PROTOCOL FOR EVALUATING RESEARCH PROPOSALS CONCERNING NONINDIGENOUS AQUATIC SPECIES

AQUATIC NUISANCE SPECIES TASK FORCE
JULY 1994

Introduction

The Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (Act; Public Law 101-646, 104 STAT. 4671, 16 U.S.C. 4701-4741 approved Nov. 29, 1990) requires that an intergovernmental Aquatic Nuisance Species Task Force (Task Force) develop and implement a protocol to ensure that research carried out under Subtitle C of the Act does not result in the introduction or dispersal of nonindigenous aquatic nuisance species to the waters of the United States. This protocol fulfills the requirements of the Act. The Task Force intends to develop the research protocol further based on experience gained through implementation of this protocol. This protocol will supplement other existing Federal protocols established to control activities with specific major classes of organisms, such as those already established for plants and insects under the Plant Quarantine Act of 1912 and the Federal Plant Pest Act of 1952, and for research involving recombinant DNA molecules under the Public Health Service Act of 1944.

This protocol must be used when research is carried out under Subtitle C of the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990. Individuals, states, corporations, and institutions not required by the Act to follow this protocol are encouraged to do so to prevent introductions and dispersal of nonindigenous aquatic nuisance species through research activities. Prevention of unintentional introductions through means other than research is addressed in the Task Force's proposed Aquatic Nuisance Species Program (which addresses prevention, detection, monitoring, and control of nonindigenous aquatic nuisance species). Intentional introductions are addressed in the Task Force's Report to Congress entitled "Findings, Conclusions, and Recommendations of the Intentional Introductions Policy Review".

A Research Protocol Committee (Appendix III) composed of representatives from the Task Force members was established to develop the required research protocol. The committee met in Gainesville, Florida, on June 25, 26, and 27, 1991, drafted the protocol, and prepared policy recommendations to the Task Force concerning implementation of

the protocol. The draft protocol was circulated to all Task Force agencies for review. A second draft was presented to the Task Force on September 27, 1991. Following a meeting of the Research Protocol Committee on April 1 and 2, 1992, and receipt of additional comments from Federal and non-Federal sources, a final draft was prepared and presented for Task Force approval on April 21, 1992. The research protocol was adopted by the Task Force on April 22, 1992 as an interim working protocol until the protocol had completed a public review. The availability of the Research Protocol for public review was announced in the Federal Register on September 24, 1992.

Research Protocol

The research protocol consists of two parts: a risk assessment (Part I) and a set of guidelines outlining preventative containment and confinement procedures (Part II). The risk assessment requires the Principal Investigator and the Research Institution to evaluate the risk that the species, if it escapes or is released, will be a nuisance, and to determine if preventative measures must be taken to prevent the species from escaping or being released. Research may be conducted with minimal special preventative measures if 1) the research site is within the present established or historic range of the species, 2) the species is free of nonindigenous diseases, parasites or other extraneous viable material, 3) the species is not likely to be a nuisance if released, and 4) the species cannot survive in the waters adjacent to the research location, or 5) only non-viable forms are used, or 6) the research does not involve actual handling or transfer of the species (e.g. computer modelling and in situ data collection). The evaluation of the proposal by the risk assessment will determine if preventative measures must be taken.

The second part of the protocol is a detailed set of preventative containment and confinement guidelines that the Principal Investigator may be required to follow to prevent the escape or release of any research species that fails to meet one or more of the conditions listed above. If directed by the risk assessment, the Principal Investigator must develop preventative measures that will contain or confine the species to the research facility or location(s).

Appendix I is a list of some of the presently existing guidelines and protocols that may be used as resources by investigators to identify the types of precautions that can be taken to prevent unintentional releases of organisms used in research or to guide research on aquatic nonindigenous species. The specific precautions needed (which include procedural and facility design and use elements) will depend on the species to be studied, its life stage and size (e.g. macroscopic and/or microscopic, and size range within each), the scope of the project, the characteristics of the research location(s) with regard to the species' critical environmental factors, and the potential of the species to survive in that locale(s) and to be a nuisance. If the species is a disease-causing organism or a parasite, or the species or the source of the species under consideration is not free of nonindigenous diseases or parasites, extra precautions may be necessary. Most of the guidelines listed require that test species be contained or confined by some combination

of physical, biological, chemical, and/or environmental barriers, or by limiting the scope of the research. The number and types of barriers needed depends on the species and the potential problems the species could create if it escapes or is released from the research site(s).

Procedures to Process Research Proposals

1. The Principal Investigator

The Principal Investigator shall determine that the research proposal complies with all applicable local, state, and national laws and regulations. The Principal Investigator will submit all research proposals concerning nonindigenous aquatic species to their Research Institution for review -- usually the Research Institution will establish a committee similar in membership, roles and responsibilities to the Institutional Biosafety Committee (IBC) described in the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (Federal Register 51, Number 88, page 16959 (51 FR 16959)). In the proposal the Principal Investigator must demonstrate a knowledge of the life history and biology of the species, provide all information necessary for preparation of a risk assessment, and provide citations for all supporting data. If the species is found to present any possibility of being a nuisance, as determined by the risk assessment, the proposal must clearly demonstrate that 1) adequate confinement and containment procedures will be in place during research and throughout the time that the species is held, and 2) the Principal Investigator has incorporated into the study plan procedures, facility design elements, and other preventative measures analogous to those in guidelines developed by NIH for research within recombinant DNA molecules, and the U.S. Department of Agriculture for research in agricultural biotechnology (49 FR 50856, 51 FR 23302, and 56 FR 4134), which are adequate to contain and confine the species and any pathogens or parasites it may contain or be infested with. Within 30 days of being notified by a Funding Agency that a nonindigenous species research proposal will be funded, the Principal Investigator must notify the appropriate state authorities in writing that the research is going to be carried out, and must submit a copy of that written notification to the Funding Agency by the end of the thirty day period. The Funding Agency will be responsible for sending a copy of the state notification document to the Research Protocol Committee before the research is initiated.

2. The Research Institution

The Research Institution accepts and reviews the research proposal, reviews and approves the risk assessment and preventative measures, agrees to support the research and signs a statement that it will ensure that the research will be conducted as planned and the preventative measures will be carried out. The Research Institution may establish an Institutional Biosafety Committee (IBC) and a Biosafety Officer (BO) position to assist it to meet its obligations. The use of an IBC or a BO is optional but the Principal

Investigator and the Research Institution should have a system in place to demonstrate that the proposal has been reviewed by a qualified independent group before submitting it to the Funding Agency. The Research Institution must determine that the proposal is complete, and that it includes an accurately completed risk assessment, all required life history and biological data, and adequate and detailed containment and confinement measures, if needed. The Research Institution should also determine that the proposal complies with all applicable local, state, and national laws and regulations. The Research Institution should determine if a species-specific containment/confinement protocol has been approved by the Research Protocol Committee for the species and if so, whether the proposal fully meets all requirements of that approved species-specific protocol (ASSP). If an ASSP exists and the Principal Investigator is proposing to deviate from that ASSP, the Research Institution should ensure that the differences and the substituted preventative measures are clearly described, since further review and approval of the proposal by the Research Protocol Committee will be required. If no ASSP exists, the Research Institution must be assured that the Principal Investigator has conducted a thorough literature review on the species, is knowledgeable of its life history, biology and ecology, and has developed and described preventative measures to adequately contain and confine the species if necessary. Proposals not conforming to an ASSP or for which no ASSP exists will require a full review by the Research Protocol Committee, and should follow guidelines similar to that outlined in Appendix I. The proposal, with the appropriate findings and a certification of compliance statement signed by the Principal Investigator and the Research Institution that states that the Principal Investigator and the Research Institution will adhere to the proposed containment and confinement procedures, must be transmitted to the Funding Agency. If the Research Institution or the IBC does not have the expertise to evaluate a particular proposal, the proposal should be transmitted to the Funding Agency accompanied by a request for a review by the Research Protocol Committee. The Principal Investigator is still responsible for providing all the information needed to fully evaluate the species.

3. The Funding Agency

The Funding Agency provides technical and programmatic review, determines if the proposal is complete and that it complies with the requirements of the National Environmental Policy Act (NEPA) and other applicable laws and regulations (Appendix IV). The Funding Agency makes all funding decisions; prioritizes and selects proposals for funding, submits the proposals to be funded to the Research Protocol Committee, and after receipt of the Research Protocol Committee's review, determines what steps must be taken, if any, before the proposals will be funded. The Funding Agency may require that the Principal Investigator make changes in the proposal before submittal to the Research Protocol Committee for initial or re-review. All proposals selected for funding will be transmitted to the Research Protocol Committee within 15 days after the proposal has been selected for funding, either for review, if the Research Institution has not already certified that the proposal is in compliance with an ASSP, or for informational purposes, if the Research Institution has certified compliance with an ASSP. The Research Protocol Committee will eventually review all proposals, but proposals following an ASSP do not have to be reviewed prior to funding.

4. The Research Protocol Committee

All proposals concerning nonindigenous aquatic species (including the risk assessment and preventative measures to be used to prevent escape or inadvertent release) selected for funding by a Funding Agency will be submitted to the Research Protocol Committee within 15 days of selection for funding. Research proposals requiring preventive/containment measures and for which the Principal Investigator and Research Institution have certified that one or more ASSPs will be followed without modification, will not have to be reviewed by the Research Protocol Committee prior to funding. However, such proposals will still be sent to the Research Protocol Committee by the Funding Agency for review to verify the risk assessment and ASSP(s), to verify compliance with the intent and provision of the Research Protocol, to obtain information that may be used to revise the Research Protocol or the ASSP(s) as appropriate, and to obtain information necessary for reporting purposes. For all other proposals, the Research Protocol Committee will review in detail the completed risk assessment, the research proposal, and the proposed containment and confinement procedures to insure that the proposed procedures are adequate to prevent the species from escaping or being released during the research. The Research Protocol Committee will review and provide comments and recommendations to the Funding agency within 90 days of receipt of the research proposals from the Funding Agency. Proposals requiring major changes must be resubmitted to the Research Protocol Committee for review. The Research Protocol Committee may call on outside expertise when necessary or may establish subcommittees to review multiple proposals for work on the same species. The Research Protocol Committee will advise the Funding Agency and make recommendations: (1) the proposal (including the completed risk assessment and preventative measures) appears to be adequate and thus funding is appropriate; (2) the proposal is not adequate in all aspects and needs to be resubmitted to the Research Protocol Committee after deficiencies identified are addressed and appropriate changes made to the proposal; or (3) the proposal has serious inadequacies that require major changes, and should not be funded until these changes are made and the proposal has been resubmitted to the Research Protocol Committee and the Research Protocol Committee has deemed the revised proposal to be adequate.

All proposals (both those complying with an ASSP and those with individualized containment and confinement plans) will be reviewed by the Research Protocol Committee to determine if there are problems in the use of the risk assessment and to improve both this research protocol and the ASSP. The Research Protocol Committee will provide an annual report to the Task Force detailing the proposals reviewed, the species involved, the number of proposals needing detailed confinement and containment procedures, the location of the research sites by species, the problems encountered, and announce the availability of ASSP's and recommend changes to the Task Force as needed.

The Research Protocol Committee will serve as an advisor to the Funding Agencies, providing comments and recommendations on the risk assessment and adequacy of preventative measures being taken by the researcher. The responsibility of ensuring

NEPA compliance, and of selecting and funding the research belongs entirely to the Funding Agency.

At every level of the processing of the proposals every effort will be taken to protect the confidentiality of the research. Genetically altered species, unless they are also nonindigenous species, should not be processed through this protocol. Research involving genetically altered species should be processed through other appropriate protocols (See Appendix I).

PART I

Risk Assessment

Completed risk assessments must be submitted in narrative form to the Funding Agency along with the preventative measures, if needed. The reasoning behind each answer must be stated. The submittal of the complete research proposal to the Research Protocol Committee is not necessary, however, the Principal Investigator is responsible for providing enough information to enable the Research Protocol Committee to understand the research, and to evaluate the risk assessment and the effectiveness of the preventative measures, if needed.

I. **Does the research concern a nonindigenous aquatic species as defined by the Nonindigenous Aquatic Nuisance Species Prevention and Control Act of 1990 (Act)?** Nonindigenous aquatic species means any species or other viable biological material that enters an ecosystem beyond its presently established or historical range, including transfers from both domestic and foreign sources. [Historical range is the territory occupied by a species at the time of European colonization of North America.]

ALL ANSWERS: go to **II.**

II. **Does the species carry any known nonindigenous diseases, parasites or any other nonindigenous species or viable biological material?** Unless there is knowledge or evidence to the contrary (e.g., oysters being transferred from an area where MSX or dermo or imported oyster drills exist, salmonid transfers from areas where IHN and VHS viruses occur, or warmwater species transfers from areas where the Asian tapeworm occurs) species transfers within the continental U.S. can be considered free of nonindigenous diseases or parasites. Any species recently imported directly or indirectly into the continental U.S., Hawaii, Alaska or a territory of the U.S. from a foreign country, or from Alaska, Hawaii, or a territory of the U.S. into the continental U.S. or the reciprocal should be considered to have nonindigenous diseases or parasites unless proven otherwise;

appropriate preventative measures must be taken (see Part II, Guideline of Preventative Measures).

YES or NOT SURE: go directly to Part **II.** (Guideline of Preventative Measures) and to **III.**
NO: go to **III.**

III. **Do or could transportation waters, media or sediments or sampling equipment carry any nonindigenous diseases, parasites, or other viable material (study or extraneous organisms)?**

YES or **NOT SURE**: transfer species to clean water and container, treat waste water to kill all organisms, disinfect original container. If this is sufficient to rid the shipment (transfer) of all extraneous organisms, go to **IV**; if not, go to Part **II** (Guideline of Preventative Measures).
NO: go to **IV**.

IV. If the research does not concern a nonindigenous aquatic species under the Act and the research could not spread nonindigenous diseases, parasites or other viable material, this protocol does not apply, however, some precautions may be necessary to avoid the spread of nonindigenous species by incidental means such as contaminated equipment. If the species falls under the Act, continue on to V.

If answers to **I**, **II**, and **III** are all **NO**: the protocol does not apply to your research organism.
If any answer to **I**, **II**, and/or **III** above is **YES** or **NOT SURE**: the species falls under the Act; go to **V**.

V. **Will live, viable, or fresh specimens be required?**

NO (specimens must be preserved in a manner to kill the organisms immediately to assure no possibility of infestation if the specimens are released): no additional procedures may be necessary.
YES: go to VI.

VI. **Will the species be transferred away from the site where collected?**

NO: The spread of the organism is unlikely therefore environmental concerns are minimal. Some precautions to avoid the incidental spread of the organism by contaminated sampling equipment may be needed. If the research will not result in the spread of live organisms the remainder of the protocol does not apply.
YES: go to VII.

VII. **Will the species be transported through areas which are free of the infestation?**

YES: adequate preventative measures must be taken to prevent escape or release during transportation; go to VIII.
NO or **NOT SURE:** go to **VIII**.

VIII. **Is the species under investigation presently established within one mile of any facility which will receive live nonindigenous species or other nonpreserved field material which may be contaminated with a nonindigenous species?** Studies may be conducted in more than one research laboratory (including field laboratories). List each laboratory in which the research will be conducted, and discuss and document for each laboratory.

YES (The species is found within one mile of a research facility or its effluent discharge point): the study may not require more than minimal measures at this facility to prevent the species' introduction. It may however require precautionary measures to ensure that nonindigenous species are not spread between collection sites, from one facility to another facility, or from a facility to noninfested sites by means of equipment or supplies used at more than one study site or used for more than one study.
NO (the species is not found within one mile of a research facility which will receive live nonindigenous species or other nonpreserved field material which may be contaminated with a nonindigenous species, or within one mile of the facility's effluent discharge point): the researcher should report the nearest known population of the species from each facility and go to IX.

IX. **Can the species survive in the surrounding waters?**

NO: only minimum preventative measures may be needed.
YES or **NOT SURE:** go to **X**.

X. **Is it absolutely certain that the species will not be a nuisance if it escapes or is released into surrounding waters?** [Note: A nuisance species threatens the diversity or abundance of native species or the ecological stability of infested waters, or commercial, agricultural, aquacultural, or recreational activities dependent on such waters.]

YES: only minimum preventative measures may be needed.
NO or **NOT SURE:** go to **XI**.

XI. **Have you previously been approved for research with this species at your present location(s) using the same facilities?**

YES: explain the changes, if any, between this proposal and previous funded studies and attach a copy of previous approval letter and submit to the Funding Agency for review by the Research Protocol Committee. Explain any changes in detail.
If major changes exist from earlier funded study or the answer is **NO:** go to **XII**.

XII. **Is there a Research Protocol Committee approved species-specific protocol (ASSP) for the nonindigenous species that is (are) the subject(s) of your research proposal, and will this ASSP be used by you for this proposal?**

YES (an ASSP exists and will be adhered to in every particular): attach the ASSP and list specifics (e.g. options to be used) that are to be used in your research. Submit to Funding Agency for review by Research Protocol Committee.
NO (no ASSP exists, or an ASSP exists but will not be used): go to **XIII**.
NO (An ASSP exists but will not be exactly adhered to, i.e. additional or different methods will be used, or parts of the ASSP will not be used): describe in detail any deviation from the ASSP, specify if any part of the ASSP will be used, and describe preventative methods to be used that differ from those in the ASSP. If any part of the ASSP is to be used, attach the ASSP: go to **XIII**.

XIII. If the proposal has reached this point in the risk assessment, a preventative containment/confinement plan must be developed and described in detail which will ensure that the species or any diseases or parasites it might carry cannot escape or be released into the surrounding waters. The species under consideration is a live or viable nonindigenous aquatic species, a nonindigenous pathogen or parasite of aquatic species, or might be carrying nonindigenous diseases or parasites of aquatic species, is not present in the waters surrounding the research site, could survive if released, and could be a nuisance. The researcher must document knowledge of the literature concerning the species and the problems which could result if released. A plan must be developed to ensure that the research does not result in the release, escape, or dispersal of the species. The investigator will be required to develop a preventative plan (PART II) and submit it with the risk assessment to the Funding Agency who will forward it to the Research Protocol Committee for review. The investigator and the supporting Research Institution must agree to comply with the preventative plan, and this protocol or an approved species-specific protocol. The Funding Agency and the Research Institution will ensure compliance.

Every investigator conducting research on a live or viable nonindigenous aquatic species which could be a nuisance, and is conducting the research outside the species' present established or historic range, is required to develop containment and confinement procedures and have a secure facility. Reference to guidelines already available (Appendix I) can be of assistance in developing a containment and confinement plan. Table I is an outline of the information and containment and confinement procedures required in most existing guidelines. In the future species-specific protocols may be developed for high visibility species (like the zebra mussel) whose life history, biology, and impacts are known and for which there are multiple studies under consideration. When reviewed and approved by the Research Protocol Committee, ASSPs may be used by investigators, however, compliance to all points of the ASSP will be mandatory if the Investigator elects to use an ASSP. Any or all protocols may be changed by the Research

Protocol Committee as new knowledge is accumulated. Deviations from an ASSP will require case by case approval of research proposals and their preventative plans. Research on nonindigenous species which may also have nonindigenous diseases and parasites will require maximum security for the species and for any diseases or parasites the species may carry. Every effort should be made to conduct research on nonindigenous species in facilities located within the existing established range of the species; in this case only one level of preventative measures may be required.

PART II

Guideline of Preventative Measures

The Research Protocol Committee cannot develop a detailed set of guidelines for every nonindigenous species under research. Investigators and Research Institutions must develop containment and confinement plans taking into consideration the species, its characteristics, diseases and parasites, and critical environmental factors, its capabilities to be a nuisance, the design of the research facilities, and the location of the test site in relationship to the species' present range. Appendix I lists guidelines which have already been developed for groups of organisms. Table I is an outline of the informational needs and preventative measures to contain or confine test species found in most guidelines. The appendix and table are included as reference materials for investigators.

If the investigator determines that live specimens must be used, that the research must be conducted in an area where the species is not already present, that the species could survive if released into surrounding waters, and that the species or its diseases or parasites could be a nuisance, major preventative measures would be required to prevent escape or release.

The preventative plan should use a combination of physical, biological, environmental, and/or chemical barriers to contain or confine all life stages of the organism. Reducing the scope of the research should also increase the safety of the research.

For containment of diseases, parasites, small species, or the early life stages of larger species, the procedures outlined in the NIH guidelines (FR 51 No. 88, May 7, 1986, pg. 16959) or guidelines developed by the U.S. Department of Health and Human Services (see references) are the most comprehensive.

For containment or confinement of larger forms, the guidelines developed for whole plants or animals by the Office of Agricultural Biotechnology, USDA, are the most appropriate, especially if the research is to be conducted outside the laboratory (see Appendix I).

Preventative measures should address all life stages present or possible during the research phase. Where feasible, use of juvenile specimens, monosex populations, or sterile individuals is recommended.

Species-Specific Confinement and Containment Protocols

The Research Protocol Committee expects to receive many research proposals on a few high profile, high risk species, such as zebra mussels. A subcommittee of the Research Protocol Committee or one of the Funding Agencies may submit a species-specific confinement/containment protocol for review by the Research Protocol Committee. When such a proposed species-specific protocol is submitted, the Research Protocol Committee will review the adequacy of proposed containment procedures to insure that the species or any associated diseases, parasites, or any other nonindigenous species or viable biological materials cannot escape or be released during research. The Research Protocol Committee will complete its review and provide a response to the appropriate Funding Agency or subcommittee within 90 days. The form of the Research Protocol Committee's response will be either: 1) the species-specific protocol is adequate as proposed and is approved for general use by the research community (i.e., the protocol has become an ASSP); or 2) the species-specific protocol is not adequate as proposed and is not approved. If the proposed species-specific protocol is not approved, the Research Protocol Committee will state reasons and may suggest modifications to correct problems seen. Since these protocols will only be prepared for species which are considered nuisance species, the risk assessment section can be reduced and the preventative plan can be standardized. Research proposals adhering to an ASSP will not need to be reviewed by the Research Protocol Committee prior to funding.

Compliance with all provisions of an ASSP must be fully accepted in writing by the Principal Investigator and the Research Institution by submitting a signed statement (certification of compliance) to that effect. Specific preventative measures to be used by the Principal Investigator must be documented in the research proposal. If all aspects of the ASSP are accepted, the Research Institution can approve confinement and containment procedures and monitor the research. All documentation, including the proposal, completed risk assessment, and preventative measures to be used, will be forwarded to the Research Protocol Committee by the Funding Agency. Any deviations from the requirements of an ASSP will require that the research proposal and confinement and containment plan be reviewed by the Research Protocol Committee before funding is approved.

The Research Protocol Committee will use the information in all research proposals (using both species-specific and non-standard protocols), to improve future protocols and to establish the location of research on nonindigenous aquatic species.

The Research Protocol Committee will report annually to the Task Force the number of proposals requiring confinement/containment measures, the species involved, and the

location of research sites. Problems will be identified and recommendations for correcting them provided to the Task Force.

Until a research proposal is funded and becomes public property the confidentiality of the contents of the proposal must be maintained at all levels. All levels of review before funding must be made aware of the legal and ethical responsibilities not to discuss, copy, or share proposals with anyone not directly involved or authorized to assist in the review.

Compliance, Inspection, Reporting

All proposals which are required to follow a confinement and containment protocol must include certification by the Principal Investigator and the Research Institution that they will comply with the requirements of the protocol, and within the proposal must document the specific containment and confinement measures to be used. The Research Institution or The Institutional Biosafety Committee and/or the Biological Safety Officer, if appointed by the Research Institution (see NIH guidelines 51 FR 16963 for specific duties), will monitor the conduct of the research and verify compliance with the containment and confinement procedures agreed to by the Principal Investigator and the Research Institution.

The Funding Agency, the Research Protocol Committee, and appropriate state agencies may inspect the facilities and containment and confinement procedures at any time. The Research Institution should inspect its research at least twice yearly.

Failure to comply with the protocol, or the escape or release of a nonindigenous aquatic species must be reported to the Funding Agency, the appropriate State agencies and the Research Protocol Committee immediately. Penalties for noncompliance with the protocol will be administered by the Funding Agency and could include suspension of research funding. The major responsibility for compliance with the protocol falls to the Principal Investigator and the Research Institution.

APPENDIX I

Existing Guidelines and Protocols

Guidelines for Recombinant DNA Molecule Research:

The following is a list of guidelines and protocols used to confine or contain nonindigenous species or organisms involved in recombinant DNA research. These can also be applied to nonindigenous aquatic species proposals. Consulting one or more of these will help investigators to identify physical, biological, chemical, and/or environmental preventative measures that may be used to confine or contain the nonindigenous aquatic species during research, transportation and storage. (Federal Register 51 No. 8, pg. 16958; FR 51 No. 123, pg. 23367; FR 52 No. 154, pg. 29800; FR 56 No. 22, pg. 4134; FR 51 No. 88, pg. 16959)

Guidelines for Microorganisms

National Institutes of Health (NIH). 1968. Guidelines for Research Involving Recombinant DNA Molecules. Published in Federal Register May 7, 1986 (51FR 16958-16961) with additional major actions August 24, 1987 (52F 31838); July 29, 1988 (53FR 28819); October 26, 1988 (53FR 43410); March 13, 1989 (54FR 10508); March 1, 1990 (55FR 7438); and August 11, 1987 (52FR 29800) with appendix P for plants and Q for animals.

Guidelines for Whole Plants and Animals

U.S. Department of Agriculture (USDA). 1984. Coordinated Framework for Regulation of Biotechnology. Federal Register December 31, 1984 (49FR 50856) and June 26, 1986 (51FR 23302+).

USDA. 1986. Advance Notice of Proposed USDA Guidelines for Biotechnology Research. Federal Register June 26, 1986 (51FR 23367-23393) and February 1, 1991 (56FR 4134-4149).

USDA. 1986. Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which are Plant Pests or for Which There is Reason to Believe are Plant Pests. Federal Register June 26, 1986 (51FR 23352-23366) and June 16, 1987 (52FR 22892-22915).

Coulson, J. R., and R. S. Soper. 1989. Protocols for the Introduction of Biological Control Agents in the U.S. Chapter I, pages 2-35 In: Kahn, R. P. (ed.). Plant Protection and Quarantine. Volume III Special Topics. CRC Press, Inc., Boca Raton, Florida.

USDA, Office of Agricultural Biotechnology. 1988. USDA Guidelines for Research Outside the Laboratory Involving Biotechnology, also Federal Register June 26, 1986 (51FR 23367-23313) and February 1, 1991 (56FR 4134-4149).

International Guidelines and Protocols:

European Inland Fisheries Advisory Commission. 1988. Code of Practice and Manual of Procedures for Consideration of Introductions and Transfers of Marine and Freshwater Organisms. FAO. EIFAC. Occasional paper No. 23. 52 pages.

International Council for the Exploration of the Sea. 1982. Proposed Guidelines for Implementing the ICES Code of Practice Concerning Introduction and Transfer of Marine Species. 23-page manuscript.

Disease Related Guidelines and Protocols:

Anonymous. 1989. Operating Procedures for the Alma Quarantine Facility. Prepared for the Alma Research Station, Guelph, Ontario, Canada. 16 pages typewritten.

Horner, R. W., and R. L. Eschenroder. 1991. Protocols to Minimize the Risk of Introducing Salmonid Disease Agents with Importation of Salmonid Fishes. Draft manuscript. 11 pages. Prepared for Great Lakes Fish Disease Control Committee. Pages 27-37.

U.S. Department of Health and Human Services. 1984. Biosafety in Microbiological and Biomedical Laboratories. 1st Edition (March 1984). U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, Georgia 30333, and National Institutes of Health, Bethesda, Maryland 20892.

An additional 17 references on laboratory disease and pathogen control methods can be found listed in the Federal Register, May 7, 1986 (51FR 16965).

Other Guidelines and Protocols:

Klingman, D. L., and J. R. Coulson. 1983. Guidelines for Introducing Foreign Organisms into the United States for Biological Control of Weeds. Bulletin of Entomological Society of America. Fall 1983:55-61.

Guidelines for the Importation, Interstate Movement, and Field Release of Foreign Arthropod-Parasitic Nematodes into the United States for Biological Control of Arthropod Pests of Plants, Man, and Domestic Animals, and Vectors of Plant, Human, and Animal Pathogens, and for the Interstate Movement and Export of Foreign and

Native Arthropod-Parasitic Nematodes for Research on Biological Control of Such Pests.

Guidelines for the Importation, Interstate Movement, and Field Release of Foreign Microbial Pathogens (Fungi, Bacteria, Rickettsia Viruses, Protozoa) into the United States for Biological Control of Arthropod Pests of Plants, Man, and Domestic Animals, and Vectors of Plant, Human, and Animal Pathogens, and for the Export of Foreign and Native Arthropod Pathogens for Research.

Guidelines for the Importation, Interstate Movement, and Field Release of Foreign Arthropods and Nematodes into the United States for Biological Control of Weeds, and for the Interstate Movement and Export of Foreign and Native Arthropod and Nematode Natural Enemies of Weeds.

Guidelines for the Importation, Interstate Movement, and Field Release in the United States of Foreign Microbial Pathogens for Biological Control of Weeds, and for the Interstate Movement and Export of Foreign and Native Pathogens of Weeds for Research.

Guidelines for the Importation, Interstate Movement, and Field Release of Foreign Beneficial Organisms (Microbial Pathogens and Antagonists) into the United States for Biological Control of Plant Nematodes and Plant Pathogens, and for the Export of Such Organisms (Foreign and Native) for Research.

Southeastern Cooperative Wildlife Disease Study. 1985. Model for State Regulations Pertaining to Captive Wild and Exotic Animals. University of Georgia, Athens, Georgia. 48-page manuscript. Prepared in response to Resolution #9. U.S. Animal Health Association, Milwaukee, Wisconsin 10/27-11/1/85.

Jennings, D. P., and J. A. McCann. 1991. Research Protocol for Handling Nonindigenous Aquatic Species. National Fisheries Research Center, U.S. Fish and Wildlife Service, Gainesville, Florida. 43-page manuscript.

Brown Tree Snake Protocol:

Pacific Basin Development Council. 1991. Recommended Protocol for Transport of Live Brown Tree Snakes (Boiga irregularis). Prepared for Plant Quarantine Branch, State of Hawaii Department of Agriculture and Biological Survey, and the U.S. Fish and Wildlife Service.

Guidelines for Animal Care and Welfare:

Guidelines for Use of Live Amphibians and Reptiles in Field Research. American Society of Ichthyologists and Herpetologists (ASIH), The Herpetologists' League (HL), and the Society for the Study of Amphibian and Reptiles (SSAR). 1987.

Interagency Research Animal Committee's Report. U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training. Published in the Federal Register. May 20, 1985.

Guidelines for the Use of Fishes in Field Research. American Society of Ichthyologists and Herpetologists (ASIH), American Fisheries Society (AFS), and American Institute of Fisheries Research Biologists (AIFRB).

APPENDIX II

Definitions

Aquatic Nuisance Species

a nonindigenous species that threatens the diversity or abundance of native species or the ecological stability of infested waters, or commercial, agricultural, aquacultural or recreational activities dependent on such waters. Aquatic nuisance species include nonindigenous species that may occur in inland, estuarine and marine waters and that presently or potentially threaten ecological processes and natural resources. In addition to adversely affecting activities dependant on waters of the United States, aquatic nuisance species adversely affect individuals, including health effects.

Biological Safety Officer (BSO)

an individual who is a member of the IBC who has the direct responsibility (after the PI) to ensure the activities and precautions stated in the research proposal are followed. See NIH guideline FR 51 No. 88, pg. 16963, for other roles and responsibilities.

Confinement

a term used primarily in the USDA guidelines meaning organisms restricted to research field facilities such as outside experimental pond areas and involving whole plants and animals.

Containment

a term used primarily in the NIH guidelines to mean restricted to laboratory environments and is usually in reference to micro-organisms, recombinant DNA molecules, or whole plants (Appendix P) or whole animals (Appendix Q).

Established

when used in reference to a species, this term means occurring as a reproducing, self-sustaining population in an open ecosystem, i.e. in waters where the organisms are able to migrate or be transported to other waters.

Institutional Biosafety Committee (IBC)

see NIH guidelines FR 51 No. 88, pg. 16962, for membership, roles, and responsibilities.

Nonindigenous Species

any species or other viable biological material that enters an ecosystem beyond its historic range, including any such organisms transferred from one country to another. Nonindigenous species include both exotics and transplants. [Note:

Historic range is interpreted to mean the territory occupied by a species at the time of European colonization of North America.]

Pathogen

as defined in USDA guidelines, is a virus or micro-organism (including its viruses and plasmids, if any) that has the ability to cause disease in another living organism.

Principal Investigator (PI)

see FR 51 No. 88, pg. 16963, for roles and responsibilities.

Research Institution

means any public or private entity (including Federal, state, or local government agencies) conducting the research.

Research Protocol Committee (RPC)

will be comprised of one or more representatives from each Federal Task Force agency who are qualified to evaluate nonindigenous species research proposals. Knowledgeable experts from other Federal, state, or private groups with different areas of expertise might be asked to assist the committee.

Surrounding Waters

means any free flowing or standing waters in the immediate vicinity of the research facility that are connected with public waters either directly or indirectly.

Survival

organism able to live in an ecosystem during its normal life span but not necessarily able to reproduce itself.

Unintentional Introduction

an introduction of nonindigenous species that occurs as a result of activities other than the purposeful or intentional introduction of the species involved, such as the transport of nonindigenous species in ballast or in water used to transport fish, mollusks or crustaceans for aquaculture or other purpose. Involved is the release, often unknowingly, of nonindigenous organisms without any specific purpose. The virtually inevitable escapement, accidental release, improper disposal (e.g., "aquarium dumping") or similar releases of intentionally introduced nonindigenous species do not constitute unintentional introductions.

Waters of the United States

the navigable waters and the territorial sea of the United States. Since aquatic nuisance species can move or be transported by currents into navigable waters, all internal waters of the United States, including its territories and possessions, are included. The Territorial Sea of the United States is that established by Presidential Proclamation Number 5928 of December 27, 1988.

APPENDIX III

Membership of the Research Protocol Committee

James A. McCann, National Fisheries Research Center-Gainesville, U.S. Fish and Wildlife Service - Chairman, May 1991-Present

Althaea Langston, Animal and Plant Health Inspection Service - Policy and Program Development, U.S. Department of Agriculture - Member, May 1991-Present

David F. Reid, Great Lakes Environmental Research Laboratory, National Oceanic and Atmospheric Administration - Member, May 1991-Present

Edwin A. Theriot, Environmental Laboratory, Waterways Experiment Station, U.S. Army Corps of Engineers - Member, August 1991-Present

J. David Yount, Environmental Research Laboratory-Duluth, U.S. Environmental Protection Agency - Member, March 1993-Present

APPENDIX IV

Other Legislation or Executive Orders Related to the Nonindigenous Aquatic Species Act

Applicable State Laws, Regulations, Permit and Notification Requirements - Must be determined on an individual basis by Principal Investigators and Research Institutions.

Lacey Act of 1900 - 16 USC 3371-3378 and 18 USC 42 Item 2,58

Endangered Species Conservation Act of 1973-16 USC 1531-1543 plus Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)-16 USC 1531-1543.

Executive Order #11987 dated March 1977 - Exotic Organisms

Plant Quarantine Act of 1912 (7 USC 151 et seq.)

Federal Plant Pest Act of 1957 (7 USC 150aa et seq.)

Federal Noxious Weed Act of 1974 (Public Law 93-629-Jan. 3, 1975) (7 USC 2801 et seq. + 21 USC 111 et seq.)

National Environmental Policy Act of 1969 (NEPA)

Occupational Safety and Health Act of 1970 - Federal Register April 12, 1984 (50FR 14468) (29 USC et seq.)

Animal Welfare Act. 7 USC 2131-2155; 80 STAT.350, 84 STAT.1560, 90 STAT.417, 99 STAT.1645.

TABLE I

Outline of Information Required by Reference Guidelines

Identification of Principal Investigator and Research Institution

Identification of Species and Source of Research Specimens

Justification for Research

Complete Description and Exact Location of Research Facility

Discussion of the Life History, Biology, Critical Environmental Factors, Ecology, Performance in Areas where Previously Introduced, Present Distribution and Status of the Study Species

Biosafety Level Based on Risk Assessment and Possible Impacts if Species Escapes or is Released

Diseases and Parasites
Identification
List of All Known Diseases and Parasites Found in Waters Where Species Were Taken
Quarantine Facilities/Procedures

Complete Description of Methods used for Physical, Biological, Chemical,and Environmental Containment and/or Scope Limitations

Fate of Surviving Specimens - Close Out Procedures

Required Permits and Related Laws and Regulations

Shipping and Transportation Precautions

Training and Qualifications of Personnel

Security

Emergency Plan and Procedures for Termination of Study

Administrative Control, Roles, Responsibilities

Frequency of Inspections, Monitoring, Compliance Evaluations and Reporting