CASE STUDY No. I

GROWTH-ENHANCED SALMON

Overview

This case study concerns the potential aquaculture production or importation of Atlantic salmon (*Salmo salar* L.) genetically engineered to contain an additional fish growth hormone gene that is intended to make the Atlantic salmon grow faster and use feed more efficiently. In general, brood stocks of such fish would be raised in conventional inland hatcheries, where brood stock would be treated to produce 100% genetically female eggs. The eggs would then be treated to cause reproductive sterility (triploidy). The reproductively sterile, all-female offspring would be grown initially in hatcheries and then to maturity in ocean net pens, before being harvested for food. The ability of hatchery managers to ensure reproductive sterility is currently high but less than 100%. Therefore, escapes of fish from net pens may include some females that are capable of reproduction.

The case study is prospective in nature, and is generalized to encompass more than one type of genetic modification. The genetic engineering causes the salmon to contain a new animal drug, which is subject to regulation by the Food and Drug Administration (FDA). Other agencies, e.g., the National Marine Fisheries Service (NMFS), Fish and Wildlife Service (FWS), Army Corps of Engineers (ACE), and Environmental Protection Agency (EPA), would be involved in regulating the actual locations and facilities for use of the salmon in aquaculture in the U.S. During the development of this case study, Atlantic salmon population segments were listed in Maine were listed under the Endangered Species Act (ESA), 16 U.S.C. § § 1531-1544, as amended by the ESA Amendments of 1978, Pub.L. 95-632 (1978) and the ESA Amendments of 1982, Pub.L. 97-304 (1982), by the FWS and NMFS. Because this is one of a series of case studies aimed at elucidating the adequacy of federal environmental regulations pertaining to transgenic organisms, more detail is provided on the FDA regulatory process.

1. Description of proposed organism/product and its use (what, where, how much, and when)

Objectives of this case study

This case study focuses on environmental oversight of the potential production of transgenic Atlantic salmon in net pens or other ostensibly-contained conditions in or near the Atlantic or Pacific coastal waters of the United States, including tank rearing and hatchery operations associated with aquaculture production. The intent of the genetic modification is to produce a variety of salmon that grows faster and uses feed more efficiently. Transgenic Atlantic salmon that are currently being developed are contained in land-locked research facilities outside of the United States. To date, FDA is aware of

no evidence that transgenic Atlantic salmon of any type have been used in commercial fish farming or have been marketed for human consumption in the U.S. There also has not been any complete application submitted to FDA for use of a transgenic fish.

This case study is not meant to apply to only one genetic construct or one variety of transgenic Atlantic salmon derived from that construct. The case study is aimed at illustrating the types of environmental safety considerations that would go into a U.S. government evaluation of a request for approval of a transgenic Atlantic salmon variety for use in aquaculture, and the government agencies and authorities involved. This case study is intended to give an overview of the federal oversight process, to point out any gaps, weaknesses, or ambiguities in that process, and to facilitate improvements in it. It is not intended to be an environmental risk assessment for transgenic Atlantic salmon in net pen aquaculture. It also does not encompass the types of environmental safety considerations that would go into a U.S. government evaluation of a request for approval of other possible uses of transgenic Atlantic salmon, for example of ocean ranching (release, return and re-capture strategies) or stocking in the open environment.

Because this is one of a series of case studies aimed at elucidating the adequacy of federal oversight of environmental risks posed by bioengineered organisms, this case study does not specifically examine food safety issues. Evaluation of food safety is, of course, an important component of the FDA approval process for transgenic food animals, such as the Atlantic salmon described in this case study.

Characteristics of the case study

Transgenic fish are fish that have been modified to contain copies of new genetic constructs introduced into their genome by modern genetic techniques (specifically, recombinant DNA techniques). The constructs consist of structural gene(s) (DNA sequences encoding a specific protein product) linked to regulatory sequence(s) (DNA sequences, e.g., a promoter, necessary for successful expression of the structural gene(s)) (Kapuscinski and Hallerman, 1991). This case study focuses on transgenic Atlantic salmon engineered to grow faster and use feed more efficiently. Such fish may be expected to contain at least one introduced structural gene for growth hormone and one introduced regulatory sequence for the control and expression of the introduced structural gene, thereby eliciting the phenotype of enhanced growth rate and feed efficiency.

The best known example of such a transgenic Atlantic salmon under investigation is the AquAdvantage variety being developed by Aqua Bounty. The AquaAdvantage gene construct uses a Chinook salmon growth hormone gene and a promoter sequence derived from another fish, called an ocean pout (C.L. Hew, G.L. Fletcher and P.L. Davies, 1995; S.J. Du et al, 1992a, 1992b). The AquAdvantage construct has been inserted into Atlantic salmon of Canadian origin. However, many constructs are possible, including constructs that contain genetic codes for human growth hormone or the growth hormone found in other animals, for example bovine somatotropin, because many growth hormones are active in Atlantic salmon and other fish (R.H. Devlin, 1997).

If the modifications work as hoped, fish farmers would find the transgenic salmon more economical to rear for sale as food than other kinds of salmon. Each variety of transgenic salmon would be descended from one transgene integration event in a newly fertilized, undivided egg. The transgene would be inherited by the offspring of reproductively capable transgenic salmon. Back-crossing (i.e., repeated inbreeding and selection) would be performed to stabilize the genetic modification so that subsequent generations would retain the genetic construct and exhibit the same accelerated growth rate. The transgenic salmon would be raised as diploid animals (i.e., animals with two sets of chromosomes, one set from each of its two parents, and thus capable of sexual reproduction) in conventional inland salmon hatcheries to serve as broodstock (parents) of the fish that would ultimately be used in food production. It is expected that the fish to be used in food production would be sterile females raised in ocean net pens.

Approximately 1.5 million tons of wild and farmed salmon are harvested each year (United Nations Food and Agriculture Organization (FAO), 1996). The U.S. accounts for approximately 500,000 tons, of which 85-90% is wild caught salmon, principally Pacific salmon species (Productivity Commission, 1997). In recent years in the U.S., the wild catch has remained stable or decreased slightly, while the amount of farmed fish, predominantly Atlantic salmon, has increased. Norway, Chile and Scotland are the major producers of farmed Atlantic salmon, jointly accounting for over 80 per cent of world supply of Atlantic salmon. Canada is also a significant producer. In the U.S., farmed Atlantic salmon are produced in northern waters on both the East and West Coast.

Despite harvesting a significant amount of wild salmon and raising increasing amounts of farmed fish, the U.S. remains a large importer of salmon. In 1998, the U.S. imported most of its farmed salmon from Canada and Chile (Price Waterhouse Coopers, 1998). The total market value of imported, farmed salmon was approximately $512 million.

Because the U.S. is a major importer of farmed salmon, the developers of transgenic salmon generally want U.S. approval of these products for human food safety. This could either come in the form of an approved new animal drug application (which allows commercial use of the transgenic animal inside the U.S.), or an import tolerance for an unapproved new animal drug's residues in imported seafood (imported food products only, no U.S. commercial production allowed). Culture locations for transgenic salmon are likely to exist both within and outside the U.S., as well as in areas of shared coastal waters, such as the Bay of Fundy on the United States and Canadian borders. As for all Atlantic salmon, culture locations for transgenic Atlantic salmon are subject to approval by NMFS, FWS, ACE, and/or EPA. The EPA is reviewing the impacts to water quality associated with aquaculture. The outcome of that review may be specific standards on discharges.

How would the transgenic fish be used, including a brief description of management practices that would be associated with it?

Management systems used for production of transgenic Atlantic salmon are likely to be the same as, or a subset of, those currently in use for non-transgenic salmon. Typically, salmon are hatched in freshwater facilities. After 12–18 months the young salmon undergo smoltification (acclimation to salt water), after which they can survive in a marine environment. These fish are then called smolts and are transferred to sea farms where they are grown in sea cages, also referred to as net pens, located in estuaries, coastal inlets, and open ocean. Additionally, Atlantic salmon can be intensively reared in raceways and circular tanks, although the economic viability of such systems for food production has not been demonstrated. Heen, Monahan and Utter (1993) contains a good overview of Atlantic salmon management in aquaculture settings, including nutrition, net pen construction, disease management, and genetic and environmental issues.

The use of only sterile female salmon has been suggested as a means to minimize environmental impacts resulting from any escapes of transgenic salmon from net pens. Technology is available for producing all-female salmon (Bye and Lincoln, 1986). It involves masculinizing females with hormones to allow the reliable production of fertile eggs that produce all-female offspring. All-female eggs can be treated with temperature and pressure to yield triploid sterile offspring (offspring with three sets chromosomes and incapable of sexual reproduction). In contrast to the reliability of producing all-female offspring, the efficiency of the induction of triploidy varies from fish species to species and with the personnel conducting the work. Sponsors using this technology as a biocontainment mitigation would be expected to provide information as to the efficiency of their induction procedures and the measures they would use to maintain that efficiency. Triploid, all-female eggs, fry and fingerlings would then be sold or contracted out to fish farmers to grow out to market size for food, in net pens or other facilities.

Is there prior experience dealing with the same varieties not genetically engineered?

Wild Atlantic salmon have been harvested as food animals for millennia and farmed Atlantic salmon have been produced for many years. Captive Atlantic salmon have been used as broodstock and selective breeding programs, over many generations, have resulted in some limited improvements in growth rate, meat quality and disease resistance. Currently, net pen aquaculture of Atlantic salmon involves several generations of breeding for net pen conditions from a stock that often includes hybrids of European origin. These stocks have been preferred to natives because they are perceived to be more productive under the stresses of net pen aquaculture. Whether it is more protective of native salmon populations for humans to use stocks in aquaculture that are more or less genetically similar to co-existing wild populations is currently the topic of debate, and will be discussed under environmental risks.

Traditional breeding practices involved selecting individuals on the basis of the trait as measured in that individual or its offspring and breeding the best ones to each other. Now it is possible to use molecular markers that are highly correlated with the desired trait to select the best more quickly and with greater accuracy than growing them to adulthood and measuring them. Even so, the selected individuals need to be crossed to each other to the point where the gene or genes involved are stably inherited. With fish,

the fastest and most precise way to make progress once the sequence of a desirable gene has been identified is to engineer it into an otherwise highly desirable stock. Once a particular combination succeeds, it can be multiplied through backcrossing and selection.

There is already a precedent for the production of Atlantic salmon in non-native environments, e.g. farmed (non-transgenic) Atlantic salmon operations in the Puget Sound and in the Pacific Ocean off the coast of Washington State, British Columbia, Canada, and Chile. The introduction of wild, reproducing populations of Atlantic salmon into the northwestern U.S. was attempted in the early part of the last century without success, and part of the rationale for using Atlantic salmon for aquaculture in that area was the failure of the species to establish there. Recently, there has been reported evidence of the first successful spawning of Atlantic salmon that escaped from net pen aquaculture in rivers in British Columbia (Rimmer, 1998; Volpe et al, 1999). However, U.S. and B.C. fisheries authorities still do not consider them "established", i.e., to be self-sustaining over the long term. Fleming et al. (2000) recently reported evidence of resource competition and competitive displacement of native salmon by farmed salmon intentionally released into a Norwegian river, although the reproductive success of the farmed fish was substantially lower than for native salmon.

While cultured Atlantic salmon might not have established themselves on the Pacific coast yet, the successful spawning of Atlantic salmon on the Pacific coast has raised concern that these fish may further jeopardize the continued existence of already fragile native Pacific salmonids through competition for food and occupation of underutilized habitat. Many of the Pacific salmon stocks have already been listed under the ESA.

Until the listing of Atlantic salmon under the ESA, the main federal regulation of net pen aquaculture associated with the production of Atlantic salmon has been through ACE permits issued for compliance with Section 10 of the Rivers and Harbors Act of 1899. Currently, applicants seeking permission to culture Atlantic salmon in state waters or waters of the US must obtain permits from both States (if in State waters) and the Federal Government (out to the edge of the Continental Shelf). Depending on the nature of the retention system, the State and Army Corps of Engineers will act as lead agencies in evaluating proposals and issuing appropriate water quality and structures permits.

The permit application must include a description of the purpose, proposed activities, location, character of the area and potential conflicting uses. The federal review process entails evaluations by the EPA, FWS and NMFS. Authority for involvement by the resource agencies is found in statutes such as the Fish and Wildlife Coordination Act (FWCA), ESA, Marine Mammal Protection Act (MMPA), 16 U.S.C. §§ 1361-1421, Clean Water Act (CWA), 33 U.S.C. §§ 1251-1387, and the National Environmental Policy Act (NEPA), 42 U.S.C. §§ 4321-4370e. Issuance of permits depends on a number of considerations usually defined as the "Public Interest Review" by the Corps of Engineers. The Public Interest Review normally includes issuance of a Public Notice regarding the proposed action. The Public Notice includes much of the information in the permit application as noted above.

Escape was not originally considered to be an important consequence of pen culturing Atlantic salmon. Initial information indicated that escapes would be minimal in number and the individuals not able to successfully compete with native stocks or form viable populations. When it was discovered that escapees could survive in the wild, some people believed that the fish would not successfully reproduce. It now appears that escaped fish can reproduce. NMFS is evaluating the consequences of escape and considering what measures might limit the ecological impact of escape. A risk management step being considered is to require the use of local, native strains as broodstock. Environmental impacts associated with unforeseen situations, such as escape, are normally covered by permit modification, suspension or revocation, when it is determined that such situations represent undesirable circumstances.

What are the projected locations and extent of production, use and disposal?

In the U.S., the principal locations for salmon culture are the northern waters of the East and West Coasts adjacent to similar fisheries in the coastal waters of Canada. Primary production is in the states of Washington and Maine where suitable habitat in the form of cold marine waters exists. In the U.S., net pen salmon production in 1997 was 33 million pounds, amounting to $75 million. These values have steadily increased since 1985 and are likely to continue to increase, based upon market demand (USDA, 1999). Atlantic salmon is a premium salmon product sold chilled, frozen or smoked.

Disposals resulting from the production, processing and consumption of salmon would be essentially the same for all salmon, transgenic or not. They would consist of disposals of waste material during production of feed and aquaculture of fish, during processing, and after consumption.

What types of adverse effects might be caused by the transgenic fish throughout its life cycle, and where might they occur?

Many of the potential adverse environmental effects that have been hypothesized for transgenic Atlantic salmon are similar to those associated with currently used farmed strains of Atlantic salmon. The potential for adverse effects is partly a function of the management systems employed for their production.

Adverse effects resulting from Atlantic salmon culture are associated with their exposure to the environment through the hatchery or the net pen and include:

1. Through escape:
    - Interbreeding with wild Atlantic salmon and gene introgression into wild salmon stocks;
    - Hybridization with brown trout (Atlantic salmon are more closely related to brown trout, a European species that has been stocked in North America, than to the various Pacific salmon species, which are close relatives of rainbow trout);

- Disturbance of habitat or displacement of wild stocks as a consequence of competition for resources, predation, or mis-matings.

2. Fouling of the hatchery effluent receiving waters and the seabed below net pens with fecal material and excess feed.
3. Spread of bacteria, viruses, and parasites such as Infectious Salmon Anemia and sea lice to wild salmon or other fauna.
4. Introduction of chemicals, e.g. those used in the treatment of fish diseases.

Currently, technologies to mitigate some of these effects, such as reducing the number of escaped fish, and increasing the effectiveness of sterility inducement, are under development. At the time of the environmental review of an application, the current status of the scientific information and technology would be assessed. Some or all of the above issues associated with the rearing and release or escape of non-engineered farm-raised Atlantic salmon, presumably would also apply to transgenic Atlantic salmon. Transgenic fishes may cause a greater or lesser magnitude of impact compared to fishes whose endogenous genes have been simply recombined through artificial selection, hybridization of closely related species, or ploidy manipulations, depending on several factors. These and similar issues are discussed in more detail in part 3, below.

What are the pathways for proliferation of those risks?

Proliferation of risk associated with gene introgression from transgenic and non-transgenic non-indigenous fish:

The amount of risk associated with gene introgression is a function of the scope of the release, the number of escaped animals and the number of potentially affected native species, the precise characteristics of the transgenic fish, and the interrelation of at least four population variables: reproductive potential of escaped individuals, frequency of introgression of the modified genes, fitness of the introgressed individuals, and potential demographic decline due to genetic load of introgressed genes.

The reproductive potential of escaped individuals is based on: (1) the survival rate and fertility of the individuals, and (2) environmental conditions affecting reproduction in the affected ecosystem, such as length of spawning season and available spawning habitat. The frequency with which introgressed genes will spread and increase within the population is related to gene flow. Several models are available to estimate this variable. Despite the prediction that introgressed individuals will exhibit lower fitness than non-introgressed individuals, not all genetic modifications will be maladaptive. Regarding the genetic load of introgressed genes, natural selection is expected to remove maladaptive genes from a population. However, depending on the severity of the maladaptation, the number of generations required for this process can be very large (USDA, 1995).

Risk of adverse events associated with introduction of triploid (both transgenic and non-transgenic) fish:

The sterility offered by inducing triploidy in some aquatic species reduces some concerns about a modified organism, and in many cases will mean that farming of a triploid transgenic species will likely pose less risk of environmental impact than similar farming of fertile non-transgenic species. Of course, to the extent that non-transgenic salmon are also made triploid prior to use for fish farming, they would obtain comparable benefits with regard to reduction in environmental, including genetic, risk. However, use of triploidy is not favored by fish farmers in currently-used Atlantic salmon stocks, as it is thought to reduce productivity and resistance to stress. Transgenic salmon, on the other hand, do not show reduced productivity when they are triploid. In addition, because of the likely enhanced productivity of the transgenic fish, small relative reductions in productivity may be more acceptable in transgenic fish than in non-transgenic fish.

However, the use of triploidy does not eliminate all environmental risk, and its ability to ensure environmental safety is complicated by three factors. First, the effectiveness of triploidy induction varies among species and the methods used. Second, although triploids are functionally sterile, the males may exhibit spawning behavior with fertile diploid females, leading to decreased reproductive success of the fertile diploid females. Third, in cases where large numbers of individuals are released, sufficient numbers of sterile triploids may survive and grow to pose heightened competition with diploid conspecifics (i.e., fish of the same species), perhaps including in some cases, predation on juvenile conspecifics (USDA, 1995).

Risk of adverse events associated with unexpected survival and persistence of escaped or intentionally released transgenic and non-native non-transgenic fish:

Despite familiarity with the unmodified Atlantic salmon, there remains some undefined degree of risk of adverse impacts associated with the unexpected survival and persistence of escaped or intentionally released transgenic and non-native (non-transgenic) fish. For example, experiences with releases of a different unmodified salmonid species, the pink salmon, suggest that genetically modified pink salmon could also survive, reproduce, and persist in a broader range of accessible ecosystems than would be expected from studies of their biology in their native range. In spite of assumptions that smolts and immature adults could not survive in fresh water, the Laurentian Great Lakes experienced population explosions of pink salmon two decades after 21,000 juveniles were flushed down the drain of a Lake Superior hatchery (United State Department of Agriculture (USDA), 1995)

What types of positive environmental impacts might occur because of this use?

If the fish can be shown to be sterile and remain that way throughout the culturing procedures, use of sterile triploid transgenic fish in conventional net pens could reduce the amount of gene introgression into wild stock that may currently be occurring as a result of escape by fertile, non-indigenous (imported) stocks that are presently being used for culture/breeding. Triploidy is available as an option for non-transgenic salmon, but

there has been resistance to its acceptance by fish farmers because they are perceived to have depressed productivity, as described above. However, the use of sterile transgenic fish in aquaculture might stimulate interest in research and a re-evaluation of this technique for use with non-transgenic salmon, which would be a benefit.

Decreased harvest pressure on wild salmon fisheries could result from increased production of highly feed-efficient farmed transgenic varieties. Since, however, there is no current recreational or commercial harvest of Atlantic salmon, this effect would not have an impact on the environment of the east coast of the U.S. It might lead to reduced demand for sustainably managed wild populations in the Pacific Northwest and Alaska and potentially have economic impacts there. Similarly, reduced pressure for use of marginal net pen culture sites could result from increased productivity in more optimal sites. However, such reduced use of marginal sites is also a function of market saturation and other economic forces. It also remains to be seen whether transgenic fish will be accepted and used commercially so as to enable evaluation of the extent that such potential benefits may be realized.

Finally, the increased production potential with transgenic fish may allow the use of land-based contained facilities to become economically viable. If contained facilities were to be used, many of the environmental issues discussed above would not be relevant.

What is the rationale for using the transgenic fish, including its advantages vis-a-vis alternatives?

If the research goes as planned, the transgenic Atlantic salmon would exhibit an accelerated rate of growth related to the expression of the added growth hormone gene construct. The improvement may be dramatic, but is expected to vary among transgenic varieties. One variety being developed is purported to reach market-weight (3-4 kg) in about 18 months, versus 24-30 months for non-transgenic salmon. Because there would be less time required to reach market weight, there presumably would be less feed required for maintenance metabolism. Thus, the transgenic variety would be expected to use feed more efficiently. In other words, less feed would be required to produce a unit of salmon meat for human consumption, compared to non-transgenic varieties. Early indications are that despite the acceleration in the growth rate over the first 18 months, the transgenic Atlantic salmon do not appear to exceed the normal weight range of adult non-transgenic salmon, although this also may vary from one transgenic variety to another.

Economic benefits of such modifications would include increases in the number of culture cycles per time at a given location, and a reduction in the amount of resources (e.g., feed used, waste produced, and space required per pound of food for humans produced) required for rearing the fish over time. Higher feed efficiency would decrease the cost of feed per unit of food produced for humans (i.e., fish meat), resulting in decreased cost of the marketed product.

2. Relevant regulatory agencies, regulatory authority and legal measures

Contained research

The National Institutes of Health (NIH) rDNA Guidelines (http://www4.od.nih.gov/oba/oct2000guide2.pdf) apply to research that is conducted at or sponsored by an institution that receives any support for recombinant DNA research from NIH, including research performed directly by NIH. NIH funding for recombinant DNA research at the institution at which the research is conducted is therefore the primary indicator as to whether a research project is covered by these guidelines.

The fundamental aspect of these guidelines is that they rate different kinds of rDNA research by the relative risks, and they determine the practices needed to safely contain the research at each stage (laboratories, and greenhouses for plants, arthropods and microorganisms, and animal rooms or securely fenced areas for animals) (Appendices P and Q, id.). Of key importance to the efficacy of these guidelines are the roles of the Institutional Biosafety Committee (IBC), the Biological Safety Officer, and the Plant, Plant Pathogen, or Plant Pest Containment Expert and the Animal Containment Expert (Section IV, ibid). Although the guidelines are voluntary for other federal agencies, the USDA Agricultural Research Service, for example, uses the IBC of the collaborating or nearby university in implementing the guidelines.

Compliance with these guidelines is monitored by a reporting process whereby any individual can present a claim of noncompliance to both the NIH/OBA and the relevant institution's IBC. If NIH or non NIH funded projects at a given institution are not in compliance, this can result in: 1) suspension, limitation or termination of NIH funds for recombinant DNA research at the institution, or 2) a requirement for prior NIH approval of any or all recombinant DNA projects at the institution. (Section I-D, ibid). If private individuals or organizations choose to use these guidelines and affiliate with an institution with an approved IBC, there are opportunities for protection of proprietary data as described in IV-D-5. To restate, the NIH guidelines are voluntary for those institutions, private organizations, and individuals that do not receive funds from NIH for recombinant DNA research. This includes other federal agencies.

Fish and Shellfish Research Performance Standards

With input from a wide range of aquatic science professionals, a U.S. Department of Agriculture-sanctioned working group developed the Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish as a tool for risk assessment and risk management. The Performance Standards were approved in 1995 by the Agricultural Biotechnology Research Advisory Committee of the USDA. These standards have been distributed widely as a two booklet set and are expected to guide evaluations of the performance and environmental safety of aquatic Genetically Modified Organisms (GMOs) in the United States and abroad. To facilitate use of the Performance Standards, a computer-based decision-support tool has been developed.

These are available on the web at http://www.nbiap.vt.edu, and select risk assessment and then Performance Standards for Fish and Shellfish.

These were established as voluntary standards. However, under Cooperative State Research Education and Extension Service (CSREES) (USDA) NEPA implementation, researchers can indicate they have utilized these standards or others. Most of the transgenic fish research funded through USDA is in contained indoor biosecure facilities. Auburn has the only pond system that has been approved by USDA for such work. These standards have been successful at raising the awareness for a variety of issues that must be considered when conducting this work.

USDA also developed an assessment of a research program, the Environmental Assessment and Finding of No Significant Impact relating to a USDA funded research program on transgenic carp (55 Fed. Reg.46661). These are believed to be the first federal NEPA documents to address environmental impacts of transgenic fish.

Authorities outside of contained facilities

Atlantic salmon farming is subject to a number of federal and state environmental controls that apply whether or not the fish being farmed are transgenic. Coastal zone management authorities in the states, the ACE, FWS, and the NMFS all are involved with site selection and permitting of net pens and hatcheries. EPA and the states enforce the CWA, regulating the potential harm that may be caused by fish wastes and disposal of new animal drugs used on fish. FDA evaluates the environmental impact of new [1]animal drugs used in fish farms, including new animal drugs contained in transgenic fish.

Several federal agencies manage the physical and social consequences of actions that encroach into public trust resources. The ACE is typically the lead Federal Agency for aquaculture projects in navigable waters of the U.S. The EPA becomes involved with discharges (National Pollution Discharge Elimination System (NPDES) permits) and has done so to varying degrees across the nation (their aquaculture management activities are now under internal review, as noted above). The U.S. Coast Guard is involved when aquaculture may affect navigation safety. For example, the Coast Guard will provide guidance on lighting or marking culture structures. The Minerals Management Service manages use of the seafloor. Requests for competing use of the seafloor/water column have not occurred but would require resolution. In the waters of the Gulf of Mexico, use of offshore petroleum production platforms is being pursued as the mooring system (the legs) for aquaculture activities. (In state waters there are counterpart elements of each of these agencies within the state government. Under the Coastal Zone Management Act (CZMA), 16 U.S.C. § § 1451-1465, States require that any federal action that can affect the State must show that the federal action is consistent with the State Coastal Zone Plan. The showing is termed a "Coastal Consistency." Additionally, EPA has the authority to delegate its water quality responsibilities to individual states. Forty-three states have

[1] ("New" with reference to animal drugs is a statutory term (21 U.S.C. § 321 (v)) that applies essentially to all animal drugs)

received EPA authority to manage the NPDES/SPDES waste discharge-permitting program.)

FWS and NMFS are routinely considered the "resource agencies" and are called upon to speak for and about fish and other aquatic resources in regulatory situations. For aquatic species, NMFS has primary purview in marine waters, and FWS in fresh water environments. These agencies are "consultants" to all federal agencies operating under a broad spectrum of federal legislation. The broadest intervention tool provided by federal legislation is the Fish & Wildlife Coordination Act. The ESA comes into play whenever listed species are suspected to occur within the impact area. The native Atlantic salmon population in Maine (see news release on the listing on the FWS website has been listed, jointly by FWS and NMFS as an endangered species under the ESA (listing of Distinct Population of Anadramous Atlantic salmon in the Gulf of Maine on November 17, 2000 (65 Fed. Reg. 69459). The last remaining wild stocks are co-managed by the FWS and the NMFS through the North Atlantic Salmon Conservation Organization (NASCO) established in 1984 under the Convention for the Conservation of Salmon in the North Atlantic Ocean. NASCO is an international body with the objective of contributing through consultation and cooperation to the conservation, restoration, enhancement and rational management of salmon stocks taking into account the best scientific information available. The Magnuson – Stevens Fishery Conservation and Management Act, as amended by the 1996 Sustainable Fisheries Act (Magnuson-Stevens Act), 16 U.S.C. § § 1801-1883, is invoked by NMFS when designated Essential Fish Habitat is present. Activities that might adversely affect those habitats must be assessed and measures taken to avoid, minimize, mitigate or compensate for such impacts. Failing that, the lead federal agency must explain why such measures will not be taken.

Most of the regulatory agencies noted above have integrated responsibilities, occasionally supplemented with Memoranda of Understanding or Agreement. They have responsibility for management and control of aquaculture to insure compatibility with wild fish management and their associated habitat. Prior experiences with environmental problems associated with aquaculture, coupled with pressures for environmental protection by various citizens groups, have heightened these agencies' concerns about, and requirements for, new uses of fish in aquaculture. The Department of Commerce (DOC) sees aquaculture as an important opportunity for the U.S. DOC recently issued an aquaculture policy (signed by Secretary Daley in 2000) that specifically targets aquaculture development, including support for new technologies and the domestication of additional species for aquaculture production in an environmentally sound manner. The Commerce goal is a $5 billion U.S. aquaculture industry by 2025 (a 5-fold increase from today).

There are a number of examples of guidance documents dealing with fish in aquaculture, including transgenic fish. NASCO, mentioned above, is the most applicable to this case study. It has published a thorough discussion of the genetic issues and potential solutions. NMFS and FWS are actively involved in the NASCO activities. The United Nation's FAO has developed a fisheries Code of Conduct (FAO, 1995). Article 9 of the document addresses aquaculture issues, including genetics. NMFS is using that

document to facilitate development of a Code of Conduct for aquaculture activities in the U.S. Exclusive Economic Zone (those waters outside State waters and extending seaward 200 miles).

In most cases the applicant for an aquaculture site permit bears the responsibility of presenting evidence of environmental compatibility, limited risk and minimal conflict with other activities or uses of the proposed culturing site. NEPA applies to major federal actions, and the lead federal agency has the responsibility for preparing the NEPA analysis of significant environmental impacts, such as those that may be caused by granting a permit for an aquaculture site. However, the applicant routinely prepares much if not all the technical information for the Environmental Assessment (EA). This is done in cooperation with the lead federal agency.

APHIS coordinates state permit programs that control interstate movement of potentially diseased or parasitized fish and shellfish. See the APHIS website for a collection of state requirements (USDA, 2000). APHIS has not so far considered fish, fish eggs, and fish gametes to be "livestock" under the Animal Quarantine Laws, 21 U.S.C. §§ 101-135. If APHIS determined that the interstate movement of Atlantic salmon needed to be controlled more actively to prevent the spread of disease, it could change the status of this species to livestock under its regulations (9 CFR 49-99), and require health certification as applied to other livestock. In this event, these same authorities would be used to provide for health certification of live transgenic Atlantic salmon intended for import into or export from the United States.

In addition to other federal, state and local oversight that pertains in general to use of Atlantic salmon for fish farming, transgenic Atlantic salmon are subject to FDA oversight because they are considered to contain a "new animal drug."[2] The Federal Food, Drug, and Cosmetic Act (FFDCA), 21 U.S.C. §§ 371-379d, defines a "drug" to include "articles … intended to affect the structure or any function of the body of man or other animals." 21 U.S.C. § 321(g). Because an introduced genetic construct will of necessity "affect the structure or . . . function" of transgenic animals, the genetic construct is a "drug." The genetic construct may also produce a protein that is a drug. Where the genetic material and the protein (when the protein is a drug) are not "generally recognized . . . as safe and effective for use under the conditions prescribed, recommended, or suggested in the labeling thereof", they are "new animal drugs." 21 U.S.C. § 321(v). ("New" is a statutory term (21 U.S.C. § 321(p)) that applies essentially to all animal drugs.)

Use of a new animal drug is considered "unsafe" under the FFDCA unless the FDA has approved an application for that particular use. 21 U.S.C. § 360b(a)(1). Thus, if the introduced genetic construct and, potentially, the protein it produces (the "articles") meet the definition of a new animal drug and were not approved by the FDA, they would be "unsafe" and subject to FDA enforcement action. The transgenic salmon's structure

[2] Fish modified to contain or produce a veterinary biologic would be subject to regulation by APHIS under the Virus-Serum-Toxin Act (VSTA), 21 U.S.C. §§ 151-159, rather than by FDA under the FFDCA. 21 U.S.C. § 902(c).

and function have been modified through insertion of the genetic construct into the genome of the salmon and the transgenic salmon therefore contains a new animal drug. In the transgenic salmon at issue, the growth hormone protein encoded by the inserted genetic construct also affects the structure and function of the salmon, and so also would be a new animal drug.

All subsequent generations of the salmon contain the inserted genetic construct and growth hormone protein, and therefore all contain a new animal drug. FDA approval of a new animal drug contained in transgenic fish would be specific to the use of the drug in the line(s) of salmon descended from the original transformation or microinjection event. Thus, FDA will evaluate the new animal drug and its intended use in the context of the fish line into which the drug has been engineered. Any conditions that FDA imposes on the new animal drug's use will apply to all fish derived from that original transgenic line.

A new animal drug enters the FDA regulatory process when the sponsor submits a Notice of Claimed Investigational Exemption (referred to as an investigational new animal drug, or INAD), before shipping the drug for clinical (effectiveness) tests in animals. 21 CFR 511.1(b)(4). Ordinarily, the agency is not permitted to disclose the existence of an INAD, unless the sponsor has publicly disclosed it. 21 CFR 514.12. For example, this case study notes that Aqua Bounty has filed an INAD for a transgenic Atlantic salmon because Aqua Bounty has previously disclosed this fact. The sponsor conducts research on the transgenic fish while the INAD is in effect. When completed, the research can become the basis of a new animal drug application (NADA). 21 U.S.C. § 360b(b)(1). FDA evaluates the NADA to determine whether the sponsor has demonstrated that the drug is safe and effective for its intended use. The burden of proving that the drug meets this standard is entirely on the sponsor.

Under the FFDCA, a new animal drug's safety is defined as having "reference to the health of man or animal." 21 U.S.C. § 321(u). The agency considers, as part of its safety assessment of the drug contained in a transgenic fish (or any other new animal drug), environmental effects that directly or indirectly affect the health of humans or animals as a result of FDA's allowing the new animal drug's "use." Only in the case of a potential adverse environmental effect that would not, directly or indirectly, pose a risk to the health of man or animals, for example an environmental impact that would detract from scenic beauty, would FDA not have authority to take such risk into account as part of its FFDCA safety assessment of a new animal drug.

Because granting an INAD and approving an NADA are federal actions under NEPA, the agency must comply with NEPA as it carries out these processes. INADs and NADAs require submission of a claim of categorical exclusion or an environmental assessment (EA). 21 C.F.R. 25.15, 21 C.F.R. 511.1(b)(10), 21 C.F.R. 514.1(b)(10). For transgenic fish, the EA will facilitate the environmental component of FDA's "safety" review under the FFDCA by providing information relevant to determining whether environmental consequences resulting from use of the new animal drug could adversely affect the health of humans or animals and possibly render the drug unsafe.

FDA conducts its environmental safety reviews for animal drug products under the broad umbrella of NEPA. NEPA provides a structure for environmental assessment that is well known as well as providing a mechanism for coordination with other Federal agencies. FDA relies on its authority under the FFDCA to require, where appropriate, environmental safety instructions on product labels, to enforce compliance with mitigations that are required as a condition of the product approval, and to refuse to approve or to withdraw approval of products that cause unexpected and unmitigatable environmental impacts that adversely affect, directly or indirectly, the health of humans or animals. Like all federal agencies, FDA must also comply with the ESA.

For example, in the pre-market environmental assessment of bovine somatotropin for dairy cows, FDA's Center for Veterinary Medicine (CVM) and the product sponsor considered among other things, the possibility that approval of the drug (1) might affect land-use patterns and water quality by affecting the types of feed ingredients grown for dairy cows, (2) might affect carbon dioxide emissions due to changed ration requirements and dairy populations, and (3) might present a used syringe disposal problem.

The first two areas did not prove to be significant. Because of concern about the risk to human health from used syringes, FDA required mitigation of the third area by an applicant-sponsored syringe collection system for customers. Had either of the first two issues proven to be significant, CVM was prepared to consider mitigations and/or refusal to approve the product because of the human and animal health impact of changes in water quality and carbon dioxide emissions. See Finding of No Significant and Environmental Assessment Impact for Sterile Sometribove Zinc Suspension for Use in Lactating Dairy Cows, NADA 140-872, May 7, 1993 (FDA, 1993), available on the CVM web site. This document also shows the scope and depth of studies that FDA required the applicants to conduct in order to assess potential environmental impacts. Such studies might be equivalent to the type of documents the sponsors of genetically engineered salmon will have to develop. The document is also an example of FDA reviews of the above information for quality and accuracy, and the agency's rationale for the decision of Finding of No Significant Impact in the case of bovine somatotropin.

For transgenic Atlantic salmon of the type being discussed in this case study, CVM plans to address the environmental assessment through the use of risk assessment approach, as described below in Section 3. This is an efficient approach, currently in use for preparing EAs for other new animal drug products. It is designed to identify likely hazards and acquire the information necessary to assess the level of risk and manage those that are significant, while at the same time reducing the burden on applicants. Unforeseen or low probability hazards are managed through post-approval monitoring by the applicant and FDA, including evaluation of new hazards that appear through that monitoring.

FDA expects the applicant to work with the scientific community to identify the reasonably anticipated hazards and either: (1) design a scientifically sound method for examining their likelihood and severity and design measures that will be taken to reduce

the severity of a low probability event, or (2) design procedures that will avoid the hazard altogether. If an NADA for a transgenic Atlantic salmon is approved, the assessment, monitoring plans, and mitigations will be available for public review at the time of approval.

FDA intends to publish draft guidance on how the new animal drug provisions of the FFDCA pertain to transgenic animals, and on procedures by which companies developing transgenic animals can comply with those provisions. FDA also intends to hold workshops or public meetings to discuss scientific issues posed by particular kinds or uses of transgenic animals (such as transgenic salmon described in this case study) and at a later date to develop draft guidances on specific scientific issues raised by particular kinds or uses of transgenic animals.

One of the goals that NEPA is intended to achieve is a public airing of an agency's consideration of significant environmental impacts posed by a prospective agency action. 42 U.S.C. §4341. At the same time, the FFDCA and the Trade Secrets Act prohibit revealing any information that is acquired as part of the new animal drug approval process and that is entitled to protection as a trade secret. 21 U.S.C. § 331(j), 18 U.S.C. § 1905. CEQ's regulations state that an agency shall comply with NEPA to the fullest extent possible unless existing law applicable to the agency's operations expressly prohibits or makes compliance impossible. 40 C.F.R. 1500.6. Under FDA's current regulations, even if the existence of an INAD or an unapproved NADA has been publicly disclosed or acknowledged, no data or information contained in that INAD or NADA are available for public disclosure before an approval has been published in the Federal Register. 21 C.F.R. 514.11(d). Thus, the agency would be precluded from making a NEPA analysis public prior to approval of an NADA because the NEPA analysis is considered part of the INAD or NADA. The agency recognizes the difficulty this poses in ensuring a public process for evaluating possible environmental risks associated with any particular transgenic modification to a fish species and is considering what options it might have to address this situation.

In any case, FDA intends to publish for comment a draft guidance document describing its approach to conducting environmental assessments of the genetic construct contained in transgenic salmon, and will involve both the public and state and federal government entities in the process of developing this guidance. The draft guidance document will describe what issues sponsors should address in order to demonstrate that use of the drug contained in each transgenic salmon line is safe in the environment. The agency expects that the approach set out in the guidance will be relevant to all new animal drug applications involving transgenic salmon.

A number of federal statutes administered by agencies with the Department of the Interior (DOI) might be applied to regulate uses of genetically engineered fish if such fish are found to be harmful to natural ecological systems.

The Lacey Act, 18 U.S.C. § 42, prohibits importation into the United States or any United States territory or possession and the shipment between the continental United

States, the District of Columbia, Hawaii, the Commonwealth of Puerto Rico, and any possession of the United States of certain categories of wild animal species – including fish – determined to be "injurious to human beings, to the interests of agriculture, horticulture, forestry, or to wildlife or the wildlife resources of the United States." Wildlife and wildlife resources are defined broadly to include all wild animals and "all types of aquatic and land vegetation upon which such wildlife resources are dependent." Id. § 42(a)(1). Thus the Lacey Act may give the Secretary of the Interior the authority, which has been delegated to the U.S. Fish and Wildlife Service, to prohibit the importation and transportation of transgenic fish if they are found to be injurious to human-related interests or ecological systems of the United States. Regulations listing species of fish found to be injurious under the Lacey Act and therefore restricted are found at 50 C.F.R. 16.13; Salmon is not currently listed. In addition, no live fish, progeny, or fish eggs may be released into the wild without written permission from the appropriate wildlife conservation agency. Id. § 16.13(a)(1).

It is not clear at this time, however, whether Lacey Act prohibitions can be applied to transgenic fish. The statute applies to "species" of mammals, birds, fish, certain aquatic invertebrates, amphibians, reptiles, and the offspring and eggs of these animals. 18 U.S.C. § 42(a)(1). DOI is currently considering whether Congress intended transgenic forms of these species to be included under the scope of the Lacey Act.

A separate part of the Lacey Act, 16 U.S.C. § 3371 et seq., also has implications for the regulation of transgenic fish. This federal law, administered by both the Secretaries of the Interior and Commerce, makes it unlawful for any person to import, export, transport, sell, receive, acquire, or purchase (or attempt to commit any such act) in interstate or foreign commerce any fish taken, possessed, transported, or sold in violation of any federal, tribal, state, or foreign law. Id. § 3372(1), (2)(A), (4). Thus, while the statute does not substantively grant authority to regulate the importation, transportation, exportation, or possession of species such as transgenic salmon, violation of another federal, state, tribal, or foreign law governing these activities would become a violation of federal law and subject to civil and criminal penalties. See id. §§ 3373, 3374.

A third federal statute, jointly administered by the Secretaries of the Interior and Commerce, potentially affecting the use and dispersal of transgenic fish is the Endangered Species Act. The Endangered Species Act (ESA) requires importers of fish (other than nonlisted fish imported for the purpose of human or animal consumption or taken in U.S. waters or on the high seas for recreational purposes) to file declarations, and limits importation to designated ports. 16 U.S.C. § 1538(d), (f). Section 7 of the ESA requires any federal agency to insure that any action authorized, funded, or carried out by the agency not jeopardize the continued existence of any endangered or threatened species or adversely modify any critical habitat of such species. Id. § 1536(a)(2). Thus, each federal agency must consult with the U.S. Fish and Wildlife Service or the National Marine Fisheries Service, depending on the species, for any action that may affect a listed species. If the action is likely to adversely affect a listed species, the appropriate Service issues a Biological Opinion, which may authorize take that is incidental to the action or, if the federal action would otherwise jeopardize the continued existence of the species,

offer alternatives to the federal action that will avoid such jeopardy. Id. § 1536(b). Any take of an endangered or threatened fish species unless otherwise authorized is unlawful under the statute. Id. § 1538. Thus, a federal agency will be held responsible for any take – unless authorized through an Incidental Take Statement issued by either the U.S. Fish and Wildlife Service or National Marine Fisheries Service – directly or indirectly caused by the authorization, funding, or other federal action associated with transgenic fish.

The Nonindigenous Aquatic Nuisance Prevention and Control Act, 16 U.S.C. § 4701 et seq., also has the potential to affect the introduction and dispersal of fish. Although the statute focuses primarily on the spread of nonindigenous species through ballast water releases, it also created a task force co-chaired by the Director of the U.S. Fish and Wildlife Service and the Undersecretary of Commerce for Oceans and Atmosphere to develop and implement a program to prevent the introduction and dispersal of aquatic nuisance species. The task force is to "establish and implement measures . . . to minimize the risk of introduction of aquatic nuisance species to waters of the United States." Id. § 4722(c). An aquatic nuisance species is defined broadly to mean "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 of such waters," with nonindigenous species defined to include "any species or other viable biological material that enters an ecosystem beyond its historic range." Id. § 4702. Thus aquatic nuisance species can include any species that is not native to that region of the United States, and are not limited to foreign species. A transgenic fish, if found to meet the definition of aquatic nuisance species, could come under the scope of the act.

Finally, various federal land management statutes give federal agencies the authority to manage and regulate species occurring on or affecting federal lands. Authority for management actions comes from each agency's general management statute (including the National Park Service's Organic Act, 16 U.S.C. § 1 et seq.; the National Wildlife Refuge System Administration Act, 16 U.S.C. §§ 668dd, 668ee; and the Bureau of Land Management's Federal Land Policy and Management Act, 43 U.S.C. § 1701 et seq.), and the USDA Forest Service's Organic Act, as well as the Property Clause of the Constitution.

3. Hazard identification and risk assessment

How are hazards/environmental safety issues associated with the transgenic fish identified?

FDA's CVM, in close cooperation with other federal, state, and tribal agencies with authorities relating to the transgenic animal in question, intends to utilize, in addition to its' considerable in-house expertise in aquaculture and environmental assessment, various sources to identify the environmental safety issues associated with investigational and commercial production of transgenic animals. CVM in-house expertise includes aquatic and microbial ecologists, veterinarians specializing in treating

aquatic organisms, fish pathologists and aquaculturists. CVM also plans to use extensively scientific expertise available in other agencies, guidelines and performance standards, public meetings, discussions with affected industry groups, consultation and interaction with experts outside the government and the scientific literature. In particular, FDA gathers information from outside groups and interested individuals when developing guidance for industry.

There are several guidelines or performance standards that have been developed recently through expert working groups that provide information pertinent to identifying environmental safety issues associated with transgenic aquatic organisms (USDA, 1995; Wheelis, 1998). For example, as noted above, the Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish (1995) were developed by the USDA through Advisory Committee meetings and a workshop attended by various experts including experts on environmental safety, biotechnology and risk management from FDA.

FDA has utilized workshops and public meetings to hear stakeholders' concerns about critical issues. For example, FDA has held workshops on developing environmental risk assessment methods for xenobiotics used as new animal drugs and an extensive workshop on determining risk associated with antimicrobial resistance. As noted above, FDA is planning a similar workshop or public meeting for transgenic Atlantic salmon. The workshop will provide stakeholders, including consumers, academics, industry, and government representatives, with an opportunity to identify environmental safety issues as well as methods and criteria for testing, risk characterization, uncertainty evaluation and risk management.

FDA also intends to involve experts from other government agencies (federal, state and local) in its identification of hazards on a national, regional and local level. For example, NMFS has an extensive background in research and assessment of environmental consequences associated with aquaculture. Guidance on compatibility of native and exotic species (irrespective of whether they are transgenic) has been formulated and has been embraced by the U.S. and adjacent nations. This background enables NMFS to offer to be a co-sponsor and participate with FDA in any approach to stakeholders for input on the environmental aspects of the use of transgenic salmon.

The environmental impacts of net pen aquaculture itself, without the use of transgenics, is currently controversial (see Naylor et al. 2000 and rejoinder). Impacts of any aquaculture activities on the management of wild stocks of salmon, be they Atlantic salmon on the east coast or native Pacific salmonids on the west coast, need to be considered. Technologies are developing to address these concerns with increased sensitivity to the environment (e.g., containment, sterility, different sources of fish feed ingredients). FDA and others would have to assess the status of these technologies in order to determine whether they could contribute to an improved environmental impact profile prior to their application to transgenic salmon culture.

There is also a growing body of literature that specifically addresses environmental concerns associated with transgenic aquatic organisms. For example,

Hindar, 1993, Kapuscinski and Hallerman, 1991, and Tiedje et al, 1989, provide extensive reviews of potential environmental and evolutionary adverse impacts associated with transgenic aquatic organisms. The National Academy of Sciences is expected to revisit this issue in 2001.

One goal of extensive cooperation at the federal, state and tribal levels is to ensure that all government entities with authorities for protecting natural resources are able to exercise their respective legal roles at the earliest possible time.

FDA will work with its federal partners in preparing draft and, after taking into account public comment, final guidance that will set out which environmental issues sponsors need to address for individual proposed products. As with all other products reviewed under its new animal drug authority, FDA is requiring data collection as part of its review of individual varieties of transgenic Atlantic salmon. The data collection should contribute to further identifying and quantifying potential adverse impacts, which in the case of a transgenic fish would principally be effects on the health of fish and other animals in the aquatic environment. The information becomes the basis for a NEPA environmental assessment of each transgenic variety and for an assessment of the safety of the new animal drugs contained in each transgenic variety.

NMFS and FWS rely primarily on in-house expertise to identify environmental safety issues, and would do so for transgenic fish. In-house expertise includes fisheries biologists, geneticists, and ecologists. ACE coordinated with the resource agencies to utilize this expertise. It is also possible that for cases as controversial and publicly sensitive as that for transgenic fish, public meetings would be warranted to identify the possible safety issues.

How are environmental safety/risks assessed for the transgenic fish?

FDA/CVM, in consultation with its federal partners, intends to apply accepted ecological risk assessment methodology for assessing the safety of transgenic Atlantic salmon. For example, the Guidelines for Ecological Risk Assessment (EPA, 1998) that were developed as part of the Risk Assessment Forum, sponsored by the EPA, may be a useful tool for the risk assessment of transgenic salmon. CVM participated in the development of this guideline as a member of the Forum and on the peer review committee. The methodology basically consists of 1) identifying possible adverse events (assessment endpoints) associated with transgenic salmon to be considered in the risk assessment, 2) determining which exposures and effects are probable, and 3) characterizing the risk associated with each adverse event that may occur as a result of the introduction of the transgenic fish. Uncertainty analysis would also be included.

Appropriate testing and information collection would occur as part of the methodology. The methodology is iterative in that if new adverse events are identified, those events must be incorporated into the environmental assessment. Additionally the methodology is flexible enough to allow incorporation of quantitative performance data as they accumulate. Once the risk of the adverse events has been characterized, a

determination can be made about conducting further testing or implementing risk management. Depending upon the adverse event, including its magnitude, uncertainties, and available risk management methods, the risk assessment may include both qualitative and quantitative determinations of risk.

How are relevant issues considered by the regulatory agency (e.g., biological factors, pathways for proliferation of risk, etc.)?

CVM intends to utilize a risk assessment process for considering possible adverse events that are identified in association with the development and commercial use of transgenic Atlantic salmon. For example, information and ideas might be obtained from a variety of sources including workshops, other experts (government, industry and academia) and the scientific literature to define potential adverse events. The relevant adverse events might be included in a conceptual model in which the studies necessary for assessing the risk associated with each adverse event would be identified. The environmental risk assessment then could enter an analysis stage, where data and information would be collected to analyze exposures and effects. This process is scientific and methodical.

After sufficient data have been collected, CVM would conduct a risk characterization. During its risk characterization, CVM would estimate the ecological risk for each adverse event, determine the overall degree of confidence in each risk estimate, cite evidence supporting the risk estimates, and provide an interpretation of the adversity of ecological effects. A good risk characterization should express results clearly, articulate major assumptions and uncertainties, identify reasonable alternative interpretations, and separate scientific conclusions from policy judgments. (Suter, 1993).

What types of risk are considered by the regulatory agency (provide definition for risk if appropriate)?

FDA intends to publish draft guidance on the kinds of information sponsors should provide to address environmental safety issues as part of a new animal drug application for the new animal drug contained in transgenic fish. FDA is providing the following discussion to illustrate an approach to review of risk associated with transgenic fish. It is derived from Hindar (1993) and Kapuscinski and Hallerman (1991).

In general, there are two themes for assessing adverse events from escaped transgenic fish that should be considered. They are (1) full spectrum of biological effects caused by the escapees on native populations whether or not the escapees spawn successfully, and (2) the reproductive success of the escaped fish.

As observed by Kapuscinski and Hallerman (1991), a gene can be completely characterized with regard to its DNA sequence; however, the primary feature of transgenic individuals that will likely determine the types of ecological questions needing attention is characterization of the nature and magnitude of specific phenotypic changes elicited by expression of the transgenes. Based on these changes, the evolutionary and

ecological factors that should be addressed include: (1) the fitness of transgenic individuals; (2) natural interactions of the unmodified species with other organisms and the related consequences of possible differences exhibited by transgenic conspecifics; (3) the natural role of the unmodified species in ecosystem processes; (4) the related consequences of possible differences exhibited by transgenic individuals; and (5) the scale and frequency of introductions into an aquatic ecosystem since these will influence the likelihood of establishment, amount of genetic diversity, amount of genetic material available for recombination, genetic adaptation, and degree of ecological risk. Phenotypic changes in one or more categories may modify life history patterns or spatial or temporal habitat distributions of transgenic fish compared to non-transgenic conspecifics. Transgenic individuals may have surprising ecological impacts associated with their degree of fitness, interaction with other organisms, role in ecosystem processes or potential for dispersal and persistence.

Among the specific phenotypic changes that ordinarily should be examined are:

1. Metabolic rates that influence nutrient and energy flow and other organisms. For example, growth hormone has been shown to modify the metabolic rate of salmonids.

2. Range of tolerance values for physical factors, such as, temperature, pH, salinity, dissolved oxygen, or turbidity effects. These effects could be pleiotropic. Growth hormone plays a role in osmoregulation.

3. Behavior changes that effect reproduction, feeding, territorial defense, migration, or other life history features that could change population dynamics, interactions with other species or genetic stocks, and possibly could lead to destabilization of the aquatic community. In some cases, the phenotypic effect and adaptive significance of particular single genes are well known but influences of polygenes, pleiotropic gene interactions and the environment may also be involved. This might lead to examining the effects of growth hormone under different environmental conditions.

4. Changes in resource or substrate use could have direct impact on nutritional requirements of the transgenic. Indirect effects on food webs such as added growth hormone increasing size at a given age that may lead to increases in the size of their selected prey. There may also be alterations in appetite and feed conversion.

5. Resistance to population regulating factors including disease, parasitism, or predation may have population effects.

If crossbreeding occurs, then the hybrids produced by crosses of transgenic and wild fish will include some that are heterozygous for the transgenic trait. The strength of natural selection for (or against) a new trait will depend on the expression of the trait in heterozygotes relative to homozygotes. It should be noted that when immigration rates into natural populations are very high, inflowing genes, irrespective of the strength of the selection might swamp the recipient populations.

It is also noted that transgenic fish in aquaculture production will usually have gone through one bottleneck more than traditionally bred fish (Kapuscinski and Hallerman, 1991). This bottleneck results from inbreeding when homozygous lines are produced from established transgenic individuals, something not always done with conventional fish breeding. Escapes of fertile transgenics can therefore lead to an even more rapid loss of genetic variation in the recipient native populations than escape of other cultured strains of the same species, other factors being equal. Transgenics could cause significant changes in the natural populations' genetic structure and lead to loss of genetic adaptation to local environmental conditions. It has also been noted that only a few fertile individuals can cause changes in the genetic structure of the wild type (Hindar, 1993; Muir and Howard, 1999). Recognition of these potential adverse events have led to consideration of various risk management methods (e.g., physical containment, sterility, etc.) that would prevent release and subsequent significant gene introgression from transgenic fish.

Lastly, the potential for the product of the genetic modification to have an impact on the environment should be included in the risk assessment. For example, if the product is additional growth hormone, the assessment should address whether the growth hormone is available to predators of the transgenic fish, whether it is metabolized or excreted and released into the environment and whether the excreted product may have effects on non-target organisms via bioaccumulation or biomagnification.

Escapes of salmon from currently designed net pen facilities are common and range from minor incidents where a few fish escape to massive escapes. Escapes may be due to operational errors, catastrophic failure of the containment systems during heavy weather events, or damage sustained from ships or large predators such as sea lions. As an example, about 4,500 farm-reared non-transgenic Atlantic salmon recently escaped into Johnstone Strait off the northeast coast of Vancouver Island from a boat transporting them to a processing plant because one of the screens in the cargo hold was not secured properly. Although salmon farm operators are attempting to prevent escapes by upgrading containment systems, installing predator deterrent devices, and taking other actions, it still must be assumed that escapes will occur.

As understanding of fish population genetics and ecology has improved, the environmental and management concerns associated with non-natives or non-local stocks breeding with and competing with native fish species, or local fish populations, have been increasingly recognized and scientific understanding further developed. Efforts need to be made to incorporate consideration of all relevant environmental issues into decisions both on fish stocking and net pen aquaculture.

Currently many states stock non-native or non-local hatchery fish, which obviously may cause some of the same concerns as net pen aquaculture in terms of breeding or competing with native populations. For example, environmental introduction and establishment of Atlantic salmon into the upper Great Lakes has been attempted. Though they were once native to Lake Ontario, after more than 100 years of trying, agencies of the governments of Canada and the United States have yet to establish these

ocean-going salmon in the fresh waters of any of the Great Lakes. Every year since 1993, the State of Michigan has planted two non-native strains of Atlantic salmon in Lakes Michigan and Huron. One of these strains, "Gullspang" Atlantic salmon, comes from the freshwater lakes of Sweden, where they have been landlocked since the Ice Ages. Michigan and Wisconsin have at times experimented with a strain of Atlantic salmon that spawns in the rivers of Quebec province, and Minnesota continues to stock this species (Wheelis et al, 1998; a Michigan website details these and other releases: http://www.dnr.state.mi.us).

Experience gained from releases and escapes of non-transgenic fish is useful, not only for helping develop new approaches to environmental oversight of fish in general, but also in predicting the consequences of escaped transgenic fish. As noted, salmonids and other fish have been both intentionally and accidentally introduced into non-native habitats. In many cases the fish have not become established. In other cases, the introduced species have become established and have even displaced native species. To date, farmed Atlantic salmon has not been proven to successfully establish in new habitats in North America, although this is currently a subject of intense study and debate. Introductions of living non-native organisms are considered to be a major cause for the loss of global biodiversity. It has been reported that introductions of non-native organisms have significantly contributed to extinctions of North American fish species during the past century (Hindar 1993, Kapuscinski and Hallerman 1991). The Invasive Species Council established pursuant to Executive Order 13112 (1999) is considering management strategies to minimize harmful introductions of non-native species.

Are possible future changes in social and ecological conditions (e.g., climate) under which the transgenic fish will be used taken into account?

As part of environmental risk assessments, agencies may consider possible future changes in social and ecological conditions, taking into account how reliably such future changes can be predicted. At present, the agencies do not consider such predictions to be reliable enough to warrant their use.

Are possible environmental risks in other countries considered?

In accordance with Executive Order 12114, "Environmental Effects Abroad of Major Federal Actions," FDA considers environmental effects abroad including environmental risks in other countries and on the global commons as part of the NEPA analysis. 21 CFR 25.60.

Atlantic salmon culture operations in both the northeast and northwest U.S. are virtually contiguous with culture operations in Canada. In view of the shared resource and shared market for food derived from farmed Atlantic salmon, coordination of the review and any conditions of approval will be important, in the event that either country becomes ready to approve net pen culture of transgenic Atlantic salmon varieties. FDA regularly contacts the various Canadian authorities under a variety of disclosure

agreements to ensure a coordinated review of this and other animal drug products for salmon to be used in shared border waters.

How are uncertainties taken into account?

Sources of uncertainty include variability, uncertainty about a quantity's true value, and data gaps. An additional source includes human error, such as mistakes in handling the fish, unclear communication, improper manipulation of data and errors in data and information collection.

In general, uncertainty is addressed by empirical data that reduce the uncertainty, in combination with various conservative assumptions, such as safety factors, that compensate for the unknown. The greater the uncertainty, the greater the value or number of the safety factors applied to each uncertainty. For each case, there may be a level of uncertainty reached that cannot be compensated for by safety factors, in which case the contemplated action could not be approved because it would not be regarded as safe.

FDA has used a variety of methods for analyzing and describing uncertainty. The methods range from simple to complex. In the simplest form, professional judgment is used in estimating the degree of uncertainty. Uncertainty has also been analyzed utilizing classical statistical methods (e.g., confidence limits, percentiles). FDA also uses models. In the recent antibiotic resistance risk assessment, Monte Carlo analysis was used. Other mathematical methods (e.g., fuzzy mathematics, Bayesian methodologies) could also be used for evaluating uncertainty in our ecological risk assessment of transgenic fish. As with other U.S. regulatory agencies, FDA is in a transition toward using more quantitative risk assessment models to analyze uncertainty (EPA, 1998; Suter, 1993).

Once uncertainties have been characterized, either additional data are collected to reevaluate the risk of an adverse event or the uncertainty is considered in risk management. Additionally, at some point in the assessment, it may be decided that safety factors should be applied to data to characterize the potential for an adverse event to occur. Safety factors are often applied to handle uncertainty when there is a lack of knowledge, or the additional data collection will not help in increasing the confidence of a decision. If additional data are to be collected, then risk assessment returns to the data collection and analysis phase. If additional data are not collected, then the uncertainties, including any safety factors, are considered in the risk management. Within risk management, there is a range of possibilities for handling uncertainties. The range would depend on the degree of the uncertainty and the magnitude of the possible adverse event. Management methods could range from limiting the use of the product, to imposing certain conditions, e.g., confinement or numerical limitations, to not approving the new animal drug product because the agency cannot determine the drug is "safe."

Like all U.S. federal agencies, NMFS uses a precautionary approach to deal with uncertainty in decision-making. NMFS follows the approach described in FAO fisheries documents on the "precautionary principle."

What is the standard that the regulatory agency uses for determining safety and what is the baseline for comparison?

Under the FFDCA, the environmental safety of a new animal drug is determined through a risk analysis. Environmental safety is to be demonstrated by "adequate tests by all methods reasonably applicable to show whether or not such drug is safe for use under the conditions prescribed, recommended, or suggested in the proposed labeling.." 21 U.S.C. § 360b(d)(2). A determination of whether the new animal drug in a transgenic fish is "safe" includes an evaluation of the direct and indirect environmental effects on the health of fish and other animals in the aquatic environment. In addition, there must be reasonable certainty of no harm from the fish to humans who consume it.

Do regulatory agencies consult on issues of mutual interest and, if so, how?

Yes. FDA consults with EPA through the NEPA process or with FWS, NMFS or Forest Service on issues where expertise in the area is needed. FDA may also consult with local or state environmental and regulatory experts on local environmental issues. Additionally, under some laws, such as the ESA or the Migratory Bird Treaties Act, FDA must consult with NMFS or FWS on issues where they have direct legislative jurisdiction. For example, if it is apparent that transgenic salmon likely would interact and impact other aquatic species, including other Atlantic salmon, that have been declared endangered or threatened, consultation with NMFS and/or FWS would be required.

The FWS, NMFS and the states manage numerous fish stocks under the Atlantic Coastal Cooperative Fisheries Act. The FWS has actively worked with the New England Fishery Management Council to determine and designate essential fish habitat for Atlantic salmon. The FWS is actively involved with NMFS and the State of Maine in management of the resource and its habitat.

The environmental agencies routinely meet and coordinate regulatory activities. Programs such as the Joint Processing Programs brings them together to discuss the issues surrounding issuance of permits pending before the ACE. This coordination would be expected for aquaculturing any species in the waters of the U.S.

Are there any significant factors that agencies don't consider / have authority to consider?

EPA, FWS and NMFS have authority to consider a wide spectrum of environmental factors when reviewing a plan to grow salmon in areas where managed fish species occur. NMFS manages more than 900 fish stocks through 40 active Fisheries Management Plans, as authorized by the Magnuson-Stevens Act. Under the essential fish habitat provision of that act, NMFS has the authority to review any activities that might negatively impact habitat. For example, the impact of salmon farms on plant or benthic communities that are part of specific fish habitats are covered by these provisions of the

Magnuson-Stevens Act, and would have to be taken into account in reviewing any plan to grow salmon in areas where managed fish species occur.

The essential fish habitat (EFH) Consultation process is coordinated among the lead federal agency, the applicant and NMFS. The consultation requires that the designated EFH be identified, its functions noted and the impacts, means of neutralizing or compensating those impacts identified and an EFH compatible course of action described (resource recommendations). The intent of all NMFS involvement in the regulatory processes is to ensure that all aspects of a proposed aquaculture activity provide adequate environmental protection.

The EFH Consultation process is not a stand-alone activity, but is incorporated into the overall regulatory process for reviewing aquaculture plans. If the Endangered Species Act (ESA) is invoked, the consultation process can be led by either the EFH or ESA procedures.

FDA likewise has authority to take into account a wide spectrum of environmental factors in its oversight of transgenic fish. Under NEPA, FDA must evaluate the full scope of environmental impacts defined under NEPA that might be associated with a major federal action and communicate and coordinate with other agencies that might be affected by the action. The FFDCA provides FDA with authority to take actions based on environmental impacts that the agency determines are likely to directly or indirectly affect the health of humans or animals. FDA anticipates that most potential adverse environmental effects from use of transgenic fish would likely have at least indirect adverse effects on fish or other animals in the food web. For example, if a new animal drug adversely affects a plant population that is a significant food source for an aquatic animal population to an extent that would adversely affect the health of that population, the use of the new animal drug would have an indirect impact on the aquatic animal by adversely affecting its food source.

4. Information and data (what, why and how is data and information collected and generated)

CVM is currently regulating transgenic fish as containing a new animal drug. CVM approves a new animal drug application (NADA) when it finds that the new animal drug is safe and effective for its intended use(s). The safety determination encompasses safety to the target animal, to humans if it is a food-producing animal, and to humans and other animals in the environment. Thus, in the case of a transgenic fish, the safety of the drug would include safety to the transgenic fish (including safety of eating the transgenic fish) as well as safety to fish and other animals in the environment that may be affected by the use of the new animal drug contained in the transgenic fish. The safety determination would also include the safety to animals of feed containing components of the transgenic fish.

In addition, as part of the demonstration of safety, CVM requires information demonstrating that the new animal drug can be manufactured in a consistent form (which,

in the case of a transgenic animal means that the genetic modification is stable and consistently expressed in the variety of transgenic animal).

The data to satisfy requirements for approval are collected from effectiveness, target animal safety, food safety, environmental safety, and manufacturing chemistry and stability studies. All data for these studies with the exception of effectiveness trials are subject to Good Laboratory Practices (GLP) standards codified under 21 CFR 58. The effectiveness trials currently are conducted under regulations (21 CFR 511) and guidance documents addressing good target animal study practices. Some of the food safety aspects of regulation are touched upon below, even though this assessment does not address food safety, to clarify relationships among these FDA authorities and those relating to other aspects of transgenic animals.

The review process for a new animal drug normally starts with a sponsor filing an INAD with CVM and, if desired, requesting authorization to slaughter and render research animals treated with the investigational drug. CVM grants an authorization to slaughter or to render investigational animals only after determining that such animals are safe for use as food or feed. New animal drugs that do not have an established INAD or an approved NADA, or are not used in accordance with the extra-label provisions of Section 512(a)(5) of the FFDCA are in violation of the FFDCA, and FDA can take or recommend enforcement action against the product, the distributor and/or the sponsor. The only exception to this is basic laboratory research: 21 CFR 511.1(a) allows new animal drug research to be conducted without an INAD as long as the animals are used solely for laboratory research, including that the animals may not be used for any food or feed purpose.

In particular, sponsors would need to establish INADs when they have a commercially viable product that they intend to eventually market to the public, when the transgenic animals are non-laboratory semi-domesticated animals that pose containment issues (such as the fish to be grown in ocean net pens), or when the animals are to be disposed of through slaughter or rendering for human food or animal feed. In some cases, sponsors may want to establish an INAD earlier in the process, because some studies in the very early stages of development are needed for product approval.

Investigations with new animal drugs are controlled under 21 CFR Part 511. The goal of investigations of a transgenic salmon intended for food use would be to gather data for an application that can be approved under 21 U.S.C. § 360 b(b)(1) as implemented by 21 CFR Part 514. The technical sections for an NADA are developed during the investigational period. All data relevant to the application must be included, whether it is considered pivotal or not by the applicant, regardless of whether it is supportive of the approval. FDA provides close oversight of the testing being conducted, the integrity of the data collected and the interpretation of results. In addition to in-house scientific experts, the agency uses contracts, advisory committees, and experts from other Federal agencies to obtain specialized expertise where needed.

The sponsor generally provides to the agency for review a proposed plan of how it intends to address all the requirements of obtaining approval of an NADA. Also, the sponsor must provide all known published documentation on the product under consideration. Some of this published documentation may be able to be used to satisfy some requirements of an NADA. Studies conducted by or behalf of the sponsor are normally needed to complete the NADA. When new studies are needed to satisfy requirements for an NADA, the sponsor is encouraged to provide study protocols for review and comment by FDA. Once concurrence is reached, a protocol provides the basis for how the study is to be conducted. All studies can be conducted by the sponsor or can be contracted out to qualified research facilities capable of conducting research under applicable regulations and statutes.

Clinical effectiveness trials are generally conducted under conditions that are representative of commercial "real life" situations. These effectiveness trials also usually provide the agency with further information about safety and conditions of use that cannot be obtained from more controlled experimental trials. In the case of transgenic salmon, conducting clinical effectiveness trials presents a particular environmental problem, because of the risk of fish escaping, in that biocontainment should be agreed upon in advance, at least for the limited trial sites. Because the results of these studies are to be used as proof of safety or effectiveness for an NADA approval, sponsors generally seek FDA agreement on study protocols in advance. An environmental problem created as a result of a clinical trial could be evidence that the product would not be safe under commercial use and could be used as grounds to refuse to approve the product.

Data collected for studies conducted under the INAD are subject to three different types of bioresearch monitoring inspections: sponsor/monitor, GLP and clinical investigator. Effectiveness studies are routinely inspected as deemed necessary by CVM personnel. Inspections are generally data audits of specific trials and are conducted by FDA field investigators who may be assisted by CVM personnel.

A sponsor generally conducts testing of a new animal drug in its final form. Changes to a product formulation occur for many reasons and are allowed as long as proper testing is done to ensure that the modified formulation is equivalent to that used in prior testing. For transgenic fish, this means that testing will generally need to be conducted on the transgenic variety, as it will be marketed in commerce, as opposed to earlier crosses and backcrosses. After a sponsor has completed testing, it submits the generated information to the agency for review. This usually entails a variety of reports, statistical analyses and copies of source data records.

FDA has authority to conduct inspections and to review records. Thus, if a reviewer suspects data integrity problems in submitted documents, he or she can request that inspections be conducted to assess the validity of the data. If the data from studies are determined to be invalid, these studies are excluded from any further consideration in the submission. When significant questions regarding data integrity are raised, FDA ordinarily will place an application under its application integrity policy, thereby

deferring substantive scientific review pending a validity assessment. Such an action could delay product approval or, if data integrity problems are confirmed, could lead to a product never being approved or to the withdrawal of a product approval.

The environmental safety review component of new animal drug applications for transgenic animals, including transgenic Atlantic salmon, follows the NEPA format in terms of scoping, development of alternatives, consideration of cumulative effects, related social and economic impacts. This facilitates organizing and coordinating the review among the several agencies that regulate environmental resources that might be affected by an approval, and so goes beyond just those areas that are directly under FDA/CVM authority.

5. Mitigation and management considerations: approvals and conditions on research, development, production, distribution, marketing, use and disposal

There is a comprehensive permitting program for non-transgenic aquaculture. Permits are required for the placement of culturing facilities in or use of waters of the U.S. The regulatory program is lead by the ACE, using two principal pieces of federal legislation: Section 10 of the Rivers and Harbors Act (structures) and Sections 401 and 404 of the CWA (fill). EPA, FWS, Department of Commerce, National Oceanic and Atmospheric Administration, NMFS and their State government counterparts are the principal parties in the program. Associated with their federal mandates, the involved State(s) provide regulatory overview through the Coastal Consistency clause of the Coastal Zone Management Act.

EPA is currently evaluating the need for requiring NPDES permits for culturing facilities and is developing guidelines and standards for all U.S. aquaculture facilities that are expected to be in place in 2004. If such permits are adopted, state agencies would ordinarily be responsible for issuing and monitoring compliance with conditions set in permits.

Disposal of materials resulting from processing is already treated as a point source discharge by EPA and is regulated under the provisions of the CWA. NPDES permits authorizing discharges from such facilities are required. The types of disposals from these facilities may include discharges of processing wastes, process disinfectants, sanitary wastewater and other wastewaters, including domestic wastewater, cooling water, boiler water, freshwater pressure relief water, refrigeration condensate, water used to transfer seafood to a facility, and live tank water. Additionally, processing facilities are required to collect and route all seafood processing wastes and wastewater to a treatment system consisting of 1 mm screens or equivalent technology. All seafood solid wastes are collected and transported to the by-product recovery facility or are recovered through an in-house fish powder plant. By-products from the salmon industry are typically processed into animal feed ingredients, including fish food (EPA, 1995).

Disposals following consumption of food derived from transgenic salmon are typically the same as other restaurant and household disposal and usually consist of

disposal in domestic wastewater, treatment in municipal or private (septic) wastewater treatment facilities, or collection for disposal as solid wastes in landfills.

NMFS' role in aquaculture is multifaceted. It assesses environmental impact and compatibility, designs and test protocols, and monitors the effects of culturing of aquatic species. NMFS is obligated to identify situations where the risk to the environment of introduction or culturing is unacceptable, and to make recommendations to the ACE as to whether a request for an aquaculture permit should be granted. NMFS has a research and regulatory program through which it gains the expertise necessary to justify recommendations regarding a proposed activity. Monitoring and routine evaluations of aquaculture operations are components of each successful permit request. Environmental monitoring is usually required of the operating company, and the data verified through site visits by NMFS. Suspension or revocation of a permit, or financial penalties, are all available where a party does not comply with its permit. In all cases, the burden of proof of environmental compatibility resides with the proponent/applicant.

NMFS and FWS have had considerable experience with the Atlantic salmon industry. The net-pen industry with which NMFS is most familiar has a routine, significant escape of salmon from their facilities. Escapes can occur *inter alia* through equipment failure, during fish handling and transport operations, through large predator intrusion into facilities, and as a result of storms. Current technologies and procedures in the industry cannot ensure that escapes will not occur. NMFS considers the escape problem as one related to operational practices. It is related, also, to the evolving nature of the culturing technology and human error. Information on the consequences of escape is being collected and NMFS is revisiting permits and the associated assumptions of impact. The regulatory tools available for insuring permit compliance or modification are found in the ACE regulations, Permit Conditions, the ESA, Magnuson – Stevens Act and Fish & Wildlife Coordination Act. Escapement is difficult to address due to the diversity of causes.

Some information exists on the behavior of salmon that escape from aquaculture facilities that indicates that they have a tendency to stay near the area of escape, probably because of a food dependency. This behavior allows the recovery of a substantial number of post-escape fish by employing seine nets or hook and line fishing.

NMFS and FWS also have considerable knowledge of reproductive sterilization techniques that might be used to mitigate interbreeding of escapees with wild stocks. To date, none of these techniques has been shown to be 100% effective, and analysis of all fish to ensure sterility of individuals may not be economically or practically viable. The present Atlantic salmon farming practices do not include a requirement that only sterile fish be cultured. A request for growing transgenic Atlantic salmon individuals may. There is a reasonably successful program in place already that is overseen by the FWS to assure triploidy in grass carp. It is possible that something similar can be developed for Atlantic salmon.

NMFS and FWS have land-based facilities that are being used to study non-transgenic Atlantic and Pacific salmon behavior and could be used as a first approach to evaluating the behavior of transgenic salmon in quasi-natural environments. Such work, however, would be costly and time consuming, and would require interpretation of results against completely natural situations.

The Gulf of Maine distinct population segment of Atlantic salmon has been listed as endangered under the ESA. NMFS and FWS have been working with the State of Maine to address the potential impacts of escapees of domestic farmed origin, including those of European origin. These potential impacts include genetic introgression, ecological competition (food, space, mates), and disease transmission. Possible measures to reduce and/or eliminate this potential impact include a phase-out of European stocks, upgraded containment systems, marking of all fish reared in net pens, and a monitoring program to document any escapes that do occur.

Various mitigation measures or management controls that will prevent or reduce the potential for adverse environmental impacts to occur can be considered during the pre-market review of transgenic Atlantic salmon under the new animal drug provisions of the FFDCA. These mitigations would have to be considered in the context of the environments where the transgenic salmon would be reared, the management procedures that would be followed, their feasibility, and the probability that properly used mitigation measures would be effective. Such measures can therefore be different for various research sites and for varying production sites, according to the environmental context and rearing systems employed.

Ensuring environmental safety is a reason why FDA might place restrictions on product development, production, transportation, distribution, and marketing for transgenic salmon as part of the new animal drug approval process. These mitigations could include physical or biocontainment performance requirements, predator exclusion design, or outright prohibition from use in certain locations. Also, because under the FFDCA food containing unapproved new animal drugs is considered adulterated and therefore may not be sold, food products derived from the transgenic variety may not be sold, imported, taste tested or test marketed without express prior approval from FDA of the new animal drug contained in the transgenic fish. Approval from FDA includes consideration of not just the genetic construct, but also the effects due to its insertion and expression.

6. Monitoring and consideration of new information

NMFS has the legal authority and an infrastructure to prescribe and evaluate monitoring of aquatic organisms held in aquaculture facilities in navigable waters of the US. These authorities are found in the legislation empowering NMFS to address ESA, EFH and the environmental consequences of authorizing culturing activities in waters of the U.S. NMFS adds such monitoring requirements to the permit requirements issued by ACE pursuant to the Rivers and Harbors Act when circumstances warrant. Because aquaculture activities represent such a wide diversity of technologies and species and the

information pool varies in adequacy, the conditions of permit issuance vary from case to case and species to species. Generally, NMFS has seen a reduction in the level of monitoring required of culturists. Often, the monitoring has revealed less than expected or a lack of adverse impacts associated with culturing practices. Typical monitoring and reporting requirements can include escapes, inventory tracking, stock tagging and environmental monitoring related to water quality and changes to the benthic environment in the area of the facility. In cases where facilities are in state waters, NMFS can transfer monitoring and reporting authorities to the state.

FDA has legal authority and existing programs to prescribe and evaluate monitoring requirements for marketed products. These requirements can be imposed on the transgenic fish sponsors as a part of approval under the new animal drug approval process. See 21 U.S.C. § 360b(l) and implementing regulations under 21 CFR 510.300. FDA requires regular product experience reporting, maintains an adverse event reporting system that obligates product sponsors to quickly report specified adverse events that might be associated with approved products, and also encourages reporting of adverse events (including adverse environmental events) by veterinarians, other government agencies and consumers into the same system. FDA follows up with inspections of product sponsors to ensure that product complaints are being addressed, that appropriate records are kept, and that labeling, promotional material, and adverse event reports received by the sponsor are being submitted to FDA on a regular basis.

If post approval-monitoring programs for environmental effects are necessary, they are ideally designed prior to product approval. However, if, subsequent to approval, FDA finds that a product cannot be safely used without a monitoring program, FDA can initiate steps to withdraw the approval unless the sponsor implements a monitoring program. FDA can utilize experts in other Federal agencies, special government employees, including experts from academia or industry and Advisory Committees and workshops to design such programs for classes of products and for specific products.

When new information is received through these monitoring programs (or is provided to FDA by other agencies or is otherwise obtained by FDA), FDA has legal authority to take a range of actions based on that information, including additional or modified information or record collection requirements, label changes, or withdrawal of approval. 21 U.S.C. §§ 360b(e), (l). Other agencies may be involved in the monitoring, either in design or use of the results for considerations under, for example, the Endangered Species Act or other resource management statutes. As the agencies involved may vary according to the species under consideration, the FDA has had to decide which agencies were likely to be interested and make contacts on a product-by-product basis.

7. Enforcement and compliance

NMFS has an enforcement office with shore-side and on-the-water presence. The U.S. Coast Guard has co-responsibility for enforcing fishing regulations, and for this purpose aquaculture has been defined as a fishing activity under the Magnuson-Stevens

Fisheries Conservation and Management Act. The resources of NFMS and the U.S. Coast Guard for fisheries and aquaculture enforcement are probably not adequate, especially if substantial new aquaculture activity occurs that requires additional enforcement intervention.

NMFS may seek revocation of ACE permits when unexpected or excessive adverse environmental impacts are identified. With the authorities provided under the ESA and Marine Mammal Protection Act and to some degree, the Magnuson – Stevens Act, NMFS has separate authorities that allow it to force cessation of unacceptable culturing activities.

The FWS has a law enforcement division that enforces Lacey Act violations and for the case of ASMFC activities also helps enforce FMPS. We anticipate a role for FWS law enforcement in enforcement of activities related to the Atlantic salmon listing.

FDA could initiate a compliance action under the FFDCA if an NADA approval for use of a genetic construct in salmon, another fish, or any other animal were to include measures aimed at mitigating environmental impacts that affect the health of man or animals, and the sponsor failed to take these mitigation measures. Under the FFDCA, if the use of a new animal drug does not conform to its approved application, it is considered "unsafe." 21 U.S.C. § 360b(a)(1)(B). Thus, if environmental mitigation measures are part of the approved application and the sponsor fails to take these measures, the use of the new animal drug would not conform to its approved application and it would be unsafe. Unsafe new animal drugs are considered adulterated drugs under the FFDCA. 21 U.S.C. § 351.

The FFDCA prohibits interstate commerce in adulterated drugs. 21 U.S.C. § 331. Violation of this provision could result in an *in rem* seizure of the violative drugs and injunction proceedings against or criminal prosecution of those responsible for distributing such drugs. 21 U.S.C. §§ 332-334.

FDA can require sponsors to keep records that are pertinent to the safety of the new animal drug and that were not previously submitted to FDA, including new studies that become available after the new animal drug approval, and to periodically submit such records to FDA. 21 CFR 510.300(a)(1), 510.300(b)(4)(1), 21 U.S.C. § 360b(l), 331(e). FDA has the authority to inspect and copy such records. 21 U.S.C. § 360b(l)(2). If new studies showing that a particular new animal drug contained in a variety of transgenic salmon causes environmental harm become available after the approval of that variety under an NADA, the sponsor would be obligated to bring the studies to FDA's attention. If the sponsor failed to do so, FDA could withdraw approval of the NADA for the new animal drug contained in the transgenic salmon, 21 U.S.C. § 360b(e)(2)(A), and/or seek penalties against the sponsor. 21 U.S.C. § 333.

Under the FFDCA, if a sponsor submits false data, FDA can withdraw approval of the NADA. 21 U.S.C. § 360b(e)(1)(E). Prosecution is also possible.

8. Public involvement and transparency

For aquaculture projects in Federal waters, a public notice of permit application is required. The notice is released by the Army Corps of Engineers. It is possible that the permit application would contain confidential commercial or trade secret information, and if so, the information would be redacted. There is less uniformity among states for notice of applications to the public. The Public Notice is used to inform the general public and adjacent property owners of the application and includes a work description. The comments stimulated by the notice are included in the record and are usually addressed in the Statement of Findings created for the acceptability determination of a proposed action. Should there be compelling issues raised by the public, the Corps of Engineers can request additional information about the proposed action, hold public hearings on the matter, modify the project design or deny the permit request. Typically, project modification is the avenue most frequently used by the regulated community.

As mentioned previously, FDA intends to hold one or more open public meetings or workshops to discuss environmental risk assessment and risk management questions posed by transgenic fish and shellfish, including Atlantic salmon. In addition, FDA is considering using an advisory committee to address any unresolved or controversial scientific questions, particularly regarding environmental issues, prior to completing its evaluation of the first NADA for a transgenic fish. Therefore, the agency believes that it will be able to provide public dialogue on the scientific foundation for making a decision on approval or limitations of such applications.

At the time of publication in the Federal Register of a notice of approval of NADA, FDA makes available through a Public Docket and increasingly as time goes on via its website, an extensive Freedom of Information summary and NEPA documentation required for the approval of the application (although information that still qualifies as trade secret information would not be disclosed). At this point, a member of the public could submit a Citizen Petition that requests withdrawal of approval of the application. For example, such a petition could point out information that should have been submitted in the application that was relevant to the approval or provide an alternative interpretation of data used in the decision. At any time after the approval, new information that has a bearing on the approval of the NADA can be brought to the agency by anyone in the form of a Citizen Petition. FDA considers the information submitted, replies to the Petition, and takes appropriate action based on its reply that could include withdrawal of approval of the NADA, following applicable procedures.

As noted earlier, FDA is not permitted to disclose the filing of an INAD or NADA, absent sponsor agreement, unless the sponsor has publicly disclosed it. 21 CFR 514.12 and 21 CFR 514.11. For example, the filing of the INAD for a transgenic Atlantic salmon has previously been disclosed, but FDA is not permitted to discuss whether or not INADs have been filed for other transgenic fish. FDA is considering whether there may be mechanisms by which it could make public its NEPA analyses of products for which there is considerable public interest and controversy over environmental issues (such as transgenic fish) and invite public comment prior to making the decision.

FDA recognizes that there are special situations (such as those described in this case study) in which it would be preferable to allow greater public access to information. In these situations, FDA encourages the sponsor to release relevant information addressing public concerns. For issues relating to a class of products, CVM uses public workshops to clarify issues that must be evaluated and the means to address them. It also utilizes the CVM advisory committee (a committee subject to the Federal Advisory Committee Act (FACA)), in public meetings where possible, to identify and advise the agency about these issues. In some cases, CVM uses other advisory committees (also subject to FACA) from elsewhere in FDA, consensus conferences in the National Institutes of Health, or requests expert reviews by the Institute of Medicine and the National Academy of Sciences. CVM also discusses issues relating to safety and effectiveness of classes of products and individual products in various international fora, including the International Conference on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) and the Codex Alimentarius Commission. In general, these meetings and/or the reports of their deliberations are open to the public.

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SIDEBAR No. I.A

ORNAMENTAL FISH (GOLDFISH)

Overview

This case study concerns the possible introduction of genes into goldfish to increase tolerance to freezing temperatures, thus allowing the fish to survive in colder water. The genes, which come from other fishes, encode production so-called antifreeze proteins that prevent the growth of ice crystals in the serum of the fish. The U.S. government is not aware of any plans to develop such fish for commercialization and such development may not be permitted in some states. Nonetheless, this sidebar illustrates some of the regulatory and environmental issues that could arise were such fish to be developed and commercialized for use as bait.

1. Description of proposed organism and its use

The goldfish (*Carassius auratus*), native to China, portions of southeast Asia,central Asia, and far eastern Europe, was introduced into the United States more than 300 years ago, making it the first introduced exotic fish in North America (Berg 1949). Today, it is one of the most popular aquarium and ornamental pond fishes throughout the world. More recently in the United States, it has been raised commercially for the fish-bait and fish-food industries. The "generalist" life history characteristics of goldfish, including its omnivorous feeding habit and ability to persist across a wide range of temperatures in various freshwater lotic (i.e., actively moving water) and lentic (i.e., still water) habitats, have allowed this species to establish wild populations in nearly every state and province of North America. Goldfish are believed to have been introduced to new bodies of water via intentional and unintentional release by aquaculturists, ornamental fish hobbyists, fishermen, and individuals desiring to free their pet fish.

Goldfish are widely regarded as a nuisance species, competing with native fish for food and habitat. This exotic species is considered to be detrimental to the fisheries industry in several states (e.g., Garling et al. 1995) and may be responsible for the extirpation of native fish species, including several listed under the Endangered Species Act (ESA), such as the Pahrump poolfish (*Empetrichthys latos latos*; Deacon et al. 1964) and the White River spinedace (*Lepidomeda albivallis*; U.S. Fish and Wildlife Service 1994).

What are the anticipated characteristics of the genetically engineered organism?

Teleost fishes (i.e., bony fishes), as well as some other plants and animals, have evolved a mechanism to reduce the freezing point of their bodily fluids without appreciably changing their osmolarity or their the ability to move as a result of osmosis (Davies, et al. 1999, Fletcher et al. 1999). Antifreeze proteins (AFPs) serve as antifreeze agents by specifically adhering to the surface of ice crystals as they form, thereby preventing their growth. This contrasts with the action of most solutes (e.g., electrolytes) that prevent freezing by colligative mechanisms (i.e., quantity of molecules). Because of the unique aspects of their tertiary structures, these proteins are up to 500 times more effective at lowering the freezing temperature than any other known solute molecule. Several distinct classes of AFPs, distinguished by their molecular structure, have been isolated from fish, insect, and plant sources (Cheng 1998, Davies and Hew 1990). To date, however, those from fish sources are perhaps best known and have been more thoroughly characterized than those from other species. Antifreeze glycoproteins (AFGPs) have been found in Antarctic *Notothenioidei* teleosts and northern cods. So-called type I AFPs are found in righteye flounder (Pleuronectidae) and in shorthorn sculpin (*Myoxocephalus scorpius*); type II AFPs are found in sea ravens, smelts, and herring; and type III AFPs are found in ocean pout and wolffishes (Davies and Hew 1990, Davies et al. 1999, Fletcher et al. 1988). Recently, a new kind of fish antifreeze, designated Type IV, was isolated from the longhorn sculpin (*Myoxocephalus octodecimspinosus)* (Deng et al. 1997, Deng and Laursen 1998, Zhao et al. 1998). The evolution of these AFPs and their genes has been reviewed (Cheng 1998, Davies et al. 1993).

Several commercial applications for AFPs have been identified and are currently being pursued (Wallace et al. 1993). These include the following:

- cold protection of mammalian cells, tissues, and organs;
- enhanced tumor cell destruction during cryosurgery;
- longer shelf life for and better quality of frozen foods;
- protection of fish and plants against cold and freezing temperatures;
- improved growth characteristics in transgenic fish by using AFP gene promoters.

The first three applications of AFPs listed above utilize purified AFP from natural sources or recombinant expression systems while the last two are implemented by gene transfer to the target organism. Genes encoding AFPs have been transferred into Atlantic salmon (Du et al. 1992, Hew et al. 1999, Hew et al. 1992), goldfish (Wang et al. 1995), and tilapia.

In research conducted outside of the United States, a gene from flounder (*Pleuronectes americanus*) that encodes AFPs has been transferred to goldfish, affording transgenic individuals higher survival rates at cold temperatures compared to non-transgenic goldfish (Wang et al. 1995). No other phenotypic traits unique to the transgenic form of goldfish have been described in the literature. However, relatively little research has been conducted on AFPs and goldfish.

AFP genes have been transferred into fish to provide freeze protection during aquaculture production. Although first attempts did not provide the level of protection

desired, new constructs consisting of more effective AFPs with stronger promoter and enhancer elements are underway.

How would the genetically engineered organism be used, including brief description of management practices that would be associated with it?

Commercialization of transgenic goldfish containing the AFP gene has not been approved in the United States, hence its intended use is not completely known at this early stage of research and development. Current biotechnology research using goldfish is not extensive in either the United States or overseas, although at least one laboratory is examining the insertion of AFP genes into goldfish oocytes (i.e., eggs before maturation) (Wang et al. 1995).

Two scenarios are likely based upon current commercial propagation of non-transgenic goldfish: (a) ornamental (aquarium and fish garden) fish, and (b) bait-fish industries. The latter use, in particular, offers a major advantage to both aquaculturists who raise the fish, and to anglers who use the fish as bait. Transgenic goldfish containing the AFP gene are likely to persist and mature under a broader range of water temperatures, allowing aquaculturists greater flexibility in the conditions under which the fish are propagated. Likewise, goldfish genetically engineered to include the AFP protein may be more active in colder waters than non-transgenic individuals, thereby enhancing the attractiveness of transgenic goldfish as a baitfish.

The actual utility of transgenics in the baitfish industry from a socioeconomic standpoint is open to debate. Some experienced with the industry consider that the use of transgenic goldfish for baitfish is not likely at all. They feel that most baitfish producers are well aware of the environmental and public concerns around transgenics and would not even consider production of transgenic fish. Many states already outlaw the use of goldfish for bait. To these individuals, it seems unlikely that the industry would invest in research, development and FDA approval for bait that would be illegal to sell in most states.

Nevertheless, if transgenic fish were to be used in the industry, management systems used for production of transgenic goldfish would likely be similar to those currently in use for non-transgenic goldfish. The goldfish is one of three major baitfish propagated in the United States, with most raised in southern states. Goldfish raised for the bait fish industry are propagated one of two ways: (a) spawning indoors in tanks, with eggs transferred from fiber spawning mats to other indoor tanks or to outdoor ponds; or (b) spawning outdoors on fiber spawning mats placed along the edges of ponds, with eggs transferred to other ponds for incubation and growth of fry. Goldfish can be harvested from ponds throughout the year with large seines, held for a short while to separate viable from unhealthy fish, then shipped via livehaul truck or plastic lined shipping boxes to bait shops (Arkansas Cooperative Extension Service). Goldfish are used as forage in the fish propagation and aquaculture industry and as live bait for sportfishing (e.g., for largemouth bass, *Micropterus salmoides*).

Is there prior experience dealing with the same varieties not genetically engineered?

Goldfish have been artificially propagated for more than 300 years in the United States, and much longer than that in other parts of the world. More than 100 varieties of *Carassius auratus* have been developed through traditional breeding technologies, and many of these are widely sold and discussed via hobbyist groups throughout the ornamental fish industry. In addition, the baitfish industry generates more than $1 billion in annual revenue in the United States, with much of these profits generated by goldfish propagation.

What are the projected locations and extent of production, use and disposal?

The aquarium fish industry operates in all fifty states, with most participants being associated with relatively small operations (pet stores, fish hobbyists, etc.). Goldfish aquaculture associated with the fish bait industry, in contrast, is concentrated in southern states from Georgia to Arkansas.

Intentional and unintentional release of non-native aquarium and bait fish have led to severe environmental problems in the United States, including serving as a primary cause in the population declines of several native fish species. Nearly 150 exotic fish species from the aquarium industry have been found in the wild in the United States (Cohen 2000). Dozens of additional species used as baitfish have established populations outside of their native ranges in the United States. Several species of escaped aquarium and baitfish have been implicated in the listing of threatened and endangered species under the ESA (Lassuy 1995). For example, released aquarium fish have been identified as a chief cause for the threatened and endangered status of the Moapa dace (*Moapa coriacea*), desert pupfish (*Cyprinodon macularius*), White River spinedace (*Lepidomeda albivallis*), and Railroad Valley springfish (*Crenichthys baileyi*).

What types of adverse environmental effects might result from the** genetically engineered **organism?

The adverse environmental effects that have been hypothesized or observed for non-transgenic goldfish introduced into the United States are likely to be similar to those exhibited by transgenic goldfish released into the environment. That is, goldfish have been documented to: exhibit competitive advantages over native fishes, including endangered species (Moyle 1976); hybridize with related species, such as the common carp (*Cyprinus carpio*; Trautman 1981); and alter aquatic vegetation and water conditions (Richardson et al. 1995).

An example of the first of these is that transgenic goldfish with the AFP gene are likely to maintain a competitive advantage over some native species if notable, periodic temperature decreases represent a demographically limiting factor for fish populations in those geographic areas. In many areas of the United States, water temperature is a limiting factor for fish distribution. These conditions can be lethal to many fishes including goldfish, and mortalities from "superchill" are frequently reported for species

such as the Atlantic salmon (Maclean et al. 1995, Martinez et al. 1996). Goldfish eggs injected with AFP genes produced offspring that were significantly more tolerant of low temperatures than controls (Wang et al. 1995). The "acquired" ability to withstand those types of "ecological crunches" may afford transgenic goldfish a competitive (demographic) advantage over other species (see also below).

Potential adverse effects that might result from the intentional or unintentional release of transgenic goldfish can outlined as follows.

Proliferation of the Transgene

The transgene may move to a related species via hybridization (e.g., goldfish-carp hybrids) or to wild populations by introgression.

Behavior and Life History Modification

Because all transgenes (by design) modify some characteristic of the target organism, transgenic organisms are expected to outperform their non-transgenic counterparts during at least some life history stage under some ecological conditions. One example of how this might result in unforeseen consequences is addressed in the so-called "Trojan gene hypothesis" (Muir and Howard 1999). Many animals (including Atlantic salmon) exhibit mate selection based on male body size. Transgenic males exhibiting larger than average adult body size, as a result of a growth hormone transgene for example, may have a mating advantage over their wild counterparts. Thus, the frequency of the transgene may increase rapidly in the wild population. However, it is generally assumed that the biological load imposed by a transgene will eventually result in a net disadvantage to the genetically modified animal thus keeping the transgene in check. For example, in transgenic medaka, Oryzias latipes, transgenic young exhibited lower fitness than the non-transgenic young. Under certain conditions, the introgression of the transgene into the wild population would cause the ultimate collapse of both the wild and transgenic populations.

Range Expansion and Increase of Invasiveness

Some transgenes such as those coding for the production of AFP may well allow escaped animals to occupy colder climes than their current range. Further, these fish may be able to remain active during cold weather while native species are dormant, thereby depleting both habitat and forage. Goldfish, for example, are already widespread and considered a nuisance in many areas. Freeze-resistant animals could potentially overwhelm many aquatic habitats. The propensity of goldfish to hybridize with carp could allow the migration of this trait into that species, thereby exacerbating the problem.

What are the pathways for proliferation of those risks?

Proliferation of risk associated with gene introgression from transgenic <u>and</u> non-indigenous (currently used, non-transgenic) fish:

The amount of risk associated with gene introgression is a function of the scope of the release, the number of escaped animals, the number of potentially affected native species, and the interrelation of at least four population variables: reproductive potential of escaped individuals, frequency of introgression of the modified genes, fitness of the introgressed individuals, and potential demographic decline due to the genetic load of introgressed genes.

The reproductive potential of escaped individuals is based on: (1) the survival rate and fertility of the individuals, and (2) environmental conditions affecting reproduction in the affected ecosystem, such as length of spawning season and available spawning habitat. The frequency with which introgressed genes will spread and increase within the population is related to gene flow. Several models are available to estimate this process. Despite the prediction that introgressed individuals will exhibit lower fitness than non-introgressed individuals, not all new genetic modifications will be maladaptive. Regarding the genetic load of introgressed genes, natural selection is expected to remove maladaptive genes from a population; however, depending on the severity of the maladaptation, the number of generations required for this process can be very large, and an introgressed population may crash before the process is completed.

Proliferation of risk associated with introduction of triploid fish:

The sterility offered by inducing triploidy in some aquatic species reduces concerns about a modified organism escaping and mating with other fish. In many cases, this will mean that aquaculture of a triploid (three sets of chromosomes in contrast to the typically occurring diploid) transgenic species will likely pose less environmental risk than similar aquaculture of fertile non-transgenic species. However, the use of triploidy does not eliminate all environmental risks and its ability to ensure environmental safety is complicated by three factors. First, the effectiveness of triploidy induction varies among species and the methods used. Second, although triploids are functionally sterile, the males may exhibit spawning behavior with fertile diploid females, leading to decreased reproductive success of the fertile diploid females. Third, in cases where large numbers of individuals are released, sufficient numbers of sterile triploids may survive and grow for an indeterminate number of years beyond the normal life span to pose heightened competition with diploid conspecifics or other species.

Proliferation of risk associated with unexpected survival and persistence of escaped or intentionally released transgenic and non-native (non-transgenic) fish:

Despite familiarity with the unmodified organism, there remains some amount of risk associated with the unexpected survival and persistence of escaped or intentionally released transgenic and non-native (non-transgenic) fish. Once colonized or persistent in new habitats, there may be resulting impacts in native population ecosystems not adapted to the presence of the species. This may also lead to the possible loss of some species.

What types of positive environmental impacts might occur because of this use?

No significant positive environmental effects are envisioned through use of transgenic goldfish containing an AFP gene.

What is the rationale for using the genetically engineered organism, including its advantages vis-a-vis alternatives?

The expected advantage of inserting an AFP gene into goldfish is development of a more cold-tolerant brood stock of aquarium and baitfish. Cold tolerance would be desirable in backyard garden ponds in colder climates because it would reduce winter mortality.

2. Relevant regulatory agencies, regulatory authority and legal measures

The regulatory process for genetically engineered goldfish would be similar to that described in the growth-enhanced Salmon case study (No. I) from the FDA and some of the Department of Interior statutes. Since there would not be net pens and goldfish are not marine, NMFS and ACE would not be involved.

EPA also has authority under the Toxic Substances Control Act to regulate animals, including genetically engineered animals, when they are used for a purpose not excluded under section 3 of the Act. Further information on TSCA regulations and biotechnology products can be found in this report in the Bioremediation and Biosensing using Bacteria case study and the EPA website.

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