

Environmental Assessment of the Effects of Chloramine-T Use in and Discharge by Freshwater Aquaculture

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Submitted to
U.S. Food and Drug Administration
Center for Veterinary Medicine
Director, Division of Therapeutic Drugs for Food Animals
Office of New Animal Drug Evaluation
7500 Standish Place
Rockville, Maryland 20855

April 2007

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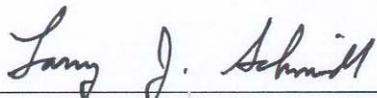
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1.0 Certification

We, the undersigned, certify that to the best of our knowledge the information and data presented in this EA concerning the use of chloramine-T in intensive aquaculture are accurate and reliable.



Signature



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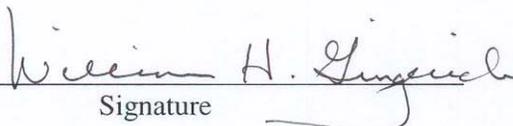


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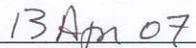


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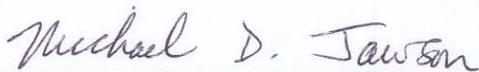


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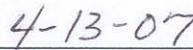


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2.0 Proposed Action and Product Label Claim

Approval is presently being sought for the use of chloramine-T as a waterborne therapeutant in aquaculture for the control of mortality resulting from external flavobacterial infections of cultured fish. More specifically, the proposed label claim would allow the therapeutic addition of chloramine-T to culture water to control mortalities associated with bacterial gill disease and external flavobacterial infections in all cultured freshwater fish. For each therapy, treatments may be administered in water as either a static or flowing immersion bath for as long as 60 min at concentrations up to 20 mg/L on consecutive or alternate days, for as many as four times.

3.0 Substance Identification for Subject of Proposed Action

Tables 1–4 present the identification and physicochemical properties of the substance of the proposed action, and the identification and physicochemical properties of its major metabolite. Its structure as well as that of its primary degradation product, para-toluenesulfonamide (p-TSA), are presented in Figure 1.

4.0 Introduction

4.1 Present Aquaculture Use - A compassionate INAD application use was in place from 1994 to 2001 to allow intensive aquaculture facilities to collect efficacy data that supports the potential label claim for chloramine-T. In the hobby fish culture industry, chloramine-T has been considered for use as a flukicide for goldfish (*Carassius auratus*) and koi (*Cyprinus carpio*, Spangenberg n.d., available online at http://www.koivet.com/html/articles/articles_details.php?article_id=71, accessed on December 2005).

The projected annual use of chloramine-T for intensive aquaculture is far less than the present aqueous chlorine use for municipal and industrial water treatment. In 2006, about 8,900 lbs of chloramine-T were used by 47 state, private, and tribal hatcheries under a compassionate INAD coordinated by the U.S. Fish and Wildlife Service (FWS) at the Bozeman National INAD Office, an average of about 189 lbs per hatchery. There are about 1,670 aquaculture facilities within the United States (70 FWS, ~400 state, and ~1,200 private intensive aquaculture facilities) that have the potential to use chloramine-T (U.S. National Agricultural Statistics Service 2000). If the percentage of hatcheries that treat or planned to treat with chloramine-T was similar to that found in a USGS survey (~60%), then an estimate of potential chloramine-T use at hatcheries would be approximately 190,000 lbs per annum (189 lbs/hatchery × 1,670 hatcheries × 0.6). This amount of chloramine-T would be equivalent to about 48,000 lbs or 24 short tons of chlorine¹. By comparison, total United States annual chlorine use is about 11,000,000 short tons, of which about 1,700,000 short tons are used for water treatment by municipalities and industry (Table 1.15 in White 1999, pages 95–96). Discharge of chlorine-treated waters is generally continuous at wastewater treatment plants and is done daily for 2 to 3 h per day at electrical power plants (Capuzzo et al. 1977). Unlike the continuous discharge of aqueous chlorine by municipal or industrial facilities, discharges of aquaculture effluent containing chloramine-T are intermittent, averaging only about 40 daily discharges per year per aquaculture facility that uses the chemical (Section 7.0).

¹ There are about 520 hatcheries in the United States that have EPA discharge permits. These are mostly high-production facilities (annual production in excess of 100,000 lbs) that discharge high volumes of water, similar to the facilities reporting chloramine-T use to the Bozeman National INAD Office. By contrast, many of the ~1,200 private intensive aquaculture facilities are small, sometimes even being operated only for part-time family income. Thus, if anything, our calculation of 190,000 lbs of chloramine-T use per annum likely over-represents the actual future use of chloramine-T in aquaculture.

4.2 Need for Action - The number of effective therapeutic agents available to control external flavobacterial diseases is limited. Formalin is a waterborne therapeutic approved for aquaculture use, but it is not effective for bacterial infections. Hydrogen peroxide is presently the only approved alternative to chloramine-T for intensive aquaculture use. The use of hydrogen peroxide to control mortality associated with bacterial gill disease in salmonids was approved by the U.S. Food and Drug Administration Center for Veterinary Medicine in January 2007. Hydrogen peroxide effectively controls mortality associated with bacterial gill disease in cold-water fish and columnaris in cool-water and some warm-water fish, but its therapeutic index—the difference between a therapeutic concentration and a toxic concentration—is much less than that of chloramine-T, especially for warm-water fish.

4.3 Other Uses - Chloramine-T has been safely used in a water-based solution throughout most of the 20th century in Europe as an antiseptic and is still used for that purpose today (http://www.osha.gov/dts/chemicalsampling/data/CH_226190.html, accessed December 2005). Perhaps the most common use in Europe is as a surface disinfectant or sanitation agent in the food, beverage, poultry, and dairy products industries. Other surface disinfectant uses are in hospitals, nursing homes, and cafeterias. It is also used as a small-scale bleaching agent in preservation work and as an analytical reagent in titrations. It is sometimes used for disinfecting small water volumes, such as whirlpools and swimming pools, but is too expensive relative to alternative treatments for large volume use, such as at wastewater treatment plants and electric power plants. A detailed account of the various uses of this chemical is given in Haneke (2002).

5.0 Intensive Aquaculture Model

The present proposed label claim for chloramine-T is to control mortality associated with bacterial gill disease and external flavobacterial infections on freshwater fish by administering a 60-min static or flow-through bath treatment once daily on consecutive or alternate days for up to four treatments. When querying hatcheries about potential chloramine-T use, we requested their treatment data to control mortality associated with either bacterial gill disease or other external flavobacterial diseases. Therefore, the data presented in this EA support use for both the original claim for bacterial gill disease and an expanded claim for other external flavobacterial infections.

The data developed on human food safety and target animal safety technical sections are for a 60-min treatment and will not support chloramine-T use in extensive aquaculture operations that are typified by fish culture in large earthen ponds with little if any water exchange capacity. Treatments conducted in most earthen ponds would not be able to meet the 60-min exposure requirement and are also likely to be cost-prohibitive. Therefore, our focus will be on the intensive aquaculture operations of private, tribal, and public (federal, state, or university) hatcheries.

Intensive aquaculture typically involves the production of various game, commercial, or threatened species of fish at relatively high densities in relatively small volumes of fresh water. Intensive aquaculture operations are frequently divided into two major categories on the basis of culture water temperature. The culture of salmonids (trout or salmon) is commonly referred to as cold-water aquaculture because water temperatures are generally maintained at $\leq 15^{\circ}\text{C}$, whereas warm-water aquaculture facilities typically maintain temperatures of $> 15^{\circ}\text{C}$ to achieve optimal growth. Families commonly cultured in warm-water aquaculture facilities include the Percidae, Esocidae, Centrarchidae, Cyprinidae, Percichthyidae, and Ictaluridae. In both cold- and warm-water aquaculture, fish are generally offered a commercially formulated semi-moist or dry diet, although some live forage may be provided at various culture stages.

Diets are generally between 30% and 50% protein with fish meal and oil and supplemented to varying degrees with alfalfa or grain meal.

Intensive aquaculture is typified by relatively high water use, although some facilities do reuse or recirculate culture water. In a single-pass culture unit, fresh water typically enters at one point of the culture unit (usually the upper or head end of a raceway or the side of a circular or square culture tank) and exits out a drain (usually at the lower tail end of a raceway or the center of a circular or square tank) that is eventually connected to the facility's water discharge system. Water reuse is generally accomplished using a series of raceways. Fresh water is provided to the head of the first raceway of a series and then is gravity fed to the next raceway in the series. The water is frequently passed through an aeration device before it enters the next raceway in the series to increase oxygen content. Water recirculation generally requires the least amount of fresh water per unit mass of fish, but is generally more technologically demanding than either single-pass or water reuse systems. Aquaculture systems using water recirculation generally have a clarification or a filtration unit to remove solids and frequently use biofilters to convert ammonia to nitrate. When therapeutic treatments are administered at a facility that incorporates water reuse, it is likely that all fish cultured in the raceways downstream of the treatment will be exposed to the therapeutic, although at concentrations that are probably much less than the initial therapeutic concentration. Treatment water at facilities that have either single-pass water use or water recirculation would generally not pass from one culture unit into another culture unit, but would instead be discharged directly into the hatchery discharge system after treatment.

Whatever type of water use is selected, intensive culture systems usually have the capability to rapidly replace or exchange the water in the culture unit after treatment, either by drainage and flushing after a static bath or by simply allowing the water to travel downstream after a flow-through treatment. Moderately hard spring or artesian water is often cited as a preferred water source for fish culture (Piper et al. 1982); however, the water sources used at intensive aquaculture facilities are as varied as the facilities themselves. Many facilities make use of surface water rather than groundwater to reduce costs associated with pumping. Dissolved oxygen is generally maintained at > 5 mg/L (Piper et al. 1982) to promote growth and reduce stress. Most facilities attempt to maintain a relatively constant temperature, although temperature is generally closely tied to the temperature of the available water supply. Fish culturists try to avoid using soft water at low pH as culture water, because of poor fish growth under these conditions. The physico-chemical properties of the water used in intensive aquaculture are extremely variable; recommended properties for several important constituents are provided in Table 5.

A wide variety of culture units are used at intensive aquaculture facilities. Culture tanks are commonly manufactured from fiberglass, plastic, aluminum, or concrete, whereas raceways and ponds are generally constructed from cement, although some older facilities continue to use earthen raceways. Culture units are frequently characterized by a small surface area to volume ratio; however, certain species such as Atlantic salmon (*Salmo salar*) and the esocids prefer a larger surface area (Piper et al. 1982) and, thus, may be cultured in appropriately sized tanks. Little if any leakage or seepage occurs from most intensive culture systems, except possibly those situations where earthen raceways are used. The potential impact of treatment water from earthen raceways entering raceway sediments or associated groundwater is subsequently discussed within this EA (Section 6.0).

Intensively cultured fish are stocked into public or private waters, used for on-site recreational fee fishing, or used for human consumption through commercial outlets. A conceptual site model for the fate of chloramine-T used at an intensive aquaculture facility is represented in Figure 2. For a typical treatment, the model involves the simple addition of chloramine-T to the water column of the tanks or raceways to be treated and adequate mixing to ensure uniform distribution throughout the treated water

body. Chloramine-T then begins to react with oxidizable materials, including a variety of amines, amino acids, humic substances, and other organic and inorganic material in the treated water body and in subsequent dilution waters. The degradation process is usually completed in receiving waters at some time well after discharge. Treatment water is typically discharged from treatment tanks or raceways and combined with other hatchery water for eventual release. Many hatcheries use holding or settling ponds to dilute, detain, or stabilize discharge water before it is released into the environment. Although there is the potential for treatment water containing chloramine-T to be discharged into brackish-water ecosystems, little useful toxicity data are available for brackish water. Therefore, we do not discuss the potential of chloramine-T to negatively affect organisms residing in brackish-water ecosystems. Discharges to public water are subject to regulation and monitoring by state or local regulatory agencies. The facility design or layout for a typical intensive aquaculture hatchery is presented in Figure 3.

6.0 Analysis of Environmental Fate and Effects

Chloramine-T is an organic *N*-chloramine. Chloramine-T is an exception to the organic chloramines because of its considerable value as a disinfectant and sanitizer. Organic chloramines in general are thought to be considerably less toxic to aquatic life than the inorganic chloramines monochloramine (NH_2Cl), dichloramine (NHCl_2), and trichloramine (NCl_3). Inorganic chloramines usually exist as monochloramine in aqueous solutions.

Both aqueous free chlorine ($\text{HOCl} + \text{OCl}^-$) and the inorganic chloramines are extremely toxic to fish and other aquatic life, to the point where concentrations of $< 10 \mu\text{g/L}$ (total of free chlorine plus inorganic chloramines) are potentially of concern (Kalmaz and Kalmaz 1981). Because of this, EPA established the criteria of $11 \mu\text{g/L}$ total residual chlorine (TRC as Cl_2) as the upper concentration limit in fresh receiving waters for chronic exposure (4 d average) and $19 \mu\text{g/L}$ TRC in fresh receiving waters for acute (1 h average) exposures (EPA 1985, 1999).

Intensive aquaculture facilities discharge into streams, rivers, and lakes. Both before and after discharge, chloramine-T can remain unchanged, release its chlorine as aqueous free chlorine, or donate its chlorine directly to produce ammonia chloramines or other chlorinated organic-N or non-N compounds. Figure 4 displays the types of covalently bonded chlorinated compounds that might be produced from chloramine-T either by direct chlorination or hydrolysis, assuming that it acts like a typical chlorine-donating organic chloramine. Chloramine-T can also react with inorganic chlorine demand to produce chlorides (Mihelcic and Hand 1999, available online at <http://www.civil.mtu.edu/~jm41/book/water.pdf>, accessed October 2005). Since chlorine demand is by far greatest in waters containing high ammonia- or organic-N, we conclude that relatively small amounts of highly oxidizable inorganic compounds (other than ammonia) exist in most waters.

Potential biological impacts exist if hatchery effluent containing either chloramine-T or any of its chlorine-exchange products are released into or produced in receiving waters after discharge. Because chloramine-T is a mild chlorinating agent, a given amount of organic and inorganic matter will always be chlorinated. As chloramine-T degrades, it also produces p-TSA, the dechlorinated remainder of the chloramine-T molecule, as its primary degradation product. The p-TSA molecule is relatively stable; therefore, the toxicity of p-TSA must also be described.

Fish are known to avoid compounds containing available chlorine (Zillich 1972). The avoidance threshold varies with fish species and also with the compound involved, with HOCl being the compound avoided at the lowest concentrations (Cherry et al. 1979). Although avoidance studies using chloramine-T have not been done, there were no anecdotal observations of fish behavior being different in its presence

during efficacy and target animal safety studies performed at UMESC (Terry Bills, Jeffrey Meinertz, Mark Gaikowski, Research Scientists, USGS, personal communications). If fish or motile invertebrates do avoid chloramine-T, then the potential toxicity of chloramine-T or its chlorine-exchange degradates may be reduced if the organism can reduce its exposure duration, especially in intermittent discharge situations.

If chloramine-T is used in an earthen raceway or when treated effluent enters an earthen pond (e.g., an unlined-detention pond), some potential exists for it or its residues to infiltrate the pore water of the bottom sediments and possibly enter the groundwater. However, it is unlikely that the presence of dilute chloramine-T 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 or synthetic liners impervious to water are commonly used for this purpose. Depending on the concentration of chloramine-T present, an effect on organisms in the bottom sediments of earthen ponds or waters receiving aquaculture effluent could possibly be realized. The potential for long-term substantial environmental impacts in groundwater or sediments after chloramine-T treatment is 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 chloramine-T contamination of groundwater or conducted a risk characterization for any organisms in sediment or groundwater.

At all application and discharge concentrations and temperatures, chloramine-T primarily has the ability to monochlorinate and to produce relatively water-soluble chlorinated compounds that may or may not have the ability to further lose their chlorine. Dichlorination will be much less frequent and polychlorination almost nonexistent except for the theoretically possible production of ultratrace concentrations of trihalomethanes. Production of electrophilic highly halogenated compounds (often suspected carcinogens) generally occurs only under conditions of relatively high temperature, pressure, and halogen or halogenating compound concentrations, conditions that usually involve the deliberate manufacture of these compounds for industrial and agricultural uses. The presence of these industrial chemicals is inconsistent with any aquaculture operations.

6.1 Fate/Effect for Intensive Aquaculture - Intensive aquaculture effluent containing trace concentrations of chloramine-T may be released into streams, rivers, or lakes. At concentrations used in aquaculture, chloramine-T can be relatively stable (half remaining in a week) under some simulated aquatic environmental conditions (fish plus softened well water; Bills et al. 1988b). Under laboratory conditions, a 0.5% aqueous solution of chloramine-T held at 40°C in a non-transparent polyethylene container was stable (99+%) for 4 weeks (Axcentive SARL, available online at <http://www.halamid.com/soluti.htm>, accessed January 2006). Chloramine-T, however, is not a refractive, bioaccumulating compound, and depending on chlorine demand, trace levels should be completely degraded in eutrophic surface waters over a period of hours, days, or weeks (details to follow in remainder of Section 6 and associated appendices).

Sections 6.1.1 and 6.1.2 discuss biodegradation of chloramine-T and its absorption to soil and sludge. Sections 6.1.3 - 6.1.5 discuss the fate of chloramine-T in relation to certain potential breakdown products that are known to be more toxic than chloramine-T. There is some discussion of effects of these substances, but our primary argument is that chloramine-T does not produce them at concentrations of concern either in hatchery waters or after discharge into public surface waters. In Sections 6.1.3 – 6.1.5, we demonstrate that degradation of chloramine-T during and after its use in aquaculture (1) does not produce aqueous free chlorine at concentrations of concern for aquatic toxicity; (2) does not produce inorganic (ammonia) chloramine in receiving waters at concentrations of concern for aquatic toxicity in

the presence of total ammonia ($\text{NH}_4^+ + \text{NH}_3$) at any environmentally possible concentration, nor does it produce inorganic chloramine in treatment water at concentrations that would be of concern at discharge; and (3) is not known to produce potentially mutagenic electrophilic organochlorines, such as the trihalomethanes.

6.1.1 Degradation - A study conducted in 1981 indicated that biodegradability of Halamid[®] (99.1% chloramine-T, see van Helvoirt 1996; Appendix H) and p-TSA by the repetitive die away (RDA) method was 80-90% per week at 25 mg/L initial Halamid[®] concentration (Blok 1981; Appendix H). The RDA test involved use of an active sludge inoculum at slightly higher concentrations than Halamid[®], since high relative concentrations of chloramine-T would have destroyed the inoculum. This study also indicated that half-lives for chloramine-T in algal growth tests exposed to light were 1-2 days. A 1998 study indicated that half-lives for chloramine-T (as Halamid[®], going by hydrolyzation to p-TSA) in 3 types of soil are much less than 1 day (van de Leur-Muttzall and Hanstveit 1998a; Appendix H). In sandy loam soil, the evolved carbon dioxide amounted to about 48% of the initial radioactivity at the end of the test (100 days). Chloramine-T was at least 90% mineralized or converted into microbial biomass within 100 days in sandy loam soil, more than 95% in low humic content sand soil, and about 60% in humic sand soil. Blok (1982; Appendix H) noted that anaerobic degradation of Halamid[®] in sludge was very slow (stable for 40 days), which is typical for aromatic compounds. The good solubility of chloramine-T in water, its octanol-water partition coefficient, its low adsorption to soil and sludge (see Section 6.1.2) and ready biodegradability indicate a low bioaccumulation potential.

6.1.2 Adsorption to Soil - van de Leur-Muttzall and Hanstveit (1998b; Appendix H) reported Halamid[®] adsorption to 3 types of soils. The distribution coefficients were 0.68 mL/g for sandy loam, 1.04 mL/g for loam, and 0.43 mL/g for low humic content sandy soil. The $K_{\text{organic matter}}$ adsorption coefficients were 31, 52, and 43 mL/g for the three respective soils, indicating only very slight adsorption to soil. Blok (1981; Appendix H) also showed no significant adsorption to one type of synthetic soil and one type of activated sludge (< 500 mg/kg of organic matter). It appears that chloramine-T biodegrades rapidly under aerobic conditions. It degrades rapidly in soils, and also adsorbs poorly to soils. It thus appears that sediment is not an important environmental compartment for chloramine-T.

6.1.3 Potential of Chloramine-T to Produce Residual Free Chlorine at Concentrations of Concern - Chloramine-T is a slow-release chlorinating agent. The hydrolysis mechanism involves the production of aqueous free-chlorine ($\text{HOCl} + \text{OCl}^-$) species that are quite toxic to aquatic life (Mattice and Tsai 1983; EPA 1985). The kinetics of chloramine hydrolysis are slow and rate-limiting compared to those where free chlorine oxidizes another organic amine or some other organic-N or non-N compound. Usually the reaction produces a compound much less toxic than free chlorine (Isaac and Morris 1983b; Mattice and Tsai 1983). Under many circumstances, chloramines also lose chlorine through a direct chlorination mechanism (i.e., no free-chlorine species is involved as an intermediate; Yoon and Jensen 1993). While the basics of chloramine chemistry are quite complex and also influenced by commonly encountered environmental conditions, no stable free-chlorine species will result until residual free chlorine is produced by sufficient addition of a chlorinating species (e.g., hypochlorite ion or a reactive organic chloramine) to water.

Breakpoint, which is different for every chlorinating compound and is also contact-time dependent, is the applied chlorinating-compound concentration at which residual chlorine species are produced. We demonstrate in Appendix C that chloramine-T will not produce free chlorine at concentrations of concern at our proposed treatment or discharge concentrations. This is partially because the amount of available chlorine present at aquaculture-use concentrations of chloramine-T (~5 mg Cl_2/L) is barely high enough to be theoretically capable of producing residual chlorine even in low chlorine

demand waters, but mostly because chloramine-T is not reactive enough to reach breakpoint within the 60-min treatment period where it is at its maximum concentration (Appendix C). By contrast, an equivalent amount of free chlorine as either sodium hypochlorite or hypochlorous acid would be much more likely to meet all of the fast-acting chlorine demand², reach breakpoint, and produce a free-chlorine residual within 60 min.

Work done by Gottardi (1992) supports our assessment that chloramine-T use in intensive aquaculture will not produce aqueous free chlorine at concentrations of concern. He found only 0.015 to 0.030 mg/L of free chlorine in an aqueous solution of chloramine-T at 1,000 mg/L at pH values that typify public waters (pH 6–8). This chloramine-T concentration (about 250 mg/L as Cl₂) would be well above breakpoint if the kinetics of chloramine-T chlorination reactions were fast. The proposed treatment (about 5 mg/L maximum as Cl₂) at hatcheries is far below Gottardi's (1992) 250-mg/L experimental concentration, which produced free chlorine at concentrations within or close to the discharge range allowed by the national EPA criteria (EPA 1985). We, therefore, feel that chloramine-T will not produce free chlorine at concentrations of concern for aquatic toxicity at either the proposed treatment concentration or the maximum environmental introduction concentration (EIC).

6.1.4 Potential of Chloramine-T to Produce Inorganic Chloramines at Concentrations of Concern - Since the inorganic chloramines are much more toxic to aquatic life than chloramine-T, it is important to understand the extent to which chloramine-T at hatchery treatment and discharge concentrations will exchange into inorganic (ammonia) chloramine in hatchery and public waters. In the presence of ammonia, chloramine-T has the potential to exchange into inorganic chloramines (mostly monochloramine) over long periods (weeks) according to the appropriate equilibrium ratios (Yoon and Jensen 1993). However, reaction rates are the most important factor in determining exchange ratios over short periods (hours, days). A detailed discussion of the potential of chloramine-T to produce inorganic chloramines is presented in Appendix D.

On the basis of the expected total ammonia and chloramine-T concentrations, it is unlikely that inorganic chloramine will be produced in amounts of concern during chloramine-T use in intensive aquaculture or that it will be produced in receiving waters after discharge. The possibility of inorganic chloramine production at concentrations of concern in treatment waters exists only in the unlikely event that high concentrations of total ammonia-N are present during treatment. However, the almost certain presence of some organic-N in treatment and other hatchery waters reduces the likelihood that substantial inorganic chloramine will survive to the time of discharge from the hatchery (Appendix D). Any production of inorganic chloramine during the 1-h treatment period will be subject to the same dilution before discharge that would occur for chloramine-T itself. Inorganic chloramine production in earthen raceway sediments is not expected to occur because of the relatively short contact time (~60 min) and the likely presence of organic-N at the water column-sediment interface. In addition, the relatively impermeable bottoms of most raceways will limit exchange between the water column and groundwater near the raceway. Production of inorganic chloramine in the sediment of receiving waters is also not likely because of the low concentrations of chloramine-T discharged relative to the probable chlorine demand within the water column.

² Note that demand could also be seen as demand for chloramine-T, and that the slow reactivity of chloramine-T relative to free chlorine is a factor in this demand. We chose to talk about chlorine demand (demand for free chlorine or equally fast-acting chlorinating substances) and about the ability of chloramine-T to meet this demand only slowly because of its slow reactivity. This is a more complicated way of saying the same thing, but the literature on inorganic chloramines seems to follow this use of language, so we did also.

6.1.5 Potential of Chloramine-T to Produce Mutagenic Electrophilic Organochlorines - In the 1970s, it was determined that chlorination of public waters in the presence of humic substances resulted in the production of hydrophobic and electrophilic mutagens or carcinogens, such as the trihalomethanes (Bellar et al. 1974; Rook 1977; Amy et al. 1984). In general, direct-acting mutagens and carcinogens are electrophilic (Cheh et al. 1983), and, thus, electrophilic organohalogens—as a category—are of special interest to mammalian and human toxicologists. Similar low molecular weight, but less hydrophobic (and probably less long-lived) organohalogens were discovered in chlorinated waters in the early to mid-1980s, and some were found to be mutagenic.

Preformed inorganic chloramine is thought to produce little organohalogen (Amy et al. 1990), suggesting that the active ingredient in forming potentially carcinogenic organochlorines is primarily the free-chlorine species. At worst, it seems that chloramine-T produces no more N or non-N electrophilic organohalogens than preformed inorganic chloramine and probably much less because of reactivity, steric, and transport considerations. Thus, chloramine-T can probably be best modeled by preformed inorganic chloramine as a worst-case surrogate to assess the risk of electrophilic organohalogen production. A complete discussion of this issue is presented in Appendix E.

At present, we cannot say that no such compounds will ever be produced by chloramine-T at concentrations of concern. However, the possibility of generating any of the presently known carcinogenic compounds from chloramine-T use in intensive aquaculture is remote.

6.2 Fate/Effect for Intensive Aquaculture - This section primarily discusses the effects of certain products that are known to be produced in significant quantities by the breakdown of chloramine-T. There is some discussion of fate of these substances, but the primary argument is that their toxicities are less than that of chloramine-T itself and, therefore, can be modeled by the toxicity of chloramine-T. They include (1) the many possible organic chloramine and other hydrophilic chlorinated organic compounds that could be produced at low individual concentrations from chloramine-T because of the presence of the many nitrogenous and non-nitrogenous organic compounds in natural waters; and (2) the relatively stable nonchlorinated breakdown product of chloramine-T (p-TSA).

6.2.1 Potential of Chloramine-T to Form Organic Chloramine, Chloramino, and Other Chlorinated Organic Compounds and Their Resultant Effect on Residual Toxicity - Even if ammonia was totally absent in waters (and, thus, the possibility of producing inorganic chloramines), it might be possible for chlorinated organic compounds to be produced from chloramine-T that are more toxic than chloramine-T and are at least as slow to degrade. Chloramine-T has the potential to react with hundreds of organic-N compounds, each at low concentrations, such as amines, amino acids, peptides, proteins, and acetonitriles, and also with non-N organics, such as humic and fulvic acids, fatty acids and esters, triglycerides, and acetic acids (Bean 1983; Stevens et al. 1990). Stability is reached when a compound is formed that is not likely to give up its acquired chlorine. Chemically, chloramine-T may produce organic chlorine-exchange degradates similar to those formed by free chlorine or *in situ* generated chloramine. However, the rate at which chloramine-T will produce those species will be much slower than that of either free chlorine or *in situ* generated chloramine.

If all chlorinated organic-N and non-N compounds are less toxic than chloramine-T, their toxicity could be appropriately modeled by that of chloramine-T. In Appendix F, we show that the aquatic toxicity of most chlorinated organic non-N compounds is not great and their net overall toxicity might be modeled by that of chloramine-T. The results of Bills et al. (1988a) suggest that overall toxicity of the typical organic-N compounds produced in waters occupied by fish is probably less than that of chloramine-T. These authors found that chloramine-T toxicity to fish significantly decreased as amounts of fish food or

fecal material were increased. Chloramine-T concentrations also significantly decreased as fish food and fecal material contact time increased. This suggests that chloramine-T is most likely exchanging into less toxic chlorinated peptides and amino acids and not into low molecular weight chlorinated amine or amino compounds, some of which are much more acutely toxic than chloramine-T.

Any individual chlorine-exchange compound would usually be at a low concentration, as chloramine-T itself would be eventually. The highly probable overall effect of chloramine-T exchange into chlorinated amine, amino, and peptide compounds would be a substantial reduction in acute aquatic toxicity. On the basis of the discussion in Appendix F, our conclusion for chloramine-T is that it will exhibit greater aquatic toxicity if it remains as chloramine-T versus exchanging into the many other organic *N*-chloramine or chloramino products that are possible. Accordingly, their toxicity can be appropriately modeled by the toxicity of chloramine-T.

6.2.2 Potential of Para-toluenesulfonamide and its Breakdown Products to be a Significant Threat to Organismal, Environmental, or Public Health - The initial breakdown product of chloramine-T in water as it loses its chlorine atom is p-TSA. It will be a component of any discharge after a chloramine-T treatment of aquaculture waters. From the available data on p-TSA (Appendix G, Table G-1), the acute and chronic toxicity seems to be considerably less than that of chloramine-T and can be conservatively modeled by the toxicity of chloramine-T. It has been shown to be the major degradation product of chloramine-T and is probably relatively stable in receiving waters, although not refractive or bioaccumulative. Details are presented in Appendix G. A review of the general toxicological literature on p-TSA is given in Haneke (2002).

6.3 Comparison of Toxicity of Chloramine-T with the Toxicity of Free Chlorine or the Inorganic Chloramines - A comparison between the toxicity of chloramine-T and the components of TRC (both expressed as Cl₂) on which the allowable limits for TRC were set by EPA is shown in Table 6. Based on the available data, the toxicity of chloramine-T is roughly 10 times less than that of TRC for fish and at least 40 times less than that for freshwater crustacean *Daphnia magna*. The TRC values are based on aqueous free chlorine plus inorganic chloramines, which have comparable toxicities. Although chloramine-T will appear as TRC in EPA's official tests, it is much less toxic than the assumed components of TRC. This is a situation also faced by the water treatment industry in general because their measured TRC in S⁺² or S⁺⁴ mitigated effluents is probably from organic chloramines. We expect that if chloramine-T or its reactive chlorine-exchange products are regarded as TRC, it will vary by local jurisdiction.

6.4 Toxicity of Chloramine-T and p-TSA to Bacteria - Since chloramine-T is used as an antiseptic and as a surface sanitizing agent, toxicity to bacteria is to be expected at some concentration level. Submitted with this EA are several studies that present data points for the toxicity of chloramine-T to various bacteria, including sewage treatment bacteria (Blok 1981; Bessems 1988, 1991, 1996; Borgmann-Strahsen 1998, 2000; Appendix H; Table 7). The EC₅₀ (concentration needed to produce a stated effect on 50% of test organisms) values for respiration inhibition of aerobic saprophytic activated sludge bacteria and nitrifying bacteria were 5 mg/L and 700 mg/L, respectively. For methogenic sewage sludge bacteria, the EC₅₀ value for inhibition of methane generation was 100 mg/L.

The toxicity of Santicizer[®] 9 (a mixture of o- and p-TSA) to sewage treatment bacteria (Cranor 1983; Appendix H) is included to represent potential toxicity of p-TSA to aquatic aerobic bacteria. The study concludes that Santicizer[®] 9 should have negligible effects on the wastewater treatment process at or below 70 mg/L. Given the results of this study, sewage treatment bacteria should be less sensitive to p-TSA than to chloramine-T (assuming the selection of aerobic saprophytic activated sludge bacteria as the

most sensitive sludge bacteria to chloramine-T). A risk assessment for sewage treatment bacteria to p-TSA will therefore not be conducted.

Most of the studies on bacterial species demonstrate effectiveness of chloramine-T against nuisance or pathological bacteria. However, members of the genus *Pseudomonas* seem to be among the most sensitive of the bacteria tested and are naturally occurring freshwater bacteria that are important in decomposition, biodegradation, and the naturally-occurring carbon and nitrogen cycles (Microbiology Video Library 2002). *Vibrio* spp. are common bacteria found in marine waters. In a standard test, chloramine-T at 10 mg/L was found to produce a 10% reduction of the O₂ uptake of *Pseudomonas putida* (Bessemers 1988; Appendix H). Chloramine-T was an effective microbicide against *Pseudomonas aeruginosa* at 300 mg/L (reduced colony forming units by 10⁵) and at 5000 mg/L against *Vibrio cholerae*.

6.5 Selection of Receptors of Interest - In general, the criteria specified in EPA guidance (EPA 1997, 1998) to determine key organisms in an aquatic food web for selection of biological receptors of interest (ROI) include two factors: (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.

Within our risk assessment, we assumed that the only chloramine-T exposure pathway of concern is that of direct contact of an organism's outer surface (integument, gills, or outer cell wall) with chloramine-T or its degradates in the water column. We did not consider chloramine-T toxicity on the basis of possible ingestion by organisms, nor do we think there are any other significant routes of exposure that would cause untoward effects (e.g., bioaccumulation). Terrestrial vegetation and wildlife were not considered for evaluation here. We find that the predominant influences of aquaculture chemicals on the surrounding ecosystem occur only through aquatic pathways where direct contact with chloramine-T occurs.

The receiving waters of most aquaculture sites are diverse and healthy ecosystems that support a variety of aquatic and terrestrial life. However, it would be unrealistic to conduct a complete risk assessment for all organisms possibly affected. Therefore, we examined effects data for four groups of ecologically important, diverse, and representative organisms or ROI. 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: (1) primary producers (algae, some bacteria), (2) primary consumers (invertebrates), and (3) secondary or tertiary consumers (fish). Effects on these ROI are presented and discussed below.

6.6 Effects on Receptors of Interest - Data available from the scientific literature on the acute effects of chloramine-T to principal ROI that are likely to reside in the receiving water at intensive aquaculture sites are presented in Table 6 and discussed in Sections 6.6.1–6.6.3 below. Effects of p-TSA, a less toxic compound, are discussed within the risk assessments for p-TSA given in Sections 8.4 and 8.5.

6.6.1 Algae - Many species of algae reside within potential receiving waters (streams, rivers, lakes) of intensive aquaculture facilities. 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 algal populations may likewise have a secondary negative effect on many other organisms higher on the food chain.

Acute - Chloramine-T was toxic to the green algae (*Scenedesmus subspicatus*) during standard acute toxicity tests. Kühn and Pattard (1990) reported the 48-h EC₅₀ (inhibition of cell multiplication / growth inhibition) for green algae to be 0.31–0.58 mg/L and the 48-h EC₁₀ to be 0.11–0.20 mg/L (Table 6). The 48-h EC₅₀ determined by Kühn and Pattard (1990) indicates that *S. subspicatus* is presently the most sensitive species for estimating potential chloramine-T risk. A 1981 study resulted in a 96-h EC₅₀ value of 4.5 mg/L (growth inhibition) for freshwater algae *Selenastrum capricornutum* (Kroon 1997; Appendix H). These results are quite divergent, and may be the result of the different species used. This study also produced a LOEC (lowest observed effect concentration) and a NOEC (no observed effect concentration) of 0.6 mg/L and 0.2 mg/L, respectively, for *S. capricornutum*.

The marine algae *Glenodinium halli* (a dinoflagellate), *Isochrysis galbana* (a microflagellate), *Skeletonema costatum* (a diatom), and *Thalassiosira pseudonana* (a diatom) were exposed to chloramine-T for 7 d and the stimulation or inhibition of cell division was compared to that of controls (Erickson and Freeman 1978). The lowest levels of chloramine-T that inhibited cell division were for *Isochrysis galbana* at 4 and 8 mg/L for 25% and 50% inhibition, respectively. These data suggest that the toxicity of chloramine-T to algae might be much less in salt water versus fresh water, but this has yet to be demonstrated.

In a report that reviewed toxicity literature on both fresh water and brackish water (EPA 1985), EPA indicated that the available data demonstrate that aquatic plants are more resistant to TRC than fish and invertebrate species. The actual data were for free chlorine in the absence or presence of ammonia (its presence would subsequently produce inorganic chloramines) and not chloramine-T or other organic chloramines. Results of their literature review indicated that freshwater exposures to TRC at concentrations of about 1.0 mg/L for periods of 1 h or less may reduce survival and inhibit physiological processes of phytoplankton. This value is much higher than corresponding TRC toxicity values for fish, but it suggests that the mode of toxic action of TRC and chloramine-T may be similar for algae.

Chronic - The data from a study on *Selenastrum capricornutum* (Kroon 1997; Appendix H) may also be used as chronic data, since an exposure duration of more than 72-h is considered to be chronic for algae (EMEA 1997), producing a 96-h EC₅₀ value of 4.5 mg/L (growth inhibition) as a chronic toxicity endpoint.

6.6.2 Invertebrates - Many different species of nektonic (waterborne) and benthic (bottom-dwelling) invertebrates reside within potential receiving waters (streams, rivers, lakes) of intensive aquaculture facilities. 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 or insectivorous fish and the juveniles of piscivorous fish. Benthic invertebrates can be an especially useful indicator of environmental quality over long periods because of their limited mobility (Pennak 1978).

Acute - Kühn et al. (1989) reported a 24-h EC₅₀ for chloramine-T of 4.8 mg/L and a 21-d NOEC of 1.3 mg/L for *Daphnia magna*. Another independent laboratory studying the acute toxicity of chloramine-T to *Daphnia magna* produced a 48-h LC₅₀ (concentration needed to produce mortality to 50% of test organisms) value of 4.5 mg/L (Blok 1981; Appendix H), closely agreeing with the 24-h EC₅₀ (immobilization) of 4.8 mg/L by Kühn et al. (1989).

The 48-h EC₅₀ for four *Ceriodaphnia dubia* studies ranged from 2.12-8.88 mg/L using four different Pennsylvania surface waters (effluent from two hatcheries and water from two receiving streams, Analytical Laboratory Services 2003).

Kroon (1995; Appendix H) reported a 72-h LC₅₀ value of 24.6 mg/L for brine shrimp *Artemia nauplii*. Brine shrimp only inhabit extremely saline waters, and toxicity values for this species should only be used as qualitative support for any risk assessment, even for brackish-water environments.

Chronic – Chronic toxicity data for invertebrates is limited. Kühn et al. (1989) reported 21-d NOEC and LOEC values of 1.3 and 2.5 mg/L, respectively for *Daphnia magna*. Putt (1993; Appendix H) reported 21-d NOEC and LOEC values of 1.1 and 3.5 mg/L, respectively for *Daphnia magna* (Table 6). These chronic toxicity values generated for chloramine-T by two independently operating laboratories are in close agreement.

6.6.3 Fish - Many species of fish may reside within potential receiving waters (streams, rivers, lakes) of intensive aquaculture facilities. 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 commercial or recreational value to man. Fish are good indicators of overall environmental health because they usually live longer than other aquatic life forms, are higher in the food chain, and therefore are susceptible to biomagnification of contaminants and prey population fluctuations.

Acute - The toxicity of chloramine-T (on the basis of several different endpoints) has been examined in a variety of fish species by several authors (Bootsma 1973; Cross and Hursey 1973; Bills et al. 1988a,b, 1993; Powell and Perry 1996; J. Bowker et al., FWS, unpublished data; M. P. Gaikowski et al., USGS, unpublished data). Of the species tested, channel catfish, rainbow trout, and striped bass were similarly sensitive when tested in soft acidic water (Bills et al. 1988b, 1993). Chloramine-T 96-h LC₅₀ values were 1.8 mg/L for channel catfish, 1.9 mg/L for rainbow trout, and 2.8 mg/L for striped bass (pH = 6.5; Table 6). The 96-h LC₅₀ values in waters of pH 7.5 for channel catfish, rainbow trout, striped bass, and fathead minnow, and in water of pH 7.7 for harlequin fish were 3.8, 2.8, 6.3, 7.3, and 60 mg/L, respectively (Table 6). The 24-h LC₅₀ for chloramine-T determined under a variety of conditions ranged from the low of 2.8 mg/L for rainbow trout to a high of 120 mg/L for harlequin fish in soft alkaline water (pH 8.0; Table 6).

Acute toxicity data generated for chloramine-T by four laboratories apparently converge. As stated in Section 6.3, the overall body of data demonstrate that chloramine-T is considerably less acutely toxic to fish than the presumed components of TRC (hypochlorous acid, hypochlorite ion, inorganic chloramines), a regulated body of substances, but much more toxic to fish than its stable degradate, p-TSA.

Chronic – A 1983 study reported a data point for the chronic toxicity of chloramine-T to fathead minnow (*Pimephales promelas*) early life stage. Data for fathead minnow indicate that the 35-d NOEC is 1.1 mg/L (Machado 1983; Appendix H). Bills et al. (1988b) presented data indicating that time-independent LC₅₀ values were statistically similar to 96-h LC₅₀ values in fish. These data suggest that 96-h LC₅₀ values may be useful in evaluating chronic toxicity of chloramine-T to fish.

6.7 Effect of pH, Temperature, Sunlight, and Hardness on Toxicity - A review of the general toxicological literature on chloramine-T is given in Haneke (2002). From the existing data, the most important physico-chemical variable controlling toxicity of chloramine-T to fish is pH (Table 6). On the basis of 96-h LC₅₀ values, chloramine-T was about 6-fold more toxic to rainbow trout and channel catfish and about 20-fold more toxic to juvenile striped bass (*Morone saxatilis*) in soft water at pH 6.5 than at pH 9.5 (Bills et al. 1988b). A similar effect for the harlequin fish (*Rasbora heteromorpha*) was noted by Cross and Hursey (1973) at pH 6.0 versus 8.0 (toxicity was 15-fold greater at the lower pH). Although

water hardness is frequently associated with pH, water hardness had little effect on chloramine-T toxicity to channel catfish, striped bass (Bills et al. 1993), or walleye (UMESC Study # CAP-99-CLT-01, M. P. Gaikowski, Study Director). Cross and Hursey (1973) noticed only slightly greater overall toxicity for harlequin fish in soft versus hard water and Bills et al. (1988b) reported about a 2-fold increase in toxicity to rainbow trout in very soft versus very hard water. The effect of pH on toxicity is demonstrated in Figure 5. The increased toxicity at lower pH's could be the result of increased rate of release of free chlorine in an acidic environment (Jean de Barbeyrac, Axcentive, personal communication). When the fish and limited invertebrate toxicity data are compared at similar pH levels (Figure 5), it appears that sensitive invertebrates (both *Daphnia* and *Ceriodaphnia*) are of similar sensitivity as sensitive fish species (catfish and rainbow trout). Chloramine-T toxicity increased with higher water temperature for channel catfish, rainbow trout, and striped bass during the initial 24 h of exposure (Figure 6). However, exposure temperature did not significantly affect toxicity at 96 h of exposure (Bills et al. 1988b, 1993).

Of the 93 hatcheries that responded to the UMESC survey that reported the pH of their culture waters, 14 (15%) reported having soft acidic waters (Appendix A). The EPA defines soft water as water having less than 75 mg/L as CaCO₃ and the pH criteria range for freshwater aquatic life is 6.5-9.0 (EPA 1976). Seven of the 14 are in the southeastern U.S. (4 in North Carolina and 3 in Georgia) and 3 are in the northeastern U.S. (2 in Pennsylvania and 1 in New York). Figure 7 shows that the regions involved are actually a relatively small 4-state region in the southeastern U.S. (the western Carolinas, northeastern Georgia, and eastern Tennessee) and a border region in the northeastern U.S. An exploded view of one region where the average pH is acidic shows that most hatcheries are actually situated in specific watersheds that are either neutral or alkaline (Figure 7). As for the percentage of rivers and streams at low pH, a separate data set, obtained from the USGS real-time water quality surveillance, produces a result similar to that of the UMESC survey (<http://waterdata.usgs.gov/nwis/current/?type=qw>, accessed December 8, 2005 for December 8, 2005). Of the 172 sites (from 28 states) reporting pH data (hardness not reported) for 149 U.S. rivers and streams on December 8, 2005, 20 (12%) reported pH values of less than 7.

Hatcheries discharging to soft acidic waters are primarily located in a specific region in the southeastern U.S. A specific region in the northeastern U.S. may also be the location of an above-average number of hatcheries discharging into soft acidic waters. Our environmental assessment assumes uniformly-distributed soft acidic receiving waters nationwide and under represent the quality of receiving waters that the majority of hatcheries discharge into.

7.0 Determining Environmental Introduction Concentrations (EICs)

Chloramine-T can only be detected as total residual chlorine by most EPA-approved methods. Therefore, intensive aquaculture use of chloramine-T may be evaluated by regulatory agencies on the basis of discharge concentrations of TRC. However, we find that the data within this EA suggest that chloramine-T toxicity is much less than that expressed as TRC-equivalent units.

Public and private aquaculture facilities were surveyed by UMESC to determine the present and projected use of chloramine-T for fish culture. A summary of the raw data collected by the USGS hatchery survey and their associated calculations are presented in Appendix A. Examples of the survey questionnaires that were sent to public and private aquaculture facilities to collect the data are provided in Appendix B. Chloramine-T EICs were estimated by using data collected from 100 public and private hatcheries representing freshwater fish culture activities in 25 states (60 hatcheries reported that they used or plan to use chloramine-T). Hatcheries that use chloramine-T could be expected to discharge less than

40 days per year according to our survey results (an average of 10 therapies per year and an average of 3.7 treatment days per therapy; Appendix A).

7.1 Water Use and Effluent Discharge - Hatchery water use was reported in the survey as average hatchery water flow (the total volume of water discharged on an average production day) and hatchery low water flow (the total volume of water discharged daily during the periods of low water use on the hatchery). Average hatchery flow reported from the 100 hatcheries ranged from a low of about 38 L/d (a facility using recirculating tanks) to a maximum of 1.88 billion L/d (a large cold-water culture facility with no water reuse). Median average hatchery flow was 12.5 million L/d and median low hatchery flow was 6.1 million L/d. Effluent from 51 of the 100 hatcheries passed through settling ponds before discharging into a river, lake, or backwater. Median settling pond volume was 3.0 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 reported effluent discharging into a river or stream, with a median typical flow of 27.4 cfs (1 cfs = 28.32 L/s) and median seasonally adjusted minimum flow of 12.0 cfs. Fourteen hatcheries discharge into lakes (median volume 4,500 acre-feet) and eight discharge into the backwaters of a river or stream (median backwater volume 55 acre-feet).

7.2 Environmental Introduction Concentration: Calculation Assumptions - The concentration of chloramine-T 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 treatments, based on a certain set of assumptions (Table 8). The typical and worst-case treatment scenarios differed only in the hatchery flow rate used to calculate the EIC. Average hatchery flow rate was used when calculating the EIC resulting from a typical treatment whereas the low hatchery flow rate was used when calculating the EIC resulting from a worst-case treatment. Environmental introduction concentration estimates are provided to predict the average discharge concentration that may be expected to occur over 1-, 5- or 21-d periods. The 1-d EIC resulting from either a typical or worst-case treatment was estimated from the following equation:

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

where C was the maximum proposed product label treatment concentration (20 mg/L), 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 raceways 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 (60 min). When we estimated the EIC for flow-through treatments, we used the treated culture unit flow rate 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 1-d EIC estimates for fish treatments assumed that a single 1-h treatment would have been administered over a 1-d period whereas the 5- or 21-d EIC estimates assumed four 1-h treatments on consecutive days over a 5- or 21-d period. The 1-d EIC calculation was modified to predict 5- or 21-d EICs for fish treatments by increasing the hatchery discharge volume (i.e., $F \times 5$ or 21 for the 5- or 21-d

EIC, respectively) and the treated volume (i.e., $V \times 4$ treatments for the 5- or 21-d EIC, respectively). The calculation would have thus produced the same results for the 21-d EIC estimates if the assumed treatments had been on alternate days. Degradation was not included in the EIC estimates presented in this EA because adequate and reliable data that represent chloramine-T degradation during hatchery discharge are not presently available.

7.3 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 EECs following chloramine-T 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 1/10th-fold dilution) to 0.99:1 (nearly a 50% dilution).

7.4 Environmental Introduction Concentration: Results and Interpretation - Two to four EIC values were developed for each of the 60 reporting hatcheries that indicated their present or planned use of chloramine-T on fish. The EICs were determined by using data unique to each 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 treatments for each time period by reporting their mean, median, and 75th and 95th percentiles (Table 9); calculations for each were completed using MS-ExcelTM. The 1-d mean typical EIC was 0.37 mg/L and the 1-d median typical EIC was 0.40 mg/L. The 1-d mean worst-case EIC was 0.42 mg/L and the 1-d median worst-case EIC was 0.40 mg/L. The 5-d EICs were essentially the same as the 1-d EICs, but the 21-d EICs were significantly lower, as expected (one third to one fifth of the 1-d EICs). There was no notable difference between typical and worst-case EICs, except in hatcheries without settling ponds. There was a notable but not dramatic difference between hatcheries with and without holding ponds for mean and median 1-d EICs, but that difference diminished for the 95th percentile EICs and for greater than 1-d EICs. The most notable difference with regard to holding ponds was the percentage of hatcheries with 1-d EICs over 0.5 mg/L; the percentage was roughly 2-fold higher for hatcheries with no ponds.

7.5 Confirmation of Environmental Introduction Concentration Estimates - There is an inherent uncertainty in the use of survey data to predict effluent concentrations at hatcheries. The simple day-to-day variation in hatchery water use provides variation in the accuracy of the estimates provided. The lack of a validated dilution model to estimate hatchery EICs could also provide an additional level of uncertainty to the EIC estimates. To improve confidence in the dilution model used to estimate hatchery chloramine-T concentrations, UMESC conducted a regulated study to compare predicted concentrations of rhodamine WT (a fluorescent dye commonly used in water flow studies) and chloramine-T in hatchery effluent after continuous flow treatments. The study was conducted within a UMESC production raceway (Gaikowski et al. 2004).

In the UMESC study, a raceway (~10 m × ~1 m × ~0.8 m, lwd) containing ~260 kg of rainbow trout (*Oncorhynchus mykiss*) was treated to maintain either 100 µg/L rhodamine WT or 20 mg/L chloramine-T for 60 min and the concentrations were determined at two locations (sample sites A and B

in Figure 1 from Gaikowski et al. 2004) in the UMESC effluent stream before discharge into UMESC's settling pond. Sample site A represented ~47% of the UMESC nonlaboratory effluent flow and is located at the approximate midpoint of the effluent system, whereas sample site B represented all of the UMESC nonlaboratory effluent flow and is located immediately upstream from the UMESC settling pond. Predicted effluent concentrations at sample site B in the UMESC study are analogous to the discharge of hatcheries without a settling pond.

Before treatment, the flow rate of every culture unit that discharged into the nonlaboratory effluent waste was measured. Effluent concentrations were predicted on the basis of the mass of chemical administered to the raceway divided by the total flow past the sample site over a 2-h period (a 2-h discharge period was used because 2 h were required to eliminate the chemical from the raceway). Rhodamine WT, a relatively nonreactive marker dye, was initially used as a volume marker to validate the dilution model in the UMESC study because chloramine-T may be degraded in the presence of organic matter. Treatments were also conducted with chloramine-T to determine how well the validated dilution model predicted chloramine-T effluent concentrations.

The predicted and observed rhodamine WT concentrations during the UMESC study are presented in Figure 8. The predicted 120-min mean rhodamine WT concentrations at sample sites A and B (14.80 and 7.35 $\mu\text{g/L}$, respectively) in the UMESC study were compared by a two-tailed t-test and were not significantly different ($|t| < 12.706$, $\alpha = 0.05$, $v = 1$; $P > 0.05$) from the observed 120-min mean concentrations (13.36 and 6.81 $\mu\text{g/L}$, respectively). Similarly, the mean observed 120-min average chloramine-T concentrations at sample sites A and B (2.68 and 1.23 mg/L, respectively) were not significantly different ($|t| < 3.182$, $\alpha = 0.05$, $v = 3$; $P > 0.05$) from the mean predicted 120-min average concentrations at sample sites A and B (2.77 and 1.31 mg/L, respectively). The predicted and observed chloramine-T concentrations are provided in Figure 9. The rhodamine WT and chloramine-T predicted and observed data from the UMESC study support the use of the dilution flow model presented in this environmental summary.

Separate from the UMESC effluent study, the actual chloramine-T discharge concentrations for one northeastern state hatchery (hatchery ID #82; Appendix A) were measured at four different times (0, 2, 4, and 6 h—actual treatment start time was not recorded by the hatchery; however, it was assumed that treatments started after the 0-h sample and before 2-h sample). Measurements were taken during 26 treatment days occurring May–July or September–December 1999 and 2000. The hatchery's intent was not to carry out a formal experiment, but rather to characterize chloramine-T concentrations in their discharge effluent soon after daily treatments. The data from this hatchery seem to support the estimated EICs determined from our hatchery survey data. The upper chloramine-T concentrations actually determined at this hatchery (6 of 25 discharges were between 5 and 7.47 mg/L) were similar to the typical and worst-case EICs that were calculated based on our hatchery survey data for this hatchery (6.7 and 8.0 mg/L, respectively). The observed chloramine-T discharge concentrations suggest that chloramine-T was completely discharged in less than 4 h after treatment, and probably much less than 4 h. These results, given the many unknowns involved, support the validity of our conservative method to estimate chloramine-T discharge concentrations after use at the hatcheries.

The higher estimated EIC predicted in the UMESC study and for hatchery ID #82 relative to the observed effluent concentrations at both locations may be partially explained by the lack of a degradation term within the dilution model-based EIC calculations. Within hatchery water, there will always be some fast-acting chlorine demand that would rapidly degrade at least a small portion of administered chloramine-T. The work of Bills et al. (1988a) suggest that rapid degradation of chloramine-T occurs in many hatchery waters through contact with fish feces or uneaten feed. Jaworske and Helz (1985)

suggested that significant amounts of oxidant demand are exerted on a millisecond time scale in the presence of bromine (or chlorine), and the work of Bills et al. (1988a) indicates that some portion of this demand can react quickly with chloramine-T as well. Before discharge from hatcheries, chloramine-T will continuously be exposed to new fast-acting chlorine demands as it passes through the hatchery system, especially at those hatcheries with settling or detention lagoons. Not accounting for this (widely varying) degradation will lead to overestimated EIC values in many instances if EIC estimates are based on dilution only. In addition, the estimated EICs are average concentrations for 1-day periods or longer and would be expected to be substantially lower than the hatchery monitoring concentrations which are essentially peak concentrations.

After discharge from a hatchery, any chloramine-T remaining will again face additional chlorine demand combined with a dilution potential that often equals or exceeds that realized in the hatchery itself. Therefore, aquatic species are not likely to be exposed to these concentrations, whether estimated or actual, for an extended period after discharge. Furthermore, most receiving waters will rapidly reestablish chlorine demand levels because chloramine-T discharges are intermittent.

8.0 Risk Characterization

We conducted a risk characterization that integrated the potential fate and effect of chloramine-T release into freshwater ecosystems. Estimates of chloramine-T release were developed to assess the risk of acute or chronic effects to biological ROI associated with chloramine-T discharge likely to occur after hatchery use. Risk assessment, when appropriate, should assess the potential acute and chronic effects associated with the release of the compound in question—in this instance, chloramine-T—in the effluent. Chloramine-T and its primary degradation product, p-TSA have relatively high water solubilities (Tables 2 and 4), and p-TSA has a relatively low octanol:water partition coefficient (Table 4). These facts, along with p-TSA's residue chemistry profiles in fish after aggressive exposure (Meinertz et al. 2004), suggest that it is unlikely that short-duration intermittent exposure to chloramine-T, the discharges expected to result from hatchery use, would cause chronic toxicity effects within aquatic ecosystems.

Chloramine-T might be administered as a 1-h static or flow-through exposure followed by subsequent hatchery discharge lasting for several hours on one to four consecutive or alternate days. Thus, the possible effects to organisms in receiving water being exposed to intermittent pulses of chloramine-T should be evaluated. For aquatic organisms other than fish, none of the toxicity data available contained any definitive information on the effects of short-duration intermittent exposures to ROI; therefore, it would be unreasonable and impossible to clearly delineate and quantify such effects for either algae or aquatic invertebrates.

Because of the paucity of available data, we did not include parameters to estimate the variance of exposure duration, the proportion of population that would respond, or the severity of the response within our quotient analysis. Rather, we chose to simply discuss the potential effects of each of those parameters on the quotient determined.

Risk characterization was based on (1) the estimated EICs of chloramine-T from aquaculture facilities as a result of chemical treatments on-site for both typical and worst-case discharge scenarios as described in Section 7.2; and (2) data from aquatic toxicity tests available for representative ROI that reside in or are similar to the resident species in surface waters at hatchery discharge sites. Where possible, data were used to conduct an acute risk quotient (RQ) analysis using selected LC₅₀ data (or EC₅₀ where the effect indicated immobilization [daphnia] or inhibition of growth [algae]) and a chronic RQ analysis using selected chronic NOEC data. The chosen LC₅₀, EC₅₀ or NOEC values are divided by an

assessment factor (AF) as specified by the VICH International Cooperation on Harmonisation (FDA 2004) to obtain a predicted no effect concentration (PNEC, see Tables 10 and 11). The acute RQ value is calculated by dividing the EIC by the acute PNEC:

$$\text{Acute RQ} = \text{EIC}/\text{acute PNEC}$$

In this analysis, an acute RQ greater than 1.0 indicates that there may be acute toxic effects to ROI. The chronic RQ value is determined by dividing the EIC by the chronic PNEC for a particular ROI:

$$\text{Chronic RQ} = \text{EIC}/\text{Chronic PNEC}$$

In this analysis, a chronic RQ greater than 1.0 indicates that there may be chronic toxic effects to ROI. By conducting both the acute RQ and chronic RQ analyses for the same ROI, we will estimate risk according to two different types of toxicity data – acute effects data (i.e. LC₅₀ or EC₅₀) and chronic NOEC values. This will help to reduce uncertainty in conclusions based on the risk analysis.

The risk assessment based on the AFs in Table 11 can be refined if a stronger toxicity database is available for a given ROI than is assumed by the VICH, or if an actual NOEC is available for the key study selected for the acute risk assessment instead of (or along with) an LC₅₀. The risk assessment will utilize such a refined assessment. The refined assessment essentially lowers the overall AF to be applied to the selected toxicity endpoint, and a justification for each lowering must be done.

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 seemed to have been designed and conducted in a manner that was 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 chloramine-T 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, data for the species that is most sensitive to chloramine-T, and for which NOEC and LC₅₀ data exist, were chosen; 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.

8.1 Potential Acute Risk of Chloramine-T Discharge - The potential acute risk (acute RQs) for the various ROI, based on 1- and 5-d EIC estimates, and using the default VICH AFs, are given in Table 11. Acute RQs based on refined AFs are given in Table 12. Key toxicity studies used in this risk assessment are summarized in detail in Appendix H.

Algae - The algal ROI and study data selected were for *Scenedesmus subspicatus*. We chose the lowest 48-h EC₅₀ value reported for *S. subspicatus* (0.31 mg/L). *Scenedesmus subspicatus* was the most sensitive species for which we have a toxicity estimate. The chloramine-T acute toxicity database for freshwater algae appears adequate, especially if marine species are included as surrogates for freshwater species. We applied an AF of 10 to extrapolate from the acute EC₅₀ to the acute PNEC, yielding a PNEC of 0.031 mg/L (Table 12). An EIC value of 0.031 mg/L would generate an acute RQ of 1. According to our refined risk assessment and hatchery survey results, maximal chloramine-T use at hatcheries would result in acute RQs of 19 for 25% of surveyed hatcheries and acute RQs of 23-26 for 5% of surveyed hatcheries (Table 12).

Although freshwater algae appear to exhibit considerable sensitivity to acute exposures to chloramine-T, any acute impacts on algae will be temporary because of the ability of their populations to rebound quickly and repopulate affected receiving waters from upstream. After the initial effect of a short exposure to TRC, algal growth and photosynthesis often recover to control levels (EPA 1985). The ability of algae to recover from TRC exposure combined with their inherent ability for rapid growth and reproduction suggests that prolonged effects on the growth and composition of natural populations are probably not likely given the short-term, intermittent discharge conditions of chloramine-T use in public aquaculture. Actual contact time and exposure concentrations will also be reduced as chloramine-T undergoes dilution and degradation in the receiving waters. Algal populations near hatcheries are also supplemented by outside introduction of organisms from upstream sources in rivers, by wind-driven circulation within lakes and backwaters, or by algal populations within the hatchery itself. Therefore, it is unlikely that freshwater algal populations will be impacted by chloramine-T discharge from hatcheries.

Invertebrates - The invertebrate data used were for *Daphnia magna*, a recognized standard test species (ASTM 1989). The acute RQs were calculated on the basis of the 24-h EC₅₀ (4.8 mg/L, Table 6). The Tier A default assessment factors (AF) include consideration of extrapolation from the laboratory to the field, which is meant to encompass such things as the effect of pH and other field variables on toxicity. Based on the available data, an assessment factor of 5X should allow for intraspecies extrapolation, reduced from a default value of 10X because data are available for multiple invertebrate species. An additional factor of 10X should allow for lab to field and acute EC₅₀ to acute PNEC extrapolation, resulting in a total AF of 50X, which would still be expected to be protective of sensitive invertebrate species residing in lower pH environments. This yields a PNEC of 0.096 mg/L (Table 12). An EIC value of 0.096 mg/L would generate an acute RQ of 1. According to our refined risk assessment and hatchery survey results, maximal chloramine-T use at hatcheries would result in acute RQs of 6.3 for 25% of surveyed hatcheries and acute RQs of 7.3-8.3 for 5% of surveyed hatcheries (Table 12). This result represents an acute risk to freshwater invertebrates that may be of some concern.

In actuality, the combination of the reduction of chloramine-T concentration by dilution and the likely degradation after introduction into streams, rivers, or lakes minimize the chance of population-level effects being realized. Of the 86 hatcheries in the UMESC hatchery survey that provided the needed data, 74 discharged into water bodies that would provide an immediate 1:1 dilution of the hatchery effluent (see Section 7.3). Some of the remaining 12 also provided for nearly an immediate 1:1 dilution. Thus, in most instances, the actual exposure concentrations would be rapidly reduced below the estimates provided, resulting in an acute RQ that quickly approaches 1, the value indicating no risk to invertebrates.

Fish - There appears to be adequate data to assess the acute toxicity of chloramine-T to freshwater fish. The definitive fish toxicity data used were the 96 h LC₅₀ value for channel catfish (1.8 mg/L). We applied an AF of 10 to extrapolate from the acute LC₅₀ to the acute PNEC, yielding a PNEC of 0.18 mg/L (Table 12). An EIC value of 0.18 mg/L will generate an acute RQ of 1. According to our refined risk assessment and hatchery survey results, maximal chloramine-T use at hatcheries would result in acute RQs of 3.3 for 25% of surveyed hatcheries and acute RQs of 3.9-4.4 for 5% of surveyed hatcheries (Table 12). This result represents an acute risk to freshwater fish that may be of concern.

The 96 h LC₅₀ value used above for channel catfish (1.8 mg/L) was for an exposure in soft, acidic water, which was demonstrated to be the most toxic chloramine-T exposure conditions for fish (Bills et al. 1988b). The combination of soft water (hardness <48 mg/L as CaCO₃) and pH <7 was reported by only 14 of the 93 hatcheries surveyed that reported pH values for their culture waters (see Section 6.7). In actuality, the combination of the reduction of chloramine-T concentration by dilution and likely

degradation after introduction into streams, rivers, or lakes minimize the chance of population-level effects being realized.

Bacteria - Direct discharge of chloramine-T from aquaculture facilities into sewage or wastewater treatment systems is not likely, since the only known discharges are to lakes, rivers, and streams. There is some chance that small indoor experimental culture facilities might discharge to public sewage, but their discharges would be relatively small in volume, thus the chloramine-T would be greatly diluted before it reached the treatment plant.

Nonetheless, we chose to do a risk assessment for sewage treatment bacteria. The most sensitive sewage treatment bacteria to chloramine-T appear to be aerobic saprophytic activated sludge bacteria. Respiration inhibition occurs at 5 mg/L. We chose to do a risk assessment for sewage sludge using 5 mg/L as the PNEC. A chloramine-T EIC of 5 mg/L would thus generate an acute RQ of 1, and chloramine-T discharges less than 5 mg/L would be of no risk to bacteria.

It is evident from Table 7 that the sensitivity of chloramine-T to bacteria varies greatly with species. Some naturally-occurring aquatic bacteria appear to be sensitive to chloramine-T. The most sensitive bacteria of those reported in Table 7 is *Pseudomonas putida*, a bacteria that is ubiquitous in the environment and occurs naturally in fresh water. Halamid[®] at an aqueous concentration of 10 mg/L produces a 10% reduction (i.e., an EC₁₀) of the O₂ uptake of *P. putida* (Bessemers 1988). Although many bacteria are apparently much less sensitive to chloramine-T than *P. putida*, we chose to do a risk assessment for naturally-occurring aquatic bacteria using 10 mg/L as the PNEC. A chloramine-T EIC of 10 mg/L would generate an acute RQ of 1, and chloramine-T discharge concentrations less than 10 mg/L would be of no risk to naturally-occurring aquatic bacteria.

In order for chloramine-T to be an effective treatment against bacterial gill disease and external flavobacterial infections in fish, it must be toxic to some pathogenic bacterial species in short-term exposures and, therefore, will likely present some risk to bacteria in receiving waters. 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 chloramine-T is intermittently introduced, its concentration is reduced by dilution and degradation. Bacteria from surrounding or incoming waters will then quickly reproduce and repopulate the affected area. It is unlikely that relatively small, isolated, and intermittent point-source discharges of chloramine-T (like those occurring after aquaculture use) could have a significant long-term impact on the numbers and types of bacteria present at any aquaculture location.

8.2 Potential Acute Risk of Repeated Chloramine-T Discharge - Data generated through target animal safety studies to support the approval of chloramine-T use suggest that the risk associated with repeated exposures of fish to chloramine-T is minimal. Cool- and warm-water fry exposed to four 1-h exposures of chloramine-T in hard water (pH 7.5) on consecutive days resulted in minimal mortality at all but the extremes of exposure duration and concentration (Table 13). In a second set of experiments, walleye and channel catfish were exposed once daily for 180 min on 12 consecutive days. Walleye experienced no mortality at concentrations of 80 mg/L, whereas channel catfish experienced no mortality when exposed to 50 mg/L. Channel catfish experienced 8% mortality when exposed to 80 mg/L for 1 h daily for 12 consecutive days. (UMESC Study # CAP-99-CLT-01, M. P. Gaikowski, Study Director). In similar studies with rainbow trout, 180-min exposures of up to 50 mg/L on consecutive or alternate days resulted in mortality similar to that of untreated controls (FWS, Bozeman National INAD Office, Study # BFTC-99-CHLT-TAS, J. Bowker, Study Director). From these data, we find that the risk of population-level effects on the basis of the estimated chloramine-T discharge profiles obtained from our hatchery discharge model are negligible.

8.3 Potential Chronic Risk of Chloramine-T Discharge - The potential chronic risk (chronic RQs) for the various ROI, based on 21-d EIC estimates, and using the default VICH AFs, are given in Table 11. Chronic RQs based on refined AFs are given in Table 14. Key toxicity studies used in this risk assessment are summarized in detail in Appendix H.

We chose to model potential chronic risks associated with prolonged chloramine-T release on the basis of chloramine-T chronic toxicity, even though chloramine-T will degrade to p-TSA and various chlorinated compounds—mainly chlorinated organic-N. We chose to do this because of the lack of data on the chronic toxicity of the numerous potential chlorinated compounds that might result from long-duration releases of chloramine-T.

Algae – Kroon (1997; Appendix H) reported a 96-h NOEC value of 0.2 mg/L (growth inhibition) for chloramine-T for freshwater algae *Selenastrun capricornutum* (see Table 6). The European Agency for the Evaluation of Medicinal Products (EMA) has stated that 72-h or longer algae tests can be considered chronic, as 72 h account for 16 life cycles (EMA 1997). Freshwater algae more sensitive to chloramine-T than *Selenastrun capricornutum* may exist, but chronic toxicity data for other species are currently unavailable. We applied an AF of 10 to account for possible interspecies variability, yielding a PNEC of 0.02 mg/L (Table 14). An EIC value of 0.02 mg/L results in a chronic RQ of 1. According to our refined risk assessment and hatchery survey results, maximal chloramine-T use at hatcheries would result in chronic RQs of 7.5-30 for 25% of surveyed hatcheries and chronic RQs of 8.0-40 for 5% of surveyed hatcheries (Table 14).

Invertebrates - The invertebrate data we chose were those for a standard test invertebrate, the small freshwater crustacean *Daphnia magna* (ASTM 1989). The toxicity value we used (1.1 mg/L) was the 21-d NOEC value reported by Putt (1993; Appendix H; Table 14). Since there appears to be limited pH data to assess the chronic toxicity of chloramine-T to freshwater invertebrates, we applied an AF of 10, yielding a PNEC of 0.11 mg/L (Table 14). An EIC value of 0.11 mg/L provides a chronic RQ of 1. According to our refined risk assessment and hatchery survey results, maximal chloramine-T use at hatcheries would result in a chronic RQ of 1.0 for 25% of surveyed hatcheries and a chronic RQ of 2.0 for 5% of surveyed hatcheries (Table 14).

Fish - An assessment of chloramine-T chronic risk can be made for freshwater fish using the results from a study by Machado (1983; Appendix H) of toxicity to the early life stage of fathead minnow (*Pimephales promelas*). Data on chloramine-T for fathead minnow indicate that the 35-d NOEC is 1.1 mg/L (Table 14). An AF of 10 was considered to be appropriate because a chronic study using the most sensitive fish species from acute study results and also using the most sensitive study conditions (e.g., pH <7) was not available. This yields a PNEC of 0.11 mg/L (Table 14). An EIC value of 0.11 mg/L would generate a chronic RQ of 1. According to our refined risk assessment and hatchery survey results, maximal chloramine-T use at hatcheries would result in chronic RQs of 1.5-6.0 for 25% of surveyed hatcheries and chronic RQs of 1.6-8.0 for 5% of surveyed hatcheries (see Table 14). It is unlikely that chloramine-T will remain at its discharge concentration for 35 days in any public receiving waters, the length of the exposure for the fish chronic toxicity test. Dilution alone in receiving waters should very rapidly bring the concentration to 0.11 mg/L, the level at which no chronic risk to fish would be indicated.

Bacteria - Chloramine-T is introduced intermittently to the aquatic environment following its use in aquaculture. Its concentration in the aquatic environment is reduced by dilution and degradation. Bacteria from surrounding or incoming waters will then quickly reproduce and repopulate the affected area. It is unlikely that relatively small, isolated, and intermittent point-source discharges of chloramine-T

(like those occurring after aquaculture use) could have a significant long-term impact on the numbers and types of bacteria present at any aquaculture location.

8.4 Potential Acute Risk of Para-toluenesulfonamide Discharge - An acute risk assessment for p-TSA was only developed in this EA for algae, the most sensitive of the four ROI. This was calculated to demonstrate that the acute toxicity of p-TSA was less than that of chloramine-T, and to demonstrate that no acute risk is indicated to any of the ROI because of exposure to p-TSA.

Algae - An acute risk assessment for p-TSA in algae, the most sensitive ROI to p-TSA, follows: The toxicity data point selected was 23 mg/L for the 72-h EC₅₀ for p-TSA to the algae *Selenastrum capricornutum* (Appendix G, Table G-1). We applied an AF of 10 to extrapolate from the acute EC₅₀ to the acute PNEC, yielding a PNEC of 2.3 mg/L, which is 65-fold higher (2.3/0.031) than the acute PNEC for chloramine-T. An EIC value of 2.3 mg/L would generate an acute RQ of 1, as compared to 0.031 mg/L for chloramine-T. This EIC of no concern for p-TSA is well above our predicted worst-case 75th and 95th percentile 1- and 5-d EICs (0.6-0.8 mg/L, Table 9).

Invertebrates - An acute RQ for p-TSA in invertebrates was not conducted, as algae, not invertebrates, are the most sensitive species to p-TSA.

Fish - An acute RQ for p-TSA for fish was not calculated, as algae, and not fish, are the most sensitive species to p-TSA.

Bacteria - As indicated in Section 6.4, a risk assessment for acute exposure of bacteria to p-TSA will not be conducted because bacteria are considerably less sensitive to p-TSA than to chloramine-T. The acute risk of p-TSA to the aquatic environment can be modeled by that of chloramine-T.

8.5 Potential Chronic Risk of Para-toluenesulfonamide Discharge - The degradate p-TSA is likely to be present in public waters long enough to warrant concern about chronic toxicity to aquatic species. The chronic RQ values (and the acute RQ values in Section 8.4 above) for p-TSA are actually somewhat lower than those presented below because the formula weight of chloramine-T is 281.69. The formula weight of the degradate p-TSA is 171.23, and the numerators given below (and in Section 8.4 above) are for chloramine-T discharge concentrations. The risk estimates assume that all of the chloramine-T is degraded to p-TSA, and in this case the numerators (discharge concentrations) would be about 171/282 or 61% of that stated, reducing the acute RQ and chronic RQ values by the same amount. The data presented in Appendix G were used to evaluate chronic risk of p-TSA to three ROI (toxicity data for bacteria were not available). This evaluation was made as follows:

Algae - The 72-h EC₅₀ for p-TSA to the algae *Selenastrum capricornutum* is 23 mg/L (Appendix G). The European Agency for the Evaluation of Medicinal Products (EMA) has stated that 72-h or longer algae tests can be considered chronic, as this period accounts for 16 life cycles (EMA 1997). We applied an AF of 10 to extrapolate from the acute EC₅₀ to the chronic PNEC, yielding a PNEC of 2.3 mg/L, which is 115-fold higher (2.3/0.02) than the chronic PNEC for chloramine-T. An EIC value of 2.3 mg/L results in a chronic RQ of 1. This EIC is identical to our acute EIC of no risk for algae and likewise results in RQs of much less than 1 for all EIC scenarios presented in Section 8.4 for acute toxicity to algae.

Invertebrates - The chronic results for p-TSA on *Daphnia magna* (21-d NOEC, static test, immobilization, and reproduction) were 47 mg/L (and a lowest-observed-effect-concentration [LOEC] of 150 mg/L, Appendix G, Table G-1). Using the NOEC, the chronic RQ value for *D. magna* suggests that

toxicity is not indicated even if all the chloramine-T is immediately converted and discharged as p-TSA at 20 mg/L (chronic RQ = 0.43; 20 mg/L / 47 mg/L). These results indicate no presumption of chronic risk to this ROI, and also suggest that p-TSA exhibits much lower chronic risk to *D. magna* than does chloramine-T.

Fish – Acute toxicity data on p-TSA for rainbow trout, the most sensitive freshwater fish tested, indicate that the 96-h LC₅₀ is 100 mg/L (Cohle and McAllister 1983a; Appendix H, also Appendix G). We applied an AF of 10 for the acute-to-chronic ratio (i.e., extrapolation of an acute LC₅₀ to a chronic NOEC), yielding a chronic NOEC of 10 mg/L. An EIC value of 10 mg/L would generate a chronic RQ of 1. This EIC is well above estimates generated for any scenario involving discharge of chloramine-T (as p-TSA) and represents a discharge concentration that is of minimal chronic risk to freshwater fish.

Bacteria - As indicated in Section 6.4, a risk assessment for chronic exposure of bacteria to p-TSA will not be conducted because bacteria are considerably less sensitive to p-TSA than to chloramine-T.

8.6 Risk Mitigation - An evaluation of the refined risk quotients in Tables 12 and 14 indicates that adverse effects on aquatic life could occur at some of the hatchery facilities that are expected to use chloramine-T 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 chloramine-T 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 chloramine-T, 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 not 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 this³. 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 chloramine-T that may have the potential to cause effects at individual facilities.

The recommended risk mitigation to insure that use of chloramine-T will not adversely impact aquatic life is to develop a water quality criterion or benchmark for the protection of aquatic life that can be used by the appropriate National Pollutant Discharge Elimination System (NPDES) or state permitting authority⁴ to establish appropriate effluent discharge limits on a facility-by-facility basis, if needed, based on site-specific conditions (e.g., effluent treatment, receiving water dilution) and in conformance with applicable state and federal water quality regulations. Environmental statements will be added to the drug label that identify the water quality benchmark for its use by NPDES permitting authorities⁵ and which require the user to report this information to the appropriate authority prior to the initial use of the drug.

8.7 Derivation of an Acute Water Quality Benchmark (Criterion) for Chloramine-T - The water quality benchmark for chloramine-T was derived using procedures in published EPA guidance, which vary depending on the amount of available and well-documented toxicity data. If the existing database is not adequate to support the use of the standard EPA (Tier I) approach (Stephan et al. 1985, EPA 1991, 1994), the Tier II

³ 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.

⁴ 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.

⁵ 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.

methodology described in the Great Lakes System guidance (21 CFR 132, Appendix A; EPA 1995) may be used for criteria development⁶. For the Tier I approach, toxicity endpoints should be available on at least eight different families to ensure a sufficient database on which to base the calculation of the “Final Acute Value” (FAV). If all eight minimum data requirements for calculating a FAV are not met, an alternate Secondary Acute Value (SAV) can be calculated using the Tier II methods described in water quality guidance for the Great Lakes System (EPA 1995). To calculate a SAV, the lowest GMAV (genus mean acute value) in the database is divided by the Secondary Acute Factor corresponding to the number of satisfied minimum data requirements. The guidance requires that a GMAV be available for one of the following 3 genera in the family Daphnidea: *Ceriodaphnia sp.*, *Daphnia sp.*, or *Simocephalus sp.* Finally, either the FAV or SAV is divided by a factor of two to give either the Criterion Maximum Concentration (CMC) or the Secondary Maximum Concentration (SMC).

The existing database for chloramine-T is inadequate to support the use of the standard EPA Tier I approach for deriving water quality criteria. However, the Tier II methodology can be used because chloramine-T toxicity data are available for *Daphnia magna*, and there are 3 other data points that meet the stipulated data requirements (Table 15). Toxicity data at pH 6.5 are used so that the resulting criterion is protective for receiving waters with higher pH values. Therefore, a SAV can be calculated by dividing 1.8 mg/L (the lowest GMAV, Tables 15 and 16) by 7.0 (the factor for 4 data requirements satisfied, Table 17) to yield a SAV of 0.26 mg/L. Using this methodology, the Secondary Maximum Concentration for chloramine-T is one-half of the SAV of 0.26 mg/L or 0.13 mg/L. This value, the SMC, is the acute benchmark value for chloramine-T.

Note that toxicity data for algae were not used to derive the acute benchmark for chloramine-T, despite the fact that algae are very sensitive to the effects of this compound. This was because EPA procedures only call for using aquatic plant toxicity data to derive chronic criteria. However, based on the 96-h NOEC for the green algae *Selenastrum capricornutum* (0.2 mg/L), the acute benchmark value of 0.13 mg/L for chloramine-T is still protective for sensitive species of algae. Also note that exposure to chloramine-T would likely result in only temporary inhibition of algal growth (see discussion in Section 8.1). Consequently, algal populations are not likely to be adversely affected by the proposed use of chloramine-T if the product labeling is followed.

Both the standard EPA procedures and Great Lakes Tier II guidance state that, if appropriate, the acute and chronic criteria/values shall be made a function of a water quality characteristic, such as pH or hardness. Initially, this would appear to be appropriate for chloramine-T, because data for several species of fish show a strong correlation between pH and toxicity (Figure 5B). Methods for developing a Final Acute Equation are described in the standard EPA procedures and Great Lakes Tier I methodologies. These methods state that a Final Acute Equation should not be developed “If useful slopes are not available for at least one fish and one invertebrate or if the available slopes are too dissimilar or if too few data are available to adequately define the relationship between acute toxicity and the water characteristic.” This is the case for chloramine-T because there are not sufficient data available for aquatic invertebrates to define the relationship between pH and toxicity. The existing invertebrate toxicity data suggest that the relationship of toxicity to pH may be similar to that for sensitive fish species (e.g., rainbow trout, channel catfish); however, the pH range of the available studies is very narrow (pH 7.5 to 8.4). Therefore, the acute water quality criterion (benchmark) for chloramine-T has been conservatively based on acute toxicity data for channel catfish at a pH of 6.5. This value is the lowest acute toxicity value in the chloramine-T database for non-plant

⁶ Criteria derived using the standard EPA approach are often referred to as Tier I criteria because the Great Lakes guidance describes several Tier I methodologies that are identical to the standard EPA approaches. The Great Lakes guidance defines these criteria as Tier I criteria while those developed using the Tier II methodologies are defined as Tier II “values” (not criteria).

species. It was generated at a pH that is at the low end of the range for receiving waters in the UMESC hatchery survey (See Section 6.7) and at the low end of the EPA pH criteria range of 6.5 to 9.0 for freshwater aquatic life (EPA 1976).

The recommended product labeling below (Section 8.8) does not contain a chronic water quality benchmark for chloramine-T. There are several reasons why a chronic water quality benchmark was not derived for chloramine-T and is not thought 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 chloramine-T from use on fish will not be chronic in nature, typically occurring over a period of only 4 to 8 days.
2. Risk quotients for chloramine-T 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* and the fathead minnow indicate a small acute to chronic ratio for toxicity. Also, Bills et al. (1988b) presented data indicating that time-independent LC50 values were statistically similar to 96-h LC50 values in fish. Therefore, the chronic benchmark, if it were derived, is not likely to be significantly lower than the acute benchmark.
4. Chloramine-T is reactive and does not bioaccumulate in tissues or environmental compartments.

8.8 Proposed Chloramine-T Product Label for Environmental Safety - 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

Before using this drug for the first time, you must inform the appropriate National Pollutant Discharge Elimination System (NPDES) permitting authority of your intentions and of the following information. A water quality benchmark for the protection of freshwater aquatic life has been derived by FDA. The acute benchmark is 0.13 mg/L, which is equivalent to the Secondary Maximum Concentration (one-half of the Secondary Acute Value). The NPDES authority may require an NPDES permit before you can discharge chloramine-T. The water quality benchmark concentration is not a discharge limit, but it may be used by the NPDES authority to derive one for the permit. The acute benchmark should be protective of aquatic life when the receiving water pH is at or above pH 6.5. Additional environmental information on chloramine-T and the benchmark value are available in an environmental assessment posted at <http://www.fda.gov/cvm/ea.htm>.

STORAGE AND DISPOSAL

Improper storage and disposal of chloramine-T could potentially result in releases that cause adverse effects on aquatic life, therefore the following storage and disposal instructions 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:

Contact your State Environmental Control Agency, or the Hazardous Waste Representative at the nearest EPA Regional Office for guidance on disposal. 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.

9.0 Alternatives to Proposed Action

Copper sulfate and potassium permanganate have traditionally been used, mainly in extensive or pond aquaculture, for treating external bacterial problems. They are marginally effective, inexpensive therapeutants, and are preferred for large-scale applications, but they too have not yet been approved to control mortalities associated with external bacterial infections on fish. The toxicity of both are influenced by water chemistry to the point that some hatcheries may not be able to achieve effective concentrations without the potential for fish mortality. Hydrogen peroxide effectively controls mortality associated with bacterial gill disease in cold-water fish and columnaris in cool-water and some warm-water fish, but its therapeutic index—the difference between a therapeutic concentration and a toxic concentration—is much less than that of chloramine-T, especially for warm-water fish. Hydrogen peroxide might be considered in applications where (1) the higher costs of chloramine-T are a significant consideration, (2) water flow in the treatment unit is sufficient to rapidly eliminate the chemical, and (3) the target species is tolerant of hydrogen peroxide treatment.

10.0 Conclusions

The use and subsequent discharge of chloramine-T from intensive aquaculture facilities is not likely to result in acute or chronic effects to populations of aquatic organisms nor is it likely to be a potential threat to public health or safety. We based this conclusion on the following: (1) that it is unlikely that chloramine-T at concentrations proposed for aquaculture use will produce either free chlorine or inorganic chloramine or other compounds more toxic than chloramine-T, (2) that the production of substantial amounts of mutagenic or electrophilic compounds from chloramine-T use or discharge is also not likely, and (3) that chloramine-T is the species on which it is appropriate to model our assessment of potential environmental risk.

Although our acute RQ analyses of chloramine-T suggests the possibility of acute risk to aquatic organisms, our use of EICs in the risk analysis did not account for the reduction in exposure concentration and contact time that would result from chlorine demand in the hatchery effluent. If analyses were conducted at intensive aquaculture sites to determine actual discharge profiles, in most instances, we would expect much lower actual discharge concentrations than the estimated values determined from the USGS hatchery survey. Our risk assessment also did not include the mitigating effects of immediate dilution and degradation that would occur in the receiving water body.

11.0 Acknowledgments and Suggested Reference

We acknowledge the following staff of the Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin, for their significant contributions in the following areas: Chelsea A. Berg, Shari L. Greseth, and Susan M. Schleis for technical assistance in helping to produce this document, David M. Soballe and William B. Richardson for technical advice on limnology, and Terrance D. Hubert for technical and editorial review. We acknowledge Rosalie A. Schnick, National NADA Coordinator, La Crosse, Wisconsin, for assistance with technical and editorial review, and Edwin C. Bisinger of Akzo-Nobel, Thomas A. Bell of U.S. Fish and Wildlife Service, John R. MacMillan of Clear Spring Foods, Inc., and James N. Jensen of the Department of Civil, Structural and Environmental Engineering, SUNY-Buffalo for external technical review of this document. We also acknowledge the States for contributing funds to the Approval of Drugs for Public Fish Production project—a project of the International Association of Fish and Wildlife Agencies, and the state, private, and federal hatcheries that participated in the survey used to determine potential usage of chloramine-T, discharge concentrations, characteristics of receiving water, and estimated introduction concentrations.

Suggested reference for this document: Schmidt, L. J., M. P. Gaikowski, W. H. Gingerich, G. R. Stehly, W. J. Larson, V. K. Dawson, and T. M. Schreier. 2007. Environmental Assessment of the Effects of Chloramine-T Use in and Discharge by Freshwater Aquaculture. U.S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin, April 2007.

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13.0 Glossary

13.1 Acronyms and Abbreviations

A	Alpha
acre-feet	volume in liters / 1,233,476
acre-foot	volume (1,233,476 L) equal to one acre x 1 foot
AF	assessment factor
ammonia-N	ammonia as nitrogen
CaCO ₃	calcium carbonate
Cfs	cubic feet per second, equal to 28.32 liters per second
CHBr ₃	Bromoform
CI	confidence interval
Cl ₂	Chlorine
D	Day
DPD	diethyl-p-phenylenediamine
EC ₁₀ or EC ₅₀	effective concentration (expected to produce the specified effect in 10% or 50% of the population within the specified time)
EEC	estimated environmental concentration
EIC	environmental introduction concentration
EMEA	European Agency for the Evaluation of Medicinal Products
EPA	U.S. Environmental Protection Agency
FAV	final acute value
FWS	U.S. Fish and Wildlife Service
g/L	grams per liter
gal	Gallon
GMAV	genus mean acute value
gpd	gallons per day
H	Hour
HOCl	hypochlorous acid, a component of aqueous free chlorine

Chronic RQ	chronic risk quotient
ID	Identification
INAD	Investigational New Animal Drug
kg	Kilogram
L	Liter
L/d	liters per day
L/s	liters per second
LC ₅₀	lethal concentration (50% of the population within the specified time)
lbs	Pounds
LOEL or LOEC	lowest-observed-effect-level or concentration
Log P _{ow}	octanol-water partition coefficient
L/m	liters per minute
lwd	length × width × depth
M ³	cubic meter
mg	Milligram
mg/L	milligram per liter
min	Minute
mL	Milliliter
MW	molecular weight
MX	3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone
N	Nitrogen
–	designates a substituent on the nitrogen in an amine
NCl ₃	Trichloramine
NH ₂ Cl	Monochloramine
NH ₃	Ammonia
NH ₄ ⁺	ammonium ion
NHCl ₂	Dichloramine

NOEC or NOEL	no-observed-effect-concentration or level
NPDES	National Pollutant Discharge Elimination System
OCI ⁻	hypochlorite ion, a component of aqueous free chlorine
OECD	Office of Economic Cooperation and Development
organic-N	organic nitrogen
p-TSA	para-toluenesulfonamide
pH	log of reciprocal of hydrogen ion concentration
PNEC	predicted no observed effect concentration
RDA	repetitive die away method
ROI	receptor of interest
RQ	risk quotient
RW	Raceway
S ⁺² , S ⁺⁴	sulfur in the +2 or +4 valence state
SAV	secondary acute value
sec	Second
SMC	secondary maximum concentration
t	absolute value of t
THM	Trihalomethane
total ammonia	NH ₄ ⁺ + NH ₃
TRC	total residual chlorine
UMESC	Upper Midwest Environmental Sciences Center
USGS	U.S. Geological Survey
μg/L	microgram per liter
μM	microMolar

Table 1. Identification of the chemical substance of the proposed action.

Chemical name	sodium <i>N</i> -chloro 4-methylbenzenesulfonamide trihydrate
Synonyms	sodium <i>p</i> -toluenesulfonchloramide trihydrate, <i>N</i> -chloro- <i>para</i> -toluene-sulfonamide sodium salt trihydrate
Common names	chloramine-T, Halamid [®] , chlorazene, halacon, aktiven, mianine
CAS registry number	127-65-1
Formula weight	281.69
Chemical formula	C ₇ H ₇ SO ₂ NNaCl • 3H ₂ O
General physical and chemical characteristics	As sodium salt trihydrate, white or faintly yellow crystals and slight chlorine odor. Loses water on drying. Decomposes slowly on exposure to air. Fairly soluble in water, nearly insoluble in benzene, chloroform or ether (the information on physical and chemical characteristics is from Budavari et al. [1989] and Lewis [1997])

Table 2. Physicochemical properties of chloramine-T.

Parameter	Value	Reference
Boiling point (°C)	not determined	Axcentive SARL (2005) ^a
Melting point (°C)	167 to 169, decomposes ~ 174	Haneke (2002)
pH	8.0–10.3 (5% solution)	Axcentive SARL (2005)
Specific gravity (kg/m ³)	1,430	Axcentive SARL (2005)
Flash point (°C)	192 (Pensky-Martens, closed cup)	Axcentive SARL (2005)
Solubility (g/L)		
Water	150 at 25 °C	Axcentive SARL (2005)
Ethanol (95%)	75 g/L (20°C)	Axcentive SARL (2005)
Benzene, chloroform, or ether	practically insoluble	Budavari et al. (1989)
<i>n</i> -Octanol/water partition coefficient	K _{ow} = 0.05	Heus 1992; Appendix H

^a Axcentive SARL (2005) Material Data Safety Sheet, available online at http://www.halamid.com/halamid_safety_sheet.pdf, accessed January 2006.

Table 3. Identification of the major degradate, p-toluenesulfonamide (OECD 1994).

Chemical name	Benzenesulfonamide, 4-methyl-
Synonyms	p-methylbenzenesulfonamide, toluene-4-sulfonamide, tolylsulfonamide, tosylamide, 4-MBSA, 4-methylbenzenesulfonamide, toluene-p-sulfonamide, p-tolylsulfonamide, p-tosylamide
Common names	p-toluenesulfonamide, p-TSA
CAS registry number	70-55-3
Formula weight	171.23
Chemical formula	C ₇ H ₉ NO ₂ S

Table 4. Physicochemical properties of the major degradate, p-toluenesulfonamide.

Parameter	Value	Reference
General physical and chemical characteristics	Nonvolatile solid, stable in acid, neutral, or alkaline solutions	OECD (1994)
Boiling point (°C)	221 at 10 mm Hg	OECD (1994)
Melting point (°C)	137.5	OECD (1994)
Flash point (°C)	202	OECD (1994)
Solubility (g/L)		
Water	3.2 at 25 °C	OECD (1994)
Alcohol	Soluble	Haneke (2002)
Vapor pressure	0.75 mm Hg at 170 °C	OECD (1994)
Octanol/water partition coefficient	Log P _{ow} = 0.84 at 25 °C	OECD (1994)

Table 5. Suggested chemical values for hatchery water supplies. Concentrations are in parts per million (ppm; Source Piper et al. 1982).

Variable	Salmonids	Warm-water species
Dissolved oxygen	>5	>5
Carbon dioxide	0–10	0–15
Total alkalinity (as CaCO ₃)	10–400	50–400
pH	6.5–8.0	6.5–9.0
Total hardness (as CaCO ₃)	10–400	50–400
Calcium	4–160	10–160
Magnesium	Needed for buffer system	Needed for buffer system
Manganese	0–0.01	0–0.01
Iron (total)	0–0.15	0–0.5
Phosphorous	0.01–3.0	0.01–3.0
Nitrate	0–3.0	0–3.0
Zinc	0–0.05	0–0.05
Hydrogen sulfide	0	0

Table 6. Summary of toxicity studies of chloramine-T to algae, aquatic invertebrates, and fish. All are freshwater- only species except for striped bass and four marine microorganisms. The chloramine-T 48- or 96-h LC₅₀ value as Cl₂ is also provided and compared, where appropriate, to available total residual chlorine (TRC) toxicity data. Key toxicity studies used in our risk assessment are indicated in bold.

Species tested	Endpoint	EC ₅₀ or LC ₅₀ - (mg/L)					Other (mg/L)	Reference	Chloramine-T as Cl ₂ ^a (mg/L)	TRC as Cl ₂ (mg/L)
		6 h	24 h	48 h	96 h					
<i>Scenedesmus subspicatus</i>	Cell multiplication / growth inhibition	–	–	0.11 (EC ₁₀)	–	–	Kühn and Pattard (1990)	–	–	
	Cell multiplication / growth inhibition	–	–	0.31 (EC₅₀)	–	–	Kühn and Pattard (1990)	–	–	
Axcentive proprietary, <i>Selenastrum capricornutum</i>	growth inhibition	–	–	–	EbC ₅₀ = 4.5	–	Kroon 1997; Appendix H	–	–	
	growth inhibition	–	–	–	ErC ₅₀ = 13	–	Kroon 1997; Appendix H	–	–	
	growth inhibition	–	–	–	LOEC = 0.6	–	Kroon 1997; Appendix H	–	–	
	growth inhibition	–	–	–	NOEC = 0.2	–	Kroon 1997; Appendix H	–	–	
Marine dinoflagellate <i>Glennodinium halli</i>	inhibition of cell division	–	–	–	–	7-d EC ₂₅ > 8 7-d EC ₅₀ > 8	Erickson and Freeman 1978	–	–	
Marine microflagellate <i>Isochrysis galbana</i>	inhibition of cell division	–	–	–	–	7-d EC ₂₅ = 4 7-d EC ₅₀ = 8	Erickson and Freeman 1978	–	–	
Marine diatom <i>Skeletonema costatum</i>	inhibition of cell division	–	–	–	–	7-d EC ₂₅ > 8 7-d EC ₅₀ > 8	Erickson and Freeman 1978	–	–	
Marine diatom, <i>Thalassiosira pseudonana</i>	inhibition of cell division	–	–	–	–	7-d EC ₂₅ > 8 7-d EC ₅₀ > 8	Erickson and Freeman 1978	–	–	
<i>Daphnia magna</i>	Immobilization	–	4.8	–	–	21-d NOEC = 1.3 ^b 21-d LOEC = 2.5	Kühn et al. (1989)	1.2 (24 h)	0.017–0.045; 96-h EC ₅₀ , EPA (1985)	
Axcentive proprietary, <i>Daphnia magna</i>	Mortality	–	–	4.5	–	–	Blok 1981; Appendix H	–	–	
Axcentive proprietary, <i>Daphnia magna</i>	Reproduction	–	–	–	–	21-d NOEC = 1.1 21-d LOEC = 3.5	Putt 1993; Appendix H	0.28 (21-d NOEC)	0.002-0.014; 7-d EC ₅₀ , EPA (1985)	
<i>Ceriodaphnia dubia</i> , in Benner Springs, PA hatchery effluent	Mortality, pH = 8.4 (8.2-8.6)	–	–	8.20, (NOEC = 3.0)	–	–	Analytical Laboratory Services 2003	–	–	
in Spring Creek PA water	Mortality, pH = 8.4 (8.2-8.6)	–	–	8.88, (NOEC = 6.0)	–	–	Analytical Laboratory Services 2003	–	–	
in Oswayo Creek, PA hatchery effluent	Mortality, pH = 7.5 (7.4-7.6)	–	–	2.12, (NOEC = 1.5)	–	–	Analytical Laboratory Services 2003	–	–	
in Oswayo Creek, PA water	Mortality, pH = 7.5 (7.4-7.6)	–	–	8.75, (NOEC = 6.0)	–	–	Analytical Laboratory Services 2003	–	–	
Axcentive proprietary, Marine, Brine shrimp, <i>Artemia nauplii</i>	Mortality	–	–	–	–	72 h EC ₅₀ = 24.6 72 h NOEC = 10.4	Kroon 1995; Appendix H	–	–	
Channel catfish <i>Ictalurus punctatus</i> juvenile	Mortality, soft water pH = 6.5	10	2.9	–	1.8	–	Bills et al. (1988b)	0.45 (96 h)	–	
	Mortality, soft water pH = 7.5	>60	10.0	–	3.8	–	Bills et al. (1988b)	0.96 (96 h)	0.09; 96-h LC ₅₀ , EPA (1985)	
	Mortality, soft water pH = 8.5	>60	51.2	–	10.5	–	Bills et al. (1988b)	2.64 (96 h)	–	
	Mortality, soft water pH = 9.5	>60	>60	–	12.3	–	Bills et al. (1988b)	3.10 (96 h)	–	
Rainbow trout <i>Oncorhynchus mykiss</i> juvenile	Mortality, soft water pH = 6.5	8.2	2.8	–	1.9	–	Bills et al. (1988b)	0.48 (96 h)	–	
	Mortality, soft water pH = 7.5	17.5	6.9	–	2.8	–	Bills et al. (1988b)	0.71 (96 h)	0.062; 96-h LC ₅₀ , EPA (1985)	

	Mortality, soft water pH = 8.5	>60	46.0	-	11.0	-	Bills et al. (1988b)	2.77 (96 h)	-
	Mortality, soft water pH = 9.5	>60	>60	-	10.8	-	Bills et al. (1988b)	2.72 (96 h)	-
Striped bass <i>Morone saxatilis</i> juvenile	Mortality, soft water pH = 6.5	14.1	4.9	-	2.8	-	Bills et al. (1993)	0.71 (96 h)	-
	Mortality, soft water pH = 7.5	44.0	14.4	-	6.3	-	Bills et al. (1993)	1.59 (96 h)	-
	Mortality, soft water pH = 8.5	>80	>80	-	31.5	-	Bills et al. (1993)	7.93 (96 h)	-
	Mortality, soft water pH = 9.5	>80	>80	-	52	-	Bills et al. (1993)	13.1 (96 h)	-
	Mortality, soft water pH = 6.0	-	8	7	7	-	Cross and Hursey (1973)	1.8 (96 h)	-
Harlequin fish <i>Rasbora heteromorpha</i> age 8-12 months	Mortality, soft water pH = 8.0	-	120	100	84	-	Cross and Hursey (1973)	21.2 (96 h)	-
	Mortality, hard water pH = 6.5	-	42	35	27	-	Cross and Hursey (1973)	6.80 (96 h)	-
	Mortality, hard water pH = 7.7	-	110	82	60	-	Cross and Hursey (1973)	15.1 (96 h)	-
	Mortality, hard water pH = 7.8	-	80	55	35	-	Cross and Hursey (1973)	8.82 (96 h)	-
Roach <i>Rutilus rutilus L</i> age 1-2 years	Mortality, hard water pH = 7.8	-	80	55	35	-	Cross and Hursey (1973)	8.82 (96 h)	-
Northern pike <i>Esox lucius L</i> fry	Mortality, hard water pH = 8.2	-	60-70	-	-	-	Bootsma (1973)	15.1 (24 h)	-
Guppy life-stage unknown	Mortality	-	-	-	31	-	Blok 1981; Appendix H	-	-
Fathead Minnow <i>Pimephales promelas</i> in reconstituted water fry	Mortality, pH = 7.5	-	-	-	7.3	-	Bills et al. (1988b)	1.84 (96 h)	-
Fathead Minnow <i>Pimephales promelas</i> in Benner Springs, PA hatchery effluent fry	Mortality, pH = 8.3 (8.2-8.4)	-	-	-	15.0 (NOEC = 10.0)	-	Analytical Laboratory Services 2003	-	-
in Spring Creek PA water fry	Mortality, pH = 7.4 (7.3-7.4)	-	-	-	28.1	-	Analytical Laboratory Services 2003	-	-
in Oswayo Creek, PA hatchery effluent fry	Mortality, pH = 7.3 (7.1-7.5)	-	-	-	10.1 (NOEC = 5.0)	-	Analytical Laboratory Services 2003	-	-
in Oswayo Creek, PA Water fry	Mortality, pH = 7.4 (7.2-7.5)	-	-	-	6.16 (NOEC = 5.0)	-	Analytical Laboratory Services 2003	-	-
Axcentive proprietary, Fathead Minnow fry	Mortality	-	-	-	-	35-d NOEC = 1.1	Machado 1983; Appendix H	0.28	0.045; 30-d LC ₅₀ , EPA (1985)

^aChloramine-T as Cl₂; calculated by dividing the chloramine-T concentration by 3.97.

^bEndpoint was reproduction.

Table 7. Summary of Axcentive proprietary toxicity studies of chloramine-T and p-TSA to bacteria. Key toxicity studies used in our risk assessment are indicated in bold.

Reference and test substance	Test	Exposure / Exposure duration	End-point	Test results
Blok 1982; Appendix H, Halamid®.	ecotoxicity: aerobic sludge bacteria.	no durations specified.	EC₅₀	for respiration inhibition of aerobic saprophytic activated sludge bacteria = 5 mg/L, nitrifying bacteria = 700 mg/L, methane generation from glucose = 1,000mg/L. Both Halamid® and p-TSA stable for 40 days under anaerobic sludge conditions.
Bessem's 1988; Appendix H, Halamid®.	bacterial toxicity to <i>Pseudomonas putida</i>.	-	-	10 mg/L Halamid® produces a 10% reduction of the O₂ uptake.
Borgmann-Strahsen 2000; Appendix H, Halamid®.	basic bactericidal activity to <i>Pseudomonas aeruginosa</i> and <i>Staphylococcus aureus</i> .	-	-	passes CEN test ^b on basic bactericidal activity at 0.03% (300 mg/L).
Bessem's 1996; Appendix H, Halamid®.	bacterial effect to 4 micro-organisms (<i>P. aeruginosa</i> , <i>S. aureus</i> , <i>Escherichia coli</i> , <i>Enterococcus hirae</i>)	-	-	passes CEN test for bacterial effect at 0.006-0.225% (60-2,250 mg/L) under clean conditions and 175-10,050 mg/L) under dirty conditions.
Borgmann-Strahsen 1998; Appendix H, Halamid®.	biocidal activity against <i>Legionella pneumophila</i> and <i>Campylobacter jejuni</i> .	-	-	passes CEN test at very low concentrations (<100 mg/L) for these micro-organisms.
Bessem's 1991; Appendix H, Halamid®.	effectiveness against <i>Vibrio cholerae</i> .	-	-	0.5% Halamid® (5000 mg/L) achieves required efficacy under dirty conditions.
Cranor 1983; Appendix H, Santicizer® 9 ^a	semi-continuous activated sludge (SCAS) biodegradation.	21 days aft. 14-d acclimation period, total 35 days.	dissolved organic carbon	Santicizer 9® has negligible effects on wastewater treatment process at or below 70 mg/L.

^a Reduces bacterial count by 10⁵ or more within 5 minutes of contact.

^b Santicizer® 9 is a mixture of o- and p-TSA.

Table 8. Assumptions made for calculation of “typical” and “worst-case” environmental introduction concentrations (EICs).

Parameter	“Typical” Treatment Scenario	“Worst-Case” Treatment Scenario
Treatment concentration	20 mg/L	20 mg/L
Treatment duration	60 min	60 min
Number of treatments	1 for 1-d EICs, 4 for 5- or 21-d EICs	1 for 1-d EICs, 4 for 5- or 21-d EICs
Hatchery flow rate	average daily water flow	low daily water flow
Receiving water flow	low flow	low 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)
Degradation	Assumed no degradation	Assumed no degradation

Table 9. 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 chloramine-T use. Data presented represent EIC estimates for the maximum daily chloramine-T 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 (60 EIC estimates); hatcheries with effluent/settling ponds (40 EIC estimates); and hatcheries without settling ponds (20 EIC estimates).

Parameter	1-d EIC		5-d EIC		21-d EIC	
	Typical	Worst-Case	Typical	Worst-Case	Typical	Worst-Case
All Hatcheries						
Mean (mg/L)	0.37	0.42	0.35	0.40	0.09	0.10
Median (50 th percentile, mg/L)	0.40	0.40	0.35	0.50	0.09	0.12
75 th Percentile (mg/L)	0.60	0.70	0.60	0.60	0.15	0.15
95 th Percentile (mg/L)	0.80	0.80	0.70	0.70	0.16	0.16
Number of EICs <0.1 mg/L	8/60	8/60	7/60	6/60	31/60	25/60
Number of EICs <0.2 mg/L	21/60	14/60	19/60	12/60	60/60	60/60
Number of EICs >0.2 mg/L	34/60	41/60	34/60	42/60	0/60	0/60
Number of EICs >0.5 mg/L	17/60	21/60	20/60	19/60	0/60	0/60
Hatcheries with a Settling Pond						
Mean (mg/L)	0.33	0.35	0.33	0.38	0.09	0.10
Median (50 th percentile, mg/L)	0.35	0.30	0.30	0.50	0.10	0.12
75 th Percentile (mg/L)	0.50	0.50	0.60	0.60	0.15	0.15
95 th Percentile (mg/L)	0.71	0.71	0.70	0.70	0.16	0.16
Number of EICs <0.1 mg/L	6/40	6/40	5/40	4/40	20/40	17/40
Number of EICs <0.2 mg/L	16/40	11/40	14/40	9/40	40/40	40/40
Number of EICs >0.2 mg/L	22/40	25/40	22/40	26/40	0/40	0/40
Number of EICs >0.5 mg/L	8/40	9/40	12/40	11/40	0/40	0/40
Hatcheries without a Settling Pond						
Mean (mg/L)	0.47	0.55	0.40	0.45	0.09	0.11
Median (50 th percentile, mg/L)	0.50	0.65	0.40	0.50	0.09	0.13
75 th Percentile (mg/L)	0.80	0.80	0.70	0.70	0.16	0.16
95 th Percentile (mg/L)	0.80	0.80	0.70	0.70	0.16	0.16
Number of EICs <0.1 mg/L	2/20	2/20	2/20	2/20	11/20	8/20
Number of EICs <0.2 mg/L	5/20	3/20	5/20	3/20	20/20	20/20
Number of EICs >0.2 mg/L	12/20	16/20	12/20	16/20	0/20	0/20
Number of EICs >0.5 mg/L	9/20	12/20	8/20	8/20	0/20	0/20

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;
Fish acute study	EC ₅₀	1,000	Extrapolation to field/community level effects
Tier B			
Algal growth inhibition (72 h)	NOEC	10	Extrapolation from lab/single species test to field/community level effects
<i>Daphnia magna</i> reproduction (fresh) / crustacean chronic study (brackish)	NOEC	10	
Fish early-life stage	NOEC	10	
Sediment invertebrate toxicity	NOEC	10	

Table 11. Risk characterization of Chloramine-T based on the VICH Phase II Tier A and Tier B assessment factors.

Species	Assessment Endpoint and Value, mg/L ^a	VICH AF ^b	PNEC (mg/L)	RQ at 1-d EIC				RQ at 5-d EIC				RQ at 21-d EIC			
				Mean	Median	75 th Percentile	95 th Percentile	Mean	Median	75 th Percentile	95 th Percentile	Mean	Median	75 th Percentile	95 th Percentile
Tier A (Acute)															
Green algae <i>Scenedesmus subspicatus</i> (acute)	48-h EC ₅₀ = 0.31	100	0.0031	119	129	194	800/ 3.1	113	113	194	226	29	29	48	52
<i>Daphnia magna</i> (acute)	24-h EC ₅₀ = 4.8	1000	0.0048	77	83	125	167	73	73	125	146	19	19	31	33
Channel catfish <i>Ictalurus punctatus</i> (acute)	96-h LC ₅₀ = 1.8	1000	0.0018	206	222	333	444	194	194	333	389	50	50	83	89
Tier B (Chronic)															
Axcentive proprietary, <i>Selenastrum capricornutum</i> (chronic)	96-h NOEC = 0.2	10	0.02	19	20	30	40	18	18	30	35	4.5	4.5	7.5	8
Axcentive proprietary, <i>Daphnia magna</i> (chronic)	21-d NOEC = 1.1	10	0.11	3.4	3.6	5.5	7.3	3.2	3.2	5.5	6.4	0.8	0.8	1.4	1.5
Axcentive proprietary, Fathead minnow. <i>Pimephales promelas</i> (chronic)	35 -d NOEC = 1.1	10	0.11	3.4	3.6	5.5	7.3	3.2	3.2	5.5	6.4	0.8	0.8	1.4	1.5

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

^a The lowest toxicity value was used for this assessment even when data reliability could not be assessed.

^b Except for channel catfish, these AFs do not account for the potential influence of lower pH conditions on the toxicity of chloramine-T.

Table 12. Acute risk characterization of Chloramine-T based on refined assessment factors.

Species	Assessment Endpoint and Value, mg/L ^a	Refined AF ^{b,c}	Acute PNEC (mg/L)	RQ at 1-d EIC				RQ at 5-d EIC			
				Mean	Median	75 th Percentile	95 th Percentile	Mean	Median	75 th Percentile	95 th Percentile
Green algae <i>Scenedesmus subspicatus</i>	48-h EC ₅₀ = 0.31	10 ^d	0.031	12	13	19	26	11	11	19	23
<i>Daphnia magna</i>	24-h EC ₅₀ = 4.8	50 ^e	0.096	3.9	4.2	6.3	8.3	3.7	3.7	6.3	7.3
Channel catfish <i>Ictalurus punctatus</i>	96-h LC ₅₀ = 1.8	10 ^f	0.18	2.1	2.2	3.3	4.4	1.9	1.9	3.3	3.9

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

^a The lowest toxicity value was used for this assessment even when data reliability could not be assessed.

^b These AFs do not account for the potential influence of lower pH conditions on the toxicity of chloramine-T.

^c The standard AFs presented are consistent with U.S. EPA methodology used to evaluate risk of pesticides to non-endangered aquatic species; Some U.S. EPA offices /divisions use different AFs in risk assessment.

^d An AF of 10 was used was applied to extrapolate from an acute EC₅₀ to an acute NOEC. Effect of pH on this endpoint is not known.

^e An AF of 10 was applied to extrapolate from an acute 24-h EC₅₀ to an acute 48-h NOEC. Because of deficiencies in the database and inconsistencies in the data, an additional AF of 5 was applied. Effect of pH on this endpoint is not known.

^f An AF of 10 was applied to extrapolate from an acute LC₅₀ to an acute NOEC. Effect of pH has been evaluated for this endpoint.

Table 13. Cumulative percent mortality of several species of fish at 96 h after the last of four exposures. Fry were exposed for 60- or 180-min chloramine-T treatments administered once daily for four consecutive days, in hard, circum-neutral pH water. Although not presented, most mortality occurred within 24 h of the initial exposure. Data represent the summed percent of treated fish in three aquaria per concentration and 10 fish per aquaria. (Data from UMESC Study # CAP-99-CLT-01, M. P. Gaikowski, Study Director).

Species	Temperature (±2 °C)	Duration (min)	Percent mortality (%) at the given chloramine-T concentration (mg/L)				
			0	20	60	100	200
Northern pike <i>Esox lucius</i>	20	60	3.7	0	3.7	3.7	96.7
Lake sturgeon <i>Acipenser fulvescens</i>	20	60	0	0	0	0	0
Walleye <i>Stizostedion vitreum</i>	15	60	0	0	0	0	0
	20	60	0	0	0	0	0
	25	60	0	0	0	0	66.7
	20	180	0	0	0	3.3	100
Channel catfish <i>Ictalurus punctatus</i>	22	60	0	0	0	0	3.3
	27	60	0	0	0	0	83.3
	32	60	0	0	0	3.3	100
	27	180	0	0	43.3	100	100
Largemouth bass <i>Micropterus salmoides</i>	27	60	0	0	0	6.7	0

Table 14. Chronic risk characterization of Chloramine-T based on refined assessment factors.

Species	Assessment Endpoint and Value, mg/L ^a	Refined AF ^{b,c}	Chronic PNEC ^a (mg/L)	RQ at 21-d EIC			
				Mean	Median	75 th Percentile	95 th Percentile
Axcentive proprietary, <i>Selenastrum capricornutum</i>	96-h NOEC = 0.2	10 ^d	0.02	4.5	4.5	7.5	8.0
Axcentive proprietary, <i>Daphnia magna</i>	21-d NOEC = 1.1	10 ^e	0.11	0.8	0.8	1.4	1.5
Axcentive proprietary, Fathead minnow. <i>Pimephales promelas</i>	35 -d NOEC = 1.1	10 ^f	0.11	0.8	0.8	1.4	1.5

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

^a The lowest toxicity value was used for this assessment even when data reliability could not be assessed.

^b These AFs do not account for the potential influence of lower pH conditions on the toxicity of chloramine-T.

^c The standard AFs presented are consistent with U.S. EPA methodology used to evaluate risk of pesticides to non-endangered aquatic species; Some U.S EPA offices /divisions use different AFs in risk assessment.

^d The 48-hour study that reported the most sensitive EC₅₀ did not report a NOEC. An AF of 10 was applied for possible interspecies variability. Effect of pH on this endpoint is not known.

^e Consistent with U.S. EPA methods. Effect of pH on this endpoint is not known.

^f An AF of 10 was considered to be appropriate because a chronic study using the most sensitive fish species in acute studies and more sensitive study conditions were not available.

Table 15. Available chloramine-T acute toxicity database for derivation of final acute value (Stephan et al. 1985, EPA 1991, 1994).

ROI [Genus Count]	Endpoint and Value, mg/L	GMAV (Rank)	Selection Comment
<i>Daphnia magna</i>	24-h LC ₅₀ = 4.8		No, 48-h LC ₅₀ is available
	48-h LC ₅₀ = 4.5	4.5 (4)	OK
<i>Ceriodaphnia dubia</i>	48-h LC ₅₀ = 2.12 – 8.88		No, done in various Pennsylvania surface waters, not lab water
Channel catfish	96-h EC ₅₀ = 1.8	1.8 (1)	OK
<i>Ictalurus punctatus</i>	Soft water, pH = 6.5		
Rainbow trout	96-h EC ₅₀ = 1.9	1.9 (2)	OK
<i>Oncorhynchus mykiss</i>	Soft water, pH = 6.5		
Striped bass	96-h EC ₅₀ = 2.8	2.8 (3)	OK
<i>Morone saxatilis</i>	Soft water, pH = 6.5		
Harlequin fish	96-h EC ₅₀ = 7		No, too many fish
<i>Rasbora heteromorpha</i>	Soft water, pH = 6.0		
Roach	96-h LC ₀ = 35		No, too many fish
<i>Rutilus rutilus L</i>	Hard water, pH = 7.8		
Northern pike	24-h LC ₅₀ = 60-70 Hard		No, too many fish
<i>Esox lucius L</i>	water, pH = 8.2		
Guppy	96-h LC ₅₀ = 31		No, too many fish
<i>Poecilia reticulata</i>			
Fathead minnow	96-h LC ₅₀ = 6.16-28.1		No, too many fish, done in various Pennsylvania surface waters, not lab water
<i>Pimephales promelas</i>			
Brine shrimp, <i>Artemia nauplii</i>	72 h EC ₅₀ = 24.6		No, brine shrimp are not to be used in this EPA calculation, even for marine, because their habitat is too salty to even represent marine species.

Table 16. Secondary Acute Factors (reprinted from EPA 1995).

Number of minimum data requirements satisfied	Adjustment factor
1	21.9
2	13.0
3	8.0
4	7.0
5	6.1
6	5.2
7	4.3

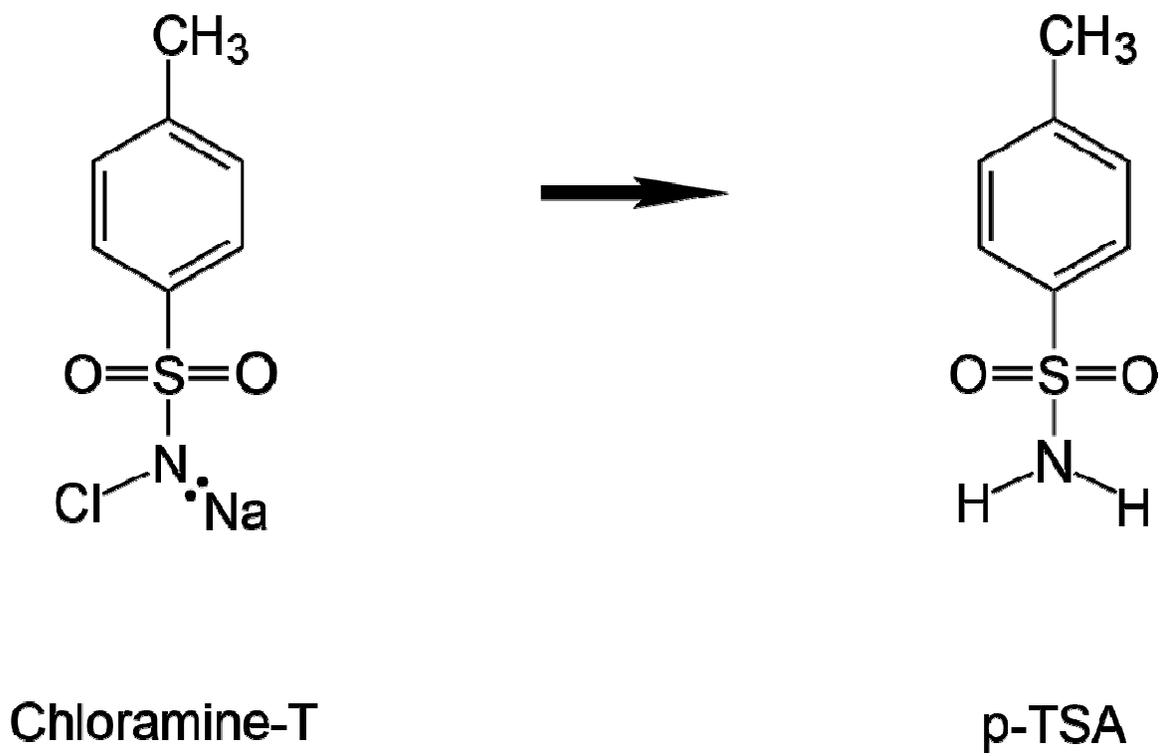


Figure 1. Chemical structure of chloramine-T (*N*-chloro-*p*-toluenesulfonamide sodium salt) and *p*-toluenesulfonamide (*p*-TSA).

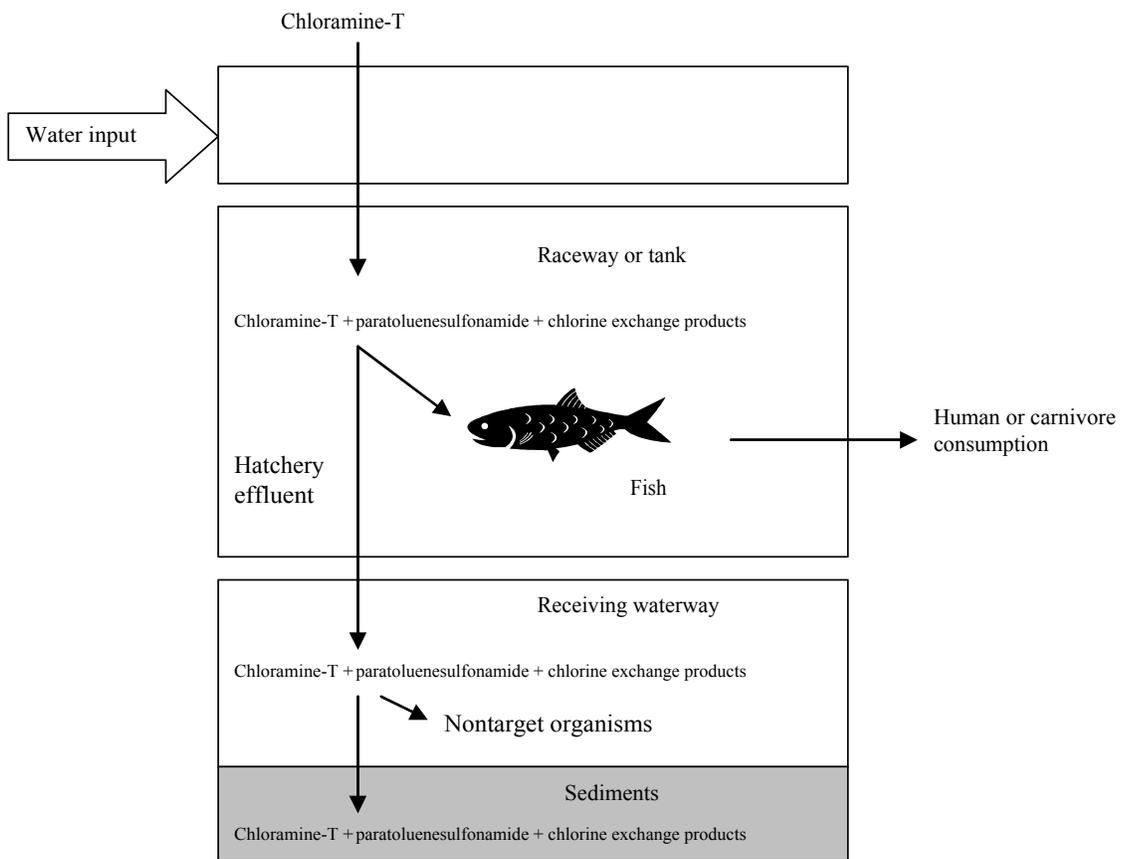


Figure 2. Conceptual model of the fate of chloramine-T used in intensive aquaculture.

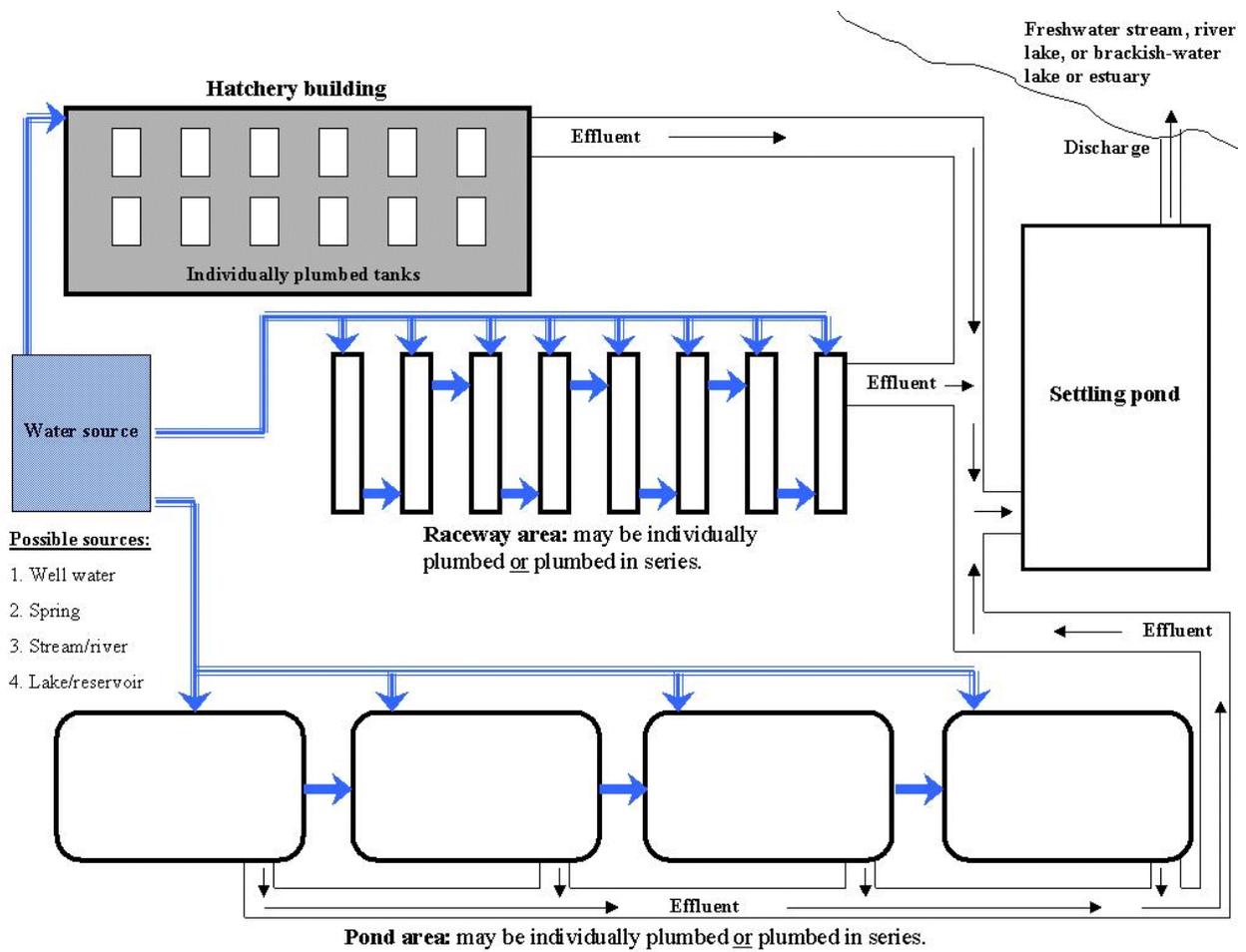
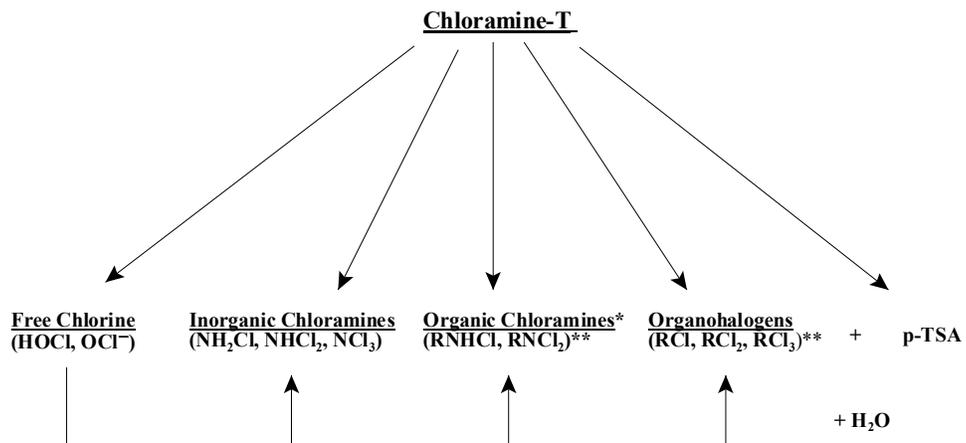


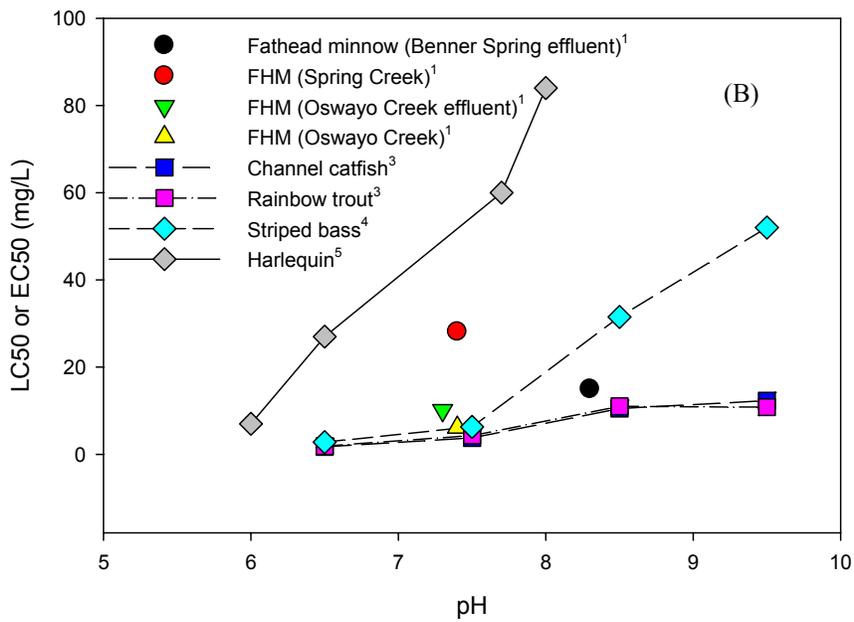
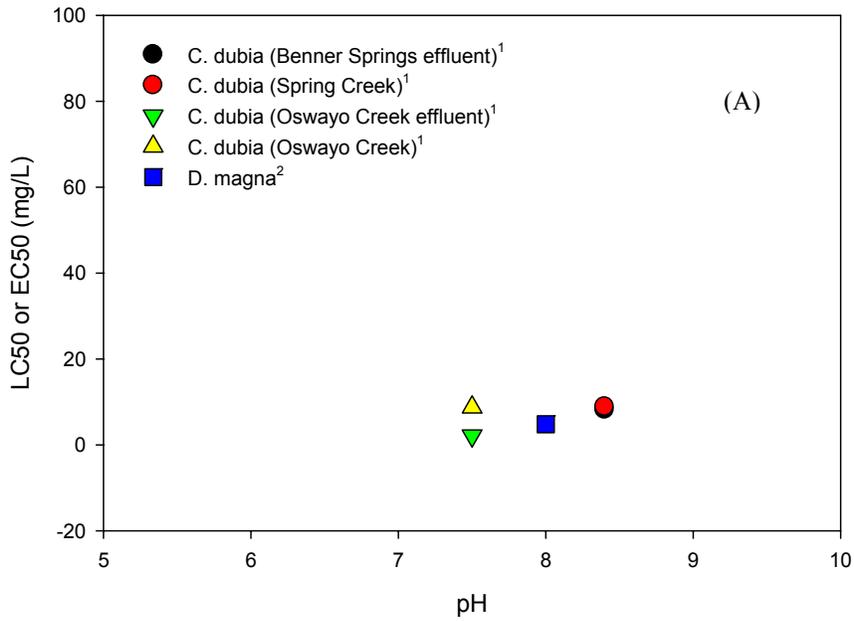
Figure 3. Conceptual diagram of a typical intensive aquaculture facility.



*Includes chlorinated amines, amides, amino acids, and peptides.

**R = an organic substituent of varying molecular size and number of carbon and hydrogen atoms. Substituents containing S, O, P, and nonamino-N in addition to carbon and hydrogen are also possible. Chlorination of organic compounds beyond monochlorination seldom occurs as a result of aqueous chloramine-T therapies.

Figure 4. Possible covalently bonded chlorine exchange or donation products of chloramine-T.



¹ Analytical Laboratory Services 2003

² Kühn

³ Bills et al. 1988b

⁴ Bills et al. 1993

⁵ Cross and Hursey 1973

Figure 5. Influence of pH on acute toxicity of chloramine-T to both invertebrates (A) and freshwater fish (B)

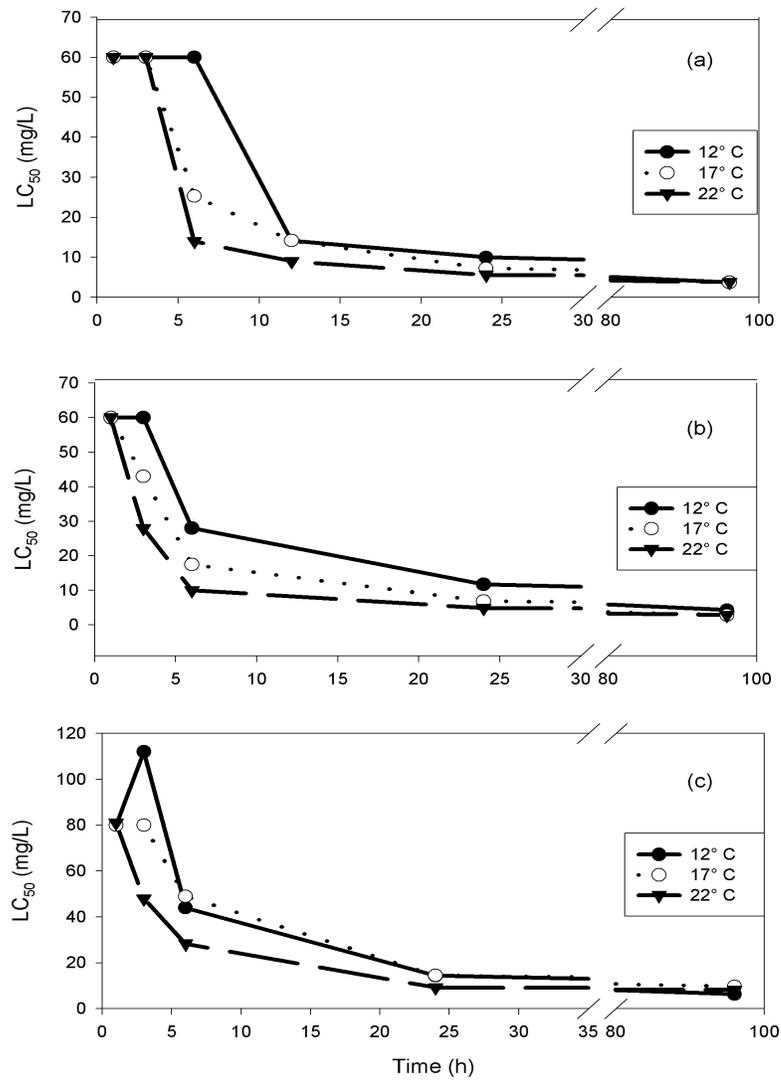


Figure 6. Toxicity of chloramine-T to channel catfish *Ictalurus punctatus* (a), rainbow trout *Oncorhynchus mykiss* (b), and striped bass *Morone saxatilis* (c) at three temperatures. Mortality (LC₅₀) was determined at 1, 3, 6, 12, 24, and 96 h for channel catfish and rainbow trout and 1, 3, 6, 24, and 96 h for striped bass (Bills et al. 1988b, 1993).

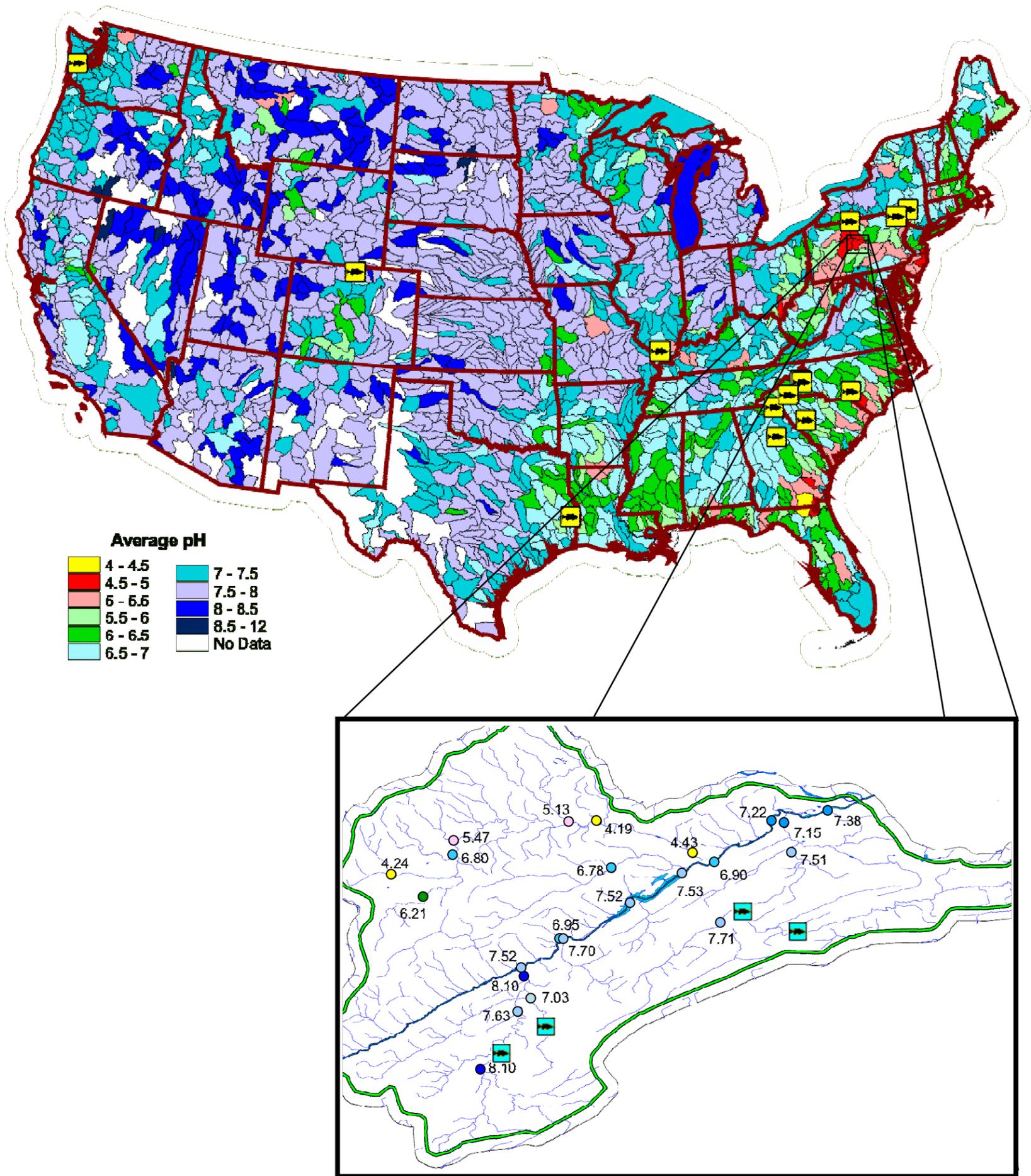


Figure 7. Average pH of continental US surface waters by hydrologic unit code (HUC). A HUC represents the generally accepted geographic boundaries of specific watershed drainage areas or distinct hydrologic features. Yellow tags identify locations of hatcheries that reported soft acidic culture water in a survey of public and private fish hatcheries. The exploded view presents the average pH of the various water sampling sites within the Bald Eagle HUC from Northcentral Pennsylvania. The overall average pH of the Bald Eagle HUC is between 4.5-5, however, all of the hatcheries located within this drainage area discharge into neutral or alkaline surface water. Hatcheries located in this HUC (blue tags) reported use of neutral to alkaline, moderately hard to hard water to culture fish in a survey of public and private fish hatcheries.

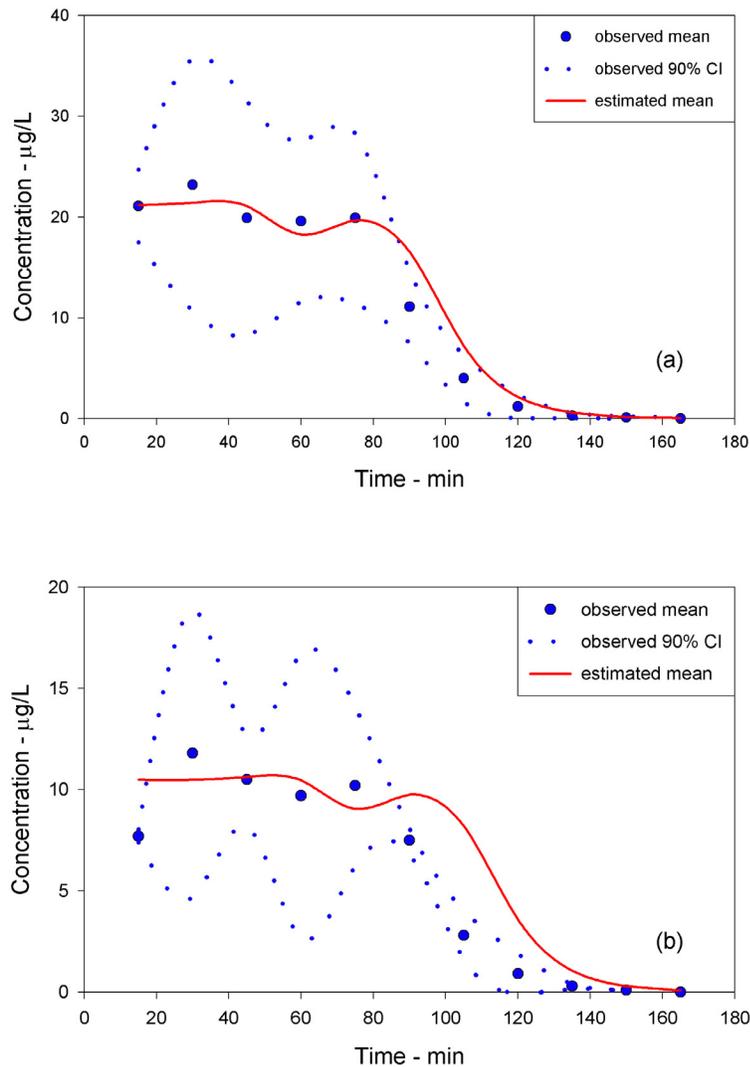


Figure 8. Mean observed rhodamine WT concentration (solid circles; 90% confidence interval [CI] dotted lines) and estimated mean rhodamine WT concentration (solid line) after continuous flow treatment of a production raceway at Upper Midwest Environmental Sciences Center (UMESC). The data are from two rhodamine WT treatments designed to maintain a rhodamine WT concentration of 100 $\mu\text{g/L}$ in the raceway for 60 min. Data presented were based on rhodamine WT concentration determined from samples withdrawn from sample sites A (a) and B (b) every 15 min following the initiation of the raceway treatment. Sample sites A and B represent $\sim 47\%$ and $\sim 100\%$ of UMESC effluent flow, respectively. Data are from Gaikowski et al. (2004).

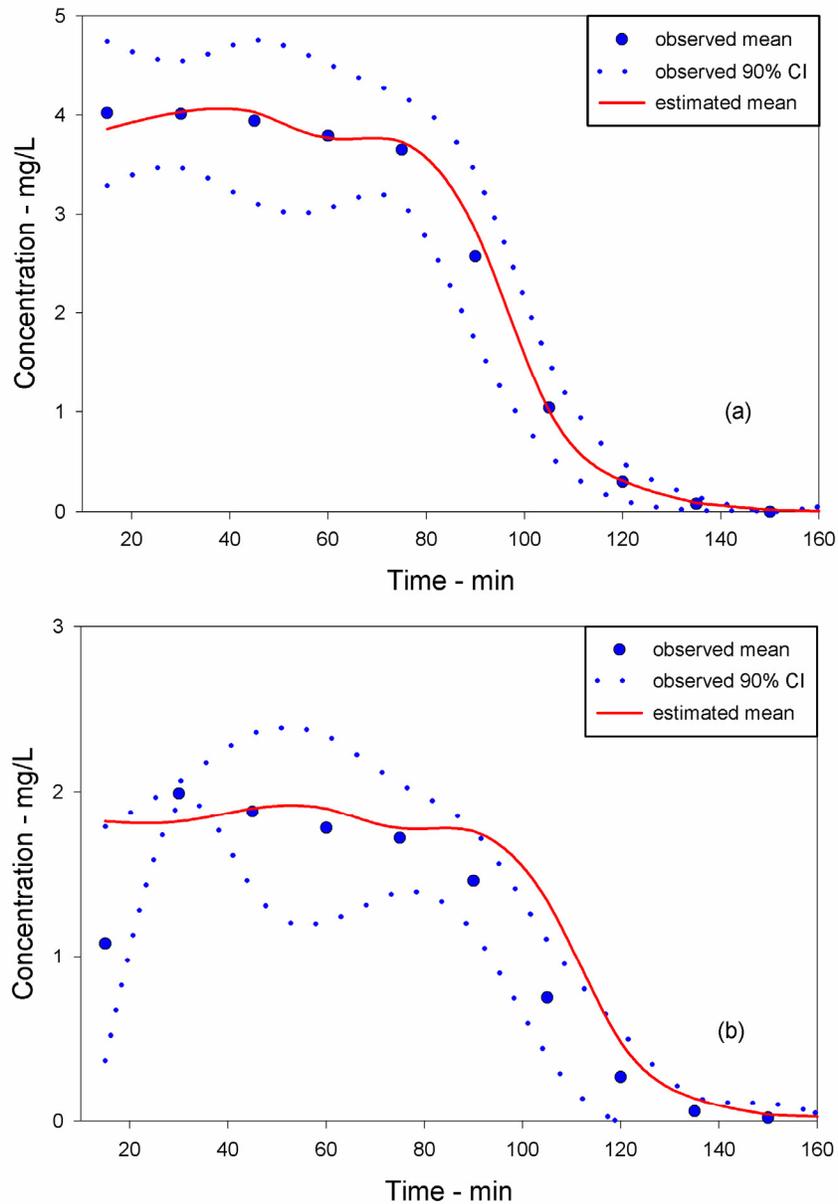


Figure 9. Mean observed chloramine-T concentration (*solid circles*; 90% confidence interval [CI] *dotted lines*) and estimated mean chloramine-T concentration (*solid line*) after continuous flow treatment of a production raceway at Upper Midwest Environmental Sciences Center (UMESC). The data are from four chloramine-T treatments designed to maintain a chloramine-T concentration of 20 mg/L in the raceway for 60 min. Data presented were based on chloramine-T concentration determined from samples withdrawn from sample sites A (a) and B (b) every 15 min following the initiation of the raceway treatment. Sample sites A and B represent ~47% and ~100% of UMESC effluent flow, respectively. Data are from Gaikowski et al. (2004).

Appendix A. Calculations Used to Estimate Hatchery Treatment and Discharge Parameters

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Appendix A. Section 1. Hatchery survey calculations. The following equations were used to estimate physical parameters of each hatchery during chloramine-T fish treatments for typical and worst-case scenarios. The survey data found in Appendix A, Sections 2 and 3 were used in the calculations to estimate chloramines-T use and generate the EIC found in Appendix A, Sections 4-6:

Hatchery discharge at average flow (L/m):

$$\text{Average hatchery water flow (gal/d)} \times 3.785 \text{ (L/gal)} / 1,440 \text{ (min/d)}$$

Hatchery discharge at minimum flow (L/m):

$$\text{Minimum hatchery water flow (gal/d)} \times 3.785 \text{ (L/gal)} / 1,440 \text{ (min/d)}$$

NOTE: Average hatchery water flow was used if no minimum water flow was reported.

Time to perform two volume exchanges (min):

$$\text{Sum of treated culture unit volume} \times 2 / \text{sum of maximum flow to the culture units}$$

NOTE: Culture unit volume and maximum flow per culture unit must have similar units (L or gal)

Settling pond volume (L):

$$\text{Pond volume (acre-feet)} \times (1,233,342 \text{ L} / \text{acre-foot})$$

Maximum daily treated volume (L):

Flow-through treatment

$$\text{Treatment duration (min)} \times \{ \{ \text{maximum number of type 1 culture units treated per day} \times \text{maximum flow per type 1 culture unit (gpm)} \} + \{ \text{maximum number of type 2 culture units treated per day} \times \text{maximum flow per type 2 culture unit (gpm)} \} + \dots \} \times 3.785 \text{ (L/gal)}$$

Static treatment

$$\text{Maximum number of culture units treated daily} \times \text{culture unit volumes (L)}$$

Maximum chloramine-T applied (mg):

$$\text{Maximum daily treated volume (L)} \times \text{Maximum treatment concentration (mg/L)}$$

Effluent concentration after settling pond (mg/L)

The term “hatchery water flow” in the following equations is replaced by hatchery average water flow (L/m) to estimate the typical EIC or hatchery low water flow (L/m) to estimate the worst-case EIC. Fish were assumed to receive four 60-min treatments at 20 mg/L as a static or flow-through treatment administered once daily on consecutive days.

1-d EIC

$$\text{Max chloramine-T (mg) applied} / \{ \{ \text{hatchery water flow (L/m)} \times 1,440 \text{ min/d} \times 1 \text{ d} \} + \text{settling pond volume (L)} \}$$

5-d EIC

$$\text{Max chloramine-T (mg) applied} \times 4 \text{ treatments} / \{ \{ \text{hatchery water flow (L/m)} \times 1,440 \text{ min/d} \times 5 \text{ d} \} + \text{settling pond volume (L)} \}$$

21-d EIC

$$\text{Max chloramine-T (mg) applied} \times 4 \text{ treatments} / \{ \{ \text{hatchery water flow (L/m)} \times 1,440 \text{ min/d} \times 21 \text{ d} \} + \text{settling pond volume (L)} \}$$

**Appendix B. 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
+++++



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 CaCO₃)

Hardness Average:

Hardness Minimum:

Hardness Maximum:

Alkalinity (mg/L as CaCO₃)

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**)

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 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 1 - hydrogen peroxide:

TR 1 - chloramine-T:

TR 1 - oxytetracycline:
TR 1 - potassium permanganate:

What is the dose administered?

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

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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.

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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 CaCO₃)

Hardness Average:
Hardness Minimum:
Hardness Maximum:

Alkalinity (mg/L as CaCO₃)

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):

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END SURVEY

**Appendix C. Potential of Chloramine-T to Produce
Residual Free Chlorine at Concentrations of Concern**

Chloramine-T is a slow-release chlorinating agent. The detailed hydrolysis mechanism of chloramine-T varies with pH and is quite complex (Agrawal and Upadhyay 1990). The hydrolysis mechanism involves the production of aqueous free chlorine ($\text{HOCl} + \text{OCl}^-$) species, which are quite toxic to aquatic life (Mattice and Tsai 1983; EPA 1985). However, the kinetics of chloramine hydrolysis are slow and rate limiting compared with those where free chlorine oxidizes another organic amine or some other organic-N or non-N compound. Usually the reaction produces a compound much less toxic than free chlorine (Isaac and Morris 1983b; Mattice and Tsai 1983). Under many circumstances, chloramines also lose chlorine through a direct chlorination mechanism (i.e., no free-chlorine species is involved as an intermediate; Isaac and Morris 1985; Yoon and Jensen 1993). While the basics of chloramine chemistry are quite complex and also influenced by commonly encountered environmental conditions, no stable free-chlorine species will result until residual free chlorine is produced by sufficient addition of a chlorinating species (e.g., hypochlorite ion or a reactive organic chloramine) to water.

From a practical empirical standpoint, the tendency for free chlorine, chloramine-T, or some other chloramine to produce concentrations of residual free chlorine follows breakpoint chlorine chemistry, which asserts that a stable free-chlorine species will not be produced until all chlorine demand is met, and that all chlorine demand is met at breakpoint (Figure C-1; White 1999, page 229). Chlorine demand is defined as the difference between the amount of chlorine added to the water (in some available form) and the amount of chlorine (free available or combined available) remaining at the end of a specified contact period (White 1999, page 376). The exact amount of chlorine needed to reach the breakpoint concentration is difficult to predict because it varies with contact time, chlorine demand level, the reactivity of the chlorinating compound added, and the reactivity of compounds (nitrogen containing or otherwise) creating the chlorine demand. In addition, as breakpoint nears, multiple chlorination of some (usually amine or amino) compounds begins to occur. Interestingly, the amount of available chlorine then actually decreases as more chlorine is added, but this condition is sharply reversed at breakpoint, after which available chlorine increases directly according to the amount added (Figure C-1). Chlorine demand as a useful concept is, therefore, empirical and almost always rate-governed and time dependent. Chlorine demands of treated or receiving waters are often expressed as 20-, 60-min, etc., demands; water treatment professionals use the term “a water” to emphasize the non-generalizability of any chlorine demand curve empirically generated for a given treatment water.

Until all chlorine demand is met, available chlorine exists as organically bound chlorine (mostly chloramines). The situation is simplest when free chlorine (e.g., hypochlorous acid or hypochlorite ion) is used to satisfy chlorine demand because it reacts relatively quickly until it reduces chlorine demand to low concentrations. Using chloramines to satisfy the demand takes more time because both the hydrolysis and direct chlorination mechanisms of chloramines are relatively slow, to the point where only a few chloramines are useful as chlorinating agents. Although the same molar quantities of free chlorine or chloramine are theoretically required to reach breakpoint in a given water, it will take a substantially larger concentration of an organic chloramine to reach breakpoint within a given short period, thereby producing residual free chlorine.

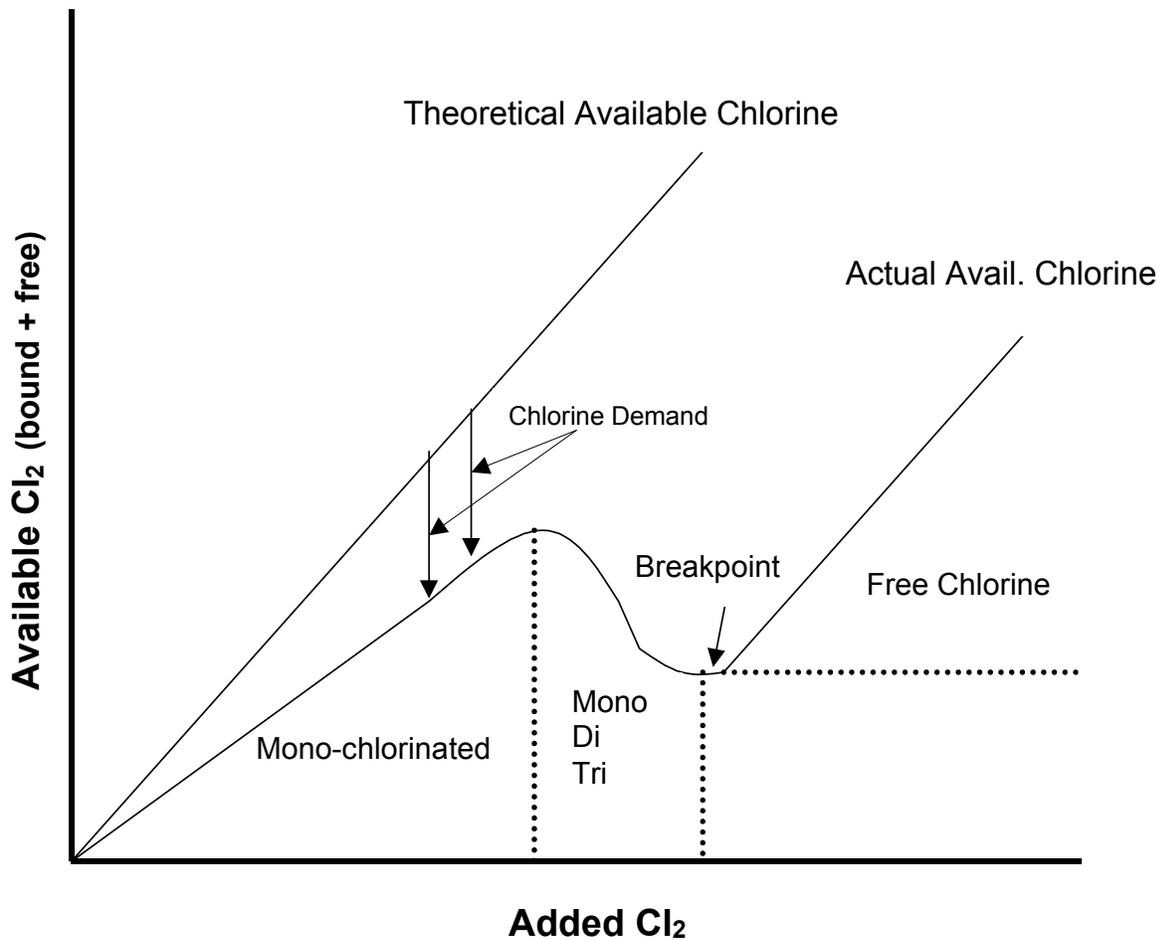


Figure C-1. Typical chlorine breakpoint curve.

Intake (source) water for potable water production is generally conceded to have a total ammonia-N ($\text{NH}_4^+ + \text{NH}_3$) concentration of 0.2 mg/L at the highest and 0.3 mg/L organic-N concentration at the highest (White 1999, page 390). In potable water, the resulting 0.5 mg/L concentration would create a minimum (assumes only monochlorination of N and no organic non-N demand) chlorine demand of about 2.5 mg/L as Cl_2 ($0.5 \text{ mg/L} \times 70.9/14$ [MW of Cl_2 / MW of N]) and would require the equivalent of about 9.9 mg/L of chloramine-T (2.5×3.97 [MW chloramine-T / MW Cl_2]) to eventually reach the theoretical breakpoint. Assuming that the cleanest hatchery waters have about the same chlorine demand as typical potable treatment intake water, some generation of residual free chlorine is possible in such water at our proposed maximum therapy concentrations (20 mg/L) if about half (9.9 mg/L) of the chloramine-T is consumed during the duration of treatment (about 1 h). This would happen if chloramine-T reacts quickly to reduce chlorine demand in a manner similar to OCl^- or HOCl . Such rapid consumptions of chloramine-T in relatively clean aquaculture waters do not take place, even in the presence of fish, as demonstrated by analytical data from Bills et al. (1988a) and in method development studies conducted at the Upper Midwest Environmental Sciences Center (Jeffrey Meinertz, Research Physiologist, U.S. Geological Survey,

personal communication). Because of the slow chlorinating behavior of chloramine-T, it would probably take several days at a 20-mg/L treatment concentration to reach breakpoint in relatively clean waters (the most likely waters to yield a free-chlorine residual because of their low chlorine demand). Once chloramine-T concentrations are diluted to less than 9.9 mg/L after treatment, reaching breakpoint is no longer even theoretically possible in our clean water model. At concentrations less than the breakpoint, chloramine-T (or any other chloramine) is unlikely to produce any measurable free chlorine because the insignificant amount of chlorine produced by chloramine hydrolysis reacts almost instantly with a myriad of compounds that constitute the remaining chlorine demand.

The literature seems to support our calculation above for the minimum chlorine demand of water containing a sum of 0.5 mg/L total ammonia plus organic N. In the literature, a breakpoint curve was empirically determined for a similar actual water containing 0.3 mg/L total ammonia nitrogen and 0.3 mg/L organic nitrogen (sum of 0.6 mg/L total ammonia plus organic N), with the latter being in about a 1:1 ratio of simple amino acids:proteinaceous material (White 1999, page 249). After 1 to 2 h of contact time with chlorine ($\text{HOCl} + \text{OCl}^-$) at pH 7–8, the breakpoint occurred at 5 mg/L as Cl_2 . The minimum chlorine demand value according to our calculation above should only be 3.0 mg/L as Cl_2 ($0.6 \text{ mg/L} \times 70.9/14 [\text{MW Cl}_2/\text{MW N}]$). The empirical value is probably higher because in the actual situation (1) multichlorination of amine or amino compounds probably took place close to the breakpoint, (2) some inorganic demand (e.g., Fe^{+2} or Mn^{+2} , which reacts with available chlorine to produce Cl^- ions) or organic non-N demand was present in the water. The 1- to 2-h contact time should be sufficient for reactions involving free chlorine with ammonia or organic-N compounds to proceed to completion. If chloramine-T had been used instead of free chlorine, much higher concentrations would have been required to reach a 1- to 2-h breakpoint at 0.6 mg/L total ammonia plus organic N because of the much slower reactivity of chloramine-T.

To summarize this discussion of chloramine behavior, even at concentrations well above potential breakpoint, the slow release of chlorine by chloramine-T (either by hydrolysis or direct chlorination) should severely limit the amount of free chlorine actually found in solution compared to that found if an equivalent amount of free chlorine were added. Work done by Gottardi (1992) supports this contention. He found only 0.015 to 0.030 mg/L of free chlorine in an aqueous solution of chloramine-T at 1,000 mg/L at pH values that typify natural waters (pH 6–8). This chloramine-T concentration (about 250 mg/L as Cl_2) would be well above breakpoint if the kinetics of chloramine-T chlorination reactions were fast. The proposed therapy concentration (about 5 mg/L maximum as Cl_2) and our proposed maximum discharge concentration limit (0.16 mg/L as Cl_2) at hatcheries are well below Gottardi's (1992) 250-mg/L experimental concentration that produced free chlorine at concentrations within or close to the discharge range allowed by the national EPA criteria (EPA 1985).

Once treatment waters are released into other hatchery waters, chloramine-T undergoes dilution as well as encountering additional chlorine demand from diluting waters. Even if the treatment water goes directly to discharge, it has been demonstrated above that no free chlorine will be present from the treatment discharge; rapid dilution in receiving waters and additional chlorine demand would assure that no free chlorine will be present thereafter.

Appendix D. Potential of Chloramine-T to Produce Inorganic Chloramines at Concentrations of Concern

Note: This appendix contains lengthy footnotes. Footnotes are used to keep the main lines of argument to the text, while still providing comprehensive information and data presentation. It is best if the text is read completely and then the footnotes are read.

Since the inorganic chloramines are much more toxic to aquatic life than chloramine-T, it is important to know the extent to which chloramine-T at treatment and hatchery discharge concentrations will exchange into inorganic (ammonia) chloramine in hatchery and public waters. In the presence of ammonia, chloramine-T has the potential to exchange into inorganic chloramines (mostly monochloramine) over long periods (weeks) according to the appropriate equilibrium ratios (Yoon and Jensen 1993). However, reaction rates will be the most important factor in determining exchange ratios over short periods (hours, days). The total ammonia-N ($\text{NH}_4^+ + \text{NH}_3$) to organic-N ratio is generally about 10:1 in nonnitrified wastewaters (Snyder and Margerum 1982) because of deliberate addition of ammonia by treatment plants. Yoon and Jensen (1993) conducted a laboratory study where they added ammonia to three pre-prepared model organic chloramines in aqueous solution at a ratio of 10:1 (total ammonia-N to organic-N). They found that chlorine transfer from these organic chloramines to ammonia to form monochloramine was small but significant. The three chloramines represent an amino acid (*N*-chloroglycine), a peptide (*N*-chloroglycylglycine), and an alkylamine (*N*-chloromethylamine). The amino acid had the highest rate constant, $3.84 \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$ (second order).

Although their model organic chloramines were capable of producing inorganic chloramine, these authors continued to demonstrate that monochloramine concentrations produced over time by each of their model compounds varied directly with total ammonia-N concentration. They varied total ammonia-N concentration from 10-fold to as much as 500-fold the concentration of each model compound's N. The 10-fold ratio is typical at wastewater plants, but higher ratios were needed to detect enough inorganic chloramine to demonstrate the effect of total ammonia-N concentration on monochloramine production. All three model compounds were prepared at 3.0 mg/L as Cl_2 for the study. The *N*-chloroglycine (the amino acid) produced much higher amounts of inorganic chloramine under these circumstances than did the other two model compounds.

The *N*-chloroglycine concentration (3.0 mg/L as Cl_2) as chloramine-T would have been 12 mg/L, and it produced 0.15 mg/L of inorganic chlorine in 60 min at a total ammonia-N to organic-N ratio of 10:1 according to the authors' results (Figure 1 in Yoon and Jensen 1993). This production roughly doubled with a tripling of total ammonia-N concentration at the levels used for their study. Since total ammonia as N is about 1.0 mg/L as a worst-case scenario in aquaculture treatment waters,¹ and 12 mg/L of chloramine-T is

¹Treatment waters represent the most severe situation with respect to presence of total ammonia in aquaculture waters. Total ammonia concentrations can be much higher in culture waters than in receiving waters because of the presence of fish excreting nitrogenous wastes as ammonia, while intake water might contain little organic-N, particularly if its source is well water. During a controlled study of benzocaine in edible tissue of rainbow trout *Oncorhynchus mykiss* conducted at the Upper Midwest Environmental Sciences Center, ammonia concentrations were measured before, during, and after exposures (Stehly et al. 1996) at 7 °C. Ammonia concentrations were monitored because the fish were maintained in a partly recirculating system that received less than 1 tank-volume exchange per hour during this study (actual fish loading values are not available). Average total ammonia concentrations were highest at 7 °C during the acclimation period and were about 1.2 mg/L (about 1 mg/L as N). Although solids (feces, excess food) were effectively removed by filtration and tank cleaning, the biofilter used to remove ammonia from the system was inefficient at the culture temperature. The 1.2-mg/L total ammonia resulted in a calculated value for NH_3 (un-ionized ammonia) of 0.0087 mg/L at the average acclimation temperature and pH (7.19 °C and 7.72). This culture situation probably represents excessive ammonia concentrations and is uncommon, although the NH_3 value is below the maximum concentration recommended by Piper et al. (1982) at 0.0125 mg/L for trout. This example is presented because it probably approximates a worst-case culture scenario for total ammonia concentration in raceways and tanks

about 0.6 mg/L as N, the 10:1 ratio of total ammonia-N:organic-N needed to produce the 0.15 mg/L of inorganic chloramine stated above would be about 6.0 mg/L total ammonia as N. The treatment water total ammonia-N concentration is sixfold less than this, which would result in a four times decrease in inorganic chloramine production according to the concentration versus production ratio of 3:2. From this, an estimate of 0.0375 mg/L (0.15 mg/L / 4) of inorganic chloramine might be produced from 12 mg/L of chloramine-T and 1.0 mg/L total ammonia-N, assuming that the reaction rate for chloramine-T is as rapid as for *N*-chloroglycine. Inorganic monochloramine (NH₂Cl) would be almost exclusively present in such dilute solutions, so the estimate would be 0.052 mg/L as Cl₂. At 20-mg/L treatments, the concentrations might be higher but probably not proportionately so. This concentration will not cause fish mortalities during the treatment period, but any discharge concentration over 0.011 mg/L as Cl₂ is of potential regulatory concern for TRC in some jurisdictions.

The 0.0375-mg/L estimate for inorganic chloramine in treatment waters is made on the basis of a worst-case total ammonia-N concentration of 1.0 mg/L. This concentration may not be reached, or nearly so, in most therapies because hatcheries usually feed fish at a reduced level during therapies to control mortality associated with bacterial gill disease. Depending on the management requirements, fish species, age, and husbandry practices, fish may be kept off food throughout the therapy period. Some species or life stages, however, may need to be fed daily throughout the therapy regimen. In those situations where feed is withheld for the entire therapy period or on treatment days, the total ammonia concentrations will be greatly reduced from even normal levels.

An element not included in the Yoon and Jensen (1993) study is the presence of other species, especially other organic-N compounds, competing for chlorine. The organic-N compounds on the average have a much higher affinity for chlorine than does ammonia. The organic-N compounds generally have faster reaction rates with chlorine compared with that for ammonia and equilibrium constants that favor organic chloramine production (Wolfe et al. 1985). The reaction kinetics of organic-N compounds produce an undesirable consequence for wastewater disinfection (poor disinfection); therefore, excess ammonia is deliberately introduced to counteract the preference of available chlorine for organic amines (Wolfe et al. 1985). The presence of organic-N compounds in aquaculture treatment water would significantly reduce

where trout are expected to remain healthy and grow. Morgan et al. (1998) considered 70 µM (about 1 mg/L as N) to be elevated total ammonia from a toxicity standpoint for rainbow trout.

We present this total ammonia concentration measured during trout culture in a recirculating system and subsequently use this concentration to estimate inorganic chloramine production, even though chloramine-T will probably be used in warm-water aquaculture as well as in cold-water aquaculture. Warm-water species may be more tolerant to ammonia than trout (Thurston and Russo 1983; Thurston et al. 1983). However, for all fish species, most fish culturists follow the limits suggested by Piper et al. (1982) to increase growth and decrease the likelihood of disease (Jim Luoma, Fish Culturist, Upper Midwest Environmental Sciences Center, personal communication). Because of the lack of domestication of many warm-water species as compared to salmonids, fish culturists often reduce the loading density of warm-water species to reduce cannibalism, thereby effectively reducing the total ammonia concentrations likely to be present during treatment. Culture water used for warm-water aquaculture is also generally more eutrophic than for the water used for salmonid culture, especially if obtained from surface water sources. Although neither warm-water nor cold-water aquaculture operations are likely to have high total ammonia loadings, the actual amount of total ammonia present (and, therefore, the potential for inorganic chloramine production) in warm-water aquaculture treatment water is probably much less than in the treatment water for salmonids because fish loading and feeding rates are generally much less (ibid). Therefore, we consider the total ammonia concentration estimates provided in the preceding paragraph to represent a maximum expected total ammonia concentration in treatment water.

the 0.0375-mg/L value calculated for inorganic chloramine from the Yoon and Jensen (1993) study.² All elements taken together, as discussed above, suggest that inorganic chloramine is not produced in concentrations of concern in treatment waters.

Other studies suggest the same conclusion. Bills et al. (1988a) investigated the effects of organic matter on the toxicity of chloramine-T to fathead minnow *Pimephales promelas*. The authors found that chloramine-T toxicity to fish either remained the same or significantly decreased as amounts of fish food or fecal material were increased in test solutions. Waterborne chloramine-T concentrations also significantly decreased during the exposure periods. The reduced toxicities elicited during chloramine-T exposures that Bills et al. (1988a) observed in the presence of organic compounds support the hypothesis that the chlorine from chloramine-T primarily reacts with organic-N compounds to produce less toxic organic chloramines, instead of the much more toxic inorganic chloramine. If even relatively small amounts of inorganic chloramine had been produced as well, greatly increased toxicities over the values for relatively pristine water would have resulted.

Once it is produced, inorganic chloramine may be reduced in the presence of organic amine or amino compounds from fish food and fecal material or from other sources by a further means. Any inorganic chloramine produced is quite reactive with most organic amine or amino or peptide compounds it subsequently encounters (Snyder and Margerum 1982). Reaction mostly occurs by direct transfer rather than by hydrolysis, with NH_3Cl^+ acting as an active chlorinating agent (ibid). It is not clear how important the subsequent interaction of organic-N with inorganic chloramine might be when treatment water is released to larger hatchery waters, although the result will always be a reduction of inorganic chloramine concentrations. Organic-N is not a parameter commonly measured in hatchery water, and, thus, we have no means of assessing its impact on inorganic chloramine produced in treatment waters.

As for public surface waters, despite the fact that ammonia is present in fish excretions and is also heavily used agriculturally, it is often not present in measurable quantities in surface waters. Nearly all intake sources for potable water production contain less than 0.2 mg/L total ammonia as N (White 1999, page 390). Even in a highly eutrophic and agriculturally influenced river like the Mississippi River, total ammonia concentration generally does not exceed 0.1 mg/L as N (Bill Richardson, U.S. Geological Survey, personal communication). This is due in part to volatilization and in part to its reactive nature and rapid uptake by living aqueous plants, including phytoplankton (ibid). Using the same calculation from Yoon and Jensen (1993) that was done for treatment water would yield an estimation of 0.0075-mg/L inorganic chloramine when total ammonia in surface water is 0.2 mg/L. This value is one-fifth of the 0.0375-mg/L inorganic chloramine concentration value estimated for treatment water at a total ammonia concentration of 1.0 mg/L. The 0.0075-mg/L concentration would not be attained in an actual discharge situation because chloramine-T would be rapidly diluted by receiving waters during the 60-min reaction period used for the calculation and would be even more diluted if a longer period had been used. Another element, in addition to continuous dilution by receiving waters, is the presence of other organic-N compounds competing for

²Again, the calculation for treatment water is not based on chloramine-T, but on a model compound that was the most reactive with ammonia of the three model organic chloramines tested by Yoon and Jensen (1993). If chloramine-T reactivity with ammonia is intermediate among the three compounds, inorganic chloramine will not be produced at concentrations of concern, even in organic-N free treatment water. However, chloramine-T could be more reactive with ammonia than any of the model chloramines. If so, the inorganic chloramine estimates for chloramine-T would be higher than those given by the above calculations. However, the important presence of organic chloramines from fish feed is still not accounted for in these estimates.

chlorine. In the Yoon and Jensen (1993) study, the large amount of ammonia-N deliberately added to yield a 10:1 total ammonia to organic-N ratio does not represent the competitive situation that exists at much lower total ammonia concentrations, when the ratio somewhat favors organic-N. This more competitive situation typifies public surface waters, where again total ammonia-N is almost always at <0.2 mg/L. Organic amino or peptide compounds are usually present at about the same concentration as this, and often higher (Zygmuntowa 1972; Gardner and Lee 1973). We consider inorganic chloramine produced by chloramine-T in public surface waters to be insignificant.

Although ammonia may be present in the sediments and sediment pore water of earthen raceways (Bill Richardson, U.S. Geological Survey, personal communication), most contact with sediments would be at the sediment surface where virtually no ammonia would be present. In situations where water percolates through sediment, the sediment surfaces involved would be quickly stripped of most of their ammonia by interactions with phytoplankton and other living matter present in the sediments of earthen raceways. Thus, no production of significant inorganic chloramine should occur in raceway sediment. Production of inorganic chloramine in the sediment of receiving waters is also unlikely because of the low concentrations of chloramine-T discharged relative to the probable chlorine demand within the water column.

From the foregoing discussion, it is unlikely that inorganic chloramine will be produced in amounts of concern during chloramine-T therapies nor will it be produced in the waters receiving hatchery discharge. With the present information, we cannot completely eliminate the possibility of inorganic chloramine production at concentrations of concern in treatment waters, especially in the unlikely event that high concentrations of total ammonia-N are present during treatment. However, the almost certain presence of some organic-N in treatment waters and other hatchery waters reduces the likelihood that substantial inorganic chloramine will survive to the time of discharge from the hatchery. Any production of inorganic chloramine during the hour-long treatment period will also be subject to the same minimum 1:20 dilution before discharge that would be required for chloramine-T itself.

Appendix E. Potential of Chloramine-T to Produce Mutagenic Electrophilic Organochlorines

In the 1970s, it was determined that chlorination of public waters in the presence of humic substances resulted in the production of hydrophobic and electrophilic mutagens or carcinogens, such as the trihalomethanes (Bellar et al. 1974; Rook 1977; Amy et al. 1984). In general, direct-acting mutagens and carcinogens are electrophilic (Cheh et al. 1983), and, thus, electrophilic organohalogens—as a category—are of special interest to mammalian and human toxicologists. Similar low molecular weight but less hydrophobic (and probably less long-lived) organohalogens were discovered in chlorinated waters in the early to mid-1980s, and some were found to be mutagenic. Examples of compounds studied include chlorinated residuals of oleic acid (Ghanbari et al. 1983), of fulvic and humic acid (Norwood et al. 1983; Kopfler et al. 1990; Thompson et al. 1990), as well as chlorinated acetic acids (DeAngelo and McMillan 1990), dihaloacetonitriles (Bieber and Trehy 1983), halo ketones, haloaldehydes, and chlorophenols (Stevens et al. 1990), and MX (3-chloro-4-[dichloromethyl]-5-hydroxy-2[5H]-furanone). This compound was found to be an especially mutagenic residual, but it has mostly been associated with paper mill effluent (Holmbloom et al. 1990). When mammalian carcinogenicity was demonstrated for the trihalomethanes, a strong movement was made starting in the 1980s toward *in situ* chloramination in wastewater treatment, where free chlorine is added in combination with large excesses of ammonia to produce inorganic chloramines (Cotruvo 1983; Scully et al. 1996; White 1999). Inorganic chloramines have reduced disinfectant power, but result in significantly lower production of trihalomethanes compared to free chlorine (Cotruvo 1983).

Although it has been demonstrated that the *in situ* chloramination process does form some organohalogens from the short exposures of humic substances to the added free chlorine, once inorganic chloramine is formed it is thought to produce little organohalogen (White 1999, pages 388–389). Likewise, preformed inorganic chloramine is thought to produce little organohalogen (Amy et al. 1990). This suggests that the active ingredient in forming potentially carcinogenic organochlorines is primarily HOCl or OCl. Organic amines like chloramine-T are even less likely to produce organohalogen than preformed inorganic chloramine. At worst, it seems that chloramine-T will produce no more N or non-N electrophilic organohalogens than preformed inorganic chloramine, and probably much less, because of reactivity, steric, and transport considerations. Thus, chloramine-T can probably be best modeled by preformed inorganic chloramine as a worst-case surrogate versus electrophilic organohalogen production. The aquaculture industry is in a position similar to other larger industries on this issue in that much more information is needed before the possibility of mutagenic or carcinogenic effects from their effluent releases can be totally ruled out. This work is ongoing, mostly by the wastewater treatment and drinking water industries,¹ and is still in the initial stages of identifying additional electrophilic compounds and determining their mammalian mutagenicity or carcinogenicity. Chemical tests for identifying individual N and non-N organohalogen compounds must also be developed to fully characterize and assess a chlorinated effluent. Concentrations of individual organohalogens from preformed inorganic or organic chloramination will be low, but we cannot say now that no such compounds will ever be produced by chloramine-T at concentrations of concern. However, the possibility of generating any of the presently known carcinogenic compounds from chloramine-T use in intensive aquaculture is remote.

¹Organochlorines are mostly formed when fulvic and humic acids compete with ammonia for the initial free chlorine (Amy et al. 1990). Chlorine is tightly held by most non-N organochlorines. Some organic N-chloramines are also electrophilic and at least potentially carcinogenic (Scully and Bempong 1982). However, they are considerably shorter-lived (days, weeks) than hydrophobic halocarbons like the trihalomethanes (months, years). The chlorine atom also becomes tightly bound to many organic amine or amino compounds, and a few have been found to be mutagenic or carcinogenic (Bull 1983; Isacson et al. 1983; Bempong et al. 1985; DeAngelo and McMillan 1990; Holmbloom et al. 1990; Horth et al. 1990; Owusu-Yaw et al. 1990). Thus, although a few specific mutagens are known to be present as the result of chlorination or chloramination, many more are unknown components of effluents that were found to be mutagenic (Cheh et al. 1983). With respect to carcinogenicity, much work needs to be done before the ultimate effects of chlorinated discharge, regardless of human-made source, is known (Keefe et al. 1997).

Appendix F. Potential of Chloramine-T to Form Organic Chloramine, Chloramino, and Other Chlorinated Organic Compounds and Their Resultant Effect on Residual Toxicity

Note: This appendix contains lengthy footnotes. The issues involved in this appendix are complex and many-faceted, as is the pertinent literature. Footnotes are used to keep the main lines of argument to the text, while still providing the comprehensive information and data presentation required by a literature search. It is best if the text is read completely and then the footnotes are read.

Even if ammonia were totally absent in waters (and, thus, the possibility of producing inorganic chloramines), it might be possible for chlorinated organic compounds to be produced from chloramine-T that are more toxic than chloramine-T and are at least as slow to degrade. Chloramine-T has the potential to react with many organic-N compounds, each at low concentrations—such as amines, amino acids, peptides, proteins and acetonitriles—and also with non-N organics, such as humic and fulvic acids, fatty acids, esters, triglycerides, and acetic acids (Bean 1983; Stevens et al. 1990). Organics such as tannins, sugars, various carboxylic acids, phenols, terpenoids, isoprenoids, and steroids are also sometimes present and will slowly react with chloramine-T or one of its secondary chlorinated products (ibid). Stability is reached when a compound is formed that is not likely to give up its acquired chlorine. The number of such possible compounds is great, and it may take an introduced organic chloramine—such as chloramine-T—several weeks to generate its final, stable end products in a given body of water. Chemically, chloramine-T may produce organic chlorine-exchange degradates similar to those formed by free chlorine or *in situ* generated inorganic chloramine. However, the rate at which chloramine-T will produce those species will be much slower than that of either free chlorine or *in situ* generated inorganic chloramine (which briefly exists as free chlorine before reacting with available ammonia). Therefore, in many instances, it is more likely that chloramine-T will produce more organic-chlorine exchange degradates within receiving waters than within treatment or hatchery waters.¹

¹Some information exists that might indicate a maximum degradation rate for chloramine-T in the presence of fast-acting organic chlorine demand. Jensen and Johnson (1990) determined the overall rate constant for reaction of chloramine-T with *N,N*-diethyl-*p*-phenylenediamine (DPD) as 1.5×10^{-4} in the presence of excess DPD, because of some combination of direct chlorination and hydrolysis. This compound is assumed to be the equivalent of any organic compound that is highly reactive with free chlorine or any inorganic or organic chloramine (the authors state that its half-time reaction with free chlorine is about 0.5 sec.). Given the presence of a fast-acting amine like DPD, the hydrolysis rate and direct reactivity of the chloramine becomes the dominant factor in overall reactivity. Of the nine organic chloramines that the authors tested, chloramine-T was one of the fastest to react with DPD. There were two organic amines that were faster and four that were slower. Two were about the same. The slowest compound was about one-third as fast as chloramine-T and the fastest was about 60 times faster. Ammonia chloramine was about six times faster. This rate constant can be used to calculate the maximum degradation rate of chloramine-T in the presence of an excess of fast-acting organic chlorine demand. The corresponding calculated half life is about 75 min. This implies that aqueous chloramine-T at 20 mg/L will degrade to 1.25 mg/L in 5 h and to 0.01 mg/L in about 14 h in the presence of a large excess of fast-acting organic chlorine demand. The calculation assumes no contribution from dilution to reach these levels at 5 and 14 hours, except for the role of dilution in introducing fresh fast-acting chlorine demand. Degradation of chloramine-T analytical standards in reagent-grade distilled water is much slower, as would be expected because of the relative lack of chlorine demand. Such an excess of fast-acting chlorine demand is easy to create in a laboratory; the most useful full-scale application of these results is to reaffirm the fate of chloramine-T in holding ponds that are often highly eutrophic and require many days to do a single water exchange. The results suggest that chloramine-T concentrations at discharge from such holding ponds would usually be extremely low, even if the dilution that occurs in holding ponds is disregarded. A second useful purpose is to estimate degradation times when chloramine-T is discharged directly from aquaculture treatment waters into relatively large and eutrophic public surface waters. Again, it seems that chloramine-T would be at low concentrations within a day in such receiving waters, even based on degradation only. The results are least applicable to discharge from treatment waters to receiving waters with minimal dilution and holding time because of the brief exposures to relatively low levels of chlorine demand usually involved.

If all chlorinated organic-N and non-N compounds are less toxic than chloramine-T, their toxicity could be appropriately modeled by that of chloramine-T. The aquatic acute toxicity of most chlorinated organic non-N compounds is not great and their net overall toxicity is probably less than that of chloramine-T.² With respect to chlorinated organic-N compounds, chloramines have especially been associated with acute toxic effects in fish (Feng 1966; Scully et al. 1996). Mattice and Tsai (1983) have shown that some organic chloramines are as toxic to fish as the assumed components of total residual chlorine (HOCl, OCl⁻, inorganic chloramines). For mosquitofish *Gambusia affinis*, four of eight organic chloramines tested had acute toxicities intermediate between the inorganic chloramines—monochloramine and dichloramine—and five were between the two species of free chlorine, HOCl and OCl⁻ (Table F-1). These tended to be low molecular weight chloramines, which are mostly from human input into waters (food and beverage waste, human excretion, etc.). Jameel and Helz (1999) also found that chloramine molecular weight is inversely correlated with toxicity, but is not closely correlated with the tendency to dechlorinate (i.e., reactivity).

Table F-1. Median lethal concentrations (LC₅₀) of residual chlorine compounds for mosquitofish *Gambusia affinis* exposed for 1 h^a (from Mattice and Tsai 1983).

Compound	Chemical formula	LC ₅₀ ^b (mg/L)
Dichloramine	NHCl ₂	0.366
Hypochlorous acid	HOCl	0.455
Cyclohexylmonochloramine	C ₆ H ₁₁ NHCl	0.547
Ethylmonochloramine	C ₂ H ₅ NHCl	0.646
N-propylmonochloramine	C ₃ H ₇ NHCl	0.673
Methylmonochloramine	CH ₃ NHCl	0.799
Monochloramine	NH ₂ Cl	1.31
N-chlorethylglycinate	C ₂ H ₅ COOCH ₂ NHCl	1.7
Hypochlorite ion	OCl ⁻	2.21
Ethanolmonochloramine	HOCH ₂ CH ₂ NHCl	15.4
N-chlorotrisamine	(HOCH ₂) ₃ CNHCl	90.4
N-chloroglycine	COOHCH ₂ NHCl	575

^aMortality assessed 48-h postexposure.

^bExpressed as total residual chlorine.

Thus, some long-lived low molecular weight organic chloramines that do not give up their chlorine easily could be of potential environmental concern, a possibility noted by a number of researchers (Gould et al.

²Fulvic acids are about 90% of the humic material in most natural waters, and account for 45% of the dissolved organic carbon in surface waters (Bean 1983, Jensen et al. 1985). They are defined by Rook (1977) as low molecular weight humic acids that remain in solution at pH 1. These as well as other organic non-N compounds will eventually acquire chlorine from chloramine-T as well as from other chloramines produced by chloramine-T. Because these compounds retain their chlorine relatively tightly, they are probably the ultimate end product of much of the chlorine from chloramine-T discharge. From both a toxicity and mutagenicity standpoint, the most important consideration is that many non-N chloroorganics will eventually be produced, each one at an extremely low concentration. Most are not electrophilic. The resulting aggregates should be of negligible harm to the environment.

1984; Bempong et al. 1985; Conyers and Scully 1997; Jensen and Helz 1998a). The question is whether any compound(s) more toxic than chloramine-T are produced at concentrations of concern. This possibility is unlikely unless a human-made precursor is also present at a relatively high concentration, given the wide variety of reactive organic-N compounds naturally present in surface water. Polypeptides (large molecules) usually constitute a sizeable proportion of organic-N in surface waters,³ at least twice that of free amino-N (Hutchinson 1957, page 893; Wolfe et al. 1985), and about the same proportion as non-amino-N (which are mostly the nonamino groups of amino acids; Hutchinson 1957, page 891). Furthermore, the resultant peptide chloramines are quite stable compared to those from other organic-N compounds (Helz and Nweke 1995; Jensen 1997). So far, no chlorinated peptides have been determined to exhibit toxicities of concern. MacCrehan et al. (1998) have recently suggested that further investigation is justified because they are the most long-lived of this class of compounds. However, some low molecular weight and potentially toxic organic chloramines are also long-lived and may account for most of the actual toxicity. Some acute aquatic toxicity probably remains when chloramine-T produces chlorinated organic-N and non-N products, be they of high or low molecular weight. The combined toxicity of these products is much less than that of free chlorine or inorganic chloramine and probably is less than that of chloramine-T itself.

The results of Bills et al. (1988a) suggest that overall toxicity of the typical organic-N compounds produced is probably less than that of chloramine-T. These authors found that chloramine-T toxicity to fish significantly decreased as amounts of fish food or fecal material were increased. Chloramine-T concentrations also significantly decreased in these periods. This suggests that chloramine-T is mostly exchanging into chlorinated peptides and similar amino acids and not into low molecular weight amine or amino compounds that can be much more toxic than chloramine-T. It follows that free chlorine or inorganic chloramine (almost always prepared *in situ*) must also exhibit reduced toxicity when used in treatments of dirty or highly eutrophic water. Studies have borne this out, at least if reduced disinfecting power is an indication of reduced toxicity (Wolfe et al. 1985; Scully et al. 1996). The highly probable overall effect of chloramine-T exchange into chlorinated amine, amino, and peptide compounds would be a significant reduction in acute aquatic toxicity,⁴ as indicated by the findings of Bills et al. (1988a). Our conclusion for

³Amino acids and other primary amines, such as peptides, are thought to be among the most environmentally significant forms of organic nitrogen in water supplies (Wolfe et al. 1985). Short chain aliphatic amines, cyclic amines—such as piperidine and pyrrolidine—as well as purine and pyrimidine bases—such as caffeine, uracil, cytosine, and 5-methylcytosine—are often present also from either natural or human-made sources (Gould et al. 1984; Jensen and Helz 1998a). Studies of individual chlorinated organic-N species or classes known to be produced have been done as well as a few studies of model waters, mostly by the wastewater treatment industry. Examples of residual chlorinated organic-N compounds studied include chlorination products of tyrosine and phenylalanine (Horth et al. 1990), of L-tryptophan (Owusu-Yaw et al. 1990), of glycine, norvaline, valine, glutamic acid, dimethylamine, and methylamine (Choshen et al. 1990), of ethylamine and piperidine (Scully and Bempong 1982), and of the nitrogenous organics alanine, glycine, histidine, leucine, phenylalanine, serine, tryptophan, creatinine, *N*-acetylglycine, glycine ethyl ester, glycylglycine, sarcosine, and sarcosine anhydride (Isaac and Morris 1983a).

⁴Chlorinated organic-N compounds are also of interest to chlorine or chloramine users and dischargers for other reasons: (1) the chlorinated organic-N compounds are their most likely immediate degradates; (2) in highly eutrophic waters, these compounds will be produced in large overall concentrations; and (3) some of them respond to official tests for TRC, and, thus, the range of their overall toxicities relative to that stated for TRC needs to be investigated.

Unfortunately, even partial characterization of specific surface waters has only been recently undertaken, including knowledge about the types and concentrations of their nitrogenous organic compounds and their most stable chlorinated products (Wolfe et al. 1985; Conyers et al. 1993). Even more unfortunately for aquaculture, the few characterizations that have been made of stable products of amine or amino compounds were done in the presence of free-chlorine residual, as this is of interest to the drinking and wastewater disinfectant industry (Nweke and Scully 1989; Conyers et al. 1993; Conyers and Scully 1997; Fox et al. 1997; Keefe et al. 1997). The findings of these authors

chloramine-T on the basis of the preceding discussion is that it will exhibit greater aquatic toxicity if it remains as chloramine-T, rather than if it exchanges into the many other organic *N*-chloramine or chloramino products that are possible. Accordingly, their aggregate toxicity can be modeled by the toxicity of chloramine-T.

would not be representative of hatchery or public waters after chloramine-T discharge from aquaculture sites where chlorination concentrations are well below breakpoint concentrations. In either instance, work on the effect (toxicity and mutagenicity) of resulting stable *N*-chloro amine and amino compounds ranges from scarce to practically nonexistent (Keefe et al. 1997). Although much could be said about research done on attempts to characterize or model the fate and toxicity of chlorine or chloramine discharges, demonstrating an aquatic toxicity prediction to be true experimentally for each individual discharge circumstance would be a toxicologist's nightmare (Mattice and Tsai 1983). This is a situation continuously being faced by the water treatment industry. In reality, the nature of the organic nitrogen present in both discharge and receiving waters must be known if accurate evaluations of aquatic toxicity of the resulting chlorinated analogs are to be made (Isaac and Morris 1983a).

Appendix G. Potential of Para-toluenesulfonamide and its Breakdown Products to be a Significant Threat to Organismal, Environmental, or Public Health

The initial breakdown product of chloramine-T in water as it loses its chlorine atom is para-toluenesulfonamide (p-TSA). This has been demonstrated routinely from analysis of chloramine-T treated waters in controlled studies at the Upper Midwest Environmental Sciences Center (Jeffrey Meinertz, Research Physiologist, U.S. Geological Survey, personal communication). During stability testing in reagent water, no compounds other than chloramine-T and p-TSA were observed by liquid chromatography or ultraviolet techniques (single wavelength detection) over a 2-week storage period in sunlight at ambient temperatures (ibid). This major degradation pathway of chloramine-T to p-TSA is probably a combination of hydrolysis and direct aqueous chlorination. Photolysis might have made some contributions as well. There seems to be no published literature on the potential products of chloramine-T from microbial action or photolysis, although Meinertz et al. (1999) reported the degradation apparently because of (at least in part) hydrolysis as did Dawson and Davis (1997). This degradate will be a component of any discharge after a chloramine-T treatment of aquaculture waters.

A substantial effort to characterize the fate and toxicity of p-TSA was undertaken by the government of Japan. Fate studies relevant to aquaculture drugs included biodegradation, photodegradation, and hydrolysis. Aquatic toxicity studies included acute toxicity to algae *Selenastrum capricornutum*, *Daphnia magna*, and orange-red killifish *Oryzias latipes*. A chronic toxicity study was also done on *Daphnia magna*. Other tests were done as well, especially on mammalian toxicology and mutagenicity. Details and results of these tests are available online from Office of Economic Cooperation and Development (OECD) at http://keyword.netscape.com/ns/boomframe.jsp?query=70-55-3.pdf&page=1&offset=0&result_url=redir%3Fsrc%3Dwebsearch%26requestId%3Dac06ce02b47e570%26clickedItemRank%3D1%26userQuery%3D70-55-3.pdf%26clickedItemURN%3Dhttp%253A%252F%252Fwww.jetoc.or.jp%252FHPSIDS%252Fpdffiles%252F70-55-3.pdf%26invocationType%3D%26fromPage%3DnsBrowserRoll%26amp%3BampTest%3D1&remove_url=http%3A%2F%2Fwww.jetoc.or.jp%2FHPSIDS%2Fpdffiles%2F70-55-3.pdf, accessed December 2005.

Fate studies from the public literature demonstrated that p-TSA is resistant to degradation and will be the major unchlorinated degradate of chloramine-T until it is diluted by receiving waters to a point far past where measurements of further degradates are possible in natural waters. Biodegradation was only 4% at 100-mg/L initial concentration after exposure to activated sludge for 28 d. Half life from photodegradation studies (estimated from degradation rate) was 132 days. Half life from hydrolysis studies (at pH 4.0, 7.0, and 9.0) was more than 1 year. The water solubility of p-TSA is 3.2 g/L at 25 °C, and the octanol/water partition log P_{ow} is 0.84 at 25 °C. A log P_{ow} of 0.84 indicates that the bioaccumulation potential of p-TSA is probably low.

These studies seemed to show that p-TSA is a stable compound in the aquatic environment, having very low rates of degradation by hydrolysis, photolysis, or biodegradation. Studies of hydrolysis or photolysis of p-TSA have not yet been completed, but studies for biodegradation listed below suggest that p-TSA might be more biodegradable than indicated by the Japanese study. One study of Santicizer[®] 9, a mixture of o- and p-TSA, produced a result similar to that of the Japanese study. Low mineralization to CO₂ was demonstrated by a mean CO₂ evolution of 3-13% of theoretical (Saeger et al. 1981; Appendix H). This suggests that complete biodegradation of Santicizer[®] 9 (to CO₂) occurs rather slowly, but the study also found high variability in results, suggesting considerable sensitivity to test conditions. An study conducted in 1981 indicated that biodegradability of both Halamid[®] and p-TSA by the RDA method (active sludge inoculum) was 80-90% per week at 25 mg/L initial Halamid[®] concentration (Blok 1981; Appendix H). Another study of Santicizer[®] 9 yielded 92.9 % degradation at 57 ppm after 21 d in the presence of activated sludge (Cranor 1983; Appendix H). A 1998 study indicated that, under aerobic conditions, p-TSA was at least 90% mineralized or converted into microbial biomass in 100 days in sandy loam soil, about 60% in

humic sand soil, and more than 95% in low humic content sand soil (van de Leur-Muttzall and Hanstveit 1998a; Appendix H). Another study (Blok 1982; Appendix H) noted that anaerobic degradation of p-TSA in sludge was very slow (stable for 40 days), which is typical for aromatic compounds. The overall results are thus inconclusive except that they indicate that p-TSA can be very stable under some conditions, but less stable under others.

van de Leur-Muttzall and Hanstveit (1998b; Appendix H) indicated only very slight adsorption of Halamid[®] to 3 types of soil. Since Halamid[®] is rapidly hydrolyzed to p-TSA in soil (van de Leur-Muttzall and Hanstveit 1998a; Appendix H), these low adsorption coefficients, taken after 18 hours of shaking in the dark, also apply to p-TSA. A 1981 study also showed no significant adsorption of Halamid[®] to one type of synthetic soil and one type of activated sludge (< 500 mg / kg of organic matter, see Blok 1981; Appendix H). Again, since there was probably substantial degradation to p-TSA during the 20.5 hours of shaking that occurred, p-TSA also did not adsorb significantly. It thus appears that sediment is not an important environmental compartment for p-TSA. The bioaccumulation potential of p-TSA appears to be somewhat greater than that of chloramine-T, based on its solubility in water and octanol-water partition coefficient, but a bioaccumulation potential of concern is not indicated.

The toxicity studies presented in Table G-1 seem to demonstrate that the toxicity of p-TSA is much less than that of chloramine-T. The toxicity tests for chloramine-T as presented in Table 6 only parallel those presented for p-TSA, but the resulting array of toxicity concentrations are much lower for chloramine-T than for p-TSA.

In a study of the sublethal effects of waterborne chloramine-T, p-TSA, and hypochlorite ion on respiratory and acid-base disturbances in rainbow trout *Oncorhynchus mykiss*, Powell and Perry (1996) noted significant effects for chloramine-T at 9 mg/L (active ingredient). There were no similar stress-indicating effects for p-TSA at 9 mg/L. This study of sublethal effects would also suggest that p-TSA is less toxic than chloramine-T.

Bacteria - Cranor (1983) describes the toxicity of Santicizer[®] 9 to sewage treatment bacteria (see Appendix H). The study concludes that Santicizer[®] 9 should have negligible effects on the wastewater treatment process at or below 70 mg/L.

One mole of dechlorinated chloramine-T will produce one mole of p-TSA, whether the breakdown is relatively slow because of environmental processes or much more rapid because of mitigation with reducing agents thiosulfate or sulfite. From the available acute toxicity data, p-TSA can be conservatively modeled by the toxicity of chloramine-T.

Table G-1. Acute and chronic toxicity of para-toluenesulfonamide (p-TSA) to select fresh- and saltwater species.¹ Key toxicity studies used in our risk assessment are indicated in bold.

Species	Period	Method or endpoint (OECD ^a guideline)	p-TSA concentration (mg/L)	Reference
semi-continuous activated sludge (SCAS)	21 days after 14-d acclimation period, total 35 days.	biodegradation, measured as dissolved organic carbon	negligible effects on SCAS at or below 70 ppm.	Cranor 1983; Appendix H
Algae, <i>Selanastrum capricornatum</i>	72-h EC₅₀	Growth inhibition	23 (reported as weight per volume)	OECD
Axcentive Proprietary, Algae, <i>Chlorella pyrenoidosa</i>	96-h EC ₅₀	Growth inhibition	80	Blok 1981; Appendix H
Water flea <i>Daphnia magna</i>	24-h EC ₀	Probit method, immobilization	32	OECD
	24-h EC ₅₀	Probit method, immobilization	150	OECD
	24-h EC ₁₀₀	Probit method, immobilization	320	OECD
	21-d NOEL^c or NOEC maximum	Static test, immobilization and reproduction	47	OECD
	21-d LOEL ^c or LOEC	Static test, immobilization and reproduction	150	OECD
Axcentive Proprietary, Water flea <i>Daphnia magna</i> (Santicizer [®] 9)	48-h EC ₅₀	Static test, immobilization	>1000	Calvert & Adams 1981; Appendix H
Axcentive Proprietary Rainbow trout,	96-h LC₅₀	Static test	100	Cohle & McAllister 1983a; Appendix H
Axcentive Proprietary, Bluegill	24, 48, 96-h LC ₅₀	Static test	All 370	Cohle & McAllister 1983b; Appendix H
Axcentive Proprietary, Rainbow trout, (Santicizer [®] 9)	24, 48, 96-h LC ₅₀	Static test	200, 120, 120	Kintner & Forbis 1983; Appendix H
Axcentive Proprietary, Bluegill, (Santicizer [®] 9)	24, 48, 96-h LC ₅₀	Static test	420, 420, 260	Calvert & Adams 1981; Appendix H
Orange-red killifish <i>Oryzias latipes</i>	LC ^c ₀ (24, 48, 72, and 96 h)	Semi-static test	324	OECD
	LC ₅₀ (24, 48, 72, and 96 h)	Semi-static test	435	OECD
	LC ₁₀₀ (24, 48, 72, and 96 h)	Semi-static test	583	OECD

^aOECD = Office of Economic Cooperation and Development, ^bEC = effective concentration, ^cNOEL = no-observed-effect-level or concentration, ^dLOEL = lowest-observed-effect-level or concentration, ^eLC = lethal concentration. Santicizer[®] 9 is a mixture of o-TSA and p-TSA.

¹The information in this table is from the Office of Economic Cooperation and Development (see text in this section for online address).

Appendix H. Summaries of Key and Proprietary Studies

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, PA 16801-8495. 408 pp.

Analytical Laboratory Services (2003) determined the 48-h EC₅₀ of chloramine-T and several other fishery chemicals for *Ceriodaphnia dubia* studies and the 96-h EC₅₀ for *Pimephales promelas* using four 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 0.75, 1.5, 3, 6, and 12 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 2.5, 5, 10, 20, and 40 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 test period. The resulting LC_{50s} were within the control limits.

The 48-h EC₅₀ for *C. dubia* ranged from 2.12 to 8.88 mg/L using the 4 surface waters. The 96-h LC₅₀ for *P. promelas* ranged from 6.16 to 28.1 mg/L. These results were not used for the 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, which was reported by Blok 1981; Appendix H.

Bessems, E. 1988. Bacterial toxicity of Halamid[®]. Research Report 88-SLM-01 of Project No. 6073 submitted by Department of Microbiology of AKZO Nobel Central Research, Duren, January 13, 1988.

The bacterial toxicity test was performed according to the Robra oxygen consumption inhibition test using *Pseudomonas putida* (Robra 1976). German law requires chemical products to be classified based on their environmental toxicity. This standard test is based on the oxygen uptake by *P. putida* during substrate consumption in the presence of the test chemical (Halamid[®]). The amount of test chemical causing a 10% reduction of the O₂ uptake (0.5 h EC₁₀) is the defining value for the classification, as a measured 10% reduction in oxygen consumption. The results of O₂ uptake of *P. putida* vs Halamid[®] concentrations were plotted. As expected, O₂ uptake decreases with increasing Halamid[®] concentrations. The EC₁₀ from the Robra test is considered a sensitive toxic endpoint for bacteria. Halamid[®] at an aqueous

concentration of 10 mg/L produces a 10% reduction of the O₂ uptake of *P. putida*. The 10 mg/L value reported in this study was a key toxicity endpoint used in our EA risk assessment. This study is proprietary and key in establishing risk assessment.

Bessems, E. 1991. Effectiveness of Na-p-toluenesulfonchloramide to *Vibrio cholerae*. Report submitted by the Department of Microbiology of AKZO Nobel Central Research, Duren, June 6, 1991.

The product Na-p-toluenesulfonchloramide, trade name Halamid[®], was tested for its killing effect to the bacteria *Vibrio cholerae* causing the cholera disease. The standard European suspension test (EST) was used. Test concentrations were 0.5 and 1.0% and contact times were 5 and 10 minutes. Protein load was 0.03% bovine albumin (BA) simulating clean conditions and 0.3% BA simulating dirty conditions. The results indicated that 0.5% product was able to kill the pathogenic bacteria *Vibrio cholerae* within 5 minutes contact time under both clean and dirty conditions.

Bessems, E. 1996. Bactericidal effect of Halamid[®] according to the CEN test for application in food, industrial, domestic and institutional areas. Report submitted by the Department of Microbiology of AKZO Nobel Central Research, Duren, August 15, 1996. 5 pp.

The efficacy of Halamid[®] was determined by means of a quantitative suspension test after the chemical disinfectants and antiseptics—quantitative suspension test for the evaluation of bacterial activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas—test method and requirements (CEN method) as described for bacteria coded prEN 1276. Microorganisms used in the testing included *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus hirae*. Test temperature was 20°C and contact time was 5 minutes. The disinfectant Halamid[®] passed the requirement of the CEN test under clean conditions at concentrations varying from 0.006% to 0.225% Halamid[®] depending on the kind of test bacteria.

Bills, T. D., L. L. Marking, V. K. Dawson and J. J. Rach. 1988b. Effects of environmental factors on the toxicity of chloramine-T to fish. Investigations in Fish Control Report 96. U.S. Fish and Wildlife Service. Available from the Publications Unit, U.S. Fish and Wildlife Service, Springfield, Virginia. 6 pp.

The critical LC₅₀ value in fish used in the EA risk assessment was obtained from Bills et al. (1988). This study was conducted to evaluate effects of various factors on the toxicity of chloramine-T. These factors include temperature, hardness, pH, and exposure conditions (static vs. flow-through). Twenty fish of each species (rainbow trout and channel catfish) were exposed per concentration for 96 hours under static or flow-through conditions. Test procedures used in this study followed those prescribed by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975), ASTM Committee E-35 on Pesticides (1980), and Guidelines for IR-4 investigations (U.S. Department of Agriculture 1986). Chloramine-T concentrations were confirmed analytically. Target hardness ranged from 10 to 320 mg/L as CaCO₃, target temperature ranged from 7°C to 17°C, and target pH ranged from 6.5 to 9.5. Numerous studies were conducted in each fish species that produced similar LC₅₀ values (range of 1.9 to 14 mg/L in rainbow trout and 1.8 to 12 mg/L in channel catfish).

The 96-h LC₅₀ reported in this study for channel catfish (1.8 mg/L) was a key toxicity endpoint used in the EA risk assessment.

Blok, J. 1981. Ecotoxicological aspects of Halamid[®] (para-toluenesulfonamide-chloramide- sodium). Report #D 81/124 submitted by Corporate Research Department Arnhem, AKZO Research. November 11, 1981. 38 pp.

The stability of the disinfectant Halamid[®] was determined in aqueous solutions under variant conditions, using high pressure liquid chromatography. In algal growth tests the half life was 1-2 days. Total organic carbon (TOC) analysis was used to determine the adsorption of Halamid[®] by soil and activated sludge. Less than 500 mg of Halamid[®]/kg was adsorbed, calculated on the organic matter in the soil and sludge. The biodegradability of both Halamid[®] and p-TSA was measured by the repetitive die away (RDA) method. It was found that the two substances are fully biodegradable at a rate of 80-90% per week. However, the Halamid[®] concentration must be low enough not to cause disinfection of the inoculum.

The acute toxicity to organisms in surface water was tested using fish, daphnids, and algae. The 96-hour LC₅₀ against guppies (*Poecilia reticulata*) was 31 mg/L and the 48-hour LC₅₀ against Daphnia (*Daphnia magna*) was 4.5 mg/L. The 96-hour EC₅₀ against algal (*Chlorella pyrenoidosa*) growth inhibition was 80 mg/L as p-TSA.

Blok, J. 1982. Ecotoxicological aspects of Halamid[®] (para-toluenesulfone-chloramide-sodium): II. CRL Report No. D 82/44 AKZO Research April 27, 1982.

The toxicity of Halamid[®] was measured on three groups of bacteria representative of biological sewage treatment plant sludge. The EC₅₀ for the respiration inhibition of aerobic saprophytic activated sludge bacteria is approximately 5 mg/L. The EC₅₀ for the respiration inhibition of nitrifying bacteria is approximately 700 mg/L. The EC₅₀ for the inhibition of methane generation from glucose is approximately 1,000 mg/L. In view of the chemical instability of Halamid[®], the ready biodegradability of para-toluenesulfonamide (p-TSA), and the low tendency to adsorption by sludge, it may be assumed that Halamid[®] would not cause significant disruption when discharged to a biological sewage treatment plant, providing the discharge is of homogeneous distribution. This study is proprietary and key in establishing risk assessment.

Borgmann-Strahsen, Renate. 1998. Biocidal activity of Halamid[®] against *Legionella pneumophila* and *Campylobacter jejuni*. Interim Research Report submitted by Department of Microbiology of AKZO Nobel Central Research, Duren, Project No. 6630, January 7, 1998. 7 pp.

The purpose of this study was to determine the biocidal activity of Halamid[®] against *Legionella pneumophila* and *Campylobacter jejuni*. *Campylobacter jejuni* was killed at low protein level by 30 ppm and high protein level by 100 ppm Halamid[®]. It was found that the active concentration of Halamid[®] passing the CEN test was below 25 ppm for *Legionella pneumophila*. *Legionella pneumophila* and *Campylobacter jejuni* are significantly more susceptible to Halamid[®] than the usual standard test bacteria.

Borgmann-Strahsen, Renate. 2000. Basic bactericidal activity of Halamid[®] according to EN 1040. Report submitted by AKZO Nobel Chemicals, Chemicals Research Duren, Department of Microbiology. February 3, 2000.

The basic bactericidal activity of Halamid[®] was determined by means of the quantitative suspension test EN 1040. Test organisms included *Pseudomonas aeruginosa*, ATCC 15442, and *Staphylococcus aureus*, ATCC 6538. Test temperature was 20°C and contact time was 5 minutes. The product Halamid[®] passed the CEN test on the basic bactericidal activity at a concentration of 0.03%.

Calvert, Cornelia and W. J. Adams. 1981. Acute toxicity of Santicizer[®] 8 and Santicizer[®] 9 to *Daphnia magna*. Report No. ES-81-SS-32 of Project No. 47-000-760.37-4382444 MIC Environmental Sciences, Monsanto Company. 22 pp.

The purpose of this study was to determine the acute toxicity of Santicizer[®] 8 and Santicizer[®] 9 to a common aquatic invertebrate *Daphnia magna*. The acute toxicity of Santicizer[®] 8 and Santicizer[®] 9 to *Daphnia magna* was assessed at the Monsanto Industrial Chemicals (MIC) aquatic laboratory, during a 48-hour static test. The 48-hour EC₅₀ values are >1,000 mg/L for both products. The no observed effect concentrations (NOEC) were 500 and >1,000 mg/L for Santicizer[®] 8 and 9, respectively.

Cohle, Paul and W. A. McAllister. 1983a. Acute toxicity of p-toluenesulfonamide to rainbow trout (*Salmo gairdneri*). Report No. 30007 submitted by Analytical Bio-chemistry Laboratories, Inc. to Monsanto Industrial Chemicals Company, St. Louis, MO. January 31, 1983. 45 pp.

The acute toxicity of p-toluenesulfonamide to rainbow trout (*Salmo gairdneri*) was assessed using the methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms. Water quality parameters of temperature, dissolved oxygen, pH, and ammonia were measured throughout the test and were within acceptable limits. As a quality check, the rainbow trout were challenged with a reference compound, antimycin A. The estimated 96 hour LC₅₀ and 95% confidence limits were within the 95% confidence limits reported in the literature, indicating that the fish were in good condition. The 24, 48, and 96 hour LC₅₀ values for p-toluenesulfonamide were 130, 110, and 100 mg/L, respectively. This study is proprietary and key in establishing risk assessment.

Cohle, Paul and W. A. McAllister. 1983b. Acute toxicity of p-toluenesulfonamide to bluegill sunfish (*Lepomis macrochirus*). Report No. 30006 submitted by Analytical Bio-chemistry Laboratories, Inc. to Monsanto Industrial Chemicals Company, St. Louis, MO. February 15, 1983. 49 pp.

The acute toxicity of p-toluenesulfonamide to bluegill sunfish (*Lepomis macrochirus*) was assessed using the methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms. Water quality parameters of temperature, dissolved oxygen, pH, and ammonia were measured throughout the test and were within acceptable limits. As a quality check, the bluegill sunfish were challenged with a reference compound, antimycin A. The estimated 96 hour LC₅₀ and 95% confidence limits were within the 95% confidence limits reported in the literature, indicating that the fish were in good condition.

The 24, 48, and 96 hour LC₅₀ values (and 95% confidence limits) for p-toluenesulfonamide were all the same at 370 (240-560) mg/L. The 96 hour no observed effect concentration (NOEC) was 42 mg/L.

Cranor, Walter. 1983. Semi-continuous activated sludge (SCAS) biodegradation of Santicizer[®] 8 and Santicizer[®] 9 (Analytical methodology and tests results). Final Report #30429 submitted to Monsanto Polymer Products Company by Analytical Bio-chemistry Laboratories, Inc. Columbia, MO. August 5, 1983.

An analytical method for Santicizer[®] 8 and Santicizer[®] 9 was developed. The method involved dichloromethane extraction with quantification by HPLC. Recoveries of Santicizer[®] 8 and Santicizer[®] 9 from water were found to be 101 ±2.3% and 97.7 ±5.6%, respectively.

A thirty-five day activated sludge study was conducted that included a fourteen day acclimation period and a twenty-one day biodegradation period. The effects of Santicizer[®] 8 and Santicizer[®] 9 on the wastewater treatment process were found to be negligible when present at or below 70 ppm. Assessment of the effect of Santicizer[®] 8 and Santicizer[®] 9 on the sludge microbial populations showed no discernable effect. During the twenty-one day biodegradation phase of the study, Santicizer[®] 8 was biodegraded >99% when initially present at 69.9 ppm; Santicizer[®] 9 was 92.9% biodegraded when initially present at 56.9 ppm. Both materials were considered to undergo rapid primary biodegradation based upon this study.

Heus, M. 1992. Partition coefficient of chloramine-T for 1-octanol/water. Report of Research Project No. 4840, Research Task No. 2114, Document Code RCD 923-309. AKZO Chemical Division.

The partition coefficient (K) of chloramine-T for 1-octanol/water was determined. The two-layer system was stirred vigorously for 15 minutes at 20° C. After separation in a separation funnel, the concentration of chloramine-T in the water layer was determined. The partition coefficient (K) was determined to be $0.47 / 8.85 = 0.05$.

Kintner, David L. and Alan D. Forbis. 1983. Acute toxicity of Santicizer[®] 9 plasticizer to rainbow trout (*Salmo gairdneri*). Report No. 29981 submitted by Analytical Bio-chemistry Laboratories, Inc. to Monsanto Industrial Chemicals Company, St. Louis, MO. January 31, 1983. 44 pp.

The acute toxicity of Santicizer[®] 9 plasticizer to rainbow trout (*Salmo gairdneri*) was assessed using the static toxicity test methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms. Water quality parameters of temperature, dissolved oxygen, pH, and ammonia were measured throughout the test and were within acceptable limits. As a quality check, the rainbow trout were challenged with a reference compound, antimycin A. The estimated 96 hour LC₅₀ and 95% confidence limits were within the 95% confidence limits reported in the literature, indicating that the fish were in good condition. The 24, 48, and 96 hour LC₅₀ values for Santicizer[®] 9 were 200, 120, and 120 mg/L, respectively.

Kroon, A. G. M. 1995. Toxicity of Halamid[®] to the brine shrimp *Artemia nauplii*. Final Report of Task No. 9028, AKZO Nobel Central Research, The Netherlands. 16 pp.

The toxicity of Halamid[®] to the brine shrimp *Artemia nauplii* was assessed in an acute toxicity test under static conditions in accordance with a slightly modified OECD test guideline for testing of chemicals. The *Artemia* were exposed to seven concentrations of the test substance for 72 hours and immobility and deviations in the behaviour or appearance were recorded after 24, 48, and 72 hours. All *Artemia* survived in the controls and up to a concentration of 10.4 mg/L during 72 hours of testing. An EC₅₀ and 95% confidence interval of 24.55 (18.99-31.74) mg/L was calculated. The NOEC (the lowest concentration causing no effect) value was 10.4 mg/L Halamid[®]. It was concluded that *Artemia mauplii* is susceptible to Halamid[®] at concentrations of 10 mg/L or higher, and that complete immobilization is accomplished at 400 mg/L or higher.

Kroon, A. G. M. 1997. Toxicity of chloramine-T to the freshwater alga *Selenastrum capricornutum*. Final Report No. RGL F97012 T 96021 AL submitted by General Analytical and Environmental Chemistry Department AKZO Nobel February 11, 1997. 29 pp.

In this study, *Selenastrum capricornutum* was exposed to chloramine-T for 96 hours under static conditions. Three replicates per concentration were tested, and appropriate water quality parameters were monitored throughout the study. The study may have underestimated the toxicity of chloramine-T because the pH ranged from 7.8 at the beginning of the test to 9.3 after 96 h.

The 96-h EC₅₀ reported in this study was 4.5 mg/L. The 96-h NOEC was much lower, at 0.2 mg/L, a value more similar to the 48-h EC₅₀ reported by Kühn and Pattard (0.31 mg/L, 1990). Kühn and Pattard (1990) also reported a 48-h EC₁₀ of 0.11 mg/L. The 96-h NOEC (0.2 mg/L) was a key toxicity endpoint used in the EA risk assessment.

Machado, Mark W. 1983. Chloramine-T - The toxicity to fathead minnow *Pimephales promelas* during an early life-stage exposure, FIFRA guideline number 72-4. Final Report SLI #93-9-4927 submitted by Springborn Laboratories, Inc. to AKZO Chemicals International, The Netherlands. 83 pp.

One of the key chronic toxicity values (35-day NOEC = 1.1 mg/L) was provided in this study. Fathead minnows (*Pimephales promelas*) were exposed to chloramine-T for 35 days under flow-through conditions. The study was GLP compliant, followed FIFRA guideline 72-4, and concentrations were analytically confirmed. Dissolved oxygen was 7.8 - 8.1 mg/L, temperature was maintained at 24 - 25°C, hardness was 19 - 20 mg/L CaCO₃, and pH ranged from 6.8 - 7.4. This study is proprietary and key in establishing risk assessment. Although the study was conducted in soft water (hardness = 19 - 20 mg/L CaCO₃), Bills et al. (1988b) observed that water hardness did not have a pronounced effect on the toxicity of chloramine-T to fish. It should be noted that rainbow trout and channel catfish appear to be the most sensitive fish species tested in acute studies (Bills 1988a, b).

Putt, Arthur E. 1993. Chloramine-T - The chronic toxicity to *Daphnia magna* under flow-through conditions. FIFRA guideline- 72-4. Final Report #93-3-4694 submitted by Springborn Laboratories, Inc. to AKZO Chemicals International, The Netherlands. May 20, 1993. 101 pp.

In this study, daphnids were exposed to chloramine-T for 21 days under flow-through conditions. The study was GLP compliant and followed FIFRA guidelines. Four replicates of 10 daphnids each were exposed to five analytically confirmed chloramine-T concentrations that ranged from 1.1 to 23 mg/L. There did not appear to be deviations in water quality parameters that would be expected to affect the results of the study. The pH in this study ranged from 8.0-8.3. The high pH range used in this study may have resulted in an underestimation of the toxicity of chloramine-T at neutral to slightly acidic pH. Also, use of flow-through conditions may also have resulted in an underestimation of the toxicity of chloramine-T.

The 21-d NOEC reported in this study for *Daphnia magna* (1.1 mg/L) was a key toxicity endpoint used in the EA risk assessment.

Saeger, V. W., R. G. Kuehnel, M. A. Lewis, C. Linck, and W. J. Adams. 1981. Ultimate biodegradation screening of Santicizer[®] 8 and 9. Report No. ES-81-SS-47 MIC Environmental Sciences, Monsanto Company.

Ultimate biodegradation screening using the shake flask carbon dioxide evolution test were carried out for two plasticizer products, Santicizer[®] 8 and Santicizer[®] 9. A low degree of mineralization to carbon dioxide was observed for both products with mean CO₂ evolution amounting to 3 percent of theoretical for Santicizer[®] 8 and 13 percent for Santicizer[®] 9. These data indicate relatively slow biodegradation or only slight alteration of the parent molecules.

van de Leur-Muttzall, P. I. and Hanstveit, A. O. 1998a. A study on the route and rate of degradation of [¹⁴C]Halamid in three soils (CTB Guideline section G.1.1). Report of Study No. IMW-97-0103-01 submitted by TNO Nutrition and Food Research Institute, The Netherlands. 46 pp.

The metabolism and rate of degradation of [¹⁴C]Halamid[®] in three soils was determined according to the guidelines of the Dutch Board for Authorization of Pesticides and in compliance with the OECD Principles of Good Laboratory Practice (GLP). In a laboratory study [¹⁴C]Halamid[®] was applied to a sandy loam soil in order to study the route and rate of degradation and to a humic sand soil and a low lumic content sand soil to study the rate of degradation. The soils were incubated under aerobic conditions in the dark at 20 ± 2° C. The application rate was 3 mg/kg on dry soil basis. For the route of degradation, the following parameters were determined after sampling times of 0, 7, 14, 28, 56, and 100 days: CO₂ evolution, (methanol) extractable radioactivity in the solids, bound residues and distribution of radioactivity between parent compound and metabolite(s) by HPLC (with the exception of the extracts obtained after 100 days). For the rate of degradation, only the extractable radioactivity in the solids and the distribution of radioactivity between parent compound and metabolite(s) was determined.

In the sandy loam soil, the evolved carbon dioxide amounted to about 48% of the initial radioactivity at the end of the test (100 days). The amount of methanol extractable radioactivity decreased from 95% at the start of the test to about 9% after 100 days. It can be assumed that at least 90% of the added [¹⁴C]Halamid[®] and transformation products(s) have been mineralized or converted into microbial biomass within the test period. Bound residue increased from about 5% at the start of the test to 36% at the end of the test. In the humic sand soil, the amount of methanol extractable radioactivity decreased from about 95% at the start of the test to 39% after 100 days of incubation. In the low lumic content sand soil, the methanol extractable radioactivity decreased from 94% to 2% after 100 days. [¹⁴C]Halamid[®] was only detected in one replicate of the sandy loam soil at the beginning of the test because it is almost instantly hydrolyzed to p-toluenesulfonamide when added to the soils.

van de Leur-Muttzall, P. I. and Hanstveit, A. O. 1998b. A study on the adsorption of [¹⁴C]Halamid to soil particles in three soil types (CTB Guideline section G.1.2/OECD 106). Report of Study No. IMW-97-0103-02 submitted by TNO Nutrition and Food Research Institute, The Netherlands. 26 pp.

The adsorption of [¹⁴C]Halamid[®] to soil particles was determined according to the Dutch Board for the Authorization of Pesticides Guidelines section G.1.2, the OECD Guideline no. 106, and in compliance with the OECD Principles of Good Laboratory Practice (GLP). The adsorption of [¹⁴C]Halamid[®] was determined by the slurry method with three soil types (i.e. sandy loam, loam, and low humic content sand soil) known to have a pH value between 6 and 8.5. Adsorption constant values for [¹⁴C]Halamid[®] based on total soil, were 0.68 ml/g for sandy loam soil, 1.04 ml/g for loam soil and 0.43 ml/g for low humic content sand soil at the reference solution concentration of 1 µg/ml. Adsorption constants calculated on organic matter base (K_{om}) were 31, 52, and 43 ml/g, respectively for these soils. These low adsorption coefficients indicated that [¹⁴C]Halamid[®] is only very slightly adsorbed to soil particles. Due to the instability of [¹⁴C]Halamid[®] in water and soil, it is assumed that its hydrolysis product p-toluenesulfonamide is only very slightly adsorbed to soil particles.

van Helvoirt, J. A. M. W. 1996. Determination of the content of Halamid in Halamid[®] – chloramine-T by titrimetry. Report of NOTOX Safety and Environmental Research B.V. Project No. 185827. Submitted to AKZO Nobel Chemicals B.V. The Netherlands September 26, 1996. 11 pp.

The content of Halamid in the technical product Halamid[®] was determined by titrimetry. The content of Halamid in the technical product Halamid[®] – chloramine-T was determined to be 990.6 g/kg, based on duplicate chemical analysis of two separate samples. The standard deviation was calculated to be 0.15%. The melting point of the amine (product after reducing the chloramine with sodium meta bisulphate) was determined to be > 134° C (136.43° C by DSC). Halamid[®] – chloramine-T is 99.1% pure and does not contain any ortho compound.