Stakeholder Workshop
"Future Directions & Research Priorities for the
USDA Biotechnology Risk Assessment Grants Program"
Washington, DC
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Research Needs & Priorities for **Fish, Shellfish, & Insects**[1]

Our group considered the state of environmental risk assessment and risk management for genetically modified aquatic species and terrestrial insects. We considered the development of predictive models, relevant data sets, analytic frameworks, and risk management protocols. Participants offered suggestions which were discussed and ranked by votes of all present into categories of high, medium, or low priority. In general, we also agreed that there should be programmatic emphasis on multidisciplinary approaches where possible, but we also recognized that budgetary constraints would not always allow this approach. All breakout group participants felt that, especially given the concerns facing regulators over the next several years, studies focusing on issues posed by genetically modified fish, shellfish, and insects should be given higher priority in the USDA Biotechnology Risk Assessment Grants Program.

In the appendix, we present the listing of prioritized concerns as voted upon at the June 2003 workshop. We recognize the overlapping scope among certain items listed and some instances of minor inconsistencies in rankings of such items. Here, we present a synthesis based on the group's rankings shown in the appendix.

High Priority

There were three areas that received consensus (unanimous) opinion of high priority:

1) **Predictive Models**: Models are needed to predict the fate of a transgene if individuals carrying the gene construct escape or are released. In this way, regulators can predict whether a given transgene construct tends to pose high or low likelihood of spread in a receiving population, and in turn poses possible genetic or ecological harm (for example, decline in population size). It was recognized that fate of the transgene is primarily determined by a transgene's effect on fitness, and hence, studies aimed at determining transgene impacts on fitness would be critical. Fitness, in this context, is more than simple survival of the fittest, but includes all life history characteristics that influence the eventual flow of a transgene to progeny. These life history characteristics could primarily be divided into juvenile and adult viability, age at sexual maturity, mating success, fecundity and fertility. Thus, data quantifying these fitness components in order to parameterize predictive models will be critical. Finally, robustness and predictive ability of these models needs to be examined. This goal will be particularly difficult as methods need to be developed which will allow extrapolation of laboratory data to field conditions. Use of surrogate animals which mimic the effect of the transgene, such as with growth hormone implants in fish, was one suggested approach to this problem, because such fish could be released into natural environments and studied, whereas genetically modified fish could not.

2) **Impacts (harms)**: Given that transgenes can spread, what will be the demographic and ecological effects of a transgene in a particular receiving population and ecosystem? In other words, the "so what?" question should be addressed. This problem will be particularly difficult to determine in a general way, as it involves large numbers of biotic interactions, many of which will be unknown *a priori*. Nevertheless, it is important to approach this difficult question with well chosen case studies. Further, this question becomes more pressing as biotechnologists are developing genetically modified organisms intended for release into the environment. In addition, comprehensive evaluation of the "so what" question begs for a multidisciplinary approach from individuals trained in applied ecology, population genetics, and evolutionary biology.

3) **Containment (exposure)**: Preventing accidental escape of transgenic animals, or spread of their genes if they do escape, minimizes exposure and risk to a large extent. Containment can be either physical or biological. Biological containment may include such measures as sterility induced by triploidization, reproductive gene knockouts using targeted genes, redundant systems (use of multiple containment methods), and suicide genes which self destruct upon escape. Many types of biological confinement have not been fully developed and evaluated. One possible approach to biological containment, the development and use of

suicide vectors, was discussed but given a medium-low level of priority for support under this grants program. Physical confinement involves use of barriers to the escape of organisms from confinement to the environment. The efficacy of biological containment and physical confinement methods for genetically modified aquatic organisms and insects needs to be assessed.

High-Medium Priority

Decision theory: Formalized risk assessment methods based on decision theory and Bayesian methods are needed to capture and account for uncertainty in the model, parameters used in the model, and possible impacts (harms). This includes better integration of risk assessment and risk management into decision-making frameworks to support the regulatory system.

Medium Priority

Decision theory: In formalized risk assessment models, where should uncertainty factors apply, and what should those factors be?

Unintended effects: Unintended effects could occur at various levels of biological organization. For example, at the organism level, transgenes could have unintended health effects (regarding resistance to diseases and parasites), and at the community level, on demographic control factors (such as predation). At the cellular level, these may include epigenetic effects. It may be possible to detect unintended effects from genomic transcriptional analysis. Another aspect of unintended effects is the possibility that for organisms used for biocontrol, the transgene may lose its intended effect.

Containment: Even though it is yet undocumented for transgenic organisms, horizontal gene transfer is a concern as a mechanism for loss of containment and spread of the transgene. Possible mechanisms through which horizontal gene transfer could occur include direct transfer, viruses, parasites, endosymbionts, or others. Such mechanisms need further study. For example, even though it is known that the transposons used to make transgenic animals can spread between species, it has never been shown how such spread occurs and with what frequencies. An associated issue is the ecological or evolutionary significance of horizontal gene transfer. There was a wide divergence of opinion within the group on the importance of this issue.

Another containment issue is how to manage distribution of cryopreserved transgenic or otherwise modified germplasm. The underlying issue is whether distribution of such material increases the likelihood of loss of control of genetically modified material.

Medium-Low Priority

Summary: There was some interest in applying invasion ecology for data, models, etc., for purposes of modeling and testing models predicting transgene fate. There was also some concern regarding the ecological effect of removal of an introduced species i.e., a pest species from the ecosystem associated with transgene biocontrol methods. Finally, there was some interest in development of protocols for eradication of escaped transgenic animals.

<u>Appendix</u>: List of priority issues (without consideration of relative priority)

Consensus High Priority

- Descriptive and predictive models on fate of transgene
- Effect of transgene on phenotype and fitness
- Data to parameterize predictive models, especially with regard to early life history and breeding; how to validate results?
- Methods to extrapolate from laboratory data to field conditions
- Demographic and ecological effects of transgene in a receiving population and ecosystem – the "So what?" question
- Assess robustness of models and predictions over large spatio-temporal scales and different genetic backgrounds
- Assess efficacy of biological containment and physical confinement methods

HIGH-MEDIUM PRIORITY

- Formalization of mathematical models for risk assessment, including Bayesian methods
- Better integration of risk assessment and risk management into decision-making frameworks
- Development of methods for redundant layers of confinement

Medium Priority

- Where should uncertainty factors apply, and what should those factors be?
- Effect of transgene on diseases, parasites, and predators
- How frequent is horizontal gene transfer, what is its mechanism (direct, viral, parasite, or endosymbiont), and what is its significance? *Note: divergence for prioritization*
- How to manage distribution of cryopreserved transgenic – or otherwise modified – germplasm
- Epigenetic effects, transcriptional analysis; importance with regard to loss of intended effect or unintended effects

Medium-Low Priority

- Development of protocols for eradication of escaped transgenics
    - What is ecological effect of removal of an introduced species – i.e., a pest species – from the ecosystem
- Development of suicide vectors; define mobility properties of vector
- Consult invasion ecology for data, models, etc., for our purposes

Fish, shellfish, and insects should be a higher priority in the program

There should be emphasis on multidisciplinary approaches.

[1] Some of the research needs and priorities listed in this document may be outside the scope of the USDA Biotechnology Risk Assessment Grants Program. This document was prepared by one or more of the individuals listed below. USDA program staff did not edit the content of this document. The USDA Biotechnology Risk Assessment Grants Program supports risk assessment and risk management research projects regarding the safety of introducing into the environment genetically modified animals, plants, and microorganisms. More information is available at: www.reeusda.gov/crgam/biotechrisk/biotech.htm. Questions regarding the suitability of research proposals should be discussed with the Program Director (dhamernik@csrees.usda.gov).

A list of people that attended this workshop is available at: http://www.isb.vt.edu/brarg/brarg_wshop/brarg_meeting.htm. The following individuals contributed to the discussion of this topic at the workshop and/or preparation of this document after the workshop:

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