Environmental Assessment for the Use of Hydrogen Peroxide in Aquaculture for Treating External Fungal and Bacterial Diseases of Cultured Fish and Fish Eggs

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1.0 EXECUTIVE SUMMARY

Introduction - This document provides an assessment of the probable environmental effects of hydrogen peroxide (H_2O_2) when used as a therapeutant in certain freshwater aquaculture operations. The assessment consists of (1) a summary of the scientific literature relevant to the natural occurrence, present uses, potential impacts, and environmental fate and effects of H_2O_2; (2) a risk characterization for certain aquaculture uses based on data from the scientific literature and results of a recent United States Geological Survey (USGS) survey detailing the projected use of H_2O_2 at public and private aquaculture facilities; and (3) tables, figures, and appendices which include toxicity data and risk results, relevant exposure and fate models, hatchery schematics, projected hatchery use data, hatchery discharge estimates, estimates of environmental dilutions of H_2O_2 immediately after discharge, and copies of supporting cited literature. Approval is sought for the use of H_2O_2 as a waterborne therapeutant in aquaculture for the control of mortalities caused by external fungal infections (saprolegniasis) on the eggs of all cultured freshwater fish, to control mortalities associated with bacterial gill disease (BGD) on all freshwater-reared salmonids, and to control mortalities associated with external columnaris disease (*Flavobacterium columnare*) on all freshwater-reared coolwater finfish and channel catfish. Environmental effects from uses or proposed uses of this compound in mariculture (e.g, on shellfish or on fish in net pens) are not addressed in this assessment.

Present uses - Hydrogen peroxide is commonly used around the world in a variety of commercial, industrial, medical, environmental, and personal hygiene applications. It is widely used in contemporary industry as a chemical intermediate in manufacturing processes, but the greatest volume of use is as a bleaching agent in the textile, pulp, and paper industries. The second highest volume of use will soon be in the environmental field for 1) treating municipal drinking and wastewater and industrial

process or wastewater; 2) *in situ* chemical remediation of contaminated groundwater, soils, and sediments; and 3) enhancing *in situ* bioremediation of contaminated groundwater, soils, and sediments.

As an aquaculture drug, H_2O_2 is considered to be of "low regulatory priority" by the U.S. Food and Drug Administration when used as a waterborne therapeutant at concentrations of 250-500 mg/L for the prevention and control of mortalities associated with external fungal infections (saprolegniasis) in cultured fish and their eggs. Hydrogen peroxide therapy also shows promise to control mortalities associated with external bacterial infections and to control parasitic infestations in cultured freshwater fish. Hydrogen peroxide is used outside the United States for treatment of external fungal and bacterial infections or parasitic infestations in cultured fish, particularly for sea lice control in marine salmon net pens in Canada, Scotland, Ireland, Norway, and Chile. The relative amount of H_2O_2 used for aquaculture purposes is virtually insignificant compared with the much larger amounts used in industrial, commercial, and municipal applications.

Aquaculture use model - For the purposes of this assessment, we model the potential environmental introduction of H_2O_2 following aquaculture use. A discussion of use-site characteristics, potential impacts, environmental fate and effects, and a risk characterization is presented for the model. The model is for intensive freshwater aquaculture operations only and includes discharge into either fresh or brackish waters.

Natural occurrence and degradation - Hydrogen peroxide exists naturally in almost all surface water. The formation of H_2O_2 results principally from ultraviolet light exciting humic substances (dissolved organic carbon, DOC) in water. The concentrations of H_2O_2 occurring naturally in freshwater are reported to range from 0.001 to 0.109 mg/L. Surface seawater concentrations of 0.001 to 0.0136 mg/L have been recorded. Higher concentrations typically occur in surface water containing high DOC. Very little H_2O_2 exists in deep marine or fresh water.

Environmental assessment of hydrogen peroxide for aquaculture use

Hydrogen peroxide naturally degrades to water and oxygen by various mechanisms, including chemical reduction and enzymatic (catalase and peroxidase) decomposition by algae, zooplankton, and heterotrophic bacteria. Microorganisms, especially bacteria, account for the majority of degradation, significantly more than all other chemical and biological mechanisms. The rate at which H_2O_2 decomposes in natural water can vary from a few minutes to more than a week, depending on numerous chemical, biological, and physical factors. The rapid degradation rates are primarily the result of microbial action, whether H_2O_2 is at naturally occurring concentrations or at concentrations 1000 to 10,000 times higher (from anthropogenic inputs during *in situ* chemical or bioremediation of groundwater). In eutrophic to somewhat oligotrophic fresh water, half-lives of 2 to 8 h are typical for H_2O_2 at naturally occurring levels, whereas the half-life may be several days or more in water devoid of microorganisms.

Environmental Fate – Upon approval, H_2O_2 will be available for use at concentrations of 50 to 1,000 mg/L to treat various diseases at aquaculture facilities. The primary mechanism for reducing treatment concentrations of hydrogen peroxide in exposure water, and in turn, its inherent toxicity before discharge to receiving water, is dilution. In most instances, dilution within the hatchery quickly reduces H_2O_2 concentrations 2- to 100,000-fold. Microbial and chemical degradation can also occur within the hatchery system, but the significance and rate of degradation relative to dilution is presently unknown because of a lack of appropriate data. For some facilities, the presence of dilution water in a holding pond that is large relative to hatchery flow rate or the deliberate reduction of hatchery flow to retain water before discharge may increase the relative contribution of degradation to reduce effluent H_2O_2 concentrations. Upon discharge to public waters, hatchery effluents are typically diluted again 2- to 100,000-fold. After discharge into most public waters, degradation by natural mechanisms would be

expected to proceed rapidly. In most circumstances, the concentration of H_2O_2 in the receiving water should be reduced to background levels within a few hours after total discharge from the hatchery.

Environmental effects - The toxicity of H_2O_2 to all organisms is concentration dependant. Fish and their eggs are relatively tolerant, and concentrations from 50 to 100 (fish) or 500 to 1,000 (eggs) mg/L are generally considered safe for brief exposures (<1 h for fish; <15 min for eggs). Other vertebrates and mammals are much more tolerant than fish. Microorganisms (i.e., bacteria, algae) and zooplankton present in aquatic ecosystems are generally less tolerant of H_2O_2 exposure than are fish or other vertebrates. The growth of some bacteria may be adversely affected by concentrations as low as 0.0034 mg/L and concentrations of 0.034 mg/L H_2O_2 may significantly decrease productivity in some algal populations after relatively long exposures. Toxicity to microorganisms from H_2O_2 discharged from aquaculture facilities is mitigated by: (1) the relatively short exposure times to potentially toxic concentrations of H_2O_2 due to rapid dilution and decay, with the microorganisms themselves being involved in degrading H_2O_2 when it is at nontoxic exposure durations or levels; (2) the ability of microorganisms to acclimate to repeated exposures of H_2O_2, and; (3) the ability of microorganisms to quickly rebound or repopulate from ubiquitous sources of microorganisms after exposures cease. Therefore, no long-term effects on populations or communities of microorganisms are expected to result from H_2O_2 use in aquaculture. Effects on terrestrial life are believed to be negligible and are not addressed in this environmental assessment.

Risk characterization and mitigation - According to the risk characterization conducted, in worst-case scenarios (highest allowable treatment levels combined with lowest subsequent internal dilution by hatcheries, and assuming no subsequent dilution or degradation in receiving waters), adverse effects or toxicity could potentially occur to populations of the most sensitive invertebrates and fish at more than 25% and 5% of intensive aquaculture facilities discharging into fresh water, respectively. In

further risk analysis, we concluded that discharge of treatment water containing H_2O_2 from aquaculture facilities into adjacent public waterways will not be a significant threat to organismal, environmental, or public health, provided that concentrations of H_2O_2 remain below 0.7 mg/L in receiving waters. This acute water quality "benchmark" was determined using EPA guidance for deriving water quality criteria. This benchmark should be included on the product label as a guide to authorities of the National Pollutant Discharge Elimination System (NPDES) to help them determine if effluent discharge limits are needed for hydrogen peroxide at individual aquaculture facilities taking into account site-specific factors and applicable state and federal water quality regulations.

Conclusion - On the basis of the toxicity and environmental exposure data examined and the risk characterizations conducted, we believe that the use of H_2O_2 as a waterborne therapeutant in intensive aquaculture operations for 1) the control of mortalities associated with external saprolegniasis on the eggs of all cultured freshwater fish; 2) the control of mortalities associated with bacterial gill disease on all freshwater-reared salmonids; and 3) the control of mortalities associated with external columnaris disease in all freshwater-reared coolwater finfish and channel catfish, constitutes no significant threat to the environment, the populations of organisms residing therein, or public health and safety when present at or less than 0.7 mg/L in receiving waters. It is not currently possible to assure that this concentration will be met at all locations using hydrogen peroxide throughout the United States, therefore, this acute water quality benchmark should be included on product labeling as a form of risk mitigation and as a guide to effluent regulatory authorities.

2.0 APPLICANT INFORMATION

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3.0 PROPOSED ACTION AND LABEL CLAIM

Approval is sought for the use of hydrogen peroxide (H_2O_2) as a waterborne therapeutant in aquaculture for the control of mortalities resulting from external saprolegniasis on the eggs of all cultured freshwater fish, for the control of mortalities associated with bacterial gill disease (BGD) caused by *Flavobacterium sp.* on all freshwater-reared salmonids, and for the control of mortalities associated with external columnaris disease (*Flavobacterium columnare*) in all freshwater-reared coolwater finfish and channel catfish. More specifically, the proposed label claim for H_2O_2 would include the following uses:

Treatment of external saprolegniasis in fish eggs - Hydrogen peroxide may be added to culture water to control mortality associated with external saprolegniasis on the eggs of all cultured freshwater fish. It may be administered once daily on consecutive or alternate days for 15 min as a flowing treatment at concentrations from 500 to 1,000 mg/L for freshwater-reared finfish eggs except channel catfish. Hydrogen peroxide concentrations may be applied to the eggs of channel catfish at concentrations of 750 to 1,000 mg/L. Therapy may be continued from fertilization through hatch, as needed (Table 1).

Treatment of bacterial gill disease on all freshwater-reared salmonids. - Hydrogen peroxide may be added to culture water to control mortalities associated with BGD on all freshwater-reared salmonids. Treatments may be administered at a concentration of 100 mg H_2O_2/L in a continuous-flow water supply or as a static bath in salmonid culture units for 30 min or at a concentration of 50 to 100 mg H_2O_2/L for 60 min once per day on alternate days for three treatments in salmonid culture units (Table 1).

Treatment of external columnaris disease on all freshwater-reared coolwater finfish and channel catfish. - Hydrogen peroxide may be added to culture water to control mortalities associated with external columnaris disease caused by *Flavobacterium columnare* on all freshwater-reared coolwater finfish and channel catfish. Treatments may be administered at a concentration ranging from 50 to 75 mg H_2O_2/L in a continuous-flow water supply or as a static bath in coolwater finfish or channel catfish culture units for 60 min once per day on alternate days for three treatments (Table 1).

4.0 SUBSTANCE IDENTIFICATION FOR SUBJECT OF PROPOSED ACTION

Tables 2 and 3 present the identification and physicochemical properties of the substance of the proposed action.

5.0 INTRODUCTION

5.1 Present Aquaculture Uses - Technical or food grade (35% active ingredient) H_2O_2 is presently considered a therapeutant of "low regulatory priority" by the U.S. Food and Drug Administration (FDA) to control mortalities associated with external fungal infections on all species and life-stages of fish when administered at concentrations ranging from 250 to 500 mg/L. The treatment concentrations on the proposed label range from as low as 50 mg/L for fish to a maximum of 1,000 mg/L for fish eggs (Table 1; Speare and Arsenault 1997, Rach et al. 1997c, 1998, 2000a, 2003, 2005b, Gaikowski et al. 1998, 1999, 2003, Lumsden et al. 1998). The disease claims presently included on the proposed H_2O_2 label include the control of mortality associated with saprolegniasis on freshwater-reared finfish eggs and the control of mortality associated with certain external bacterial infections on freshwater-reared finfish (Table 1). Preliminary studies and hatchery field trials with H_2O_2 suggested that H_2O_2 was also efficacious for the control of external parasitic infestations (Rach et al. 2000b) and fungal infections (Rach et al. 2005b) in a variety of cultured fish. Additional supporting efficacy data is being collected for these uses by aquaculture facilities under an Investigational New Animal Drug application (INAD #10-023) established by the Upper Midwest Environmental Sciences Center (UMESC, La Crosse, Wisconsin).

Hydrogen peroxide is also used internationally for treatment of external parasitic infestations of cultured fish, particularly to control sea lice (*Lepeophtheirus* and *Caligus* spp.) in marine salmon net pens in Canada, Scotland, Ireland, Norway, and Chile. Sea lice treatments are applied by enclosing the fish net pen in an impervious tarpaulin bag and adding H_2O_2 to achieve a treatment concentration of approximately 1,500 mg/L for about 20 min (Johnson et al. 1993). Environmental effects of this usage are not addressed by this environmental assessment, nor will the proposed label claim cover this usage.

The projected total amount of H_2O_2 to be used annually for aquaculture purposes in the near future is less than 500 tons in North America (personal communication with industry representatives). This amount is relatively insignificant (less than 0.1%) compared with the much larger amounts used by industrial, commercial, and municipal users (see section 5.3).

5.2 Need for Action - External fungal (saprolegniasis) and bacterial diseases present major problems in nearly all fish hatcheries in the United States, as well as in some brood-stock fish collected from the wild. These diseases can significantly diminish the ability of hatcheries to produce adequate numbers of healthy fish. If left untreated, the diseases can eradicate entire stocks of cultured fish or their eggs. As recreational and commercial fishing pressures continue to increase across the public water of the United States, the need for large quantities of high quality hatchery-raised fish also increases. Public and private aquaculture desperately needs safe, effective, and legal therapeutants to meet continually increasing public demands. The number of effective, legal therapeutants has diminished over the last 20 years. The use of malachite green, a highly effective and once heavily used therapeutant, is no longer allowed to treat fish because of concerns over teratogenicity, undesirable tissue residues, and user safety (Meyer and Jorgenson 1983; Alderman and Clifton-Hadley 1993). Formalin is used as a parasiticide on fish and as a fungicide for fish eggs, but it is not yet approved for use as a fungicide on fish. Copper sulfate and potassium permanganate are effective and inexpensive therapeutants for large-scale pond use, but approval of their use on fish is also pending. Because of its simple chemical composition and its relatively rapid degradation to water and oxygen, H_2O_2 seems to be a desirable therapeutant for aquaculture use.

5.3 Other Legal and Possible Uses - Global use of H_2O_2 was estimated at about 2.5 million tons annually in 1997, with 690,000 tons being used in North America alone (Institute of Applied Catalysis 1997). Although most commonly used as a bleaching agent in the textile, pulp, and paper

industries (Pardieck et al. 1992; Institute of Applied Catalysis 1997), it is also an often-used chemical intermediate in manufacturing processes (McGraw 1994). Various environmental applications will soon become the second largest market, surpassing use as a chemical intermediate. Environmental use accounted for about 12% of total usage in the United States in 1997 (Institute of Applied Catalysis 1997). Hydrogen peroxide is an effective disinfectant in treating municipal water supplies (Baldry 1983; Pedazhur et al. 1995) and municipal wastewater treatment effluents (Elizardo 1992). Hydrogen peroxide has been successfully used to oxidize and remove various toxic organic pollutants from (1) natural water (Beltran et al. 1996); (2) public drinking water (Baldry 1983; Fiessinger 1992; Pedazhur et al. 1995); (3) groundwater (McGuire and Davis 1988; Singh and Medlar 1992); (4) contaminated soils (Pardieck et al. 1992; Fagan 1994); and (5) contaminated river or lake sediments (Anid et al. 1993). It is used at low concentrations (milligrams per liter) for enhancing the *in situ* bioremediation (primarily microbial) of contaminated soils, sediments, and groundwater (Pardieck et al.; 1992 Fagan 1994). It is also used at higher concentrations (hundreds of milligrams per liter) for *in situ* chemical remediation by direct oxidation of contaminants in soils, sediments, or groundwater (Ravikumar and Gurol 1990; Tyre et al. 1991; Fagan 1994; Miller and Valentine 1999).

Hydrogen peroxide is widely used in human health as a disinfecting and sanitizing agent (McGraw 1994). It can be purchased over-the-counter in dilute form (3%) for personal or household use as a bleaching, cleansing, sanitizing, or antiseptic agent. It has been approved for use in a variety of food processing and preparation industries and as a food additive by the U.S. Food and Drug Administration (Pedazhur et al. 1995).

Hydrogen peroxide is an effective algicide (Kay et al. 1982). It has been suggested as a possible control measure for unwanted aquatic vegetation (Quimby 1981). Although not a current aquaculture

practice, H_2O_2 has also been shown to be safe and effective as a source of oxygen for the transportation or shipping of live fish (Taylor and Ross 1988).

5.4 Natural Occurrence - Hydrogen peroxide is formed and occurs naturally in aquatic environments. It exists at various natural levels in water as the result of several large-scale processes involving its natural production and decay. Hydrogen peroxide is produced naturally in surface water by a photochemical process involving dissolved light-absorbing organic matter and molecular oxygen (Cooper and Zika 1983; Szymczak and Waite 1989). More specifically, the primary means of natural production occurs when dissolved organic carbon (DOC) from humic substances is excited by ultraviolet light in freshwater and marine environments, and the superoxide anion (O_2^-) formed disproportionates and protonates to form H_2O_2 and oxygen (Cooper et al. 1994). A large number of organic compounds, such as glycerol, benzoic acid, aniline, tryptophan, and humic acid can serve as promoters of H_2O_2 generation by this mechanism (Draper and Crosby 1983).

Large scale natural production of H_2O_2 is believed to be limited to the depth of ultraviolet light penetration into water (Cooper et al. 1988), usually no more than 1 m (Cooper and Lean 1992; Scully et al. 1995). In shallow water, H_2O_2 is often distributed downward in the water column by various convective mixing processes, primarily wind-induced turbulence (Cooper et al. 1994). Hydrogen peroxide is usually not found in deep water under natural conditions (Johnson et al. 1989). However, laboratory experiments using deep water (250 m) and surface-water samples from the Mediterranean Sea showed similar H_2O_2 production rates of 1 to 10 nmol/L/h after sunlight-simulating illumination (Johnson et al. 1989). Thus, it seems that light penetration is the primary limiting factor. Rain can physically input notable quantities of H_2O_2 over highly localized areas (Cooper and Lean 1989, Willey et al. 1999, Yuan and Shiller 2000). Contributions can also come from dry atmospheric deposition, but these are usually minimal (Thompson and Zafiriou 1983). Hydrogen peroxide does occur naturally in the earth's

atmosphere, where the concentrations found vary with temperature, solar radiation, humidity, and the presence of precursors (CH_4 and CO) and inhibitors such as SO_2 and NO_x (Thompson et al. 1989).

Other chemical and biological means of H_2O_2 production in natural water are considered to be less important than photochemical production (Cooper et al. 1994). Hydrogen peroxide is produced naturally by some living organisms, including algae (Stevens et al. 1973; Zepp et al. 1987; Johnson et al.1989). Metabolites surrounding the organism may act as promoters of H_2O_2 formation (Moffett and Zika 1987; Mopper and Zika 1987). In the absence of light, H_2O_2 may be formed through the oxidation of iron and copper in groundwater, however the contribution to surface water H_2O_2 concentration from metal oxidation in groundwater is believed to be relatively insignificant (Holm et al. 1987). In both fresh water and marine water, a steady background concentration of H_2O_2 typically exists as a result of these large-scale processes of natural production, as well as equally large-scale natural decay processes (see detailed discussion in sections 7.1-7.2). The production processes are greatest in highly eutrophic freshwater bodies because of the larger concentration of DOC present, and lowest for the open ocean. Resulting equilibrium freshwater concentrations range from 0.001 to 0.109 mg/L (Cooper and Lean 1989; Cooper et al. 1989; Price et al. 1992; Moore et al. 1993) and surface seawater concentrations of 0.001 to 0.0136 mg/L have been recorded, mostly in coastal and estuarine areas (Zika et al. 1985; Johnson et al. 1989; Price et al. 1992; Fujiwara et al. 1993). Surface-water ambient concentrations are typically 50-100 times lower than that discharged in a typical hatchery situation.

6.0 ENVIRONMENTAL DESCRIPTION OF SITES OF INTRODUCTION

6.1 Freshwater Aquaculture Model - Freshwater aquaculture typically involves the production of various game, commercial, or threatened species of fish in intensive and extensive freshwater aquaculture between 4 and 35 °C. The raising of salmonids (trout or salmon) in fresh water is commonly referred to as cold-water aquaculture because it is conducted at water temperatures lower than

15 °C. Water pH is variable and typically ranges from 6.7 to 8.2. Optimal conditions for most salmonid species are water temperatures of 12 to 15 °C, approximately neutral pH, and high dissolved oxygen concentrations (above 5.0 mg/L; Piper 1982). The most common coldwater culture system used is an "intensive aquaculture" system where fish or eggs are cultured at relatively high densities in tanks, raceways, or egg incubators. Although many coldwater aquaculture facilities use single-pass culture systems in which water is used only once before discharge, water reuse, the process of passing water from one culture unit to the next lower unit (typically by gravity) before being discharged from the facility, is becoming increasingly common at coldwater facilities.

Freshwater aquaculture facilities using culture water temperatures greater than 15 °C are typically referred to as warmwater aquaculture. These operations usually involve the production of various game, commercial, or threatened species of fish in relatively warm fresh water. The culture water is often supplied from well or surface water sources. Culture water typically has a lower dissolved oxygen concentration than the water in cold-water aquaculture, and the pH is usually >7.0. The most common culture system used is an "extensive aquaculture" system, a pond environment where fish density is relatively low. Ponds are usually managed as static systems during most culture activities but are usually designed to have some flow-through capabilities (incoming fresh water and discharge capabilities). In some instances earthen raceways may be used, and for the purposes of this report we have grouped them with earthen ponds in the model because of the similar potential for therapeutants to enter sediments or groundwater.

The model also includes situations where earthen raceways or hatchery ponds may receive effluent water containing H_2O_2 from treatments administered to intensive culture units (tanks, raceways, or egg incubators) upstream. This occurs at hatcheries where all culture water flows from a single source (well or surface water) through a series of tanks, raceways, or ponds, and is eventually discharged into a

receiving water body. At these hatcheries, treatment water flows through the entire system and may affect nontarget fish and various other organisms.

Freshwater culture facilities may be owned and operated by Federal, State, tribal, or private entities. Fish are usually raised for eventual stocking into public water but may also be cultured for recreational fishing on-site, stocking into private ponds, or food fish sold to restaurants or supermarkets. A conceptual site model for the fate of H_2O_2 used at a typical freshwater aquaculture facility can be viewed in Figure 1. For a typical treatment, the model involves the simple addition of H_2O_2 to the water column of a tank, raceway, or egg incubator and adequate mixing to ensure uniform distribution throughout the water body of that culture unit. Hydrogen peroxide then reacts with a variety of living and nonliving substrates (i.e., oxidizeable matter) or is enzymatically reduced to water and oxygen (see sections 7.1-7.2), usually within a relatively short period after discharge. Treatment water is typically discharged from treatment tanks, raceways, or egg incubators and combined with other hatchery water for eventual release into receiving water. Many hatcheries use holding or settling ponds to dilute, detain, or stabilize discharge water for various reasons. The effluent water is eventually discharged directly into public water (streams, rivers, or lakes). Discharges to public water are usually subject to regulation and monitoring by state or local regulatory agencies. The facility design or layout for a typical freshwater hatchery is presented in Figure 2.

Although this EA is being written for discharge from freshwater aquaculture facilities, some may discharge into brackish water. Therefore toxicity data were collected and a risk assessment was determined for potential discharge from aquaculture facilities into brackish receiving water. Two types of facilities are identified: (1) private facilities that supply restaurants or supermarkets with food fish; and (2) public facilities that raise fingerlings to stock in public water.

6.2 Potential Impacts of Discharge into Fresh Water - There exists some potential for a variety of biological and chemical impacts to be realized if treatment water containing H_2O_2 is released from a freshwater fish hatchery into a receiving stream, river, or lake. For a typical freshwater hatchery situation, the release of a large amount of treatment water containing H_2O_2 into any type of freshwater body (stream, river, lake) may have some short-term effects on the resident biota. As we will discuss and document in the following sections (7.4-7.6), some bacterial, algal, zooplankton, and invertebrate populations could potentially be impacted by H_2O_2 discharge depending on the concentration and duration of exposure. However, H_2O_2 concentration at most of the sites surveyed (Appendix A, Section 7) is rapidly reduced to concentrations unlikely to cause detrimental effects to most aquatic organisms.

The chemistry of receiving water may also be impacted slightly depending on the ultimate fate of the released H_2O_2. Hydrogen peroxide may enzymatically degrade through the action of catalase, producing oxygen and water (Spain et al. 1989), or it may decompose through its actions as an oxidizing agent. As an oxidizing agent, it can work through several pathways including direct oxidation, peroxide-catalyzed oxidation, and free radical oxidation initiated by photochemical or metal-catalyzed decomposition (Watts et al., 1999; Zepp et al., 1987). A given amount of organic and/or inorganic matter would likely be oxidized (Bielski et al. 1985) if a release occurs (see sections 7.1-7.2). This oxidation has the potential to cause adverse effects if the material being oxidized is associated with a living organism and this, in fact, may account for most, if not all, of the toxicity of H_2O_2 to bacteria and other aquatic life. On the other hand, if H_2O_2 degrades enzymatically, this may lead to slight increases in dissolved oxygen concentrations in the water column. We proceed under the assumption that the production of oxygen by H_2O_2 in hatchery effluents after treatment would have only positive effects on individual organisms and the ecosystem at large.

Environmental assessment of hydrogen peroxide for aquaculture use

Hydrogen peroxide use in extensive aquaculture systems (i.e., large ponds with no or little water flow) will not be included on the present proposed label. Target animal safety data for fish are insufficient to allow therapy beyond a 60 min exposure (an exposure period that would be all but impossible to produce in extensive aquaculture). Although it is unlikely that H_2O_2 would be used in extensive aquaculture operations or pond environments, its effects would be quite similar, in general, to those of intensive aquaculture operations. Some obvious differences from intensive culture situations would be that (1) ponds are usually managed as static water environments and therefore, rapid discharge of H_2O_2 into public surface water following treatment is unlikely; (2) H_2O_2 will probably contact natural sediments in an earthen pond or raceway and, therefore, degrade more rapidly (see sections 7.1-7.2); (3) the organisms residing in ponds (and their sensitivity to H_2O_2 exposure) may differ somewhat, especially at higher water temperatures; (4) it is unlikely that exposure concentrations greater than 20 mg/L would be used in ponds because prolonged exposure to higher concentrations may be toxic to the target animals in a static system; and (5) the cost of treating a large volume of water with H_2O_2 would likely be prohibitive.

In a hatchery situation where H_2O_2 is introduced into an earthen pond or raceway, some potential exists for it to infiltrate the pore-water of the bottom sediments and possibly the groundwater. However, it is unlikely that the presence of dilute H_2O_2 in earthen ponds or raceways would lead to a significant release into adjacent sediments or groundwater because most ponds or raceways are constructed to hold water with minimal leakage. Bentonite clay, synthetic, or rubber liners impervious to water are commonly employed for this purpose. Depending on the concentration of H_2O_2 present, an effect on organisms in the bottom sediments could possibly be realized. Research conducted in this area, although limited, seems to indicate that significant long-term adverse effects would be unlikely. Decomposition in soil or sediments usually takes only minutes to a few hours, depending on initial H_2O_2

concentrations, the numbers and types of microorganisms in the soil, and the mineral content (Spain et al. 1989; Cooper and Zepp 1990; Pardieck et al. 1992; Cooper et al. 1994).

The potential for long-term substantial environmental impacts in groundwater or sediments after H_2O_2 treatment is extremely unlikely because of its rapid degradation by sediment, the relatively low treatment concentrations used, the relative impermeability of the pond wall liner, and the dilution by groundwater. Therefore, we have not further explored H_2O_2 contamination of groundwater or conducted a risk characterization for any organisms in sediment or groundwater.

6.3 Potential Impacts of Discharge into Brackish water - The potential impacts of H_2O_2 release from freshwater aquaculture into brackish water would be quite similar, in general, to those already discussed for fresh water. The notable differences would be that (1) in a brackish-water environment, there exists a greater potential for dilution upon discharge because the water volume of estuarine systems is generally greater than in most freshwater streams, rivers, or lakes; (2) the organisms residing in brackish water and their sensitivities to H_2O_2 exposure may differ somewhat from those residing in fresh water; and 3) the potential for rapid microbial degradation of H_2O_2 should be greater in brackish waters since these waters are generally more eutrophic than most fresh waters. Although salinity is unlikely to significantly alter the fate of H_2O_2, there is little information describing the effects of salinity on H_2O_2 toxicity to target and non-target species.

7.0 ANALYSIS OF ENVIRONMENTAL FATE AND EFFECTS

7.1 Fate of Aquaculture Discharge Containing H_2O_2 into Fresh Water - In freshwater aquaculture, hatchery effluent water containing dilute to trace concentrations of H_2O_2 may be released into local receiving streams, rivers, lakes, or estuaries. The fate of H_2O_2 released into such waters is simple compared with that of many anthropogenic pollutants or contaminants. As H_2O_2 is naturally produced or introduced by man into an aquatic environment, it is constantly decomposing into water and

oxygen (Spain et al. 1989), hydroxyl radicals (Watts et al., 1999), or directly reacting with oxidizable matter. The ambient concentration of H_2O_2 in a specific aquatic environment at any given time is the result of a dynamic equilibrium between large-scale natural production (see section 5.4) and the various natural degradation processes discussed here.

The typical products of H_2O_2 decomposition--water and oxygen--do not harm aerobic nontarget organisms in the environment. Nontarget organisms in small, confined water bodies could be affected by H_2O_2 itself, or by reactive hydroxyl radicals ($OH\cdot$) formed when it reacts with metal catalysts in the water such as iron (II) sulfate (Watts et al., 1999). This would need to occur before H_2O_2 decomposes or dilutes to background levels in the environment (see sections 7.4-7.6). No persistent contaminants are released into or accumulate in the environment as a result of H_2O_2 release into aquatic ecosystems (Spain et al. 1989; Boyd and Massaut 1999). Hydrogen peroxide discharged into public waters from intensive aquaculture should rapidly dilute and simultaneously decompose until natural background levels are reached, which in fresh water range from 0.001-0.109 mg/L (Johnson et al. 1987; Cooper et al. 1989; Cooper and Lean 1989; Price et al. 1992; Moore et al. 1993).

Time-to-degradation studies of H_2O_2 are scarce, but the few that have been conducted suggest that the rate of environmental degradation varies considerably. Degradation rates depend primarily on contact with enzymes (from microorganisms) and various catalytic materials (Moffett and Zika 1987; Spain et al. 1989; Cooper and Zepp 1990; Moffett and Zifarou 1993; Cooper et al. 1994). These researchers found that microorganisms were responsible for the bulk of H_2O_2 decay, with other mechanisms in the natural environment making relatively insignificant contributions. Cooper et al. (1994) examined the biologically mediated decay of H_2O_2 present in lake water by filtering water samples to remove various-sized organisms. They observed a half-life of 4.4 h for unfiltered water, 4.7 h for water filtered to 64 μm (zooplankton removed), 6.4 h for water filtered to 12 μm (large algae removed), 19.1 h

for water filtered to 1.0 μm (small algae removed), and 58.7 h for water filtered to 0.2 μm (bacteria removed). In a similar study Cooper and Lean (1989) observed the half-lives to be 7.8 h for unfiltered lake water, 8.6 h for water filtered to 5 μm, and 31 h for water filtered to 1 μm. No decay over 24 h was found in water filtered to 0.45 μm. The conclusion in both studies was that half-life decreases significantly as microorganisms increase.

In surface water, natural concentrations of H_2O_2 show an exponential decrease with time when experimentally deprived of sunlight (Moore et al. 1993). The half-life of H_2O_2 may range from several hours to several days or more, depending on the characteristics of receiving water (Herut et al. 1998). The longer half-lives occur in extremely clear, pristine, oligotrophic water that is nearly devoid of microorganisms, algae, and organic matter. Much shorter half-lives occur in nutrient-rich eutrophic water containing a larger biomass of microorganisms. Even at much higher than natural concentrations, decay can be rapid in surface water. Kay et al. (1984) observed that in culture water containing freshwater algae (*Raphidiopsis* spp), 94% of an initial 4.7 mg/L H_2O_2 treatment disappeared within 4 h after treatment. Water temperature, pH, alkalinity, and the presence of transitional metals and other catalysts can also have a minor influence on decomposition rates in natural water (FMC Corporation 1992).

A similar degradation trend also occurs in soil and groundwater. Decomposition in soil or groundwater typically takes minutes to several hours, depending on the concentrations of microorganisms present. This is true whether H_2O_2 is initially present at relatively low naturally occurring concentrations (Cooper and Zepp 1990; Cooper et al. 1994), or at much higher concentrations (several thousand fold) characteristic of *in situ* soil and groundwater remediation treatments (Spain et al. 1989; Pardieck et al. 1992). Difficulty has been encountered in maintaining H_2O_2 at the desired *in situ* treatment concentrations (above 100 mg/L) because of its rapid environmental decomposition (Morgan and Watkinson 1992). Although no direct studies are known on the effect of such breakdown to the microbial organisms

themselves, the literature seems to suggest that when microbial density and biomass are high compared with the concentration and total amount of available H_2O_2, or if oxygen demand is high, there are no adverse effects to microbial populations (Larisch and Duff 1997). In the opposite situation, short-term toxicity to microorganisms is evident, but acclimation and rebound of the populations always takes place (Balvay 1981; Spain et al. 1989; Xenopoulos and Bird 1997). No long-term or irreversible damage to a given microbial biomass as the result of such exposure has been recorded.

Rates of hydrogen peroxide decomposition are much slower in environmental systems with little or no microbial biomass present. In model subsurface systems composed of silica sand-goethite slurries, Watts et al. (1999) found half-lives for unstabilized H_2O_2 on the order of 4 to 5 days and sometimes more depending on the pH and iron concentration of the system. In these systems, potentially toxic hydroxyl radicals were generated through the mineral-catalyzed decomposition of H_2O_2.

Hydrogen peroxide use in extensive aquaculture systems will not be included on the present proposed label. Because of this, we did not further examine the fate, effects, or risks of using H_2O_2 in extensive aquaculture situations beyond the information presented in the following three paragraphs.

Using H_2O_2 in an "extensive" fish culture situation (ponds) should be a lesser risk to the surrounding environment than use in intensive aquaculture systems because the chemical is almost completely confined to the pond environment. In general, the fate of H_2O_2 applied in this situation would be similar to that already described, except that dilution would generally not be a significant factor. Unlike in tanks, egg incubators, and concrete raceways, degradation of H_2O_2 in earthen ponds is also facilitated by organisms and processes associated with pond sediments, in addition to microbes in the water column. Decomposition in the culture pond could take up to several days, based on results of studies on the stability of hydrogen peroxide in static aquaria (Tort et al., 2003). In these systems, in the

presence of aeration and/or organic matter, it took 48 to 72 hours for concentrations of hydrogen peroxide to decrease to below the level of detection when initially starting at 10 mg/L.

The use of H_2O_2 in extensive culture units may entail some initial toxicity to the most sensitive organisms, such as certain types of algae, bacteria, and zooplankton. The toxicity is likely to persist since there is no easy way to dilute the treatment by flushing water from the pond, as is the case, e.g., in raceway culture. However, aquaculture ponds are not public water, and short-lived adverse effects on algal and zooplankton populations in an aquaculture pond should have no effects on the surrounding natural environment, and therefore pose no threat to environmental or public health. Boyd and Massaut (1999) conducted a study to determine the risk associated with the use of various chemicals in pond aquaculture; they concluded that H_2O_2 was a "low risk" compound and that the use of oxidants in general (including H_2O_2) poses no environmental or public health risks.

Any use of H_2O_2 in extensive aquaculture situations would have to be conducted as an "extra-label use" under the supervision of a veterinarian (assuming the eventual withdrawal of LRP use after the initial label claim is approved). The veterinarian would be exclusively responsible for all aspects of the application, including the discharge of treatment water into the environment and any subsequent effects. Additionally, the user may be required to ensure the discharge is authorized by their state or federal discharge permitting agency.

Only one study of actual H_2O_2 discharge concentrations from a hatchery is available from the literature. Saez and Bowser (2001) conducted a H_2O_2 fate study at a freshwater hatchery in upstate New York. They administered roughly 3,400 grams of H_2O_2 over a 60 min period to an approximately 4,200 L raceway that had a flow of 113 L/min during each of two trials. This application rate (500 mg/L) simulated the simultaneous treatment of five similar-size raceways in a hatchery at 100 mg/L. Fish were not present in the raceway during treatment. The actual discharge for the entire hatchery was 3,907 L/min

during trial 1 and 5,072 L/min during trial 2 (Saez 1999). Stream flow was 8,840 L/min during trial 1 and 6,907 L/min during trial 2 (Saez 1999). Hydrogen peroxide concentrations were measured in the raceway and at the hatchery outflow pipe every 20 min over the first 2 h then every hour or at multi-hour intervals to 96 h after treatment. Midstream concentrations downstream from the hatchery were also measured. This facility did not have a detention pond at the time the study was conducted.

The maximum mean concentration (2 trials, three replicates per trial) at the hatchery outflow pipe at the end of the 1-h treatment was 9 mg/L, compared to approximately 400 mg/L in the raceway at 1 h. Hydrogen peroxide decay curves (concentration vs. time) for the treated raceway and the outflow pipe were very similar in shape and nearly overlapping in time. The half-life of elimination from the treated raceway was 28.4 min, indicating rapid flushing. From the information presented, the difference between the raceway and outflow-pipe concentrations indicate that degradation was insignificant in the reduction of H_2O_2 at this facility, as the theoretical dilutions based on hatchery versus raceway flow for trials 1 and 2 (about 35-fold and 45-fold, respectively) were similar to the dilution observed between the raceway and outflow pipe. The influence due to degradation that fish (and fish feces) might have had on H_2O_2 hatchery discharge concentrations if fish had been present in the raceway is not known.

Reportable concentrations were found at the hatchery outflow pipe at 60 and 120 min (mean of 9 and 2 mg/L, respectively) while samples collected at $\geq$180 min were at or below the detection limit 1.0 mg/L of the method used by Saez and Bowser (2001). The reduction in concentration at the outflow pipe is assumed to have been solely due to dilution and passing of the treatment slug through the facility as the degradation rate at this facility is presently unknown. The midstream concentrations at 60 min (3 mg/L) indicated a 3-fold dilution by stream water 7.5 m downstream from the hatchery outflow pipe. This is reasonable given the ratios between discharge and stream flows during trials 1 and 2.

The observed 24-h discharge average concentration for this facility could be calculated from the following: [1 h x 9 mg/L + 1 h x 2 mg/L + (22 h x 1 mg/L) / 24 h] or 1.4 mg/L, substituting the method detection limit of 1-mg/L for time points ≥180 min post treatment. Background measurements collected from the facility water supply and the receiving water had a background reading of 1-mg/L according to the analytical methods used (Saez 1999). Applying the 24-h average concentration calculation methods described in section 8.1 to the data from Saez (1999), the expected 24-h average concentration would be 0.5-0.6 mg/L. Although about half the estimate from their reported results, the discrepancy is likely the result of the 1-mg/L detection limit for the test method used. Most of the samples collected more than 180 min after treatment would likely have approached zero instead of the 1-mg/L used in the calculation. Use of a holding pond would likely have substantially reduced the observed discharge concentrations because of both dilution and degradation.

7.2 Fate of Aquaculture Discharge Containing H_2O_2 into Brackish Water- For discharge into brackish water, treatment water containing dilute to trace concentrations of H_2O_2 may be released into a receiving estuary. The fate of the released H_2O_2 would be similar to the scenario described for the freshwater site, typically involving rapid dilution and degradation to substantially lower concentrations. Hydrogen peroxide discharged would degrade to water and oxygen; no persistent contaminants would be released into the environment or accumulate in aquatic organisms. The degradation rate of H_2O_2 discharged into brackish water may be greater than into fresh water because estuaries are typically warmer and more eutrophic than fresh receiving waters. Additionally, the volume of the receiving water would typically be much greater for brackish water, and should result in greater dilution and dispersion of H_2O_2 after discharge. Salinity should not be a factor in the fate of H_2O_2 (Moore et al. 1993).

The concentrations of H_2O_2 naturally occurring in seawater are reported to range from 0.001 to 0.0136 mg/L (Zika et al. 1985; Johnson et al. 1989; Price et al. 1992; Fujiwara et al. 1993). The lowest

concentrations are in the open ocean, where water has the lowest dissolved organic carbon concentration. The degradation rate for H_2O_2 in brackish water would primarily depend on the factors previously described for fresh water, with microbial action being the dominant degradation mechanism. At ambient temperatures and concentrations, the degradation rate of naturally occurring H_2O_2 in seawater varies widely from 0.00034 to 0.017 mg/L per h (Johnson et al. 1987). The half-life of naturally occurring H_2O_2 in seawater samples from the Bay of Biscay filtered to 0.2 µm (microorganisms removed) was 60 h (Petasne and Zika 1987). Florence and Stauber (1986) observed relatively rapid degradation of H_2O_2 added to seawater samples while testing its toxicity to algae at concentrations similar to our predicted discharge concentrations from hatchery effluent. An initial exposure concentration of 2.72 mg/L degraded to just 0.19 mg/L in 24 h and to < 0.1 mg/L in 48 h when the initial algal cell densities were approximately 3 x 10^4 cells/ml.

The recommended maximum treatment concentration for H_2O_2 is 100 mg/L for fish and 1,000 mg/L for fish eggs. The combination of dilution and degradation should ensure that concentrations 1,000- to 1,000,000-fold lower will be reached within a few hours after discharge into brackish water.

7.3 Selection of Receptors of Interest - In general, the criteria for selection of biological receptors of interest (ROI) include two factors as specified in U.S. Environmental Protection Agency guidance (U.S. EPA 1997 and 1998) for determining "key organisms" in an aquatic food web: (1) resident communities or species exposed to the highest chemical concentrations in sediments or surface water; and (2) species or functional groups considered to be essential to, or indicative of, the normal functioning of the affected habitats. Other selection factors may include the organism's trophic level, feeding habits, abundance, and the availability of appropriate life history and toxicity data.

For this environmental assessment we chose to proceed under the following three assumptions. First, terrestrial vegetation and wildlife were not considered for evaluation here because we believe the

predominant influences of chemical introduction on the surrounding ecosystem occur only through aquatic pathways where direct contact with H_2O_2 occurs. Second, the only exposure pathway that was considered is that of direct contact of an organism's outer surface (integument, gills, or outer cell wall) with H_2O_2 in the water column. Third, we did not consider H_2O_2 toxicity based on possible ingestion by organisms, nor do we believe there are any other significant routes of exposure (e.g. bioaccumulation).

The receiving waters of most aquaculture sites are diverse and healthy ecosystems that support a variety of aquatic and terrestrial life. It would be unrealistic, however, to conduct a complete risk assessment for all organisms possibly affected, and we therefore examined effects data for four groups of ecologically important and representative organisms or receptors of interest. Within the aquatic ecosystem, the emphasis of this assessment was on selected species of algae, invertebrates, fish, and bacteria. By selecting these groups, the analysis included data for organisms from three separate and important trophic levels: primary producers (algae, some bacteria), primary consumers (invertebrates), and secondary or tertiary consumers (fish). Populations of many bacterial species are also important in ecosystem nutrient cycling, while others are used in municipal sewage treatment plants. In addition, data from the scientific literature should usually be available for organisms from these groups, a consideration that is essential for risk assessment.

Data on the effects of H_2O_2 from the scientific literature were selected and are presented in the sections following (sections 7.4.-7.6). Toxicity data were selected for presentation according to the following criteria: (1) data chosen were from peer-reviewed studies that were judged to have been conducted in a scientifically sound manner and whose methods roughly conformed to those outlined by the American Society of Testing and Materials (ASTM 1989); (2) when toxicity data for various life-stages of an organism were reported in a given study, we reported only data for the most sensitive life-stage; (3) when toxicity data were presented for various exposure durations in a given study, we chose a

duration that was the most likely to occur from an actual hatchery discharge; (4) when toxicity data were presented for a given test organism at various water temperatures, we reported only data for the temperatures listed as standard test water temperatures for that organism, according to standard methods (ASTM 1989); and (5) we chose toxicity data that allowed us to present or easily derive lethal concentration point estimates (LC_0s, LC_{50}s, or LC_{100}s) or No Observed Effect Concentrations (NOECs) from the mortality data presented.

7.4 Effects of Discharge into Fresh Water on Receptors of Interest - The maximum recommended treatment concentration of H_2O_2 is 100 mg/L for fish and 1,000 mg/L for fish eggs. Dilution and degradation to concentrations much lower than this (100- to 100,000-fold) should occur within a few hours after treatment and discharge at most freshwater aquaculture sites. From the standpoint of receiving waters, discharges into small oligotrophic streams and ponds receiving treatment effluents would likely be a worst-case freshwater scenario. Discharges into rivers and medium to large sized lakes would be of the least concern, because dilution and degradation of H_2O_2 to nontoxic levels would occur relatively quickly. In most rivers and streams, mobile and nonmobile organisms (algae, invertebrates, fish, bacteria, and others) would be exposed to H_2O_2 for a relatively brief time.

As was discussed in sections 6.2-6.3 (potential impacts), the release of water containing even dilute concentrations of H_2O_2 into an aquatic environment may potentially impact a wide variety of flora and fauna on a short-term basis. The discharge of H_2O_2 into surface water may especially entail some initial toxicity to the most sensitive organisms, such as certain types of algae and bacteria. We present data available from the scientific literature on the effects of H_2O_2 to ROI that are likely to reside in the receiving water at aquaculture sites.

7.4.1 Algae - Many species of algae reside within all likely receiving waters of aquaculture discharge (streams, rivers, lakes). They are primary producers and serve as the basis for the

entire food web in most aquatic ecosystems (Smith 1950). Any significant negative effect on resident algae populations may likewise have a secondary negative effect on many other organisms higher on the food chain. Table 4 summarizes the toxicity data available for algae that may be found in fresh water.

Hydrogen peroxide is a natural growth inhibitor for most algae if concentrations are high enough. Nearly all species of algae exposed to H_2O_2 in toxicity tests appear to be adversely affected. The degree of effect is both concentration and time dependent. Kay et al. (1982) evaluated H_2O_2 as a potential algicide in freshwater aquaculture. At a concentration of 9.9 mg/L, the chlorophyll level of a dense bloom of *Anabaena* spp. was reduced to 20% of that observed for the control after 24 h. "Threshold toxicities" (the lowest exposure concentration to elicit an adverse effect) under laboratory conditions were 6.8 to 10.0 mg/L for *Ankistrodesmus* spp., <3.4 mg/L for *Raphidiopsis* spp., and <1.7 mg/L for *Microcystis* spp. after 24-h exposures. Hydrogen peroxide exposures of 24-h at concentrations of 17, 6.8, and 1.7 mg/L reduced the optical densities of chlorophyll extracts to <5% of that observed for the controls in *Ankstrodesmus*, *Raphidiopsis*, and *Microcystis*, respectively. Because these were the lowest concentrations tested, the "threshold toxicities" were also nearly LC_{100}s. The 24-h NOEC (no observable effect concentration, or the highest concentration that elicited no adverse effect on primary production) for three phytoplankton, *Dinobryon* spp., *Ochromonas* spp., and *Chrysochromulina* spp., in a mesohumic lake (Lac Cromwell, Quebec, Canada) ranged from 0.34 to 34 mg/L (Xenopoulos and Bird 1997). The green algae *Scenedesmus subspicatus* was relatively insensitive to H_2O_2, exhibiting a 7-d EC_{03} for proliferation of 7.3 mg/L (Trenel and Kühn 1982). By contrast, the 1.5-h and 22-h EC_{50} (effective concentrations for eliciting a given effect in 50% of test organisms) for nitrogen fixation by the blue-green algae *Aphanizomenon flos-aquae* were 3.4 mg/L at high cell densities and 0.9 mg/L at low cell densities, respectively (Peterson et al. 1995, see Appendix D for study summary). One of the valued blue-green algae (used as a human nutritional supplement), *A. flos-aquae* can also generate geosmin, an

undesirable odor compound in drinking water. It is the most sensitive reported freshwater algae species to H_2O_2, based on nitrogen fixation. Nitrogen fixation is not a lethal endpoint. Therefore we are not using these results as key data point for algal risk assessment.

Even though relatively low concentrations of H_2O_2 may adversely affect the growth of a small percentage of the total algae in receiving water temporarily, it is not likely that any long-term adverse effects on algal populations would be realized. Environmental exposures are likely to be relatively brief and pulsed, especially in large-volume fresh waters, compared with the prolonged, continuous exposures associated with the laboratory studies. In most circumstances, the dilution by receiving water would be considerable and degradation significant, thus reducing H_2O_2 concentrations rapidly within a few hours (see sections 7.1-7.2 and also discussion in section 8.1.2 of H_2O_2 degradation in water from Jack's Lake). Algae initially affected by brief exposures are likely to rebound quickly after the exposure ends, and long-term effects such as altered species composition or population densities would not be expected (Balvay 1981; Xenopoulos and Bird 1997). Freshwater algae have resistant spores or cysts (Smith 1950) that would likely survive a short exposure to H_2O_2 and then reproduce quickly once the H_2O_2 had degraded. Algae and algal spores are ubiquitous in receiving waters and air (Smith 1950). They would quickly repopulate any affected waters, especially in flowing waters where the upstream input of drifting organisms into an affected area would be constantly occurring.

7.4.2 Invertebrates - Many different species of nektonic (waterborne) and benthic (bottom dwelling) invertebrates reside within all likely receiving waters of freshwater aquaculture discharge (streams, rivers, lakes). As primary or secondary consumers, they represent an integral part of the food web (Pennak 1978). These organisms are often the primary food of planktivorous fish or the juveniles of larger piscivorous game fish. Benthic invertebrates can be an especially useful indicator of

environmental quality over long periods because of their limited mobility (Pennak 1978). Table 5 contains data on the toxicity of H_2O_2 to various invertebrates that may be found in fresh receiving waters.

Several researchers have investigated the toxicity of H_2O_2 to *Daphnia* spp., a recognized, standard, representative aquatic invertebrate appropriate for characterizing chemical toxicity (ASTM 1989). Gannon and Gannon (1975) found that *Daphnia pulex* could be immobilized by exposures of 3,000 mg/L H_2O_2 for 5 min. Shurtleff (1989) calculated a 48-h LC_{50} value (the lethal concentration to 50% of test organisms after 48 h exposure) of 2.4 mg/L for *Daphnia pulex* exposed to H_2O_2. The sensitivity of a similar but larger daphnid, *Daphnia magna*, was determined by Bringmann and Kuehn (1982). They determined the 24-h EC_0, EC_{50}, and EC_{100} values for immobilization after 24-h exposures to be 3.8, 7.7, and 15 mg/L, respectively. Other endpoints for *D. Magna* have been reported (Trenel and Kühn 1982, USEPA 2000, see Table 5), but we were unable to obtain reports or abstracts of the original studies. The 48-h EC_{50} for four *Ceriodaphnia dubia* studies ranged from 8.1-11.2 mg/L using four different Pennsylvania surface waters (effluent from two hatcheries and water from two receiving streams, Analytical Laboratory Services 2003). *Ceriodaphnia dubia* mortality was not observed in 3 of the 4 waters tested when exposed to 3.75 mg/L H_2O_2 for 48-h (the fourth water was evidently not tested at 3.75 mg/L).

The aquatic invertebrate *Gammarus* spp., an amphipod commonly known as "scuds," are another standard aquatic invertebrate used for characterizing toxicity (ASTM 1989). *Gammarus* spp. were found to be moderately sensitive to H_2O_2 (Kay et al. 1982), with an estimated 96-h LC_{50} value of 4.42 mg/L. In tests with the larvae of other common aquatic insects, Kay et al. (1982) found that *Chironomid* spp. larvae and *Stratiomys* spp. larvae exhibited no mortality even after exposures to 218 mg/L for 96 h. Kay et al. (1982) determined the sensitivity of a freshwater snail (*Physa* spp.) to H_2O_2. They estimated the

96-h LC_{50} value at 17.7 mg/L. Kay et al. (1982) also determined that exposures of 170 mg/L H_2O_2 for 96 h caused no mortality in dragon fly naiads (*Pachydiplx longipennis*).

Hydrogen peroxide concentrations of 30, 20, and 12 mg/L, at 22 °C, resulted in 100% mortality of zebra mussels (*Dreissena polymorpha*) after 72, 120, and 408 h, respectively, and exposures to 30.0 and 20.0 mg/L at 12 °C resulted in 100% mortality after 576 and 684 h, respectively (Martin et al. 1993). After 10 or 70 h of exposure at 22 °C, the approximate LC_{50}s were 30 or 6 mg/L, respectively. Approximate NOEC exposure concentration by exposure duration combinations were 4.5 mg/L at 48 h or 1.5 mg/L at 120 h. Zebra mussels are generally considered an invasive, nuisance species in the United States; however, they are the only mussels for which we have data, and the data may have some value because zebra mussels are similar in some ways to native mussel species.

A 21-d chronic study of H_2O_2 toxicity to *Daphnia magna* was conducted at UMESC under flow-through conditions with nominal exposure concentrations of 0, 0.32, 0.63, 1.25, 2.5 and 5.0 mg/L. The full study (Meinertz, et al. 2005) is included in the EA submission as Appendix E. The study is summarized in detail in Appendix D. *Daphnia magna* is considered to be a sensitive aquatic invertebrate and is recommended by the American Society for Testing and Materials (ASTM) for conducting macro invertebrate acute and life-cycle toxicity tests (ASTM Designation E 1193-97 1997, Standard Guide for Conducting *Daphnia magna* Life Cycle Toxicity Tests). The continuous exposure regimen selected represents the worst-case exposure scenario that could occur during intensive aquaculture operations, one that would occur only rarely, if at all (see discussion below in this section). The summary data from Meinertz et al. (2005) are presented in Table 6 and the major study conclusions are that H_2O_2 concentrations of:

$\leq$ 1.25 mg/L did not increase the probability of death;

$\geq$ 0.32 mg/L reduced daphnia growth relative to untreated controls;

≤ 1.25 mg/L had no effect on the time to first brood production;

≤ 1.25 mg/L had no effect on the number of broods produced;

≤ 0.63 mg/L had no effect on the total number of young produced.

The study, conducted in an aqueous medium not typical of the receiving waters of most fish hatcheries (UMESC well water), provides an example of the tendency of H_2O_2 to quickly degrade even in waters containing minimal amounts of oxidizable organic matter (i.e., only daphnia feed). In this study, the concentrations of hydrogen peroxide were found to be extremely unstable during and after the addition of daphnia food to individual test chambers during continuous flow testing. In preliminary studies, hydrogen peroxide concentrations in one test chamber from each test group (0.36, 0.68, 1.42, 2.73, and 4.05 mg/ L) were monitored during presentation of a simulated feeding regimen in order to assess he magnitude and length of depression of hydrogen peroxide concentrations in the test chambers over a feeding event. The hydrogen peroxide concentrations in test chambers were measured before a ration of food was dispensed into test chambers, and 30, 60, and 85 min thereafter. A second ration of food was dispensed into test chambers 95 min after the first ration was dispensed. The hydrogen peroxide concentrations in test chambers were measured 30, 60, 120, and 180 min after the second ration was dispensed. The hydrogen peroxide concentrations in all test groups fell below 65% of initial concentrations within 85 min after the first ration was dispensed. Hydrogen peroxide concentrations recovered only to within about 70% of the initial concentrations 180 min after the second ration was dispensed. Because of the sensitivity of hydrogen peroxide stability to the daphnia food ration, the flow through the daphnia test chambers had to be increased from 4 to ~36 volume-exchanges/d to maintain H_2O_2 at 70-100% of the nominal concentration during the continuous-flow chronic exposure study. Even at this flow rate, the organic matter resulting from the introduction of daphnia feed caused a rapid reduction of H_2O_2. The microorganisms and organic matter present in a hatchery settling pond or in the

final receiving water (lake, pond, river/stream, or estuary) would therefore likely provide an environment that would even more rapidly degrade H_2O_2 released from aquaculture facilities.

Meinertz et al. (2005, Appendix E) present an adequate well-controlled *Daphnia magna* chronic toxicity study and there are no apparent reasons to suspect that its results are not valid for *D. magna* exposed to H_2O_2 in high-quality well water at a high flow rate. However, Analytical Laboratory Services (2003) reported H_2O_2 48-h EC_{50}s for *Ceriodaphnia dubia* ranging from 8.1-11.2 mg/L in four Pennsylvania surface waters. Shurtleff (1989) reported 48-h LC_{50}s for *Daphnia pulex* of 1.0 or 2.4 mg/L following exposure to H_2O_2 in ultrapure, Milli-Q reconstituted water or in a 50:50 mixture of distilled and lake water, respectively. Shurtleff (1989) discounted the lower LC_{50}s obtained in reconstituted water for H_2O_2 and sodium percarbonate because of the "detrimental" nature of the high purity water to both test and control daphnia. With respect to the 21-d chronic exposure time used for the Meinertz et al. study, it is possible that H_2O_2 administrations due to product use could occasionally occur that could result in a time-averaged discharge of 1 mg/L and greater over a 21-d period according to simple hatchery calculations (mass of H_2O_2 used per day / hatchery water discharge volume per day) for a worst-case scenario (see section 8.1). These calculations assume no degradation of H_2O_2 prior to discharge. Breakdown in hatchery waters should be at least as rapid as it was in the laboratory situation, especially if a settling pond is present. Except for the pulsed discharges following treatment, replenishment of H_2O_2 would not occur. Identification of a discharge scenario where a hatchery could discharge a constant 1 mg/L of H_2O_2 for 21 d in effluent is extremely unlikely because of the pulsed use pattern and internal dilution, the mass of chemical required, and the amount of oxidizable material present in any hatchery effluent stream.

Even though relatively low concentrations of H_2O_2 may have temporary sublethal adverse affects, it is not likely that any long-term adverse effects on populations or health of invertebrates would

be realized. Environmental exposures are likely to be relatively brief, especially in larger volume receiving water bodies, compared with the prolonged exposures associated with the laboratory studies. In most circumstances, the dilution by large receiving water bodies would be considerable and degradation significant, thus reducing H_2O_2 concentrations rapidly within a few hours (see sections 7.1-7.2). Invertebrates initially affected by brief exposures would probably rebound quickly after exposure ended, and resident populations would probably not exhibit adverse long-term effects with respect to species composition or numbers. It is also important to note that most freshwater zooplankters (like Daphnids) have highly resistant resting stages (Pennak 1978) that are designed to withstand periods of drought or other environmental stresses. This allows these organisms to transition from a resting to an active stage and repopulates the aquatic environment once the stress has passed.

7.4.3 Fish - Many species of fish may reside within waters receiving H_2O_2 from freshwater aquaculture discharge (streams, rivers, lakes). They may be primary, secondary, or tertiary consumers depending on species and life stage (Lee et al. 1980). They are important ecologically as a food source for higher level carnivores and some have great value to mankind both commercially and for recreation. Fish are good indicators of overall aquatic environmental health because they usually live longer than other aquatic life forms, are higher in the food chain, and are, therefore, susceptible to biomagnification of contaminants and population fluctuations of prey. Table 7 summarizes the toxicity data available for fish that may be found in fresh receiving waters of hatchery discharges. Table 8 includes data on several species of anadromous salmonids also found in fresh receiving waters.

Rach et al. (1997c) investigated the toxicity of H_2O_2 to various species of freshwater fish and observed that most species are quite tolerant to exposure. Rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), and lake trout (*Salvelinus namaycush*) fingerlings showed no mortality at exposure concentrations of 283, 283, and 1,132 mg/L, respectively, after 45-min exposures, every other day, for

four consecutive treatments. In additional tests with fathead minnows (*Pimphales promales),* bluegill sunfish (*Lepomis macrochirus*), and channel catfish (*Ictalurus punctatus*) fingerlings, no mortality was observed for exposures of 566, 1,132, and 1,132 mg/L, respectively, after 45-min exposures. Walleye (*Sander vitreum*) were the most sensitive species tested, with two fish mortalities being observed even at the lowest exposure concentration (113 mg/L).

Rach et al. (1997c) also conducted tests on the same species of fish using 15-min exposures, for which the NOEC values for mortality were approximately 2 to 3 times as great (1,132 to 3,396 mg/L). All of the above treatments were "dip" treatments, where fish were immersed in treatment water for the desired exposure period, then removed and placed into well water for recovery immediately after the exposure period. In the same study, the 24-h LC_{50} values for rainbow trout, channel catfish, and bluegill sunfish were 48, 63, and 81 mg/L, respectively.

Gaikowski et al. (1999) determined the acute toxicity of longer exposures (60 min), administered every other day, for three consecutive daily treatments, to the fingerlings and fry of various freshwater fish. They found that the freshwater species tested--rainbow trout, lake trout, Atlantic salmon (*Salmo salar*), and largemouth bass (*Micropterus salmoides*)--could be safely treated for 60 min at exposure concentrations as high as 150 mg/L without mortality occurring. All muskellunge (*Esox masquinongy*), walleye, bluegill sunfish, channel catfish, yellow perch (*Perca flavescens*), pallid sturgeon (*Scaphirhynchus albus*) fingerlings, fathead minnow fingerlings, white sucker fingerlings (*Catostomus commersoni*), and northern pike fry (*Esox lucius*) could be treated for 60 min at exposure concentrations as high as 100 mg/L without mortality occurring. Northern pike fingerlings and white sucker, yellow perch, and fathead minnow fry could be treated for 60 min at $\leq$50 mg/L without adverse effects. These exposures were static bath treatments, and the treatment was gradually flushed-out with well water at the end of the 60 min exposure period. The majority of the H_2O_2 was eliminated within 60 min; however,

some additional exposure beyond 60 min occurred, and this may have lead to an NOEC estimate for mortality that is artificially low.

Other researchers have studied the toxicity of H_2O_2 to various species of salmonids. McKee and Wolf (1963) reported that 48-h exposures of greater than 40 mg/L caused mortality in rainbow trout. Arndt and Wagner (1997) estimated that the 1-h LC_{50} values for rainbow trout fry and fingerlings were 322 and 329 mg/L, respectively, at 15 °C. They also conducted similar tests with cutthroat trout (*Oncorhynchus clarki*) and estimated that the 1-h LC_{50} values at 15 °C for fry and fingerlings were 377 and 506 mg/L, respectively. Speare and Arsenault (1997) reported that twice-weekly H_2O_2 treatments of 200 mg/L for 60 min administered to juvenile (6.2 g) rainbow trout over seven weeks caused no change in fish weight or gill histology compared to untreated controls. Growth was suppressed during the first 3 weeks of treatment, but was followed by a compensatory growth phase the final 4 weeks of the study.

Kay et al. (1982) estimated that the 96-h LC_{50} for channel catfish was 37 mg/L. Clayton and Summerfelt (1996) estimated that the 1-h LC_{50} for walleyes was 145 mg/L and identified them as the most sensitive freshwater fish species they tested. Their estimates are probably artificially low because H_2O_2 was not rapidly flushed from the system after treatments ended; thus the actual time that fish were exposed to chemical was greater than the 1 h reported.

The effects of H_2O_2 on certain aspects of fish biochemistry have also been studied. Hydrogen peroxide did not affect glutamic oxalacetic transaminase activity in the blood plasma of white suckers after in vitro exposure to 2,000 mg/L for 2 weeks, but the lactic dehydrogenase activity was inhibited (Christensen 1971). Olson and Christensen (1980) observed that H_2O_2 did not have an effect on the activity of acetylcholinesterase prepared from the muscle of fathead minnows.

7.5 Effects of Discharge into Brackish Water on Receptors of Interest - As was the case for fresh receiving waters, the release of water containing even dilute concentrations of H_2O_2 into brackish

water may potentially affect a wide variety of flora and fauna. The recommended maximum treatment concentration for H_2O_2 is 100 mg/L for fish and 1,000 mg/L for fish eggs. Although our survey of hatcheries did not provide data on discharge into brackish water, we assume that most brackish receiving waters would have a combination of dilution and degradation similar to or greater than that of fresh receiving waters. Based on this assumption, H_2O_2 treatments would be diluted by 100- to 100,000-fold or more within a few hours after discharge into most brackish receiving waters. We present here data available from the scientific literature on the effects of H_2O_2 to ROI that are likely to reside in brackish receiving water.

7.5.1 Algae - Many species of algae may reside in brackish water that may receive some aquaculture discharge. They are primary producers and form the base of the entire food web of the estuarine ecosystem (Remane and Schlieper 1971; Gross 1977). Any significant deleterious effect on resident algal populations would result in negative effects on many other organisms. Some species of freshwater algae, for which we have already presented effects data (see section 7.4.1), may also reside in brackish water (Remane and Schlieper 1971; Gross 1977). Those data are not re-presented here. Table 4 contains the available data on the toxicity of H_2O_2 to various algae that may be found in brackish or marine waters.

In brackish-water or marine environments, H_2O_2 may at times act as a natural algal growth-inhibitor. Florence and Stauber (1986) observed that a 72-h exposure of 0.85 mg/L H_2O_2 caused a 50% decrease in the growth rate of the marine unicellular diatom *Nitzschia closterium*. They also observed that the 72-h NOEC for growth was less than 0.68 mg/L. Cysts of *Polykrikos schwartzii*, a red tide dinoflagellate, would not germinate after exposure to H_2O_2 at 100 mg/L for 48 h (Ichikawa et al. 1993). Cysts of *Alexandrium catenella* and *A. tamarense*, dinoflagellates which produce the toxin that causes paralytic shellfish poisoning, showed a fatal change of appearance after exposure to 30 mg/L H_2O_2 for 48-

h. The results indicated that treatment with H_2O_2 at 100 mg/L for 96 h was effective in destroying algal cysts (Ichikawa et al. 1993). For the algae *Oscillatoria* spp., found in shrimp ponds, H_2O_2 at 4.19 mg/L and 7.18 mg/L could reduce 42.19% and 46.77% of chlorophyll after a 72-h exposure (Srisapoom et al. 1999).

7.5.2 Invertebrates - Many different species of nektonic and benthic invertebrates typically reside within brackish water (Remane and Schlieper 1971; Gross 1977). As primary or secondary consumers, they are an integral part of the food web (Remane and Schlieper 1971; Gross 1977). These organisms are often the primary food of planktivorous fish or the early life stages of larger piscivorous game fish. Benthic invertebrates can be an especially useful indicator of environmental quality over long periods because of their limited mobility. Some species of freshwater invertebrates, for which we have already presented effects data (see section 7.4.2), may also reside in brackish water (Remane and Schlieper 1971; Gross 1977). Those data are not presented here again. Table 5 contains the available data on the toxicity of H_2O_2 to various invertebrates that may be found in brackish water.

The larvae of a euphausiid (*Euphausia pacifica*) and an oyster (*Crassostrea gigas*) were both sensitive to H_2O_2 (EVS Environment Consultants 1992). The 96-h LC_{50} for the euphausiid was 0.24 mg/L (although both the 24-h and 48-h LC_{50} values were estimated at >1.5 mg/L), whereas the 48-h EC_{50} (abnormal shell development) for the Pacific oyster larvae was 1.2 mg/L. In the same study, a 48-h NOEC (abnormal shell development) of 0.47 mg/L was also found for oyster larvae. Srisapoom et al. (1999) reported a 24-h LC_{50} of 30.6 mg/L for *Penaeus monodon* (tiger prawn) postlarva. Matthews (1995) reported a 24-h IC_{50} (concentration needed to reach 50% inhibition of mobility in nauplii) of 918 mg/L for *Artemia salina* (brine shrimp). Johnson et al. (1993) studied the toxicity of H_2O_2 to several life-stages of the parasitic sea lice (*Lepeophtheirus salmonis*). Exposure concentrations of 1,500 mg/L for 20 min resulted in 57% mortality for sea lice eggs. Forty-one percent died when the chalimus stage was exposed

to 4,000 mg/L for 24 h. In tests with adult sea lice, 68% mortality was observed after exposures to 3,000 mg/L for 24 h. Parasitic sea lice are not generally considered a desirable species; however, these data are of use because they are thought to be similar phylogenetically, morphologically, and physiologically in some ways to other desirable species of aquatic invertebrates (such as copepods) that commonly inhabit brackish and marine water and are important components in aquatic food webs (Remane and Schlieper 1971; Gross 1977).

Morse et al. (1976) observed that the addition of H_2O_2 to seawater at a concentration of 170 mg/L caused synchronous spawning in male and female red abalones (*Haliotis rufescens*). The authors suggested that H_2O_2, or some product derived from it, may act on or with prostaglandin endoperoxide-forming cyclooxygenase (or on some substrate formed as a consequence of the activity of this enzyme), to induce spawning. Kuzirian et al. (2001) demonstrated that 1 mg/L of H_2O_2 can produce 100% mortality (measured as immobilization) of plankton in mixed marine plankton samples (collected from local coastal waters off Woods Hole, Massachusetts, USA) in less than 35 min at a pH of 8.5, which is within the typical pH range of brackish water. Times to produce 100% mortality decreased as pH were increased further (to 9.0, 9.5, 10.0). This makes H_2O_2 a potential candidate for treating the ballast water of ships. The authors performed the same test on a single species, the ctenophore *Mnemiopsis leidyi*, which was considerably more sensitive to H_2O_2 than the mixed plankton (Table 5).

Since zebra mussels may reside in brackish as well as fresh water (Walton 1996), it is appropriate for us to reference the effects data previously presented for zebra mussels in section 7.4.2. Toxicity values reported in this section for brackish-water invertebrates seem to indicate that they are quite sensitive to H_2O_2.

7.5.3 Fish - Numerous species of fish reside within brackish waters. They are primary, secondary, or tertiary consumers depending on the species and life stage (Remane and Schlieper 1971;

Gross 1977). They are extremely important ecologically as a food source for higher level carnivores and have great value commercially and recreationally to humankind. Fish are good indicators of overall environmental health because they usually live longer than lower life forms and are higher in the food chain, where they are susceptible to bioaccumulation problems and the population fluctuations of their prey. We conducted risk characterizations for discharge into brackish water using the data available for species of fish that are the most common or representative possible. Tables 7 and 8 summarize the toxicity data available for freshwater and marine fish that may be found in brackish receiving waters. Since some freshwater fish may also reside in brackish water, we refer to our previous discussion of effects for fish species found in fresh receiving waters (see section 7.4.3). We present only new data for anadromous and other marine fish here.

The toxicity of H_2O_2 to various species of anadromous fish has been documented for several species of salmon (Table 8). Boutillier (1993) estimated the 96-h LC_{50} for juvenile chinook salmon (*Oncorhynchus tshawytscha*) at 105 mg/L. Johnson et al. (1993) estimated the 20-min LC_0 at 14 °C and the 40-min LC_{100} at 11 °C, both at 1,500 mg/L. Thomassen and Poppe (1992) calculated a 1-h LC_{50} of 2,500 mg/L for Atlantic salmon. For shorter exposures of 20 min, Johnson et al. (1993) and Bruno and Raynard (1994) observed mortalities of 7.7% and 35%, respectively, after exposure of Atlantic salmon to 1,500 mg/L H_2O_2.

Kiemer and Black (1997) concluded that there was a significant correlation between H_2O_2 exposure concentration and duration with sublethal damage to gill tissues and mortality of Atlantic salmon. Exposures of 2,580 mg/L H_2O_2 for 20 min at 10.4 °C caused significant gill tissue damage and complete mortality of test fish (n = 18). Fish exposed to the same concentration and temperature but for only 10 min had only minor gill damage and one mortality (n = 18). Exposures of 1,370 mg/L for 20 min at 10.4 °C resulted in no significant damage to gill tissues and no mortalities.

Toxicity data are also available for marine fish (Table 8). Hiatt et al. (1953) found that exposure to as little as 20 mg/L H_2O_2 for 2 min caused dispersal of the Hawaiian aholehole *(Kuhlia sandvicenis)*, a marine schooling fish. Bruno and Raynard (1994) exposed goldsinny wrasse (*Ctenolabrus rupestris*) to H_2O_2 and estimated that the 20-min LC_0 was 1,260 mg/L. Kanda et al. (1989) reported a 24-h LC_{50} of 224 mg/L for dusky spinefoot (*Siganus fuscescens*) and a 24-h LC_{50} of 89 mg/L for jack mackerel (*Trachurus japonicus*). They also reported a 24-h LC_{50} of 155 mg/L for chameleon goby (*Tridentiger trigonocephalus*).

7.6 Effects on Bacteria - Hydrogen peroxide is used in aquaculture to control external bacterial infections and fungal infestations on fish and is widely used throughout the world in human health for its antimicrobial properties. It is therefore logical to assume that it may be more toxic to bacteria than other freshwater organisms. Extensive amounts of data on the toxicity of H_2O_2 to bacteria are available from the literature. Much of the literature is in the form of H_2O_2 efficacy studies on pathologic or nuisance bacteria. Toxicity data for aquatic bacteria are presented in Table 9. Toxicity endpoints are available for non-aquatic bacteria and bacteria that are not common in the environment, however these data were not included in Table 9. The minimum inhibitory concentration (MIC) and EC data indicate that H_2O_2 toxicity varies widely among bacteria species (Garcia-Mendoza et al. 1993) with MICs ranging from 5.1 to 2,500 mg/L. Contact time, pH, and water quality are important as well (Wolfe et al. 1989, Larsen and White 1995). The most sensitive species presently appears to be *Pseudomonas aeruginosa* (MIC 5.1 mg/L, Baldry 1983) whereas *Escherichia coli* are the least sensitive bacteria identified to date (MIC 2,505 mg/L, Penna et al. 2001). The data indicate that bacteria are not the most sensitive aquatic species to H_2O_2.

Sewage treatment by anaerobic (mainly methane-producing) bacteria to reduce BOD and COD in wastewater often precedes treatment by aerobic bacteria (Welander 1988, He et al.1995). Hydrogen

peroxide is well known to be highly toxic to anaerobic bacteria (Welander and Andersson 1985, Welander 1988, Cohen 1992, He et al.1995) and is widely recognized as potentially problematic when present at harmful concentrations in intake waters of sewage treatment plants. Hydrogen peroxide was toxic to anaerobic sludge bacteria at the lowest concentration (18 mg/L) tested by Cohen (1992) with no methane production even after 63 h. Cocci et al. (1985) recommend a reduction of peroxide concentration to 8 mg/L or less for the safe operation of an anaerobic treatment system. However, even strictly anaerobic bacteria can become acclimated to otherwise normally lethal doses of H_2O_2 (see also section 7.7). The wastewater treatment industry actually takes advantage of anaerobic bacterial acclimation to H_2O_2 through the use of single floc sludge in which the sludge microfuana is alternated from anaerobic to aerobic populations by the addition of H_2O_2 (Smith 1979, McCue et al. 2003). Our survey of public and private aquaculture facilities did not identify any hatcheries that directly discharge to a municipal wastewater treatment facility.

A similar concern for toxicity to aerobic sludge bacteria was not identified from the available literature. Occasionally H_2O_2 is used to maintain a purely aerobic environment to enhance aerobic bacterial treatment (Cole et al. 1973, Spain et al. 1989, Taylor and Jaffe 1991). Toxicity to aerobic sludge bacteria does exist, and excessive H_2O_2 exposures may result in toxicity rather than promotion of bacterial sludge population growth. The toxicity to aerobic bacteria was generally reported to be much less than to anaerobic bacteria, even though the lowest MIC presented in Table 9 (5.1 mg/L) was for an aerobic species (*Pseudomonas aeruginosa*, an opportunistic human pathogen used in wastewater treatment plants because of its ability to degrade many industrial organic compounds). The aerobic bacteria *Pseudomonas putida*, a species valuable in hydrocarbon remediation, has a 16-18 h EC_{10} of 11 mg/L (Knie et al. 1983). Although a conservative endpoint, it indicates that *P. putida* may be one of the more sensitive bacterial species.

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Nitrifying bacteria (*Nitrosomonas* and *Nitrobacter* spp), are an important group of aquatic and soil bacteria that oxidize ammonium to nitrite and then to nitrate (Schwartz, et al. 2000). Jones (1987) found that H_2O_2 concentrations of as much as 680 mg/L only inhibited *Nitromonas* spp ammonium oxidation by 12%. Nitrifying bacteria also seem to acclimate to the presence of H_2O_2 (Siedlecka et al. 2002). Other literature also seems to indicate that the presence of H_2O_2 at low concentrations does not inhibit the efficacy of nitrifying bacteria in sewage treatment plants, although additional MIC or EC values could not be found (Neyens, et al. 2002). Aquaculture systems using water recirculation generally have a clarification or a filtration unit to remove solids and use biofilters with nitrifying bacteria to convert ammonia to nitrate. Pedersen et al. (2006) studied the fate of H_2O_2 in a small-scale recirculation system with an active bio filter and found that decomposition rates were significantly related to the amount of organic matter (BOD_5) and the initial dosage of H_2O_2. Decomposition rate constants ranged from 0.451 to 3.686 h^{-1} which is equivalent to half lives of 0.188 to 1.537 h. We have had no anecdotal feedback that the aquaculture use of H_2O_2 reduces recirculating system biofilter efficiency although almost total impairment of biofilter nitrification resulted after a 100 mg/L static bath in an experimental recirculation system (Schwartz, et al. 2000).

Hydrogen peroxide is often used to remediate sludge bulking (failure of sludge to settle adequately) in wastewater treatment plants by reducing the growth of filamentous bacteria during aerobic treatment (Cole et al. 1973, Strunk and Shapiro 1976). The efficacy/safety limits for administration are 20-400 mg/L; concentrations below 20 mg/L are not effective and over 400 mg/L will cause partial deflocculation (Cole et al. 1973, Sona and Kyushin 1974). It has also been observed that sludge bacteria can acclimate rapidly to H_2O_2 exposure (Larisch and Duff 1997, see also Section 7.7). Thus, the practical aerobic bacterial tolerance of H_2O_2 is quite high for purposes of wastewater treatment.

In summary:

1. Bacteria are not the most sensitive ROI.

2. As a group, anaerobic bacteria are more sensitive than aerobic bacteria.

3. Sub-lethal H_2O_2 concentrations present in an anthropogenic-influenced environment will often induce considerable resistance in bacteria to otherwise lethal concentrations of H_2O_2. This is true to the extent that a single floc sludge [alternating from anaerobic to aerobic (by H_2O_2 addition), back to anaerobic, etc.] can be successfully used at treatment plants. There are also other uses of added H_2O_2 in aerobic bacterial treatment systems.

7.7 Effects of Acclimation to H_2O_2-

There is evidence that bacteria and other organisms (worms, sea lice, fish) acclimate and become less sensitive to H_2O_2 with time after initial exposure. When pre-exposed to sublethal concentrations of H_2O_2, the concentrations required for H_2O_2 to be acutely toxic increase. High levels of reactive oxygen species lead to DNA, protein, and membrane damage in enteric bacteria (Demple and Amábile-Cuevas 1991) and the cells of higher organisms (Kotze 2003). Various organisms respond to oxidative stress by increasing the production of antioxidant enzymes (e.g., catalase and superoxide dimutase, Kotze 2003) to degrade various toxic reactive oxygen species (ibid). Such induction is known from bacteria, yeast, and mammalian cells, as well as from nematodes. Mammalian cells (mice) also have been reported to increase catalase and superoxide dimutase, resulting in an increased ability to expel parasite infections (ibid). Oxidant induced protective responses often result from a coordinated activation of genes involved in oxidant detoxification and repair (Demple and Amábile-Cuevas 1991, Vattanaviboon and Mongkolsuk 2001). These include genes for enzymes such as catalase, alkyl hydroperoxide reductase and methionine sulfoxide reductase (Vattanaviboon and Mongkolsuk 2001). These processes are coordinated by oxidant sensitive regulatory proteins such as OxyR and SoxRS (ibid). For most organisms, exposure to sublethal H_2O_2 also induces new protein synthesis that likely results in the production of

catalase and possibly some other enzymes as a defense mechanism to destroy H_2O_2. Vattanaviboon and Mongkolsuk (2001) demonstrated that exposure of the prawn pathogen *Vibrio harveyi* to sublethal H_2O_2 induced subsequent protection against lethal concentrations of H_2O_2. The protective responses involved new protein synthesis and were abolished by addition of a protein synthesis inhibitor (ibid). Rao et al. (2003) identified a major catalase gene in *Edwardsiella tarda* (a fish and mammal pathogen) that provides this pathogen resistance to H_2O_2.

There is ample evidence that acclimation to H_2O_2 occurs in bacteria, including sludge bacteria (Larisch and Duff 1997). Catalase activity is often described as essential for aerobic life (del Carmen Vargas et al. 2003). With respect to aerobic bacteria, exposure to H_2O_2 initially results in selection against bacteria lacking functional catalase. For example, Klotz and Anderson (1994) concluded that the activity levels of catalase in the aerobic bacteria *Pseudomonas putida* are positively correlated with its resistance to H_2O_2. They found a 16-fold difference in toxicity between *P. putida* containing functional catalase and *P. putida* that did not (Table 9, also del Carmen Vargas et al. 2003). Extensive studies with *Escherichia coli* and *Salmonella typhimurium* have shown that the resistance of these enteric bacteria to H_2O_2 is correlated with the activity of catalase (Klotz and Anderson 1994). Virulence and catalase activity were correlated in *Staphylococcus aureus* (ibid). A positive correlation between the presence of catalase isoenzymes and survival of exposure to H_2O_2 was reported for *Pseudomonas syringae* (ibid) and for biofilm bacteria (Armon et al. 2000). Ohwada et al. (1999) demonstrated that root nodule bacteria have higher susceptibility to H_2O_2 than other aerobic or facultative anaerobic bacteria because of their lower catalase activity in the cells. In general, increased catalase activities correlated positively with H_2O_2 resistance among all bacteria that they tested. Del Carmen Vargas et al. (2003) found that *Rhizobium etli*, an aerobic nitrogen-fixing symbiotic bacteria that interacts with the roots of beans, can also survive higher concentrations of H_2O_2 after pre-exposure to a sub-lethal concentration.

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Bacterial resistance levels to oxidants vary with growth phase. In general, stationary growth phase cells are more resistant to oxidant killing than exponential growth phase cells (Vattanaviboon and Mongkolsuk 2001). Katsuwon and Anderson (1989) demonstrated that unacclimated exponential growth phase *Pseudomonas putida* bacteria were killed by 1 mM of H_2O_2. However, protection of these bacteria in exponential growth phase against 5 mM of H_2O_2 was apparent after a previous exposure to 30-300 nM of the chemical, representing a 5-fold increase in tolerance because of acclimation. Extracts of the protected cells showed increased catalase activity relative to cells killed by 1 mM of H_2O_2. For *Escherichia coli*, Pietersen et al. (1996) found that acclimation to H_2O_2 due to sub-inhibitory oxidizing stress occurred during the stationary growth phase only, not the exponential growth phase. They also found that cellular catalase increased by about 50% because of pre-exposure to H_2O_2.

Even many anaerobic bacteria are evidently capable of induced resistance to H_2O_2. McCue et al. (2003) found that both methanogenic and sulfidogenic dechlorination of organic solvent contaminants could resume after transient exposures to either oxygen or H_2O_2. For cycles as frequent as 10 days between aerobic treatment cycles, reductive dechlorination was found to be at least as rapid as it was without the aerobic cycle. Rocha et al. (1996) demonstrated that inducible resistance could be achieved in the aerotolerant anaerobic bacteria *Bacteroides fragilis*. They showed that catalase production might be responsible for such resistance in these bacteria. The lack of protective mechanisms against oxygen activity in anaerobic bacteria is seen as an explanation for their sensitivity to oxygen exposure (ibid). However, anaerobic bacteria exhibit a broad range of tolerance to oxygen activity and the ones that are able to remain viable might do so by induced production of catalase or superoxide dismutase or reductase (Rocha et al. 1996, Jenney et al. 1999). Briukhanov et al. (2002) found that strictly anaerobic bacteria all possessed superoxide dismutase activity, an enzyme necessary for protection from the toxic products of oxygen reduction and some anaerobic bacteria also possess catalase activity. Hemin produced a strong

positive effect on the catalase activity in many anaerobic microorganisms (ibid). In methanogens, antioxidant enzyme activities varied widely depending on the stage of growth and energy source (ibid).

With respect to other ROI, Tort et al. (1998) demonstrated significantly increased tolerance of walleye exposed to 100 mg/L H_2O_2 for 60 min following weekly 60-min bath exposures of 10 mg/L (94% survival following pretreatment vs 37% survival without pretreatment). Tripi and Bowser (2001) found that pre-exposure of young walleye to sublethal H_2O_2 induced resistance to higher exposures only under hard water conditions. Furthermore, pre-exposure seemed to be detrimental to the youngest (50-d post-hatch) walleye tested. Treasurer et al. (2000) reported that a fish farm that had previously used H_2O_2 41 times experienced greatly reduced efficacy against sea lice compared to a farm that had never used it (15-16% vs 87-90% mortality), indicating possible tolerance through induction of catalase from sub-therapeutic exposure. Kotze (2003) found that the sheep parasite *Haemonchus contortus* (barber pole worm) showed increases of catalase activity of 2.3-fold (adult) and 4.6-fold (L4 stage) when exposed to sublethal H_2O_2. Adult worms were then exposed to toxic concentrations of H_2O_2 and possessed an increased ability to tolerate these levels (LC_{50} 3-fold higher than controls). Thus, toxic concentrations can be up to 3 to 5-fold higher for acclimated worms and sea lice.

8.0 RISK CHARACTERIZATION

General - We conducted a risk characterization that integrates the results of the fate and effects assessments (sections 7.1-7.7) and presents an evaluation of adverse effects or risk to biological ROI associated with exposure to H_2O_2 discharged into fresh water or brackish water from aquaculture facilities. Risk assessments were developed for a typical and a worst-case scenario that are likely to occur. Risk assessments were based on (1) the estimated H_2O_2 environmental introduction concentrations (EICs) from use at aquaculture facilities (section 8.1) and (2) data from aquatic toxicity tests available for representative ROI that reside in or are similar to species that reside in U.S. surface waters that may be

impacted by aquaculture discharge. These data were used to conduct an acute risk quotient (RQ) analysis using selected LC_{50} data (or EC_{50} where the effect indicated was different than mortality) or a chronic RQ analysis using selected chronic NOEC data. The chosen LC_{50}, EC_{50}, or NOEC values were divided by assessment factors as specified by the International Cooperation on Harmonization (VICH, International Cooperation on Harmonization of Technical Requirements for Regulation of Veterinary Medical Products 2004; Table 10) to obtain a predicted no effect concentration (PNEC). Acute or chronic RQ values were calculated by dividing the EIC by the acute or chronic PNEC:

$$RQ = EIC/PNEC$$

In this analysis, RQ values greater than 1.0 indicate that acute or chronic effects to ROI are probable (Suter 1995). By conducting both acute and chronic RQ analyses for the same ROI, we estimated risk according to two different types of toxicity data -- LC_{50} and chronic NOEC values -- to reduce uncertainty in conclusions based on the risk analysis.

The risk assessment based on the VICH assessment factors (Table 10) may be refined if a robust toxicity database is available for a given ROI or ROI category or if actual NOEC data are available for the key studies selected. The risk assessment completed for H_2O_2 will utilize such a refined assessment because the toxicity database is relatively strong for all ROI discussed and several key NOEC values are available. The refined assessment includes a justification for lowering the overall assessment factor applied to the selected toxicity endpoint.

Several criteria were used to select toxicity data that were utilized for the risk characterization. These items are presented in the order of their importance as follows: (1) data were chosen from a given study only if the study seems to have been designed and conducted in a manner that is scientifically sound, and the methodologies employed reasonably conform with those outlined by standard procedures (ASTM 1989); (2) each ROI selected must be an organism that is broadly distributed and typically resides

in aquatic environments where discharges of H_2O_2 from an aquaculture facility occur, or could be a probable surrogate for that organism; (3) the ROI chosen must be "ecologically relevant" or an important component in the normal functioning of the ecosystem in question, or could be a probable surrogate for that ROI; (4) in the event that acceptable data exist for multiple ROI, select data for the species that is most sensitive to H_2O_2, and for which NOEC and LC_{50} data exist; and (5) data were selected from a study where the exposure regimen (exposure concentration, duration, repetition, and interval) most closely resembles that which is likely to occur in the natural environment.

Typical hatchery use of H_2O_2 on fish includes treatments and subsequent discharges on alternate days over a five day period. Treatments on eggs typically results in discharges on consecutive or alternate days over the period from fertilization until hatching. Thus, the possible effects to organisms in receiving water being repeatedly exposed to H_2O_2 are of concern. The risk characterization conducted here does not consider simultaneous treatment of multiple culture units. Very little of the toxicity data currently available contained any definitive information on the effects of repeated exposures on ROI; therefore, it would be impossible to clearly delineate and quantify such effects. The few studies that do provide information on repeated exposures show that some organisms tend to become tolerant to H_2O_2 with repeated exposure (Pardieck et al. 1992; Larisch and Duff 1997; Tort et al. 1998, see also section 7.7). Therefore, we chose to proceed under the assumption that the effects of repeated exposures are not incremental or cumulative.

8.1 Determination of Estimated Environmental Introduction Concentrations - Public and private aquaculture facilities were surveyed by UMESC to determine the present and projected use of H_2O_2 for fish and fish egg culture. The EICs of H_2O_2 were estimated from data collected from 100 public and private hatcheries representing fish culture in 22 states. The surveyed hatcheries represent a mix of 9 federal, 80 state, and 11 private fish hatcheries and reported culturing a diverse mixture of 253 different

fish species. Commonly cultured species included rainbow trout (49 hatcheries), brown trout (34 hatcheries), channel catfish (30 hatcheries), brook trout (*Salvelinus fontinalis*; 29 hatcheries), walleye (25 hatcheries), bluegill (24 hatcheries), largemouth bass (23 hatcheries), muskellunge (18 hatcheries), fathead minnow (16 hatcheries) and striped bass (15 hatcheries). The data collected to support the H_2O_2 environmental assessment and the calculations performed are included electronically on CD-ROM (MS-Excel™) in Appendix A.

8.1.1 Water Use and Effluent Discharge - Hatchery water use was reported in the survey as "average daily water flow" (the total volume of water discharged on an average production day), and "low daily water flow" (the total volume of water discharged daily during the periods of low water use on the hatchery). Average daily water flow reported from the 100 hatcheries ranged from about 38 L/d, a facility using recirculating tanks, to 1,881 million L/d, a large cold-water culture facility (Appendix A, Section 2). Median average daily water flow was 12.5 million L/d and median low daily water flow was 6.1 million L/d (Appendix A, Section 2). Effluent from 51 of the 100 hatcheries passed through settling ponds before discharge into a river, lake, or backwater (Appendix A, Section 2). For the purpose of this environmental assessment, we assume these are in-line settling ponds. Median settling pond volume was 3 acre-feet and the average settling pond volume was 10.6 acre-feet (1 acre-foot equals 1,233,476 L). Seventy-seven of the hatcheries discharge into a river or stream, with a median average flow of 27.4 cfs (one cfs = 28.32 L/s) and median low flow of 12.0 cfs (Appendix A, Section 2). Fourteen hatcheries discharge into lakes (median volume 4,500 acre-feet) and eight discharge into the backwater of a river or stream (median backwater volume 55 acre-feet) (Appendix A, Section 2).

Of the 100 hatcheries that responded, 39 treat or anticipate treating fish eggs, whereas 32 treat or anticipate treating fish (Appendix A, Section 3 and 5). Thirty-four hatcheries reported administering flow-through treatments to eggs, whereas five reported administering static bath treatments to eggs

(Appendix A, Section 3). The median number of treatments administered during an egg treatment regimen was 15, with most hatcheries administering consecutive daily treatments (Appendix A, Section 3). Most hatcheries treated eggs in either spring (25 of 39) or fall (15 of 39), although egg treatment in summer (8 of 39) or winter (13 of 39) is not unlikely (Appendix A, Section 3). Eleven hatcheries administered static fish treatments whereas twenty hatcheries administered flow-through treatments (Appendix A, Section 5). The median number of treatments administered to fish was three, with most hatcheries administering treatments every other day (Appendix A, Section 5). Fish treatments were distributed equally throughout the year; 18 hatcheries would administer at least one fish treatment in spring, 22 in summer, 23 in fall, and 15 in winter (Appendix A, Section 5).

8.1.2 EIC Calculation Assumptions - The concentration of H_2O_2 in hatchery effluent, as a result of treatment water discharge, was estimated for both the "typical" and "worst-case" treatment scenarios that might reasonably occur following fish or egg treatments based on a certain set of assumptions (Table 11). Although some facilities reported use of H_2O_2 to treat both fish and eggs, we assumed these were separate treatment scenarios and calculated separate EIC estimates for fish or egg treatments. Two recirculating aquaculture facilities reported present or proposed H_2O_2 use at their facility. Both hatcheries were excluded from the calculations described below because the model presently used to predict EIC's at hatcheries with minimal water reuse does not fit the information available for recirculating systems. These two recirculating systems reuse a substantial portion of the total system volume (>95% recirculation), resulting in an apparent concentration of H_2O_2 in the effluent. Intensive recirculation technology requires the use of extensive water treatment to remove uneaten fish feed, fecal matter, fish metabolites, and other waste materials from production water (Wedemeyer 2001). The water in these filtration systems would further dilute H_2O_2 applied and discharged from the system and would also provide extensive contact with biological material that could be oxidized by H_2O_2. Data are not

presently available to adequately model the dilution or degradation that would occur in intensive recirculating aquaculture systems like the two included in our survey. Although not included in our EIC estimates, it is likely that recirculating aquaculture systems would be able to meet the same discharge limitations (if needed) placed on traditional flow-through aquaculture facilities through engineering controls or modification of treatment application.

The typical and worst-case treatment scenarios differed in the hatchery flow rate used to calculate the EIC (Table 11). Average hatchery flow rate was used when calculating the EIC resulting from a typical treatment whereas the hatchery low flow rate was used when calculating the EIC resulting from a worst-case treatment. Environmental introduction concentrations estimates are provided to predict the average discharge concentration that may be expected to occur over 1-, 2-, 5, or 21-d periods. The 1-d EIC resulting from either a typical or worst-case treatment day was estimated from the following equation:

$$EIC = \frac{C \times V}{F + E}$$

where C was the maximum proposed label concentration (100 mg/L for fish or 1,000 mg/L for eggs; Section 3.0), V was the maximum daily treated volume, F was the total hatchery discharge over 24 h (typical = average daily water flow; worst-case = low daily water flow), and E was the effluent pond volume. The parameter V was estimated by summing the maximum daily treated tank or raceway volumes for the various culture unit sizes (i.e, tanks size 1, 2, or 3, or raceway size 1, 2, or 3). For static treatments, V was estimated by multiplying the number of culture units that a hatchery reported treating by the culture unit volume whereas V for flow-through treatments was determined by multiplying the number of culture units that a hatchery reported treating by the maximum flow rate to the culture unit times the maximum treatment duration allowed on the present proposed label (15 min for eggs; 60 min for fish). When estimating the EIC for flow-through treatments, the treated culture unit flow rate was used to estimate F

in those cases where the treated culture unit flow rate exceeded the average or low daily water flow. Similarly, the average hatchery flow rate was substituted for F if the hatchery did not report a low daily flow. The 2-d EIC estimates for fish treatments assumed one treatment would have been administered over a 48-h period whereas the 5- or 21-d EIC estimates assumed three treatments over a 5 or 21-d period. The 1-d EIC calculation was thus modified to predict 2-, 5-, or 21-d EICs for fish treatments by increasing the hatchery discharge volume (i.e., $F \times 2$, 5, or 21 days for the 2-, 5-, or 21-d EIC, respectively) and the treated volume (i.e., $V \times 1$, 3, or 3 treatments for the 2-, 5-, or 21-d EIC, respectively). Since egg treatments were expected to be administered on consecutive days, the 2-d and 5-d EIC estimates were assumed to be equal to the 1-d EIC estimate, therefore the 1-d EIC estimate was substituted for those estimates in EIC summaries. The 21-d EIC estimate for egg treatments calculated by modifying the 1-d EIC calculation by multiplying V by 15 (the median number of days eggs were reported to be treated) and by multiplying F by 21 (equal to the hatchery flow over 21 d).

Degradation was not included in the EIC estimates presented in this EA because relevant data for H_2O_2 degradation within hatcheries are not presently available. Results of the hatchery study of Saez and Bowser (2001) suggest that dilution will account for most of the decline in H_2O_2 concentrations with hatcheries prior to discharge; however, this study did not include fish (and associated organic matter) within the system and therefore may have had less degradation than normally would occur.

8.1.3 Describing EIC Tendencies - Two to four EIC values were developed for each reporting hatchery that indicated their present or planned use of H_2O_2 on eggs or fish. The EICs were determined by using data unique to that hatchery and represent our understanding of their potential typical and worst-case treatments. Rather than conduct separate risk analyses for each EIC from each hatchery and each time point, we chose to summarize the EIC values for typical and worst-case fish and fish egg treatments for each time period by reporting the mean, median, and 75th and 95th percentiles (Table 12);

calculations for each were completed using MS-Excel™. We chose to include the median because the mean of our relatively small sample size (n = 69) could be skewed by a relatively small number of extreme data points. The histogram in Figure 3 describes the frequency of typical 24-h EICs calculated based on present or expected use of H_2O_2 on fish or eggs. Examination of Figure 3 indicates that the sample mean of 1.2 is likely skewed by the relatively few extreme data points on the upper end of the distribution relative to the median of 0.6. Although the median is a poor estimate of the mean when data do not fit a normal distribution (Zar 1984), we believe it is a better representation of the central tendency of our EIC data because it is less likely to be skewed by extreme, atypical values than is the mean. We also summarized the available EIC data based on the presence or absence of a holding pond (Table 13).

8.1.4 Describing Available Environmental Dilution of Hatchery Effluent - Estimated Environmental Concentrations (EECs) were not developed for the present EA because of the lack of an accepted model that could predict EEC following H_2O_2 use at hatcheries. Instead, the relative immediate dilution power of a hatchery's receiving water was estimated by dividing the receiving water volume available for effluent dilution by the hatchery's average daily water flow. The receiving water volume available for discharge was assumed to be the daily flow of a river or stream at the low flow rate or the lake or backwater volume, depending on whether the hatchery discharged to a river/stream or a lake/backwater. A 50% dilution of hatchery water is thus represented by a ratio of 1:1 by our estimation methods. Of the 100 hatcheries surveyed, data were available to estimate this ratio for 86 hatcheries. Of these 86 hatcheries, 74 discharged into water bodies that would provide an immediate 1:1 dilution of the hatchery effluent. Dilution ratios at the remaining 12 ranged from 0.1:1 (i.e., only a $1/10^{th}$-fold dilution) to 0.99:1 (i.e., nearly 1:1 dilution).

8.2 Risk Estimation for Fresh Receiving Waters - Risk estimation for discharge into fresh water from aquaculture sites is based on selected data from aquatic toxicity tests available for

representative ROI that most typically reside in fresh receiving waters of aquaculture discharge (Tables 4, 5, and 7). A summary of the VICH Phase II default Tier A and Tier B assessment factors used are given in Table 10. The initial RQs calculated based on the default VICH assessment factors are included in Table 14. The refined acute and chronic RQs calculated based on refined assessment factors are presented in Tables 15 and 16, respectively. The acute RQs have been determined using EICs time-averaged over 1 to 5 days, while the chronic RQs are based on only the 21-d average EICs. The refined RQs calculated based on the refined assessment factors are used in the risk assessments described in this section, and the section also includes a discussion of justifications for use of refined assessment factors.

8.2.1 Acute Risk Quotient Analysis: Fresh Receiving Waters - For this analysis, it was necessary to substitute the lowest concentration tested for LC_{50} for certain ROI (i.e., algae).

Algae Acute – The data selected for the acute risk assessment were the lowest concentration tested for a 24-h exposure for *Microcystis* spp (Kay et al.1982; Table 4, 1.7 mg/L). At 1.7 mg/L, the lowest H_2O_2 concentration tested, chlorophyll production was <5% of the control. Thus it is nearly a LC_{100} as well as a "threshold toxicity" and an application factor should be used to derive an acute NOEC for this species in the refined risk assessment. *Microcystis* spp are undesirable blue-green algae that only occur in very eutrophic surface waters. However, it is the most sensitive algal species for which we have a toxicity point estimate and may represent the sensitivity of desirable and widely distributed species for which no data are available. The H_2O_2 acute toxicity database for freshwater algae appears to be adequate, especially if marine species are included as surrogates for freshwater species. An assessment factor of 10 to extrapolate from the acute LC_{100} to the acute PNEC was applied, plus another factor of 10 to extrapolate laboratory data to the field (single species effects to multiple species / community level effects), yielding a PNEC of 0.017 mg/L (Table 15). A PEC value of 0.017 mg/L would generate an acute RQ of 1. According to our refined risk assessment and hatchery survey results, maximal H_2O_2 use at

hatcheries would result in acute RQs of 65-88 for 25% of surveyed hatcheries and acute RQs of 129-241 for 5% of surveyed hatcheries (Table 15).

Invertebrate Acute – The definitive invertebrate toxicity data used were the 48-h LC_{50} value for *Daphnia pulex* (Shurtleff 1989; Table 5, 2.4 mg/L). Several *Daphnia* species are recognized as standard test subjects to assess aquatic toxicity to invertebrates (ASTM 1989). The H_2O_2 acute toxicity database for freshwater invertebrates appears to be adequate, especially if marine species are included as surrogates for freshwater species. An assessment factor of 2 to extrapolate from the acute EC_{50} to the acute PNEC was applied[1] plus a factor of 10 to extrapolate laboratory data to the field (single species effects to multiple species / community level effects), yielding a PNEC of 0.12 mg/L (Table 15). A PEC value of 0.12 mg/L would generate an acute RQ of 1. According to our refined risk assessment and hatchery survey results, maximal H_2O_2 use at hatcheries would result in acute RQs of 9.2-13 (RQs of 13 and 9.2 for 24- and 48-h exposures, respectively) for 25% of surveyed hatcheries and acute RQs of 18-34 for 5% of surveyed hatcheries (Table 15).

Fish Acute – The definitive fish toxicity data used were the 24 h LC_{50} value for fingerling rainbow trout (Rach et al. 1997c; Table 7, 48 mg/L). There appears to be ample data to assess the acute toxicity of H_2O_2 to freshwater fish. An assessment factor of 3 to extrapolate from the acute EC_{50} to the acute PNEC was applied[2] plus a factor of 10 to extrapolate laboratory data to the field (single species effects to multiple species / community level effects), yielding a PNEC of 1.6 mg/L (Table 15). A PEC value of 1.6 mg/L will generate an acute RQ of 1. According to our refined risk assessment and hatchery

[1] A VICH assessment factor of 10 is typically used to extrapolate an acute LC_{50} to an acute NOEC, however a factor of 2 was used based on H_2O_2 toxicity data in Shurtleff, 1989, Bringmann, 1982, Trenel and Kuhn, 1982 and Meinertz et al. 2005).

[2] A VICH assessment factor of 10 is typically used to extrapolate an acute LC_{50} to an acute NOEC, however a factor of 3 was used based on H_2O_2 toxicity data in Clayton and Summerfelt, 1996 and Gaikowski, et al. 1999.

survey results, maximal H_2O_2 use at hatcheries would result in acute RQs of 0.7-0.9 for 25% of surveyed hatcheries and acute RQs of 1.4-2.6 for 5% of surveyed hatcheries (Table 15).

8.2.2 Chronic Risk Quotient Analysis: Fresh Receiving Waters - Chronic risk analyses are based on extrapolated NOECs for algae and fish because no chronic NOEC data are available for these ROI.

Algae Chronic - The algal ROI and study data chosen were the lowest concentration tested for a 24-h exposure of *Microcystis* spp. (Kay et al. 1982; Table 4, 1.7 mg/L). At 1.7 mg/L, the lowest H_2O_2 concentration tested, chlorophyll production was <5% of the control. Thus it is very nearly an LC_{100} as well as a "threshold toxicity" and an application factor should be used to derive a chronic NOEC for this species in the refined risk assessment. *Microcystis* spp are undesirable blue-green algae that occur in very eutrophic surface waters. However, it is the most sensitive algal species for which we have a toxicity point estimate and may represent the sensitivity of desirable or widely distributed species for which data are not available. The H_2O_2 chronic toxicity database for freshwater algae appears to be adequate, especially if marine species are included as surrogates for freshwater species. An assessment factor of 20 for the acute-to-chronic ratio (i.e., extrapolation of acute LC_{100} to chronic NOEC) was applied plus a factor of 10 to extrapolate laboratory data to the field (single species effects to multiple species / community level effects), yielding a PNEC of 0.0085 mg/L (Table 16). A PEC value of 0.0085 mg/L results in a chronic RQ of 1. According to our refined risk assessment and hatchery survey results, maximal H_2O_2 use at hatcheries would result in a chronic RQ of 71 for 25% of surveyed hatcheries and a chronic RQ of 212 for 5% of surveyed hatcheries (Table 16).

Invertebrate Chronic – *Daphnia* spp. are common in fresh receiving waters (Pennak 1978) and are an integral component in the aquatic food web (Pennak 1978). *Daphnia* spp. are typically more sensitive to chemicals than other invertebrates (Table 5; ASTM 1989) and are considered to be standard

test invertebrates (ASTM 1989). A controlled study on the chronic toxicity of H_2O_2 to *Daphnia magna* is summarized in Section 7.4.2 and Appendix D (the complete study is included as Appendix E). As discussed in Section 7.4.2., this study represents a conservative toxicity estimate because of the considerably higher than recommended flow rate used to maintain constant H_2O_2 concentrations and because of the test water's low organic content (low BOD/COD) relative to natural surface waters. Daphnia would not likely be exposed to H_2O_2 under similar conditions in the field. Nonetheless, 21-d NOEC (reproduction, total young produced; Table 6, 0.63 mg/L) was used for the chronic risk assessment to freshwater invertebrates. An assessment factor of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects) was applied, yielding a PNEC of 0.063 mg/L (Table 16). A PEC value of 0.063 mg/L provides a chronic RQ of 1. According to our refined risk assessment and hatchery survey results, maximal H_2O_2 use at hatcheries would result in a chronic RQ of 9.5 for 25% of surveyed hatcheries and a chronic RQ of 29 for 5% of surveyed hatcheries (Table 16).

Fish Chronic – The definitive fish toxicity data used were the 96-h LC_{50} value for fingerling channel catfish (Kay et al. 1982; Table 7, 37 mg/L). There appears to be adequate data to assess the risk of chronic H_2O_2 exposure to freshwater fish. An assessment factor of 10 for the acute-to-chronic ratio (i.e., extrapolation of acute LC_{50} to chronic NOEC) was applied plus a factor of 10 to extrapolate laboratory data to the field (single species effects to multiple species / community level effects), yielding a NOEC of 0.374 mg/L (Table 16). A PEC value of 0.374 mg/L would generate a chronic RQ of 1. According to our refined risk assessment and hatchery survey results, maximal H_2O_2 use at hatcheries would result in a chronic RQ of 1.6 for 25% of surveyed hatcheries and a chronic RQ of 4.8 for 5% of surveyed hatcheries (Table 16).

8.3 Risk Estimation for Brackish Receiving Waters - Risk estimation for brackish receiving waters was based on data from aquatic toxicity tests available for representative ROI that most typically

reside in brackish receiving waters. We assume that most brackish receiving waters are usually larger bodies of water (coastal estuaries, bays, large rivers, or large salt lakes) than fresh receiving waters and are more eutrophic overall than fresh waters; therefore, we believe that an additional mitigating factor with regard to the PEC is likely to be present when assessing risk to brackish-water species. A summary of the acute risk assessments for brackish water using the VICH default Tier A and Tier B assessment factors is given in Table 14. A summary of acute and chronic risk assessments for brackish water using refined assessment factors is given in Tables 15 and 16. The refined factors are used in the risk assessments described in this section, and this section includes a discussion of justifications for any use of refined VICH assessment factors.

8.3.1 Acute Risk Quotient Analysis: Brackish Receiving Waters- Acute toxicity values were available for all ROI in brackish receiving water.

Algae Acute – The definitive algal toxicity data were the 72-h NOEC (growth inhibition) of *Nitzschia closterium* (Florence and Stauber 1986; Table 4, $\leq$ 0.68 mg/L). The definitive algal toxicity data were the lowest test concentration administered in a 72-h growth reduction study of *Nitzschia closterium* (Florence and Stauber 1986; Table 4, $\leq$ 0.68 mg/L [algal growth decreased 31% relative to controls]). For simplicity, we assumed that the 0.68 mg/L value was the best available LC_{50} estimate even though the reported LC_{50} was 0.85 mg/L. The H_2O_2 acute toxicity database for brackish-water algae appears to be adequate, especially if freshwater species are included as surrogates for brackish-water species. An assessment factor of 10 was applied to extrapolate laboratory data to the field (single species effects to multiple species / community level effects), yielding a PNEC of 0.068 mg/L (Table 15). A PEC value of 0.068 mg/L will generate an acute RQ of 1. According to our refined risk assessment and hatchery survey results, maximal H_2O_2 use at hatcheries would result in acute RQs of 16-22 for 25% of surveyed

hatcheries and acute RQs of 32-60 for 5% of surveyed hatcheries, if they discharged into brackish water (Table 15).

Invertebrate Acute – The definitive invertebrate toxicity data were the 48-h NOEC (mortality) for the Pacific oyster larvae *Crassostrea gigas* (EVS Environment Consultants 1992; Table 5, 0.94 mg/L). The H_2O_2 acute toxicity database for brackish-water invertebrates appears to be adequate, especially if freshwater species are included as surrogates for brackish-water species. An assessment factor of 10 was applied to extrapolate laboratory data to the field (single species effects to multiple species / community level effects), yielding a PNEC of 0.094 mg/L (Table 15). A PEC value of 0.094 mg/L would generate an acute RQ of 1. According to our refined risk assessment and hatchery survey results, maximal H_2O_2 use at hatcheries would result in acute RQs of 12-16 for 25% of surveyed hatcheries and acute RQs of 23-44 for 5% of surveyed hatcheries, if they discharged into brackish water (Table 15).

Fish Acute – The definitive fish toxicity data were the 96-h LC_{50} for chinook salmon (Boutillier 1993; Table 8, 105 mg/L). The H_2O_2 acute toxicity database for brackish-water fish appears to be adequate, especially if freshwater species are included as surrogates for brackish-water species. An assessment factor of 6 to extrapolate from the acute EC_{50} to the acute NOEC was applied[3] plus a factor of 10 to extrapolate laboratory data to the field (single species effects to multiple species / community level effects), yielding a PNEC of 1.75 mg/L (Table 15). A PEC value of 1.75 mg/L will generate an acute RQ of 1. According to our refined risk assessment and hatchery survey results, an estimated 25% of hatchery discharges would result in acute RQs of 0.6-0.9, and 5% would result in acute RQs of 1.3-2.3, if they discharged into brackish water (Table 15).

[3] A VICH assessment factor of 10 is typically used to extrapolate an acute LC_{50} to an acute NOEC, however a factor of 6 was used based on H_2O_2 toxicity data in Thomassen and Poppe 1992 and Johnson et al. 1993).

8.3.2 Chronic Risk Quotient Analysis: Brackish Receiving Waters - Chronic risk analyses are based on extrapolated NOECs for algae, invertebrates, and fish, because no chronic NOEC data are available for these ROI.

Algae Chronic – The European Agency for the Evaluation of Medicinal Products (EMEA) states that 72-h algae tests may be considered chronic because this period provides for 16 life cycles (EMEA 1997). The definitive algal toxicity data were the lowest test concentration from a 72-h growth reduction study of *Nitzschia closterium* (Florence and Stauber 1986; Table 4, $\leq$ 0.68 mg/L [algal growth decreased 31% relative to controls]). We assumed that the 0.68 mg/L value was the best available NOEC estimate even though the true NOEC is somewhat less than 0.68 mg/L. Although there was only one 72-h algal toxicity study, there appears to be adequate data for chronic toxicity to algae in brackish water if the numerous data for 48-h exposures are considered as supporting data. Most 48-h toxicity values were several-fold larger than the 72-h endpoint for *Nitzschia closterium*. An assessment factor of 10 was applied to extrapolate laboratory data to the field (single species effects to multiple species / community level effects), yielding a PNEC of 0.068 mg/L (Table 16). A PEC value of 0.068 mg/L would generate a chronic RQ of 1. According to our refined risk assessment and hatchery survey results, an estimated 25% of hatchery discharges would result in a chronic RQ of 8.8, and 5% would result in a chronic RQ of 27, if they discharged into brackish water (Table 16).

Invertebrate Chronic – *Euphausia pacifica*, an ecologically important oceanic krill was used as a surrogate for brackish-water invertebrates. The definitive toxicity value used was the 96-h LC_{50} (EVS 1992; Table 5, 0.24 mg/L). The H_2O_2 chronic toxicity database for brackish-water invertebrates appears to be adequate, especially if data for freshwater species are included. Applying an assessment factor of 10 for the acute-to-chronic ratio (i.e., extrapolation of acute LC_{50} to chronic NOEC) plus a factor of 10 to extrapolate laboratory data to the field (single species effects to multiple species / community level

effects) to the 96-h LC_{50} yields a PNEC of 0.0024 mg/L (Table 16). A PEC value of 0.0024 mg/L will generate a chronic RQ of 1. According to our refined risk assessment and hatchery survey results, an estimated 25% of hatchery discharges would result in a chronic RQ of 250, and 5% would result in a chronic RQ of 750, if they discharged into brackish water (Table 16).

Fish Chronic – The definitive fish toxicity data used were the 96 h LC_{50} for chinook salmon (Boutillier 1993; Table 8, 105 mg/L). The H_2O_2 chronic toxicity database for brackish-water fish appears to be adequate, especially if toxicity data for freshwater species are included. An assessment factor of 10 for the acute-to-chronic ratio (i.e., extrapolation of acute LC_{50} to chronic NOEC) was applied plus a factor of 10 to extrapolate laboratory data to the field (single species effects to multiple species / community level effects), yielding a PNEC of 1.05 mg/L (Table 16). A PEC value of 1.05 mg/L would generate a chronic RQ of 1. According to our refined risk assessment and hatchery survey results, an estimated 25% of hatchery discharges would result in a chronic RQ of 0.6, and 5% would result in a chronic RQ of 1.7, if they discharged into brackish water (Table 16).

8.4 Risk Estimation for Bacteria -

Direct discharge from aquaculture facilities into sewage or wastewater treatment systems is unlikely; none of the 100 hatcheries surveyed discharged into municipal wastewater treatment systems (Section 8.1.1). Although some small experimental culture facilities may discharge to municipal wastewater treatment systems, their discharge volumes are likely to be miniscule relative to the total flow into the wastewater system. Any aquaculture discharge of H_2O_2 into a municipal sewage system would likely be substantially diluted before reaching a treatment plant. Although municipal drinking water plants do not use bacteria in their treatment processes, it is possible that a hatchery could discharge into a municipal water supply. However, most hatcheries are not situated upstream of municipal drinking water intakes and in those situations where hatcheries discharge into a municipal water supply, any H_2O_2

discharged would likely be diluted to background levels before reaching the intake. We are presently unaware of any hatcheries that discharge upstream of municipal drinking water plants.

Although the available data indicate that exposure or discharge into municipal wastewater treatment plants are unlikely, we completed a risk assessment for sewage treatment bacteria as follows. Published toxicity studies using standard methods (ASTM, OECD) for aerobic sludge bacteria, nitrifying bacteria, and anaerobic (methane-generating) bacteria do not appear to be available. The most sensitive sewage sludge bacteria to H_2O_2 are the anaerobic bacteria (Section 7.6) with a recommended H_2O_2 exposure limit of 8 mg/L to anaerobic sludge bacteria in municipal wastewater treatment plants. The most sensitive freshwater bacteria to H_2O_2, however, is *Pseudomonas aeruginosa* (Table 9, MIC = 5.1 mg/L), a bacteria that is ubiquitous in the environment and occurs naturally in fresh water. Using 5.1 mg/L as the PNEC for sewage treatment plant bacteria, PECs of $\leq$5.1 mg/L would result in a RQ of $\leq$1 and should pose no risk to aerobic or anaerobic sewage treatment bacteria. Furthermore, bacterial acclimation is known to occur following sublethal H_2O_2 exposures (ca. 1-10 mg/L; Katsuwon and Anderson 1989, Vattanaviboon and Mongkolsuk 2001). We conclude that H_2O_2 does not appear to be harmful to sewage treatment bacteria at exposure levels predicted from aquaculture effluents.

The sensitivity of naturally-occurring aquatic bacteria (fresh and marine) appears to be widely variable (Table 9) with *Pseudomonas aeruginosa* presently the most sensitive species. Based on its MIC, a H_2O_2 PEC of 5.1 mg/L would result in an acute RQ of 1. Hydrogen peroxide discharges of $\leq$5.1 mg/L should therefore pose no risk to naturally-occurring bacteria. Countless types of bacteria are abundant in nearly all surface water and are also ubiquitous worldwide on land, in other waters, and in the air. Once H_2O_2 from a short intermittent discharge has been degraded, bacteria from surrounding or incoming waters will quickly reproduce and repopulate the affected area. For example, Xenopoulos and Bird (1997) found an approximate 50% decrease of normal bacterial production in lake water (average of four

experiments) at 0.034 mg/L and an approximate 30% decrease of normal production at 0.0034 mg/L. Background H_2O_2 levels were not measured but the concentrations tested were thought to commonly occur in the lake from natural H_2O_2 sources. Although acutely toxic to these bacteria, H_2O_2 exposure did not result in long-term depletion of the lake bacterial population. Because bacteria acclimate and desensitize to H_2O_2 quickly after an initial exposure (Section 7.7), it is unlikely that relatively small, isolated, and intermittent point-source discharges of H_2O_2 could have a significant long-term effect on the numbers and types of bacteria fauna present at any freshwater location.

The H_2O_2 toxicity database for brackish-water or marine bacteria is limited (Table 9). Given the wide range in sensitivity of freshwater bacteria, inclusion of the freshwater bacteria toxicity data seems appropriate since the range of sensitivity of freshwater bacteria would likely be protective of most brackish-water bacteria. The most sensitive marine species for which we have data is *Vibrio harveyi* (MIC = 9.57 mg/L). A H_2O_2 PEC of 9.57 mg/L would thus generate an acute RQ of 1. Although slightly higher than the PNEC used for freshwater bacteria (5.1 mg/L), the limited information available for brackish-water bacteria suggest sensitivity similar to freshwater species. Brackish-water bacterial populations should be at least as capable as freshwater species of rapid recovery following H_2O_2 exposure. It is unlikely that relatively small, isolated, and intermittent point-source discharges of H_2O_2 would have a significant long-term effect on the numbers and types of bacteria fauna present at any brackish-water location.

8.5 Risk Characterization and Proposed Mitigation – An evaluation of the risk quotients in Tables 15 and 16 indicates that there is a potential for adverse effects on aquatic life at a significant fraction of the hatchery facilities that are expected to use hydrogen peroxide once it is approved. Although these risk quotients are "worst-case" in that the exposure estimates that they are based on do not take into account any potential degradation of hydrogen peroxide prior to discharge, the exposure

estimates do account for internal dilution and site-specific use conditions such as the number and frequency of treatments. These risk quotients are also "worst case" in that they are based on estimated end-of-the pipe effluent concentrations of hydrogen peroxide, and not on predicted concentrations in receiving waters below the points of effluent discharge. Receiving water concentrations for most hatcheries will be well below the effluent concentrations due to subsequent dilution and degradation. However, many states do no allow the discharge of toxic substances in toxic amounts, therefore, it is inappropriate to automatically factor in dilution in receiving waters for all facilities without some assurance that state and local water quality regulations allow this[4]. This is not possible when evaluating drugs that are to be approved on a nationwide basis; therefore, a different approach is needed for drugs like hydrogen peroxide that may have the potential to cause effects at individual facilities.

The recommended risk mitigation to insure that use of hydrogen peroxide will not adversely impact aquatic life is to develop a water quality criterion or benchmark that can be used by the appropriate National Pollutant Discharge Elimination System (NPDES) or state permitting authority[5] to establish appropriate effluent discharge limits on a facility-by-facility basis, if needed, based on site-specific conditions (e.g., receiving water dilution) and in conformance with applicable state and federal water quality regulations. Environmental statements should be added to the drug label that identify the water quality benchmark for its use by NPDES permitting authorities[6] and which require the user to report this information to the appropriate authority prior to initial use of the drug.

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[4] The Clean Water Act allows individual states to set water quality standards and regulations that are more restrictive than national standards and regulations. For example, some states allow toxicity in the mixing zone, while others do not. Those that do not, evaluate toxicity at the end-of-the-pipe without consideration of dilution.

[5] The U.S. EPA is responsible for implementing the NPDES system, but may authorize individual States, Territories, or Tribes to implement all or parts of the national system, including issuing permits.

[6] Under Clean Water Act regulations (see 40 CFR 122.44(d)(1)(vi)(A)), information provided by FDA (such as water quality benchmarks) can be used by permitting authorities to derive numerical water quality criteria and establish appropriate effluent discharge limits.

8.6 Calculation of Acute Water Quality Benchmark[7] (Criterion) – The procedures used to calculate the acute benchmark value for H_2O_2 were those described in the EPA guidelines for deriving numerical national water quality criteria (Stephan et al. 1985, EPA 1991 and 1994). Appropriate toxicity endpoints (LC_{50}s for specific exposure durations) must be available for at least eight different specific families to ensure a sufficient database on which to base the calculation of the "Final Acute Value" (FAV). Flow-through toxicity tests are preferred but static or static-renewal data are acceptable. Many of the H_2O_2 toxicity endpoints for fish and invertebrates may be used to calculate the FAV. Species-specific data are collated and the geometric mean calculated for those species with two or more toxicity endpoints (Species Mean Acute Value, SMAV). *Daphnia magna* were the only species with 2 H_2O_2 endpoints so all other SMAV values were simply the toxicity endpoint for that species (for N = 2, the geometric mean is simply the square root of the product of the 2 endpoints). After determining SMAVs, genus toxicity endpoints were similarly collated to determine the Genus Mean Acute Value (GMAV; Table 17). As with the SMAV, the geometric mean was determined for each genus with two or more endpoints. *Daphnia* were the only genus with 2 endpoints so the GMAV for each other genus was equal to the one toxicity endpoint for that genus.

GMAVs were ranked (R) from most sensitive to least sensitive; identical GMAVs were arbitrarily assigned successive ranks. The FAV value is an estimate of the concentration of a chemical corresponding to a cumulative probability of 0.05 in the toxicity values for the genera for which acceptable acute tests have been conducted on the chemical. The cumulative probability (P) for each GMAV was calculated as:

$$R / (N+1)$$

[7] The term "benchmark" is being used here instead of "criterion" because this value has not been officially promulgated by the EPA in compliance with all of the appropriate Clean Water Act regulations (e.g., with public notice and comment).

The four GMAVs with cumulative probabilities closest to 0.05 (typically the four lowest-ranked GMAVs) were selected to reduce skewness, following Erickson and Stephan (1988). The FAV was calculated by substituting the selected GMAVs and Ps into the following formulae:

$$S = \frac{\sum((\ln GMAV)^2 - ((\sum(\ln GMAV))^2 / 4)}{\sum(P) - ((\sum(\sqrt{P}))^2 / 4)}$$

where S is the slope of the geometric mean functional relationship between ln GMAV and $\sqrt{P}$ (ibid). The ln-transformation of GMAV is used to reduce skewedness and the $\sqrt{}$transformation of P is used to provide the best estimate corresponding to P = 0.05. The intercept on the GMAV axis (the y axis) is given by L as follows (ibid):

$$L = (\sum(\ln GMAV) - S(\sum(\sqrt{P}))) / 4$$

These slope (S) and intercept (L) values are then used to calculate A, the ln-transformed toxicity value corresponding to P = 0.05 (ibid):

$$A = S(\sqrt{0.05}) + L$$

A is then back-transformed to yield the FAV (ibid):

$$\text{Final Acute Value (FAV)} = e^A$$

The FAV was divided by a safety factor of 2 to determine the Continuous Maximum Concentration (CMC), which is also the acute benchmark. Substitution of the available freshwater GMAV data into the preceding equations (Table 18) results in a FAV of 1.4. If for a commercially or recreationally important species the geometric mean of the acute values from tests in which the concentrations of test material were measured is lower than the FAV, then that geometric mean should be used as the FAV instead of the calculated FAV. However, the FAV of 1.4 mg/L is lower than any value

for the freshwater fish and invertebrates for which we have data. Therefore, the CMC or acute benchmark is 1.4 mg/L / 2 or 0.7 mg/L.

8.7 Incorporation of the Proposed Risk Mitigation on the Drug Label - The drug label should provide information that would enable its safe use in the environment and inform appropriate effluent regulatory authorities. The following label language is proposed:

"**LIMITATIONS AND CAUTIONS FOR ALL USES**

Prior to the initial use of this drug, you must inform the appropriate National Pollutant Discharge Elimination System (NPDES) permitting authority of your intentions and the information below. A NPDES permit may be required before you can discharge hydrogen peroxide. Effluent discharge limits may also be needed because of its toxicity to aquatic life. Water quality benchmarks have been derived by FDA for use by the NPDES authority. For freshwater aquatic life, the acute benchmark is 0.7 mg/L (equivalent to the Criteria Maximum Concentration or one-half the Final Acute Value). Additional environmental information is available at http://www.fda.gov/cvm/ea.htm."

Note that the recommended labeling above does not contain a chronic water quality benchmark for hydrogen peroxide. There are several reasons why a chronic water quality benchmark was not derived for hydrogen peroxide and is not believed to be necessary to mitigate potential risks. Many of these factors have been previously discussed in the environmental assessment. These include:

1. Most discharges of hydrogen peroxide from use on fish and eggs will not be chronic in nature, typically occurring over a period of only 5 to 15 days.
2. Risk quotients for hydrogen peroxide are based on toxicity data from laboratory studies with relatively constant exposures, while the actual exposures in the field will be short and pulsed.
3. Data for *Daphnia magna* indicate a small acute to chronic ratio for toxicity; therefore, the chronic benchmark, if it were derived, is not likely to be significantly lower than the acute benchmark.

4. Many organisms including fish, invertebrates, and bacteria have shown acclimation to sublethal exposures of hydrogen peroxide.
5. Hydrogen peroxide is reactive and does not bioaccumulate in tissues.

9.0 ALTERNATIVES TO PROPOSED ACTION

The major alternative to H_2O_2 as a waterborne fungicide on cultured fish or fish eggs is formalin (a mixture of 37% formaldehyde gas dissolved in water). As a fungicide, formalin is effective for treating saprolegniasis on fish eggs (Rach et al. 1997b, Rach et al. 2005a, Rach et al. 2005b). Formalin is generally considered to be similarly effective as H_2O_2 to control saprolegniasis on fish and eggs (Marking et al. 1994, Rach et al. 2005a, Rach et al. 2005b). Although approved for use as a fungicide for all fish eggs by the FDA, it is not presently approved as a fungicide for fish. Formaldehyde is a human carcinogen and poses serious worker health issues (UMESC search results from various web sites). Additionally, several permitting agencies have recently required hatcheries to reduce formalin effluent discharge concentrations.

10.0 STORAGE AND DISPOSAL

Improper storage and disposal of hydrogen peroxide could potentially result in releases that cause adverse effects on aquatic life, therefore, storage and disposal instructions are recommended for the product label. The following language is recommended in addition to statements that may already be included on product labeling:

Storage:

Store in a manner designed to prevent spills that may result in discharge to surface waters. Implement procedures for properly containing, cleaning, and disposing of any spilled material.

Disposal:

"Hydrogen peroxide is a strong oxidizer and a characteristic hazardous waste as defined by RCRA (40 CFR 261). Contact your State Environmental Control Agency, or the Hazardous Waste Representative at the nearest EPA Regional Office for guidance on disposal. DO NOT flush to sewer unless diluted to 1% or less concentration due to explosion hazard. Do not contaminate surface water when disposing of equipment washwaters or rinsate. Empty containers may contain residues and should be washed with water prior to disposal."

11.0 CONCLUSIONS

On the basis of the toxicity and environmental exposure data examined and the risk characterizations conducted, we believe that the use of H_2O_2 as a waterborne therapeutant in intensive and extensive freshwater aquaculture operations constitutes no significant threat to the environment, the populations of organisms residing there, or public health and safety if receiving water concentrations do not exceed 0.7 mg/L on a short-term basis. This acute water quality benchmark should be included on the product label to alert effluent regulatory authorities of the potential need to establish discharge limits at individual facilities using hydrogen peroxide based on site-specific conditions. Monitoring of effluent concentrations should only be required for those facilities that discharge to receiving water with either minimal flow relative to the hatchery discharge or that have minimal oxidizable material in the receiving water. Because H_2O_2 undergoes rapid degradation in eutrophic waters, most freshwater facilities with large holding ponds will probably discharge H_2O_2 at concentrations far below the proposed 0.7 mg/L acute benchmark.

The following mitigating factors were not included when estimating the acute water quality benchmark:

1) Hydrogen peroxide is not likely to pose an imminent threat to the aquatic environment because dilution by receiving water will reduce exposure concentrations.

2) Degradation by oxidizable organic matter in receiving water will reduce the exposure concentration and duration.

3) Organisms acclimate to H_2O_2 exposure through increased catalase production.

4) Intermittent H_2O_2 use in aquaculture will result in pulsed environmental exposures, not the continuous exposures used in the available laboratory toxicity studies.

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14. CERTIFICATION

We, the undersigned, certify that to the best of our knowledge, the information and data presented in this environmental assessment concerning the use of H_2O_2 in U.S. aquaculture are accurate and reliable.

William H. Gingerich 8 June 2006
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15. REFERENCES (Note: the citations in bold were not referenced in Appendix B of the original EA. The literature associated with most bolded citations is included in Appendix F of the revised EA.)

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Appendix A. Projected Use of Hydrogen Peroxide at Various Hatcheries Surveyed, Estimates for Hatchery Flow Rates, Dilution Factors, and Discharge Concentrations over Time for each Site.

APPENDIX A: TABLE OF CONTENTS

Appendix A. Section 1. Revised hatchery survey calculations. The following equations were used to estimate physical parameters of each hatchery during hydrogen peroxide egg and fish treatments for typical and worst-case scenarios. These equations support the data found in Appendix A, Sections 4, 6, and 7.

Hatchery average water flow (Lpm)

Average hatchery water flow (gal/d) × 3.785 (L/gal) / 1,440 (min/d)

Hatchery low water flow (Lpm)

Minimum hatchery water flow (gal/d) × 3.785 (L/gal) / 1,440 (min/d)
NOTE: Average hatchery water flow was used if no minimum water flow was reported

Time to perform two volume exchanges (min)

Sum of treated culture unit volume × 2 / sum of maximum flow to the culture units
NOTE: Culture unit volume and maximum flow per culture unit must have similar units (L or gal)

Settling pond volume (L)

Pond volume (acre-feet) × (1,233,342 L / acre-foot)

Maximum daily treated volume (L)

Flow-through treatment

Treatment duration (min) {{maximum number of treated culture unit 1 per day × maximum flow per culture unit 1 (gpm)} + {maximum number of treated culture unit 2 × maximum flow per culture unit 2 (gpm)) + ...}} × 3.785 (L/gal)

Static treatment

Maximum number of culture units treated daily × culture unit volumes (L)

Maximum H_2O_2 applied (mg)

Maximum daily treated volume (L) × Maximum treatment concentration (mg/L)

Effluent concentration after settling pond (mg/L)

The term "hatchery water flow" in the following equations is replaced by hatchery average water flow (Lpm) to estimate the typical EIC or hatchery low water flow (Lpm) to estimate the worst-case EIC. Fish were assumed to receive three 60-min treatments at 100 mg/L as a static or flow-through treatment administered once daily on alternate days. Fish eggs were assumed to receive fifteen 15-min treatments at 1000 mg/L as a flow-through treatment administered daily on consecutive days.

1-d EIC (fish or eggs)

Max H_2O_2 (mg) applied / {{hatchery water flow (L/min) × 1,440 min/d} + settling pond volume (L)}

2-d EIC (fish)

Max H_2O_2 (mg) applied / {{hatchery water flow (L/min) × 1,440 min/d x 2 d} + settling pond volume (L)}

2-d EIC (eggs)
Max H_2O_2 (mg) applied x 2 treatments / {{hatchery water flow (L/min) × 1,440 min/d x 2 d} + settling pond volume (L)}

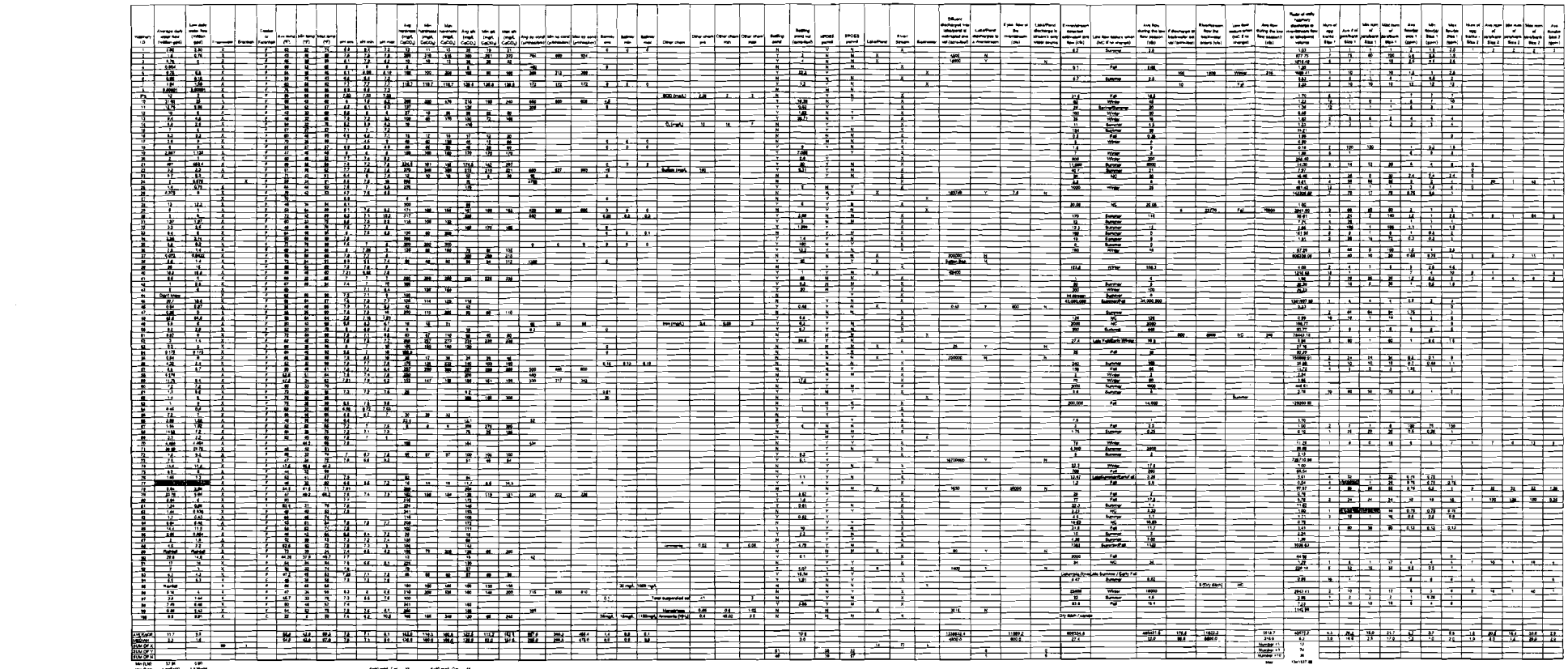
5-d EIC (fish)
Max H_2O_2 (mg) applied x 3 treatments / {{hatchery water flow (L/min) × 1,440 min/d x 5 d} + settling pond volume (L)}

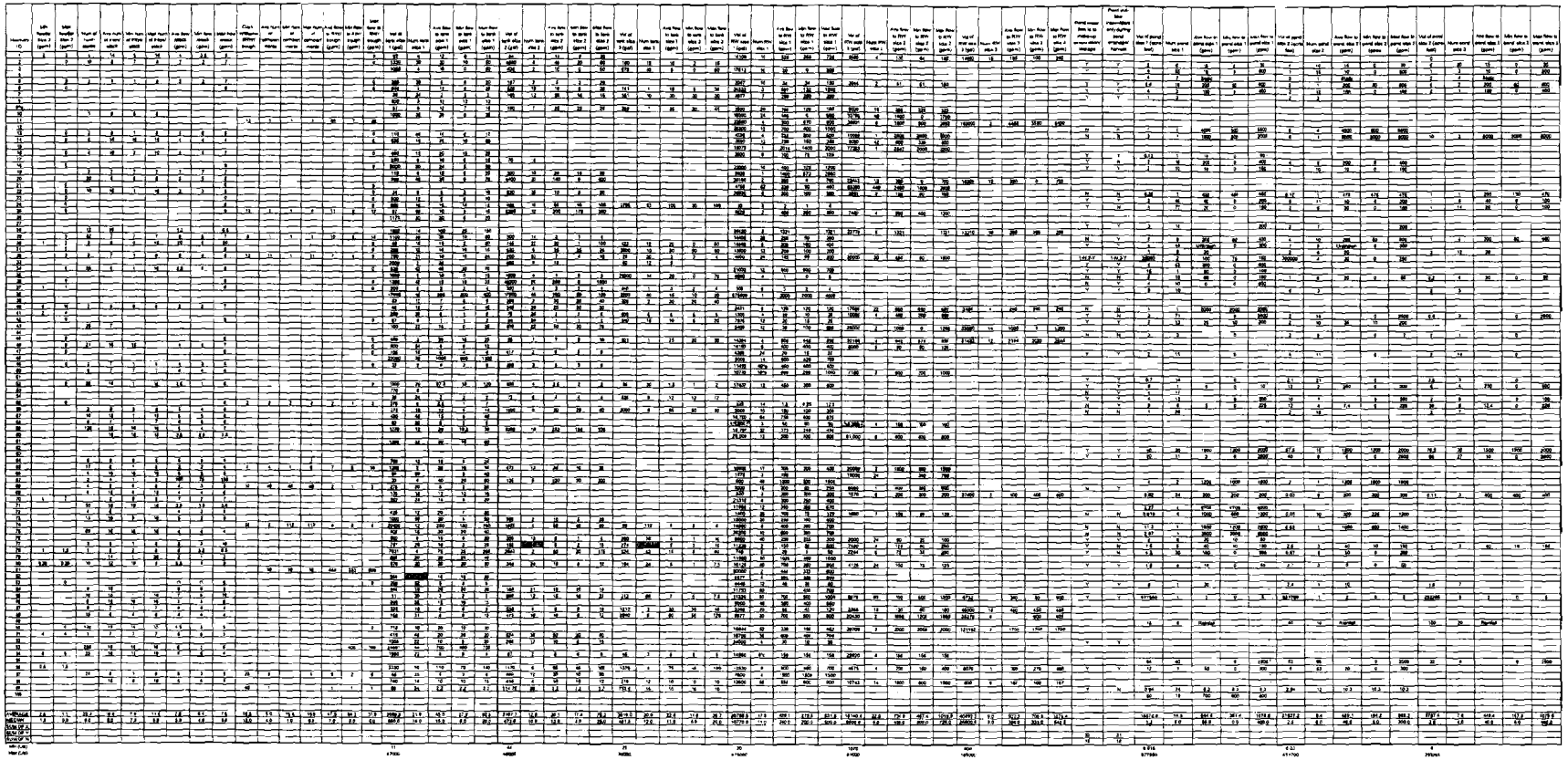
5-d EIC (eggs)
Max H_2O_2 (mg) applied x 5 treatments / {{hatchery water flow (L/min) ×1,440 min/d x 5 d} + settling pond volume (L)}

21-d EIC (fish)
Max H_2O_2 (mg) applied x 3 treatments / {{hatchery water flow (L/min) × 1,440 min/d x 21 d} + settling pond volume (L)}

21-d EIC (eggs)
Max H_2O_2 (mg) applied x 15 treatments / {{hatchery water flow (L/min) × 1,440 min/d x 21 d + settling pond volume (L)}

Hatchery water flows, water chemistry parameters, and fish culture unit information





Appendix A. Section 3. Hydrogen peroxide treatment regimens for eggs.

Hatchery I.D.	Hydrogen peroxide	Minimum (mg/L)	Maximum (mg/L)	Static bath	Flow-through	Number days treated	Treatments on consecutive or alternate days?	Treatment duration static minimum (min)	Treatment duration static maximum (min)	Treatment duration flow-through - minimum (min)	Treatment duration flow-through - maximum (min)
1e	X	500	1000		X	40	C			15	15
2e	X	500	500		X	14	C			14	16
3e											
4e											
5e	X	500	1000		X	10	A			15	30
6e	X	50	250	X		10	C	30	60		
7e											
8e											
9e											
10e											
11e											
12e											
13e	X	800	800		X	21	A			10	10
14e	X	500	1000		X	42	C			15	30
15e											
16e	X	500	1000		X	10	C			15	15
17e											
18e	X	250	250	X				30	60		
19e											
20e											
21e	X	500	1000		X	3	A			15	30
22e	X	500	1000		X	30	C			15	15
23e	X	200	500	X		3	C	15	15		
24e	X	300	300	X		30	C	30	30		
25e	X	500	500		X	15	A			15	15
26e											
27e											
28e											
29e	X	100	500		X	8	C			2	5
30e	X	500	500	X		2	C	60	60		
31e	X	250	600		X	10	C			15	15
32e	X	500	1000		X	23	A			15	30
33e											
34e	X	500	500		X	45	C			30	30
35e											
36e	X	100	500		X	12	C			10	11
37e	X	350	500		X	6	C			15	20
38e											
39e											
40e											
41e											
42e	X	250	250		X	4	C			30	30
43e											
44e											
45e	X	500	500		X	30	C			15	15
46e	X	1000	1000		X	30	A				19
47e	X	500	500		X	5	C			15	16
48e											
49e	X	250	250		X		C			15	30
50e											
51e											
52e	Y	500	500		X	60	C			15	15
53e											
54e											
55e	X	500	1000		X	3	A				15
56e											
57e											
58e	X	500	500		X	12	A			15	15
59e											
60e											
61e											
62e											
63e											
64e											
65e											
66e											
67e	X	500	1000		X	35	C			30	30
68e											
69e											
70e											
71e											
72e											
73e	X	500	1000		Y	60	C			15	30
74e	X	250	350		X	15	A			30	30
75e											
76e											
77e	X	250	500		X	40	C			15	15
78e	X	200	500		X	8	A			15	30
79e											
80e											
81e											
82e											
83e	X	500	500		X	20	C			15	15
84e											
85e											
86e	X	100	500		X	100	C			15	30
87e											
88e											
89e											
90e	X	50	250		X	48	A			15	15
91e											
92e											
93e	X	500	500		X	5	A			15	15
94e	X	500	1000		X					30	30
95e											
96e											
97e	X	500	750		X	60	A			15	15
98e											
99e											
100e											
AVERAGE						24.1					
MEDIAN						15					
SUM of X	39			5	34						
SUM of Y											
SUM of N											

Hatchery I.D.	Fungus	BGD	Columnaris BCWD	Furunculosis/Aeromonas hydrophila	BKD/ERM	Trematodes, protozoans, copepods	Other	If you checked other, enter disease name	Cold-water	Cool-water	Warm-water	Max concurrent egg jars size 1	Max concurrent egg jars size 2	Max concurrent heath stacks	Max concurrent clark-williams	Max concurrent tank size 1	Max concurrent tank size 2	Max concurrent tank size 3	Max concurrent raceway size 1	Max concurrent raceway size 2	Max concurrent raceway size 3
1e	X								X			1	2	4	0						
2e	X									X		200	0	0	0						
3e																					
4e																					
5e	X								X	X	X	10	0	2	0						
6e	X						X			X		24	0	0	0						
7e																					
8e																					
9e																					
10e																					
11e																					
12e																					
13e	X								X			1	0	1	0						
14e	X								X			8	0	5	0						
15e																					
16e	X								X			0	0	4	0						
17e																					
18e	X									X	X	10	0	0	0						
19e																					
20e																					
21e	X								X			12	0	0	0						
22e	X								X			0	0	8	0						
23e	X										X	20	0	0	0						
24e	X										X	42	0	0	0				8		
25e	X								X			12	0	0	20						
26e																					
27e																					
28e																					
29e	X									X	X	3	0	0	0						
30e	X										X	5	0	0	0		10				
31e	X									X	X	104	0	0	0						
32e	X								X	X		100	0	16	4						
33e																					
34e	X								X	X		108	0	14	0						
35e																					
36e	X									X	X	150	0	0	0						
37e	X									X		0	11	0	0						
38e																					
39e																					
40e																					
41e																					
42e	Y									X	X	10	0	0	0	4					
43e																					
44e																					
45e	X								X			4	0	0	0						
46e	X								X			0	0	21	0						
47e	X										X	18	0	0	0						
48e																					
49e	X								X			0	0	2	0						
50e																					
51e																					
52e	X								X	X		80	0	20	0						
53e																					
54e																					
55e	X										X	0	0	0	4						
56e																					
57e																					
58e	X								X					2							
59e																					
60e																					
61e																					
62e																					
63e																					
64e																					
65e																					
66e																					
67e	X								X					2							
68e																					
69e																					
70e																					
71e																					
72e																					
73e	X								X					13							
74e	X								X						2						
75e																					
76e																					
77e	X								X	X	X	112		8							
78e	X									X	X	56	32	1							
79e																					
80e																					
81e																					
82e																					
83e	X									X	X	48	0	0	0						
84e																					
85e																					
86e	X								X					8							
87e																					
88e																					
89e																					
90e	X								X			0	0	132	0						
91e																					
92e																					
93e	X								X					288							
94e	X								X					24							
95e																					
96e																					
97e	X								X	X		2		24							
98e																					
99e																					
100e																					
AVERAGE																					
MEDIAN																					
SUM of X	39								24	16	14										
SUM of Y																					
SUM of N																					

Hatchery I.D.	Max daily egg jars size 1	Max daily egg jars size 2	Max daily heath stacks	Max daily clark-williams	Max daily tank size 1	Max daily tank size 2	Max daily tank size 3	Max daily raceway size 1	Max daily raceway size 2	Max daily raceway size 3	Max daily pond size 1	Max daily pond size 2	Max daily pond size 3	Percent of the treated volume drained from the culture unit after treatment (%)	Percent flow rate increase after treatment (%)	Flow rate increase maintained for X min	Times per year	Spring	Summer	Fall	Winter
1e	1	2	3	0										0	0	0	40			X	X
2e	700	0	0	0										100	0		1	X			
3e																					
4e																					
5e	5	0	1	0										0	0	0	16	X		X	
6e	24	0	0	0													16	X			
7e																					
8e																					
9e																					
10e																					
11e																					
12e																					
13e	10	0	2	0										100	0		5	X			
14e	8	0	5	0													120			X	X
15e																					
16e	0	0	4	0																	
17e																					
18e	80	0	0	0										0	0	0	8	X	X	X	
19e																					
20e																					
21e	12	0	0	0													158	X	X	X	X
22e	0	0	8	0													2	X		X	
23e	20	0	0	0													4	X			
24e	42	0	0	0				8						100	0			X			
25e	12	0	0	10										100	0		5	X	X	X	X
26e																					
27e																					
28e																					
29e	20	0	0	0										0	0	0	30	X			
30e	5	0	0	0		2								0	300	30	30	X			
31e	120	0	0	0										100			50	X			
32e	150	0	32	10													5	X	X	X	X
33e																					
34e	108	0	14	0													100	X		X	X
35e																					
36e	100	0	0	0										100	0	0	6	X	X		
37e	0	30	0	0										10	0		28	X			
38e																					
39e																					
40e																					
41e																					
42e	10	0	0	0	4									100	0		50	X	X		
43e																					
44e																					
45e	4	0	0	0													4			X	X
46e	0	0	6	0																	
47e	18	0	0	0													6	X			
48e																					
49e	0	0	2	0																	
50e																					
51e																					
52e	240	0	20	0																	
53e																					
54e																					
55e	0	0	0	4													5	X			
56e																					
57e																					
58e	0	0	6	0										100	0		6	X		X	
59e																					
60e																					
61e																					
62e																					
63e																					
64e																					
65e																					
66e																					
67e	0	0	2	0													180	X			X
68e																					
69e																					
70e																					
71e																					
72e																					
73e	0	0	13	0										0	0	0	80			X	X
74e	0	0	0	10										0	0		15				X
75e																					
76e																					
77e	112	0	8	0													2	X		X	
78e	1	1	1	0													12	X			
79e																					
80e																					
81e																					
82e																					
83e	48	0	0	0								0		0	0		25	X			
84e																					
85e																					
86e	0	0	8	0													120		X	X	
87e																					
88e																					
89e																					
90e	0	0	132	0													2		X	X	X
91e																					
92e																					
93e	5	0	288	0										0			5			X	
94e	3	0	24	0											0	0	60			X	X
95e																					
96e																					
97e	2	0	24	0										0	0		75			X	X
98e																					
99e																					
100e																					
AVERAGE																	36.4				
MEDIAN																	13.5				
SUM of X																		25	6	16	13
SUM of Y																					
SUM of N																					

Appendix A. Section 4. Environmental Introduction Concentrations for eggs.

Hatchery I.D.	Conducts egg treatments (Y/N)	Max daily egg jars size 1	Max flow/jar size 1 (L/m)	Total treated volume - egg jars - size 1	Max daily egg jars size 2	Max flow/jar size 2 (L/m)	Total treated volume - egg jars - size21	Max daily heath stacks	Max flow - heath stack	Total treated volume - heath stack	Max daily clark-williams	Max flow clark-williams	Total vol clark-williams	Maximum treated volume (L)	Max trt conc (mg/L)	Max H_2O_2 Applied (mg)	Settling pond vol (acre-feet)	Settling pond vol (L)	Total flow during treatment (L/min)	Hatchery average water flow (L/min)	Hatchery average water flow (L/min) greater than treated flow rate (Y/N)	Hatchery low water flow (L/min)	Hatchery low water flow (L/min) greater than treated flow rate (Y/N)	Typical 24 hr avg conc (mg/L)	Worst case 24 hr avg conc (mg/L)	Typical 21-d avg conc (mg/L)	Worst case 21-d avg conc (mg/L)	number of days treatment administered
1e	Y	1	9	135	2	11	330	3	23	1035	0	0	0	1500	1000	1500000			100	10300	Y	6000	Y	0.10	0.17	0.07	0.12	40
2e	Y	700	6	63000	0	0	0	0	26	0	0	0	0	63000	1000	63,000,000	2	2466000	4200	3900	N	2000	N	7.40	7.40	7.30	7.30	14
3e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	4	4932000		15100	Y	0	N					
4e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0				100	Y	0	N					
5e	Y	5	9	675	0	0	0	1	26	390	0	0	0	1100	1000	1100000	22.2	27372600	73	2000	Y	1300	Y	0.04	0.04	0.19	0.25	10
6e	Y	24	8	2880	0	0	0	0	0	0	0	0	0	2900	1000	2900000			193	2300	Y	500	Y	0.88	4.03	0.63	2.88	10
7e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	1.2	1479600		5100	Y	2800	Y					
8e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0				0	N	0	N					
9e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0				31500	Y	5300	Y					
10e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	16.26	20046580		83100	Y	65700	Y					
11e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0.03	36990		36100	Y	26200	Y					
12e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	1.62	1997460		26300	Y	15800	Y					
13e	Y	10	15	2250	0	0	0	2	23	690	0	0	0	2900	1000	2900000	30.71	37865430	193	22100	Y	11800	Y	0.04	0.05	0.06	0.11	21
14e	Y	8	15	1800	0	0	0	5	15	1125	0	0	0	2900	1000	2900000			193	15200	Y	6800	Y	0.13	0.30	0.09	0.21	42
15e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0				18400	Y	13100	Y					
16e	Y	0	0	0	0	0	0	4	26	1560	0	0	0	1600	1000	1600000			107	800	Y	500	Y	1.39	2.22	0.99	1.59	10
17e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0				9500	Y	0	N					
18e	Y	40	6	3600	0	0	0	0	0	0	0	0	0	3600	1000	3600000	9	11097000	240	15800	Y	0	N	0.11	0.11	0.11	0.11	
19e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	7.086			7500	Y	3000	Y					
20e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	2.6			5300	Y	2600	Y					
21e	Y	12	30	5400	0	0	0	0	0	0	0	0	0	5400	1000	5400000	20	24660000	360	1306400	Y	1270600	Y	0.00	0.00	0.00	0.00	3
22e	Y	0	0	0	0	0	0	6	19	1710	0	0	0	1700	1000	1700000	0.31	382230	113	8700	Y	6000	Y	0.13	0.19	0.10	0.14	30
23e	Y	20	9	2700	0	0	0	0	0	0	0	0	0	2700	1000	2700000			180	1800	Y	800	Y	1.04	2.34	0.74	1.67	3
24e	Y	42	15	9450	0	0	0	0	0	0	0	0	0	9500	1000	9500000			633	5300	Y	1500	Y	1.24	4.40	0.89	3.14	30
25e	Y	12	19	3420	0	0	0	0	0	0	10	45	6750	10200	1000	10200000	2	2466000	680	3700	Y	2100	Y	1.31	1.86	1.34	2.32	15
26e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0				1000	Y	0	N					
27e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0					N	0	N					
28e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	5			34200	Y	32100	Y					
29e	Y	20	11	3300	0	0	0	0	26	0	0	57	0	3300	1000	3300000			220	13100	Y	3700	Y	0.17	0.62	0.12	0.44	6
30e	Y	5	9	675	0	9	0	0	189	0	0	0	0	700	1000	700000	2.86	3526380	47	7900	Y	0	N	0.05	0.05	0.04	0.04	2
31e	Y	120	4	7200	0	0	0	0	0	0	0	0	0	7200	1000	7200000	3	3699000	480	5200	Y	5200	Y	0.64	0.64	0.67	0.67	10
32e	Y	150	6	13500	0	0	0	32	30	14400	10	34	5100	33000	1000	33000000	1.964	2421612	2200	8400	Y	6600	Y	2.27	2.77	1.93	2.45	23
33e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0				1100	Y	0	N					
34e	Y	108	4	6480	0	0	0	14	23	4830	0	0	0	11300	1000	11300000	1.4	1726200	753	8900	Y	7200	Y	0.78	0.93	0.63	0.77	45
35e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	100	123300000		2100	Y	500	Y					
36e	Y	100	9	13500	0	0	0	0	0	0	0	0	0	13500	1000	13500000	13.2	16275600	900	18900	Y	3700	Y	0.31	0.62	0.34	1.56	12
37e	Y	0	4	0	30	4	1800	0	0	0	0	0	0	1800	1000	1800000			120	200	Y	100	N	6.25	10.42	4.46	8.93	6
38e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	30	36990000		15200	Y	3700	Y					
39e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0				52600	Y	39400	Y					
40e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	1	1233000		44200	Y	44200	Y					
41e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	50			1600	Y	0	N					
42e	Y	10	6	900	0	0	0	0	0	0	0	0	0	900	1000	900000	0.3	369900	60	2600	Y	1300	Y	0.22	0.40	0.17	0.34	4
43e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	20			21000	Y	15800	Y					
44e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0				0	N	0	N					
45e	Y	4	11	660	0	0	0	0	0	0	0	0	0	700	1000	700000			47	54400	Y	49400	Y	0.01	0.01	0.01	0.01	30
46e	Y	0	0	0	0	0	0	6	26	2340	0	0	0	2300	1000	2300000	0.45	554850	153	1700	Y	1500	Y	0.77	0.85	0.66	0.75	30
47e	Y	16	8	1920	0	0	0	0	0	0	0	0	0	1900	1000	1900000			127	700	Y	700	Y	1.88	1.88	1.35	1.35	5
48e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	5.5			219500	Y	170300	Y					
49e	Y	0	0	0	0	0	0	2	19	570	0	0	0	600	1000	600000	0.2	246600	40	17100	Y	15800	Y	0.02	0.03	0.02	0.02	
50e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0.7			16300	Y	7400	Y					
51e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0				200	Y	0	N					
52e	Y	240	6	21600	0	0	0	20	23	6900	0	0	0	28500	1000	28500000	94.5	116518500	1900	7900	Y	3700	Y	0.22	0.23	1.20	1.87	60
53e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0				800	Y	0	N					
54e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0				500	Y	500	Y					
55e	Y	0	0	0	0	0	0	0	0	0	4	11	660	700	1000	700000			47	1400	Y	1400	Y	0.35	0.35	0.25	0.25	3
56e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0				11400	Y	6300	Y					
57e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0				22600	Y	15000	Y					
58e	Y	0	0	0	0	0	0	6	19	1710	0	0	0	1700	1000	1700000	0		113	1500	Y	1500	Y	0.79	0.79	0.56	0.56	12
59e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	17.5			30900	Y	16800	Y					
60e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			18900	Y	18900	Y					
61e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			3400	Y	1600	Y					

Appendix A. Section 4. Environmental Introduction Concentrations for eggs.

Hatchery I.D.	Conducts egg treatments (Y/N)	Max daily egg jars size 1	Max flow/jar size 1 (L/m)	Total treated volume - egg jars - size 1	Max daily egg jars size 2	Max flow/jar size 2 (L/m)	Total treated volume - egg jars - size21	Max daily heath stacks	Max flow - heath stack	Total treated volume - heath stack	Max daily clark-williams	Max flow clark-williams	Total vol clark-williams	Maximum treated volume (L)	Max trt conc (mg/L)	Max H_2O_2 Applied (mg)	Settling pond vol (acre-feet)	Settling pond vol (L)	Total flow during treatment (L/min)	Hatchery average water flow (L/min)	Hatchery average water flow (L/min) greater than treated flow rate (Y/N)	Hatchery low water flow (L/min)	Hatchery low water flow (L/min) greater than treated flow rate (Y/N)	Typical 24 hr avg conc (mg/L)	Worst case 24 hr avg conc (mg/L)	Typical 21-d avg conc (mg/L)	Worst case 21-d avg conc (mg/L)	number of days treatment administered
62e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			3700	Y	0	N					
63e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			2600	Y	0	N					
64e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	1			1200	Y	1100	Y					
65e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			18900	Y	18400	Y					
66e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			7600	Y	4200	Y					
67e	Y	0	568	0	0	0	0	2	568	17040	0	0	0	17000	1000	17000000	4	4932000	1133	5100	Y	4300	Y	1.38	1.53	1.60	1.89	35
68e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			30300	Y	18900	Y					
69e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			6000	Y	5800	Y					
70e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			11700	Y	11700	Y					
71e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			102200	Y	62500	Y					
72e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0.2			4700	Y	1300	Y					
73e	Y	0	0	0	0	0	0	13	30	5850	0	0	0	5900	1000	5900000	5.1	6288300	393	19700	Y	7900	Y	0.17	0.33	0.15	0.36	60
74e	Y	0	0	0	0	0	0	0	0	0	10	30	4500	4500	1000	4500000	0		300	37900	Y	30000	Y	0.08	0.10	0.06	0.07	15
75e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			18100	Y	13100	Y					
76e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	1.1			4100	Y	3400	Y					
77e	Y	112	3	5040	0	0	0	8	38	4560	0	0	0	9600	1000	9600000	4	4932000	640	3800	Y	2300	Y	0.92	1.16	1.20	1.93	40
78e	Y	1	4	60	1	6	90	1	25	375	0	0	0	500	1000	500000	0		33	14600	Y	6800	Y	0.02	0.05	0.02	0.04	8
79e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	3.52	4340160		62500	Y	13200	Y					
80e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	1.8	2219400		22700	Y	15800	Y					
81e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0.81			3300	Y	2500	Y					
82e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			3800	Y	2500	Y					
83e	Y	48	3	2160	0	0	0	0	0	0	0	0	0	2200	1000	2200000	0.82	1011060	147	4500	Y	1100	Y	0.29	0.85	0.24	0.96	20
84e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			22700	Y	17000	Y					
85e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	10	12330000		37900	Y	30200	Y					
86e	Y	0	0	0	0	0	0	6	19	1710	0	0	0	1700	1000	1700000	2.2	2712600	113	7600	Y	2300	Y	0.12	0.28	0.11	0.35	100
87e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			5300	Y	4700	Y					
88e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	4.79	5906070		12100	Y	5800	Y					
89e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			0	N	0	N					
90e	Y	0	0	0	0	0	0	132	23	45540	0	0	0	45500	1000	45500000	0.1	123300	3033	75700	Y	39200	Y	0.42	0.80	0.30	0.58	48
91e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			44700	Y	36800	Y					
92e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	4.07	5018310		5300	Y	2600	Y					
93e	Y	0	0	0	0	0	0	288	23	99360	0	2650	0	99400	1000	99400000	15.34	18914220	6627	11300	Y	11300	Y	2.82	2.82	4.13	4.13	5
94e	Y	0	23	0	0	23	0	24	19	6840	0	0	0	6800	1000	6800000	1.91	2355030		14800	Y	13900	Y	0.29	0.30	0.23	0.24	0
95e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			0	N	0	N					
96e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			13600	Y	10500	Y					
97e	Y	2	4	120	0	0	0	24	19	6840	0	23	0	7000	1000	7000000	0		467	9500	Y	3800	Y	0.51	1.28	0.37	0.91	60
98e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	3.08	3797640		19700	Y	17000	Y					
99e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			2300	Y	1100	Y					
100e	N	0	0	0	0	0	0	0	0	0	0	0	0	0	1000	0	0			2400	Y	0	N					

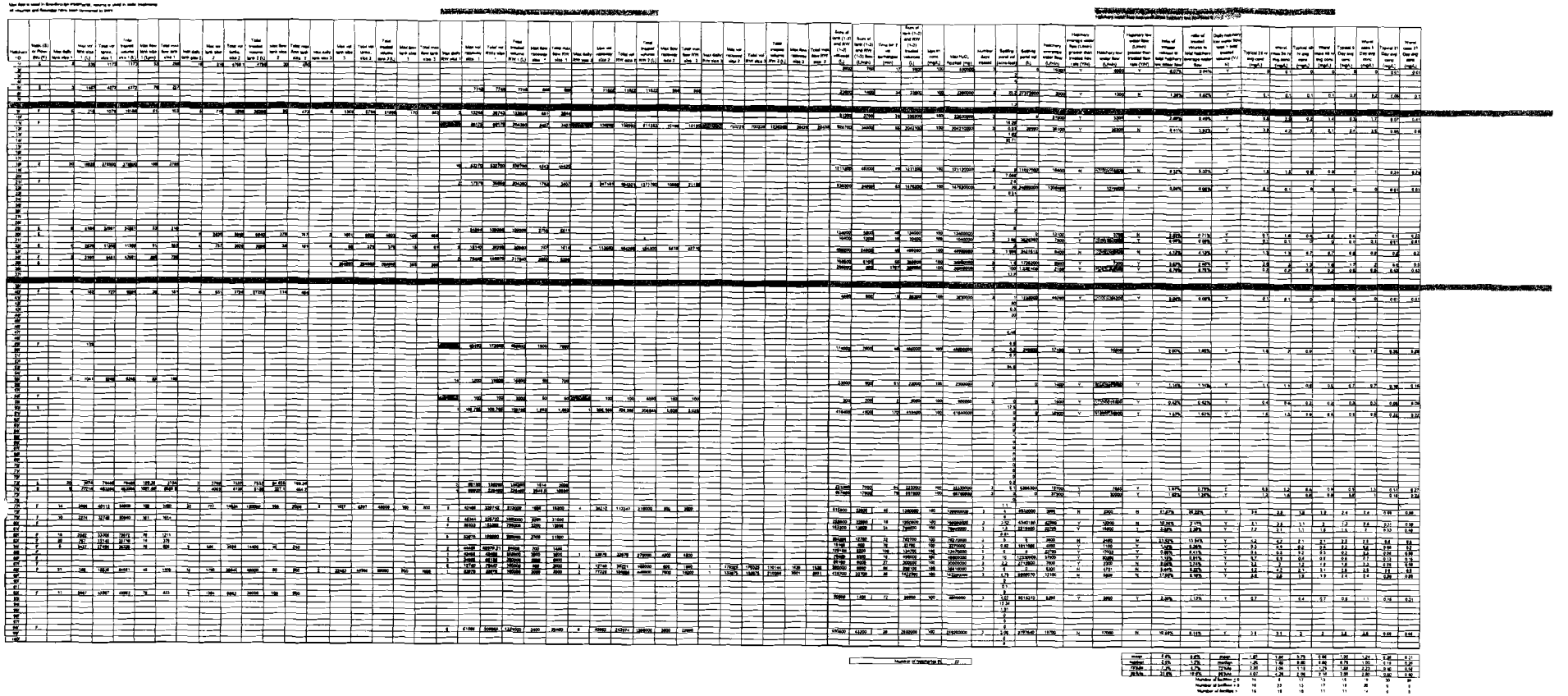
mean	0.91	1.36	0.85	1.32	23.43
median	0.31	0.62	0.30	0.58	15.00
75%ile	0.982194	1.693075	0.940588	1.772885	
95%ile	3.167473	4.698291	4.167456	4.450904	
Number ≤ 0.7 mg/L	24	21	27	21	
Number > 0.7 mg/L	15	18	12	18	
Number > 1 mg/L	10	13	9	14	

Appendix A. Section 5. Hydrogen peroxide treatment regimens for fish.

Hatchery I.D.	Hydrogen peroxide	CHT	OTC	Potassium permanganate	Minimum (mg/L)	Maximum (mg/L)	Minimum (mg/L)	Maximum (mg/L)	Static bath	Flow-through	Number days treated	Treatment on consecutive or alternate days?	Treatment duration static - minimum (min)	Treatment duration static - maximum (min)	Treatment duration flow through - minimum (min)	Treatment duration flow through - maximum (min)	Fungus	BGD	Columnaris/BCWD	Furunculosis/Aeromonas hydrophila	BKD/ERM	Trematodes, protozoans, ectoparasites	Other	If you checked other, write disease name
1f	X						60	250	X		3	A	15	60			X							
2f																								
3f																								
4f																								
5f	X						50	250	X		3	A	15	60			X	X	X			X		
6f																								
7f																								
8f	X						250	500		X	1				1	2	X	X				X		
9f	X						150	150		X	1				60	60		X				X		
10f																								
11f	X						100	300		X	3	C			30	60		X						
12f																								
13f																								
14f																								
15f																								
16f																								
17f																								
18f	X						250	250	X				30	60			X		X			X		
19f																								
20f																								
21f	X						50	250		X	3	A			15	30	X	X	X					
22f																								
23f																								
24f																								
25f																								
26f																								
27f																								
28f																								
29f	X						100	400	X		1		10	15			X							
30f	X						100	150	X		3	C	60	60			X							
31f																								
32f	X						50	75	X		3	A	15	30			X	X						
33f																								
34f	X						50	200		X	2	D			60	60		X						
35f	X						25	25	X		3	A					X					X	X	Streptococcus
36f																								
37f																								
38f	X						40	120		X	3	C			90	240	X	X	X			X		
39f																								
40f	X						50	250		X					60	60		X						
41f																								
42f																								
43f																								
44f																								
45f																								
46f																								
47f																								
48f																								
49f	X						25	100		X	19	A			15	60	X		X			X		
50f																								
51f																								
52f																								
53f																								
54f																								
55f	X						50	250	X		3	A	15	60			X		X			X		
56f																								
57f																								
58f	X						250	250		X	3	A			60	60	X							
59f																								
60f	X						50	125	X		90	A	60	60			X					X		
61f																								
62f																								
63f																								
64f																								
65f																								
66f																								
67f																								
68f																								
69f																								
70f																								
71f																								
72f																								
73f	X						50	250	X				15	60			X	X	X	X		X		
74f	X						35	60	X		2	C	60	60				X				X		
75f																								
76f																								
77f	X						50	600		X	3	C			30	60	X	X	X			X		
78f																								
79f	X						250	250		X	2	C			17	60	X							
80f	X						400	400		X	4	C			30	30	X	X				X		
81f																								
82f	X						250	250		X	3	A			30	30	X							
83f	X						200	300		X	3	C			30	60	X							
84f	X						200	350		X					30	60	X							
85f	X						500	750		X	2	C			30	60	X							
86f	X						150	250		X	3	A			30	30	X							
87f	X						50	250		X	3	A			30	60	X							
88f	X						50	250		X	3	A			30	60	X	X				X		
89f																								
90f																								
91f																								
92f	X						100	100		X	1	C			14	60	X	X	X			X		
93f																								
94f																								
95f																								
96f																								
97f																								
98f	X						250	750		X	12	A			90	90	x							
99f																								
100f																								
AVERAGE																								
MEDIAN																								
SUM of X	32								11	20							28	15	9	1	0	15	1	
SUM of Y																								
Sum of "A"												14												
Sum of "C"												11												
SUM of N																								

Hatchery I.D.	Cold-water	Cool-water	Warm-water	Max concurrent egg jars size 1	Max concurrent egg jars size 2	Max concurrent heath stacks	Max concurrent clark-williams	Max concurrent tank size 1	Max concurrent tank size 2	Max concurrent tank size 3	Max concurrent raceway size 1	Max concurrent raceway size 2	Max concurrent raceway size 3	Max daily tank size 1	Max daily tank size 2	Max daily tank size 3	Max daily RW size 1	Max daily RW size 2	Max daily RW size 3	Percent treated volume drained from culture unit after treatment (%)	Percent flow rate increase after treatment (%)	Flow rate increase maintained for X min	Times per year	Spring	Summer	Fall	Winter
1f	X										1	1	1	5	15	2				50	0	0	2		X	X	
2f																											
3f																											
4f																											
5f	X	X	X					3			1	1		3			1	1		50	0	0	6		X	X	
6f																											
7f																											
8f			X					1						1						0	0		3				
9f	X							1	1	1	1			5	5	5	3			100	0		10	X	X	X	X
10f																											
11f	X										2	2	1				4	6	2	100	0		2	X			
12f																											
13f																											
14f																											
15f																											
16f																											
17f																											
18f		X	X					5			2			20			10			0	0	0	5	X	X	X	
19f																											
20f																											
21f	X										2	2					2	2		100	0		50	X	X	X	X
22f																											
23f																											
24f																											
25f																											
26f																											
27f																											
28f																											
29f		X	X					6			2			6			2			50	0	0	12	X			
30f			X						2	3					2	3				50	200	30	6	X			X
31f																											
32f	X	X						1	3	3	1	2		4	4	4	2	4						X	X	X	X
33f																											
34f	X							3			2			3			2			100	0		10		X		X
35f			X							2						1							3				
36f																											
37f																											
38f			X					12	12	1	1			8	8	8	1			0	0		75	X	X	X	X
39f																											
40f	X							4	8		1			4	4					0	0		5			X	
41f																											
42f																											
43f																											
44f																											
45f																											
46f																											
47f																											
48f																											
49f	X										32						32			0			50		X	X	
50f																											
51f																											
52f																											
53f																											
54f																											
55f			X					6			14			6			14			75	100	3	2	X	X	X	
56f																											
57f																											
58f	X										[illegible]	[illegible]					1	1		100	0		8	X	X	Y	X
59f																											
60f	X										1	1					1	1		0	0	0	45		X		
61f																											
62f																											
63f																											
64f																											
65f																											
66f																											
67f																											
68f																											
69f																											
70f																											
71f																											
72f																											
73f	X							2	2		2			20	2		2			30	0	0	10	X	X	Y	
74f	X							4	4		4			8	2		4			0	0		4	X	X	X	X
75f																											
76f																											
77f	X	X	X					26	25	8	30	16		14	20	8	8	4					4	X	X	X	
78f																											
79f	X							20			5			10			5			0	0		60	X		X	
80f	X										4						4			0	0		10	X		X	X
81f																											
82f	X							16			5			16			5			100	0		as needed	X	X	X	X
83f		X	X					40						20	-					0	0		4	X	X	X	
84f	X							18	21					8	8		4			0	0		50		X	X	X
85f	X										1	1					1	1		0	0	0	20		X	X	X
86f	X										2						2			100	0		32		X	X	X
87f	X										15	3	1				6	3	1	0	0		48			X	X
88f	X							31	16	2	4	2	1	31	16	2	1	2	1	0	0	0	60		X	X	X
89f																											
90f																											
91f																											
92f		X	X	96				11	3					11	8						0		14	X	X		
93f																											
94f																											
95f																											
96f																											
97f																											
98f	X										3	3					6	6		100	0	0	1			X	X
99f																											
100f																											
AVERAGE																							29.4				
MEDIAN																							10				
SUM of X	22	7	11																					18	22	23	15
SUM of Y																											
Sum of "A"																											
Sum of "C"																											
SUM of N																											

Appendix A: Section 6. Environmental Introduction Concentrations for Fish.



Appendix A. Section 7a. Hydrogen peroxide environmental introduction concentration estimates following egg therapy of 1000 mg/L for 15 min or fish therapy at 100 mg/L for 60 min.

Hatchery I.D.	Fish (f) or egg (e) treatment	Settling pond vol (L)	Typical 24 hr avg conc (mg/L)	Worst case 24 hr avg conc (mg/L)	Typical 48 hr avg conc (mg/L)	Worst case 48 hr avg conc (mg/L)	Typical 5 Day avg conc (mg/L)	Worst case 5 Day avg conc (mg/L)	Typical 21-d avg conc (mg/L)	Worst case 21-d avg conc (mg/L)
1f	f		0	0.1	0	0	0	0	0.01	0.01
21e	e	24660000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
45e	e		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
78e	e		0.02	0.05	0.02	0.05	0.02	0.05	0.02	0.04
49e	e	246600	0.02	0.03	0.02	0.03	0.02	0.03	0.02	0.02
5e	e	27372600	0.04	0.04	0.04	0.04	0.04	0.04	0.19	0.25
13e	e	37865430	0.04	0.05	0.04	0.05	0.04	0.05	0.06	0.11
30e	e	3526380	0.05	0.05	0.05	0.05	0.05	0.05	0.04	0.04
74e	e		0.08	0.10	0.08	0.10	0.08	0.10	0.06	0.07
40f	f	1233000	0.1	0.1	0	0	0	0	0.01	0.01
30f	f	3526380	0.1	0.1	0	0	0.1	0.1	0.01	0.01
21f	f	24660000	0.1	0.1	0	0	0	0	0.01	0.01
5f	f	27372600	0.1	0.1	0.1	0.1	0.2	0.2	0.08	0.1
1e	e		0.10	0.17	0.10	0.17	0.10	0.17	0.07	0.12
18e	e	11097000	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
86e	e	2712600	0.12	0.28	0.12	0.28	0.12	0.28	0.11	0.35
22e	e	382230	0.13	0.19	0.13	0.19	0.13	0.19	0.10	0.14
14e	e		0.13	0.30	0.13	0.30	0.13	0.30	0.09	0.21
73e	e	6288300	0.17	0.33	0.17	0.33	0.17	0.33	0.15	0.36
29e	e		0.17	0.62	0.17	0.62	0.17	0.62	0.12	0.44
35f	f	123300000	0.2	0.2	0.2	0.2	0.6	0.6	0.43	0.43
42e	e	369900	0.22	0.40	0.22	0.40	0.22	0.40	0.17	0.34
52e	e	116518500	0.22	0.23	0.22	0.23	0.22	0.23	1.20	1.87
94e	e	2355030	0.29	0.30	0.29	0.30	0.29	0.30	0.23	0.24
63e	e	1011060	0.29	0.85	0.29	0.85	0.29	0.85	0.24	0.96
83f	f	1011060	0.3	0.9	0.2	0.5	0.2	0.8	0.05	0.2
36e	e	16275600	0.31	0.62	0.31	0.62	0.31	0.62	0.34	1.56
55e	e		0.35	0.35	0.35	0.35	0.35	0.35	0.25	0.25
58f	f		0.4	0.4	0.2	0.2	0.3	0.3	0.06	0.06
84f	f		0.4	0.5	0.2	0.3	0.2	0.3	0.06	0.08
90e	e	123300	0.42	0.80	0.42	0.80	0.42	0.80	0.30	0.58
9f	f		0.5	2.9	0.2	1.4	0.3	1.7	0.07	0.41
97e	e		0.51	1.28	0.51	1.28	0.51	1.26	0.37	0.91
73f	f	6288300	0.6	1.3	0.4	0.8	0.5	1.1	0.11	0.27
31e	e	3699000	0.64	0.64	0.64	0.64	0.64	0.64	0.67	0.67
85f	f	123300000	0.7	0.9	0.4	0.5	0.5	0.7	0.13	0.16
92f	f	5018310	0.7	1	0.4	0.7	0.6	1.1	0.16	0.31
29f	f		0.7	1.6	0.4	0.8	0.4	1	0.1	0.23
46e	e	554850	0.77	0.85	0.77	0.85	0.77	0.85	0.66	0.75
34e	e	1726200	0.78	0.93	0.78	0.93	0.78	0.93	0.63	0.77
58e	e		0.79	0.79	0.79	0.79	0.79	0.79	0.56	0.56
6e	e		0.86	4.03	0.86	4.03	0.86	4.03	0.63	2.86
77e	e	4932000	0.92	1.16	0.92	1.16	0.92	1.16	1.20	1.93
23e	e		1.04	2.34	1.04	2.34	1.04	2.34	0.74	1.67
55f	f		1.1	1.1	0.6	0.6	0.7	0.7	0.16	0.16
24e	e		1.24	4.40	1.24	4.40	1.24	4.40	0.89	3.14
32f	f	2421612	1.3	1.3	0.7	0.7	0.8	0.8	0.2	0.2
74f	f		1.3	1.6	0.6	0.8	0.8	1	0.18	0.23
25e	e	2466000	1.31	1.86	1.31	1.86	1.31	1.86	1.34	2.32
67e	e	4932000	1.38	1.53	1.38	1.53	1.38	1.53	1.60	1.89
16e	e		1.39	2.22	1.39	2.22	1.39	2.22	0.99	1.59
18f	f	11097000	1.5	1.5	0.8	0.8	1	1	0.24	0.24
60f	f		1.5	1.5	0.8	0.8	0.9	0.9	0.22	0.22
49f	f	246600	1.8	2	0.9	1	1.1	1.2	0.26	0.29
47e	e		1.88	1.88	1.88	1.88	1.88	1.88	1.35	1.35
79f	f	4340160	2.1	3.8	1.1	2	1.3	2.5	0.31	0.59
86f	f	2712600	2.2	3	1.2	1.8	1.6	2.3	0.39	0.58
80f	f	2219400	2.2	3.1	1.1	1.6	1.4	2	0.33	0.46
32e	e	2421612	2.27	2.77	2.27	2.77	2.27	2.77	1.93	2.45
34f	f	1726200	2.5	3	1.3	1.6	1.7	2	0.4	0.5
93e	e	18914220	2.82	2.82	2.82	2.82	2.82	2.82	4.13	4.13
77f	f	4932000	3.6	3.6	1.9	1.9	2.4	2.4	0.59	0.59
88f	f	5906070	3.6	3.6	1.9	1.9	2.4	2.4	0.59	0.59
96f	f	3797640	3.9	3.9	2	2	2.5	2.5	0.59	0.59
11f	f	36990	3.9	4.2	2	2.1	2.4	2.5	0.56	0.6
82f	f		4.2	4.2	2.1	2.1	2.5	2.5	0.6	0.6
87f	f		4.2	4.2	2.1	2.1	2.5	2.5	0.6	0.6
37e	e		6.25	10.42	6.25	10.42	6.25	10.42	4.46	8.93
2e	e	2466000	7.40	7.40	7.40	7.40	7.40	7.40	7.30	7.30
mean		12206643.95	1.18	1.58	0.86	1.20	0.95	1.31	0.59	0.88
median		3612690.00	0.64	0.90	0.40	0.79	0.51	0.80	0.24	0.36
75%ile		11097000.00	1.50	2.34	1.10	1.80	1.30	2.00	0.60	0.75
95%ile		36291505.50	4.08	4.20	2.20	3.55	2.50	3.55	1.80	3.04
maximum		123300000.00	7.40	10.42	7.40	10.42	7.40	10.42	7.30	8.93
		Number of facilites ≤ 0.7	38	29	41	34	39	31	57	51
		Number of facilites > 0.7	31	40	28	35	30	38	12	18
		Number of facilites > 1	26	32	20	24	21	27	9	14

Appendix A. Section 7b. Summary of hydrogen peroxide environmental introduction concentration estimates following egg therapy of 1000 mg/L for 15 min or fish therapy at 100 mg/L for 60 min for facilities with a settling pond.

Hatchery I.D.	Settling pond vol (L)	Typical 24 hr avg conc (mg/L)	Worst case 24 hr avg conc (mg/L)	Typical 48 hr avg conc (mg/L)	Worst case 48 hr avg conc (mg/L)	Typical 5 Day avg conc (mg/L)	Worst case 5 Day avg conc (mg/L)	Typical 21-d avg conc (mg/L)	Worst case 21-d avg conc (mg/L)
11f	36990	3.9	4.2	2	2.1	2.4	2.5	0.56	0.6
90e	123300	0.42	0.80	0.42	0.80	0.42	0.80	0.30	0.58
49e	246600	0.02	0.03	0.02	0.03	0.02	0.03	0.02	0.02
49f	246600	1.8	2	0.9	1	1.1	1.2	0.26	0.29
42e	369900	0.22	0.40	0.22	0.40	0.22	0.40	0.17	0.34
22e	382230	0.13	0.19	0.13	0.19	0.13	0.19	0.10	0.14
46e	554850	0.77	0.85	0.77	0.85	0.77	0.85	0.66	0.75
83e	1011060	0.29	0.85	0.29	0.85	0.29	0.85	0.24	0.96
83f	1011060	0.3	0.9	0.2	0.5	0.2	0.8	0.05	0.2
40f	1233000	0.1	0.1	0	0	0	0	0.01	0.01
34e	1726200	0.78	0.93	0.78	0.93	0.78	0.93	0.63	0.77
34f	1726200	2.5	3	1.3	1.6	1.7	2	0.4	0.5
80f	2219400	2.2	3.1	1.1	1.6	1.4	2	0.33	0.48
94e	2355030	0.29	0.30	0.29	0.30	0.29	0.30	0.23	0.24
32f	2421612	1.3	1.3	0.7	0.7	0.8	0.8	0.2	0.2
32e	2421612	2.27	2.77	2.27	2.77	2.27	2.77	1.93	2.45
25e	2466000	1.31	1.86	1.31	1.86	1.31	1.86	1.34	2.32
2e	2466000	7.40	7.40	7.40	7.40	7.40	7.40	7.30	7.30
86e	2712600	0.12	0.28	0.12	0.28	0.12	0.28	0.11	0.35
86f	2712600	2.2	3	1.2	1.8	1.6	2.3	0.39	0.58
30e	3526380	0.05	0.05	0.05	0.05	0.05	0.05	0.04	0.04
30f	3526380	0.1	0.1	0	0	0.1	0.1	0.01	0.01
31e	3699000	0.64	0.64	0.64	0.64	0.64	0.64	0.67	0.67
98f	3797640	3.9	3.9	2	2	2.5	2.5	0.59	0.59
79f	4340160	2.1	3.8	1.1	2	1.3	2.5	0.31	0.59
77e	4932000	0.92	1.16	0.92	1.16	0.92	1.16	1.20	1.93
67e	4932000	1.38	1.53	1.38	1.53	1.38	1.53	1.60	1.89
77f	4932000	3.6	3.6	1.9	1.9	2.4	2.4	0.59	0.59
92f	5018310	0.7	1	0.4	0.7	0.6	1.1	0.16	0.31
88f	5906070	3.6	3.6	1.9	1.9	2.4	2.4	0.59	0.59
73e	6288300	0.17	0.33	0.17	0.33	0.17	0.33	0.15	0.36
73f	6288300	0.6	1.3	0.4	0.8	0.5	1.1	0.11	0.27
18e	11097000	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
18f	11097000	1.5	1.5	0.8	0.8	1	1	0.24	0.24
85f	12330000	0.7	0.9	0.4	0.5	0.5	0.7	0.13	0.16
36e	16275600	0.31	0.62	0.31	0.62	0.31	0.62	0.34	1.58
93e	18914220	2.82	2.82	2.82	2.82	2.82	2.82	4.13	4.13
21e	24660000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
21f	24660000	0.1	0.1	0	0	0	0	0.01	0.01
5e	27372600	0.04	0.04	0.04	0.04	0.04	0.04	0.19	0.25
5f	27372600	0.1	0.1	0.1	0.1	0.2	0.2	0.08	0.1
13e	37865430	0.04	0.05	0.04	0.05	0.04	0.05	0.06	0.11
52e	116518500	0.22	0.23	0.22	0.23	0.22	0.23	1.20	1.87
35f	123300000	0.2	0.2	0.2	0.2	0.6	0.6	0.43	0.43
mean	12206643.95	1.19	1.41	0.85	1.01	0.96	1.15	0.64	0.82
median	3612690.00	0.62	0.87	0.40	0.70	0.55	0.80	0.25	0.40
75%ile	11097000.00	1.88	2.19	1.13	1.60	1.33	1.89	0.59	0.69
95%ile	36291505.50	3.86	3.89	2.23	2.67	2.49	2.73	1.88	2.43
maximum	123300000.00	7.40	7.40	7.40	7.40	7.40	7.40	7.30	7.30
	Number of facilites ≤ 0.7	25	19	26	23	25	20	37	33
	Number of facilites > 0.7	19	25	18	21	19	24	7	11
	Number of facilites > 1 r	16	18	13	14	14	17	7	8

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Appendix A. Section 7c. Summary of hydrogen peroxide environmental introduction concentration estimates following egg therapy of 1000 mg/L for 15 min or fish therapy at 100 mg/L for 60 min for facilities without a settling pond.

Hatchery I.D.	Settling pond vol (L)	Typical 24 hr avg conc (mg/L)	Worst case 24 hr avg conc (mg/L)	Typical 48 hr avg conc (mg/L)	Worst case 48 hr avg conc (mg/L)	Typical 5 Day avg conc (mg/L)	Worst case 5 Day avg conc (mg/L)	Typical 21-d avg conc (mg/L)	Worst case 21-d avg conc (mg/L)
1f		0	0.1	0	0	0	0	0.01	0.01
58f		0.4	0.4	0.2	0.2	0.3	0.3	0.06	0.06
84f		0.4	0.5	0.2	0.3	0.2	0.3	0.06	0.08
9f		0.5	2.9	0.2	1.4	0.3	1.7	0.07	0.41
29f		0.7	1.6	0.4	0.8	0.4	1	0.1	0.23
55f		1.1	1.1	0.6	0.6	0.7	0.7	0.16	0.16
74f		1.3	1.6	0.6	0.8	0.8	1	0.18	0.23
60f		1.5	1.5	0.8	0.8	0.9	0.9	0.22	0.22
82f		4.2	4.2	2.1	2.1	2.5	2.5	0.6	0.6
87f		4.2	4.2	2.1	2.1	2.5	2.5	0.6	0.6
45e		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
78e		0.02	0.05	0.02	0.05	0.02	0.05	0.02	0.04
74e		0.08	0.10	0.08	0.10	0.08	0.10	0.06	0.07
1e		0.10	0.17	0.10	0.17	0.10	0.17	0.07	0.12
14e		0.13	0.30	0.13	0.30	0.13	0.30	0.09	0.21
29e		0.17	0.62	0.17	0.62	0.17	0.62	0.12	0.44
55e		0.35	0.35	0.35	0.35	0.35	0.35	0.25	0.25
97e		0.51	1.28	0.51	1.28	0.51	1.28	0.37	0.91
58e		0.79	0.79	0.79	0.79	0.79	0.79	0.56	0.56
6e		0.88	4.03	0.88	4.03	0.88	4.03	0.63	2.88
23e		1.04	2.34	1.04	2.34	1.04	2.34	0.74	1.67
24e		1.24	4.40	1.24	4.40	1.24	4.40	0.89	3.14
16e		1.39	2.22	1.39	2.22	1.39	2.22	0.99	1.59
47e		1.88	1.88	1.88	1.88	1.88	1.88	1.35	1.35
37e		6.25	10.42	6.25	10.42	6.25	10.42	4.46	8.93
mean	NA	1.17	1.88	0.88	1.52	0.94	1.59	0.51	0.99
median	NA	0.70	1.28	0.51	0.80	0.51	0.90	0.18	0.25
75%ile	NA	1.30	2.34	1.04	2.10	1.04	2.22	0.60	0.91
95%ile	NA	4.20	4.36	2.10	4.32	2.50	4.32	1.28	3.09
maximum	NA	6.25	10.42	6.25	10.42	6.25	10.42	4.46	8.93
	Number of facilites ≤ 0.7	13	10	15	11	14	11	20	18
	Number of facilites > 0.7	12	15	10	14	11	14	5	7
	Number of facilites > 1 r	10	14	7	10	7	10	2	6

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Appendix A. Section 7d. Summary of hydrogen peroxide environmental introduction concentration estimates following egg therapy of 1000 mg/L for 15 min.

Hatchery I.D.	Fish (f) or egg (e) treatment	Settling pond vol (L)	Typical 24 hr avg conc (mg/L)	Worst case 24 hr avg conc (mg/L)	Typical 48 hr avg conc (mg/L)	Worst case 48 hr avg conc (mg/L)	Typical 5 Day avg conc (mg/L)	Worst case 5 Day avg conc (mg/L)	Typical 21-d avg conc (mg/L)	Worst case 21-d avg conc (mg/L)
21e	e	24660000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
45e	e		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
78e	e		0.02	0.05	0.02	0.05	0.02	0.05	0.02	0.04
49e	e	246600	0.02	0.03	0.02	0.03	0.02	0.03	0.02	0.02
5e	e	27372600	0.04	0.04	0.04	0.04	0.04	0.04	0.19	0.25
13e	e	37865430	0.04	0.05	0.04	0.05	0.04	0.05	0.06	0.11
30e	e	3526380	0.05	0.05	0.05	0.05	0.05	0.05	0.04	0.04
74e	e		0.08	0.10	0.08	0.10	0.08	0.10	0.06	0.07
1e	e		0.10	0.17	0.10	0.17	0.10	0.17	0.07	0.12
18e	e	11097000	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
86e	e	2712600	0.12	0.28	0.12	0.28	0.12	0.28	0.11	0.35
22e	e	382230	0.13	0.19	0.13	0.19	0.13	0.19	0.10	0.14
14e	e		0.13	0.30	0.13	0.30	0.13	0.30	0.09	0.21
73e	e	6288300	0.17	0.33	0.17	0.33	0.17	0.33	0.15	0.36
29e	e		0.17	0.62	0.17	0.62	0.17	0.62	0.12	0.44
42e	e	369900	0.22	0.40	0.22	0.40	0.22	0.40	0.17	0.34
52e	e	116518500	0.22	0.23	0.22	0.23	0.22	0.23	1.20	1.87
94e	e	2355030	0.29	0.30	0.29	0.30	0.29	0.30	0.23	0.24
83e	e	1011060	0.29	0.85	0.29	0.85	0.29	0.85	0.24	0.96
36e	e	16275600	0.31	0.62	0.31	0.62	0.31	0.62	0.34	1.58
55e	e		0.35	0.35	0.35	0.35	0.35	0.35	0.25	0.25
90e	e	123300	0.42	0.80	0.42	0.80	0.42	0.80	0.30	0.58
97e	e		0.51	1.28	0.51	1.28	0.51	1.28	0.37	0.91
31e	e	3699000	0.64	0.64	0.64	0.64	0.64	0.64	0.67	0.67
46e	e	554850	0.77	0.85	0.77	0.85	0.77	0.85	0.66	0.75
34e	e	1726200	0.78	0.93	0.78	0.93	0.78	0.93	0.63	0.77
58e	e		0.79	0.79	0.79	0.79	0.79	0.79	0.56	0.56
6e	e		0.88	4.03	0.88	4.03	0.88	4.03	0.63	2.88
77e	e	4932000	0.92	1.16	0.92	1.16	0.92	1.16	1.20	1.93
23e	e		1.04	2.34	1.04	2.34	1.04	2.34	0.74	1.67
24e	e		1.24	4.40	1.24	4.40	1.24	4.40	0.89	3.14
25e	e	2466000	1.31	1.86	1.31	1.86	1.31	1.86	1.34	2.32
67e	e	4932000	1.38	1.53	1.38	1.53	1.38	1.53	1.60	1.89
16e	e		1.39	2.22	1.39	2.22	1.39	2.22	0.99	1.59
47e	e		1.88	1.88	1.88	1.88	1.88	1.88	1.35	1.35
32e	e	2421612	2.27	2.77	2.27	2.77	2.27	2.77	1.93	2.45
93e	e	18914220	2.82	2.82	2.82	2.82	2.82	2.82	4.13	4.13
37e	e		6.25	10.42	6.25	10.42	6.25	10.42	4.46	8.93
2e	e	2466000	7.40	7.40	7.40	7.40	7.40	7.40	7.30	7.30
mean		12204850.50	0.91	1.36	0.91	1.36	0.91	1.36	0.85	1.32
median		3119490.00	0.31	0.62	0.31	0.62	0.31	0.62	0.30	0.58
75%ile		12391650.00	0.98	1.69	0.98	1.69	0.98	1.69	0.94	1.77
95%ile		36291505.50	3.17	4.70	3.17	4.70	3.17	4.70	4.17	4.45
maximum		116518500.00	7.40	10.42	7.40	10.42	7.40	10.42	7.30	8.93
	Number of facilites ≤ 0.7		24	21	24	21	24	21	27	21
	Number of facilites > 0.7		15	18	15	18	15	18	12	18
	Number of facilites > 1		10	13	10	13	10	13	9	14

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Appendix A. Section 7e. Summary of hydrogen peroxide environmental introduction concentration estimates following fish therapy at 100 mg/L for 60 min.

Hatchery I.D.	Fish (f) or egg (e) treatment	Settling pond vol (L)	Typical 24 hr avg conc (mg/L)	Worst case 24 hr avg conc (mg/L)	Typical 48 hr avg conc (mg/L)	Worst case 48 hr avg conc (mg/L)	Typical 5 Day avg conc (mg/L)	Worst case 5 Day avg conc (mg/L)	Typical 21-d avg conc (mg/L)	Worst case 21-d avg conc (mg/L)
1f	f		0	0.1	0	0	0	0	0.01	0.01
40f	f	1233000	0.1	0.1	0	0	0	0	0.01	0.01
30f	f	3526380	0.1	0.1	0	0	0.1	0.1	0.01	0.01
21f	f	24660000	0.1	0.1	0	0	0	0	0.01	0.01
5f	f	27372600	0.1	0.1	0.1	0.1	0.2	0.2	0.08	0.1
35f	f	123300000	0.2	0.2	0.2	0.2	0.6	0.6	0.43	0.43
83f	f	1011060	0.3	0.9	0.2	0.5	0.2	0.8	0.05	0.2
58f	f		0.4	0.4	0.2	0.2	0.3	0.3	0.06	0.06
84f	f		0.4	0.5	0.2	0.3	0.2	0.3	0.06	0.08
9f	f		0.5	2.9	0.2	1.4	0.3	1.7	0.07	0.41
73f	f	6288300	0.6	1.3	0.4	0.8	0.5	1.1	0.11	0.27
85f	f	12330000	0.7	0.9	0.4	0.5	0.5	0.7	0.13	0.16
92f	f	5018310	0.7	1	0.4	0.7	0.6	1.1	0.16	0.31
29f	f		0.7	1.6	0.4	0.8	0.4	1	0.1	0.23
55f	f		1.1	1.1	0.6	0.6	0.7	0.7	0.16	0.16
32f	f	2421612	1.3	1.3	0.7	0.7	0.8	0.8	0.2	0.2
74f	f		1.3	1.6	0.6	0.8	0.8	1	0.18	0.23
18f	f	11097000	1.5	1.5	0.8	0.8	1	1	0.24	0.24
60f	f		1.5	1.5	0.8	0.8	0.9	0.9	0.22	0.22
49f	f	246600	1.8	2	0.9	1	1.1	1.2	0.26	0.29
79f	f	4340160	2.1	3.8	1.1	2	1.3	2.5	0.31	0.59
86f	f	2712600	2.2	3	1.2	1.8	1.6	2.3	0.39	0.58
80f	f	2219400	2.2	3.1	1.1	1.6	1.4	2	0.33	0.48
34f	f	1726200	2.5	3	1.3	1.6	1.7	2	0.4	0.5
77f	f	4932000	3.6	3.6	1.9	1.9	2.4	2.4	0.59	0.59
88f	f	5906070	3.6	3.6	1.9	1.9	2.4	2.4	0.59	0.59
98f	f	3797640	3.9	3.9	2	2	2.5	2.5	0.59	0.59
11f	f	36990	3.9	4.2	2	2.1	2.4	2.5	0.56	0.6
82f	f		4.2	4.2	2.1	2.1	2.5	2.5	0.6	0.6
87f	f		4.2	4.2	2.1	2.1	2.5	2.5	0.6	0.6
mean		12208796.10	1.53	1.86	0.79	0.98	1.00	1.24	0.25	0.31
median		4068900.00	1.20	1.50	0.60	0.80	0.75	1.00	0.19	0.26
75%ile		7490475.00	2.20	3.08	1.18	1.75	1.55	2.23	0.40	0.56
95%ile		32168970.00	4.07	4.20	2.06	2.10	2.50	2.50	0.60	0.60
maximum		123300000.00	4.20	4.20	2.10	2.10	2.50	2.50	0.60	0.60
	Number of facilites ≤ 0.7		14	8	17	13	15	10	30	30
	Number of facilites > 0.7		16	22	13	17	15	20	0	0
	Number of facilites > 1		16	19	10	11	11	14	0	0

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Appendix A. Section 7f. Summary of hydrogen peroxide environmental introduction concentration estimates following egg therapy of 1000 mg/L for 15 min at hatcheries with a settling pond.

Hatchery I.D.	Fish (f) or egg (e) treatment	Settling pond vol (L)	Typical 24 hr avg conc (mg/L)	Worst case 24 hr avg conc (mg/L)	Typical 48 hr avg conc (mg/L)	Worst case 48 hr avg conc (mg/L)	Typical 5 Day avg conc (mg/L)	Worst case 5 Day avg conc (mg/L)	Typical 21-d avg conc (mg/L)	Worst case 21-d avg conc (mg/L)
90e	e	123300	0.42	0.80	0.42	0.80	0.42	0.80	0.30	0.58
49e	e	246600	0.02	0.03	0.02	0.03	0.02	0.03	0.02	0.02
42e	e	369900	0.22	0.40	0.22	0.40	0.22	0.40	0.17	0.34
22e	e	382230	0.13	0.19	0.13	0.19	0.13	0.19	0.10	0.14
46e	e	554850	0.77	0.85	0.77	0.85	0.77	0.85	0.66	0.75
83e	e	1011060	0.29	0.85	0.29	0.85	0.29	0.85	0.24	0.96
34e	e	1726200	0.78	0.93	0.78	0.93	0.78	0.93	0.63	0.77
94e	e	2355030	0.29	0.30	0.29	0.30	0.29	0.30	0.23	0.24
32e	e	2421612	2.27	2.77	2.27	2.77	2.27	2.77	1.93	2.45
25e	e	2466000	1.31	1.86	1.31	1.86	1.31	1.86	1.34	2.32
2e	e	2466000	7.40	7.40	7.40	7.40	7.40	7.40	7.30	7.30
86e	e	2712600	0.12	0.28	0.12	0.28	0.12	0.28	0.11	0.35
30e	e	3526380	0.05	0.05	0.05	0.05	0.05	0.05	0.04	0.04
31e	e	3699000	0.64	0.64	0.64	0.64	0.64	0.64	0.67	0.67
77e	e	4932000	0.92	1.16	0.92	1.16	0.92	1.16	1.20	1.93
67e	e	4932000	1.38	1.53	1.38	1.53	1.38	1.53	1.60	1.89
73e	e	6288300	0.17	0.33	0.17	0.33	0.17	0.33	0.15	0.36
18e	e	11097000	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
36e	e	16275600	0.31	0.62	0.31	0.62	0.31	0.62	0.34	1.58
93e	e	18914220	2.82	2.82	2.82	2.82	2.82	2.82	4.13	4.13
21e	e	24660000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5e	e	27372600	0.04	0.04	0.04	0.04	0.04	0.04	0.19	0.25
13e	e	37865430	0.04	0.05	0.04	0.05	0.04	0.05	0.06	0.11
52e	e	116518500	0.22	0.23	0.22	0.23	0.22	0.23	1.20	1.87
mean		12204850.50	0.86	1.01	0.86	1.01	0.86	1.01	0.95	1.22
median		3119490.00	0.29	0.51	0.29	0.51	0.29	0.51	0.27	0.62
75%ile		12391650.00	0.81	0.99	0.81	0.99	0.81	0.99	1.20	1.88
95%ile		36291505.50	2.74	2.82	2.74	2.82	2.74	2.82	3.80	3.88
maximum		116518500.00	7.40	7.40	7.40	7.40	7.40	7.40	7.30	7.30
		Number of facilites ≤ 0.7	16	14	16	14	16	14	17	13
		Number of facilites > 0.7	8	10	8	10	8	10	7	11
		Number of facilites > 1 r	5	6	5	6	5	6	7	8

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Appendix A. Section 7g. Summary of hydrogen peroxide environmental introduction concentration estimates following egg therapy of 1000 mg/L for 15 min at hatcheries without a settling pond.

Hatchery I.D.	Fish (f) or egg (e) treatment	Settling pond vol (L)	Typical 24 hr avg conc (mg/L)	Worst case 24 hr avg conc (mg/L)	Typical 48 hr avg conc (mg/L)	Worst case 48 hr avg conc (mg/L)	Typical 5 Day avg conc (mg/L)	Worst case 5 Day avg conc (mg/L)	Typical 21-d avg conc (mg/L)	Worst case 21-d avg conc (mg/L)
45e	e		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
78e	e		0.02	0.05	0.02	0.05	0.02	0.05	0.02	0.04
74e	e		0.08	0.10	0.08	0.10	0.08	0.10	0.06	0.07
1e	e		0.10	0.17	0.10	0.17	0.10	0.17	0.07	0.12
14e	e		0.13	0.30	0.13	0.30	0.13	0.30	0.09	0.21
29e	e		0.17	0.62	0.17	0.62	0.17	0.62	0.12	0.44
55e	e		0.35	0.35	0.35	0.35	0.35	0.35	0.25	0.25
97e	e		0.51	1.28	0.51	1.28	0.51	1.28	0.37	0.91
58e	e		0.79	0.79	0.79	0.79	0.79	0.79	0.56	0.56
6e	e		0.88	4.03	0.88	4.03	0.88	4.03	0.63	2.88
23e	e		1.04	2.34	1.04	2.34	1.04	2.34	0.74	1.67
24e	e		1.24	4.40	1.24	4.40	1.24	4.40	0.89	3.14
16e	e		1.39	2.22	1.39	2.22	1.39	2.22	0.99	1.59
47e	e		1.88	1.88	1.88	1.88	1.88	1.88	1.35	1.35
37e	e		6.25	10.42	6.25	10.42	6.25	10.42	4.46	8.93
mean		NA	0.99	1.93	0.99	1.93	0.99	1.93	0.71	1.48
median		NA	0.51	0.79	0.51	0.79	0.51	0.79	0.37	0.56
75%ile		NA	1.14	2.28	1.14	2.28	1.14	2.28	0.82	1.63
95%ile		NA	3.19	6.20	3.19	6.20	3.19	6.20	2.28	4.88
maximum		0.00	6.25	10.42	6.25	10.42	6.25	10.42	4.46	8.93
		Number of facilites ≤ 0.7	8	7	8	7	8	7	10	8
		Number of facilites > 0.7	7	8	7	8	7	8	5	7
		Number of facilites > 1 r	5	7	5	7	5	7	2	6

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Appendix A. Section 7h. Summary of hydrogen peroxide environmental introduction concentration estimates following fish therapy at 100 mg/L for 60 min at hatcheries with a settling pond.

Hatchery I.D.	Fish (f) or egg (e) treatment	Settling pond vol (L)	Typical 24 hr avg conc (mg/L)	Worst case 24 hr avg conc (mg/L)	Typical 48 hr avg conc (mg/L)	Worst case 48 hr avg conc (mg/L)	Typical 5 Day avg conc (mg/L)	Worst case 5 Day avg conc (mg/L)	Typical 21-d avg conc (mg/L)	Worst case 21-d avg conc (mg/L)
11f	f	36990	3.9	4.2	2	2.1	2.4	2.5	0.56	0.6
49f	f	246600	1.8	2	0.9	1	1.1	1.2	0.26	0.29
83f	f	1011060	0.3	0.9	0.2	0.5	0.2	0.8	0.05	0.2
40f	f	1233000	0.1	0.1	0	0	0	0	0.01	0.01
34f	f	1726200	2.5	3	1.3	1.6	1.7	2	0.4	0.5
80f	f	2219400	2.2	3.1	1.1	1.6	1.4	2	0.33	0.48
32f	f	2421612	1.3	1.3	0.7	0.7	0.8	0.8	0.2	0.2
86f	f	2712600	2.2	3	1.2	1.8	1.6	2.3	0.39	0.58
30f	f	3526380	0.1	0.1	0	0	0.1	0.1	0.01	0.01
98f	f	3797640	3.9	3.9	2	2	2.5	2.5	0.59	0.59
79f	f	4340160	2.1	3.8	1.1	2	1.3	2.5	0.31	0.59
77f	f	4932000	3.6	3.6	1.9	1.9	2.4	2.4	0.59	0.59
92f	f	5018310	0.7	1	0.4	0.7	0.6	1.1	0.16	0.31
88f	f	5906070	3.6	3.6	1.9	1.9	2.4	2.4	0.59	0.59
73f	f	6288300	0.6	1.3	0.4	0.8	0.5	1.1	0.11	0.27
18f	f	11097000	1.5	1.5	0.8	0.8	1	1	0.24	0.24
85f	f	12330000	0.7	0.9	0.4	0.5	0.5	0.7	0.13	0.16
21f	f	24660000	0.1	0.1	0	0	0	0	0.01	0.01
5f	f	27372600	0.1	0.1	0.1	0.1	0.2	0.2	0.08	0.1
35f	f	123300000	0.2	0.2	0.2	0.2	0.6	0.6	0.43	0.43
mean		12208796.10	1.58	1.89	0.83	1.01	1.07	1.31	0.27	0.34
median		4068900.00	1.40	1.40	0.75	0.80	0.90	1.10	0.25	0.30
75%ile		7490475.00	2.28	3.23	1.23	1.83	1.63	2.33	0.41	0.58
95%ile		32168970.00	3.90	3.92	2.00	2.01	2.41	2.50	0.59	0.59
maximum		123300000.00	3.90	4.20	2.00	2.10	2.50	2.50	0.59	0.60
		Number of facilites ≤ 0.7	9	5	10	9	9	6	20	20
		Number of facilites > 0.7	11	15	10	11	11	14	0	0
		Number of facilites > 1 r	11	12	8	8	9	11	0	0

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Appendix A. Section 7i. Summary of hydrogen peroxide environmental introduction concentration estimates following fish therapy at 100 mg/L for 60 min at hatcheries without a settling pond.

Hatchery I.D.	Fish (f) or egg (e) treatment	Settling pond vol (L)	Typical 24 hr avg conc (mg/L)	Worst case 24 hr avg conc (mg/L)	Typical 48 hr avg conc (mg/L)	Worst case 48 hr avg conc (mg/L)	Typical 5 Day avg conc (mg/L)	Worst case 5 Day avg conc (mg/L)	Typical 21-d avg conc (mg/L)	Worst case 21-d avg conc (mg/L)
1f	f		0	0.1	0	0	0	0	0.01	0.01
58f	f		0.4	0.4	0.2	0.2	0.3	0.3	0.06	0.06
84f	f		0.4	0.5	0.2	0.3	0.2	0.3	0.06	0.08
9f	f		0.5	2.9	0.2	1.4	0.3	1.7	0.07	0.41
29f	f		0.7	1.6	0.4	0.8	0.4	1	0.1	0.23
55f	f		1.1	1.1	0.6	0.6	0.7	0.7	0.16	0.16
74f	f		1.3	1.6	0.6	0.8	0.8	1	0.18	0.23
60f	f		1.5	1.5	0.8	0.8	0.9	0.9	0.22	0.22
82f	f		4.2	4.2	2.1	2.1	2.5	2.5	0.6	0.6
87f	f		4.2	4.2	2.1	2.1	2.5	2.5	0.6	0.6
mean		NA	1.43	1.81	0.72	0.91	0.86	1.09	0.21	0.26
median		NA	0.90	1.55	0.50	0.80	0.55	0.95	0.13	0.23
75%ile		NA	1.45	2.58	0.75	1.25	0.88	1.53	0.21	0.37
95%ile		NA	4.20	4.20	2.10	2.10	2.50	2.50	0.60	0.60
maximum		0.00	4.20	4.20	2.10	2.10	2.50	2.50	0.60	0.60
		Number of facilites ≤ 0.7	5	3	7	4	6	4	10	10
		Number of facilites > 0.7	5	7	3	6	4	6	0	0
		Number of facilites > 1 r	5	7	2	3	2	3	0	0

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Appendix A. Section 8a. Risk quotients determined based on the application of VICH Phase II Tier A and Tier B assessment factors to available acute or chronic toxicity data.

	1-d EIC				2-d EIC				5-d EIC				21-d EIC			
	Mean (mg/L)	Median (50th percentile, (mg/L)	75th Percentile (mg/L)	95th Percentile (mg/L)	Mean (mg/L)	Median (50th percentile, (mg/L)	75th Percentile (mg/L)	95th Percentile (mg/L)	Mean (mg/L)	Median (50th percentile, (mg/L)	75th Percentile (mg/L)	95th Percentile (mg/L)	Mean (mg/L)	Median (50th percentile, (mg/L)	75th Percentile (mg/L)	95th Percentile (mg/L)
17	70.58824	35.29412	88.23529	241.1765	52.94118	23.52941	64.70588	129.4118	58.82353	29.41176	76.47059	147.0588	35.29412	11.76471	35.29412	105.8824
2.4	500	250	625	1708.333	375	166.6667	458.3333	916.6667	416.6667	208.3333	541.6667	1041.667	250	83.33333	250	750
63	19.04762	9.52381	23.80952	65.07937	14.28571	6.349206	17.46032	34.92063	15.87302	7.936508	20.63492	39.68254	9.52381	3.174603	9.52381	28.57143
48	25	12.5	31.25	85.41667	18.75	8.333333	22.91667	45.83333	20.83333	10.41667	27.08333	52.08333	12.5	4.166667	12.5	37.5
37	32.43243	16.21622	40.54054	110.8108	24.32432	10.81081	29.72973	59.45946	27.02703	13.51351	35.13514	67.56757	16.21622	5.405405	16.21622	48.64865
6.8	176.4706	88.23529	220.5882	602.9412	132.3529	58.82353	161.7647	323.5294	147.0588	73.52941	191.1765	367.6471	88.23529	29.41176	88.23529	264.7059
6.8	176.4706	88.23529	220.5882	602.9412	132.3529	58.82353	161.7647	323.5294	147.0588	73.52941	191.1765	367.6471	88.23529	29.41176	88.23529	264.7059
0.24	5000	2500	6250	17083.33	3750	1666.667	4583.333	9166.667	4166.667	2083.333	5416.667	10416.67	2500	833.3333	2500	7500
9.4	127.6596	63.82979	159.5745	436.1702	95.74468	42.55319	117.0213	234.0426	106.383	53.19149	138.2979	265.9574	63.82979	21.2766	63.82979	191.4894
105	11.42857	5.714286	14.28571	39.04762	8.571429	3.809524	10.47619	20.95238	9.52381	4.761905	12.38095	23.80952	5.714286	1.904762	5.714286	17.14286
105	11.42857	5.714286	14.28571	39.04762	8.571429	3.809524	10.47619	20.95238	9.52381	4.761905	12.38095	23.80952	5.714286	1.904762	5.714286	17.14286

Parameter	1-d EIC		2-d EIC		5-d EIC		21-d EIC	
	Typical	Worst-Case	Typical	Worst-Case	Typical	Worst-Case	Typical	Worst-Case
Mean (mg/L)	1.2	1.6	0.9	1.2	1	1.3	0.6	0.9
Median (50th percentile, (mg/L)	0.6	0.9	0.4	0.8	0.5	0.8	0.2	0.4
75th Percentile (mg/L)	1.5	2.3	1.1	1.8	1.3	2	0.6	0.8
95th Percentile (mg/L)	4.1	4.2	2.2	3.6	2.5	3.6	1.8	3

Appendix A. Section 8b. Risk quotients determined based on the application refined VICH assessment factors to acute toxicity data.

	1-d EIC				2-d EIC				5-d EIC				21-d EIC			
	Mean (mg/L)	Median (50^{th} percentile, (mg/L)	75^{th} Percentile (mg/L)	95^{th} Percentile (mg/L)	Mean (mg/L)	Median (50^{th} percentile, (mg/L)	75^{th} Percentile (mg/L)	95^{th} Percentile (mg/L)	Mean (mg/L)	Median (50^{th} percentile, (mg/L)	75^{th} Percentile (mg/L)	95^{th} Percentile (mg/L)	Mean (mg/L)	Median (50^{th} percentile, (mg/L)	75^{th} Percentile (mg/L)	95^{th} Percentile (mg/L)
17	70.58824	35.29412	88.23529	241.1765	52.94118	23.52941	64.70588	129.4118	58.82353	29.41176	76.47059	147.0588	35.29412	11.76471	35.29412	105.8824
120	10	5	12.5	34.16667	7.5	3.333333	9.166667	18.33333	8.333333	4.166667	10.83333	20.83333	5	1.666667	5	15
1600	0.75	0.375	0.9375	2.5625	0.5625	0.25	0.6875	1.375	0.625	0.3125	0.8125	1.5625	0.375	0.125	0.375	1.125
68	17.64706	8.823529	22.05882	60.29412	13.23529	5.882353	16.17647	32.35294	14.70588	7.352941	19.11765	36.76471	8.823529	2.941176	8.823529	26.47059
94	12.76596	6.382979	15.95745	43.61702	9.574468	4.255319	11.70213	23.40426	10.6383	5.319149	13.82979	26.59574	6.382979	2.12766	6.382979	19.14894
1750	0.685714	0.342857	0.857143	2.342857	0.514286	0.228571	0.628571	1.257143	0.571429	0.285714	0.742857	1.428571	0.342857	0.114286	0.342857	1.028571

Parameter	1-d EIC		2-d EIC		5-d EIC		21-d EIC	
	Typical	Worst-Case	Typical	Worst-Case	Typical	Worst-Case	Typical	Worst-Case
Mean (mg/L)	1.2	1.6	0.9	1.2	1	1.3	0.6	0.9
Median (50^{th} percentile, (mg/L)	0.6	0.9	0.4	0.8	0.5	0.8	0.2	0.4
75^{th} Percentile (mg/L)	1.5	2.3	1.1	1.8	1.3	2	0.6	0.8
95^{th} Percentile (mg/L)	4.1	4.2	2.2	3.6	2.5	3.6	1.8	3

Appendix A. Section 8c. Risk quotients determined based on the application refined VICH assessment factors to chronic toxicity data.

	1-d EIC				2-d EIC				5-d EIC				21-d EIC			
	Mean (mg/L)	Median (50^{th} percentile, (mg/L)	75^{th} Percentile (mg/L)	95^{th} Percentile (mg/L)	Mean (mg/L)	Median (50^{th} percentile, (mg/L)	75^{th} Percentile (mg/L)	95^{th} Percentile (mg/L)	Mean (mg/L)	Median (50^{th} percentile, (mg/L)	75^{th} Percentile (mg/L)	95^{th} Percentile (mg/L)	Mean (mg/L)	Median (50^{th} percentile, (mg/L)	75^{th} Percentile (mg/L)	95^{th} Percentile (mg/L)
28.3	42.40283	21.20141	53.00353	144.8763	31.80212	14.13428	38.86926	77.73852	35.33569	17.66784	45.9364	88.33922	21.20141	7.067138	21.20141	63.60424
63	19.04762	9.52381	23.80952	65.07937	14.28571	6.349206	17.46032	34.92063	15.87302	7.936508	20.63492	39.68254	9.52381	3.174603	9.52381	28.57143
374	3.208556	1.604278	4.010695	10.96257	2.406417	1.069519	2.941176	5.882353	2.673797	1.336898	3.475936	6.684492	1.604278	0.534759	1.604278	4.812834
68	17.64706	8.823529	22.05882	60.29412	13.23529	5.882353	16.17647	32.35294	14.70588	7.352941	19.11765	36.76471	8.823529	2.941176	8.823529	26.47059
2.4	500	250	625	1708.333	375	166.6667	458.3333	916.6667	416.6667	208.3333	541.6667	1041.667	250	83.33333	250	750
1050	1.142857	0.571429	1.428571	3.904762	0.857143	0.380952	1.047619	2.095238	0.952381	0.47619	1.238095	2.380952	0.571429	0.190476	0.571429	1.714286

Parameter	1-d EIC		2-d EIC		5-d EIC		21-d EIC	
	Typical	Worst-Case	Typical	Worst-Case	Typical	Worst-Case	Typical	Worst-Case
Mean (mg/L)	1.2	1.6	0.9	1.2	1	1.3	0.6	0.9
Median (50^{th} percentile, (mg/L)	0.6	0.9	0.4	0.8	0.5	0.8	0.2	0.4
75^{th} Percentile (mg/L)	1.5	2.3	1.1	1.8	1.3	2	0.6	0.8
95^{th} Percentile (mg/L)	4.1	4.2	2.2	3.6	2.5	3.6	1.8	3

Section 9. Appendix definitions.

The following is a list of common abbreviations and their definitions as used in the calculations and descriptions throughout Appendix A.

Abbreviation	Definition
L	Liter
mg	milligram
Lpm	Liter per minute
gpd	gallon per day
gal	gallon
d	day
h	hour
min	minute
cfs	cubic feet per second
°F	°Fahrenheit
°C	°Celsius
RW	Raceway
acre-feet	1,233,476 L

Appendix B. Copies of Literature Cited in Original Environmental Assessment for Use of Hydrogen Peroxide in Aquaculture (Cited literature has been previously submitted to CVM as Appendix B of the original EA)

Appendix C. Environmental Assessment Survey Questionnaire Sent to Public and Private Aquaculture Facilities

The following Upper Midwest Environmental Sciences Center Environmental Assessment Survey was provided to public (State, Federal and tribal) and private fish hatcheries to gather hatchery information:

BEGIN SURVEY
++++++++++

USGS
science for a changing world

Answers to questions within Sections 1 through 4 of the survey provide general information about your hatchery, the fish cultured, its water use, and the water body your hatchery effluent enters. Sections 1 through 4 are vitally important because they serve as the reference point for all of the treatment regimen information requested within Section 5 of the survey.

In Section 5, we ask you to provide treatment regimen information to describe treatment regimens you currently use or would anticipate using to prevent or control pathogens in the next five years. ***We understand that the answers provided in Section 5 are based on the assumption that the chemicals are, or will be, legally available for use either with an approved label or via INAD.***

Remember to keep all answers to the right of the colon. Answers are not case-sensitive, and answers are not required for each question (i.e., blank lines are acceptable).

All main headings of sections are in bold Italics and section subheadings are in Italics. All header and administrative portions of the survey are separated from data entry lines by a series of asterisks (*). Survey questions are in bold (**i.e., the text to the left of the colon**), if a suggested response example or unit of measure is included, it is presented as an **underlined bold response suggestion or unit of measure (e.g., million gpd)**.

Please be sure to periodically save your file.

Section 1 - Hatchery Information
****

Hatchery Name:
Contact Person:
Address:
City:
State:
Zip Code:
Phone number:
Fax number:
E-mail address:

Section 2 - Species Cultured

Please enter the name and life stage of the species most commonly cultured at your facility, even those you typically would not treat. Species held at your facility for only a brief period (i.e., less than a week) before transfer or those brought in for forage (other than fish routinely cultured on site for forage) do not need to be included.

****

Species 1 (name):
Species 1 (life stage cultured; E, F, or B):

Species 2 (name):
Species 2 (life stage cultured; E, F, or B):

Species 3 (name):
Species 3 (life stage cultured; E, F, or B):

Species 4 (name):
Species 4 (life stage cultured; E, F, or B):

Species 5 (name):
Species 5 (life stage cultured; E, F, or B):

Species 6 (name):
Species 6 (life stage cultured; E, F, or B):

Species 7 (name):
Species 7 (life stage cultured; E, F, or B):

Species 8 (name):
Species 8 (life stage cultured; E, F, or B):

Species 9 (name):
Species 9 (life stage cultured; E, F, or B):

Species 10 (name):
Species 10 (life stage cultured; E, F, or B):

Species 11 (name):
Species 11 (life stage cultured; E, F, or B):

Species 12 (name):
Species 12 (life stage cultured; E, F, or B):

Species 13 (name):
Species 13 (life stage cultured; E, F, or B):

Species 14 (name):
Species 14 (life stage cultured; E, F, or B):

****

Section 3 - Hatchery Water Source and Use

Describe the physical and chemical characteristics of your hatchery water, including how the water is treated before it leaves the hatchery and what type of water body it enters after leaving the hatchery. Also, please provide the amount of water your hatchery uses throughout the year.

Total Hatchery Water Use

Please estimate average hatchery water use.

****

Average Total Hatchery Daily Water Flow? (million gpd):
Lowest probable flow (million gpd):

In general, how would you describe your hatchery water? (**X only one**)
****

Freshwater?:
Brackish?:

Water Chemistry Characteristics

Temperature
****

Celcius or Farenheit? (Enter C or F):
Temperature Average:
Temperature Minimum:
Temperature Maximum:

pH
****

pH Average:
pH Minimum:
pH Maximum:

Hardness (**mg/L as $CaCO_3$**)
****

Hardness Average:
Hardness Minimum:
Hardness Maximum:

Alkalinity (**mg/L as $CaCO_3$**)
****

Alkalinity Average:
Alkalinity Minimum:
Alkalinity Maximum:

Specific Conductivity (**μmhos/cm**)
****

Specific Conductivity Average:
Specific Conductivity Minimum:
Specific Conductivity Maximum:

Salinity (ppt)
****

Salinity Average:
Salinity Minimum:
Salinity Maximum:

Enter in the other water chemistry parameters not listed in the above
****

Other Chemistry Type:
Other Chemistry Type Average:

Other Chemistry Type Minimum:
Other Chemistry Type Maximum:

Effluent Water Treatment and Discharge
The following units of measure are used within this section of the survey;
acre-foot - the volume of water that would cover one acre one foot deep; also equals 325850 gallons
cfs - cubic feet per second

****

Does hatchery effluent pass through a settling pond before discharge?(Y/N):
If yes, what is the settling pond volume? (acre-feet):

Hatchery has a National Pollution Discharge Elimination System (NPDES) permit? (Y/N):
Hatchery has a State Pollution Discharge Elimination System (SPDES) permit? (Y/N):

What type of water body does your hatchery effluent enter? (**X only one**)
****

Lake/Pond:
River/Stream:
Backwater of a River/Stream:

In general, how would you describe the water body you discharge into? (**X only one**)
****

Freshwater?:
Brackish?:
Estuary?:

If your effluent enters a Lake/Pond, estimate the following.
****

If Lake/Pond selected, what is the estimated average volume? (acre-feet)?:
Does the Lake/Pond discharge to a river or stream?(Y/N):
If yes, what is the estimated flow of the river/stream (cfs):
Is the Lake/Pond discharge the stream's only water source? (Y/N):

If your effluent enters a River/Stream, answer the following.
****

If River/Stream selected, what is the estimated average flow? (cfs):
The lowest flow occurs during what season? (NC if no change):
What is the estimated average flow during the low flow season? (cfs):

If your effluent enters a River/Stream Backwater, answer the following.
****

What is the Backwater volume in a typical year (acre-feet)?:
What is the flow of the river/stream the backwater enters? (cfs):
The lowest flow occurs during what season? (NC if no change):
What is the estimated average flow during the low flow season? (cfs):

Section 4 - Hatchery Culture Units

Please describe the number and types of fish culture units (egg incubators, tanks, raceways, and ponds) your hatchery uses to incubate eggs or culture fish. We understand that, unlike egg incubators, tanks, raceways, and ponds come in a plethora of shapes and sizes. In the spaces provided please provide

information describing each of your three most representative tanks, raceways, and ponds, particularly those in which you would anticipate treating fish. For lack of a better label, the fish culture units are referred to as Tank size 1, Tank size 2, Tank size 3; Raceway size 1, Raceway size 2, Raceway size 3; Pond size 1, Pond size 2, and Pond size 3. Survey questions seeking to describe your hatchery treatment regimens will request the numbers of a given tank, raceway, or pond treated of a given size. Please refer back to this section when completing the treatment regimen descriptions.
This information will allow us to estimate "worst-case" treatment scenarios in a typical hatchery.

Egg Jars – Size 1
****

Number of egg banks - Size 1:
Average number of jars/bank - Size 1:
Minimum number of jars/bank - Size 1:
Maximum number of jars/bank - Size 1:
Average flow rate/jar - Size 1 (gpm):
Minimum flow rate/jar - Size 1 (gpm):
Maximum flow rate/jar - Size 1 (gpm):

Egg Jars – Size 2
****

Number of egg banks - Size 2:
Average number of jars/bank - Size 2:
Minimum number of jars/bank - Size 2:
Maximum number of jars/bank - Size 2:
Average flow rate/jar - Size 2 (gpm):
Minimum flow rate/jar - Size 2 (gpm):
Maximum flow rate/jar - Size 2 (gpm):

Heath Trays
****

Number of stacks:
Average number of trays/stack:
Minimum number of trays/stack:
Maximum number of trays/stack:
Average flow rate/stack (gpm):
Minimum flow rate/stack (gpm):
Maximum flow rate/stack (gpm):

Clark-Williams (trough incubators)
****

Number of raceways or troughs:
Average number of compartments:
Minimum number of compartments:
Maximum number of compartments:
Average flow rate / raceway or trough (gpm):
Minimum flow rate / raceway or trough (gpm):
Maximum flow rate / raceway or trough (gpm):

Fish Culture Units – Tanks and Raceways
****

What is the volume of Tank size 1 (gallons):
Number of tanks at Tank size 1:

Average flow rate to Tank size 1 (gpm):
Minimum flow rate to Tank size 1 (gpm):
Maximum flow rate to Tank size 1 (gpm):

What is the volume of Tank size 2 (gallons):
Number of tanks at Tank size 2:
Average flow rate to Tank size 2 (gpm):
Minimum flow rate to Tank size 2 (gpm):
Maximum flow rate to Tank size 2 (gpm):

What is the volume of Tank size 3 (gallons):
Number of tanks at Tank size 3:
Average flow rate to Tank size 3 (gpm):
Minimum flow rate to Tank size 3 (gpm):
Maximum flow rate to Tank size 3 (gpm):

What is the volume of Raceway size 1 (gallons):
Number of raceways at Raceway size 1:
Average flow rate to Raceway size 1 (gpm):
Minimum flow rate to Raceway size 1 (gpm):
Maximum flow rate to Raceway size 1 (gpm):

What is the volume of Raceway size 2 (gallons):
Number of raceways at Raceway size 2:
Average flow rate to Raceway size 2 (gpm):
Minimum flow rate to Raceway size 2 (gpm):
Maximum flow rate to Raceway size 2 (gpm):

What is the volume of Raceway size 3 (gallons):
Number of raceways at Raceway size 3:
Average flow rate to Raceway size 3 (gpm):
Minimum flow rate to Raceway size 3 (gpm):
Maximum flow rate to Raceway size 3 (gpm):

Fish Culture Units – Ponds
acre-foot - the volume of water that would cover one acre one foot deep; also equals 325850 gallons
****

Is water flow to Pond size 1, 2, or 3 to make-up evaporation/leakage? (Y/N):
Is Pond out-flow intermittent, e.g., only during pond drainage/harvest? (Y/N):

What is the volume of Pond size 1 (acre-feet):
Number of ponds at Pond size 1:
Average flow rate to Pond size 1 (gpm):
Minimum flow rate to Pond size 1 (gpm):
Maximum flow rate to Pond size 1 (gpm):

What is the volume of Pond size 2 (acre-feet):
Number of ponds at Pond size 2:
Average flow rate to Pond size 2 (gpm):
Minimum flow rate to Pond size 2 (gpm):
Maximum flow rate to Pond size 2 (gpm):

What is the volume of Pond size 3 (acre-feet):
Number of ponds at Pond size 3:
Average flow rate to Pond size 3 (gpm):
Minimum flow rate to Pond size 3 (gpm):
Maximum flow rate to Pond size 3 (gpm):

Section 5- Chemical Treatments

From the list of chemicals provided below, please describe your typical treatment and anesthetic practices. ***Also include those treatments you would use provided you have legal access to the drug through an approved label or an INAD.*** If you do not have experience with these drugs but anticipate needing to use them, supply your best guess at the dose or concentration based on prior knowledge with similar drugs.

The following chemicals will likely be approved for use on both fish and fish eggs. Please place an **E (eggs), F (fish), or B (both)** to indicate the life stages you will treat or hope to treat using these chemicals in the next 5 years at your hatchery. ***We understand that the answers provided to this question and in treatment regimen descriptions are based on the assumption that the chemicals are, or will be, legally available for use (either with an approved label or via an INAD).***
****

hydrogen peroxide (fish – 50 to 250μL/L; eggs – 500 to 1000 μL/L)? (E, F, or B):
potassium permanganate (0.25 to 8 mg/L)? (E, F, or B):

The following chemicals will likely be approved for use only on fish. Please place a **Y/N** to indicate whether or not you will use or hope to use these chemicals in the next 5 years to treat fish at your hatchery. ***We understand that the answers provided to this question and in treatment regimen descriptions are based on the assumption that the chemicals are, or will be, legally available for use (either with an approved label or via an INAD).***
****

Aqui-S (should be from 25 to 50 mg/L) (Y/N):
Chloramine-T (allowable limit is 10 to 20 mg/L for four treatments) (Y/N):
Florfenicol (allowable limit is 10 mg/kg for 10 d) (Y/N):
Oxytetracycline (static immersion bath; 10 to 50 mg/L) (Y/N):

Treatment Regimens

The treatment regimen information you will provide at this point in the survey is one of the most important portions of the survey. The treatment regimens are separated into an Oral Drug Treatment Regimen (OR), eight Water-borne Treatment Regimens (TR), and two Anesthetic Regimens (AR). Florfenicol is the only oral drug that we currently anticipate writing a portion of the Environmental Assessment.

Please describe your treatments as thoroughly as possible. Although the survey attempts to consolidate as many different treatment scenarios as possible into one treatment regimen, some cases require submission of multiple treatment regimens for one chemical. For instance, hydrogen peroxide is administered at much greater concentrations and for a greater number of exposures to control fungus on eggs than when used to control fungus, bacteria, or parasites on fish. Your responses will form the basis of our Environmental Assessment that tells the U.S. Food and Drug Administration how chemicals are used, how often they are administered, and potentially how much may enter the environment.

Please see the examples for water borne and oral drug treatment regimens in the completed example surveys attached as "example.doc" (MS Word97) or "example.wpd" (WordPerfect 6/7/8).

If you wish to describe additional treatment regimens, copy the information from one of the treatment regimens and paste it at the end of the document. Please state that additional treatment regimens were added to the survey in the body of your e-mail message when you return the survey to UMESC (applies only to electronically submitted surveys).

########## **Please Enter Oral Drug Treatment Regimens on the following page** ##########

Oral Drug Treatment Regimen (OR) 1 - ***Florfenicol at 10 mg/kg for 10 days***
Disease treated (**X all that apply**)
****

OR 1 - BGD:
OR 1 - Columnaris / BCWD:
OR 1 - furunculosis / Aeromonas hydrophilia:
OR 1 - BKD / ERM:
OR 1 - other:
If checked OR 1 - other, enter disease name:

What types of fish are treated (**X all that apply**)?
****

OR 1 - Coldwater:
OR 1 - Coolwater:
OR 1 - Warmwater:

Please give the maximum number of culture units treated on a given day and the average fish mass (**kg**) treated in a given culture unit. (Note - you entered culture unit size information beginning on page 10 {depending on printer})
****

OR 1 - tank size 1:
OR 1 - average treated biomass in tank size 1 (kg):
OR 1 - tank size 2:
OR 1 - average treated biomass in tank size 2 (kg):
OR 1 - tank size 3:
OR 1 - average treated biomass in tank size 3 (kg):
OR 1 - raceway size 1:
OR 1 - average treated biomass in raceway size 1 (kg):
OR 1 - raceway size 2:
OR 1 - average treated biomass in raceway size 2 (kg):
OR 1 - raceway size 3:
OR 1 - average treated biomass in raceway size 3 (kg):
OR 1 - pond size 1:
OR 1 - average treated biomass in pond size 1 (kg):
OR 1 - pond size 2:
OR 1 - average treated biomass in pond size 2 (kg):
OR 1 - pond size 3:
OR 1 - average treated biomass in pond size 3 (kg):

How often would you typically administer this treatment regimen?
****

OR 1 - times per year (enter number):

When do you typically treat? (**X all that apply**)
****

TR 1 - For this regimen, on how many days would you administer treatment?:
TR 1 - Are treatments administered on consecutive (C) or alternate (A) days?:

How long does a typical treatment (exposure) last? (**minutes**)
****

TR 1 - Static - minimum:
TR 1 - Static - maximum:
TR 1 - Flow-through - minimum:
TR 1 - Flow-through maximum:

Disease treated (**X all that apply**)
****

TR 1 - fungus:
TR 1 - BGD:
TR 1 - Columnaris / BCWD:
TR 1 - furunculosis / Aeromonas hydrophilia:
TR 1 - BKD / ERM:
TR 1 - trematodes, protozoans, or copepods:
TR 1 - other:

If you checked TR 1 - other, enter disease name:

What types of fish are treated (**X all that apply**)?
****

TR 1 - Coldwater:
TR 1 - Coolwater:
TR 1 - Warmwater:

Maximum number of culture units treated simultaneously
(Note - you entered culture unit size information beginning on page 10 {depending on printer})
****

TR 1 - egg jars size 1:
TR 1 - egg jars size 2:
TR 1 - heath stacks:
TR 1 - clark-williams:
TR 1 - tank size 1:
TR 1 - tank size 2:
TR 1 - tank size 3:
TR 1 - raceway size 1:
TR 1 - raceway size 2:
TR 1 - raceway size 3:
TR 1 - pond size 1:
TR 1 - pond size 2:
TR 1 - pond size 3:

Maximum number of culture units treated on a typical day
****

TR 1 - egg jars size 1:
TR 1 - egg jars size 2:
TR 1 - heath stacks:
TR 1 - clark-williams:
TR 1 - tank size 1:
TR 1 - tank size 2:
TR 1 - tank size 3:
TR 1 - raceway size 1:
TR 1 - raceway size 2:
TR 1 - raceway size 3:
TR 1 - pond size 1:
TR 1 - pond size 2:
TR 1 - pond size 3:

Answer the following for tank/raceway/pond treatments.
****

TR 1 - What percent of the treated volume is drained from the culture unit after treatment? (%):
TR 1 - By what percent is the flow rate increased after treatment (%):
TR 1 - If flow rate is increased, how long is it maintained? (min):

How often would you typically administer this treatment regimen?
****

TR 1 - times per year (enter number):

When do you typically treat? (**X all that apply**)
****

TR 1 - spring:

TR 1 - summer:
TR 1 - fall:
TR 1 - winter:

Water-borne Chemical Treatment Regimen (TR) 2

Please select the chemical described in this treatment regimen (only one chemical per treatment regimen description) and identify the life stage treated by placing an **E (eggs) or F (fish)** to the right of the colon for the appropriate chemical.
****

TR 2 - hydrogen peroxide:
TR 2 - chloramine-T:
TR 2 - oxytetracycline:
TR 2 - potassium permanganate:

What is the dose administered?
****

TR 2 - water minimum (mg/L):
TR 2 - water maximum (mg/L):
TR 2 - water minimum (uL/L):
TR 2 - water maximum (uL/L):

How is the dose administered? (**X only one**)
****

TR 2 - Water static bath?:
TR 2 - Water flow-through?:

TR 2 - For this regimen, on how many days would you administer treatment?:
TR 2 - Are treatments administered on consecutive (C) or alternate (A) days?:

How long does a typical treatment (exposure) last? (**minutes**)
****

TR 2 - Static - minimum:
TR 2 - Static - maximum:
TR 2 - Flow-through - minimum:
TR 2 - Flow-through maximum:

Disease treated (**X all that apply**)
****

TR 2 - fungus:
TR 2 - BGD:
TR 2 - Columnaris / BCWD:
TR 2 - furunculosis / Aeromonas hydrophilia:
TR 2 - BKD / ERM:
TR 2 - trematodes, protozoans, or copepods:
TR 2 - other:

If you checked TR 2 - other, enter disease name:

What types of fish are treated (**X all that apply**)?
****

TR 2 - Coldwater:

TR 2 - Coolwater:
TR 2 - Warmwater:

Maximum number of culture units treated simultaneously
(Note - you entered culture unit size information beginning on page 10 {depending on printer})
****

TR 2 - egg jars size 1:
TR 2 - egg jars size 2:
TR 2 - heath stacks:
TR 2 - clark-williams:
TR 2 - tank size 1:
TR 2 - tank size 2:
TR 2 - tank size 3:
TR 2 - raceway size 1:
TR 2 - raceway size 2:
TR 2 - raceway size 3:
TR 2 - pond size 1:
TR 2 - pond size 2:
TR 2 - pond size 3:

Maximum number of culture units treated on a typical day
****

TR 2 - egg jars size 1:
TR 2 - egg jars size 2:
TR 2 - heath stacks:
TR 2 - clark-williams:
TR 2 - tank size 1:
TR 2 - tank size 2:
TR 2 - tank size 3:
TR 2 - raceway size 1:
TR 2 - raceway size 2:
TR 2 - raceway size 3:
TR 2 - pond size 1:
TR 2 - pond size 2:
TR 2 - pond size 3:

Answer the following for tank/raceway/pond treatments.
****

TR 2 - What percent of the treated volume is drained from the culture unit after treatment? (%):
TR 2 - By what percent is the flow rate increased after treatment (%):
TR 2 - If flow rate is increased, how long is it maintained? (min):

How often would you typically administer this treatment regimen?
****

TR 2 - times per year (enter number):

When do you typically treat? (**X all that apply**)
****

TR 2 - spring:
TR 2 - summer:
TR 2 - fall:
TR 2 - winter:

Water-borne Chemical Treatment Regimen (TR) 3

Please select the chemical described in this treatment regimen (only one chemical per treatment regimen description) and identify the life stage treated by placing an **E (eggs) or F (fish)** to the right of the colon for the appropriate chemical.

****

TR 3 - hydrogen peroxide:
TR 3 - chloramine-T:
TR 3 - oxytetracycline:
TR 3 - potassium permanganate:

What is the dose administered?

****

TR 3 - water minimum (mg/L):
TR 3 - water maximum (mg/L):
TR 3 - water minimum (uL/L):
TR 3 - water maximum (uL/L):

How is the dose administered? (**X only one**)

****

TR 3 - Water static bath?:
TR 3 - Water flow-through?:

TR 3 - For this regimen, on how many days would you administer treatment?:
TR 3 - Are treatments administered on consecutive (C) or alternate (A) days?:

How long does a typical treatment (exposure) last? (**minutes**)

****

TR 3 - Static - minimum:
TR 3 - Static - maximum:
TR 3 - Flow-through - minimum:
TR 3 - Flow-through maximum:

Disease treated (**X all that apply**)

****

TR 3 - fungus:
TR 3 - BGD:
TR 3 - Columnaris / BCWD:
TR 3 - furunculosis / Aeromonas hydrophilia:
TR 3 - BKD / ERM:
TR 3 - trematodes, protozoans, or copepods:
TR 3 - other:

If you checked TR 3 - other, enter disease name:

What types of fish are treated (**X all that apply**)?

****

TR 3 - Coldwater:
TR 3 - Coolwater:
TR 3 - Warmwater:

Maximum number of culture units treated simultaneously

(Note - you entered culture unit size information beginning on page 10 {depending on printer})
****

TR 3 - egg jars size 1:
TR 3 - egg jars size 2:
TR 3 - heath stacks:
TR 3 - clark-williams:
TR 3 - tank size 1:
TR 3 - tank size 2:
TR 3 - tank size 3:
TR 3 - raceway size 1:
TR 3 - raceway size 2:
TR 3 - raceway size 3:
TR 3 - pond size 1:
TR 3 - pond size 2:
TR 3 - pond size 3:

Maximum number of culture units treated on a typical day
****

TR 3 - egg jars size 1:
TR 3 - egg jars size 2:
TR 3 - heath stacks:
TR 3 - clark-williams:
TR 3 - tank size 1:
TR 3 - tank size 2:
TR 3 - tank size 3:
TR 3 - raceway size 1:
TR 3 - raceway size 2:
TR 3 - raceway size 3:
TR 3 - pond size 1:
TR 3 - pond size 2:
TR 3 - pond size 3:

Answer the following for tank/raceway/pond treatments.
****

TR 3 - What percent of the treated volume is drained from the culture unit after treatment? (%):
TR 3 - By what percent is the flow rate increased after treatment (%):
TR 3 - If flow rate is increased, how long is it maintained? (min):

How often would you typically administer this treatment regimen?
****

TR 3 - times per year (enter number):

When do you typically treat? (**X all that apply**)
****

TR 3 - spring:
TR 3 - summer:
TR 3 - fall:
TR 3 - winter:

Water-borne Chemical Treatment Regimen (TR) 4

Please select the chemical described in this treatment regimen (only one chemical per treatment regimen description) and identify the life stage treated by placing an **E (eggs) or F (fish)** to the right of the colon for the appropriate chemical.

****

TR 4 - hydrogen peroxide:
TR 4 - chloramine-T:
TR 4 - oxytetracycline:
TR 4 - potassium permanganate:

What is the dose administered?

****

TR 4 - water minimum (mg/L):
TR 4 - water maximum (mg/L):
TR 4 - water minimum (uL/L):
TR 4 - water maximum (uL/L):

How is the dose administered? (**X only one**)

****

TR 4 - Water static bath?:
TR 4 - Water flow-through?:

TR 4 - For this regimen, on how many days would you administer treatment?:
TR 4 - Are treatments administered on consecutive (C) or alternate (A) days?:

How long does a typical treatment (exposure) last? (**minutes**)

****

TR 4 - Static - minimum:
TR 4 - Static - maximum:
TR 4 - Flow-through - minimum:
TR 4 - Flow-through maximum:

Disease treated (**X all that apply**)

****

TR 4 - fungus:
TR 4 - BGD:
TR 4 - Columnaris / BCWD:
TR 4 - furunculosis / Aeromonas hydrophilia:
TR 4 - BKD / ERM:
TR 4 - trematodes, protozoans, or copepods:
TR 4 - other:

If you checked TR 4 - other, enter disease name:

What types of fish are treated (**X all that apply**)?

****

TR 4 - Coldwater:
TR 4 - Coolwater:
TR 4 - Warmwater:

Maximum number of culture units treated simultaneously
(Note - you entered culture unit size information beginning on page 10 {depending on printer})

****

TR 4 - egg jars size 1:
TR 4 - egg jars size 2:
TR 4 - heath stacks:
TR 4 - clark-williams:
TR 4 - tank size 1:
TR 4 - tank size 2:
TR 4 - tank size 3:
TR 4 - raceway size 1:
TR 4 - raceway size 2:
TR 4 - raceway size 3:
TR 4 - pond size 1:
TR 4 - pond size 2:
TR 4 - pond size 3:

Maximum number of culture units treated on a typical day
****

TR 4 - egg jars size 1:
TR 4 - egg jars size 2:
TR 4 - heath stacks:
TR 4 - clark-williams:
TR 4 - tank size 1:
TR 4 - tank size 2:
TR 4 - tank size 3:
TR 4 - raceway size 1:
TR 4 - raceway size 2:
TR 4 - raceway size 3:
TR 4 - pond size 1:
TR 4 - pond size 2:
TR 4 - pond size 3:

Answer the following for tank/raceway/pond treatments.
****

TR 4 - What percent of the treated volume is drained from the culture unit after treatment? (%):
TR 4 - By what percent is the flow rate increased after treatment (%):
TR 4 - If flow rate is increased, how long is it maintained? (min):

How often would you typically administer this treatment regimen?
****

TR 4 - times per year (enter number):

When do you typically treat? (**X all that apply**)
****

TR 4 - spring:
TR 4 - summer:
TR 4 - fall:
TR 4 - winter:

Water-borne Chemical Treatment Regimen (TR) 5

Please select the chemical described in this treatment regimen (only one chemical per treatment regimen description) and identify the life stage treated by placing an **E (eggs) or F (fish)** to the right of the colon for the appropriate chemical.

****

TR 5 - hydrogen peroxide:
TR 5 - chloramine-T:
TR 5 - oxytetracycline:
TR 5 - potassium permanganate:

What is the dose administered?
****

TR 5 - water minimum (mg/L):
TR 5 - water maximum (mg/L):
TR 5 - water minimum (uL/L):
TR 5 - water maximum (uL/L):

How is the dose administered? (**X only one**)
****

TR 5 - Water static bath?:
TR 5 - Water flow-through?:

TR 5 - For this regimen, on how many days would you administer treatment?:
TR 5 - Are treatments administered on consecutive (C) or alternate (A) days?:

How long does a typical treatment (exposure) last? (**minutes**)
****

TR 5 - Static - minimum:
TR 5 - Static - maximum:
TR 5 - Flow-through - minimum:
TR 5 - Flow-through maximum:

Disease treated (**X all that apply**)
****

TR 5 - fungus:
TR 5 - BGD:
TR 5 - Columnaris / BCWD:
TR 5 - furunculosis / Aeromonas hydrophilia:
TR 5 - BKD / ERM:
TR 5 - trematodes, protozoans, or copepods:
TR 5 - other:

If you checked TR 5 - other, enter disease name:

What types of fish are treated (**X all that apply**)?
****

TR 5 - Coldwater:
TR 5 - Coolwater:
TR 5 - Warmwater:

Maximum number of culture units treated simultaneously
(Note - you entered culture unit size information beginning on page 10 {depending on printer})
****

TR 5 - egg jars size 1:
TR 5 - egg jars size 2:
TR 5 - heath stacks:

TR 5 - clark-williams:
TR 5 - tank size 1:
TR 5 - tank size 2:
TR 5 - tank size 3:
TR 5 - raceway size 1:
TR 5 - raceway size 2:
TR 5 - raceway size 3:
TR 5 - pond size 1:
TR 5 - pond size 2:
TR 5 - pond size 3:

Maximum number of culture units treated on a typical day
****

TR 5 - egg jars size 1:
TR 5 - egg jars size 2:
TR 5 - heath stacks:
TR 5 - clark-williams:
TR 5 - tank size 1:
TR 5 - tank size 2:
TR 5 - tank size 3:
TR 5 - raceway size 1:
TR 5 - raceway size 2:
TR 5 - raceway size 3:
TR 5 - pond size 1:
TR 5 - pond size 2:
TR 5 - pond size 3:

Answer the following for tank/raceway/pond treatments.
****

TR 5 - What percent of the treated volume is drained from the culture unit after treatment? (%):
TR 5 - By what percent is the flow rate increased after treatment (%):
TR 5 - If flow rate is increased, how long is it maintained? (min):

How often would you typically administer this treatment regimen?
****

TR 5 - times per year (enter number):

When do you typically treat? (**X all that apply**)
****

TR 5 - spring:
TR 5 - summer:
TR 5 - fall:
TR 5 - winter:

Water-borne Anesthetic Regimen (AR) 1 – Aqui-S Use at Hatcheries
anticipated dose - 25 to 50 mg/L

What types of fish are treated (**X all that apply**)?
****

AR 1 - Coldwater:
AR 1 - Coolwater:
AR 1 - Warmwater:

What is the anesthesia purpose (**X all that apply**)?
****

AR 1 - spawning:
AR 1 - tag/release/mark:
AR 1 - transportation:
AR 1 - collection:
AR 1 - other:

What is the dose administered?
****

AR 1 - water minimum (mg/L):
AR 1 - water maximum (mg/L):

How is the dose administered?
****

AR 1 - On an annual basis, on how many days would you administer treatment?:
AR 1 - What volume of anesthetic bath would you typically prepare? (L):
AR 1 - How many times per day would you prepare the above volume?:

When do you typically treat? (**X all that apply**)
****

AR 1 - spring:
AR 1 - summer:
AR 1 - fall:
AR 1 - winter:

Water-borne Anesthetic Regimen (AR) 2 – Aqui-S Use Away from the Hatchery
anticipated dose - 25 to 50 mg/L

What types of fish are treated (**X all that apply**)?
****

AR 2 - Coldwater:
AR 2 - Coolwater:
AR 2 - Warmwater:

What is the anesthesia purpose (**X all that apply**)?
****

AR 2 - spawning:
AR 2 - tag/release/mark:
AR 2 - transportation:
AR 2 - collection:
AR 2 - other:

What is the dose administered?
****

AR 2 - water minimum (mg/L):
AR 2 - water maximum (mg/L):

How is the dose administered?
****

AR 2 - On an annual basis, on how many days would you administer treatment?:

AR 2 - What volume of anesthetic bath would you typically prepare? (L):
AR 2 - How many times per day would you prepare the above volume?:

When do you typically treat? (**X all that apply**)
****

AR 2 - spring:
AR 2 - summer:
AR 2 - fall:
AR 2 - winter:

What type of water body is the anesthetic bath discharged to? (**X only one**)
****

AR 2 - Lake/Pond:
AR 2 - River/Stream:
AR 2 - Backwater of a River/Stream:

If the anesthetic enters a Lake/Pond, estimate the following.
****

AR 2 - What is the estimated average volume? (**acre-feet**)?:

If the anesthetic enters a River/Stream, answer the following.
****

AR 2 - If River/Stream selected, what is the estimated average flow? (**cfs**):
AR 2 - The lowest flow occurs during what season? (**NC if no change**):
AR 2 - What is the estimated average flow during the low flow season? (**cfs**):

++++++++++
END SURVEY

The following Upper Midwest Environmental Sciences Center Environmental Assessment Survey was provided to the private catfish producers:

Dear Private Catfish Producer:

As the National Coordinator for Aquaculture New Animal Drug Applications, I am asking you to fill out the attached survey to help gain approvals of aquaculture drugs for your use. I am acting on behalf of the Upper Midwest Environmental Sciences Center (UMESC) and the Stuttgart National Aquaculture Research Center (SNARC) who will provide important information from this survey to the Center for Veterinary Medicine (CVM) in the form of environmental assessments (EAs) that are needed for approvals of three aquaculture drugs under the Federal-State Aquaculture Drug Approval Partnership. UMESC and SNARC will summarize the information from this survey in EAs to provide an overview of projected drug use patterns anticipated in the next five years. Your response is an important component of this overview. All the information you provide will be confidential.

Your responses to this one survey will enable UMESC to develop EAs for AQUI-S and florfenicol and SNARC to develop an EA for potassium permanganate. Because it is important for UMESC and SNARC to describe both current and projected use, please provide information for treatment regimens you currently use or would anticipate using to prevent or control infectious diseases or to anesthetize fish in the next five years. ***I understand that the answers provided are based on the assumption that the drugs are, or will be, legal to use either with an approved label or via an investigational new animal drug (INAD) exemption or regulatory discretion.***

UMESC and SNARC need treatment regimen information from you for as many of the following drugs and their use patterns as possible:

AQUI-S –anesthetic with potential for a zero withdrawal period
Florfenicol – broad-spectrum oral antibacterial for control of gram-negative and gram-positive systemic bacteria
Potassium permanganate – external microbicide for control of fungus, bacterial gill disease, external flavobacteriosis, and external parasites

UMESC and SNARC need detailed facility information from you in the following areas:

Identification of species to be treated
Description of the treatment facilities, such as the total production facility water flow, number of culture units, and culture unit volume
Description of the treatment environments including pond volume and treatment concentration
Characterization of the body of water that ultimately receives the treatment effluent including water body volume and/or flow

Your answers to the questions below will help UMESC or SNARC describe the typical and worst-case environmental concentrations that could be expected after drug treatments. Although you may not have all of the information for all of the survey questions, please answer as much of the survey as possible. My goal and that of UMESC and SNARC is to develop databases that support the broadest approvals possible.

When you have completed the survey, please return an electronic copy to Mark Gaikowski mgaikowski@umesc.er.usgs.gov by e-mail, or a hard copy of the questionnaire to his attention at Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Road, La Crosse, Wisconsin 54603

Please return completed electronic or hardcopy surveys as soon as you can. Thank you in advance for taking the time to fill out this survey.

Rosalie (Roz) Schnick, National Coordinator for Aquaculture New Animal Drug Applications, Michigan State University, 3039 Edgewater Lane, La Crosse, WI 54603-1088; Telephone: 608-781-2205; Fax: 608-783-3507; E-mail: RozSchnick@aol.com; Website: http://ag.ansc.purdue.edu/aquanic/jsa/Aquadrugs/index.htm

HOW TO FILL OUT THIS SURVEY

1. If you have any questions regarding the survey, contact:

    a. Mischelle Mrozek for technical questions regarding e-mail attachments, editing attached files, or returning completed electronic surveys at 608-781-6235 or via e-mail at mmrozek@umesc.er.usgs.gov. If Mischelle is not available, contact Mike Caucutt at 608-783-7550 extension 702.

    b. Jeff Rach (jeff_rach@usgs.gov 608-781-6322), Verdel Dawson (verdel_dawson@usgs.gov 608-781-6223), or Mark Gaikowski (mgaikowski@umesc.er.usgs.gov 608-781-6284) for survey content questions. They will be glad to discuss the survey questions and the data they hope to gather.

1. If you would prefer to complete a hardcopy of the survey, please print the file "CatfishSurvey.doc" (Word97) and send the completed survey to:

   Mark Gaikowski, Upper Midwest Environmental Sciences Center
   2630 Fanta Reed Road, La Crosse, WI 54603

2. To complete the survey, please save "CatfishSurvey.doc" (Word97) to your PC's local hard drive or server. Open the file and complete the survey.

3. If you have trouble saving the file from your e-mail client, the survey and examples of a completed survey can also be retrieved from the internet at:

   http://www.umesc.usgs.gov/cvm_survey/cvm_survey.html

4. Please be careful to ensure that all answers (usually number or letter) are placed to the right of the colon.

5. All main headings of sections are in ***bold Italics*** and section subheadings are in *Italics*. All header and administrative portions of the survey are separated from data entry lines by a series of asterisks (*). Survey questions are in bold (i.e., **the text to the left of the colon**), if a suggested response example or unit of measure is included, it is presented as an underlined bold response suggestion or unit of measure (e.g., **million gpd**).

6. Please be sure to periodically save your file.

7. After you have completed the survey, save the file. Then e-mail the completed file to Mark Gaikowski (email address: mgaikowski@umesc.er.usgs.gov). UMESC will parse your responses into a spreadsheet to facilitate data analysis.

NOTE: It is important that you keep your answers to the right of the colon and on the same line as the corresponding question so that the program can correctly identify your answers.

BEGIN SURVEY OF CATFISH PRODUCTION FACILITIES
++++++++++

Answers to questions within Sections 1 through 4 of the survey provide general information about your catfish production facilities, its water use, and the water body your effluent enters. Sections 1 through 4 are vitally important because they serve as the reference point for all of the treatment regimen information requested within Section 5 of the survey.

In Section 5, we ask you to provide treatment regimen information to describe treatment regimens you currently use or would anticipate using to prevent or control pathogens or use an anesthetic in the next five years. ***We understand that the answers provided in Section 5 are based on the assumption that florfenicol, potassium permanganate, and AQUI-S are, or will be, legally available for use either with an approved label or via INAD or regulatory discretion.***

Remember to keep all answers to the right of the colon. Answers are not case-sensitive, and answers are not required for each question (i.e., blank lines are acceptable).

All main headings of sections are in bold Italics and section subheadings are in Italics. All header and administrative portions of the survey are separated from data entry lines by a series of asterisks (*). Survey questions are in bold (**i.e., the text to the left of the colon**), if a suggested response example or unit of measure is included, it is presented as an **underlined bold response suggestion or unit of measure (e.g., million gpd)**.

Please be sure to periodically save your file.

Section 1 – Production Facility Information

****

Name of Production Facility:
Contact Person:
Address:
City:
State:
Zip Code:
Phone number:
Fax number:
E-mail address:

Section 2 - Species Cultured

Please enter **F (fish)** for the species and life stage of catfish cultured at your facility.

****

Blue x Channel Catfish - BXC:
Channel Catfish - CCF:

If a species you culture was not listed above, please enter its common name and the life stages you culture below. If you have more than 2 other species to enter, copy and paste the text below and change the number.

****

Other Species 1 (name):
Other Species 1 (life stage cultured; F):

Other Species 2 (name):
Other Species 2 (life stage cultured; F):

Section 3 – Production Facility Water Source and Use

Describe the physical and chemical characteristics of your production water, including how the water is treated before it leaves your facility and what type of water body it enters after leaving your facility. Also, please provide the amount of water your production facility uses throughout the year.

Total Production Facility Water Use

Please estimate average production facility water use.

****

Average Total Production Facility Daily Water Flow? (million gpd):
Lowest probable flow (million gpd):

Water Chemistry Characteristics

Temperature
****

Celsius or Fahrenheit? (Enter C or F):
Temperature Average:
Temperature Minimum:
Temperature Maximum:

pH
****

pH Average:
pH Minimum:
pH Maximum:

Hardness (**mg/L as $CaCO_3$**)
****

Hardness Average:
Hardness Minimum:
Hardness Maximum:

Alkalinity (**mg/L as $CaCO_3$**)
****

Alkalinity Average:
Alkalinity Minimum:
Alkalinity Maximum:

Specific Conductivity (**mhos/cm**)
****

Specific Conductivity Average:
Specific Conductivity Minimum:
Specific Conductivity Maximum:

Salinity (ppt)
****

Salinity Average:
Salinity Minimum:
Salinity Maximum:

Enter in the other water chemistry parameters not listed in the above
****

Other Chemistry Type:
Other Chemistry Type Average:
Other Chemistry Type Minimum:
Other Chemistry Type Maximum:

Effluent Water Treatment and Discharge

The following units of measure are used within this section of the survey;
acre-foot - the volume of water that would cover one acre one foot deep; also equals 325850 gallons
cfs - cubic feet per second

****

Does the production facility effluent pass through a settling pond before discharge? (Y/N):
If yes, what is the settling pond volume? (acre-feet):

Production Facility has a National Pollution Discharge Elimination System (NPDES) permit? (Y/N):
Production Facility has a State Pollution Discharge Elimination System (SPDES) permit? (Y/N):

What type of water body does your production facility effluent enter? (**X only one**)
****

Lake/Pond:
River/Stream:
Backwater of a River/Stream:

If your effluent enters a Lake/Pond, estimate the following.
****

If Lake/Pond selected, what is the estimated average volume? (acre-feet)?:
Does the Lake/Pond discharge to a river or stream? (Y/N):
If yes, what is the estimated flow of the river/stream (cfs):
Is the Lake/Pond discharge the stream's only water source? (Y/N):

If your effluent enters a River/Stream, answer the following.
****

If River/Stream selected, what is the estimated average flow? (cfs):
The lowest flow occurs during what season? (NC if no change):
What is the estimated average flow during the low flow season? (cfs):

If your effluent enters a River/Stream Backwater, answer the following.
****

What is the Backwater volume in a typical year (acre-feet)?:
What is the flow of the river/stream the backwater enters? (cfs):
The lowest flow occurs during what season? (NC if no change):
What is the estimated average flow during the low flow season? (cfs):

Section 4 – Production Facility Culture Units

Please describe the number and types of fish culture ponds your production facility uses to culture fish. We understand that ponds can come in a plethora of shapes and sizes. In the spaces provided please provide information describing each of your three most representative ponds, particularly those in which you would anticipate treating fish. For lack of a better label, the fish culture units are referred to as Pond size 1, Pond size 2, and Pond size 3. Survey questions seeking to describe your production facility treatment regimens will request the numbers of a pond treated of a given size. Please refer back to this section when completing the treatment regimen descriptions.

This information will allow us to estimate "worst-case" treatment scenarios in a typical catfish production facility.

Fish Culture Units – Ponds
acre-foot - the volume of water that would cover one acre one foot deep; also equals 325850 gallons
****

Is water flow to Pond size 1, 2, or 3 to make-up evaporation/leakage? (Y/N):
Is Pond out-flow intermittent, e.g., only during pond drainage/harvest? (Y/N):

What is the volume of Pond size 1 (acre-feet):
Number of ponds at Pond size 1:
Average flow rate to Pond size 1 (gpm):
Minimum flow rate to Pond size 1 (gpm):
Maximum flow rate to Pond size 1 (gpm):

What is the volume of Pond size 2 (acre-feet):
Number of ponds at Pond size 2:
Average flow rate to Pond size 2 (gpm):
Minimum flow rate to Pond size 2 (gpm):
Maximum flow rate to Pond size 2 (gpm):

What is the volume of Pond size 3 (acre-feet):
Number of ponds at Pond size 3:
Average flow rate to Pond size 3 (gpm):
Minimum flow rate to Pond size 3 (gpm):
Maximum flow rate to Pond size 3 (gpm):

Section 5- Chemical Treatments

From the list of drugs provided below, please describe your typical treatment and anesthetic practices. ***Also include those treatments you would use provided you have legal access to the drug through an approved label, an INAD or regulatory discretion.*** If you do not have experience with these drugs but anticipate needing to use them, supply your best guess at the dose or concentration based on prior knowledge with similar drugs.

The following drugs will likely be approved for use on fish. Please place an **Y/N** to indicate whether or not you will use or hope to use florfenicol, AQUI-S, or potassium permanganate in the next 5 years to treat fish at your production facility. ***We understand that the answers provided to this question and in treatment regimen descriptions are based on the assumption that these drugs are, or will be, legally available for use (either with an approved label, an INAD, or regulatory discretion).***
****

AQUI-S (should be from 25 to 50 mg/L) (Y/N):
Florfenicol (allowable limit is 10 mg/kg for 10 d) (Y/N):
Potassium permanganate (0.25 to 8 mg/L)? (E, F, or B):

Treatment Regimens

The treatment regimen information you will provide at this point in the survey is one of the most important portions of the survey. The treatment regimens are separated into an Oral Drug Treatment Regimen (OR), Water-borne Treatment Regimen (TR), and two Anesthetic Regimens (AR).
Please describe your treatments as thoroughly as possible. Although the survey attempts to consolidate as many different treatment scenarios as possible into one treatment regimen, some cases require submission of multiple treatment regimens for one drug. Your responses will form the basis of our Environmental Assessment that tells the U.S. Food and Drug Administration how the drugs are used, how often they are administered, and potentially how much may enter the environment.

If you wish to describe additional treatment regimens, copy the information from one of the treatment regimens and paste it at the end of the document. Please state that additional treatment regimens were added to the survey in the body of your e-mail message when you return the survey to UMESC (applies only to electronically submitted surveys).

########## **Please Enter Oral Drug Treatment Regimens on the following page** ##########

Oral Drug Treatment Regimen (OR) 1 - ***Florfenicol at 10 mg/kg for 10 days***
Disease treated (**X all that apply**)
****

OR 1 –Bacterial gill disease:
OR 1 - Columnaris:
OR 1 - other:
If checked OR 1 - other, enter disease name:

Please give the maximum number of culture units treated on a given day and the average fish mass (**kg**) treated in a given culture unit.
****

OR 1 - pond size 1:
OR 1 - average treated biomass in pond size 1 (kg):
OR 1 - pond size 2:
OR 1 - average treated biomass in pond size 2 (kg):
OR 1 - pond size 3:
OR 1 - average treated biomass in pond size 3 (kg):

How often would you typically administer this treatment regimen?
****

OR 1 - times per year (enter number):

When do you typically treat? (**X all that apply**)
****

OR 1 - spring:
OR 1 - summer:
OR 1 - fall:
OR 1 - winter:
######## **Please Enter Water-borne Chemical Treatment Regimens on the following page** ########

Water-borne Chemical Treatment Regimen (TR) 1

Please identify the life stage treated by placing an **F (fish)** to the right of the colon.
****

TR 1 - potassium permanganate (0.25 to 8 mg/L):

What is the dose administered?
****

TR 1 - water minimum (mg/L):
TR 1 - water maximum (mg/L):

How is the dose administered? (**X only one**)
****

TR 1 - Water static bath?:
TR 1 - Water flow-through?:

TR 1 - For this regimen, on how many days would you administer treatment?:
TR 1 - Are treatments administered on consecutive (C) or alternate (A) days?:

How long does a typical treatment (exposure) last? (**minutes**)
****

TR 1 - Static - minimum:
TR 1 - Static - maximum:
TR 1 - Flow-through - minimum:
TR 1 - Flow-through maximum:

Disease treated (**X all that apply**)
****

TR 1 - fungus:
TR 1 – Bacterial gill disease:
TR 1 - Columnaris:
TR 1 - trematodes, protozoans, or copepods:
TR 1 - other:

If you checked TR 1 - other, enter disease name:

Maximum number of culture units treated simultaneously
****

TR 1 - pond size 1:
TR 1 - pond size 2:
TR 1 - pond size 3:

Maximum number of culture units treated on a typical day
****

TR 1 - pond size 1:
TR 1 - pond size 2:
TR 1 - pond size 3:

Answer the following for pond treatments.
****

TR 1 - What percent of the treated volume is drained from the culture unit after treatment? (%):
TR 1 - By what percent is the flow rate increased after treatment (%):
TR 1 - If flow rate is increased, how long is it maintained? (min):

How often would you typically administer this treatment regimen?
****

TR 1 - times per year (enter number):

When do you typically treat? (**X all that apply**)
****

TR 1 - spring:
TR 1 - summer:
TR 1 - fall:
TR 1 - winter:

Water-borne Anesthetic Regimen (AR) 1 – Aqui-S Use at Production Facilities
anticipated dose - 25 to 50 mg/L

What is the anesthesia purpose (**X all that apply**)?
****

AR 1 - spawning:
AR 1 - transportation:
AR 1 – collection/harvest:
AR 1 - other:

What is the dose administered?
****

AR 1 - water minimum (mg/L):
AR 1 - water maximum (mg/L):

How is the dose administered?
****

AR 1 - On an annual basis, on how many days would you administer treatment?:
AR 1 - What volume of anesthetic bath would you typically prepare? (L):
AR 1 - How many times per day would you prepare the above volume?:

When do you typically treat? (**X all that apply**)
****

AR 1 - spring:
AR 1 - summer:
AR 1 - fall:
AR 1 - winter:

Water-borne Anesthetic Regimen (AR) 2 – Aqui-S Use Away from the Production Facility
anticipated dose - 25 to 50 mg/L

What is the anesthesia purpose (**X all that apply**)?
****

AR 2 - spawning:
AR 2 - transportation:
AR 2 – collection/harvest:
AR 2 - other:

What is the dose administered?
****

AR 2 - water minimum (mg/L):
AR 2 - water maximum (mg/L):

How is the dose administered?
****

AR 2 - On an annual basis, on how many days would you administer treatment?:
AR 2 - What volume of anesthetic bath would you typically prepare? (L):
AR 2 - How many times per day would you prepare the above volume?:

When do you typically treat? (**X all that apply**)
****

AR 2 - spring:
AR 2 - summer:
AR 2 - fall:
AR 2 - winter:
What type of water body is the anesthetic bath discharged to? (**X only one**)
****

AR 2 - Lake/Pond:
AR 2 - River/Stream:
AR 2 - Backwater of a River/Stream:

If the anesthetic enters a Lake/Pond, estimate the following.
****

AR 2 - What is the estimated average volume? (**acre-feet**)?:

If the anesthetic enters a River/Stream, answer the following.
****

AR 2 - If River/Stream selected, what is the estimated average flow? (**cfs**):
AR 2 - The lowest flow occurs during what season? (**NC if no change**):
AR 2 - What is the estimated average flow during the low flow season? (**cfs**):

++++++++++
END SURVEY

Appendix D. Summaries of Key Toxicity Studies Used for the Risk Assessment

Analytical Laboratory Services, Inc. 2003. Results of acute toxicity tests with *Ceriodaphnia dubia* and *Pimephales promelas* and chronic toxicity tests with *Selenastrum capricornutum* on pure products using effluent and receiving waters as dilution water. Prepared for the Pennsylvania Fish and Boat Commission, 1225 Shiloh Road, State College, Pennsylvania16801-8495. 408 pp.

Analytical Laboratory Services (2003) determined the 48-h EC_{50} of H_2O_2 and several other fishery chemicals for *Ceriodaphnia dubia* studies and the 96-h EC_{50} for *Pimephales promelas* using 4 different Pennsylvania surface waters for dilution (effluent from two hatcheries and water from two receiving streams). *C. dubia* were cultured in-house and *P. promelas* were obtained from Aquatox, Inc., Hot Springs, Arkansas. For *C. dubia*, there were 5 replicates per concentration and 10 organisms per replicate for a total of 50 organisms per concentration. Test chambers were 30 mL disposable beakers and the test volume was 25 mL. The test was static with no renewal. The photoperiod was 16 h light, 8 h dark over the test duration. The nominal test concentrations were 3.75, 7.5, 15, 30, 60, and 120 mg/L.

For *P. promelas*, there were 4 replicates per concentration and 10 organisms per replicate for a total of 40 organisms per concentration. Test chambers were 400 mL beakers and test volume was 200 mL. The test was static with renewal after 48 h. The photoperiod was 16 h light, 8 h dark over the test duration. The nominal test concentrations were 6.25, 12.5, 25, 50, and 100 mg/L.

There was no mention of dose confirmation for either study. The dilution waters for both studies were Benner Springs (PA) hatchery effluent, Spring Creek (PA) receiving water, Oswayo Creek (PA) hatchery effluent and Oswayo Creek (PA) receiving water. For both studies water quality determinations were made on the 4 dilution waters for alkalinity, hardness, conductivity, total residual chlorine, ammonia-N, and pH. Temperature, pH, dissolved oxygen, and conductivity were measured during the test period. Dilutions were chosen to preferably obtain 100% survival at the lower concentrations, partial mortalities at 2 or more concentrations, and 100% mortality at the highest concentration. For both studies, reference toxicity tests using potassium chloride were run during the test period. The resulting LC_{50}s were within the control limits.

The 48-h EC_{50} for *C. dubia* ranged from 8.1 to 11.2 mg/L using the 4 surface waters. The 96-h LC_{50} for *P. promelas* ranged from 23 to 72 mg/L. These results were not used for the revised EA risk assessment calculations because the tests were not done in laboratory water, but the data for *C. dubia* are useful supportive data for the critical acute toxicity data point for daphnids by Shurtleff (1989).

Boutillier, J. A. 1993. The efficacy of hydrogen peroxide against the salmon louse, *Lepeophtheirus salmonis*, its toxicological effects on Atlantic and chinook salmon, its stability in seawater, and its toxic effects on some non-target marine species. Aquaculture Update, No. 63. Bureau of Fisheries and Oceans. Pacific Biological Station, Nanaimo, British Columbia.

This report refers to H_2O_2 toxicity tests for chinook salmon conducted by the Environmental Protection Service of Environment Canada (EPS). Although no formal citation to the EPS study was given, the 96-h LC_{50} for juvenile chinook salmon (~ 12 g) was reported as 105 mg/L in sea water at 12 °C. Additional information describing test procedures were not available but Environment Canada toxicity testing for fish was likely in accordance with standardized testing procedures (e.g., ASTM).

This study on chinook salmon produced a key data point for our risk assessment.

EVS Environment Consultants. 1992. Toxicity testing with hydrogen peroxide contract no. FP92-5132. EVS Project No.:9/064-36. 41 pp.

The 96-h acute toxicity of H_2O_2 to larval euphausiid krill (*Euphausia pacifica*, an oceanic krill) was determined according to methods modified from ATSM (1989) and the 48-h acute toxicity of H_2O_2 to Pacific oyster (*Crassostrea gigas*) larvae was determined according to standard methods (ATSM 1989).

Krill toxicity tests: Krill larvae were collected at night during times of cloud cover or no moonlight from surface waters of Howe Sound, British Columbia. Krill larvae were held at 7 ± 1 °C in complete darkness until tested. Tests were performed in glass beakers containing 1-L of test solution with 10 organisms per test chamber and 3 replicates per treatment concentration. Treatment concentrations were at 0.09, 0.19, 0.38, 0.75, and 1.5 mg/L H_2O_2. Percent survival, dissolved oxygen, pH, and temperature were measured for each concentration at 24-h intervals. Salinity of the test solutions was measured at test initiation and test termination (96 h). Test chambers were kept at 23 ppt salinity, 7 ± 1 °C in total darkness throughout the test period, and the organisms were not fed. Because krill larvae are extremely sensitive, a mean control survival of at least 80% was considered the limit for test acceptability. Subsamples were removed for dose confirmation but dose confirmation results were not provided. The 96-h LC_{50} was determined to be 0.24 mg/L.

Oyster toxicity tests: Oysters obtained from a commercial supplier were maintained in spawning condition by thermal conditioning, increased photoperiod, and increased feeding. Spawning of conditioned oysters was induced by thermal and biological stimulation. The test was conducted in clean 250-mL polyethylene beakers containing 200 mL of test solution. A series of seven test concentrations (0.47, 0.94, 1.9, 3.8, 7.5, 15, and 30 mg/L) was prepared from a stock solution, plus a negative (clean) control with three replicates per treatment. Each container was inoculated within 2-h of egg fertilization to give a concentration of about 30 embryos per mL. Test vessels were not aerated and larvae were not fed during the test. "Zero-time" controls were used to establish the initial density of embryos and to monitor larval development without disturbing the real test controls. A positive (toxic) control was also conducted using a reference toxicant, sodium dodecyl sulphate. Toxicity in the oyster larvae toxicity test was based on abnormal shell development; larvae which failed to transform to the fully shelled, straight, hinged "D" shaped prodissoconch I stage were considered abnormal. Subsamples were removed for dose confirmation but dose confirmation results were not provided. Water quality data were not presented. The 48-h EC_{50} (abnormal shell development) for Pacific oyster larvae was 1.2 mg/L, the 48-h NOEC (abnormal shell development) was 0.47 mg/L and the 48-h NOEC (mortality) was 0.94 mg/L.

The studies on *Euphausia pacifica* and *Crassostrea gigas* produced key data points for our risk assessment.

Florence, T. M., and J. L. Stauber. 1986. Toxicity of copper complexes to the marine diatom *Nitzschia closterium*. Aquatic Toxicology 8:11-26.

Laboratory studies were conducted to determine the toxicity of H_2O_2 to the unicellular marine diatom *Nitzschia closterium* obtained from an Australian Commonwealth algal collection. Lighting was controlled during both culture and toxicity testing. Axenic cultures were maintained and assays were

conducted in filtered unsupplemented sea water. Water quality data were not reported nor was the use of replicates mentioned. Initial cell density in each flask was 2 to 4 x 10^4 cells per mL. Growth inhibition at six H_2O_2 concentrations (20-80 μM) relative to an untreated control was assessed. Hydrogen peroxide concentrations were measured spectrophotometrically and growth was expressed as a percentage of the control. Growth was decreased by 50% relative to controls following 72-h exposure to H_2O_2 at an initial concentration of 0.85 mg/L (2.5 x 10^{-5} M). The lowest concentration tested (20 μM) resulted in a 31% growth decrease, so the 72-h NOEC (growth) was less than 0.68 mg/L H_2O_2 (2.0 x 10^{-5} M initial concentration).

This study on *Nitzschia closterium* produced a key data point for our risk assessment.

Kay, S. H., P. C. Quimby, Jr,. and J. D. Ouzts. 1982. Hydrogen peroxide: A potential algicide for aquaculture. Proceedings of the Southern Weed Science Society 35:275-289.

Kay et al. (1982) evaluated H_2O_2 as a potential algicide in freshwater aquaculture. Field and laboratory toxicity studies were conducted with four algal genera. Laboratory toxicity studies were conducted with channel catfish, amphipods (*Gamarus sp.*,), snails (*Physa sp.*), and stratiomyid fly larvae (*Stratiomys sp.*).

Algal field studies: Field exposures to hydrogen peroxide (H_2O_2) were conducted with *Anabaena sp.* in polyethylene tanks placed in a commercial catfish pond. *Anabaena sp.* were collected from commercial catfish ponds. Channel catfish were present in tanks during testing and all tanks were evaluated in triplicate. Four separate experimental designs were evaluated and H_2O_2 concentrations tested ranged from 0 to 10 mg/L. Toxic effects were expressed as reduction of chlorophyll optical density relative to controls at 24 and 48-h. Chlorophyll concentrations were determined spectrophotometrically. Hydrogen peroxide concentrations were not verified nor were other water quality parameters assessed.

Algal laboratory studies: Three algal genera, *Ankistrodesmus* sp., *Raphidiopsis*, sp. and *Microcystis* sp. were selected for laboratory evaluation. *Ankistrodesmus* sp. were obtained from Carolina Biological Supply, *Raphidiopsis*, sp. were taken from an aquarium containing goldfish, and *Microcystis* sp. were collected from a commercial catfish pond. Lighting was controlled during culture and experimentation. Each treatment was replicated 3 times. Three 10-mL aliquots were taken for chlorophyll extraction at test initiation and at 24- and 48-h post-exposure for each species. Optical densities of all extracts were measured spectrophotometrically. Toxicity was expressed as a decrease in optical density compared to untreated controls. Hydrogen peroxide concentrations were not verified nor were other water quality parameters assessed.

The relative reduction of chlorophyll following exposure of *Ankistrodesmus*, *Raphidiopsis*, *Microcystis*, and *Anabaena* to hydrogen peroxide is tabulated following this paragraph.

Algal species	hydrogen peroxide concentration (mg/)	chlorophyll production (percent [%] of control chlorophyll production)	
		24-h	48-h
Field trial – *Anabaena*			
trial 1 (4 fish per tank)	10	not reported	2.5
trial 2 (3 fish per tank)	10	not reported	23
trial 2 (1 fish per tank)	10	not reported	32
trial 3	2.4	61	17
	5.1	44	6
	7.5	28	11
trial 4	2.4	88	29
	5.1	86	41
	7.5	81	26
Laboratory trials			
Ankistrodesmus	17	<5	not given
Microcystis	1.7	<5	not given
Raphidiopsis	6.8	not reported	<6

Fish and invertebrate laboratory studies: Channel catfish fingerlings were obtained from a commercial catfish farm and held in polyethylene tanks for ~two months before being tested. Ten fish each were placed in glass aquaria containing aerated tap water maintained at 22 ± 2 °C and allowed to acclimate to the test chamber overnight. The following morning H_2O_2 was added to provide concentrations of 0 (control), 0.06, 0.12, 0.24, 0.35, 0.47, 0.71, 0.94, 1.41, or 1.88 mM. Replicates were not mentioned. Water was changed and fresh H_2O_2 added daily for 4 days (96 h). Dead fish were removed as soon as they were observed. The 96-h LC_{50} values were determined by probit analysis. Hydrogen peroxide concentrations were not verified nor were water quality data reported. The 96-h LC_{50} for channel catfish was 37.4 mg/L and the 96-h LC_{01} estimate was 17 mg/L.

The results for *Microcystis* and channel catfish were key data points for our risk assessment.

Meinertz, J. R., Greseth, S.L., and M.P. Gaikowski. 2005. Chronic Toxicity of Hydrogen Peroxide to the Cladocera, *Daphnia magna*, in a Flow-Through Continuous Exposure System.

A 21-d chronic study of H_2O_2 toxicity to *Daphnia magna* was conducted at UMESC under flow-through conditions. The full study (Meinertz, et al. 2005) is included in the EA submission as Appendix D. *Daphnia magna* is considered to be a sensitive aquatic invertebrate and is recommended by the American Society for Testing and Materials (ASTM) for conducting macro invertebrate acute and life-cycle toxicity tests (ASTM Designation E 1193-97 1997, Standard Guide for Conducting *Daphnia magna* Life Cycle Toxicity Tests). The continuous exposure regimen selected represents the worst-case exposure scenario that could occur during intensive aquaculture operations, one that would occur only rarely, if at all (see discussion in section 7.4.2 of the EA).

The study objective was to determine H_2O_2 concentrations that have no effect on the time to death, growth rate, time to production of the first brood, numbers of broods, total number of young produced, and gender ratio of young produced from *Daphnia magna* during 21 d of continuous exposure. The research protocol was reviewed by the FDA Center for Veterinary Medicine (CVM) for comments

and concurrence and the study was conducted according to FDA good laboratory practice (GLP) regulations (21 CFR Part 58).

The experimental design included six test groups with target H_2O_2 concentrations of 0.0, 0.32, 0.63, 1.25, 2.5, and 5.0 mg/L. Each test group consisted of 10 test chambers. Each chamber was randomly assigned to one of ten blocks so that each test group was represented in each block; a randomized block design in a 2 x 3 configuration. Flow through the 205-mL chambers was maintained at ~5.0 mL/min and provided ~36 volume-exchanges/d. Although about nine times the maximum recommended flow (4 volume-exchanges/d; ASTM 1997), this flow was required to maintain H_2O_2 at 70-100% of the nominal concentration. Since the required flow was almost an order of magnitude greater than the recommended flow, the study likely resulted in more conservative effect estimates than if recommended flows had been used. The increased flow likely increased metabolic demands that required increased energy consumption and may have ultimately decreased growth and production. Even at a flow rate of 5 mL/min, the organic matter present in the test jar caused a rapid reduction of H_2O_2 (temporarily as much as 50%) before returning to within 70% of the nominal concentration. Therefore, we consider the test conditions to be artificial compared to those that would likely be encountered in the environment.

The study was initiated when one <24 h old *Daphnia magna* was distributed to each test chamber (1 daphnid/chamber) and then was continuously exposed to H_2O_2 for 21 d. Water temperature was maintained at 17.7-20.4 °C (mean = 20.1 °C). A light cycle of 16-h light (44-152 lux):8-h dark was maintained throughout the study. The daily pH values ranged from 7.45 to 7.99. Alkalinity and hardness ranged from 123 to 127 mg/L as $CaCO_3$ and from 168 to 172 mg/L, respectively. Daily dissolved oxygen concentrations were from 7.93 to 10.0 mg/L in all groups. Daphnia were fed an algal food designed for aquatic invertebrates five times daily during the week and three times daily during the weekends. Hydrogen peroxide concentration was confirmed daily. Mortality of first generation daphnia and number of young produced were enumerated daily. First-generation daphnia length at 21-d, time to death and time to first brood, number of broods, and total number of young produced were compared among treatment groups. The summary data from Meinertz et al. (2005) are presented in Table 6 and the major study conclusions are that H_2O_2 concentrations of:

$\leq$ 1.25 mg/L did not increase the probability of death;
$\geq$ 0.32 mg/L reduced daphnia growth relative to untreated controls;
$\leq$ 1.25 mg/L had no effect on the time to first brood production;
$\leq$ 1.25 mg/L had no effect on the number of broods produced;
$\leq$ 0.63 mg/L had no effect on the total number of young produced.

Rach, J. J., T. M Schreier, G. E. Howe, and S. D. Redman. 1997c. Effect of species, life stage, and water temperature on the toxicity of hydrogen peroxide to fish. The Progressive Fish-Culturist 59:41-46.

The acute toxicity of hydrogen peroxide to rainbow trout was determined. Fish were cultured at UMESC and were acclimated to test conditions for 96 h. Twenty-four-hour acute toxicity tests were conducted in duplicate glass jars containing 15-L test solution and each jar contained 10 fish (0.9 to 1.2 g/fish). Hydrogen peroxide concentrations ranged from 0 (control) to 5,660 mg/L (concentrations were originally reported as µL/L but were converted to mg/L by multiplying by 1.132 mg H_2O_2/µL). Tests were conducted at temperatures of 7, 12, 17, and 22 °C and dissolved oxygen, temperature, pH, and H_2O_2 concentrations were measured throughout the study. Mean percent mortality was calculated and pooled

mortality data were used to calculate the LC_{50} and the 95% confidence interval estimates. The 24-h LC_{50} for rainbow trout fingerlings ranged from 35 to 78 mg/L depending on exposure temperature. The 24-h LC_{50} was 48 mg/L when tested at 12 °C.

This study on rainbow trout fingerling produced a key data point for our risk assessment.

Shurtleff, L. E. 1989. Interox America sodium percarbonate and hydrogen peroxide--Acute toxicity to the freshwater invertebrate *Daphnia pulex*. Burlington Research, Burlington North Carolina.

The acute toxicity of H_2O_2 to *Daphnia pulex* was characterized in four water qualities: a reconstituted water of known hardness, Milli-Q ultrapure water, Triton® distilled water, and buffered water from a lake whose water quality was monitored routinely. The reconstituted water was diluted to the needed volume for testing with either Milli-Q ultrapure water or with a 50:50 mixture of Triton® distilled water and buffered lake water. Daphnids were cultured according to carefully documented procedures. Dissolved oxygen, pH and temperature were monitored in each test chamber at the beginning of testing and again at 24 and 48 h. Static-renewal tests (24-h renewals) with hydrogen peroxide concentrations of 1000, 500, 100, 50, 10, and 1 mg/L were conducted; replication was not mentioned. Concentrations were determined by standard titrimetric methods and showed considerable H_2O_2 degradation during the study. The LC_{50} values were determined using Spearman-Karber estimates and a mean 48-h LC_{50} (the lethal concentration to 50% of test organisms after 48 h exposure) of 2.4 mg/L for *Daphnia pulex* determined for studies using a 50:50 mixture of distilled and lake surface water.

This study on *Daphnia pulex* produced a key data point for our risk assessment.

Appendix E. Meinertz, J. R., S.L. Greseth, and M.P. Gaikowski. 2005. Chronic Toxicity of Hydrogen Peroxide to the Cladocera, *Daphnia magna*, in a Flow-through Continuous Exposure System (submitted with the revised draft EA as separate volumes)

Appendix F. Copies of Literature Cited in the Revised Draft Environmental Assessment for Use of Hydrogen Peroxide in Aquaculture (Cited literature has been previously submitted to CVM as Appendix F of the revised draft EA)

Table 1. Hydrogen peroxide therapies administered to control mortalities associated with various diseases of freshwater-reared finfish and eggs.

Species	Life stage	Disease	Duration
All freshwater-reared finfish (except channel catfish)	eggs	saprolegniasis	administer continuous-flow treatments of 500-1,000 mg/L for 15 min once daily on consecutive or alternate days from fertilization through hatch
Channel catfish	eggs	saprolegniasis	administer continuous-flow treatments of 750-1,000 mg/L for 15 min once daily on consecutive or alternate days from fertilization through hatch
All freshwater-reared salmonids	all fish	bacterial gill disease	administer continuous-flow or static bath treatments of 100 mg/L for 30 min or 50-100 mg/L for 60 min once daily on consecutive or alternate days for a total of three treatments
Channel catfish and all freshwater-reared coolwater finfish (except northern pike)	fingerlings or adults	external columnaris disease	administer continuous-flow or static bath treatments of 50-75 mg/L for 60 min once daily on consecutive or alternate days for a total of three treatments
Channel catfish and all freshwater-reared coolwater finfish (except northern pike or pallid sturgeon)	fry	external columnaris disease	administer continuous-flow or static bath treatments of 50 mg/L for 60 min once daily on consecutive or alternate days for a total of three treatments

Table 2. Identification of the chemical substance of the proposed action.

Chemical name	**Hydrogen peroxide (35% active ingredient)**
Synonyms	hydrogen dioxide, hydroperoxide, albone, superoxol
Common names	hydrogen peroxide, peroxide
CAS Registry Number	7722-84-1
Formula Weight	34.01
Chemical formula	H_2O_2
Physical and chemical characteristics	Clear, colorless liquid; specific gravity of 1.13 at 35% active ingredient; miscible in water; strong oxidant; degrades gradually to water and oxygen in the absence of stabilizers at sufficient concentrations.

Table 3. Physicochemical properties of hydrogen peroxide.

Parameter	Value	Reference
Boiling Point (° C)	152 °C 108 °C (35% soln.)	HSDB[a] MSDS[b]
Melting Point (° C)	-0.43 °C -33 °C (35% soln.)	HSDB MSDS
Density	1.44 @ 25 °C 1.13 (35% soln.)	HSDB MSDS
Dissociation constant (pK_a)	11.75	HSDB
pH	4.6 (35% soln.)	HSDB
Solubility in water	1×10^6 mg/L @ 25 °C	HSDB
Vapor pressure	24 mm Hg (35% soln.)	MSDS
Henry's Law constant	7.04×10^{-9} atm•m3/mol @ 25 °C	HSDB
Storage stability	Very stable under normal conditions	MSDS
Other	Oxidizer, corrosive	MSDS

[a] HSDB: Hazardous Substance Data Bank (2004).
[b] MSDB: BHS Marketing / Western Briquette (2003).

Table 4 Toxicity values (receptors of interest – ROI) for freshwater and marine algae exposed to hydrogen peroxide Values used in the risk assessment are bolded

Organism	Effect Measured	Concentration (mg/L)	Duration (hours)	Reference
FRESHWATER				
Dinobryon spp., *Ochromonas* spp., *and Chrysochromulina* spp (a mixture)	NOEC [a] (primary productivity)	0 34-34	24	Xenopoulos and Bird 1997
Anabaena spp	Reduced chlorophyll	9 9	24	Kay et al 1982
Ankistrodesmus spp	Reduced chlorophyll	17 0	24	
Raphidopsis spp	Reduced chlorophyll	6 8	24	
Ankistrodesmus spp.	Threshold toxicity [b]	6 8-10	24[c]	
Raphidiopsis spp	Threshold toxicity	< 3 4	24	
Microcystis spp	Threshold toxicity [c]	< 1 7	24	
Scenedesmus subspicatus	EC_{50}, proliferation	7 3	7 days	Trenel and Kühn 1982
Aphanizomenon flas-aquae	EC_{50} [d], inhibition of nitrogen fixation	0.9	22	Peterson et al. 1995
	EC_{15}, inhibition of nitrogen fixation	3 4	1.5	
MARINE				
Gyrodinium spp	No cysts germinated	6 0	48	Montani et al. 1995
Chattonella spp	No cysts germinated	90	48	
Alexandrium spp	No cysts germinated	150	48	
Scrippsiella spp	No cysts germinated	150	48	
Gymnodinium spp.	No cysts germinated	150	48	
Protoperidinium spp	No cysts germinated	150	48	
Nitzschia spp.	EC_{50} growth decrease	0.85	72	Florence and Stauber 1986
	NOEC (growth)	≤0.68	72	
Polykrikos spp.	No cysts germinated	100	48	Ichikawa et al. 1993
Alexandrium catenella	Mortality	30	48	
Alexandrium tamarense	Mortality	30	48	
Oscillatoria spp.	42.19% reduction in chlorophyll	4.19	72	Srisapoom et al 1999
	46.77% reduction in chlorophyll	7.18	72	

[a] NOEC = the highest exposure concentration that elicited no observable adverse effect.
[b] Threshold toxicity = the lowest exposure concentration that elicited an adverse effect.
[c] Threshold toxicity will be substituted for both the acute and chronic LC_{100} values due to the lack of any better data
[d] EC_{50} = effective concentration for eliciting a particular effect in 50% of test organisms.

Table 5. Toxicity values (receptors of interest – ROI) for freshwater and marine invertebrates exposed to hydrogen peroxide. Values used in the risk assessment are bolded.

Organism	Effect Measured	Concentration (mg/L)	Duration (hours)	Reference
FRESHWATER				
Daphnia pulex (water flea)	LC_{50} [a]	2.4	48	Shurtleff 1989
Daphnia magna (water flea)	EC_0 (immobilize)	3.8	24	Bringmann 1982
	EC_{50} [b] (immobilize)	7.7	24	
	EC_{50} (equilibrium)	2.3	24	Trenel and Kühn 1982
	EC_{50} (immobilize)	18	48	EPA Pesticide Ecotoxicity Database, AQUIRE ref. #344, only endpoint is available
	NOEC [c] (mortality)	1.25	21 d	Meinertz et al. 2005
	NOEC (time to first brood)	1.25	21 d	
	NOEC (number of broods produced	1.25	21 d	
	NOEC (total number of young produced)	0.63	21 d	
	reduced growth	> 0.32	21 d	
Ceriodaphnia dubia in Benner Springs, PA. effluent	LC_{50}, NOEC	11.2, 7.5	48	Anal. Lab. Services, Inc. 2003
in Spring Creek, PA. water	LC_{50}, NOEC,	8.1, 3.75	48	
in Oswayo Creek FCH, PA. effluent	LC_{50}, NOEC,	10.8, 7.5	48	
in Oswayo Creek water	LC_{50}, NOEC,	9.23, 3.75	48	
Procambarus clarkii (Crayfish)	LC_0	64.6	96	Kay et al. 1982
Gammarus spp. (scuds)	LC_{50}	4.42	96	
Physa spp. (snail)	LC_{50}	17.7	96	
Pchydiplax spp.(dragon fly nymph)	LC_0	170	96	
Stratiomys spp. (fly larvae)	LC_0	218	96	
Dreissena polymorpha (zebra mussel)	NOEC	4.5	48	Martin et al. 1993
	NOEC	1.5	120	
	LT_{50} [d]	6	70	
	LT_{50}	12	approx. 36	
	LT_{50}	30	10	
	LC_{100}	30	72	
	LC_{100}	20	120	
	LC_{100}	12	408	
Chironomid larvae (midge)	LC_{50}	125	72	Alexander et al. 1997
MARINE				
Rhepoxynius abronius (amphipod)	LC_{50}	75	96	EVS Consultants 1992
Euphausia pacifica (euphaushiid)	LC_{50}	0.24	96	
Crassostrea gigas (oyster larvae)	EC_{50} (abnormal shell development)	1.2	48	
	NOEC (abnormal shell development)	0.47	48	
	NOEC (mortality)	0.94	48	
Lepeophtheirus salmonis, (sea lice), eggs,	57% mortality	1500	20 min	Johnson et al. 1993
chalimus stage,	41% mortality	4000	20 min	
adults	68% mortality	3000	20 min	
Artemia salina (brine shrimp) nauplii	IC_{50} [e] (immobilize)	918	24	Matthews 1995
Penaeus monodon.(tiger prawn) postlarva	LC_{50}	30.6	24	Srisapoom et al. 1999
Mixed plankton	LT_{100}	1.0 @ pH 8.5	<35 min	Kuzirian et al. 2001
Mnemiopis leidyi	LT_{100}	1.0 @ pH 8.5	<10 min	

[a] LC_{50} = lethal concentration to 50% of test organisms.
[b] EC_{50} = concentration for eliciting a particular effect in 50% of test organisms.
[c] NOEC = the highest exposure concentration that elicited no observable adverse effect.
[d] LT_{50} = time to 50% lethality.
[e] Concentration needed to reach 50% inhibition of mobility in uauplii.

Table 6. *Daphnia magna* survival (F_0 generation; number survivors/number exposed), growth (mean length of F_0 generation), and reproduction (mean time to first brood, mean total number of broods, mean total number of young) determined after continuous exposure to hydrogen peroxide for 21 days (Meinertz et al. 2005, Appendix E). Data within the same column with a common letter are not statistically different ($P \geq 0.05$; na = not applicable).

Concentration (mg/L)	Survival	Length (mm)	Days to first brood	Number of broods	Total young
0	10/10	4.62 (a)	11 (a,b,c)	41 (a)	1,516 (a)
0.32	9/10	4.46 (b)	12 (a,d)	40 (a)	1,564 (a)
0.63	9/10 (a)	4.39 (b)	10 (a,b,c)	39 (a)	1,388 (a)
1.25	8[a]/10 (a)	3.90 (c)	10 (a,b,c)	40 (a)	1,000 (b)
2.5	0/10 (b)	na	16 (d)	1 (b)	1 (c)
5.0	0/10 (c)	na	na	0	0

[a] The chamber of one daphnid in this test group was found overflowing on day 21. This daphnid was counted as a mortality because it could not be found.

Table 7 Toxicity values (receptors of interest – ROI) for freshwater fish exposed to hydrogen peroxide. Values used in the risk assessment are bolded.

Organism	Effect	Concentration (mg/L)	Duration	Reference
Rainbow trout (*Oncorhynchus mykiss*); juvenile	Mortality	>40	48 h	McKee and Wolf 1963
fry	LC_{50} [a]	322	1 h	Arndt and Wagner 1997
juvenile	LC_{50}	329	1 h	
	NOEC [b] (mortality)	283	45 min [c]	Rach et al. 1997c
	LC_{50}	48	24 h	
	NOEC (<10% mort.)	162	1 h [d]	Gaikowski et al. 1999
	EC_0 [e] (growth)	200	7 wk [f]	Speare and Arsenault 1997
Cutthroat trout (*O. clarki*); fry	LC_{50}	377	1 h	Arndt and Wagner 1997
juvenile	LC_{50}	506	1 h	
Brown trout (*Salmo trutta*); juvenile	NOEC (mortality)	283	45 min [c]	Rach et al. 1997c
Lake trout (*Salvelinus namaycush*); juvenile	NOEC (mortality)	1132	45 min [c]	
	NOEC (<22% mort.)	298	1 h [d]	Gaikowski et al. 1999
Muskellunge (*Esox masquinongy*); juvenile	NOEC (<10% mort.)	104	1 h [d]	
Northern pike (*E. lucius*); juvenile	NOEC (<37% mort.)	76	1 h [d]	
Pallid sturgeon (*Scaphirhynchus albus*); juvenile	NOEC (<10% mort.)	93	1 h [d]	
White sucker (*Catostomus commersoni*); juvenile	NOEC (<10% mort.)	78	1 h [d]	
Fathead minnow (*Pimephales promelas*); juvenile	NOEC (mortality)	566	45 min [c]	Rach et al. 1997c
	NOEC (<13% mort.)	78	1 h [d]	Gaikowski et al. 1999
in Benner Springs, PA. effluent; fry	LC_{50}, NOEC	72, 50	96-h	Anal. Lab. Services, Inc. 2003
in Spring Creek, PA. water; fry	LC_{50}, NOEC	71, 50	96-h	
in Oswayo Creek FCH, PA. effluent; fry	LC_{50}, NOEC	39, 25	96-h	
in Oswayo Creek water; fry	LC_{50}, NOEC	23, 12.5	96-h	
Bluegill sunfish (*Lepomis macrochirus*); juvenile	NOEC (mortality)	1132	45 min [c]	Rach et al. 1997c
	LC_{50}	81	24 h	
	NOEC (<13% mort.)	78	1 h [d]	Gaikowski et al. 1999
Channel catfish (*Ictalurus punctatus*); juvenile	NOEC (mortality)	1132	45 min [c]	Rach et al. 1997c
	LC_{50}	63	24 h	
	LC_{50}	37	96 h	Kay et al. 1982
	NOEC (<17% mort.)	78	1 hd	Gaikowski et al. 1999
Walleye (*Sander vitreum*); juvenile	LC_{50}	145	1 h	Clayton and Summerfelt 1996
	NOEC (<20% mort.)	96	1 h [d]	Gaikowski et al. 1999
Largemouth bass (*Micropterus salmoides*); juvenile	NOEC (<10% mort.)	130	1 h [d]	
Yellow perch (*Perca flavescens*); juvenile	NOEC (<13% mort.)	130	1 h [d]	
Common carp (*Cyprinus carpio*); life stage not given	LD_{50} [g]	42	48 h	Miyazaki et al. 1990
Western mosquito-fish (*G. affinis*); life stage not given	NOEC	10	48 h	Kay et al. 1982
Guppy (*L. reticulatus*); various ages (male and female)	NOEC	34	5 d	Quimby 1981

[a] LC_{50} = lethal concentration to 50% of test organisms.
[b] NOEC = the highest exposure concentration that elicited no observable adverse effect.
[c] Four consecutive exposures were administered every-other-day; concentrations reported by Rach et al. (1997c) were multiplied by 1.132 to convert hydrogen peroxide concentration from µL/L to mg/L.
[d] Three consecutive exposures were administered every-other-day.
[e] EC_0 = the effeetive concentration that resulted in 0% change in the observed effect relative to the control organisms.
[f] Exposures were administered twice weekly.
[g] LD_{50} = lethal dose to 50% of test organisms.

Table 8. Toxicity values (receptors of interest – ROI) for brackish-water or marine fish exposed to hydrogen peroxide. Values used in the risk assessment are bolded.

Organism	Effect	Concentration (mg/L)	Duration	Reference
Chinook salmon (*O. tschawytscha*); juvenile	LC_{50} [a]	105	96 h	Boutillier 1993
juvenile	LC_0 [b]	1500	20 min	Johnson et al. 1993
juvenile	LC_{100} [c]	1500	40 min	
Atlantic salmon (*Salmo salar*);	LC_{50}	2,500	1 h	Thomassen and Poppe 1992
juvenile				
juvenile	LC_{50}	>8,800	0.5 h	
juvenile	LC_0	1500	20 min	Johnson et al. 1993
juvenile	NOEC [d] (<10% mort.)	221	1 h	Gaikowski et al. 1999
Hawaiian aholehole (*Kuhlia sandvicenis*); juvenile	NOEC (dispersal) [e]	20	2 min	Hiatt, et al. 1953
Goldsinny wrasse (*Ctenolabrus rupestris*); adult	LC_0	1260	20 min	Bruno and Raynard 1994
Dusky spinefoot (*Siganus fuscescens*); life stage not given	LC_{50}	224	24 h	Kanda et al. 1989
Jack mackerel (*Trachurus japonicus)*; life stage not given	LC_{50}	89	24 h	
Chameleon goby (*Tridentiger trigonocephalus)*; life stage not given	LC_{50}	155	24 h	

[a] LC_{50} = lethal concentration to 50% of test organisms.
[b] LC_0 = lethal concentration to 0% of test organisms.
[c] LC_{100} = lethal concentration to 100% of test organisms.
[d] NOEC = the highest exposure concentration that elicited no observable adverse effect.
[e] Dispersal = caused dispersal of schooling fish.

Table 9. Toxicity values (receptors of interest – ROI) of hydrogen peroxide to various microbial species that may occur in aquatic environments. Values used in the risk assessment are bolded.

Organism	Effect	Concentration (mg/L)	Endpoint / Duration	Reference
FRESHWATER				
Pseudomonas putida (functional catalase present)	mortality	≤136	15-min NOEC (100% survival)	Klotz and Anderson 1994
Pseudomonas putida (functional catalase absent)	mortality	≥8.5	15-min EC_{75} (75% mortality)	
Pseudomonas putida	reduction of O_2 uptake	11	16-18h EC_{10}	Knie et al. 1983
Pseudomonas aeruginosa	no visible growth	5.1	MIC (minimum inhibitory concentration)	Baldry 1983
Nitrosomonas sp.	inhibition of ammonia oxidation	680	12% inhibition	Jones
Anaerobic bacterial sludge	0% methane in headspace gas	≤18	63-h exposure	Cohen 1992
Fecal coliform	reduction of initial fecal coliform level (10^6 to 10^7 cfu[a]/100 mL) to 10^4 cfu/100 mL	213-493	60-min minimum effective concentration to reduce coliform level to 10^4 cfu/100 mL	Wagner et al. 2002
		106-285	120-min minimum effective concentration to reduce coliform level to 10^4 cfu/100 mL	
Escherichia coli	no visible growth	40-160	MIC	Aarestrup and Hasman 2004
	no visible growth in tryptic soy broth	3000-4000	Minimum bacteriostatic concentration	Vijayakumar, et al. 2002
	no visible growth in tryptic soy broth or on tryptic soy agar	4000	Minimum bactericidal concentration	
	reduced bacteria populations over 08 log_{10}	2505	MIC	Penna et al. 2001
Enterobacter cloacae	reduced bacteria populations over 08 log_{10}	1250	MIC	
Acinetobacter calcoaceticus	reduced bacteria populations over 08 log_{10}	469	MIC	
Serratia marcescens	reduced bacteria populations over 08 log_{10}	625	MIC	
enterococci	no visible growth	80-160	MIC	Aarestrup and Hasman 2004
Streptococcus faecalis	no visible growth	15.3	MIC	Baldry 1983
Klebsiella pneumoniae	no visible growth	15.3	MIC	
Candida, various strains (yeast)	growth inhibition	150- 2990	MIC	Larsen and White
Penicillium expansum (fungus)	no visible growth	250	MIC	Venturini et al. 2002
MARINE				
Vibrio alginolyticus	not known from abstract, probably growth inhibition	19.41 (0.6 in 1.5% NaCl)	MIC	Srisapoom et al. 1999
Vibrio harveyi	probably growth inhibition	9.57 (0.6 in 1.5% NaCl)	MIC	
Vibrio parahaemolyt-icus	probably growth inhibition	38.27 (2.39 in 1.5% NaCl)	MIC	
Vibrio vulnificus	probably growth inhibition	38.27 (2.39 in 1.5% NaCl)	MIC	
Vibrio fischeri	Microtox, probably fluorescence reduction	41.5	15 min. EC_{50} (probably 50% reduction of fluorescence)	GloxoSmith Kline 2003
probably *Vibrio fischeri*	Not known, probably Microtox	30	EC_{50} bacteria (probably 50% reduction of fluorescence)	BHS Marketing / Western Briquette 2003

Table 10. Assessment factors recommended in VICH Phase II guidance for Tier A and Tier B (International Cooperation on Harmonization of Technical Requirements for Regulation of Veterinary Medical Products 2004).

Type of Aquatic Study	Toxicity Endpoint	Assessment Factor	Basis for Factor
Tier A			
Algal growth inhibition	EC_{50}	100	Interspecies variability; Extrapolation to field/community level effects
Daphnia acute study (fresh) / crustacean acute study (brackish)	EC_{50}	1,000	Extrapolation to NOEC; Interspecies variability; Extrapolation to field/community level effects
Fish acute study	EC_{50}	1,000	
Tier B			
Algal growth inhibition (72 h)	NOEC	10	Extrapolation from lab/single species test to field/community level effects
Daphnia magna reproduction (fresh) / crustacean chronic study (brackish)	NOEC	10	
Fish early-life stage	NOEC	10	
Sediment invertebrate toxicity	NOEC	10	

Table 11. Assumptions made by applicant for calculation of "Typical" and "Worst-Case" EICs.

Parameter	"Typical" Treatment Scenario	"Worst-Case" Treatment Scenario
Treatment concentration	eggs - 1000 mg/L; fish - 100 mg/L	eggs - 1000 mg/L; fish - 100 mg/L
Treatment duration	eggs - 15 min; fish - 60 min	eggs - 15 min; fish - 60 min
Number of treatments; 1-d; 2-d; 5-d; 21-d	eggs - 1, 2, 5, 15; fish - 1, 1, 3, 3	eggs - 1, 2, 5, 15; fish - 1, 1, 3, 3
Hatchery flow rate	average daily water flow	low daily water flow
Number of culture units treated	Maximum number of culture units treated daily	Maximum number of culture units treated daily
Treated culture unit flow rate	At the maximum flow rate	At the maximum flow rate
Settling pond volume	Per survey (if present)	Per survey (if present)
Receiving water flow	low flow	low flow

Table 12. Summary statistics for the 1-, 2-, 5- and 21-d Estimated Introductory Concentration (EIC) calculated based on information provided by fish hatcheries in a survey of present and projected hydrogen peroxide use. Data presented represent EIC estimates for the maximum daily hydrogen peroxide treatment use under average hatchery water flow (typical) or low water flow conditions (worst-case). The EIC summaries are segregated into three categories: all hatcheries (69 EIC estimates); hatcheries with effluent/settling ponds (44 EIC estimates); hatcheries without settling ponds (25 EIC estimates); hatcheries conducting egg treatments (39 EIC estimates); hatcheries conducting fish treatments (30 EIC estimates).

Parameter	1-d EIC		2-d EIC		5-d EIC		21-d EIC	
	Typical	Worst-Case	Typical	Worst-Case	Typical	Worst-Case	Typical	Worst-Case
All Hatcheries								
Mean (mg/L)	1.2	1.6	0.9	1.2	1.0	1.3	0.6	0.9
Median (50th percentile, (mg/L)	0.6	0.9	0.4	0.8	0.5	0.8	0.2	0.4
75th Percentile (mg/L)	1.5	2.3	1.1	1.8	1.3	2	0.6	0.8
95th Percentile (mg/L)	4.1	4.2	2.2	3.6	2.5	3.6	1.8	3.0
Maximum	7.4	10.4	7.4	10.4	7.4	10.4	7.3	8.9
Number < 0.7 mg/L	38	29	41	34	39	31	57	51
Number > 0.7 mg/L	31	40	28	35	30	38	12	18
Number > 1.0 mg/L	26	32	20	24	21	27	9	14
Hatcheries with settling ponds								
Mean (mg/L)	1.2	1.4	0.9	1.0	1.0	1.2	0.6	0.8
Median (50th percentile, (mg/L)	0.6	0.9	0.4	0.7	0.6	0.8	0.3	0.4
75th Percentile (mg/L)	1.9	2.2	1.1	1.6	1.3	1.9	0.6	0.7
95th Percentile (mg/L)	3.9	3.9	2.2	2.7	2.5	2.7	1.9	2.4
Maximum	7.4	7.4	7.4	7.4	7.4	7.4	7.3	7.3
Number < 0.7 mg/L	25	19	26	23	25	20	37	33
Number > 0.7 mg/L	19	25	18	21	19	24	7	11
Number > 1.0 mg/L	16	18	13	14	14	17	7	8
Hatcheries without a settling pond								
Mean (mg/L)	1.2	1.9	0.9	1.5	0.9	1.6	0.5	1.0
Median (50th percentile, (mg/L)	0.7	1.3	0.5	0.8	0.5	0.9	0.2	0.3
75th Percentile (mg/L)	1.3	2.3	1.0	2.1	1.0	2.2	0.6	0.9
95th Percentile (mg/L)	4.2	4.4	2.1	4.3	2.5	4.3	1.3	3.1
Maximum	6.3	10.4	6.3	10.4	6.3	10.4	4.5	8.9
Number < 0.7 mg/L	13	10	15	11	14	11	20	18
Number > 0.7 mg/L	12	15	10	14	11	14	5	7
Number > 1.0 mg/L	10	14	7	10	7	10	2	6
Egg treatments								
Mean (mg/L)	0.9	1.4	0.9	1.4	0.9	1.4	0.9	1.3
Median (50th percentile, (mg/L)	0.3	0.6	0.3	0.6	0.3	0.6	0.3	0.6
75th Percentile (mg/L)	1.0	1.7	1.0	1.7	1.0	1.7	0.9	1.8
95th Percentile (mg/L)	3.2	4.7	3.2	4.7	3.2	4.7	4.2	4.5
Maximum	7.4	10.4	7.4	10.4	7.4	10.4	7.3	8.9
Number < 0.7 mg/L	24	21	24	21	24	21	27	21
Number > 0.7 mg/L	15	18	15	18	15	18	12	18
Number > 1.0 mg/L	10	13	10	13	10	13	9	14
Fish treatments								
Mean (mg/L)	1.5	1.9	0.8	1.0	1.0	1.2	0.3	0.3
Median (50th percentile, (mg/L)	1.2	1.5	0.6	0.8	0.8	1.0	0.2	0.3
75th Percentile (mg/L)	2.2	3.1	1.2	1.8	1.6	2.2	0.4	0.6
95th Percentile (mg/L)	4.1	4.2	2.1	2.1	2.5	2.5	0.6	0.6
Maximum	4.2	4.2	2.1	2.1	2.5	2.5	0.6	0.6
Number < 0.7 mg/L	14	8	17	13	15	10	30	30
Number > 0.7 mg/L	16	22	13	17	15	20	0	0
Number > 1.0 mg/L	16	19	10	11	11	14	0	0

Table 13. Comparisons of exposure estimates for hydrogen peroxide use in fish hatcheries.

Treatment	Flow and Treatment Conditions	Number of EIC estimates	Holding Pond	Environmental Introduction Concentration (EIC - mg/L)							
				1-d		2-d		5-d		21-d	
				Max.	No. >1 mg/L	Max.	No. >1 mg/L	Max.	No. >1 mg/L	Max.	No. >1 mg/L
Eggs	Typical	15	No	6.3	5	6.3	5	6.3	5	4.5	2
	Worst-case	15	No	10.4	7	10.4	7	10.4	7	8.9	6
	Typical	24	Yes	7.4	5	7.4	5	7.4	5	7.3	7
	Worst-case	24	Yes	7.4	6	7.4	6	7.4	6	7.3	8
Fish	Typical	10	No	4.2	5	2.1	2	2.5	2	0.6	0
	Worst-case	10	No	4.2	7	2.1	3	2.5	3	0.6	0
	Typical	20	Yes	3.9	11	2	8	2.5	9	0.6	0
	Worst-case	20	Yes	4.2	12	2.1	8	2.5	11	0.6	0

n of VICH Phase II Tier A and Tier B assessment factors to available acute or chronic toxicity data and "Typical – All Hatcheries" EIC summaries from Table 12.

	Risk Quotient (Table 12 EIC divided by the PNEC)															
	For a 1-d EIC				For a 2-d EIC				For a 5-d EIC				For a 21-d EIC			
(EC g/L)	Mean	Median	75th percentile	95th percentile	Mean	Median	75th percentile	95th percentile	Mean	Median	75th percentile	95th percentile	Mean	Median	75th percentile	95th percentile
.7	71	35	88	241	53	24	65	129	59	29	77	147	35	12	35	106
7	71	35	88	241	53	24	65	129	59	29	77	147	35	12	35	106
.4	500	250	625	1,710	375	167	458	917	417	208	542	1,040	250	83	250	750
.3	19	10	24	65	14	6.3	17	35	16	7.9	21	40	9.5	3.2	9.5	29
8	25	13	31	85	19	8.3	23	46	21	10	27	52	13	4.2	13	38
7	32	16	41	111	24	11	30	59	27	14	35	68	16	5.4	16	49
.8	176	88	221	603	132	59	162	324	147	74	191	368	88	29	88	265
.8	176	88	221	603	132	59	162	324	147	74	191	368	88	29	88	265
.4	128	64	160	436	96	43	117	234	106	53	138	266	64	21	64	192
24	5,000	2,500	6,250	17,080	3,750	1,670	4,580	9,170	4,170	2,080	5,420	10,420	2,500	833	2,500	7,500
)5	11	5.7	14	39	8.6	3.8	10	21	9.5	4.8	12	24	5.7	1.9	5.7	17

entration (Assessment Endpoint Value / VICH AF); EIC = Environmental Introduction Concentration; RQ = Risk Quotient.
NOEC

Table 15. Risk quotients determined based on the application refined VICH assessment factors to acute toxicity data for selected fresh and brackish-water ROI and "Typical – All Hatcheries" EIC summaries from Table 12.

				Acute Risk Quotient (Table 12 EIC divided by the PNEC)											
				For a 1-d EIC				For a 2-d EIC				For a 5-d EIC			
Species	Assessment Endpoint (Value, mg/L)	AF	PNEC (µg/L)	Mean	Median	75th percentile	95th percentile	Mean	Median	75th percentile	95th percentile	Mean	Median	75th percentile	95th percentile
FRESH															
Microcystis spp.	lowest concentration tested (24-h) (1.7 mg/L)	100 [a]	17	71	35	88	241	53	24	65	129	59	29	76	147
Daphnia pulex	48-h EC_{50} (2.4 mg/L)	20 [b]	120	10	5	13	34	7.5	3.3	9.2	18	8.3	4.2	11	21
Rainbow trout fingerling	24-h LC_{50} (48 mg/L)	30 [c]	1,600	0.8	0.4	0.9	2.6	0.6	0.3	0.7	1.4	0.6	0.3	0.8	1.6
BRACKISH															
Nitzschia closterium	72-h NOEC (≤ 0.68 mg/L)	10 [d]	68	18	8.8	22	60	13	5.9	16	32	15	7.4	19	37
Pacific oyster larvae	48-h NOEC [mortality] (0.94 mg/L)	10 [e]	94	13	6.4	16	44	9.6	4.3	12	23	11	5.3	14	27
Chinook salmon	96 h LC_{50} (105 mg/L)	60 [f]	1,750	0.7	0.3	0.9	2.3	0.5	0.2	0.6	1.3	0.6	0.3	0.7	1.4

AF = Assessment Factor; PNEC = Predicted No Effect Concentration; EIC = Environmental Introduction Concentration; RQ = Risk Quotient.

[a] Value of 10 for the extrapolation of the acute LC_{100} to the acute NOEC; Value of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects).

[b] Value of 2 for the extrapolation of the acute EC_{50} to the acute NOEC; Value of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects).

[c] Value of 3 for the extrapolation of the acute LC_{50} to the acute NOEC; Value of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects).

[d] Value of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects).

[e] Value of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects).

[f] Value of 6 for the extrapolation of the acute LC_{50} to the acute NOEC; Value of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects).

Table 16. Risk quotients determined based on the application refined VICH assessment factors to chronic toxicity data for selected fresh and braekish-water ROI and "Typical – All Hatcheries" EIC summaries from Table 12.

Species	Assessment Endpoint (Value, mg/L)	AF	PNEC (µg/L)	Chronic Risk Quotient (Table 12 EIC divided by the PNEC) For a 21-d EIC			
				Mean	Median	75th percentile	95th percentile
FRESH							
Mierocyrtis spp.	lowest concentration tested (24-h) (1.7 mg/L)	200 [a]	8.5	71	24	71	212
Daphnia magna	21-d NOEC (0.63 mg/L)	10 [b]	63	9.5	3.2	9.5	29
channel catfish	96-h LD_{50} (37.4 mg/L)	100 [c]	374	1.6	0.5	1.6	4.8
BRACKISH							
Nitzschia closterium	72-h NOEC (≤ 0.68 mg/L)	10 [d]	68	8.8	3.0	8.8	27
Euphausia pacifica	96-h LC_{50} (0.24 mg/L)	100 [e]	2.4	250	83	250	750
chinook salmon	96-h LC_{50} (105 mg/L)	100 [f]	1,050	0.6	0.2	0.6	1.7

AF = Assessment Factor; PNEC = Predicted No Effect Concentration; EIC = Environmental Introduction Concentration; RQ = Risk Quotient

[a] Value of 20 for the acute-to-chronic ratio (i.e., extrapolation of acute LC_{100} to chronic NOEC); Value of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects).

[b] Value of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects).

[c] Value of 10 for the acute-to-chronic ratio (i.e., extrapolation of acute LC_{50} to chronic NOEC); Value of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects).

[d] Value of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects)

[e] Value of 10 for the acute-to-chronic ratio (i.e., Extrapolation of acute LC_{50} to chronic NOEC); Value of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects).

[f] Value of 10 for the acute-to-chronic ratio (i.e., Extrapolation of acute LC_{50} to chronic NOEC); Value of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects).

Table 17. Available freshwater toxicity data, Genus Mean Acute Value (GMAV) data, and selection criteria used in the calculation of a proposed discharge limitation for hydrogen peroxide use in aquaculture (sources of data and other details of toxic endpoints, etc. are given in Tables 5, 7, and 8). GMAV ranks 1-4 are bolded.

ROI [GENUS COUNT]	ENDPOINT (VALUE, mg/L)	GMAV (RANK)	MEETS SELECTION CRITERIA?
Daphnia pulex	48-h LC_{50} (2.4)	-	YES
Daphnia magna	48-h EC_{50} (18)	-	No, suspect value, deviant from other values
	24-h EC_{50} (7.7)	-	YES, no 48-h EC_{50} available
	24-h EC_{50} (2.3)	-	YES, no 48-h EC_{50} available
Daphnia [1]		3.2 [a] **(1)**	
Ceriodaphnia dubia	48-h LC_{50}s, NOECs (various)	-	No, tests not done in lab water
Crayfish (crustacean) [2]	96-h LC_0 (64.6)	64.6 (8)	YES, a LC_0 is more conservative than a LC_{50}
Gammarus spp. (scuds, crustacean) [3]	96-h LC_{50} (4.42)	4.42 **(2)**	YES
Dreissena polymorpha (zebra mussel, a bivalve mollusc) [4]	70-h LT_{50} (6)	6 **(3)**	YES
Physa spp. (snail, a mollusk) [5]	96-h LC_{50} (17.7)	17.7 **(4)**	YES
Pchydiplax spp.(dragon fly nymph)	96-h LC_0 (170)	-	No, sufficient data available for other species
Stratiomys spp. (fly larvae)	96-h LC_0 (218)	-	No, sufficient data available for other species
Chironomid larvae (midge) [6]	72-h LC_{50} (125)	-	No, sufficient data available for other species
Rainbow trout [7]	24-h LC_{50} (48)	48 (7)	YES, no 48/96-h LCs for salmonids
Bluegill sunfish fingerling [8]	24-h LC_{50} (71.5)	-	No, sufficient data available for other species
Channel catfish fingerling [9]	96-h LC_{50} / 24-h LC_{50} (37.4 / 55.5)	37.4, 55.5 (5)	YES, use 96-h LC_{50}
Common carp [9]	48-h LD_{50} (42)	42 (6)	YES, no 48/96-h LCs for carp
Western mosquito-fish	48-h NOEC (9.9)	-	No, sufficient data available for other species
Guppy	5-d NOEC (34)	-	No, sufficient data available for other species

[a] Geometric mean of 4.21 (= species geometric mean acute value of 7.7 mg/L and 2.3 mg/L) for *Daphnia magna* and **2.4** for *Daphnia pulex*.

Table 18. Calculation of the freshwater Final Acute Value (FAV) for hydrogen peroxide (GMAV data from Table 17, calculations from Stephan et al. [1985] and Erickson and Stephen [1988]).

Rank	GMAV (mg/L)	lnGMAV	$(\ln GMAV)^2$	P = R/ (N+1)	$\sqrt{P}$
4	17.7	2.8736	8.2576	0.44444	0.66667
3	6	1.7918	3.2105	0.33333	0.57735
2	4.42	1.4861	2.2085	0.22222	0.47140
1	3.2	1.1632	1.3530	0.11111	0.33333
	Sum	**7.3147**	**15.0296**	**1.11110**	**2.04875**

$$S^2 = \frac{\sum((\ln GMAV)^2 - ((\sum(\ln GMAV))^2 / 4)}{\sum(P) - ((\sum(\sqrt{P}))^2 / 4)}$$

$$S^2 = \frac{15.0296 - (7.3147)^2 / 4}{1.11110 - (2.04875)2 / 4} = 26.7714$$

$$S = 5.1741$$

$$L = (\sum(\ln GMAV) - S(\sum(\sqrt{P}))) / 4$$

$$L = (7.3147 - 5.1741 \times 2.0485) / 4 = -0.8211$$

$$A = S(\sqrt{0.05}) + L$$

$$A = 5.1741 \times (\sqrt{0.05}) + -0.8211 = 0.3358$$

$$\text{Final Acute Value (FAV)} = e^A = e^{0.3358} = 1.3991$$

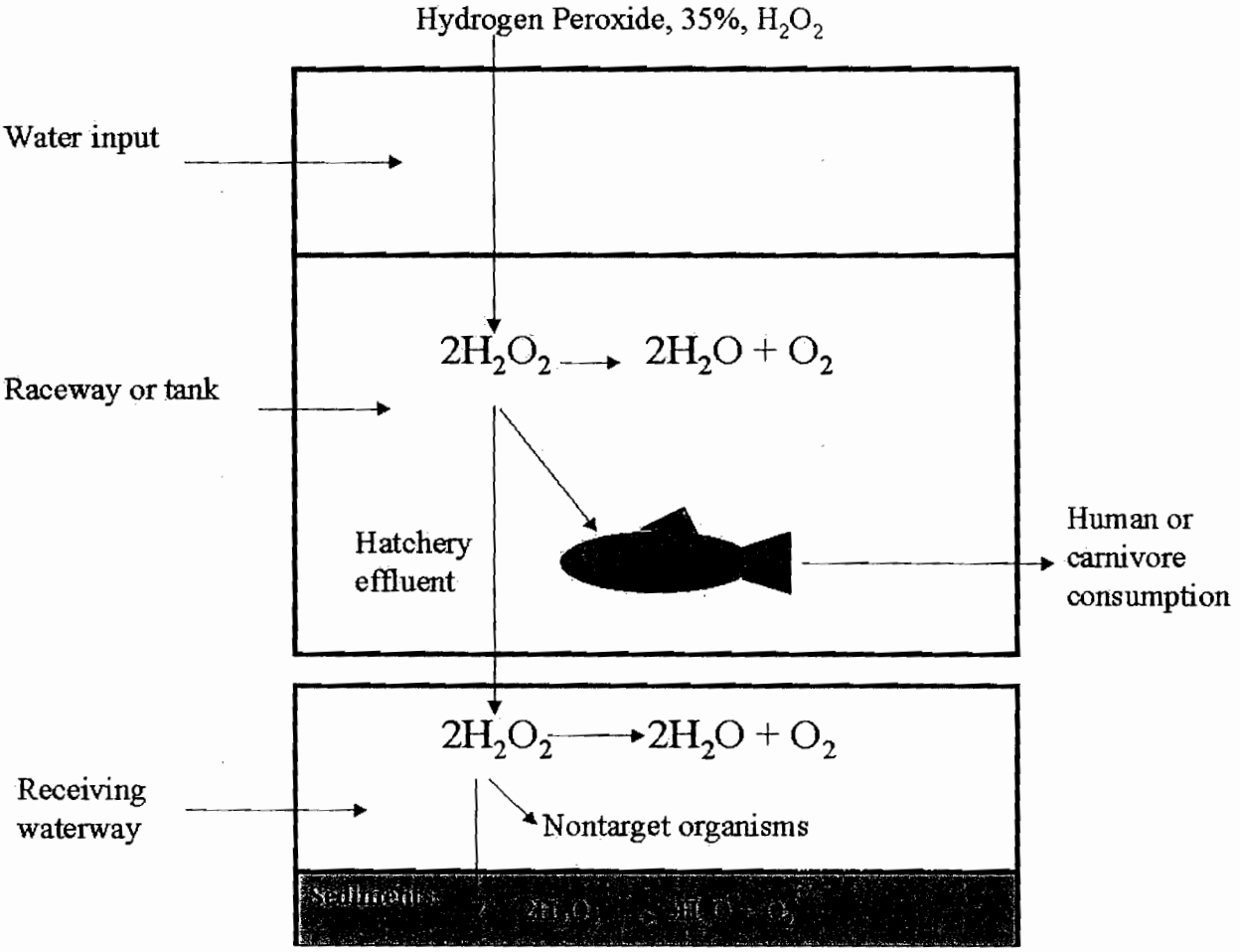

Figure 1. Conceptual model for the fate of hydrogen peroxide used in a typical fish culture situation. Hydrogen peroxide used at a typical aquaculture facility would be added to water flowing into a culture unit where freshwater fish or eggs are present. The culture water containing hydrogen peroxide would then flow into either a fresh or brackish-water body where it would degrade into water and oxygen either in the water column or after interaction with sediments. Hydrogen peroxide may affect nontarget organisms present in the water column or sediments before it is degraded.

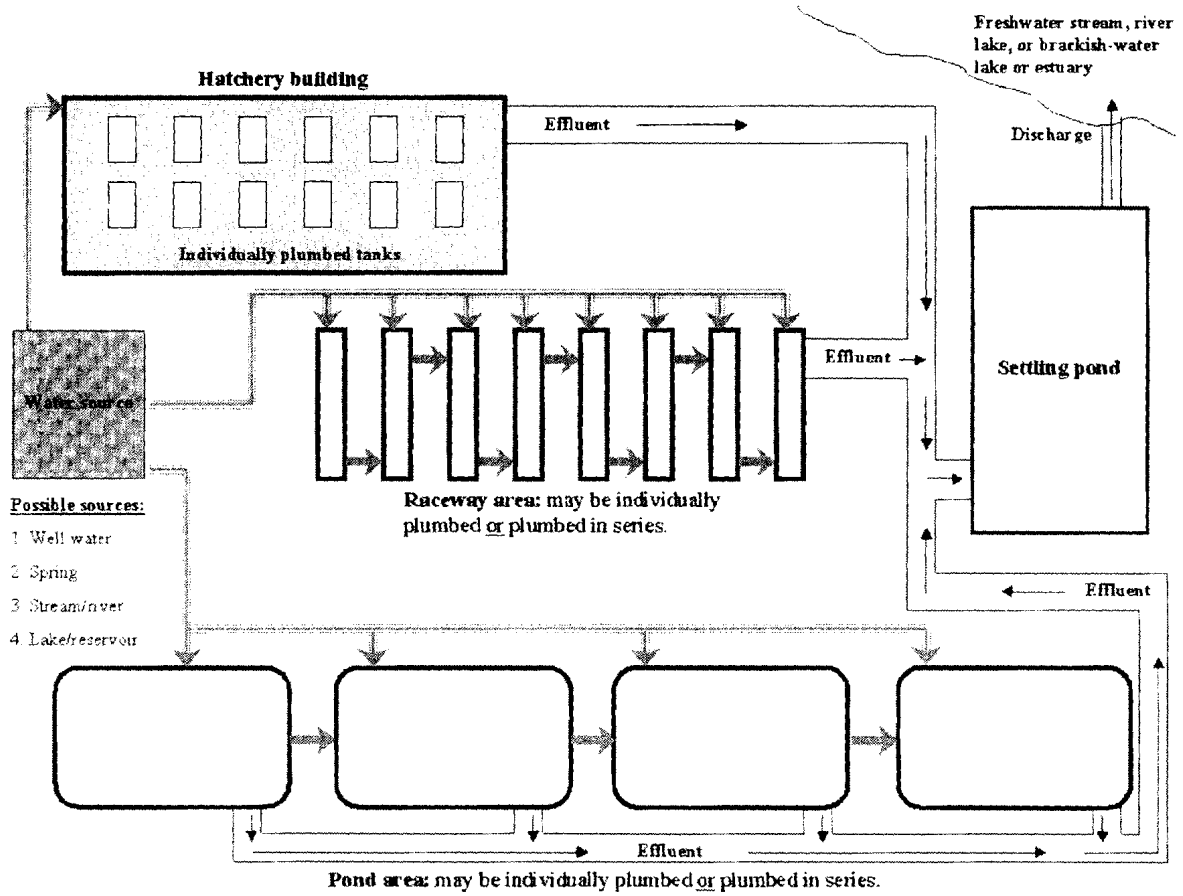

Figure 2.C onceptual design of a typical freshwater hatchery facility.

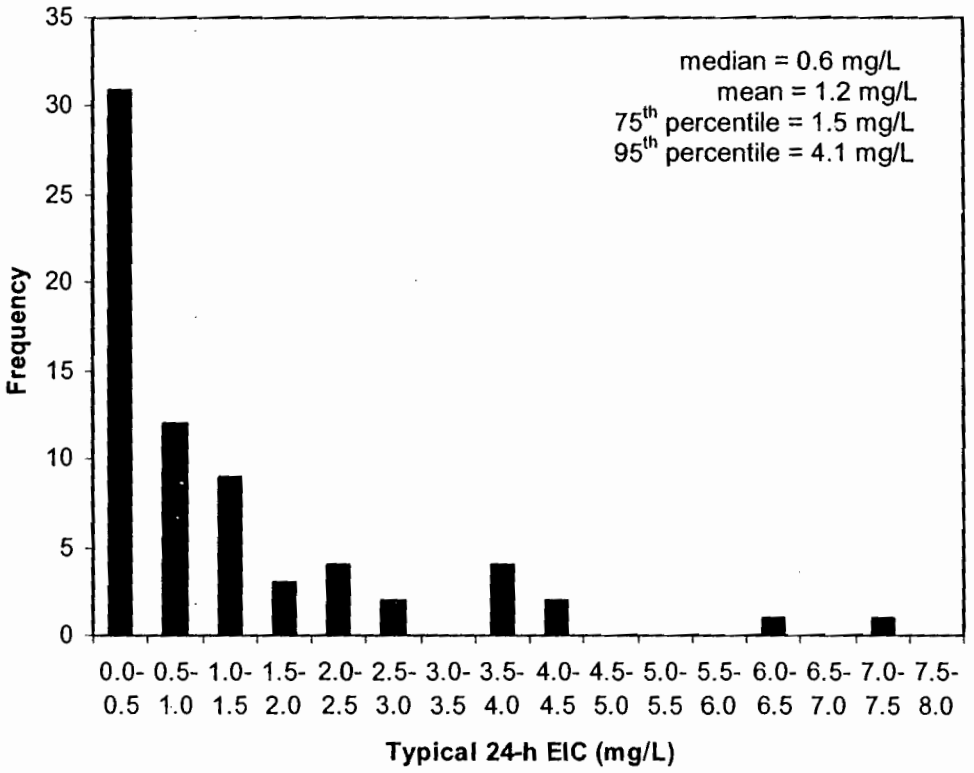

Figure 3. Frequency of typical 24-h EICs calculated based on the hatchery survey response of present or expected use of hydrogen peroxide on fish or fish eggs.