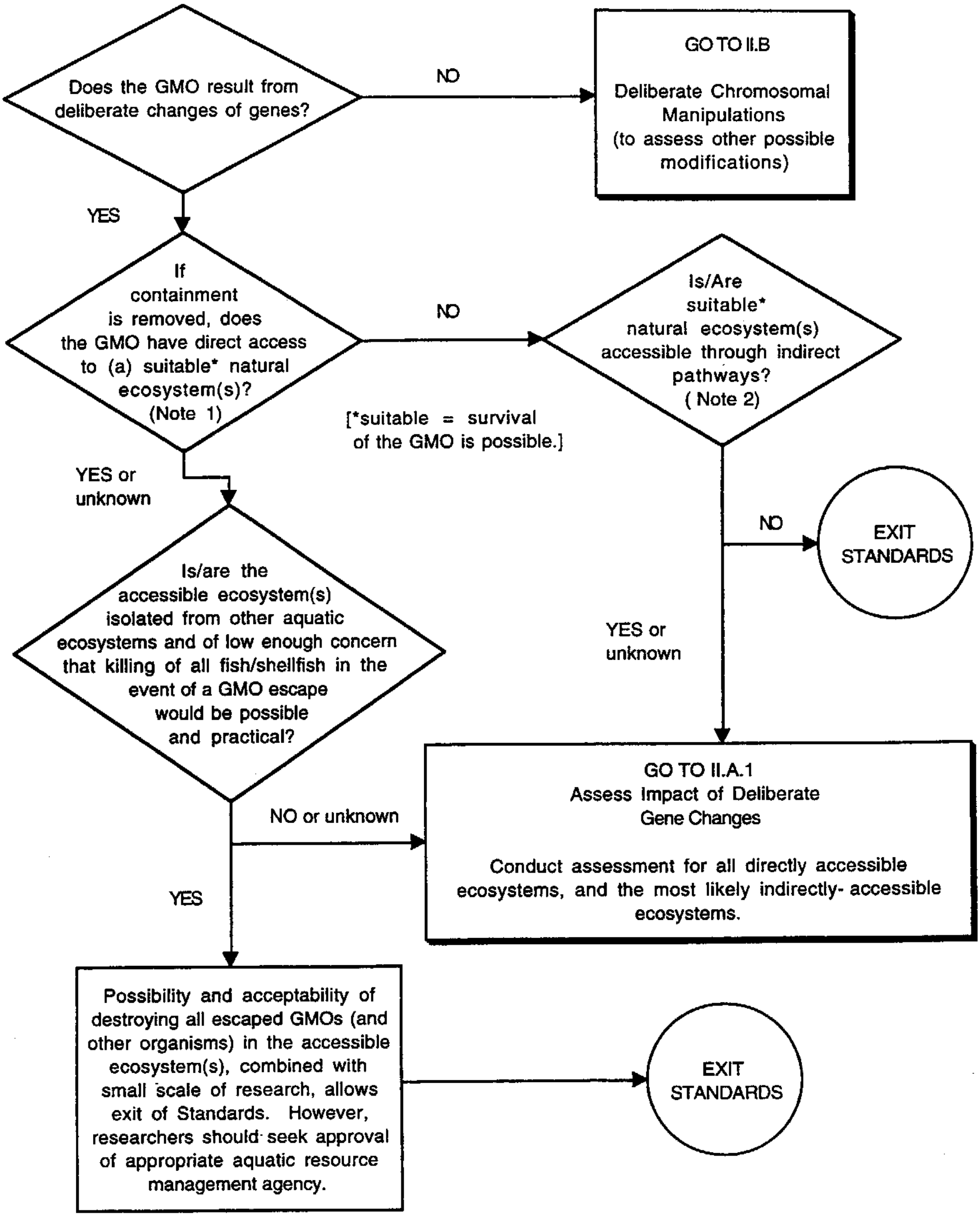
I. Applicability of Performance Standards
for Research with Genetically Modified Finfish And Shellfish

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



II.A Survival and Reproduction Assessment - Deliberate Gene Changes

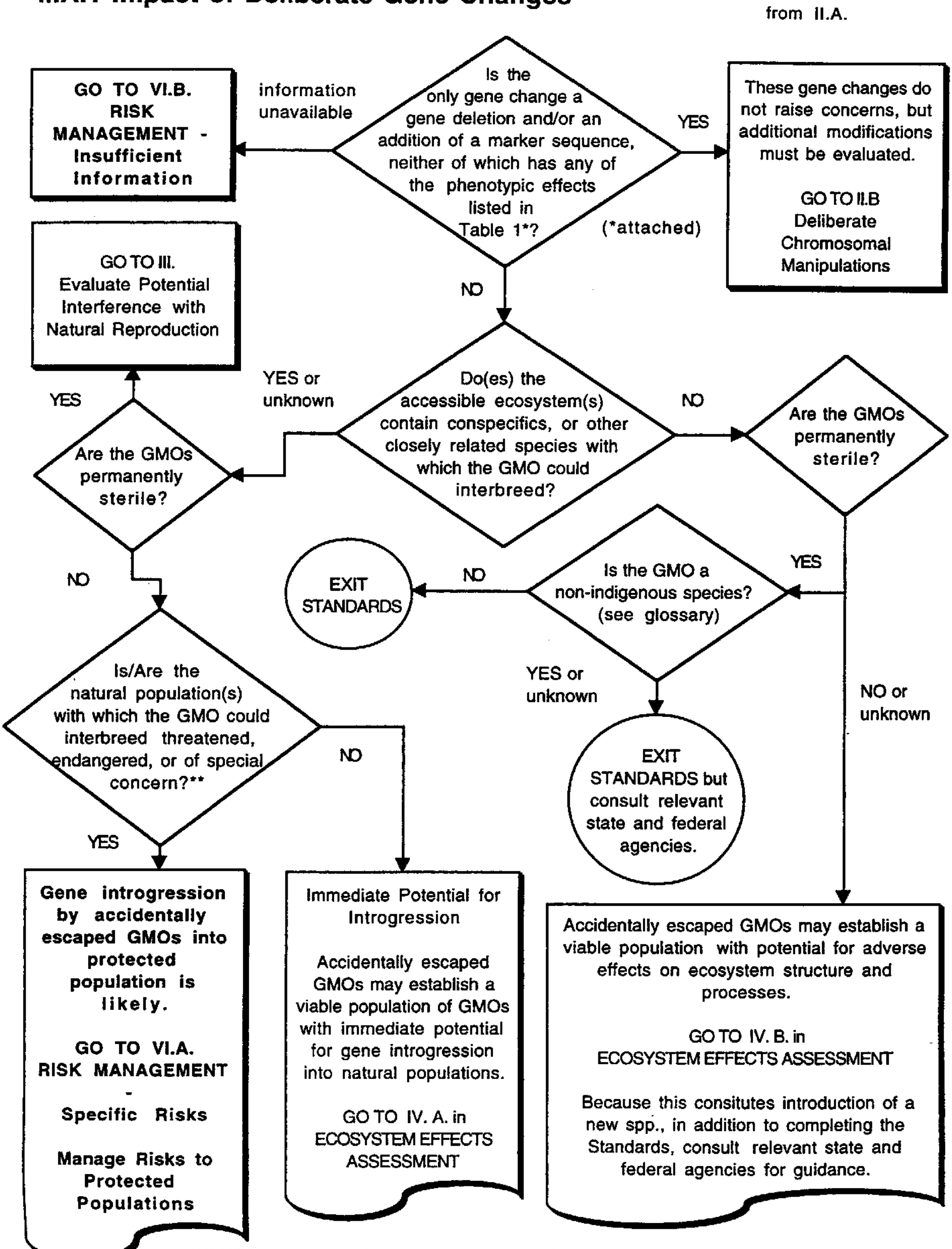
from I.



Note 1: Direct access is possible through natural waterbodies and human-created physical pathways, including navigation canals, and interbasin water transfers (e.g. irrigation, municipal water supply, etc.) See Appendix A: Table 2.

Note 2: See Appendix A: Table 2 for full list of such pathways.

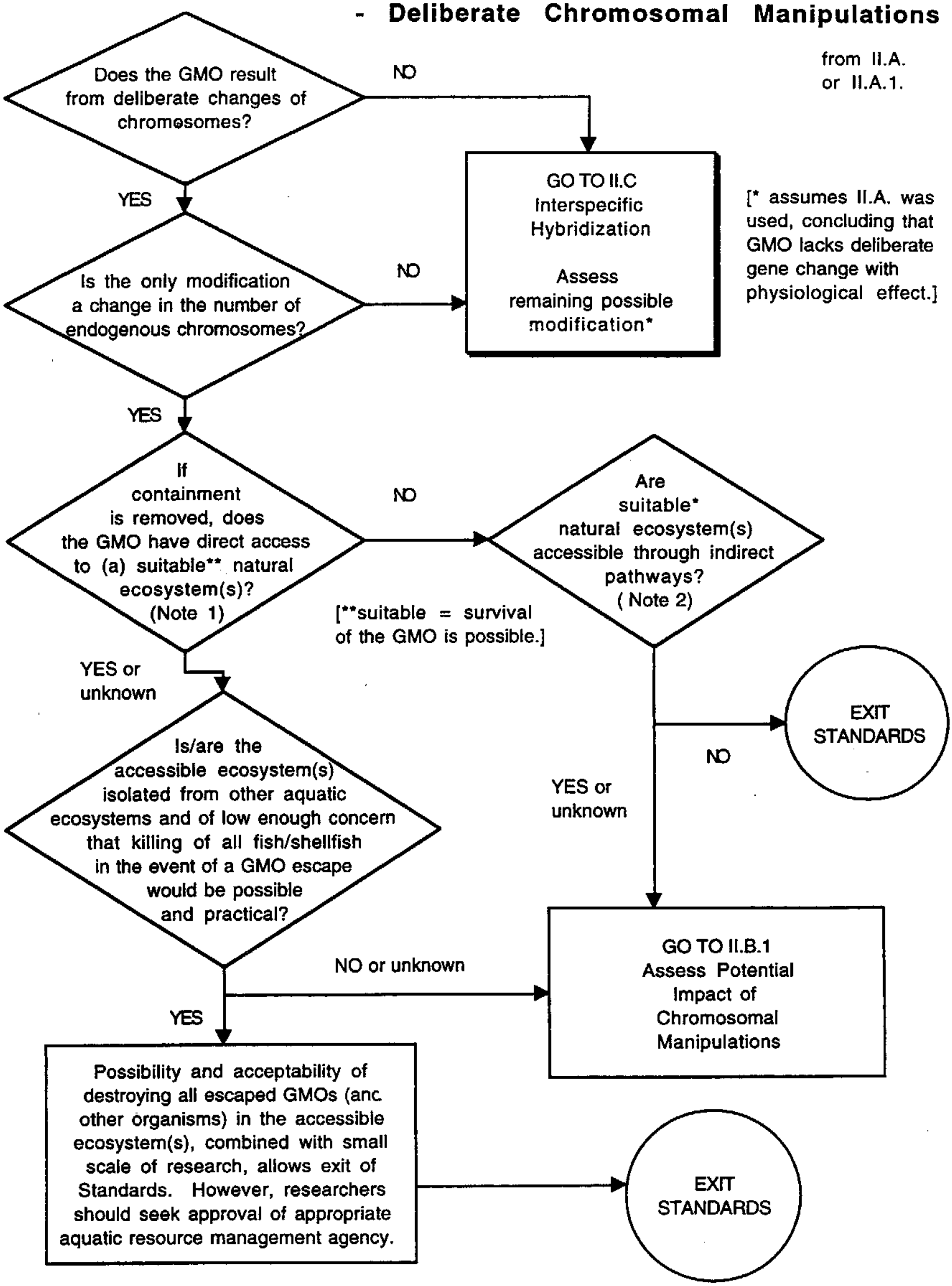
II.A.1 Impact of Deliberate Gene Changes



(**If YES, one option is to move to a site where no protected spp. are present. However, if this is considered, other topics in the Standards must be addressed. To explore the potential implications of site relocation, answer NO here and continue.)

II.B. Survival and Reproduction Assessment

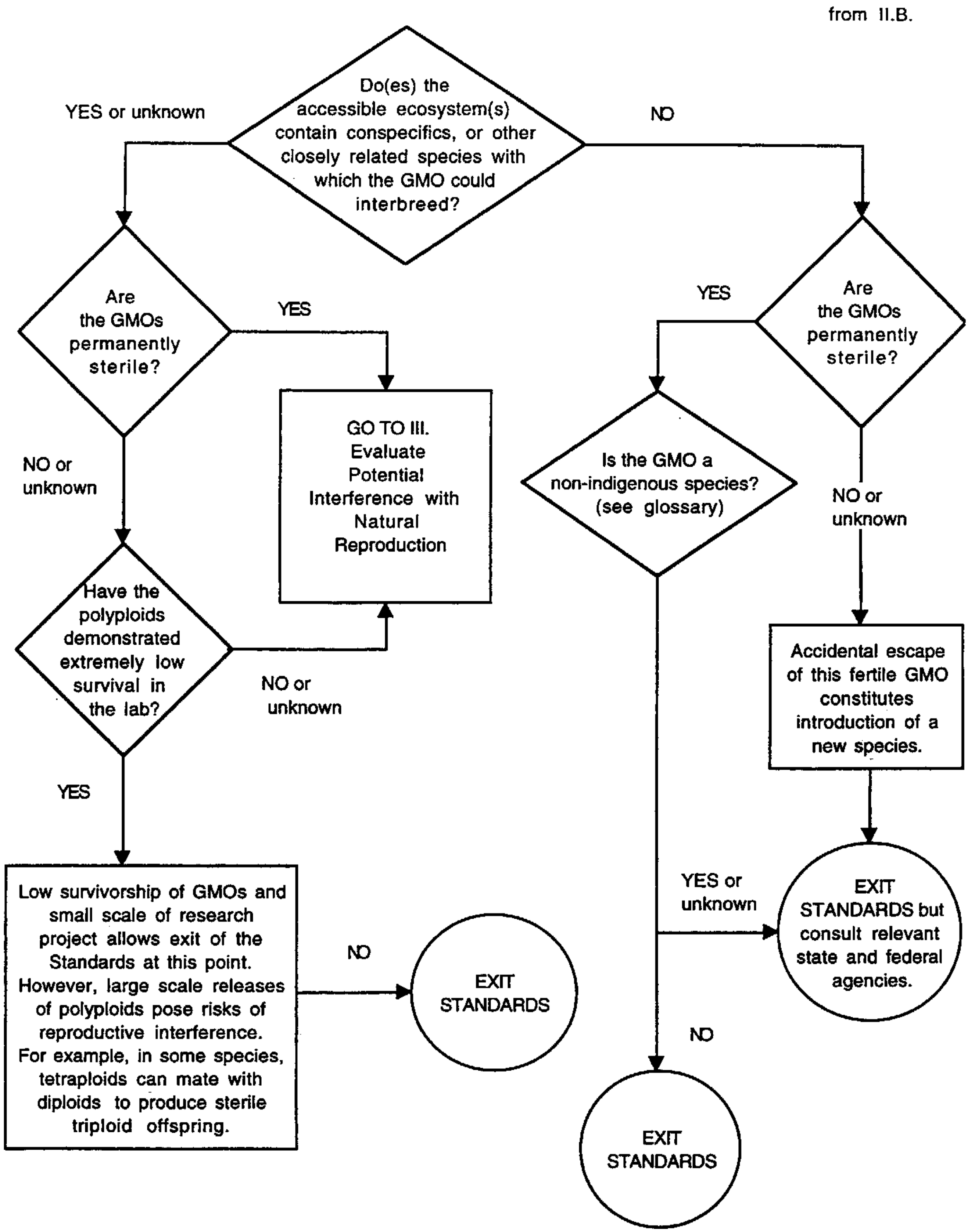
- Deliberate Chromosomal Manipulations



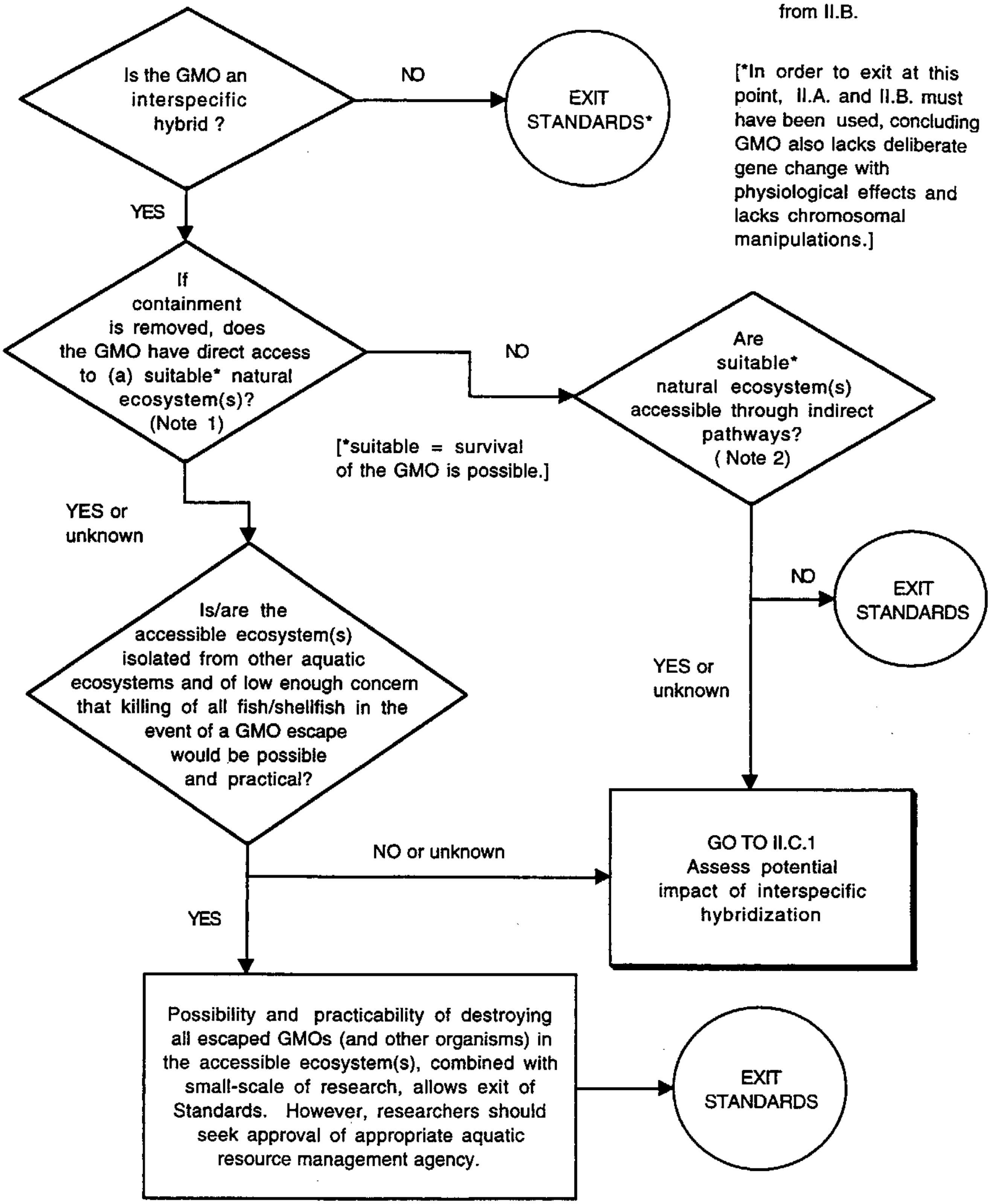
Note 1: Direct access is possible through natural waterbodies and human-created physical pathways, including navigation canals, and interbasin water transfers (e.g. irrigation, municipal water supply, etc.). See Appendix A: Table 2.

Note 2: See Appendix A: Table 2 for full list of such pathways.

II.B.1 Impact of Deliberate Chromosomal Manipulations



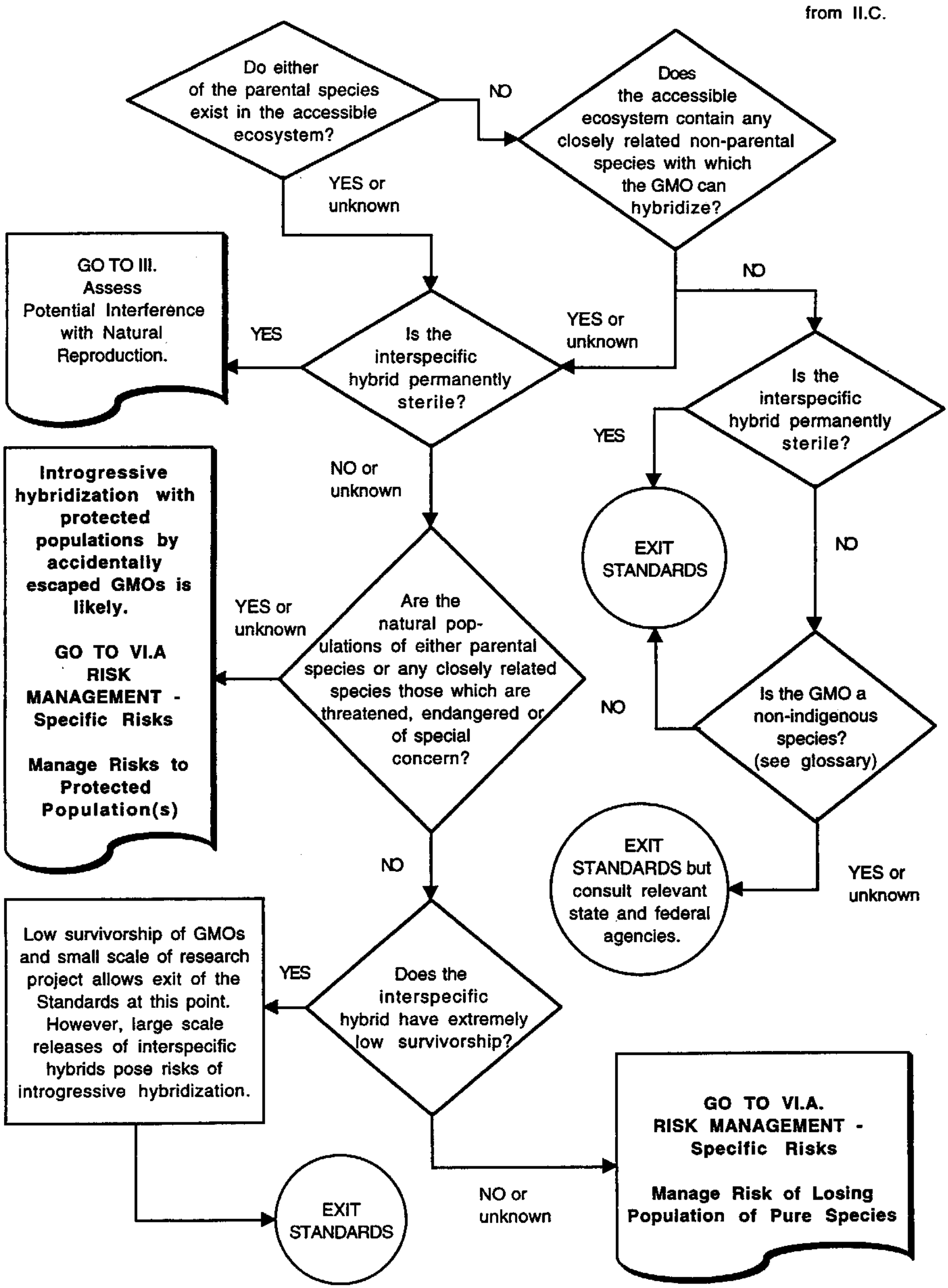
II.C. Survival and Reproduction Assessment - Interspecific Hybridization



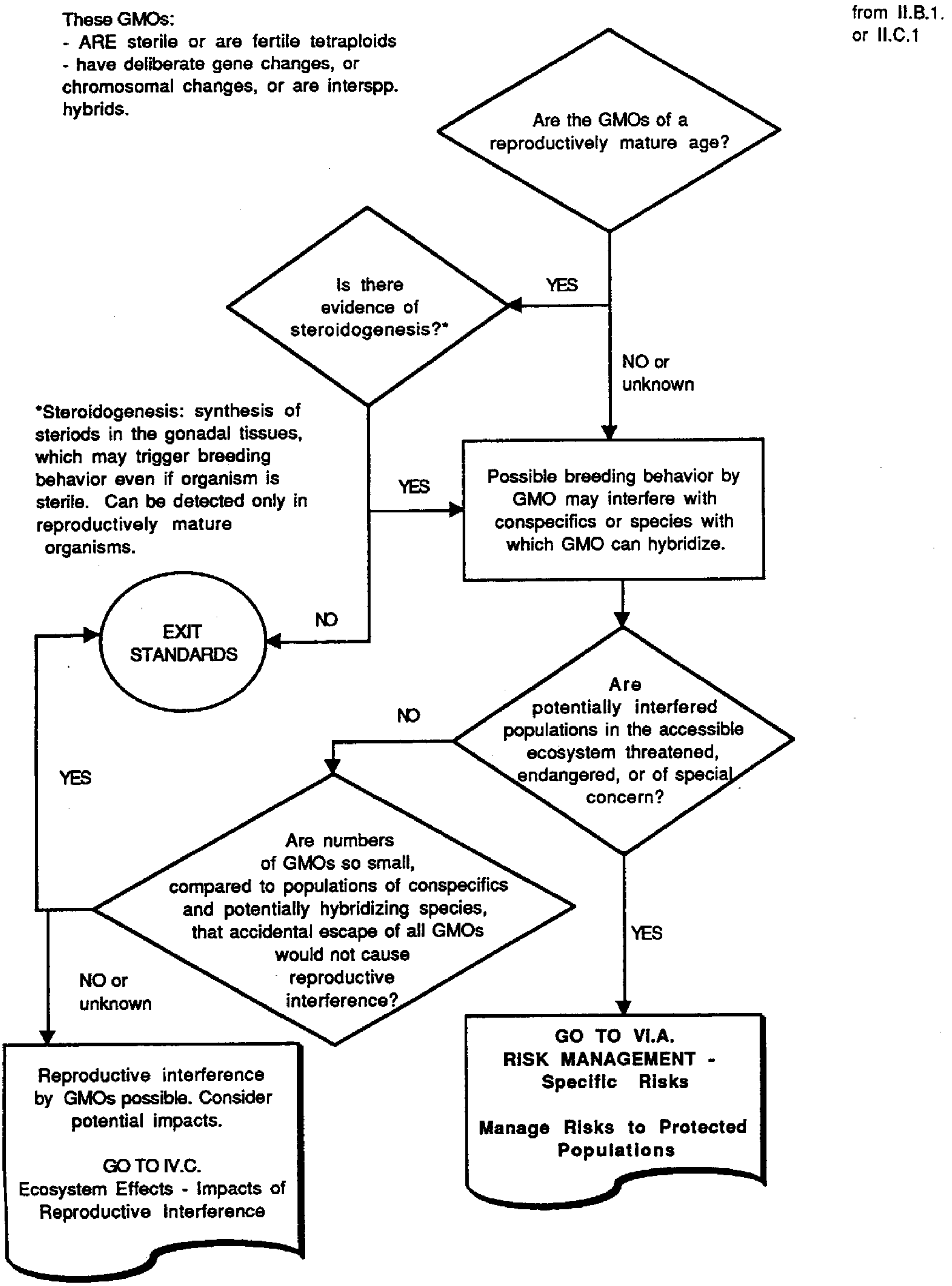
Note 1: Direct access is possible through natural waterbodies and human-created physical pathways, including navigation canals, and interbasin water transfers (e.g. irrigation, municipal water supply, etc.) See Appendix A: Table 2.

Note 2: See Appendix A: Table 2 for full list of indirect pathways.

II.C.1 Impact of Interspecific Hybridization

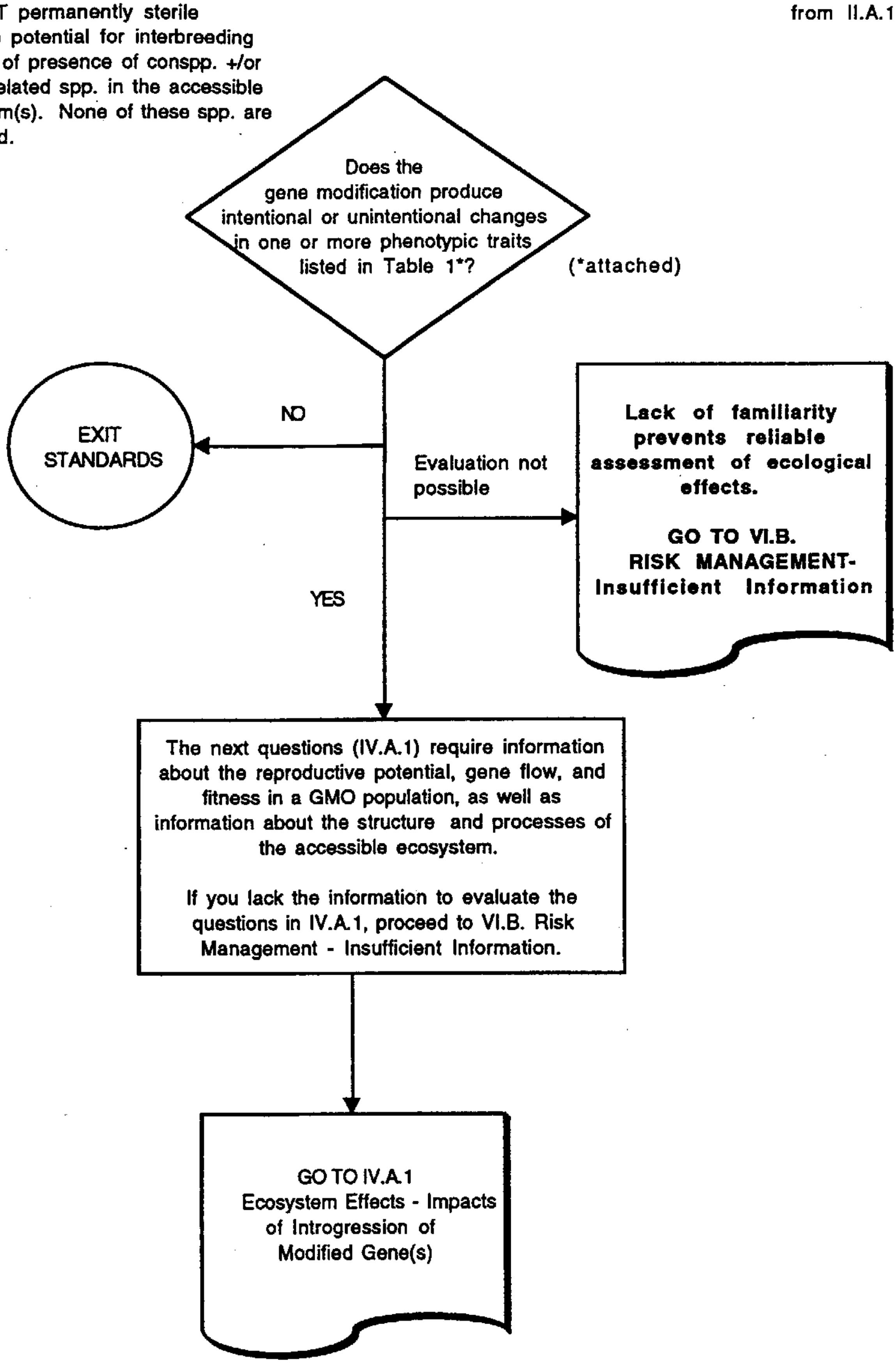


III. Potential Interference with Natural Reproduction



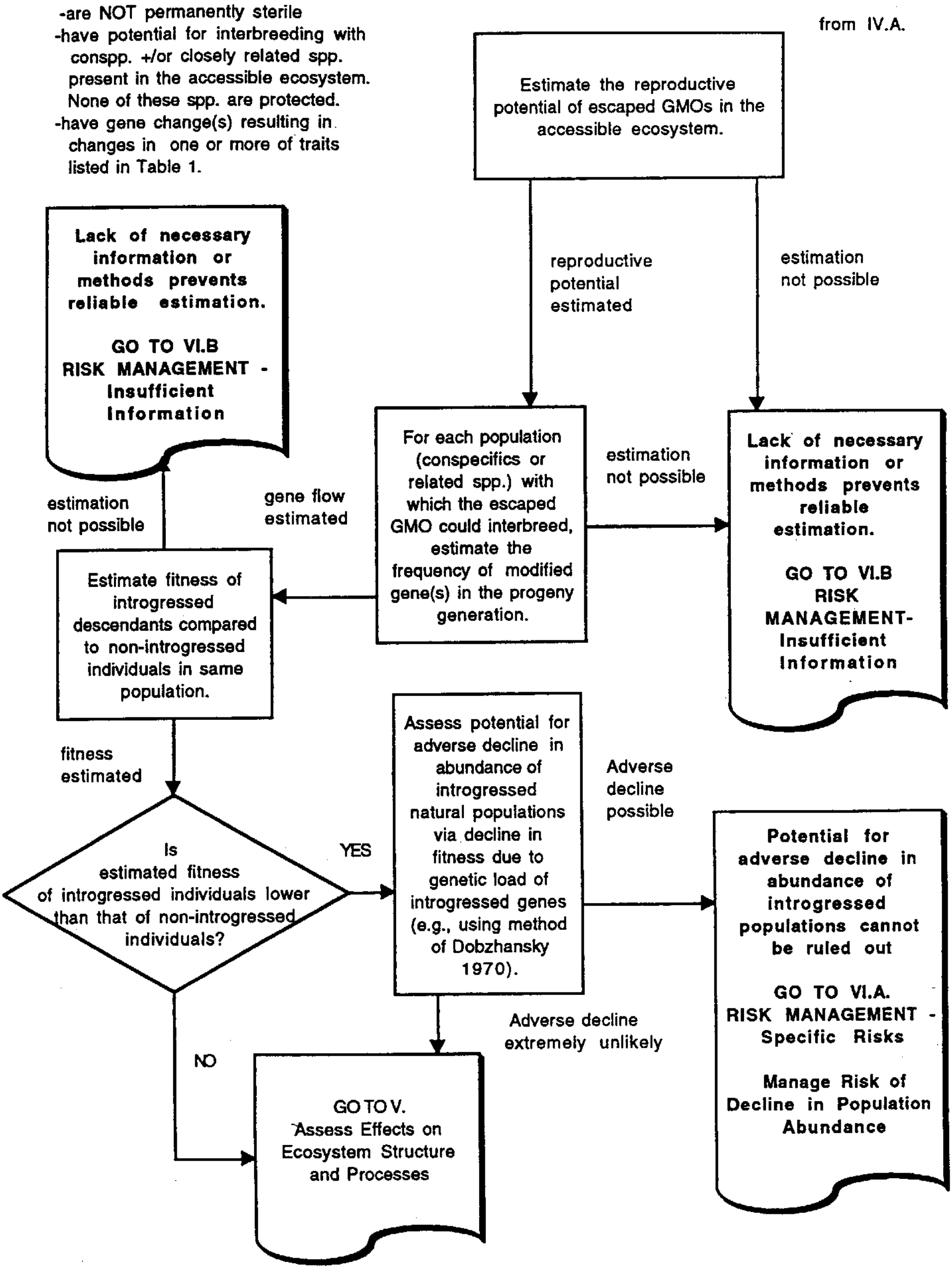
IV.A. Ecosystem Effects - Deliberate Gene Changes

These GMOs:
-are NOT permanently sterile
-do have potential for interbreeding because of presence of conspp. +/or closely related spp. in the accessible ecosystem(s). None of these spp. are protected.

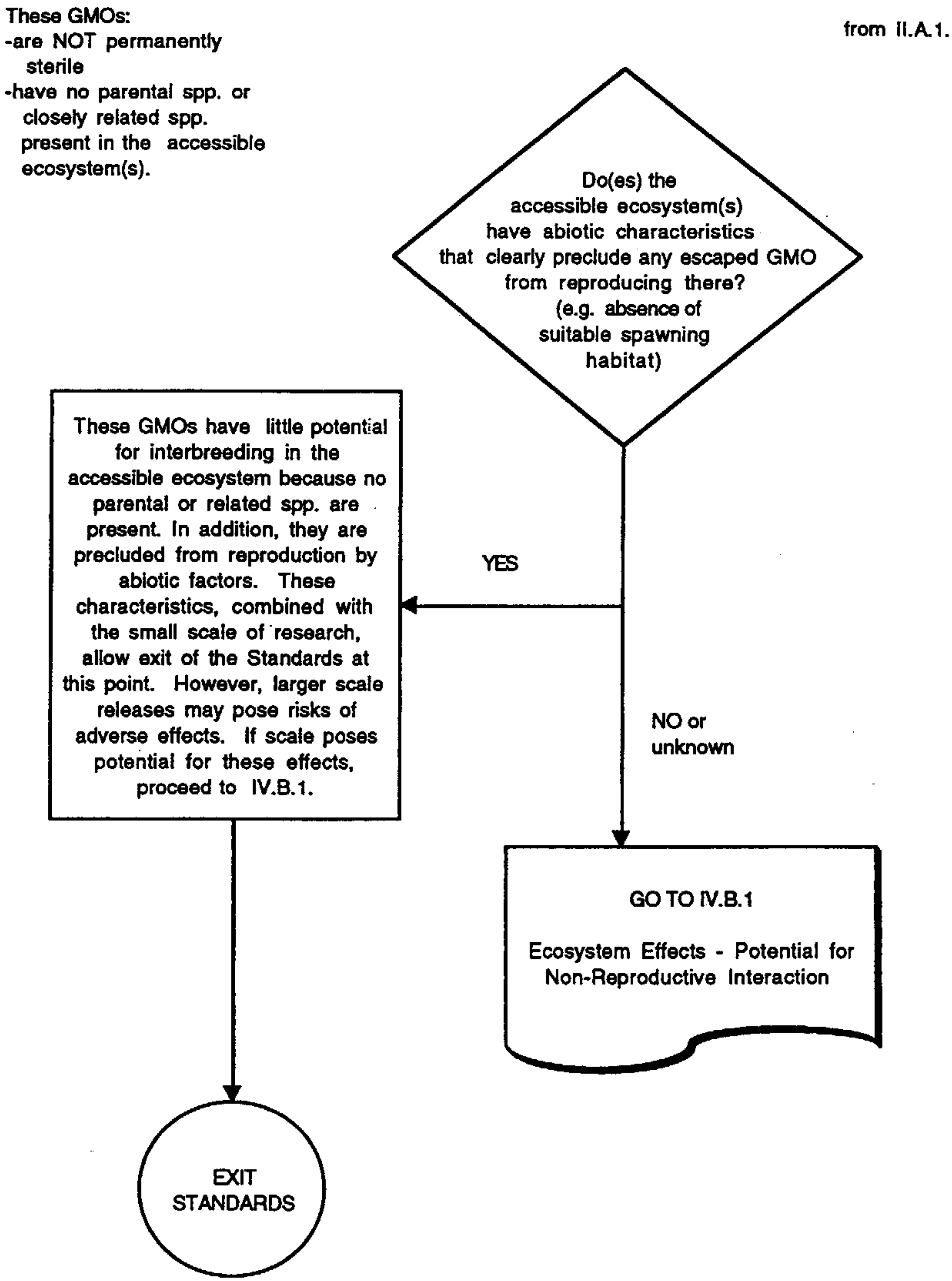


IV.A.1 Ecosystem Effects - Impacts of Introgression of Modified Gene(s)

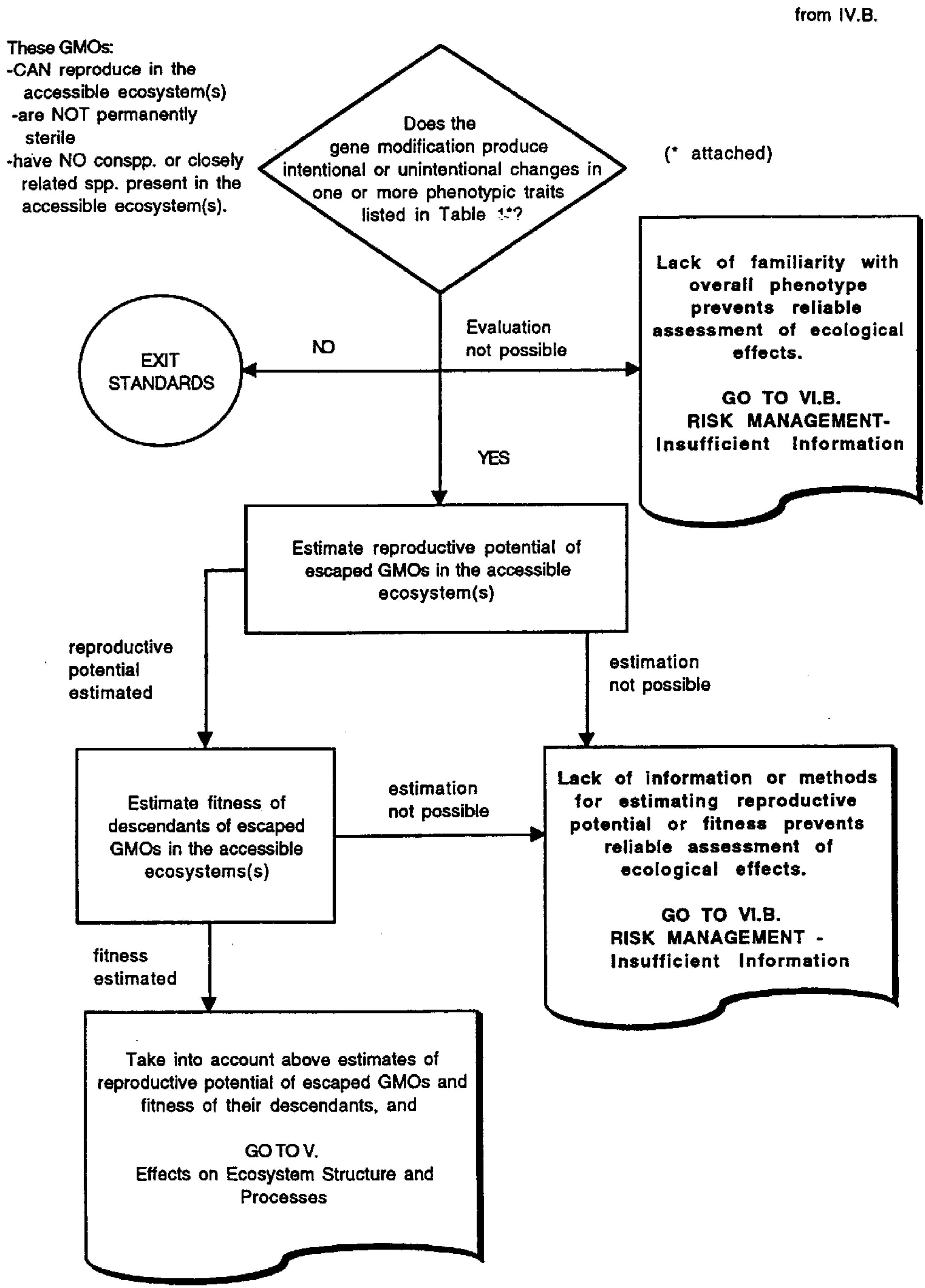
These GMOs:
- are NOT permanently sterile
- have potential for interbreeding with conspp. +/or closely related spp. present in the accessible ecosystem. None of these spp. are protected.
- have gene change(s) resulting in changes in one or more of traits listed in Table 1.



IV.B. Potential Barriers Associated with Accessible Ecosystem



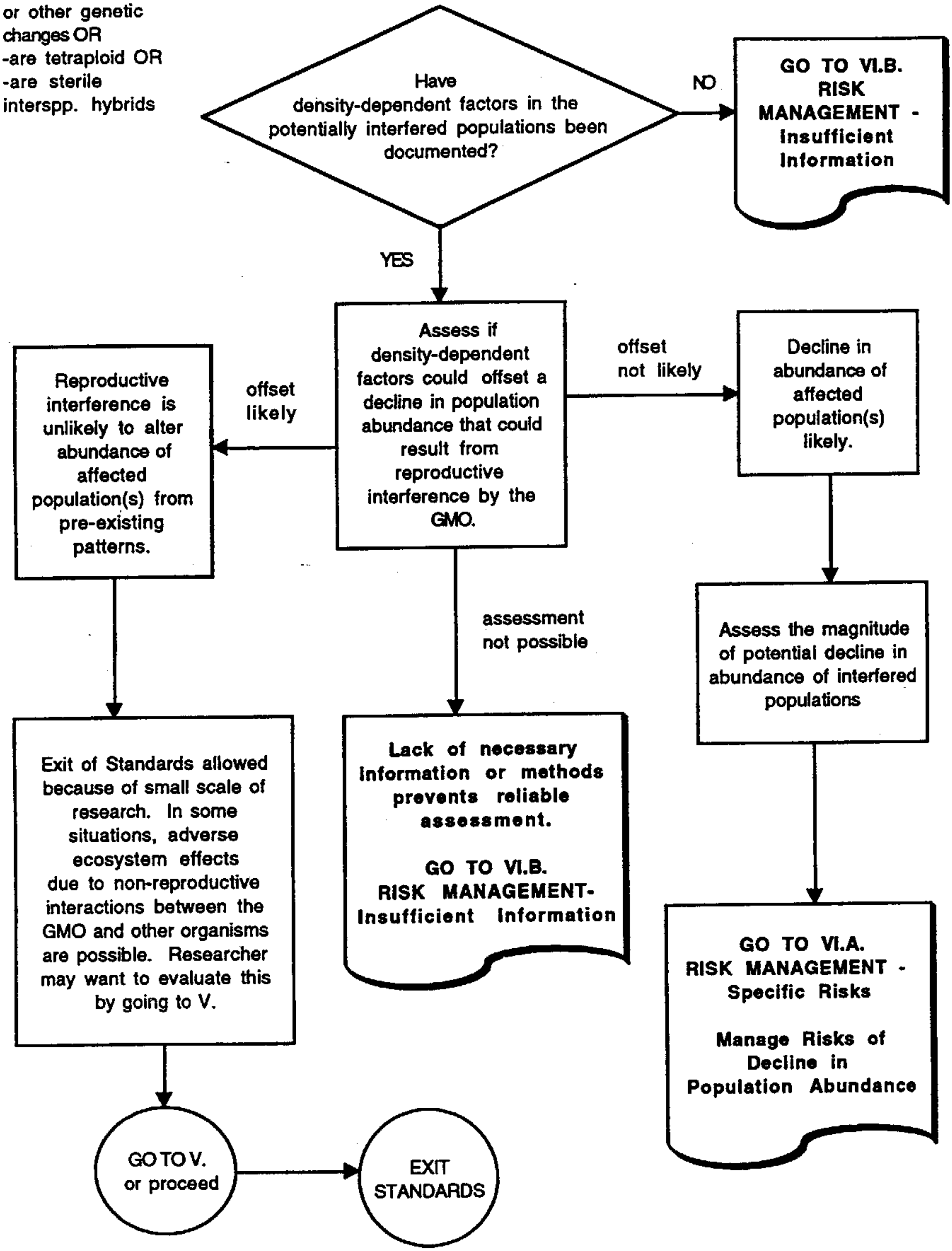
IV.B.1 Ecosystem Effects - Potential for Non-Reproductive Interaction



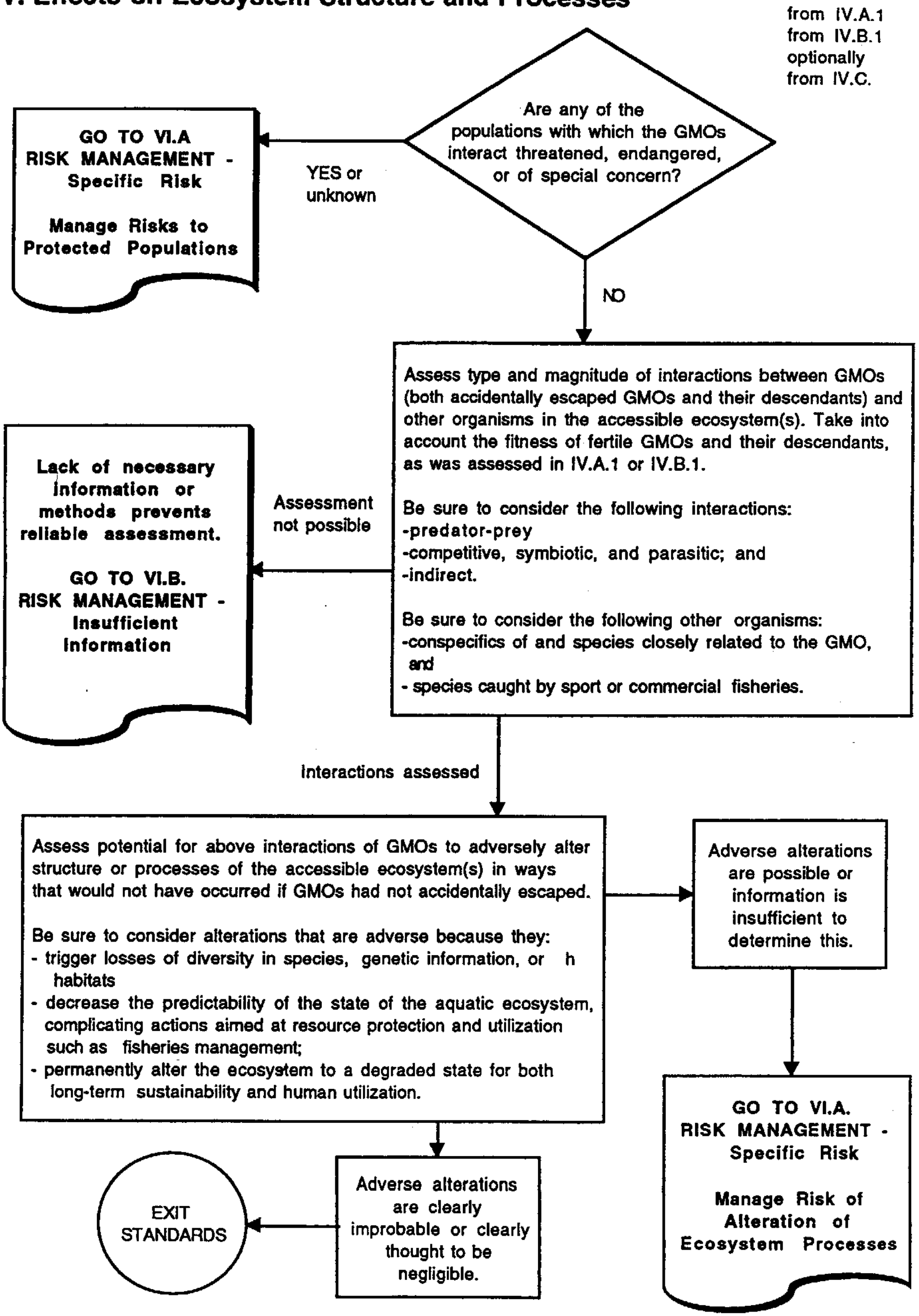
IV.C. Ecosystem Effects - Impacts of Reproductive Interference

These GMOs:
- are sterile and have chromosomal or other genetic changes OR
- are tetraploid OR
- are sterile interspp. hybrids

from III.



V. Effects on Ecosystem Structure and Processes



VI.A. Risk Management - Specific Risks

Select from the list below the particular risk you have been instructed to manage. For each risk (or set of risks) the acceptable number of accidental escapees* is indicated in bold.

<u>Manage Risks to Protected Populations</u> - from II.A.1. or

no/negligible accidental escape.

Protected populations contain species which are threatened, endangered, or of special concern. Risks are gene flow, reproductive interference, or introgressive hybridization in these populations.

*Accidental escapees = combined outcome of scale of experiment and effectiveness of barriers.

<u>Manage Risk of Losing Population of Pure Species</u> - from II.C.1

no/negligible accidental escape.

These GMOs are NOT sterile, and have parental/related spp. present, but none are protected spp. Concern is that populations of parental or related species will become introgressed 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.

<u>Manage Risk of Decline in Population Abundance</u> - from IV.A.1 or IV.C.

acceptable number is one that ensures that accidental escapees are fewer than the number that will avoid a decline in the abundance of the affected population(s) resulting from lowered fitness or introgressed descendents (IV.A.1), or from reproductive interference (IV.C.)

From IV.A.I.: These GMOs are not sterile, and do have conspp. +/or closely related spp. present, but none are protected spp.

From IV.C.: These GMOs are sterile and have chromosomal or other genetic changes, or they are fertile/sterile tetraploids, or are sterile interspp. hybrids.

<u>Manage Risk of Alteration of Ecosystem Processes</u> - from V.

no/negligible accidental escape

These GMOs CAN reproduce in the accessible ecosystem(s), are NOT sterile, and have no conspp. or closely related spp. present. Risks of adverse alteration(s) in ecosystem processes exist.

Select sufficient barriers from the categories listed below to assure that accidental escapees are fewer than the acceptable number for your research project. Consult text of Risk Management Recommendations for details about project siting and design of barriers.

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

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

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

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

SCALE OF EXPERIMENT
Maintain an experimental size small enough so that accidental escape of all organisms would not have an 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.

→ STANDARDS ARE COMPLETED

VI.B. Risk Management - Insufficient Information

The precautionary approach of the Standards requires that in the absence of information to evaluate risk, the goal of risk management must be no/negligible accidental escape of GMOs.

*Accidental escapees = combined outcome of scale of experiment and effectiveness of barriers.

Insufficient Information at II.A.1.

no/negligible accidental escape

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

Insufficient Information at IV.A. or IV.A.1.

no/negligible accidental escape.

These GMOs are NOT sterile . Conspp. or closely related spp. ARE present in the accessible ecosystem(s), but none are protected spp. Because the GMOs 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.1.

no/negligible accidental escape.

These GMOs are NOT sterile, and have NO conspp. or closely related spp. present in the accessible ecosystem(s). No barriers to their reproduction in accessible ecosystem(s) are known to exist. Because the GMOs 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.C.

no/negligible accidental escape.

These GMOs are either sterile with chromosomal or other genetic changes, or are sterile intraspecific hybrids, or are tetraploid. Conspecifics or closely related species are present in the accessible ecosystem(s), but none are protected spp.

Information is insufficient to assess the effect of reproductive interference on the affected population(s), or to assess the combined outcome of density-dependent factors and reproductive interference .

Select sufficient barriers from the categories listed below to ensure no/negligible accidental escape of GMOs for your research project. Consult text of Risk Management Recommendations for details about project siting and barrier design.

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

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

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

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

SCALE OF EXPERIMENT
Maintain an experimental 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.

→ STANDARDS ARE COMPLETED

Table 1. Classes and examples of possible phenotype changes in genetically modified fish, crustaceans, and molluscs.

Class	Examples of Phenotypic Change	Ecological Effect
Metabolism	- Growth rate - Enery metabolism - Food Utilization	- Shift to different prey size - Alter nutrient and energy flows
Tolerance of Physical Factors	- Temperature - Salinity - pH - Pressure	- Shift preferred habitats - Alter geographic range
Behavior	- Reproduction - Territoriality - Migration - Chemosensory (including pheromones, allelochemicals) - Swimming/navigation	- Alter life history patterns - Alter population dynamics - Alter species interactions
Resource/Substrate Use	- Food utilization	- Release from ecological limits - Alter food webs
Population Regulating Factors	- Novel disease resistance - Reduced predation/parasitism - Habitat preference	- Alter population and communtiy dynamics - Release from ecological limits
Reproduction	- Mode - Age at maturation and duration - Fecundity - Sterility	- Alter population and community dynamics - Interfere with reproduction of related organisms
Morphology	- Shape and size - Color - Fin/appendage form	- Alter species interactions
Life History	- Embryonic and larval development - Metamorphosis - Life span	- Alter life history patterns - Alter population and community dynamics

WORKSHEET ACCOMPANYING PERFORMANCE STANDARDS

Worksheet Accompanying
Performance Standards for Safely Conducting Research
with Genetically Modified Finfish and Shellfish

Introduction

The Performance Standards for Safely Conducting Research with Genetically Modified Finfish and Shellfish are voluntary guidelines intended to aid researchers and institutions in assessing the genetic and ecological effects of research activities involving genetically modified fish, crustaceans, and molluscs, and in determining appropriate procedures and safeguards so that the research can be conducted without causing adverse impacts on the environment. The Flowcharts of the Performance Standards guide researchers in identifying, assessing and managing specific risks. This Worksheet accompanies the Flowcharts. Once completed by the researcher, the Worksheet will document both the decision path taken through the flowcharts of the Performance Standards, and any risk management measures. It is designed to assist researchers and reviewers in evaluating the project. Until the Performance Standards are incorporated into a computerized expert system with the capability of producing a hard-copy trace of the decision path, this worksheet should be used.

Principal
Investigator: ____
Proposed
project: ____
____
____

Please mark your response to a question by checking "Yes," "No," "Don't know," "EXIT," or by indicating your routing to a subsequent flowchart. Marking of more than one blank may be appropriate in particular situations. *Attach written explanatory materials as directed below.*

<u>Flowchart Documentation</u>

Please list the numbers of all flowcharts that you used:
____
____

Flowchart No.

I. Do the performance standards apply to the proposed experiment?
- ____ Yes or don't know. Where were you routed?
  - ____ Continue to flowchart II.A.
  - ____ Consult Appendix B.
- ____ No. EXIT the standards.

II.A. Does the GMO result from deliberate gene changes?
- ____ Yes. Where does flowchart II.A. route you?
  - ____ II.A.1. Assess impact of deliberate gene changes.
  - ____ EXIT the standards. *Attach your rationale.*
  - ____ No. Continue to flowchart II.B.

Flowchart
No.

II.A.1. Where are you directed following completion of the flowchart regarding possible impact of deliberate gene changes? *Attach a written description of any identified risks.*

____ II. Assess potential interference with natural reproduction.
____ IV.A. in Ecosystem effects assessment.
Accidentally escaped GMOs may establish population posing potential for introgression.
____ IV.B. in Ecosystem effects assessment.
Accidentally escaped GMOs may establish population posing adverse effects on ecosystem structure or processes.
____ VI.A. Risk management - identified risks: manage risks to protected populations.
____ VI.B. Risk management - insufficient information.
____ EXIT the standards. *Attach your rationale.*
____ EXIT but consult relevant federal and state agencies regarding use of non-indigenous species. *Attach your rationale.*

II.B. Does the GMO result from deliberate chromosomal manipulations?

____ Yes. Where does flowchart II.B. route you?
 ____ II.B.1. Assess potential impact of chromosomal manipulations.
 ____ II.C. Assess impact of additional modifications.
 ____ EXIT the standards. *Attach your rationale.*
 ____ No. Continue to flowchart II.C.

II.B.1. Where are you directed following completion of the flowchart regarding possible impacts of deliberate chromosomal changes? *Attach a written description of any identified risks.*

____ III. Evaluate potential interference with natural reproduction.
____ EXIT the standards. *Attach your rationale.*
____ EXIT but consult relevant federal and state agencies regarding use of non-indigenous species. *Attach your rationale.*

II.C. Does the GMO result from interspecific hybridization?

____ Yes. Where does flowchart II.C. route you?
 ____ II.C.1. Assess potential impact of interspecific hybridization.
 ____ EXIT the Standards. *Attach your rationale.*
 ____ No. EXIT the standards. *Attach your rationale.*

II.C.1. Where are you directed following completion of the flowchart regarding potential impact of interspecific hybridization? *Attach a written description of identified risks* .

____ III. Evaluate potential interference with natural reproduction.
____ VI.A. Risk management - specific risks: Manage risks to protected populations.
____ VI.A. Risk management - specific risks: Manage risks of losing population of pure species.
____ EXIT the standards. *Attach your rationale.*
____ EXIT but consult relevant federal and state agencies regarding use of non-indigenous species. *Attach your rationale.*

III. If you were directed to use the flowchart regarding potential interference of a sterile GMO with natural reproduction, where were you routed? *Attach a written description of identified risks.*

____ IV.C. Ecosystem effects - impacts of reproductive interference.
____ VI.A. Risk management - specific risks. Manage risks to protected populations.
____ EXIT the standards. *Attach your rationale.*

Flowchart
No.

IV.A. If you were directed to use the flowchart regarding potential ecosystem effects of GMOs expressing deliberate gene changes, where were you routed? *Attach material describing risks identified.*

____ IV.A.1. Ecosystem effects - impacts of introgression of modified gene(s)
____ VI.B. Risk management - insufficient information.
____ EXIT the standards. *Attach your rationale.*

IV.A.1. If you were directed to use the flowchart regarding potential impacts of introgression of the modified gene into natural populations, where were you routed? *Attach a written description of identified risks.*

____ V. Assess effects on ecosystem structure and processes.
____ VI.A. Risk management - specific risks. Manage risk of decline in population abundance.
____ VI.B. Risk management - insufficient information.

IV.B. If you were directed to use the flowchart regarding potential barriers to reproduction of the GMO associated with the accessible ecosystem, where were you routed? *Attach a written description of identified risks.*

____ IV.B.1. Ecosystem effects - potential for non-reproductive interaction.
____ EXIT the standards. *Attach your rationale.*

IV.B.1. If you were directed to use the flowchart regarding the potential for non-reproductive interaction of the GMO with conspecifics or closely related species, where were you routed? *Attach a written description of identified risks.*

____ V. Effect on ecosystem structure and processes.
____ VI.B. Risk management - insufficient information.
____ EXIT the standards. *Attach your rationale.*

IV.C. If you were directed to use the flowchart regarding potential ecosystem impacts of reproductive interference by sterile GMOs, where were you routed? *Attach a written description of identified risks.*

____ VI.A. Risk management - specific risks. Manage risks of decline in population abundance.
____ VI.B. Risk management - insufficient information.
____ EXIT the standards. *Attach your rationale.*

V. If you were directed to use the flowchart regarding potential effects of the GMO on ecosystem structure and processes, where were you routed?

____ VI.A. Risk management - specific risks Manage risks to protected populations.
____ VI.A. Risk management - specific risks. Manage risks of alteration of ecosystem processes.
____ VI.B. Risk management - insufficient information.
____ EXIT the standards. *Attach your rationale.*

VI.A. If you were directed to use the flowchart regarding risk management when there are identified risks, what measures do you plan to adopt to manage these potential risk(s)? *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.*

Flowchart
No.

VI.B. If you were directed to use the flowchart regarding risk management when there is insufficient information to assess risks, what measures do you plan to adopt to effectively confine the proposed experiment? *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.

Additional Questions

1. Are you working with a non-indigenous species?
   ____ Yes.
   ____ No.

2. If yes, have you consulted the state and federal agencies which oversee uses of non-indigenous fish, crustaceans, and molluscs 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 experiment and in designing adequate safety measures.

____

____

____

____

________________________________ ____________________

Signature of researcher Date

Address and Phone No.

____

____

____

____

____

Risk Management Documentation

As part of compliance with the voluntary Performance Standards, the researcher must 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 must 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 GMOs in uncovered outside tanks or ponds, 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 GMOs 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 GMOs by water spray or waves into nearby natural water bodies? (Explanatory diagrams may be useful).

Design of Barriers

The Standards identify four types of barriers: (1) physical or chemical; (2) mechanical; (3) biological; and (4) scale of experiment as a barrier.

2. Was the project site chosen because the surrounding accessible ecosystems are lethal to all life stages of the GMO?
   ___ 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 GMO.
   (b) Explain how the siting reduces the need for barriers on-site.

3. Could the project's GMOs potentially escape through any of the paths (aquatic and non-aquatic) listed below? Answer "Yes" if there is potential for escape or uncertainty about potential escape of GMOs 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 GMOs in effluent, at least one additional barrier is necessary.)
   ___ c. Waste slurries?
   ___ d. Disposal of experimental animals?
   ___ e. Aerosols (applies only to shellfish with small larvae)?
   ___ f. Equipment cleaning and storage?

4. Have you identified additional, potential escape paths? 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 slurries; disposal of experimental animals; and cleaning and storage of equipment.

6. Describe how the types and numbers of barriers in series are sufficient to achieve the "acceptable number of accidental escapees" 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 GMO 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 animals 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 a water level alarm (required for all projects)?
   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 federal, state, and local 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
______________________________	_____	_____	_____
______________________________	_____	_____	_____
______________________________	_____	_____	_____
______________________________	_____	_____	_____
______________________________	_____	_____	_____

Worksheet Accompanying
Performance Standards for Safely Conducting Research with Genetically Modified Finfish and Shellfish

Introduction

The Performance Standards for Safely Conducting Research with Genetically Modified Finfish and Shellfish are voluntary guidelines intended to aid researchers and institutions in assessing the genetic and ecological effects of research activities involving genetically modified fish, crustaceans, and molluscs, and in determining appropriate procedures and safeguards so that the research can be conducted without causing adverse impacts on the environment. The Flowcharts of the Performance Standards guide researchers in identifying, assessing and managing specific risks. This Worksheet accompanies the Flowcharts. Once completed by the researcher, the Worksheet will document both the decision path taken through the flowcharts of the Performance Standards, and any risk management measures. It is designed to assist researchers and reviewers in evaluating the project. Until the Performance Standards are incorporated into a computerized expert system with the capability of producing a hard-copy trace of the decision path, this worksheet should be used.

Principal Investigator: M. Saxatilis

Proposed project: Investigation of growth performance of hybrid striped bass (*Morone saxatilis* x *M. chrysops*) in Lake Rend, a southern Illinois reservoir.

Please mark your response to a question by checking "Yes," "No," "Don't know," "EXIT," or by indicating your routing to a subsequent flowchart. Marking of more than one blank may be appropriate in particular situations. *Attach written explanatory materials as directed below.*

Flowchart Documentation

Please list the numbers of all flowcharts that you used: I

Flowchart No.

I. Do the performance standards apply to the proposed experiment?
- ____ Yes or don't know. Where were you routed?
  - ____ Continue to flowchart II.A.
  - ____ Consult Appendix B.
- ✓ No. EXIT the standards. **Optional rationale attached.**

List names addresses, telephone numbers, and area of expertise of the experts you contacted for substantial advice in assessing effects of a proposed experiment and in designing adequate safety measures.

M. Saxatilis

Signature of researcher

August 1, 1995

Date

Address and Phone No.

Fisheries Research Laboratory
Southern Illinois University
Carbondale, IL 62901
(618) 453-4131

Optional Rationale Attached to:

Worksheet Accompanying Performance Standards
for Safely Conducting Research with
Genetically Modified Finfish and Shellfish

Proposed Project: Investigation of growth performance of hybrid striped bass (*Morone saxatilis*) x white bass (*M. chrysops*) in Lake Rend, a southern Illinois reservoir.

The proposed work involves stocking of a fish modified only through interspecific hybridization into a flood control/recreation reservoir. The hybrid has been stocked in the reservoir over the past 15 years. I exited from the Performance Standards at flowchart I because there is no documentation of adverse effect of this hybrid in the accessible ecosystem. However, I opt to attach the following information to explain the value of the research, given that no studies have yet addressed potential adverse effects.

Risks identified: Should the project go forward, marked hybrids will be released directly into the reservoir, which is an ecosystem suitable for their survival. It would not be practical to treat the reservoir to remove the hybrids once they were stocked.

Regarding potential risks, one of the parental species, white bass, is native to the watershed at issue. Because the hybrid is fertile, there is a possibility of its back-crossing to this species. Indeed, such backcrosses were observed in a similar reservoir, Lake Palestine, in Texas (Forshage et al. 1986. Natural reproduction of white bass x striped bass in a Texas reservoir. Proc. Ann. Conf. Southeast. Assoc. Fish & Wildl. Agencies 40:9-14).

Risk management considerations: Although this proposed experiment presents the potential for introgressive hybridization, the hybrid has been stocked in the reservoir for the past 15 years. The proposed project differs from fishery management practice only inasmuch as growth performance of the hybrid will be monitored. Because of this context, it would not be appropriate to adopt additional confinement measures.

Risk assessment research: In order to evaluate the potential for introgressive hybridization - i.e., to support better-informed fishery management decisions - a genetic monitoring component will be incorporated into the proposed project. Two years after stocking of the hybrids, 200 young-of-the-year *Morone* sp. will be collected and examined for species-specific genetic markers. Presence of individuals which are neither white bass nor F_1 hybrids will be taken as evidence of reproduction of the F_1 hybrids. Further experiments with hybrid striped bass, as well as future fishery management practice, will be evaluated in light of the results.

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Principal Investigator: C. Gigas

Proposed project: Investigation of resistance of triploid Pacific oysters to the disease MSX and dermo in Chesapeake Bay.

Please mark your response to a question by checking "Yes," "No," "Don't know," "EXIT," or by indicating your routing to a subsequent flowchart. Marking of more than one blank may be appropriate in particular situations. *Attach written explanatory materials as directed below*.

Flowchart Documentation

Please list the numbers of all flowcharts that you used:
I, Appendix B, VI.B, if taken to be capable of selfing; otherwise I, II.A, II.B, II.B.1, if selfing is ruled out.

Flowchart No.

I. Do the performance standards apply to the proposed experiment?

- ✓ Yes or don't know. Where were you routed?
  - ✓ Continue to flowchart II.A.
  - ____ Consult Appendix B.
- ____ No. EXIT the standards.

II.A. Does the GMO result from deliberate gene changes?

- ____ Yes. Where does flowchart II.A. route you?
  - ____ II.A.1. Assess impact of deliberate gene changes.
  - ____ EXIT the standards. *Attach your rationale.*
  - ✓ No. Continue to flowchart II.B.

Flowchart
No.

II.B. Does the GMO result from deliberate chromosomal manipulations?

_✓_ Yes. Where does flowchart II.B. route you?

_✓_ II.B.1. Assess potential impact of chromosomal manipulations.
____ II.C. Assess impact of additional modifications.
____ EXIT the standards. *Attach your rationale.*
____ No. Continue to flowchart II.C.

II.B.1. Where are you directed following completion of the flowchart regarding possible impacts of deliberate chromosomal changes? *Attach a written description of any identified risks.*

____ III. Evaluate potential interference with natural reproduction.
____ EXIT the standards. *Attach your rationale.*
_✓_ EXIT but consult relevant federal and state agencies regarding use of non-indigenous species. *Attach your rationale.*
Rationale attached

Additional Questions

1. Are you working with a non-indigenous species?

_✓_ Yes.
____ No.

2. If yes, have you consulted the state and federal agencies which oversee uses of non-indigenous fish, crustaceans, and molluscs 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 experiment and in designing adequate safety measures.

Local official, Virginia Marine Resources Commission

Marine ecologist

Reproductive Physiologist

C. Gigas
Signature of researcher

August 1, 1995
Date

Address and Phone No.

Department of Fisheries and Aquaculture
Virginia Institute of Marine Science
Gloucester Point, VA
(804) 234-2222

Rationale Attached to:

Worksheet Accompanying Performance Standards
for Safely Conducting Research with
Genetically Modified Finfish and Shellfish

Proposed Project: Investigation of resistance of triploid Pacific oysters to the diseases MSX and dermo in Chesapeake Bay

Risks identified: 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. Can. J. Fish. Aquat. Sci. 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 ends abruptly. Bay Journal 4 (5): 1-4). Therefore, application of the precautionary principle would have me consult Appendix B of the Performance Standards and practice stringent confinement.

Should it be possible to rule out the possibility of selfing or conventional reproduction, a different pathway through the Performance Standards would lead me to identify much the same set of risks. With exit from the Performance Standards 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 below). 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: 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.

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Principal Investigator: I. Punctatus

Proposed project: Field testing of channel catfish expressing an introduced growth hormone gene in Alabama

Please mark your response to a question by checking "Yes," "No," "Don't know," "EXIT," or by indicating your routing to a subsequent flowchart. Marking of more than one blank may be appropriate in particular situations. *Attach written explanatory materials as directed below.*

Flowchart Documentation

Please list the numbers of all flowcharts that you used:
I, II.A, II.A.1, IV.A, VI.B

Flowchart No.

I. Do the performance standards apply to the proposed experiment?

- ✓ Yes or don't know. Where were you routed?
  - ____ Continue to flowchart II.A.
  - ____ Consult Appendix B.
- ____ No. EXIT the standards.

II.A. Does the GMO result from deliberate gene changes?

- ✓ Yes. Where does flowchart II.A. route you?
  - ✓ II.A.1. Assess impact of deliberate gene changes.
  - ____ EXIT the standards. *Attach your rationale.*
  - ____ No. Continue to flowchart II.B.

Flowchart No.

II.A.1. Where are you directed following completion of the flowchart regarding possible impact of deliberate gene changes? *Attach a written description of any identified risks.*

- ____ II. Assess potential interference with natural reproduction.
- _✓_ IV.A. in Ecosystem effects assessment. Accidentally escaped GMOs may establish population posing potential for introgression.
- ____ IV.B. in Ecosystem effects assessment. Accidentally escaped GMOs may establish population posing adverse effects on ecosystem structure or processes.
- ____ VI.A. Risk management - identified risks: manage risks to protected populations.
- ____ VI.B. Risk management - insufficient information.
- ____ EXIT the standards. *Attach your rationale.*
- ____ EXIT but consult relevant federal and state agencies regarding use of non-indigenous species. *Attach you rationale.*

IV.A. If you were directed to use the flowchart regarding potential ecosystem effects of GMOs expressing deliberate gene changes, where were you routed? *Attach material describing risks identified.*

- ____ IV.A.1. Ecosystem effects - impacts of introgression of modified gene(s)
- _✓_ VI.B. Risk management - insufficient information.
- ____ EXIT the standards. *Attach your rationale.*

Additional Questions

1. Are you working with a non-indigenous species?
   - ____ 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 experiment and in designing adequate safety measures.

Local Fish and Game Department Official

Evolutionary biologist

Aquatic ecologist

I. Punctatus
Signature of researcher

August 1, 1995
Date

Address and Phone No.

Department of Fisheries and Aquaculture
Auburn University
Auburn, AL 36820
(205) 826-4444

Risk Management Documentation

As part of compliance with the voluntary Performance Standards, the researcher must 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 must 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 GMOs in uncovered outside tanks or ponds, 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 GMOs 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 GMOs by water spray or waves into nearby natural water bodies? (Explanatory diagrams may be useful).

Design of Barriers

The Standards identify four types of barriers: (1) physical or chemical; (2) mechanical; (3) biological; and (4) scale of experiment as a barrier.

2. Was the project site chosen because the surrounding accessible ecosystems are lethal to all life stages of the GMO?
   ___ 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 GMO.
   (b) Explain how the siting reduces the need for barriers on-site.

3. Could the project's GMOs potentially escape through any of the paths (aquatic and non-aquatic) listed below? Answer "Yes" if there is potential for escape or uncertainty about potential escape of GMOs 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 GMOs in effluent, at least one additional barrier is necessary.)
   No c. Waste slurries?
   Yes d. Disposal of experimental animals?
   No e. Aerosols (applies only to shellfish with small larvae)?
   No f. Equipment cleaning and storage?

4. Have you identified additional, potential escape paths? 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 slurries; disposal of experimental animals; and cleaning and storage of equipment.

6. Describe how the types and numbers of barriers in series are sufficient to achieve the "acceptable number of accidental escapees" specified in Flowcharts VI.A. or VI.B.

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 GMO 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 animals 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 a water level alarm (required for all projects)?
   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 federal, state, and local 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
	____	____	____

Supporting Material Attached to:

Worksheet Accompanying Performance Standards for Safely Conducting Research with Genetically Modified Finfish and Shellfish

Proposed Project: Field testing of channel catfish expressing an introduced growth hormone gene in Alabama.

Risks identified: 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: 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 1). 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.

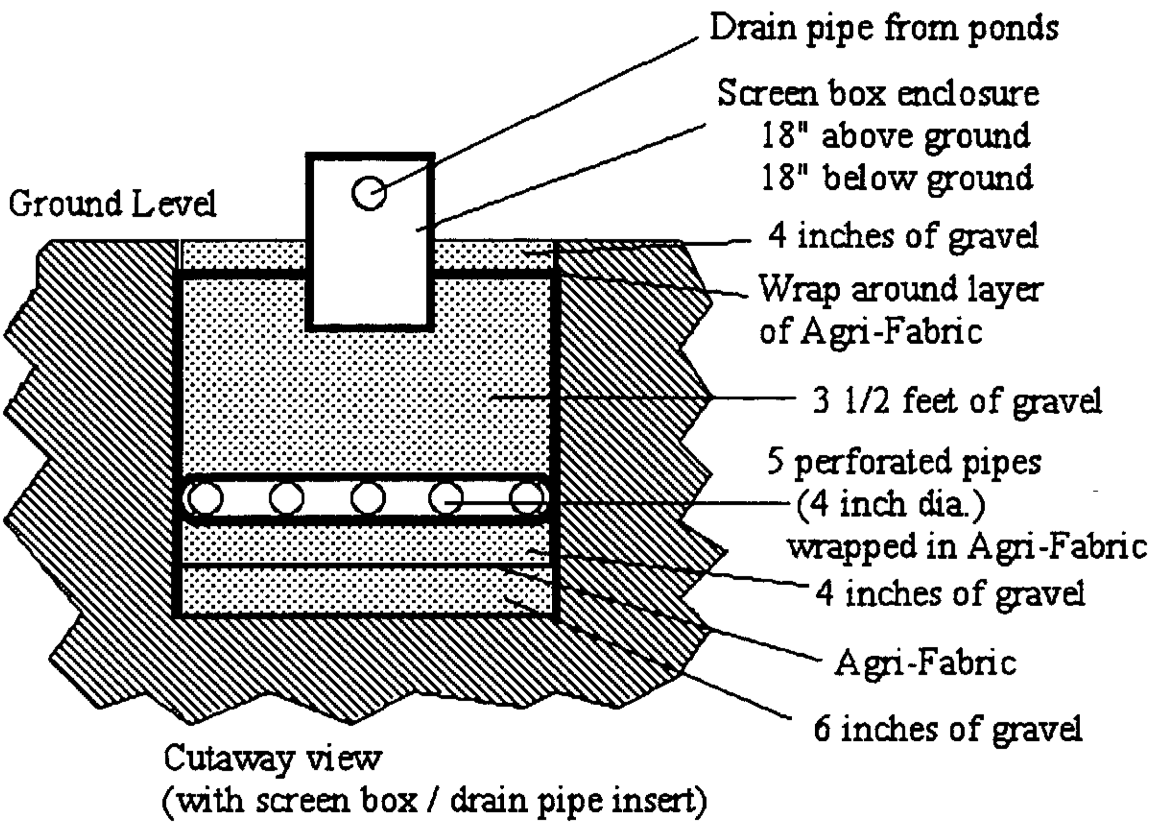

Figure 1. Schematic drawing of a French drain installed for the effluent of each outdoor pond used in transgenic fish experiments. The French drain is designed to retain the smallest possible size of fish reared in the pond. Water discharged from this drain eventually reaches surface waters. (Adapted from Cooperative State Research Service 1990, as cited in Cooperative State Research Service 1990a.)

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