An Environmental Assessment of the Proposed Use of Oxytetracycline-Medicated Feed in Freshwater Aquaculture

Prepared by:

Larry J. Schmidt, Mark P. Gaikowski, William H. Gingerich, Verdel K. Dawson, and Theresa M. Schreier

U.S. Geological Survey, Biological Resources Division
Upper Midwest Environmental Sciences Center
2630 Fanta Reed Road
La Crosse, Wisconsin 54603
(608) 783-6451

Prepared for submission to the Center for Veterinary Medicine
U.S. Food and Drug Administration
[redacted]

July 27, 2007

Table of Contents

Tables

Figures

Appendices

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 oxytetracycline in intensive aquaculture are accurate and reliable.

William H. Gingerich for Larry J. Schmidt 26 July 07
Signature Date

Larry J. Schmidt, B.A.
Chemist, Chemistry and Physiology Branch
Upper Midwest Environmental Sciences Center

Signature 26 Jul 07
Date

Mark. P. Gaikowski, M.A.
Research Physiologist, Chemistry and Physiology Branch
Upper Midwest Environmental Sciences Center

William H. Gingerich 26 July 07
Signature Date

William H. Gingerich, Ph.D
Supervisory Research Physiologist and Branch Chief, Chemistry and Physiology Branch
Upper Midwest Environmental Sciences Center

Verdel Dawson 8/6/07
Michael D. Jawson 8/1/07
Signature Date

Verdel K. Dawson, M.S.
Research Chemist, Chemistry and Physiology Branch
Upper Midwest Environmental Sciences Center

Theresa Schreier 7/26/07
Signature Date

Theresa M. Schreier, B.A.
General Biologist, Chemistry and Physiology Branch
Upper Midwest Environmental Sciences Center

Michael D. Jawson 8/1/07
Signature Date

Michael D. Jawson, Ph.D., Center Director
Upper Midwest Environmental Sciences Center

2. Proposed Action and Label Claim

This environmental assessment (EA) describes the environmental risk associated with several label expansions and extensions that are being sought from the U.S. Food and Drug Administration (FDA). The request also changes the water temperature at which salmonids may be treated to include all water temperatures used for culture of freshwater fish species. The recommended therapy and potential label claim (Table 1) is to add OTC to fish feed to control mortalities associated with (1) columnaris disease in all freshwater-reared finfish; (2) bacterial hemorrhagic septicemia and pseudomonas disease in scaled warm freshwater-reared finfish; (3) bacterial hemorrhagic septicemia, furunculosis, and pseudomonas disease in cool freshwater-reared finfish; and (4) coldwater disease in freshwater-reared salmonids. A compassionate INAD application has been in place since 1994 to allow intensive aquaculture facilities to collect efficacy data that support potential label claims for oral OTC therapy.

3. Substance Identification for Subject of Proposed Action

Oxytetracycline is a member of the tetracycline family of antibiotics and has the basic 2-naphthacenecarboxamide structure (Figure 1). Hydroxy substitution at the C-5 position (Figure 1) characterizes OTC. The OTC free base (Figure 1) is highly insoluble in distilled water but readily forms soluble salts (Wassef, 1983 p. 13). Tables 2 and 3 present the identification and physicochemical properties, respectively, of OTC. Three OTC salts (OTC hydrochloride, monoalkyl (C_8-C_{18}) trimethyl ammonium OTC, or OTC dihydrate) are presently or have previously been used in aquaculture. The OTC hydrochloride water solubility is about sevenfold greater than that of the alkyl ammonium salt and 11.5-fold greater than the dihydrate (Table 3). While the hydrochloride salt is the most commonly used form in Europe, the monoalkyl trimethyl ammonium salt and now the dihydrate salt are approved for incorporation into fish feed for use in US aquaculture. The FDA has previously considered the monoalkyl trimethyl ammonium salt and the dihydrate salt to be equivalent for effectiveness based on the assumption that the OTC component confers the effectiveness of the formulation. This EA is written based on the following two assumptions: (1) that the bioavailability and the environmental introduction concentration are equal regardless of which OTC form is administered and (2) that the environmental risk associated with OTC use at aquaculture facilities will be equivalent regardless of which OTC form is used. Furthermore, it is expected that at acceptance of this EA, OTC dihydrate will be the only salt used in the medicated premix in US aquaculture.

4. Introduction

4.1 Present Aquaculture Use - Oxytetracycline is a broad spectrum antibiotic approved by FDA as a feed additive to control certain gram-negative bacteria in salmonids, catfish, and lobster and as a skeletal marking agent in Pacific salmon (http://www.fda.gov/cvm/4401.htm.; accessed January 2006). To treat these diseases in salmonids (bacterial hemorrhagic septicemia, ulcer disease, pseudomonas disease, and furunculosis) and catfish (bacterial hemorrhagic septicemia and pseudomonas disease), OTC is incorporated into feed which is then administered for 10 days at a rate of 2.5-3.75 g OTC per 100 lb body weight/day (BW/d) at water temperatures equal to or greater than 9 °C in salmonids and 16.7 °C in catfish. This dose is equal to 55-82.5 mg/kg BW/d. In addition to the use patterns listed on the present label, OTC historically has been the drug of choice when diagnostic evidence shows salmonids to have enteric redmouth, coldwater disease, columnaris, or vibriosis. Oxytetracycline has also been used to control columnaris in several other families of fishes, including Acipenseridae (sturgeons), Centrarchidae (sunfish), and Percidae (perch; Post 1987). Oxytetracycline has been used in intensive aquaculture under a compassionate INAD permit (9332)

issued to the U.S. Fish and Wildlife Service to control mortality associated with enteric redmouth, coldwater disease, columnaris, and vibriosis.

On May 21, 2002, water-solubilized OTC hydrochloride was approved by the Center for Veterinary Medicine (CVM) for skeletal marking of all larval or juvenile finfish. It also may be used in feed as a skeletal marking agent in Pacific salmon. Oxytetracycline immersion therapy shows promise to control mortalities associated with various external fish diseases as well (Stehly 2002 and Rach 2007).

Table 4 presents data on OTC use in the United States during the years 1997 through 2003. In 2001, 2002 and 2003, 15,200 kg (33,480 lb), 7,990 kg (17,600 lb) and 14,880 kg (33,780 lb), respectively, of OTC were sold to the U.S. aquaculture industry. Presumably all OTC sold was used during these respective years. These figures include the use of OTC by more than 60 hatcheries under INAD 9332 for the new purposes for which approval is being sought. This represents a minor use (about 0.4%) compared to the major market for tetracyclines (as a terrestrial species veterinary drug; Section 4.3). In 2001-2002, a total of 51,080 lb of OTC was used for aquaculture in the United States. Use under INAD 9332 (including marine species and treatments to control *Streptococcus iniae*) was 1,439 lb for the same 2-year period (calculated from the INAD data in Appendix A). At most, OTC use in aquaculture (presently about 25,540 lbs per annum) might be expected to increase by about three-fold the present annual INAD use (Dave Erdahl, U.S. Fish and Wildlife Service, 2004, personal communication; estimated to be about 562 lbs at a dose rate of 82.5 mg/kg BW/d), resulting in a potential total aquaculture use of about 27,700 lbs when all potential future label claims are approved. Annual use of OTC by aquaculture facilities for present and proposed new feed label claims would still be <0.5% of total annual tetracycline use for both aquatic and terrestrial veterinary use.

4.2 Need for Action - There are only a few FDA-approved antibiotics for use in aquaculture and these are limited to narrow disease- or species-specific situations. Besides OTC, approved antibiotics include only sulfadimethoxine/ormetoprim (Romet-30) and florfenicol (Aquaflor® and Aquaflor® - CA1). The approval of florfenicol is limited to use in salmonids and catfish. It is also presently being used under an INAD in the United States. Since losses of cultured fish intended for sale or distribution can range from 50% to 90% because of disease, the need for an approved broad-spectrum therapeutant that will reduce disease-related mortalities for many species is of paramount importance.

4.3 Other Uses - Oxytetracycline use in the United States is both as an antibiotic and as a pesticide (Table 4). By far the greatest use of OTC is as a veterinary antibiotic in both companion and farm animals and as a growth promoter in farm animals. Another agricultural use is as a fungicide to prevent diseases in fruit trees. In the hobby fish culture industry, OTC is used to treat bacterial diseases such as vibriosis, Malawi bloat, hemorrhagic septicemia, and those caused by *Aeromonas* sp. It is used both in feed preparations and as a bath (UMESC staff search of multiple hobby fish Web sites, September 2003). Insignificant uses that have been reported are as an algaecide in marine paint formulations and as a medicinal drug to treat human diseases.

5. Environmental Description of Sites of Introduction

5.1 Intensive Aquaculture Model - Intensive aquaculture typically involves the public and private 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 coldwater aquaculture because water temperatures are generally maintained at ≤15 °C, whereas warmwater aquaculture facilities typically maintain temperatures of >15 °C to achieve optimal growth. In this simplified classification scheme, coolwater species (e.g.,

percids or esocids such as walleye [*Sander vitreus*] or northern pike [*Esox lucius*]) are included in the warmwater category. Families commonly cultured in warmwater aquaculture facilities include the Percidae, Esocidae, Centrarchidae, Cyprinidae, Percichthyidae, and Ictaluridae. In both cold- and warmwater aquaculture, fish are generally offered a commercially formulated semi-moist or dry diet, although some live forage may be provided at various culture stages. A Type-A medicated article such as Terramycin® 200 for fish (OTC) or Aquaflor® (florfenicol) would be incorporated into the diet at the feed mill before use in a treatment situation.

Intensive aquaculture is typified by relatively high volume water use, although some facilities do reuse or recirculate water (especially private facilities). In a single-pass culture unit, fresh water enters at one point of the culture unit (usually the upper head of a raceway or the side of a circular or square culture tank) and exits out a drain (usually at the lower tail of a raceway or the center of a circular or rectangular tank) that is 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 fed by gravity 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 with nitrifying bacteria to convert ammonia to nitrate (OTC might be harmful to nitrifying bacteria; Section 7.3.4). When therapeutic treatments are administered at facilities that use water reuse or recirculation, it is possible that fish cultured in culture units downstream of the treatment or in the same recirculating circuit may be exposed to the therapeutant, albeit at concentrations that are probably much less than the initial therapeutant concentration. Treatment water at facilities that have single-pass water use would generally not pass treatment water from one culture unit into another culture unit, but would discharge it 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 culture unit water after treatment, either by drainage and flushing after a static bath or by simply allowing the bolt to travel downstream after a flow-through treatment. Moderately hard spring or artesian water is often cited as a preferred water source (Piper et al. 1982); however, the water sources used at intensive aquaculture facilities are varied. 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. The physicochemical 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). 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 groundwater is discussed in Section 6.6.

Intensively cultured fish are stocked into public or private water, used for on-site recreational fee fishing, or used as food fish sold to restaurants or supermarkets. A conceptual model for the fate of OTC-medicated feed used in intensive aquaculture is represented in Figure 2.

For a typical treatment, OTC-medicated feed (medicated feed is produced by incorporation of the Type-A medicated article directly into the feed mash by the feed mill before pelleting) replaces part or all of regular diet. If the mass of medicated diet offered to achieve the required daily OTC dose is not sufficient to maintain fish growth, unmedicated feed may also be offered. However, medicated and unmedicated feed are not offered at the same time to ensure complete consumption of the medicated diet and to prevent preferential consumption of the unmedicated diet. Fish are fed the medicated feed one or more times daily (depending on the size of the fish) for 10 consecutive treatment days. Fish may either be fed by hand or by an automated feeder. Regardless of feeding method, feed is generally distributed in a fashion appropriate to the species cultured and the feed type offered. For example, rainbow trout (*Oncorhynchus mykiss*) are frequently offered a semi-moist "sinking" diet that is distributed evenly across the water surface. Conversely, lake trout (*Salvelinus namaycush*) or Atlantic salmon offered the same diet may have the diet "floated" across the tank surface to allow the fish greater opportunities to consume the offered feed. Care is taken to minimize uneaten feed because feed, especially medicated feed, is a major expense in cultured fish production.

Most culture units are designed to concentrate uneaten feed and feces near the culture unit drain; these wastes may be temporarily resuspended when the culture unit is cleaned. Most facilities clean culture units by temporarily removing a standpipe in the culture unit and brushing debris down the culture unit drain. Depending on the culture unit design, fish loading, and water flow, biosolids (uneaten feed and feces) may be removed daily, weekly, or at some other interval designed to maximize fish health and growth while minimizing labor and other costs. The resuspended biosolids are transported by culture water flow out of the culture unit to a quiescent zone or a settling pond, the latter being used by many hatcheries to dilute, detain, or stabilize discharge water before it is released into the environment. Some hatcheries use vacuums to remove accumulated biosolids from culture units to minimize effluent biosolids loading into the settling ponds, and at least one State (Washington) prohibits discharge of accumulated solids from settling ponds to public surface waters. Quiescent zones and settling ponds downstream from culture units are typically cleaned once or twice yearly, if they are cleaned at all. Removed biosolids are dewatered and disposed of in an approved manner. Recirculating systems, in addition to biosolids removal as previously described, frequently use specially designed filters and clarifiers to reduce/remove biosolids from the culture units. Biosolids captured on these filters/clarifiers are typically removed by "backwashing" the filters with the backwash water carrying the captured solids into the hatcheries' effluent stream. Effluent ponds were reported to be used for solids settling prior to effluent discharge from 51 of 100 hatcheries that responded to a UMESC hatchery survey (Appendix A; USGS hatchery survey.otc ea.xls, "hatchery information" tab). Of these hatcheries with settling ponds, the median settling pond volume was 3 acre-feet, equivalent to ~3.7 million liters of water.

Treatment water is typically discharged from the culture unit and combined with other hatchery water for eventual release into streams, rivers, or lakes. Although there is the potential for treatment water containing OTC to be discharged into brackish-water ecosystems, none of the 100 hatcheries that responded to a UMESC hatchery survey indicated that their effluent would be discharged into brackish-water ecosystems. Likewise, none of the hatcheries that reported OTC use in INAD 9332 categorized their receiving watershed as a brackish-water ecosystem. Nonetheless, discharge from freshwater aquaculture into brackish water might occur; therefore, our risk assessment evaluates the potential risk associated with discharge into both fresh- and brackish-water lentic or lotic systems.

Discharges to public water are subject to regulation and monitoring by state or local regulatory agencies. A conceptual facility design or layout for an intensive aquaculture hatchery is presented in Figure 3.

6. Analysis of Environmental Fate in Intensive Freshwater Aquaculture

6.1 Previous EAs - Several EAs have been prepared for various OTC uses (Wassef 1983; Katz 1984, 1985, 1995). The most recent EA (Katz 1995) was for use of OTC in buffered water for the marking of skeletal tissue of fish fry and fingerlings. Katz also prepared EAs for OTC to control gaffkemia infections in lobsters (Katz 1984) and to treat/control respiratory and digestive tract infections in captive-reared alligators (Katz 1985). Katz's earlier studies (1984 and 1985) do not add to the fate information in his 1995 EA for marking fry and fingerlings. An EA for OTC use in terrestrial livestock animals was prepared by Wassef (1983). The EA covered most of what was known about OTC physical-chemical properties and environmental fate/effects at the time and included about 150 citations from the literature. These EAs are included in our literature section and their major findings are incorporated into this EA.

6.2 Physical-Chemical Properties - Oxytetracycline is ionized throughout the pH range, existing in the cationic form below pH 3.3, as a zwitterion between pH 3.3 and 7.7, and an anion above pH 7.7 (Wassef 1983, p. 13). The apparent partition coefficients for OTC between octanol and aqueous buffers at 25 °C are presented in Table 6 (Collaizzi and Klink 1969; Wassef 1983, p.35). When partition coefficient calculations were performed, it was concluded that OTC best transfers into n-octanol at pH 3.9, where it exists as a zwitterion. At the pH range normally related to aquatic systems, 5.5-8.5, there would be a limited capacity for OTC to partition into lipid materials.

Aqueous tetracyclines form complexes with metallic ions at the C-10, 11 and 12 positions (Figure 1). At pH 8.0, tetracyclines chelate iron (Fe^{++}), copper (Cu^{++}), magnesium (Mg^{++}), and calcium (Ca^{++}, Wassef 1983, p. 14). Most of the chelates are considered to be water insoluble at the molar ratio of 1:1 drug:metal (Wassef 1983, p. 14), but they might be soluble at ultra-trace concentrations. Wassef (1983, pp. 22-23, 29-31) also stated that OTC sorbs to sand, clay, and organic matter and is deactivated to varying degrees when bound to these substances. Chelation is discussed in detail in Section 6.8 and sorption to clay and association with organic matter in Section 6.9.

After the work by Wassef (1983), some additional work has been done on the chemical properties of OTC. A modern treatise on tetracycline chemistry and degradation products at various pHs and in the presence of light and chelating metals was given by Halling-Sørensen et al (2002). They also presented an excellent summary of previous work in this area.

6.3 Introduction into the Environment - Wassef's (1983) EA suggests that most of the introduction to the environment from terrestrial veterinary use is from unmetabolized OTC in animal excrement. This is true for fish as well (see below in this section). Up to 75% of ingested tetracyclines pass through livestock and is excreted in unmetabolized form (Wassef 1983, p. 22). Oxytetracycline required up to 144 h to disappear from bovine body fluids (Wassef 1983, p. 22).

Pharmacokinetic studies of OTC have been conducted in a variety of fish species over the past several decades. Several factors emerge from these studies that are of direct relevance to an environmental assessment of an amended use of OTC in freshwater aquaculture. First, OTC is not known to be metabolized by any of the fish studied. Second, when administered intravenously or orally, OTC is distributed readily throughout the body (Grondel et al. 1987; Plakas et al. 1988; Björklund and Bylund 1990; Rogstad et al. 1991; Rigos et al. 2003). Highest tissue concentrations are found in organs of clearance (i.e., liver and kidney) and both renal and hepatobiliary clearances seem

to be major avenues of loss from the vascular system (Plakas et al 1988; Björklund and Bylund 1990; Namdari et al. 1998; Rigos et al. 2002). For example, about 50% of intravenous doses are accounted for in urine and bile of channel catfish (*Ictalurus punctatus*) within 48 h of dosing (Plakas et al. 1988). Third, both within and among the species studied, absorption into tissues and elimination from tissues are directly influenced by temperature with greater absorption and faster elimination occurring at higher temperatures (Tables 7 and 8; Lane and Jackson 1969; Rigos et al. 2002). Fourth and lastly, a major confounding factor in the use of OTC as an oral therapeutant has been the poor bioavailability of the drug in fish. While some studies have documented the bioavailability as great as 30%, most reported values have been less than 10% (Table 8). This is generally attributed to poor gastrointestinal absorption. The significance of this last factor is that substantial amounts of OTC are excreted as fecal wastes without entering the body. These residues are then lost to the aquatic environment en masse without having ever been available to the animals for therapeutic activity. Such excretion would result in the mass release of substantial quantities of OTC, bound to fecal material, into culture water after passage through the gut. With gut transit times in fish usually being about 48 h (Table 7), the release of OTC into water from fish will typically begin and end about 2 d after the beginning and end of therapy.

Smith et al. (1994) suggest that OTC elimination from fish can begin at least as early as 8 h after consumption of medicated feed. Releases of OTC occur from fish feces, fish urine and bile fluid (Cravedi et al. 1987; Björklund and Bylund 1990). In aquaculture, introduction from OTC-medicated feed also occurs through leaching from uneaten feed (15-40% of administered feed in salmon net pen culture; Gowen et al. 1989; Capone et al. 1996). Losses from uneaten feed may increase during a disease outbreak, especially if the disease or the lower palatability of medicated feed results in loss of appetite (Hustvedt et al. 1991; Sandaa et al. 1992).

Smith (1996) speculated that some uneaten feed may possibly be consumed by nontarget organisms, and several studies support this conclusion (Björklund et al. 1990; Weston et al. 1994). Oxytetracycline has been found in varying amounts in invertebrates near net pens from trace levels in oysters and Dungeness crab (*Cancer magister*) to levels of up to 3.8 μg/g (mg/kg) in red rock crab (*Cancer productus*; Capone et al. 1996). Studies have documented the presence of OTC residues in wild fish, crabs, and mussels near these marine sites for up to 2 weeks after treatment (Capone et al. 1996). It is possible that such organisms eventually reintroduce biologically available OTC to the environment through their excrement.

Oxytetracycline from flow-through freshwater aquaculture discharge is thus potentially introduced to the environment adsorbed to fish feed and feces biosolids, and in total-dissolved form (freely-dissolved ionic OTC in water [chelated or unchelated] or OTC sorbed to dissolved organic matter [DOM]) from fish urine and by desorption from fish feed and feces. To some extent, total-dissolved OTC can also re-adsorb to biosolids, or adsorb to other hatchery suspended solids (Doi and Stoskopf 2000). Oxytetracycline can potentially re-enter the aquatic environment in biologically available form from wild fauna excretions, from leaching from suspended solids, and from leaching from solids depositions in sediment.

6.4 Environmental Fate: Stability - In this EA, discussion of stability refers to the continued existence of OTC in its biologically available form, or in a form that could revert to its biologically available form. Katz's literature review (1995) indicated that OTC is stable in terrestrial soils and Gonsalves and Tucker (1977) reported that OTC persisted at measurable levels in soils for as long as 1.5 years. No reported microbial mechanism of degradation is known that is significant enough to reduce OTC concentrations present in soil or sediment as the result of therapeutic treatments. Oxytetracycline is not a refractive compound in any environmental compartment under any known circumstances, but its degradation rate in sediment is very slow and from unknown mechanisms

(Lunestad and Goksøyr 1990; Lai et al. 1995). Aqueous OTC solutions are relatively stable at neutral pH, but readily degrade as a function of pH, temperature, and light (Wassef 1983, p. 23; Sections 6.4.1-6.4.3 give details). Oxytetracycline's half-life is considerably shorter in seawater than in marine sediment (Samuelsen 1989; Pouliquen et al. 1993), a characteristic that is probably true for fresh water. Oxytetracycline's half-life in the water column is affected by temperature, pH, and sunlight (Doi and Stoskopf 2000) with photolysis and hydrolysis as the primary mechanisms for degradation. Total-dissolved concentrations are also reduced by the presence of suspended bentonite clay and organic matter particulates (Doi and Stoskopf 2000), but this is from mobility into these compartments rather than degradation.

6.4.1 Environmental Fate: Temperature - Aqueous OTC solutions degrade faster at higher temperatures. A 1% solution of OTC-hydrochloride in water will maintain potency for at least 30 d at 25 °C, but for only 5.5 d at 38 °C (Wassef 1983, p. 23). Katz (1995) also found that OTC degrades as a function of temperature in brackish water, having only about a quarter as much remaining activity at 28 °C than at 4 °C after 20 d (nonsterile water) and about half as much after 31 d (in sterile water; the method for determining activities was not specified, but probably used microbial inhibition assays). Katz's (1995) overall half-life estimate for brackish water was 12 d (nonsterile water, pH 7.0, 20 °C). Doi and Stoskopf (2000), using HPLC analysis, found a half-life of 0.26 d for OTC at 43 °C in deionized water, 54-fold shorter than at 25 °C (14.04 d). At 4 °C, OTC concentrations did not decline significantly after 77 d. Using HPLC, seawater half-lives of samples held at 4 °C in the dark (7 d when illuminated and 16.3 d when not illuminated) were found to be substantially longer than those held at 15 °C (5.3 d when illuminated and 9.8 d when not illuminated; Samuelsen 1989). Assuming that the typical half-life of aqueous OTC is near 10-12 d at neutral pH and warm water (about 24-27 °C), temperature is a relatively insignificant factor in determining OTC discharge concentrations from aquaculture facilities.

6.4.2 Environmental Fate: Photodecomposition - According to Wassef (1983, pp. 23-26), tetracyclines (TC) in general are decomposed to biologically unavailable components by exposure to strong sunlight or to near ultraviolet (UV) radiation. Photodecomposition products are the peroxide, the hydroperoxide, the anhydro compound, and the epimer -deoxy-TC (Wassef 1983, p. 26). Although the photodecomposition mechanism was not established when Wassef produced his EA, Halling-Sørensen et al. (2002) stated that tetracyclines can undergo direct photolysis. An excellent schematic of tetracycline degradation pathways and products is available in Figure 2 of Halling-Sørensen et al. (2002). For OTC, the 4-epi-OTC is probably the most significant, by far, of all degradates produced by photolysis or hydrolysis (Halling-Sørensen et al. 2003).

Using HPLC, Samuelsen (1989) recorded OTC half-lives in seawater of 7 d and 5.3 d at 5 and 15 °C, respectively, under illumination for 24 h/d with a 40-W fluorescent tube, and 16.3 and 9.8 d, respectively, in aquaria maintained in darkness. Under natural lighting, Choo (1994), using HPLC, found OTC half-lives of 12.4 d in seawater (pH 7.9, 27 °C) and 2.4 d in fresh water (pH 7.3, 27 °C). He stated that his findings were the reverse of those of Lunestadt (1991, whose method of analysis was not clearly stated), who calculated OTC half-life to be 24 times longer in fresh water than seawater, but were in approximate agreement with those of Samuelsen (1989) and the Merck Index (Windholz et al. 1983). Using HPLC, Lunestad et al. (1995) reported a 96% reduction of initial OTC concentration (50 mg/kg) in seawater after 7 d in natural light at a depth of 1 m. Using HPLC, Doi and Stoskopf (2000) found that OTC degraded threefold faster in light than in the dark in distilled water under laboratory conditions (in a 600 mL beaker). They calculated a half-life of 3.94 d (at 25 °C) under constant irradiation from a 15-W fluorescent lamp.

Ultraviolet light penetrates deeper in clear fresh water than in seawater (Lunestad et al. 1995), but Doi and Stoskopf (2000) also note that attenuation of light might often be greater in fresh water

because of more eutrophic conditions. However, brackish water is usually at least as eutrophic as fresh water. Photodecomposition should be more important in fresh water than in marine or brackish water because many lakes and streams (or parts of streams) are relatively shallow. Studies in seawater are important because most OTC is probably chelated by divalent cations in seawater yet it continues to degrade much faster in light than in the dark. Thus both chelated and unchelated OTC degrade by photolysis. Photodecomposition may be the major mechanism by which OTC eventually degrades in fresh water, especially in ponds, rivers, and streams.

The shortest reported half-life value because of photolysis for fresh water at environmentally relevant temperatures (~27 °C) is 2.4 d (Choo 1994). The half-life is probably longer in turbid fresh water, but most OTC is probably bound to solids under these circumstances and thus biologically unavailable. Therefore, photodecomposition is probably an insignificant factor in determining OTC peak discharge concentrations from freshwater aquaculture facilities, especially if no holding pond is present.

6.4.3 Environmental Fate: Hydrolysis - Tetracyclines are sensitive to both acids and bases, and produce anhydro-TCs or iso-TCs, respectively, by hydrolysis. This is primarily because of the nature of the hydroxyl group at the C-6 position (Wassef 1983, p. 26; Figure 1). It is well known that basic aqueous conditions promote more rapid OTC breakdown than neutral or acidic conditions (Halling-Sørensen et al. 2002). Clive (1968, from Wassef 1983) reported that the half-life of OTC (at 37 °C) was 14 h at pH 10 but increased to 134 h at pH 2.5, with half-lives almost uniformly increasing as pH decreased; Wassef 1983, p. 27, Table XI). Similarly, Doi and Stoskopf (2000) reported a pH dependent decrease in the half-life of OTC (at 25°C) from 46.36 d at pH 3.0 to 14.04 d at pH 7.0 to 9.08 d at pH 10.0. The relatively long half-lives observed across the environmentally relevant pH ranges tested suggest that hydrolysis is a relatively insignificant factor in determining peak OTC discharge concentrations from freshwater aquaculture. Although not as important to the immediate fate of OTC as photolysis, hydrolysis may be the most important means of eventual OTC degradation in relatively deep bodies of fresh or brackish water.

6.5 Environmental Fate: Biodegradation - Wassef (1983, p. 26) speculated that since OTC is a natural metabolic product of the fungus *Streptomyces rimosus* a biological process would probably exist that biodegrades OTC and prevents its buildup in nature. However, at that time, no such organisms had been identified, and Katz (1995) made the same statement more than a decade later. A study by Lai et al. (1995) did indicate that biological transformation of OTC occurred when pond sediments were continuously aerated and stirred, but they found no such degradation under quiet, anaerobic conditions. A recent study of tetracycline (TC), a closely related compound, suggested that biodegradation of tetracycline did occur in ventilated liquid pig manure (Kühne et al. 2000), but the results were not conclusive. Likewise, in another recent study, OTC appeared to slowly biodegrade using a shake-flask procedure and natural surface water, especially in the presence of oxygen (Ingerslev et al. 2001). However, abiotic controls were not used and a mechanism was not proposed.

6.6 Environmental Fate: Mobility - Oxytetracycline is mobile between two major aqueous compartments-sediment and the water column. In the water column, OTC is either dissolved in water, attached to DOM, or attached to suspended solids. Section 6.9 presents a discussion of OTC sorption to inorganic and organic solids and association with DOM.

Oxytetracycline mobility into sediment is thought to largely originate from deposits of uneaten medicated feed and also fecal matter (Smith et al. 1994). Loss of appetite that often occurs during a disease outbreak results in significantly higher concentrations of OTC in sediment. This is because of increased accumulation from the rapid settling rate of feed and also to increased OTC half-life under the resultant anoxic conditions (Coyne et al. 1994b). Mobility into sediment occurs to a significant

extent underneath marine net pens. Oxytetracycline concentrations in marine sediment are subsequently reduced primarily by outwashing, followed by relatively rapid dilution and possibly some degradation in the water column. The reduction of OTC in sediment under marine net pens is highly variable and depends as much on physical conditions (e.g., agitation and current flow) as it does on sediment chemical composition (Coyne et al. 1994a). For example, the presence of fauna (especially burrowing fauna) in sediment affects sediment turbulence in both salt water and fresh water and will affect the rate of leaching from sediment into the water column.

Although freshwater hatchery situations differ substantially from those of marine net pens, studies of OTC fate in marine sediments under net pens may be helpful in indicating the fate of OTC in freshwater or brackish water sediments. Smith (1996) demonstrated through calculations using simple models, with exaggerated worst-case assumptions, that sediment is the repository for only a tiny fraction (1-2%) of administered OTC. Actual concentrations in the top 2 cm of sediment were 10.9 mg/kg, but that only represented 2% (at most) of administered OTC, according to his calculations. Thus, even though OTC shows by far the most chemical magnification in sediments, sediments account for little of the mass balance of the administered chemical. Another small amount is absorbed by cultured fish (7-9%; Cravedi et al. 1987), and the rest is diluted by and goes to an unknown fate in the water column.

Unlike marine sediments under net pens, the role of sediment deposition in the fate of OTC after use as medicated feed in freshwater intensive aquaculture has not been characterized from actual data. An initial semi-quantitative model was developed based on the best available literature-based input parameters using the Water Quality Analysis Simulation Program version 6.0 (WASP-6) software (Wool et al., no date). The WASP-6 simulation model presently indicates that sediment deposition is probably the dominant fate of OTC in freshwater aquaculture (Rose and Pedersen 2005). However, the WASP-6 discharge model for OTC has not yet been validated. For example, if the leaching rates from fish biosolids observed in seawater are much higher than those in fresh water, then sediment partitioning may be of greater importance in fresh water relative to marine or brackish water ecosystems.

Concentrations, not amounts, are of most interest in environmental toxicity. The concentrations of OTC are highest in sediment, even though the chemical is almost totally in a biologically unavailable form while in sediment (based on microbial toxicity; no toxicity data are available for benthic invertebrates; Section 6.9). The lack of degradability of OTC in sediment and its solubility indicate that it might take considerable time for OTC to completely leach from sediments (Coyne et al. 1994a). Studies of OTC half-lives were conducted in the late 1980s and early 1990s. Almost all of these involved sediments below marine net pens. Because degradation of OTC in sediment is considered negligible, especially under anaerobic conditions (Lunestad and Goksøyr 1990; Samuelsen et al. 1994; Lai et al. 1995), the half-life in sediments depends on the rate of re-entry into the water column (Lunestad 1991; Samuelsen 1992; Coyne et al. 1994a; Smith and Samuelsen 1996). Burrowing and movement of fauna associated with aerobic sediments may disturb/stir the sediment and thus hasten the re-entry of OTC into the water column (Coyne et al. 1994a).

Katz (1995) stated that "many of the calculated half-lives published for OTC in sediments vary considerably based on three factors: (1) depth of sediment, (2) the redox potential of the sediment, and (3) losses from water flows. Oxytetracycline half-lives in marine sediment from published literature are summarized in Table 9. Björklund et al. (1990) listed half-lives of 9 to 419 d, a wider range than that of any of the other authors. Their stated half-life range was a function of whether the sediment was exposed to currents or stagnant water. Katz (1995) also stated that a "problem in the results cited in the literature comes from the extraction techniques used. Use of pH 4.0 buffers and solvent has never been shown to break the binding of OTC to the Group II or Group III cations, soil

components, or proteinaceous matter. Thus, shorter half-lives or results indicating diminishing OTC levels over time in sediments are not unexpected, if the extraction techniques cannot break the binding." Thus, OTC might be longer lived and at higher concentrations in sediments than the data from the literature imply.

When OTC is used in an earthen raceway or when treated effluent enters an earthen pond (e.g., an unlined detention pond), a question arises as to its potential to infiltrate the pore water of the bottom sediments and its possible mobility into groundwater. However, it is unlikely that the presence of dilute OTC in earthen ponds or raceways would lead to a significant release into 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. The potential for long-term substantial environmental impacts in groundwater after OTC treatment is unlikely. Therefore, we have not further explored OTC contamination of groundwater or conducted a risk characterization for groundwater.

Studies of actual OTC residue in sediment were few in number until the early to mid 1990s, when considerable work was done to characterize OTC concentrations in sediments under marine net pens. There has been little similar work done for sediment in freshwater aquaculture flow-through applications, although there have been some data generated for aqueous OTC residues inside and outside of freshwater hatcheries, as discussed later in this section.

When detected, OTC in sediments below marine net pens has been observed to range from 0.1 to 11 mg/kg to as much as 260 mg/kg (Samuelsen et al. 1992), usually depending on sediment depth. Oxytetracycline concentrations in marine sediment from published literature are summarized in Table 10. Surface sediments (the top ~2 cm) yield the highest immediate concentrations (Samuelsen et al. 1992; Coyne et al. 1994a; Weston et al. 1994), but deeper sediments have the longest time to depletion to below detection limits following cessation of treatment (Hektoen et al. 1995). Concentrations of 4 to 10 mg/kg are commonly recorded in top sediment under net pens (Table 10). Under quite disparate conditions, essentially no OTC was found below 10 cm in two studies of sediment deposition below marine net pens (Samuelsen et al. 1992; Smith and Samuelsen 1996). Detectable amounts of OTC have been found in sediment 3-6 months following feeding of the antibiotic (Kerry et al. 1994). By contrast, Romet-30 appears to be short lived in marine sediments (Capone et al. 1996).

In net pen farming, OTC is transported to sediment primarily by the settling of OTC-containing organic waste (suspended feces and uneaten feed) present in the net pen (Gowen et al. 1989). Some horizontal advection of OTC occurs from marine net pens to adjacent sediments, mostly in the direction of the dominant current flow (Coyne et al. 1994a; Kerry et al. 1996), but concentrations drop steeply to non- or nearly non-detectable levels not far (> 20-35 m) from the pens. The estimated OTC distribution outside the area below the cages by these authors was from less than the area of the cage block to twice that area. Their estimations were based on OTC levels actually found adjacent to the cage blocks, which would be influenced by dosage levels and solids settling rates, as well as by OTC leaching rates from the various solids involved.

Leaching by seawater (including brackish water) might be at a markedly different rate than by fresh water. Samuelsen et al. (1992) detected OTC in sediment under an adjacent down-current net pen that was about 90% reduced in concentration from that found directly under a treated net pen. Capone et al. (1996) detected OTC in sediments at 30 m but not at 100 m down-current from OTC-treated marine cage areas. Oxytetracycline in feces should result in a somewhat longer sediment plume than that in feed, because of a slower settling rate (Coyne et al. 1994a). However, Coyne et al. (1994a) did not find any OTC in sediments that were predicted to be associated with fecal deposits

only. Although no known studies of horizontal advection have been done for freshwater aquaculture, net pen plumes indicate that OTC migration to sediments at places outside a freshwater aquaculture facility is only likely to occur to the extent that suspended solids and biosolids travel from inside to outside the facility. Oxytetracycline would have to remain attached to the solids for the time it takes to do so and the time it takes to settle to the sediment bed.

As stated previously, studies of actual residue concentrations after use and discharge in freshwater flow-through aquaculture operations are virtually nonexistent. Bebak-Williams et al. (2002) did investigate the fate of OTC in a freshwater recirculating system. They administered OTC-HCl in feed at 3 g OTC/lb of feed (assayed at 6,100 μg/g [mg/kg] as OTC-HCl), and offered the feed at 1% BW/d for 10 d. They then measured OTC in the various compartments of the system from day 1 to 31. The greatest concentrations by far were in sediment (consisting mostly of fish feces and uneaten feed), which reached 1,900 μg/g by day 10. The next highest compartment was the biofilter sand at 13.7 μg/g at day 10. By day 15 (5 d after the end of treatment), the sediment concentration had been reduced to 22 μg/g, which is more than an 85-fold reduction from 1,900 μg/g. Using generous estimates for the weights of sediment and sand present in the system (the fish and water weights were given or could be calculated), our estimates from the information presented in this paper lead to the conclusion that most of the OTC mass was lost through the water column, even when OTC loss by sediment removal (every other day) was all assigned to the sediment compartment[1]. If the sediment had not been removed, much of this OTC would have potentially been lost to the water column. The Bebak-Williams et al. (2002) study also suggests that while surface sediments below net pens have usually shown the highest concentrations of OTC, in most instances they may have already experienced considerable outwashing of OTC into the water column, since their concentrations are much lower than those found by these authors for biofilter sediment.

In a similar study, Smith et al. (1994) used a circulating drum filter of a nominal porosity of 50 μm to capture solids-bound OTC residues of the administered OTC hydrochloride in feed from culture water over 2 days of treatment at a freshwater hatchery. The filter processed the combined downstream effluent (mostly water and suspended biosolids) from the 19 ponds comprising the hatchery and was designed to remove the suspended solids. There was no mention of a settling pond upstream of the filter, and thus most solids probably reached the filter. Their hourly analyses of samples taken over a 24-h period indicated that more than 60% (1,250 g out of 2,008 g total administered over a 2-d period) of OTC was removed by the filter during the second day of treatment. Since sampling was not begun until the second day, 24 h after the initial administration, the filter had probably already removed a considerable mass of OTC by the time of first sampling. The filter was continuously back-flushed into a sedimentation tank, effecting continuous removal of the collected solids-bound OTC. In this instance outwashing of the OTC-rich solids was minimized and a sizeable

---

[1]Based on the information presented in Bebak-Williams et al. (2002), the volume of water in the system was 2,160 L (water was added to the system at 3 L/min x 1,440 min/d = 4,320 L, and there were 2 turnovers/d of H_2O). We estimated from this information, and from the average OTC concentration in water (0.36 mg/L), that 20 g of OTC is carried out in the water column in 13 d. We estimated that 400 g (about 40% of the weight of administered feed) of sediment was removed during each sediment removal, which took place every 2 to 4 d. Five such removals would remove 3.1 g of OTC (400 g x first five removals during Trial 1 x an apparent typical concentration of 1,550 μg/g, at most, in the sediment). We estimated that the sand filter contained 50 kg of sand and that 0.7 g total OTC are transported to the sand (50,000 g of sand x 13.7 μg/g, the average analysis result on day 10 = 0.7 g). A known weight of fish (108 kg) was treated during the experiment and the typical analysis result for trout muscle was 1.25 μg/g (for hydrophilic compounds this approximates the concentration for whole fish). This results in an estimated 0.135 g of OTC transported to fish. Doubling the OTC masses for all the compartments other than water would not alter the conclusions as to the fate of most OTC.

percentage of OTC remained with solids, which could then be dewatered and discarded. This seems to suggest that, at least initially, most of OTC present in the water column of treatment and other hatchery waters is attached to suspended solids.

It is likely that raceway sediment would be a compartment containing OTC, but these sediments are not left undisturbed, often being roiled by sweeping and being moved out of the raceway (along with fish feces) on almost a daily basis as part of the typical cleaning operation. Such sediments usually deposit themselves in quiescent places or holding ponds within the aquaculture facility. It has not been established whether or not significant amounts of solids containing OTC ever reach sediments beyond the point of discharge.

Some analyses for aqueous hatchery and ambient stream OTC in fresh water have been reported. Thurman et al. (2002) randomly collected 175 water samples during an 18-month period in 2001-2002 from 13 fish hatcheries that used antibiotics. The samples were then analyzed for various antibiotics. Oxytetracycline was found in seven samples, from five hatcheries, at a range from 0.17 to 10 µg/L, with the median concentration being close to the low end of the range. Trace concentrations for the antibiotics found, including OTC, were mostly detected in the 0.1 to 2 µg/L range. It was not stated whether any samples were collected during treatments. The highest concentration was 10 µg/L and was found in the settling pond water of a single hatchery. Dietze et al. (2005) reported that dissolved OTC was found at 0.35 to 9 µg/L in the treatment raceway water during a 10-d therapy at label dosage at "intensive fish hatchery A". The higher concentrations occurred between days 5 and 8 of the therapy. No OTC was detected at any time in the hatchery's effluent.

Analyses have been conducted for ambient OTC (along with other emerging contaminants) in the surface water of streams throughout the United States. A study by Kolpin et al. (2002) indicated that only one sample out of 84 analyzed for OTC contained detectable levels of OTC (0.34 µg/L) in surface water. Using a similar method, another laboratory reported no detectable OTC in 115 surface water samples (Koplin et al. 2002). Lindsey et al. (2001) reported the same 0.34 µg/L value, as well as values of 0.07-1.34 µg/L from surface waters in Kansas. From these data, it appears that ambient OTC levels in surface water are at very low concentrations, if they are detected at all.

6.7 Environmental Fate: Bioaccumulation - Bioconcentration of a compound in tissue can be estimated by calculating the ratio of the concentration in tissue to the concentration in water. If the bioconcentration factor (BCF) is 1,000 or greater, accumulation is of considerable significance and concern. If the BCF is 100-1,000, the accumulation is still possibly of concern. If it is below 100, bioconcentration of concern is unlikely (Wassef 1983). Wassef (1983, pp. vii, 34-35) calculated the BCF of OTC in tissues of aquatic animals at various pHs. All BCFs were below 25, including at pH 3.9 where the maximum zwitterion occurs, indicating that substantial bioaccumulation of OTC is not likely to occur. However, a bioaccumulation factor of 450 for OTC was found for the bryophyte *Fontinalis antipyretica*, a species known for its ability to accumulate polycyclic aromatic hydrocarbons and metals as well (Delépée et al. 2004). Le Bris et al. (1995) studied the bioaccumulation of OTC in 3 marine species. The 7-d BCFs in Pacific oyster (*Crassostrea gigas*), short-necked clam (*Ruditapes philippinarum*), and a bivalve, *Scrobicularia plana*, were all < 2.0.

The low bioaccumulation potentials are consistent with OTC oral bioavailability data for fish (<10% for most fish evaluated; Table 8). Oxytetracycline may be incorporated into calcified tissues but is biologically unavailable in this state. This property is often used to "mark" scales or otoliths in hatchery-reared fish. Other than the work by Delépée et al. (2004), there is little to add to the findings of Wassef (1983) on the bioaccumulation of OTC. It appears that little potential exists for bioaccumulation in aquatic organisms from exposure to total-dissolved OTC.

6.8 Environmental Fate: Chelation - Tetracyclines form complexes with metallic ions at C-11 and 12 (Figure 1; Halling-Sørensen et al. 2002). This chelation is most strongly pronounced with hydrophilic tetracyclines (mainly TC and OTC; Lunestad and Goksøyr 1990). Chelation of tetracyclines is pH dependent. At pH 8.0, TC chelates iron (Fe^{++}), copper (Cu^{++}), magnesium (Mg^{++}), and calcium (Ca^{++}) and at pH 5.5 it chelates aluminum (Al^{+++}) and cobalt (Co^{++}). However, at pH 1.0, no chelation of metal ions by TC occurs (Chin and Lach 1975, from Wassef 1983, p. 24). Halling-Sørensen et al. (2002) noted that chelation by trivalent cations Fe^{+++} and Al^{+++} is possible as well. Most of the chelates are considered to be water insoluble at the molar ratio of 1:1 drug:metal (Wassef 1983, p. 14), but they may be soluble at environmentally relevant trace levels.

Oxytetracycline is largely biologically unavailable when chelated by divalent cation (Lunestad and Goksøyr 1990). This may be because such chelation is possibly involved in the mechanism of biological activity (Wassef 1983, p. 14; Schmitt and Schneider 2000). The primary mode of action of OTC is inhibition of protein synthesis on ribosomes, although OTC is known to inhibit many other cellular processes (Wassef 1983, p. iv). Reduced antibacterial effect could also be caused by any alteration of the molecular charge that diminishes the ability of OTC to cross lipid-rich biological membranes (Lunestad and Goksøyr 1990).

Poor OTC bioavailablilty because of chelation is more important in seawater (including brackish water) than in fresh water. In seawater, only 5% of up to 100 ppm (mg/L) of total-dissolved OTC exists in the free form, the rest being chelated and strongly deactivated by divalent cation (Lunestad and Goksøyr 1990). They found that even 70% seawater in their test medium increased minimum inhibitory concentrations (MICs) to the various organisms tested (strains of *Escherichia coli* and *Yersina ruckeri*) by an average of almost tenfold (range 4- to 32-fold) over fresh water and that the effect of magnesium was greater than that of calcium. These authors stated that seawater, whether natural or synthetic, contains roughly 54 mM Mg^{++} and 10 mM Ca^{++}. This corresponds to about 1,300 mg/kg Mg^{++} and 400 mg/kg Ca^{++}. Not surprisingly, the 1:1 OTC:magnesium complex predominates in seawater. Compared to calcium and magnesium, the concentration of other di- or trivalent ions in seawater is negligible (Lunestad and Goksøyr 1990).

Oxytetracycline bioavailability at the concentrations of total-dissolved OTC being released from aquaculture facilities will probably be somewhat reduced by chelation in hard fresh water. Calcium and magnesium are the main divalent ions found in hard fresh water, and the term hardness actually refers to the quantity of dissolved calcium and magnesium. These minerals, which come primarily from limestone type rock formations, are found to some degree in all natural waters. Typically, rain water and surface fresh water are soft (<17 mg/L [ppm] hardness as $CaCO_3$) and groundwater is slightly hard (17-60 mg/L) to very hard (>180 mg/L; UMESC staff search of water treatment Web sites). Some aquaculture receiving waters could be described as moderately hard (60-120 mg/L) to very hard (>180 mg/L), especially spring-fed streams in rocky areas (UMESC search of various water quality Web sites). Hard fresh water is 120-180 mg/L as $CaCO_3$, or 48-72 mg/L as Ca^{++} or 29-43 mg/L as Mg^{++}. Overall, such water is still about an order of magnitude lower in divalent cation concentration than seawater. Nonetheless, at high pH, chelation may be important in hard fresh water, and perhaps in most other fresh water as well, because concentrations of total-dissolved OTC appear to be very low (low µg/L [ppb]) compared to typical divalent cation concentrations (low mg/L) in fresh water. These circumstances might mean that dissolved OTC in receiving waters exists largely in chelated form long after discharge from aquaculture, until it is finally degraded (see Sections 6.4.2 and 6.4.3 for discussion of OTC degradation by photolysis and hydrolysis). In our risk assessment, all total-dissolved OTC at discharge is assumed to be biologically available. However, little data are available on the amount of biologically available OTC in hard vs soft freshwater, on rates of chelation at extremely low reactant concentrations, or on rates of reversion from chelated OTC to the

biologically available form. Therefore, chelation will not be a factor in our risk assessment for total-dissolved OTC at discharge.

6.9 Environmental Fate: Sorption to Solids - Oxytetracycline sorbs to a variety of solids including suspended hatchery biosolids (mostly fish feed and fish feces), river solids, and hatchery and river sediments. It is biologically available in the freely-dissolved form when unchelated, but is tightly bound to and largely biologically unavailable when sorbed to either organic or inorganic solids or associated with DOM (Pinck et al. 1961a,b; Gonsalves and Tucker 1977; Sithole and Guy 1987a,b; Smith et al. 1996; Vaughan and Smith 1996; Doi and Stoskopf 2000).

Oxytetracycline adsorbs to most common clays, but much more strongly to some than others. For example, OTC was sorbed at 300 mg OTC/g clay to montmorillonite (bentonite) clay, but at only 10 mg OTC/g clay to kaolite clay (Pinck et al. 1961a, from Wassef 1983, p. 30). This is because of differences in specific surface areas and cation exchange capacity of these clays (Joel Pedersen, Department of Soil Science, University of Wisconsin-Madison, 2004, personal communication). Tolls (2001) also indicates that sorption of tetracyclines is strongly influenced by the specific surface areas of clay particles, much more than by the hydrophobicity of the particular tetracycline species. Trends in absorption to soils predominantly composed of one or another type of clay followed the trends in absorption for the pure clays. Rabølle and Spliid (2000) found that OTC was strongly adsorbed on all four types of Danish soil tested, with K_d values between 417 in sand soil and 1,026 in sandy loam. All of the soils contained at least 5 % clay and 1% organic carbon. The soil with the highest clay content (16.9%) had the highest K_d value, and a soil containing 5.8% clay had the lowest K_d of the soils tested, but there was little difference between the K_d values of soils containing 11.35% clay and 5.2% clay. Thus, the relation between K_d value and clay content was inconclusive, probably because of variation in clay type among the soils tested. All four soils had similar organic carbon content; thus, the relation between K_d value and organic carbon was also inconclusive. Furthermore, no significant desorption of OTC from the soils was observed using 0.01M $CaCl_2$, nor was any leaching observed using a standard EPA leaching test with the same solution. Thus, even the presence of divalent cation is not sufficient to desorb OTC from solids, although higher cation concentrations might have been able to displace OTC from cation exchange sites (Joel Pedersen, Department of Soil Science, University of Wisconsin-Madison, 2004, personal communication). Nonetheless, even the most deliberate laboratory efforts have resulted in only 35-60% claimed recoveries of OTC from soils (ibid). It is not known whether the recalcitrant 40+% of OTC is mostly attached to clays or organic carbon.

Sithole and Guy (1987a) reported a steady decline in bentonite adsorptive capacity for tetracycline as pH was increased from 5 to 6.5 despite the chemical existing primarily (>90%) as a zwitterion[2]. Figueroa et al. (2004) indicate that even at pH values at which the cation is not the

[2]Oxytetracycline differs from TC only by the former's hydroxylation of C-5 carbon (Figure 1). Tetracycline adsorption to clay minerals is pH-dependent. Porubcan et al. (1978) examined the mechanism of TC adsorption to montmorillonite clays by X-ray diffraction (XRD) and infrared (IR) spectroscopy at pH 1.5, 5.0, 8.7, and 11.0. The XRD indicated that TC is held in the interlayer space in unexchanged montmorillonite (containing ~10% exchangeable calcium) at pHs 5.0 and 8.7, and IR spectroscopy suggested complexation to interlayer cations occurred at pHs 5.0 and 8.7. Maximum adsorptive capacity of unexchanged montmorillonite at pH 8.7 was approximately 33% of that at pH 5.0. Exchange of sodium for calcium at cation exchange sites resulted in no complexation at pHs 5.0 and 8.7, and TC was present in the interlayer space at pH 5.0 but not at pH 8.7. The IR spectra of Ca-TC complexes were identical to TC adsorbed to Ca-montmorillonite. The zwitterion appears to undergo a combination of calcium bridging and cation exchange, whereas monoanion adsorption occurs exclusively by calcium bridging. Thus, two known sorptive mechanisms are operative for the zwitterion,

predominant species in solution, the cation may still dominate overall sorption. In a bioassay to demonstrate the effects of sorption of OTC to sediment, Vaughan and Smith (1996) demonstrated that in the presence of 8% (w/v) freshwater river sediment in nutrient medium, the activity (against *Yersinia ruckeri*) of OTC was only a maximum of 15% of the control, and a minimum of <1% (against *Staphylococcus aureus*). The sediment consisted of clay particles mixed with fine gravel. Organic material was not mentioned. They also determined that the inactivity was probably because of mechanisms other than chelation. In another study, marine sediments exerted an even greater inhibitory effect on OTC than divalent cation (against the bacteria *Listonella anguillarum*, Smith et al. 1996). Pinck et al. (1961b) found that OTC had to be released from various clay soils to exhibit bioactivity. Phosphate or citrate buffers (0.07 M, pH 6.1) can release OTC from all the clay and soil types tested, including bentonite clay (Martin and Gottlieb 1952; Pinck et al. 1961a,b; Soulides et al. 1961, from Wassef 1983, Table XIV). Distilled water would not release OTC in the same clays and soils tested. Thus, significant amounts of OTC might be released under some natural environmental conditions, although more recent thinking attributes the presence of a chelating agent as the most probable cause of OTC release from solids, since OTC sorption is higher at low pH (Joel Pedersen, Department of Soil Science, University of Wisconsin-Madison, 2004, personal communication). However, if this is true, the net result is transfer of OTC from one state of bio-unavailability to another.

Most soils strongly inhibit the bioavailability of OTC, unless the soil consists almost entirely of sand. One study demonstrated that OTC remained highly active for weeks in sandy (>99% sand) soil (Gonsalves and Tucker 1977). However, accumulations of OTC in sandy soils (or sediments) are unlikely because OTC does not readily attach to sand (Gonsalves and Tucker 1977).

Oxytetracycline is also largely biologically unavailable when sorbed to sedimented organic matter, when associated with DOM such as humic acids, and when sorbed to biosolids. The chemically similar compound tetracycline (TC) rapidly sorbs to peat and becomes associated with humic acid (Sithole and Guy 1987b; Smith and Samuelsen 1996). Doi and Stoskopf (2000) determined, using liquid chromatography, that bentonite clay resulted in a 17% decrease in dissolved OTC concentration from distilled water in 5 min but a combination of bentonite clay and organic matter (fish feed) resulted in a 41% decrease in OTC concentration in the same amount of time. These removals were believed to be because of sorption to or partitioning into such suspended material. Sithole and Guy (1987b) examined the equilibrium distribution of TC to non-soluble peat and soluble humic acid at pHs 4.6 and 6.1. Sorption coefficients were similar between these types of organic matter and bentonite clay (~104; Sithole and Guy 1987a). Thus, TC also adsorbs to natural organic matter strongly. Tolls (2001) stated that association to DOM is much stronger than that indicated by hydrophobicity alone and suggested that hydrogen bonding and possibly cation bridging are also involved. Less is known about means of releasing OTC from organic matter under environmental conditions than releasing OTC from clays, or even if it can be readily released from organic matter. Adsorption to organic matter is more important at lower pH, as Sithole and Guy (1987b) found an inverse relation between pH and TC sorptive capacities for both peat and humic acid (although the relation was less strong for humic acid[3]). Such association with DOM and sorption to insoluble

---

whereas only one is active for the monoanion, with the contribution of each to total sorption unknown. All potential OTC sorption mechanisms are discussed in Tolls (2001).

[3] The existing data indicate that sorption mechanisms in addition to hydrophobic partitioning appear operative in the association of tetracyclines with DOM and suspended solids. Hydrogen bonding and cation exchange have been invoked to explain the higher-than-expected distribution coefficients relative to K_{ow}; cation bridging

organic matter substantially reduces the biological activity of OTC, as Gonsalves and Tucker (1977) found that OTC was completely inactivated in organic muck soil. Soluble and insoluble organic material are often present in eutrophic waters and thus act to make a portion of OTC biologically unavailable in such waters.

Oxytetracycline is probably not of toxic concern while sorbed to sediment, or when attached to suspended solids. Oxytetracycline introduction to sediment is thought to largely originate from deposition of uneaten medicated feed and fecal matter (Smith et al. 1994). The bulk of these solids probably migrate to the sediment bed relatively quickly, at least according to the biosolids settling rates used for the WASP-6 model (Rose and Pedersen 2005). However, highly fractionated fecal material may settle slowly. Since OTC is rapidly taken up by and remains tightly sorbed to most solids, the present WASP-6 model suggests that this mechanism alone results in OTC being largely unavailable under most hatchery, riverine, and estuarine conditions, as only a small portion (<20%) of OTC exists in total-dissolved form at peak discharge concentrations. Oxytetracycline released as the total-dissolved form can also re-adsorb to biosolids and hatchery suspended solids (Doi and Stoskopf 2000), thus distribution coefficients become important (Rose and Pedersen 2005).

A second issue is the ability of OTC attached to solids or DOM to remain a reservoir of biologically available OTC. With regard to suspended solids and DOM-dissolved OTC, a return to a biologically available form would only occur slowly in fresh or brackish receiving waters and after extreme dispersion of these substances far from their point of discharge because they travel at essentially the velocity of the stream flow. The same is true for chelated OTC, even though we chose to disregard chelation of OTC in our risk assessment for total-dissolved OTC.

Settleable solids containing OTC could potentially accumulate in sediment beds, especially near the point of discharge. Such solids have been found with OTC concentrations as high as 260 mg/kg in marine sediments directly under net pens, although 4-10 mg/kg is more typical (Table 10). Katz (1995) stated that the extent to which OTC in such sediments remains a reservoir of potentially biologically available OTC in water is unclear and that "since stream water is not distilled water and will contain or solubilize many cations, there is a potential for movement and leach of OTC from bottom muds or sediments." Calculations by Smith and Samuelsen (1996) indicate that even at high OTC sediment concentrations (10.9 mg/kg) at the marine water-sediment interface, concentrations of dissolved OTC in the lowest 1 cm of the seawater column (immediately adjacent to the sediment, worst-case estimation of 16 µg/L) never reach levels of toxic concern because of desorption from marine sediments.

Desorption from freshwater sediments (and therefore water column concentrations above freshwater sediments) should be less than desorption from seawater sediments because seawater (including brackish water) has a greater concentration of divalent cation, which facilitates extraction of OTC from solids into water. Estimates by Smith and Samuelsen (1996) of OTC concentration in the seawater column 1 cm above sediments containing OTC were based on OTC half-lives in seawater, and are therefore probably higher than the maximums for fresh water. Their estimated maximum OTC concentration for seawater (16 µg/L) from sediments containing OTC at about 11 mg/kg does not represent a concern for fresh water. The present WASP-6 simulations suggest that very little OTC will leach from the OTC-containing top-layer sediment (the ratio of water column to upper benthic layer median concentrations was $< 10^{-7}$; Rose and Pedersen [2005]). Thus, a reservoir

---

may also play a role under some conditions (Porubcan et al. 1978; Sithole and Guy 1987a,b, from Rose and Pedersen 2005).

of available OTC of concern does not exist even in the most likely compartment - settled solids in the sediment bed near points of hatchery discharge.

Oxytetracycline is thus tightly sorbed to inorganic and organic solids and associates strongly with DOM, and in these states it is largely biologically unavailable. In fast-flowing waters, the bottom material will consist of mostly pebbles, rocks, and some sand. In this instance, OTC attached to solids will most likely be to biosolids that will travel quickly down the river at near stream velocity. Slower moving streams will have a sediment mixture of sand, gravel, clay, and biosolids just outside the hatchery with biosolids decreasing with distance from the outfall. Organic material, biosolids, and clays will be most important where discharge is to slow-moving streams, estuaries, lakes and ponds, especially if their waters are eutrophic.

Since we do not have data on OTC concentrations in freshwater sediment and suspect that they are much lower than under net pens, we did not include any assessment of OTC leached from sediments in our risk assessment. For summary of OTC sorption to solids as it pertains to the WASP-6 model, see Rose and Pedersen (2005).

6.10 General Discussion of OTC Fate - Oxytetracycline fate is complex and involves a number of factors. All known factors act to reduce concentrations and lengthen discharges of freely-dissolved and therefore biologically available (if unchelated) OTC released from a hatchery. The factors that act to broaden the plume of biologically available OTC from medicated feed are as follows (in the probable order of importance):

1. The time required for OTC consumed by fish to pass through the fish gut (the large majority of consumed OTC mass passes through fish unabsorbed and is excreted as unmetabolized parent).

2. The time required for complete leaching of OTC from uneaten feed and from fish feces.

3. The time required to release OTC once it sorbs to bentonite clay or water soluble or insoluble organic materials in water or sediment. An assumption (Doi and Stoskopf 2000) is that the sorption occurs relatively quickly with available bentonite clay or organic material. Oxytetracycline released from sediment or suspended particles will be in biologically available form unless it resorbs again to these compartments or chelates with divalent cation.

4. Breakdown of total-dissolved OTC by photolysis, hydrolysis, or biodegradation.

5. The time required to release OTC from its chelated form (assuming that its reaction with available divalent cations in water is relatively fast) to its biologically available un-chelated form. Note this is not assumed to be an important factor for OTC fate in fresh water. It may actually be important in harder fresh waters or in brackish waters, but no data exist to support or refute this.

These factors all have the effect of reducing freely-dissolved OTC discharge concentrations; thus, lessening the risk of toxicity. All of the factors might be operative in an aquaculture facility that has a large holding pond. As for bound OTC, whether in solution, suspension, or in sediment, it is the opinion of most researchers that OTC in this state is largely biologically unavailable and not of environmental concern, based on studies of (1) sorption to clays and other inorganic material; (2) sorption to sedimented organic matter (e.g., peat) and to biosolids; and (3) association with humic matter and other dissolved organic material.

The WASP-6 model predicted that the large majority of OTC administered at an aquaculture facility is deposited into the settling pond attached to hatchery solids and subsequently buried there by additional hatchery solids (Rose and Pedersen 2005). When no settling pond is present, these predictions would apply to the nearest receiving water segments outside the aquaculture facility.

Inside an aquaculture facility, the model predicted that only a very small percentage of OTC will be attached to DOM during treatment and discharge, even compared to freely-dissolved OTC in the water column. Oxytetracycline attached to DOM is largely bio-inactive, but according to the model will not be a large component of OTC at discharge regardless of availability. The tendency of OTC to become chelated or released from chelation in either sediment or the water column was not modeled.

Oxytetracycline fate in brackish water would parallel that for fresh receiving water, except that dilution by estuaries would be generally greater than for typical freshwater rivers and streams, and OTC deactivation by divalent-cation chelation would probably play a greater role in brackish water. Solids might also release OTC to the water column more readily in brackish water, but this would be mostly in the chelated form.

6.11 Determining Environmental Introduction Concentrations - Oxytetracycline enters the freshwater environment in one of three fractions: 1) in the freely-dissolved form; 2) associated with dissolved organic materials (DOM); and 3) sorbed to organic and inorganic solids. In the freely-dissolved form it can exist as unchelated or chelated OTC (because we are unable to assess the extent of chelation of OTC and because it would vary significantly in actual situations, we assume that freely-dissolved chelated OTC is biologically available in our risk assessment). Although chelated OTC is thought to be water insoluble, we assume that it is soluble at the low parts per billion (µg/L) level or less. Oxytetracycline other than in the ionic form is mostly not bioavailable (Section 6.9) because it is tightly sorbed or associated with DOM or solids, and is also largely biologically unavailable in the chelated form. Because of the nature of our available data, we chose to estimate environmental introduction concentrations (EICs) based on the total-dissolved OTC fraction, which includes the free ionic, chelated, and DOM-sorbed forms[4]. Total-dissolved OTC concentrations are then used as EICs in our subsequent risk assessments. The EICs we provide represent the predicted facility discharge concentrations, that is, the "end of pipe" concentrations. Our EICs will usually be decreased immediately by dilution in the receiving waters. According to 86 hatcheries that responded to the hatchery survey, 74 (86%) discharged into water bodies that would provide at least an immediate 1:1 dilution of the hatchery effluent.

From 2000 to 2007, the U.S. Fish and Wildlife Service's National INAD Office in Bozeman, Montana, collected use and discharge data for OTC medicated feed administered under the compassionate INAD permit 9332. Data from 1,135 administrations were reported by 88 hatcheries. Reported data included fish species treated, treatment intent (disease agent or marking), mass of OTC used in 24 h, total hatchery flow (24 h), treatment date, and total number and weight of fish treated (Appendix A). The INAD data were parsed at UMESC to eliminate facilities that did not treat

---

[4] "Truly dissolved" is the term used by Rose and Pedersen (2005) to represent aqueous OTC in the ionic form. This could be chelated or unchelated OTC because the authors did not include chelation in their model assessment. Furthermore, the aqueous OTC concentrations reported from the WASP-6 model are actually aqueous OTC in the ionic form + DOM sorbed OTC. The ionic fraction (chelated and unchelated) does represent 95% of this sum during treatment and peak discharge and the 5% DOM-sorbed fraction is not biologically active. We chose the conservative option of reporting the sum as "total-dissolved", whether referring to estimates from the WASP-6 model or from a simple hatchery calculation.

freshwater fish (several facilities treated abalone or marine fish species), that did not discharge water (i.e., 100% recirculation), or those that reported use to control streptococosis caused by *Streptococcus spp.* Parsing this database resulted in a subset of 838 administrations from 74 hatcheries. Four treatments were identified as being unrealistic because of the extreme loading densities reported. The treatments conducted at facility 520 on 3/24/03 and 4/16/03 and at facility 302 on 2/17/00 and 3/14/00 were excluded from the summary because the estimated loading density for these four treatments were six to over one hundred-fold greater than the estimated loading densities of any other facility (Appendix A; spreadsheet INAD 9332 2000-2007 extracted data.xls, "raw data" page). This substantially larger loading density is indicative of either unreported water reuse or recirculation or the existence of an error in the data provided. The data for these four treatments were thus excluded because of the uncertainty regarding these treatments, resulting in a final subset of 834 administrations from 72 hatcheries. The remaining data were then further categorized as: (1) all freshwater-reared finfish, (2) all freshwater-reared finfish by calendar year (except CY 2007 during which there were only seven data points), (3) all freshwater-reared salmonids, and 4) all other freshwater-reared finfish treatments.

Because the reported dose administered during INAD 9332 was frequently not within the 55 to 82.5 mg/kg BW/d range at which OTC is expected to be labeled, we recalculated the mass of OTC administered per treatment based on a dose of 82.5 mg/kg BW/d to estimate the daily mass of OTC used. Although several facilities had administrations that may have overlapped, we treated each administration as a separate discharge event since we lacked exact information on each facility's physical characteristics. Similarly, some treatments might also involve considerable water reuse and possible filtration as part of a semi-recirculating system; therefore, the simple hatchery calculation may produce a much higher concentration than what would actually be realized in the effluent. Table 11 summarizes the OTC therapies applied by hatcheries participating in INAD 9332.

Data reported by hatcheries under INAD 9332 include treatments administered for a variety of disease conditions in a broad range of freshwater-reared finfish species including cold and warmwater fish. Of the 834 administrations reported from 2000 to 2007, 642 were administered to 9 salmonid species (including steelhead trout) from three genuses with the remaining 192 administered to 10 non-salmonid freshwater-reared finfish species (including three hybrids) representing six different families. The treatment indications ranged from use to leave a skeletal mark (126 salmonid treatments) to control of mortality associated with a variety of Gram-negative and one Gram-positive bacteria (*Renibacterium salmoninarum*). Salmonid disease control treatments were administered to control gram-negative bacterial diseases caused by pathogenic members of the aeromonads, pseudomonads, various flavobacteria, *Yersina ruckerii*, and *Renibacterium salmoninarum*. Disease treatments in warmwater fish were administered to control mortality associated with aeromonads, flavobacteria, and *Edwardsiella tarda*. Over the INAD reporting period (2000-2007), drug administration (excluding salmonid marking administrations) to all freshwater-reared finfish was more frequent during the spring (213 [159 salmonid; 54 non-salmonid]) and summer (239 [176 salmonid; 63 non-salmonid]) than during the fall (127 [79 salmonid; 48 non-salmonid]) or winter (129 [102 salmonid; 27 non-salmonid]).

Unlike a bath treatment with a water-soluble drug, OTC incorporated into medicated feed will not dissolve immediately into the culture waters. Instead, it will either slowly leach from uneaten medicated feed or from OTC bound to fish feces. After ingestion and gastro-intestinal tract absorption, OTC may also be eliminated from fish either across the gill membrane or through urine or bile. Gut passage times for fish range from 1 to 4 d, suggesting that medicated feed consumed on the first treatment day may not be released as feces until the fourth day. Therefore, rather than a simple 10-d discharge period, the total dose will probably be discharged over a period equal to the treatment period plus the gut transit period (we used 2 d as a typical gut passage). Some total-dissolved OTC

following treatment will probably reattach or sorb to solids present in hatchery water (feces, feed, inorganic solids). A qualitative examination of OTC fate suggests that OTC total-dissolved peak concentrations at discharge are substantially reduced from concentrations derived from a simple discharge calculation (24-h OTC use/24-h total hatchery discharge), a calculation method that is most appropriate to model effluent concentrations of water-soluble therapeutants administered as an immersion-bath treatment. However, the estimation of a suitable reduction factor to apply to a simple discharge calculation for an OTC medicated feed application is not easy, given the complex environmental fate of OTC. The WASP-6 model described in Appendix B provides an estimate of a reduction factor that might be used to predict total-dissolved OTC concentrations likely to occur following OTC application and also our most quantitative evaluation of the compartmentalization of OTC before discharge from aquaculture facilities. According to the WASP-6 model, the peak concentration of total-dissolved OTC in the settling pond water occurs during dosing. Total-dissolved OTC EICs were developed by applying the reduction factors discussed in Appendix B to the OTC use and hatchery flow data reported by hatcheries that treated cold-, cool- or warmwater fish in INAD 9332. The 95th percentiles for the most probable (simple hatchery calculation divided by 25) and maximum likely (simple hatchery calculation divided by 3.4) OTC discharge concentrations for all fish treatments were estimated to be 0.77 and 5.6 µg/L[5], respectively.

6.12 Selection of a total-dissolved EIC at discharge - For total-dissolved OTC at discharge, an estimation can be made from four independent studies. One involves estimates and three involve actual data. They are as follows:

6.12.1 Estimation using a simple hatchery calculation – The simple estimate of a facility's mean daily OTC discharge concentration was made from the information reported during participation in INAD 9332 (Appendix A, INAD 9332 2000-2007 extracted data.xls, "All fw fish w dis (no strep)" tab). The simple estimate was calculated by dividing the daily mass of OTC (ug) administered by the daily total hatchery flow (L/d):

Example: Facilty 136, treatment date 1/7/00; 325,470,000 µg OTC / (1.12 m^3/s x 1,000 L/m^3 x 86,400 s/d) = 325,470,000/96,768,000 L = 3.36 µg OTC/L.

According to the simple hatchery calculation, the 95th percentile (actually the 792nd highest EIC out of 834 INAD data points) for OTC discharges for all fish treatments was estimated to be 19.1 µg/L (Table 11). The 95th percentile EIC estimate following all administrations to salmonids was 20.0 µg/L whereas the nonsalmonid 95th percentile EIC was 13.5 µg/L. The by-year EIC estimates were surprisingly consistent over the course of INAD 9332 reporting (Figure 4), ranging from a low 95th percentile EIC of 7.2 µg/L in 2005 to a high of 43.7 µg/L in 2001, a variation of about 6-fold. All calculations with INAD 9332 data assume a treatment rate of 82.5 mg/kg BW/d, the maximum proposed label rate and do not include any information regarding predischarge effluent amendment by the hatchery. Slightly over half of the hatcheries that responded to a UMESC survey reported the use of settling ponds to amend effluent predischarge to the environment. The median settling pond volume was ~3.7 million liters and could provide (1) substantial dilution of OTC discharge depending

[5]The 5.8- µg/L exposure concentration is an OTC peak effluent concentration leaving the settling pond and entering the receiving water. It is an estimation of the maximum total-dissolved concentration based on the WASP-6 model and using the 95th percentile (actually the 792nd highest EICs out of 834 INAD data points) of the OTC therapies listed in the INAD data for all fish. Concentration values near (either above and below) this peak value are expected to be reached at some time each day during therapy, but fall off to insignificance within 4 to 5 days after the end of therapy.

on total hatchery water flow or (2) settling of suspended solids and concomitant removal of sorbed or bound OTC (Appendix A; USGS hatchery survey.otc ea.xls, "hatchery information" tab).

6.12.2 Measured OTC Concentrations at Hatcheries - Three separate studies, using different approaches, reported measurable OTC concentrations in hatchery water following OTC administration. Thurman et al. (2002) reported a maximum dissolved OTC concentration of 10 µg/L (collected from a New York State fish hatchery settling pond) in 175 random samples of hatchery water collected from 13 hatcheries across seven States. They filtered their water samples through a 0.7 micron glass-fiber filter and used a liquid chromatograph/mass spectrometer (LC/MS) for analysis. In a more recent study by the same laboratory, Dietze et al. (2005) reported that dissolved OTC (water samples were filtered through a 0.7 micron glass-fiber filter) was found at 0.35 to 9 µg/L in the treatment raceway water during a 10-d therapy at label dosage at "intensive fish hatchery A". The higher concentrations occurred between days 5 and 8 of the therapy. No OTC was detected at any time in the hatchery's effluent.

In the third study, Smith et al. (1994) measured samples from total farm effluent every hour during a 24-h period during (and after) a treatment with OTC-hydrochloride medicated feed on a freshwater salmon hatchery. Smith et al.'s (1994) detection limit of 20 µg/L was similar to their estimated OTC concentration (21.4 µg/L; total daily mass of OTC administered [904 g] divided by the daily total effluent flow [42,192,000 L]). They found measurable OTC in only 2 of 24 water samples analyzed, once at the beginning of treatment (30 µg/L) and again 8 h later (60 µg/L), when the fish were being fed unmedicated feed during their final feeding. Their analysis method was high performance liquid chromatography (HPLC) and they clearly stated that particulates were present in some of their water samples, which they did not remove before sample extraction. The authors surmised that these represented relatively transient peak concentrations, one from initial dose of medicated feed at the beginning of treatment and the other from expulsion of OTC-containing feces shortly after final feeding. The two concentrations above the detection limit appeared to exist for only short periods (less than an hour). The initial peak (30 µg/L) was probably because of leaching of OTC-hydrochloride from feed by water. The OTC alkyl ammonium salt or the dihydrate would probably not leach as readily from the medicated feed given their significantly lower solubility in water (Table 3). Smith et al. (1994) stated that the second peak (60 µg/L) was probably OTC leaching from fish feces. We do not know whether leaching from feces is influenced by the form of OTC administered. With both peaks, some influence from solids-bound OTC is possible. The feed consumption of the healthy fish used in this experiment may or may not be representative of sick fish. However, if water concentrations of OTC are maximized by leaching from feces relative to uneaten feed, it probably represents the worst-case since feed consumption by healthy fish is probably greater than that of sick fish.

6.12.3 Selection of a total-dissolved EIC - The data available to develop an EIC to assess the risk of OTC use at hatcheries (Sections 6.12.1-6.12.2) are at best sparse. Measured concentrations are of uncertain quality. For example, Smith et al.'s (1994) quantitation limit may have been as high as 60 µg/L, at or above the OTC concentrations reported during dosing. The maximum value of 10 µg/L reported by Thurman et al. (2002) and 9 µg/L reported by Dietz et al. (2005) were well above their detection limits. The sampling time relative to the initiation of dosing is not reported by Thurman et al. (2002) but was by Dietz et al. (2005). Although the input parameters for the WASP-6 model are thought to be conservative, many of the values are not accurately known nor has the model been calibrated against actual discharge concentrations measured at any aquaculture facility. Furthermore, the WASP-6 model assumed a settling pond and therefore might not satisfactorily predict total-dissolved OTC EICs of aquaculture facilities without one. Application of the reduction factors developed from the present WASP-6 model resulted in an EIC estimate less than the maximum measured concentration reported (5.8 µg/L vs 60 µg/L). This concentration is about an order of

magnitude less than the maximum value measured by Smith et al. (1994) but similar to the maximum concentration reported by Thurman et al. (2002). The 95th percentile EIC value produced by the simple hatchery calculation (19.1 µg/L) is intermediate of the measured values reported in the literature. Though less than that reported by Smith et al. (1994), the 95th percentile EIC is very similar to the maximum values reported by Thurman et al. (2002) and Dietz et al. (2005). Combining the closeness of the latter two reported values to the 95th percentile EIC predicted from INAD 9332 with the lack of validation of the WASP-6 model, there is insufficient justification to preferably use the lower values derived from the WASP-6 model. Therefore the EIC value used in all risk assessments in this EA will be the 95th percentile EIC calculated for freely – total dissolved OTC from the INAD 9332 use data. It should be noted that the simple hatchery calculation also assumes that all OTC will be discharged as freely-dissolved OTC. To the extent that OTC is discharged in a biologically unavailable form, a mitigating factor exists as to the risk caused by the discharge of this chemical from hatcheries at the estimated concentrations stated.

6.13 Anticipated Patterns of Multiple OTC Uses and Discharges and their Effect on OTC EICs – The proposed label calls for daily use (and therefore discharge) of OTC for up to 10 d for a given therapy. By itself, this results in multiple discharges of OTC over a 10-d period. From the use data developed under INAD 9332, several facilities reported therapies that may have overlapped, and others had multiple therapies within a short period of time (skeletal marking was one of the most frequent uses which resulted in significant treatment overlap reported in the INAD 9332 data). This means that multiple (as many as two dozen or more) daily discharges may occur over less than several months time, and more discharges than that over a half year or longer period of time. Total-dissolved OTC EICs will decrease with time, unless therapies are essentially continuous and consecutive, or unless further ambient total-dissolved OTC is supplied from OTC partitioned to suspended solids and sediment within the hatchery. A qualitative review of several overlapping treatments indicates that use of the 95th percentile EIC from a single administration is still protective relative to most overlapping treatments (e.g. facility 12 administered seven overlapping treatments which when combined results in an EIC of ~6 µg/L relative to the 95th percentile EIC for all freshwater-reared fish – 19.1 µg/L; Appendix A, INAD 9332 2000-2007 extracted data.xls, "All fw fish w dis (no strep)" tab).

Most of the factors affecting the environmental fate of OTC seem to broaden the potential total-dissolved OTC plume and reduce the concentration of total-dissolved OTC released from hatcheries into public receiving waters. Gastrointestinal transit times and partitioning into solids are the primary means by which the potential total-dissolved OTC concentration is reduced after treatment. Because of this, the total-dissolved OTC concentrations at discharge should be substantially less than those resulting from a simple dilution of a completely dissolved substance. This substantially reduces the predicted biologically available OTC concentrations at discharge.

Most receiving water bodies have a dilution potential at least equal to, if not much greater than, that of the hatchery. After discharge, total-dissolved OTC is expected to be rapidly diluted to concentrations well below those that can be reliably detected by current analytical instrumentation. Eventually, OTC at such concentrations will be degraded by physicochemical mechanisms.

It has already been estimated, using the most conservative assumptions, that OTC in marine sediments below net pens does not result in appreciable concentrations of dissolved OTC in the first centimeter of the water column above the sediments. Calculations by Smith and Samuelsen (1996) indicate that even OTC sediment concentrations of up to 10.9 mg/kg at the water-sediment interface result in a worst-case estimation of 16 µg/L of dissolved OTC in the lowest 1 cm of marine water, similar to the 19.1 µg/L 95th percentile EIC used for our risk assessment. The semi-quantitative predictions of the WASP-6 model yielded a similar concentration for fresh water (Rose and Pedersen

2005). These low estimates of OTC concentration in surface waters above sediments bearing OTC residues seem to be the result of a number of factors: 1) absolute OTC quantities in surface sediments are relatively low compared to the water masses above them even though concentrations in surface sediments can be high (e.g., at the mg/kg level); 2) OTC partitions from solids into water, especially into fresh water, at a relatively slow rate; and 3) surface sediments containing OTC are quickly buried by sediment deposits free of OTC. It therefore appears that total-dissolved OTC discharge from sources other than those producing the daily peak discharge of total-dissolved OTC from hatcheries are negligible, as are the total-soluble OTC discharge concentration increases resulting from multiple (more than 10 in a year) consecutive daily OTC uses and discharges. If the 95th percentile EIC indicates no risk of concern for total-dissolved OTC discharges to fresh receiving waters for 10 consecutive days of treatment, no further risk should be indicated for multiple 10-d treatments and discharges.

6.14 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 OTC use at hatcheries. Instead, the relative immediate dilution effect 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 by USGS, data were available to estimate this ratio for 86 hatcheries (Appendix A; USGS hatchery survey.otc ea.xls, "hatchery information" tab). Of these 86 hatcheries, 74 (86%) discharged into water bodies that would provide at least 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. Analysis of Environmental Effects in Intensive Fresh-Water Aquaculture

7.1 Introduction - Considerable toxicity data have been generated on OTC during the last 50 years, mostly on terrestrial species. Much of the earlier work was summarized by Wassef (1983) and again by Katz (1995). As of 1990, little had been done to assess toxicity to aquatic organisms. Because of this, the EPA Office of Pesticide Programs had definitive acute waterborne toxicity studies performed on daphnia and fish (bluegill [*Lepomis macrochirus*] and rainbow trout) in the early 1990s (Bellantoni et al. 1991; Murphy and Peters 1991a,b). These studies were conducted with OTC in its presumably biologically available form.

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

For this EA, we chose to proceed under the following three assumptions. First, terrestrial vegetation and wildlife were not considered for evaluation because the predominant influences of OTC introduction for aquaculture purposes on the surrounding ecosystem occur only through aquatic pathways where direct contact with OTC occurs. Second, the only exposure pathway considered is that of direct contact of an organism's outer surface (integument, gills, or outer cell wall) with OTC in

the water column (although we do present toxicity data on dietary exposure for fish; Section 7.3.3). Third, there are no other significant routes of exposure that would cause untoward effects (e.g., bioaccumulation).

The receiving waters of most aquaculture sites are diverse and healthy ecosystems that support a variety of aquatic and terrestrial life. We examined effects data for four groups of ecologically important, diverse, and representative organisms or ROI because it would be unrealistic to conduct a risk assessment of all organisms possibly affected. 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 four separate and important trophic levels: primary producers (algae and some bacteria), primary consumers (invertebrates), and secondary or tertiary consumers (fish). It should be noted that algae and bacteria do not have the same status with regard to the type of screening risk assessments used in this EA as do invertebrates and fish. Because of the ubiquitous presence of algae and bacteria in the environment and their ability to quickly repopulate an aquatic area that has been temporarily affected by exposure to a discharge of OTC from aquaculture, permanent population effects on these ROI are unlikely from such exposures. Consequently, results from screening risk assessments that are of concern for invertebrates and fish are of less concern for algae or bacteria.

A fair amount of aquatic toxicity data on OTC are now available from the public literature. Fortunately, a significant number of toxicity data points are available that do focus on these important ROI.

7.3 Effects on Receptors of Interest - Data available from the scientific literature on the effects of OTC to principal ROI that are likely to reside in the receiving water at freshwater aquaculture sites are presented in Table 12 for discharge into fresh water and in Table 13 for discharge into brackish water. Summaries of the key studies used in the risk assessment are presented in Appendix C.

7.3.1 Algae - Many species of algae reside within potential receiving waters (streams, rivers, estuaries, lakes) of intensive aquaculture facilities, are primary producers, and serve as the basis for the entire food web in most aquatic ecosystems (Smith 1950). Any significant direct negative effect on resident algae populations may likewise have a secondary negative effect on many other organisms higher on the food chain.

Oxytetracycline was more toxic to algae during standard acute toxicity tests than to other ROIs (Tables 12 and 13), except for bacteria. The 72-h EC_{50} (effective concentration that results in 50% of test organisms to have reached the specified toxic endpoint within 72 h) for *Scenedesmus quadricuada* was about eightfold greater than the EC_{50} reported for *Selenastrum capricornutum* (Table 12), indicating that the latter was substantially more sensitive to OTC. Several authors (Rojíčková et al. 1998; Holten Lützhøft et al. 1999; De Liguoro et al. 2003) reported similar 72-h EC_{50} values for *S. capricornutum* tests (1.99 to 4.5 mg/L; Table 12). Holten Lützhøft et al. (1999) also reported that when exposed to OTC for at least 72 h, the cyanobacteria *Microcystis aeruginosa* was substantially more sensitive (0.207 mg/L EC_{50}) than either the freshwater green algal species (Table 12) or the marine cryptophycean *Rhodomonas salina* (1.6 mg/L EC_{50}, Table 13).

Wilson et al. (2004) evaluated the effects of a mixture of four tetracyclines, including OTC, in an experimental freshwater mesocosm with phytoplankton and zooplankton present. They reported an LOEC of 0.218 µM for phytoplankton total abundance and species richness but no significant adverse effects for zooplankton at a total mixture concentration of 2.29 µM. The lowest treatment tested was 0.080 µM (equivalent to concentrations of 9.10, 8.32, 11.2, and 10.6 µg/L for tetracycline, oxytetracycline, chlortetracycline and doxytetracycline) exhibited no adverse effects on either

zooplankton or phytoplankton in this experimental microcosm. In their antibiotic reconniassance, Kolpin et al. (2002) detected chlortetracycline, OTC, and tetracycline at 0.69, 0.34, and 0.11 µg/L, respectively, in various freshwater sampling sites (doxycycline was not detected).

7.3.2 Invertebrates - Many different species of nektonic (waterborne) and benthic (bottom-dwelling) invertebrates reside within potential receiving waters (streams, rivers, estuaries, lakes) of intensive freshwater 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/insectivorous fish and the juveniles of piscivorous game fish. Several authors reported the acute and chronic toxicity of OTC to *Daphnia magna* (Bellantoni et al. 1991; Wollenberger et al. 2000; Table 12). Oxytetracycline appears to be relatively non toxic to ***D. magna*** as the 21-d EC_{50} based on reproduction was about half the 48-h EC_{50} for immobilization. The only data available for marine invertebrates is for whiteleg shrimp, (*Penaeus vannamei*). The 48-h LC_{50} ranged from 136 to 238 mg/L depending on life stage (Williams et al. 1992; Table 13).

Although no data are available on the toxicity of sediment-bound OTC to sediment fauna, Baguer et al. (2000) seem to indicate that OTC applied to two soils was extremely non toxic to three soil invertebrates: earthworm (*Aporrectodea caliginosa*), springtails (*Folsomia fimetaria*), and enchytraeids (*Enchytraeus crypticus*). One soil contained 6.2% clay, 2.7% humus, and 1.5% total carbon and the other contained 13.0% clay, 2.8% humus, and 1.6% total carbon (source of soils not stated, probably from Denmark or Spain). The 21-d no observed effect concentration (NOEC) values for survival and reproduction were 3,000-5,000 and 2,000-5,000 mg/kg dry weight, respectively (Baguer et al. [2000] also report other OTC toxic endpoints). These invertebrates could be very resistant to OTC, but given the clay, etc. content of the soils, OTC was more likely sorbed to clay or attached to organic carbon, causing it to be biologically unavailable to the test organisms.

Benthic invertebrates can be an especially useful indicator of environmental quality for long periods because of their limited mobility (Pennak 1978). Pelagic organisms (e.g., *Daphnia magna*) appear to be relatively tolerant of OTC and are more likely to be exposed to biologically available OTC (the freely-dissolved fraction that is not chelated) than are benthic organisms. Benthic organisms are more likely to be exposed to OTC bound to either organic (uneaten feed, feces, etc.) or inorganic (clay) solids. Oxytetracycline bound to these substances is likely in a biologically unavailable state, as it appeared to be in soils in Baguer et al. (2000), and its toxicity is likely to be substantially less than when in the freely-dissolved (and unchelated) form.

7.3.3 Fish - Many species of fish may reside within potential receiving waters (streams, rivers, estuaries, lakes) of intensive aquaculture facilities and may be primary, secondary, or tertiary consumers depending on species and life stage (Lee et al. 1980). They are important ecologically as a food source for higher level carnivores and some have great value to humankind both commercially and for recreation. 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 are, therefore, susceptible to biomagnification of contaminants and population fluctuations of prey. Neither bioaccumulation nor biomagnification are probable given the OTC low K_{ow} at the environmentally relevant pH ranges typically observed in freshwater ecosystems (Table 6) and the apparent low bioavailability of OTC from feed (Cravedi et al. 1987).

The acute toxicity of OTC in solution has been examined in a variety of fish species by several authors (Wellborn 1969; Hughes 1973; Murphy and Peters 1991a,b). Toxicity data of bluegill, rainbow trout, and striped bass (*Morone saxatilis*), Table 12, indicated that fish are not the most sensitive species to environmental levels of OTC.

On May 21, 2002, water-solubilized OTC hydrochloride was approved by CVM for skeletal marking of all larval or juvenile fish at water bath concentrations of up to 700 mg/L for up to 6 h. Data on target animal safety were generated to support this usage (U.S. Food and Drug Administration Public Master File 5667 Freedom of Information Summary, available online at http://www.fda.gov/OHRMS/DOCKETS/98fr/PMF5667-fois001.doc.; accessed October 2005). These data further support the conclusion that fish are not the most sensitive species with respect to OTC aquatic environmental effects.

Toxicity data also exist for OTC fed to fish as part of determination of OTC safety to target animals (Gaikowski et al. 2003). Oxytetracycline was fed to hybrid striped bass (*Morone saxatilis x Morone chrysops*), yellow perch (*Perca flavescens*), and walleye at 0 to 5-fold the recommended dose rate of 82.5 mg/kg for 10 d (20 d for walleye). No mortalities were observed during the study because of OTC. Dose-related effects were limited to minimal to mild reduction of hematopoetic and lymphopoetic tissue in walleye (413 mg/kg BW/d dose group) and growth suppression of hybrid striped bass (413 mg/kg BW/d dose group).

7.3.4 Bacteria - The toxicity of OTC on environmentally relevant bacteria was investigated by Halling-Sørensen (2001) using activated sludge from wastewater treatment plants as a surrogate bacterial population. The effect of OTC on inhibition of growth and nitrification were investigated by Halling-Sørensen (2001). Oxytetracycline inhibited growth of sludge bacteria (Table 12). Triplicate tests to assess nitrification inhibition gave indications of a decreased rate. The 10 d EC_{50} was 1.7 mg/L for growth inhibition of *Nitrosomonas europaea*. Growth inhibition of activated sludge bacteria and *N. europaea* was also assessed using a pour-plate method; the sludge bacteria 48-h EC_{50} was 0.14 mg/L and the *N. europaea* 5 to 6-d EC_{50} was 0.32 mg/L. Oxytetracycline degradation product toxicity was also investigated using activated sludge (Halling-Sørensen et al. 2002). All OTC degradates studied were less toxic to activated sludge bacteria than the parent compound, with 48-h EC_{50} values ranging from about 2- to 88-fold greater than that of OTC (Table 14). For growth inhibition of activated sludge bacteria, the 48-h EC_{50} for OTC reported in this study was 0.08 mg/L. The toxicity of OTC to activated sludge bacteria was thus consistent between both studies (two 48-h EC_{50} values, 0.14 mg/L and 0.08 mg/L, Table 12).

8. Risk Characterization

We conducted a risk characterization that integrated the potential fate and effects of total-dissolved OTC release into fresh-water ecosystems even though it is not intended for direct application to the aquatic environment. Risk characterization was based on (1) the estimated EICs of OTC from aquaculture facilities as a result of chemical treatments on-site for both typical and worst-case discharge scenarios as described in Section 6.11; 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 LC_{50} data (or EC_{50} where the effect indicated immobilization [daphnia] or inhibition of growth [algae]) and a chronic RQ analysis using selected chronic NOEC data. The chosen LC_{50}, EC_{50} or NOEC values are divided by an assessment factor (AF) as specified by the International Cooperation on Harmonization (VICH) to obtain an initial predicted no effect concentration (PNEC, International Cooperation on Harmonization of Technical Requirements for Regulation of Veterinary Medical Products 2004, see Tables 15 and 16). The initial acute RQ value is calculated by dividing the EIC by the initial acute PNEC:

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

In this analysis, an acute RQ greater than 1.0 indicates that acute toxic effects to ROI are probable, although if the acute RQ value is greater than 1.0 there is a presumption of hazard that may be mitigated by restricted use or dilution / degradation or perhaps sorption or chelation by receiving waters. The initial chronic RQ value is determined by dividing the EIC by the initial chronic PNEC for a particular ROI:

Chronic RQ = EIC/Chronic PNEC

In this analysis, a chronic RQ greater than 1.0 indicates that chronic toxic effects to ROI are probable, although in that case there is a presumption of hazard that may be mitigated by restricted use or dilution / degradation or perhaps sorption or chelation by receiving waters. 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 LC_{50} and chronic NOEC values. This will help to reduce uncertainty in conclusions based on the risk analysis.

Typically, the risk assessment is an iterative process beginning with an initial assessment using default assessment factors (AF). If the initial RQs are greater than 1.0, indicating possible toxic effects to the ROI, the assessment based on the AFs in Table 15 can be refined using the AFs in Table 17 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 LC_{50}. 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 seems to have been designed and conducted in a manner that is scientifically sound, and the methodologies employed reasonably conform with those outlined by standard procedures (ASTM 1989); (2) each ROI selected must be an organism that is broadly distributed and typically resides in aquatic environments where discharges of OTC 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 OTC, and for which NOEC and LC_{50} 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.

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 if the quotient suggests an unacceptable risk to the selected ROI.

Our risk assessment for OTC will emphasize potential OTC worst-case discharge events following use at cold-, cool- and warm freshwater aquaculture facilities. Actual medicated-feed use data were obtained from use reported by facilities participating in INAD 9332. The estimated total-dissolved OTC environmental introduction concentrations (EICs) were determined assuming dosing at 82.5 mg/kg BW/d. The 95th percentile EIC determined for all freshwater fish administrations that were for proposed label expansions in INAD 9332 was selected to represent the worst-case EIC. We realize that some hatcheries whose discharges are greater than the 95th percentile may have circumstances where, in the future, they might have to mitigate OTC concentrations in their effluent or manage OTC use before discharge. Because of this, the 95th percentile seemed the best

representation of the "worst-case" EIC for the entire data set of 834 uses and discharges. The risk characterization presented in this EA is based on the selection of the 95th percentile EIC for all fish treatments (19.1 µg/L [Section 6.12.1; Table 11]), to represent the worst-case potential discharge concentration following aquaculture use and is used to predict acute and chronic risks associated with OTC use at cold-, cool- and warm freshwater hatcheries. After determining the worst-case environmental introduction concentration, initial RQs will be calculated. If no risk is indicated (i.e., an RQ less than 1.0), this will demonstrate that discharge of total-dissolved OTC from aquaculture facilities is not a cause for concern. If the initial RQ is greater than 1.0, a refined assessment will be attempted.

8.1 Acute Risk of Worst-Case Total-dissolved OTC EICs in Fresh Water- Assessment factors recommended in VICH Phase II guidance for Tier A (International Cooperation on Harmonization of Technical Requirements for Regulation of Veterinary Medical Products 2004) are presented in Table 15. Risk calculations for the selected aquatic ROI are presented in Table 16 (based on VICH Phase II Tier A assessment factors) and Table 17 (based on refined assessment factors) and are discussed below.

Algae – Because algae reproduce so rapidly, the typical effects on growth (biomass and cell number) measured in algal toxicity tests are considered to be "chronic" effects even though the test exposures are short-term or acute in nature, usually less than 96 hours. Therefore, available data on the toxicity of OTC to algae in fresh water will be reported under the section on chronic risk (Section 8.2) below.

Invertebrates - The definitive toxicity data we chose for invertebrates were for *Daphnia magna*, a recognized standard test species (American Society for Testing and Materials [ASTM] 1989). The 48-h LOEC was 100 mg/L (Table 12). The 48-h LOEC divided by an AF of 1,000 (Table 15) results in an initial PNEC of 100 µg/L (Table 16). At the estimated 95th percentile hatchery discharge concentration by cold-, cool- or warmwater fish hatcheries in INAD 9332 (19.1 µg/L), the initial acute RQ for *D. magna* is 0.19 (Table 16). Since the RQ was less than 1.0, OTC use at intensive-culture aquaculture facilities does not seem to pose an acute environmental risk to freshwater aquatic invertebrates. Actual contact time with OTC at the selected EIC is usually less than what we have stated because OTC discharged into a stream, river, estuary, or lake would be immediately diluted. Actual contact time and exposure concentrations might also be reduced as OTC becomes biologically unavailable by sorption to suspended particles in the receiving waters, or possibly by chelation with divalent cations in very hard waters, or as OTC undergoes degradation in the receiving waters.

Fish - Several toxicity data points are available for freshwater fish. The definitive toxicity data point we chose for freshwater fish was the 96-h LC_{50} for striped bass fingerlings (75 mg/L; Table 12). An assessment factor of 1,000 was applied resulting in an initial PNEC of 75 µg/L. At the estimated 95th percentile hatchery discharge concentration of 19.1 µg/L, the initial acute RQ for freshwater fish is 0.25. Since the RQ was less than 1.0, OTC use at intensive-culture aquaculture facilities does not pose an acute environmental risk to freshwater fish. Actual contact time and exposure concentrations will also be reduced as discussed for acute risk to freshwater invertebrates.

8.2 Chronic Risk of Worst-Case Total-dissolved OTC EICs in Fresh Water – Chronic assessment factors recommended in VICH Phase II guidance are presented in Table 15. Chronic risk calculations for the selected aquatic ROI are presented in Table 16 (based on VICH Phase II assessment factors) and Table 18 (based on refined assessment factors) and are discussed below.

Algae - Algal tests may be considered chronic if conducted for a 72-h or longer period, as 72 h accounts for 16 life cycles (EMEA 1997). The 7-d EC_{50} for OTC to the blue-green algae *Microcystis aeroginosa* is 0.207 mg/L (Table 12). The 7-d EC_{50} was therefore divided by an assessment factor of 100 to estimate an initial chronic PNEC of 2.1 µg/L (Table 16). At the estimated 95th percentile hatchery discharge concentration by cold-, cool- or warmwater fish hatcheries in INAD 9332 (19.1 µg/L), the initial chronic RQ for *M. aeroginosa* is 9.10. This initial chronic RQ analysis indicates that exposures to predicted total-dissolved OTC discharge concentrations after hatchery treatment pose a potential chronic risk to this ROI. A refined assessment using an assessment factor of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects) and another factor of 10 for extrapolation of the chronic EC_{50} to the chronic PNEC resulted in the same chronic RQ of 9.10 for *M. aeroginosa* (Table 18). However, because long-term exposures will be at concentrations considerably reduced from the EIC values by dilution within the receiving waters, and because of the ability of algae to quickly repopulate after a temporary depletion, the actual chronic risk to an environmentally significant algal population will be much less than that indicated here. Actual contact time and exposure concentrations might also be reduced as OTC becomes biologically unavailable by sorption to suspended particles in the receiving waters, or possibly by chelation with divalent cations in very hard waters, or as OTC undergoes degradation in the receiving waters.

Invertebrates - Two chronic toxicity endpoints (EC_{50} and EC_{10}) based on reproduction are available from a 21-d *Daphnia magna* toxicity test (Table 12). The 21-d EC_{10} (7.4 mg/L) was selected for use in the risk assessment. An assessment factor of 10 was applied for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects). The 21-d EC_{10} was therefore divided by a factor of 10 to estimate an initial PNEC (7.4 mg/L/10 = 740 µg/L, Table 16). At the estimated 95th percentile hatchery discharge concentration by cold-, cool- or warmwater fish hatcheries in INAD 9332 (19.1 µg/L), the initial chronic RQ for *D. magna* is 0.026. This result indicates no presumption of chronic risk to this ROI from total-dissolved OTC discharge concentrations after hatchery treatment. Since long-term exposures will be at concentrations considerably reduced from the EIC values by dilution within the receiving waters, the actual chronic risk to an environmentally significant population will be much less than that indicated here.

Fish - Chronic OTC toxicity data for total-dissolved OTC exposures to freshwater fish are not available. Acute toxicity studies may be used as a first step to conservatively predict no-effect concentrations for chronic toxicity by using extrapolation factors (EMEA 1997). If this results in a risk indication, chronic studies may need to be run. Several acute toxicity data points are available for freshwater fish. The definitive toxicity data point we chose for freshwater fish was the 96-h LC_{50} for striped bass fingerlings (75 mg/L; Table 12). Assessment factors were applied for the acute to chronic ratio (i.e., extrapolation of the acute LC_{50} to the chronic NOEC); additional factors were applied for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects). The initial PNEC estimate for striped bass fingerlings is therefore 75 µg/L (75 mg/L/1,000, Table 16). At the estimated 95th percentile hatchery discharge concentration by cold-, cool- or warmwater fish hatcheries in INAD 9332 (19.1 µg/L), the initial chronic RQ for striped bass fingerlings is 0.25. Based on the present toxicity data, OTC use at intensive-culture aquaculture facilities does not pose a chronic environmental risk to freshwater fish. Actual contact time and exposure concentrations will also be reduced as discussed for acute risk to freshwater algae.

Bacteria - The 10 d EC_{50} was 1.7 mg/L for growth inhibition of *Nitrosomonas europaea*. Growth inhibition of activated sludge bacteria and *N. europaea* was also assessed using a pour-plate method; the 48-h EC_{50} for sludge bacteria was 0.14 mg/L and the 5 to 6-d EC_{50} for *N. europaea* was 0.32 mg/L. For growth inhibition of activated sludge bacteria, another study reported that the 48-h EC_{50} for

OTC was 0.08 mg/L (see Section 7.3.4). The toxicity of OTC to activated sludge bacteria was thus consistent between both studies (two 48-h EC_{50} values, 0.14 mg/L and 0.08 mg/L, Table 12).

We also chose to do a risk assessment for natural bacterial populations (we assume that an assessment for fresh water would also apply to brackish water, however we have no toxicity data for brackish water bacteria). The 0.32 mg/L value for *N. europaea* (5 to 6-d EC_{50}) will be used for the risk assessment to natural bacterial populations. An assessment factor of 10 was applied for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects) and another factor of 10 was applied to estimate a refined PNEC (32 µg/L, Table 18). At the estimated 95th percentile hatchery discharge concentration by cold-, cool- or warmwater fish hatcheries in INAD 9332 (19.1 µg/L), the refined chronic RQ for *N. europaea* is 5.97 (Table 18). This result indicates that OTC discharged from aquaculture might represent a risk to natural bacterial populations. However, countless types of microorganisms are abundant in nearly all surface waters and are also ubiquitous worldwide on land, in other waters, and in the air. Therefore, it is unlikely that relatively small, isolated, and intermittent point-source discharges of OTC (like those occurring after aquaculture use) could have a significant long-term effect on the numbers and types of microflora/fauna present at any location.

Direct discharge of OTC from aquaculture facilities into sewage or wastewater treatment systems is not likely, as the only known discharges are to lakes, rivers, estuaries, 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 OTC would be greatly diluted before it reached the treatment plant. Nonetheless, we chose to do a risk assessment for sewage treatment bacteria. The 0.08 mg/L value (see discussion above) will be used for the risk assessment to sewage treatment bacteria. An assessment factor of 10 was applied for extrapolation of the EC_{50} to the PNEC of 0.008 mg/L (8 µg/L). At the estimated 95th percentile hatchery discharge concentration by cold-, cool- or warmwater fish hatcheries in INAD 9332 (19.1 µg/L), the refined chronic RQ for sewage treatment bacteria is 2.39 (Table 18). Again, this would assume direct discharge from an aquaculture facility into a sewage treatment plant without dilution, which is unlikely.

8.3 Acute Risk of Worst-Case Total-dissolved OTC EICs in Brackish Water - Although this EA is being written for discharge from freshwater aquaculture facilities, some may discharge into brackish water. Therefore a risk assessment will be made in this EA for total-dissolved OTC discharge from aquaculture into brackish water. The majority of this aquaculture type is composed of striped bass (*Morone saxatilis*) and hybrid striped bass (*M. saxatilis* x *M. chrysops*) culture, and perhaps salmonid facilities located near coastal waters. Two types of facilities are identified: (1) private facilities that supply restaurants or supermarkets with food fish; and (2) public facilities that raise fingerlings to stock in public waters.

The potential impacts of total-dissolved OTC release into brackish water would be quite similar, in general, to those already discussed for fresh water. The notable differences would be that (1) in a brackish-water environment, there exists a greater potential for dilution upon discharge because of the greater volumes of water and water exchange in an estuarine system than in smaller streams, rivers, or lakes; (2) the organisms residing in brackish water and their sensitivities to total-dissolved OTC exposure may differ somewhat from those residing in fresh water; and 3) the potential for deactivation by chelation should be greater in brackish water since it generally will have a higher divalent cation concentration than fresh water (depending on brackish-water salinity, which can vary from 1 part-per-thousand to 29 parts per thousand, almost the typical salinity of seawater).

The recommended maximum treatment concentration for OTC is 82.5 mg/kg BW/d for fish. The combination of attachment to solids and dilution of any total-dissolved OTC should ensure that very

low concentrations of total-dissolved OTC will be reached within a few hours after discharge into brackish water. We assume that the concentration decreases after discharge into brackish water would result in bioavailable OTC concentrations equal to or lower than the estimates presented in Section 6.14 for discharge into fresh water, and that the discharged total-dissolved OTC in many brackish waters would soon be in the chelated form.

Algae - Because algae reproduce so rapidly, the typical effects on growth (biomass and cell number) measured in algal toxicity tests are considered to be "chronic" effects even though the test exposures are short-term or acute in nature, usually less than 96 hours. Therefore, available data on the toxicity of OTC to algae in brackish water will be reported under the section on chronic risk (Section 8.5) below.

Invertebrates - The only data available for marine invertebrates is for whiteleg shrimp, *Penaeus vannamei*. The 48-h LC_{50} was reported to be 136 mg/L (Table 13). This will be the definitive test result for acute toxicity to brackish water invertebrates. Using an application factor of 1,000, the initial PNEC for whiteleg shrimp is 136 µg/L. At the estimated 95th percentile hatchery discharge concentration by cold-, cool- or warmwater fish hatcheries in INAD 9332 (19.1 µg/L), the initial acute RQ for whiteleg shrimp is 0.14. This indicates very little acute risk to brackish water invertebrates when exposed to OTC. Whiteleg shrimp, *Penaeus vannamei* is a marine species native to the Pacific coast from Mexico to Peru that is also farmed in several American states, primarily in Texas (http://www.oceansalive.org/eat.cfm?subnav=fishpage&fish=120.; accessed in December 2005). Oxytetracycline is actually used as an antibiotic therapeutant for this species in the U.S. (Williams et al. 1992). There is little information to indicate whether whiteleg shrimp are a good surrogate for even the more sensitive brackish-water invertebrates, and thus the acute risk to this ROI cannot be determined from the available data.

Fish – The 96-h LC_{50} for striped bass fingerlings is 75 mg/L. The initial PNEC estimate is 75 µg/L (Table 16). This is the identical species used for acute risk assessment in fresh water, since striped bass can survive in both fresh and brackish water, and the same assessment factors are used. At the estimated 95th percentile hatchery discharge concentration by cold-, cool- or warmwater fish hatcheries in INAD 9332 (19.1 µg/L), the initial acute RQ for striped bass fingerlings is 0.25 (Table 16). Based on the present toxicity data, OTC use at intensive-culture aquaculture facilities does not pose an acute environmental risk to brackish water fish. Actual contact time and exposure concentrations will also be reduced as discussed in the beginning of this section.

8.4 Chronic Risk of Worst-Case Total-dissolved OTC EICs in Brackish Water – Most of the statements presented in the introductory paragraphs of Section 8.4 for OTC acute risk in brackish waters also apply to this section on chronic OTC risk in brackish waters.

Algae – Algal tests may be considered chronic if conducted for a 72-h or longer period, as 72 h accounts for 16 life cycles (EMEA 1997). The definitive toxicity test selected was the 72-h EC_{50} for *Rhodomonas salina* of 1.6 mg/L (Table 13). Using an application factor of 100, as was done for *Microcystis aeroginosa* in fresh water, the initial PNEC would be 1.6 mg/L/100 or 16 µg/L (Table 16). At the estimated 95th percentile hatchery discharge concentration by cold-, cool- or warmwater fish hatcheries in INAD 9332 (19.1 µg/L), the initial chronic RQ for *R. salina* is 1.19 (Table 16). A refined assessment using an assessment factor of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects) and another factor of 10 for extrapolation of the chronic EC_{50} to the chronic PNEC resulted in the same chronic RQ of 1.19 for *Rhodomonas salina* (Table 18). This chronic RQ analysis indicates that exposures to total-dissolved OTC discharge concentrations into brackish water after hatchery treatment pose a potential chronic risk to this ROI. However, because long-term exposures will be at concentrations considerably

reduced from the EIC values by dilution and perhaps chelation within an estuary, and because of the ability of algae to quickly repopulate after a temporary depletion, the actual chronic risk to an environmentally significant algal population will be much less than that indicated here. We do not consider OTC to be of chronic risk to brackish-water algal populations.

Invertebrates – The only toxicity data available for marine invertebrates is for whiteleg shrimp, *Penaeus vannamei*. The 48-h LC_{50} was reported to be 136 mg/L (Table 13). Since this organism has a relatively short life cycle, the 48-h LC_{50} will be used in the assessment of chronic toxicity to brackish water invertebrates. Using an application factor of 1,000, the initial PNEC for whiteleg shrimp is 136 µg/L. At the estimated 95th percentile hatchery discharge concentration by cold-, cool- or warmwater fish hatcheries in INAD 9332 (19.1 µg/L), the refined acute RQ for whiteleg shrimp is 0.14. This indicates minimal chronic risk to brackish water invertebrates when exposed to OTC, especially considering dilution, chelation, and other mitigating factors present in brackish waters

Fish - Chronic OTC toxicity data for total-dissolved OTC exposures to brackish-water fish are not available. Acute toxicity studies may be used as a first step to conservatively predict no-effect concentrations for chronic toxicity by using extrapolation factors (EMEA 1997). If this results in a risk indication, chronic studies may have to be run. Several acute toxicity data points are available for brackish water fish. The definitive toxicity data point we chose for brackish water fish was the 96-h LC_{50} for striped bass fingerlings (75 mg/L; Table 12). This is the identical species used for chronic risk assessment in fresh water, since striped bass can survive in both fresh and brackish water. The initial PNEC estimate for striped bass is 75 µg/L (Table 16). At the estimated 95th percentile hatchery discharge concentration by cold-, cool- or warmwater fish hatcheries in INAD 9332 (19.1 µg/L), the initial chronic RQ for striped bass fingerlings is 0.25. Based on the present toxicity data, OTC use at intensive-culture aquaculture facilities does not pose a chronic environmental risk to brackish water fish. Actual contact time and exposure concentrations will also be reduced as discussed in the beginning of this section.

9. Alternatives to Proposed Action and Mitigation Options

9.1 Alternatives to Proposed Action - Sulfadimethoxine/ormetoprim (Romet-30), administered in feed at 50 mg/kg/d for 5 d, is approved for use to control furunculosis in salmonids or enteric septicemia in catfish. Feed medicated with Romet-30 is less palatable than other medicated feeds and OTC is already approved for these uses. In addition, the withdrawal time for sulfadimethoxine is undesirably long at a minimum of 42 d.

Florfenicol (Acetamide, 2,2-dichloro-N-[1-(flouromethyl) -2-hydroxy-2-[4-(methylsulfonyl) phenyl]ethyl]-[R-(R*,S*)]-) is approved for use in feed by veterinary feed directive (VFD) to control mortality in freshwater-reared salmonids due to coldwater disease associated with *Flavobacterium psychrophilum*. Florfenicol (as Aquaflor®) is also approved for control of catfish mortality due to enteric septicemia (ESC) associated with *Edwardsiella ictaluri*, and (as Aquaflor® -CA1) for control of mortality in catfish due to columnaris disease associated with *Flavobacterium columnare*. In addition, Florfenicol is presently being used in the United States under an INAD.

For antibiotics, approval of several oral drugs is highly desirable so that the use of the drugs may be rotated to reduce disease resistance to one or more therapeutants. Oxytetracycline, because of its broad usage as a fishery drug, was an important addition to the number of available fishery antibiotics and further expanding and extending its label will allow it to be used for a number of important new disease indications. The need for an approved broad-spectrum therapeutant that will reduce disease-related mortalities for many species is of paramount importance.

9.2 Treatment Management and Post-treatment Mitigation - *Management:* Public and commercial freshwater aquaculture uses far less OTC than land-based agriculture (beef, pork, poultry) because it is used intermittently for short periods to control disease outbreaks, not continuously at sub-therapeutic levels to promote growth. Recent developments could potentially limit the need for OTC in aquaculture, thus reducing potential OTC environmental discharge. Vaccines have recently been developed to reduce the susceptibility of the larger life-stages of some fish species to certain diseases that OTC has the potential to treat. Improved husbandry techniques (e.g., all fish in-all same fish out of pond water before water replacement; higher flow rates; improved aeration; lower animal densities; exposing incoming water to ozone) also may decrease disease outbreaks.

Mitigation: It seems that in almost all aquaculture facilities, OTC can be used to treat cultured fish and then resulting total-dissolved OTC concentrations can be adequately diluted within the hatchery system to concentrations of no toxic concern. No additional mitigation would be required before subsequent discharge to receiving water. Mitigation of total-dissolved OTC would probably only occur if much lower total-dissolved OTC discharge concentrations than those anticipated by this EA are someday required. Two recent papers have shown that freely-dissolved OTC as well as several other aquaculture drugs (chloramine-T and formalin) can be removed by activated carbon adsorption (Aitcheson et al. 2000; 2001). At present, few hatcheries nationwide are using this technology (Jim Luoma, Fish Culturist, USGS, 2004, personal communication). Total-dissolved OTC aquaculture discharges to fresh water might also be temporarily reduced by addition of chelating salts (rich in divalent cations) to hatchery waters before discharge, thus postponing any potential activity until OTC is far below any detectible concentration in receiving waters.

For OTC sorbed to solids, one alternative may be to use a rotary drum filter designed to retain hatchery solids, including OTC bound to biosolids, particularly if the hatchery is already using these filtering systems to remove other unwanted solid substances from their effluents. Such a system was described by Smith et al. (1994) for a facility using recirculating culture waters. At present, few hatcheries nationwide are using this expensive technology (Jim Luoma, Fish Culturist, USGS, 2004, personal communication).

10. Conclusions

Oxytetracycline use at aquaculture facilities is substantially less than that at concentrated animal feedlot operations. Breakdown products of OTC discharged from freshwater aquaculture facilities will not be a significant threat to organismal, environmental, or public health because they are formed only after substantial dilution by receiving waters has taken place. The algal, invertebrate, fish, and bacterial acute and chronic toxicity data for OTC solubilized in water suggest that there is little if any potential risk to populations of aquatic organisms from total-dissolved OTC discharged from intensive aquaculture facilities, nor is it likely to be a potential threat to public health or safety. Discharge of OTC sorbed to solids, associated with DOM, or chelated by divalent cations is also unlikely to result in risk of concern to waterborne organisms because of the poor biological availability of OTC under these circumstances.

11. Acknowledgments

This document is prepared in memory of Dr. Guy R. Stehly, a friend and colleague taken from us too soon.

We acknowledge the following UMESC staff for their significant contributions in the following areas: Susan M. Schleis, Theresa M. Schreier and Shari L. Greseth for technical assistance in helping to produce this document and Terry D. Hubert for technical and editorial review. We acknowledge

Peter E. Rose and Joel A. Pedersen of the Department of Soil Science, University of Wisconsin, Madison, for adoption of the WASP-6 simulation model for use in predicting OTC discharge concentrations as well as running the simulations and preparing the paper (Rose and Pedersen 2005). We acknowledge the state Natural Resource Agencies 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 submission of OTC use and discharge data for administration of the chemical under the compassionate INAD #9332 granted by CVM. We also acknowledge the National INAD Office in Bozeman, Montana, for their efforts in collecting and collating the OTC use and discharge data. Preparation of this EA was partially funded by a grant (contract # 301811N032) from the U.S. Fish and Wildlife Service (reverted Dingell-Johnson funds).

12. References

Abedini S., R. Namdari, and F. C. P. Law. 1998. Comparative pharmacokinetics and bioavailability of oxytetracycline in rainbow trout and chinook salmon. Aquaculture 162:23–32.

American Society for Testing and Materials (ASTM). 1989. Standard guide for conducting acute toxicity testing with fishes, macroinvertebrates, and amphibians. American Society for Testing and Materials Report E 729-88a, Philadelphia, Pennsylvania. 20 pp.

Aitcheson, S. J., J. Arnett, K. R. Murray, and J. Zhang. 2000. Removal of aquaculture therapeutants by carbon adsorption 1: Equilibrium adsorption behaviour of single components. Aquaculture 183:269–284.

Aitcheson, S. J., J. Arnett, K. R. Murray, and J. Zhang. 2001. Removal of aquaculture therapeutants by carbon adsorption 2: Multicomponent adsorption and the equilibrium behaviour of mixtures. Aquaculture 192:249–264.

Anderson, T. A., and H. Braley. 1993. Appearance of nutrients in the blood of the golden perch *Macquaria ambigua* following feeding. Comparative Biochemistry and Physiology 104A:349–356.

Baguer, A. J., J. Jensen, and P. H. Krogh. 2000. Effects of the antibiotics oxytetracycline and tylosin on soil fauna. Chemosphere 40:751–757.

Beamish, F. W. H. 1972. Ration size and digestion in largemouth bass, *Micropterus salmoides* Lacépède. Canadian Journal of Zoology 50:153–164.

Bebak-Williams, J., G. Bullock, and M. C. Carson. 2002. Oxytetracycline residues in a freshwater recirculating system. Aquaculture 205:221–230.

Bellantoni, D. C., C. M Holmes, and G. T. Peters. 1991. Oxytetracycline hydrochloride: A 48-hour static acute toxicity test with the cladoceran (*Daphnia magna*). Laboratory Study No. 260A-101. Prepared by Wildlife International, Ltd., Easton, Maryland. Submitted by Pfizer, Inc., New York. EPA MRID 417832-03. Data Evaluation Record (DER) provided by EPA under EFED Document Number E9001100.

Björklund, H.V., and G. Bylund. 1990. Temperature-related absorption and excretion of oxytetracycline in rainbow trout (*Salmo gairdneri* R.). Aquaculture 84:363–372.

Björklund, H., J. Bondestam, and G. Bylund. 1990. Residues of oxytetracycline in wild fish and sediments from fish farms. Aquaculture 86:359–367.

Björklund, H. V., C. M. I. Råbergh, and G. Bylund. 1991. Residues of oxolinic acid and oxytetracycline in fish and sediments from fish farms. Aquaculture 97:85–96.

Brain, R. A., D. J. Johnson, S. M. Richards, H. Sanderson, P. K. Sibley, and K. R. Solomon. 2004. Effects of 25 pharmaceutical compounds to *Lemna gibba* using a seven-day static renewal test. Environmental Toxicology and Chemistry 23:371–382.

Budavari, S., M. J. O'Neil, A. Smith, and P. E. Heckelman. 1989. Merck Index: Eleventh Edition. Merck & Co., Rahway, New Jersey. 1606 pp.

Capone, D. G., D. P. Weston, V. Miller, and C. Shoemaker. 1996. Antibacterial residues in marine sediments and invertebrates following chemotherapy in aquaculture. Aquaculture 145:55–75.

Chin, T. -F., and J. L. Lach. 1975. Drug diffusion and bioavailability: tetracycline metallic chelation. American Journal of Hospital Pharmacy 32:625–629.

Choo, P. S. 1994. Degradation of oxytetracycline hydrochloride in fresh- and seawater. Asian Fisheries Science 7:195–200.

Clive, D. L. J. 1968. Chemistry of tetracyclines. Quarterly Review of the Chemical Society. 22:435–457.

Collaizzi, J. L., and P. R. Klink. 1969. pH-partition behavior of tetracyclines. Journal of Pharmaceutical Science 58:1184–1189.

Coyne, R., M. Hiney, B. O'Connor, J. Kerry, D. Cazabon, and P. Smith. 1994a. Concentration and persistence of oxytetracycline in sediments under a marine salmon farm. Aquaculture 123: 31–42.

Coyne, R., M. Hiney, and P. Smith. 1994b. Evidence associating overfeeding on a salmon farm with a prolonged half-live of oxytetracycline in under cage sediments. Bulletin of the European Association of Fishery Patholol gists 14:207–210.

Cravedi, J. P., G. Choubert, and G. Delous. 1987. Digestibiltiy of chloramphenicol, oxolinic acid and oxytetracycline in rainbow trout and influence of these antibiotics on lipid digestibility. Aquaculture 60:133–141.

De Liguoro, M., V. Cibin, F. Capolongo, B. Halling-Sørensen, and C. Montesissa. 2003. Use of oxytetracycline and tylosin in intensive calf farming: Evaluation of transfer to manure and soil. Chemosphere 52:203–212.

Delépée, R., H. Pouliquen, and H. Le Bris. 2004. The bryophyte *Fontinalis antipyretica* Hedw. bioaccululates oxytetracycline, flumequine and oxolinic acid in the freshwater environment. Science of the Total Environment 322:243–253.

Dietze, J.E., E.A. Scribner, M.T. Meyer, and D.W. Kolpin. 2005. Occurrence of antibiotics in water from 13 fish hatcheries, 2001-2003. International Journal of Environmental Analytical Chemistry 85: 1141-1152.

Doi, A.M., and M.K. Stoskopf. 2000. The kinetics of oxytetracycline degradation in deionized water under varying temperature, pH, light, substrate, and organic matter. Journal of Aquatic Animal Health 12: 246–253.

Dvořáková, D., R. Rojíčková-Padrtová, and B. Maršálek. 1999. Is the replacement of *Scenedesmus quadricauda* L. by *Selenastrum capricornutum* L. in toxicity tests reasonable? Polish Archives of Hydrobiology 46:345–352.

Elema, M. O., K. A. Hoff, and H. G. Kristensen. 1996. Bioavailability of oxytetracycline from medicated feed administered to Atlantic salmon (*Salmo salar* L.) in seawater. Aquaculture 143:7–14.

European Agency for the Evaluation of Medicinal Products (EMEA). 1997. Environmental risk assessment for veterinary medical products other than GMO containing and immunological products. EMEA/CVMP/055/96-FINAL. EMEA, London, United Kingdom, England. 42 pp. Available online at http://www.emea.eu.int/pdfs/vet/regaffair/005596en.pdf.; accessed October 2005.

Figueroa, R. A., A. Leonard, and A. A. Mackay. 2004. Modeling tetracycline antibiotic sorption to clays. Environmental Science and Technology 38:476–483.

Fribourgh, J. H., F. P. Meyer, and J. A. Robinson. 1969. Oxytetracycline leaching from medicated fish feeds. U.S. Department of Interior, Washington, D.C. Bureau of Sport Fisheries and Wildlife Technical Paper #40. 6 pp.

Gaikowski, M. P., J. C. Wolf, S. M. Schleis, and W. H. Gingerich. 2003. Safety of oxytetracycline (Terrramycin, TM-100F) administered in feed to hybrid striped bass, walleye, and yellow perch. Journal of Aquatic Animal Health 15:274–286.

Gonsalves, D., and D. P. H. Tucker. 1977. Behavior of oxytetracycline in Florida citrus and soils. Archives of Environmental Contamination and Toxicology 6:515–523.

Gowen, R. J., N. B. Bradbury, and J. R. Brown. 1989. The use of simple models in assessing two of the interactions between fish farming and the marine environment. Pages 1071–1080 *in* N. De Pauw, et al., editors. Aquaculture; a biotechnology in progress. European Aquaculture Society, Bredene, Belgium.

Grondel, J. L., J. F. M. Nouws, M. De Jong, A. R Schutte, and F. Driessens. 1987. Pharmacokinetics and tissue distribution of oxytetracycline in carp, *Cyprinus carpio* L., following different routes of administration. Journal of Fish Diseases 10:153–163.

Grove, D. J., L. G. Loizides, and J. Nott. 1978. Satiation amount, frequency of feeding and gastric emptying rate in *Salmo gairdneri*. Journal of Fish Biology 12:507–516.

Halling-Sørensen, B. 2001. Inhibition of aerobic growth and nitrification of bacteria in sewage sludge by antibacterial agents. Archives of Environmental Contamination and Toxicology 40:451–460.

Halling-Sørensen, B., A. Lykkeberg,, F. Ingerslev, P. Blackwell, and J. Tjørnelund. 2003. Characterization of the abiotic degradation pathways of oxytetracyclines in soil interstitial water using LC-MS-MS. Chemosphere 50:1331–1342.

Halling-Sørensen, B., G. Sengeløv, and J. Tjørnelund. 2002. Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria, including selected tetracycline-resistant bacteria. Archives of Environmental Contamination and Toxicology 42:263–271.

Hansen, P. K., B. T. Lunestad, and O. B. Samuelsen. 1992. Effects of oxytetracycline, oxolinic acid, and flumequine on bacteria in an artificial marine fish farm sediment. Canadian Journal of Microbiology 39:1307–1312.

Haug, T. and P. A. Hals. 2000. Pharmacokinetics of oxytetracycline in Arctic charr (*Salvelinus alpinus* L.) in freshwater at low temperature. Aquaculture 186:175–191.

Hektoen, H., J. A. Berge, V. Hormazabal, and M. Yndestad.1995. Persistence of antibacterial agents in marine sediments. Aquaculture 133:175–184.

Hoar, W. S., D. J. Randall, and J. R. Brett. 1979. Fish Physiology; Vol. 8, Bioenergetics and growth. Academic Press, New York. 786 pp.

Holten Lützhøft, H. C., B. Halling-Sørensen, and S. E. Jørgensen. 1999. Algal toxicity of antibacterial agents applied in Danish fish farming. Archives of Environmental Contamination and Toxicology 36:1–6.

Hughes, J. S. 1973. Acute toxicity of thirty chemicals to striped bass (*Morone saxatilis*). Presented at the Western Association of State Game and Fish Commissioners, Salt Lake City, Utah, July 1973. 15 pp.

Hustvedt, S. O., T. Storebakken, and R. Salte. 1991. Does oral administration of oxolinic acid or oxytetracycline affect feed intake of rainbow trout? Aquaculture 92:109–113.

Idaho Division of Environmental Quality. 1997. Waste management guidelines for aquaculture operations, Idaho Division of Environmental Quality, Twin Falls, Idaho, 87 pp. Available online at http://www.deq.state.id.us/water/prog_issues/waste_water/pollutant_trading/aquaculture_guidelines.pdf. Accessed February 2006.

Ingerslev, F., L. Toräng, M-L. Loke, B. Halling-Sørensen, and N. Nyholm. 2001. Primary biodegradation of veterinary antibiotics in aerobic and anaerobic surface water simulation systems. Chemosphere 44:865–872.

International Cooperation on Harmonization of Technical Requirements for Regulation of Veterinary Medical Products (VICH). 2004. Environmental impact assessment for veterinary medical products, phase II guidance, VICH-CL38. Available one-line at http://vich.eudra.org/pdf/10_2004/GL38_st7.pdf.; accessed on June 30, 2005.

Jacobsen, P., and L. Berglind. 1988. Persistence of oxytetracycline in sediments from fish farms. Aquaculture 70:365–370.

Jobling, M. 1980. Gastric evacuation in plaice, *Pleuronectes platessa* L.: effects of temperature and fish size. Journal of Fish Biology 17:547–551.

Katz, S. E. 1984. Environmental impact assessment for the use of oxytetracycline to control gafkemia infections in lobsters. Department of Biochemistry and Microbiology, Cook College/New Jersey Agricultural Experiment Station, Rutgers-the State University of New Jersey, New Brunswick, New Jersey, November 1984. 16 pp.

Katz, S. E. 1985. Environmental impact assessment for the use of oxytetracycline to treat/control respiratory and digestive tract infections in captively-reared alligators. Department of Biochemistry and Microbiology, Cook College/New Jersey Agricultural Experiment Station, Rutgers-the State University of New Jersey, New Brunswick, New Jersey, February 1985. 15 pp.

Katz, S. E. 1995. Environmental impact assessment: Use of oxytetracycline hydrochloride in buffered water for the marking of skeletal tissue of fish fry and fingerlings. Department of Biochemistry

and Microbiology, Cook College/New Jersey Agricultural Experiment Station, Rutgers-the State University of New Jersey, New Brunswick, New Jersey, August 1995. 27 pp.

Kerry, J., R. Coyne, D. Gilroy, M. Hiney, and P. Smith. 1996. Spatial distribution of oxytetracycline and elevated frequencies of oxytetracycline resistance in sediments beneath a marine salmon farm following oxytetracycline therapy. Aquaculture 145:31–39.

Kerry, J., M. Hiney, R. Coyne, D. Cazabon, S. NicGabhainn, and P. Smith. 1994. Frequency and distribution of resistance to oxytetracycline in micro-organisms isolated from marine fish farm sediments following therapeutic use of oxytetracycline. Aquaculture 123:43–54.

Kerry. J., M. Hiney, R. Coyne, S. NicGabhainn, D. Gilroy, D. Cazabon, and P. Smith. 1995. Fish feed as a source of oxytetracycline resistant bacteria in the sediments under fish farms. Aquaculture 131: 101–113.

Kolpin, D. W., E. T. Furlong, M. T. Meyer, E. M. Thurman, S. D. Zaugg, L. B. Barber, and H. T. Buxton. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnaissance. Environmental Science and Technology 36:1202–1211.

Kühne, M., D. Ihnen, G. Möller, and O. Agthe. 2000. Stability of tetracycline in water and liquid manure. Journal of Veterinary Medicine A 47:379–384.

Lai, H. T., S. M. Liu, and Y. H. Chien. 1995. Transformation of chloramphenicol and oxytetracycline in aquaculture pond sediments. Journal of Environmental Science and Health A30: 1897–1923.

Lane, T. H., and H. M. Jackson. 1969. Voidance time for 23 species of fish. U.S. Fish and Wildlife Service. Washington, D.C. Investigations in Fish Control 33. 4 pp.

Le Bris, H., H. Pouliquen, J.M. Debernardi, V. Buchet, and L. Pinault. 1995. Preliminary study on the kinetics of oxytetracycline in shellfish exposed to an effluent of a land-based fish farm: Experimental approach. Marine Environmental Research. 40: 171-180.

Lee, D. S., C. R. Gilbert, C. H. Hocutt, R. E. Jenkins, D. E. McAllister, and J. R. Stauffer, Jr. 1980. Atlas of North American freshwater fishes. North Carolina State Museum of Natural History, Raleigh, North Carolina. 853 pp.

Lewis, R. J., Sr., editor. 1997. Hawley's condensed chemical dictionary. John Wiley & Sons, New York. 1229 pp.

Lindsey, M., M. Meyer, and E. M. Thurman. 2001. Analysis of trace levels of sulfonamide and tetracycline antimicrobials in groundwater and surface water using solid-phase extraction and liquid chromatography/mass spectrometry. Analytical Chemistry 73:4640–4646.

Lunestad, B. T. 1991. Fate and effects of antimicrobial agents in aquatic environments. Pages 152–161 *in* C. M. Michael and D. J. Alderman, editors. Chemotherapy in aquaculture: From theory to reality. Office International des Epizooties, Paris, France. 567 pp.

Lunestad, B. T, and J. Goksøyr. 1990. Reduction in the antibacterial effect of oxytetracycline in the sea water by complex formation with magnesium and calcium. Diseases of Aquatic Organisms 9:67–72.

Lunestad, B. L., O. B. Samuelsen, S. Fjelde, and A. Ervik. 1995. Photostability of eight antibacterial agents in seawater. Aquaculture 134:217–225.

Marking, L.L., G.E. Howe, and J.R. Crowther. 1988. Toxicity of erythromycin, oxytetracycline, and tetracycline administered to lake trout in water baths, by injection, or by feeding. The Progressive Fish-Culturist 50:197–201.

Martin, M. and D. Gottlieb. 1952. The production and role of antibiotics in the soil: III. Phytopathology. 42:407–408.

Micromedex, Inc. 2003. Tetracyclines veterinary-systemic. Available online at http://www.usp.org/pdf/EN/veterinary/tetracyclines.pdf.; accessed Febraury 2006.

Murphy, D., and G. T. Peters. 1991a. Oxytetracycline hydrochloride: A 96-hour static acute toxicity test with the bluegill (*Lepomis macrochirus*). Project No. 260A-102. Performed by Wildlife International, Ltd., Easton, Maryland. Submitted by Pfizer, Inc., New York, EPA MRID 417832-01. . Data Evaluation Record (DER) provided by EPA under EFED Document Number E9001098.

Murphy, D., and G. T. Peters. 1991b. Oxytetracycline hydrochloride: A 96-hour static acute toxicity test with the rainbow trout (*Oncorhynchus mykiss*). Laboratory Study No. 260A-103. Prepared by Wildlife International, Ltd., Easton, Maryland. Submitted by Pfizer, Inc., New York, EPA MRID 417832-02. . Data Evaluation Record (DER) provided by EPA under EFED Document Number E9001099.

Namdari, R., S. Abedini, L. Albright, and F. C. P. Law. 1998. Tissue distribution and elimination of oxytetracycline in sea-pen cultured chinook salmon, *Oncorhynchus tshawytscha*, and Atlantic salmon, *Salmo salar*, following medicated-feed treatment. Journal of Applied Aquaculture 8:39–51.

Organisation for Economic Cooperation and Development (OECD). 1996. Guidelines for testing of chemicals. Proposed for updated Guideline 211, *Daphnia magna* reproduction test. Revised draft document. OECD, Paris, France.

Pennak, R.W. 1978. Freshwater invertebrates of the United States. Second edition. John Wiley & Sons, New York. 803 pp.

Pinck, L. A., W. F. Holton, and F. E. Allison. 1961a. Antibiotics in soils: 1. Physico-chemical studies of antibiotic-clay complexes. Soil Science 91:22–28.

Pinck, L. A., D. A. Soulides, and F. E. Allison. 1961b. Antibiotics in soils: II. Extent and mechanism of release. Soil Science 91:94–99.

Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard. 1982. Fish hatchery management. U.S. Department of Interior, Fish and Wildlife Service, Washington, D.C. 517 pp.

Plakas, S. M., R. M. McPhearson, and A. M. Guarino. 1988. Disposition and bioavailability of 3H-tetracycline in the channel catfish (*Ictalurus punctatus*). Xenobiotica 18:83–93.

Porubcan, L. S., C. J. Serna, J. L. White, and S. L. Hem. 1978. Mechanism of adsorption of clindamycin and tetracycline by montmorillonite. Journal of Pharmaceutical Sciences 67:1081–1087.

Post, G. 1987. Textbook of fish health. T.F.H. Publications, Neptune City, New Jersey. 288 pp.

Pouliquen, H., H. Le Bris, and L. Pinault. 1993. Experimental study on the decontamination kinetics of seawater polluted by oxytetracycline contained in effluents released from a fish farm located in a salt marsh. Aquaculture 112:113–123.

Pye-MacSwain, J., D. Rainnie, E. Cawthorn, and G. Johnson. 1992. Plasma pharmacokinetics and bioavailability of oxytetracycline in Atlantic salmon held in seawater. Bulletin of the Aquaculture Association of Canada. 92:70–72.

Rach, J. J. 2007. Summary report: Efficacy of oxytetracyline bath immersion to control mortality associated with external flavobacter infections on cool- and warmwater fish cultured at hatcheries. U.S. Geological Survey. Upper Midwest Environmental Sciences Center. La Crosse, Wisconsin. 159 pp.

Rabølle, M., and N. H. Spliid. 2000. Sorption and mobility of metronidazole, olaquindox, oxytetracycline and tylosin in soil. Chemosphere 40:715–722.

Rigos, G., M. Alexis, A. Andriopoulou, and I. Nengas. 2002. Pharmacokinetics and tissue distribution of oxytetracycline in sea bass, *Dicentrarchus labrax*, at two water temperatures. Aquaculture 210:59–67.

Rigos, G., I. Nengas, M. Alexis, and F. Athanassopoulou. 2004. Bioavailability of oxytetracycline in sea bass, *Dicentrarchus labrax* (L.). Journal of Fish Diseases 27:119–122.

Rigos, G., I. Nengas, A. E. Tyrpenou, M. Alexis, and G. M. Troisi. 2003. Pharmacokinetics and bioavailability of oxytetracycline in gilthead sea bream (*Sparta aurata*) after a single dose. Aquaculture 221:75–83.

Rogstad, A., V. Hormazabal, O. F. Ellingsen, and K. E. Rasmussen. 1991. Pharmacokinetic study of oxytetracycline in fish. I. Absorption, distribution and accumulation in rainbow trout in freshwater. Aquaculture 96:219–226.

Rojíčková, R., D. Dvořáková, and B. Maršálek. 1998. The use of miniaturized algal bioassays in comparison to the standard flask assay. Environmental Toxicology and Water Quality 13:235–241.

Rose, P.E., and J. A. Pedersen. 2005. Fate of oxytetracycline in streams receiving aquaculture discharges: Model simulations. Environmental Toxicology and Chemistry 24: 40-50.

Ross, B., and K. Jauncey. 1981. A radiographic estimation of the effect of temperature on gastric emptying time in *Sarotherodon niloticus* (L.) x *S. aureus* (Steindachner) hybrids. Journal of Fish Biology 19:333–344.

Samuelsen, O. B. 1989. Degradation of oxytetracycline in seawater at two different temperatures and light intensities, and the persistence of oxytetracycline in the sediment from a fish farm. Aquaculture 83:7–16.

Samuelsen, O.B. 1992. The fate of antibiotics/chemotherapeutics in marine aquaculture sediments. Pages 162–173 *in* C. M. Michael and D.J. Alderman, editors. Chemotherapy in aquaculture: From theory to reality. Office International des Epizooties, Paris, France. 567 pp.

Samuelsen, O. B., B. T. Lunestad, A. Ervik, and S. Fjelde. 1994. Stability of antibacterial agents in an artificial marine aquaculture sediment studied under laboratory conditions. Aquaculture 126:283–290.

Samuelsen, O. B., V. Torsvik, and A. Ervik. 1992. Long-range changes in oxytetracycline concentration and bacterial resistance towards oxytetracycline in a fish farm sediment after medication. The Science of the Total Environment 114:25–36.

Sandaa, R. A., V. L. Torvik, and J. Goksøyr. 1992. Transferable drug resistance in bacteria from fish-farm sediments. Canadian Journal of Microbiology 38:1061–1065.

Schmitt, M. O., and S. Schneider. 2000. Spectroscopic investigation of complexation between various tetracyclines and Mg2+ or Ca2+. PhysChemComm 9. Available online at http://www.rsc.org/ej/qu/2000/b005722n/b005722n.pdf.; accessed October 2005.

Sithole, B. B., and R. D. Guy. 1987a. Models of tetracycline in aquatic environments. I. Interaction with bentonite clay systems. Water, Air, and Soil Pollution 32:303–314.

Sithole, B. B., and R. D. Guy. 1987b. Models of tetracycline in aquatic environments. II. Interaction with humic substances. Water, Air, and Soil Pollution 32:315–321.

Smith, G. M. 1950. The freshwater algae of the United States. Second edition. McGraw-Hill Book Company, New York. 719 pp.

Smith, P. 1996. Is sediment deposition the dominant fate of oxytetracycline used in marine salmonid farms: a review of available evidence. Aquaculture 146:157–169.

Smith, P. and O. B. Samuelsen. 1996. Estimates of the significance of out-washing of oxytetracycline from sediments under Atlantic salmon sea-cages. Aquaculture 144:17–26.

Smith, P., J. Donlon, R. Coyne, and D. J. Cazabon. 1994. Fate of oxytetracycline in a fresh water fish farm: influence of effluent treatment systems. Aquaculture 120:319–325.

Smith, P., T. Niland., F. O'Domhnaill, G. O'Tauthaigh, and M. Hiney. 1996. Influence of marine sediment and divalent cations on the activity of oxytetracycline against *Listonella anguillarum*. Bulletin of the European Association of Fish Pathologists 16:54–57.

Soulides, D. A., Pinck, L. A., and F. E. Allison. 1961. Antibiotics in soil. 3. Further studies on release of antibiotics from clays. Soil Science 92:90–93.

Stehly, G. R. 2002. Summary report: Efficacy of immersion oxytetracycline treatments for diseases of finfish species cultured on public fish facilities. U.S. Geological Survey. Upper Midwest Environmental Sciences Center. La Crosse, Wisconsin. 455 pp.

Talbot, C., P. J. Higgins, and A. M. Shanks. 1984. Effects of pre- and post-prandial starvation on meal size and evacuation rate of juvenile Atlantic salmon, *Salmo salar* L. Journal of Fish Biology 25:551–560.

Thurman, E. M., J. E. Dietz, and E. A. Scribner. 2002. Occurrence of antibiotics in water from fish hatcheries. U.S. Geological Survey Fact Sheet 120-02, November 2002.

Tolls, J. 2001. Sorption of veterinary pharmaceuticals in soils: A review. Environmental Science and Technology 35:3397–3406.

Uno, K. 1996. Pharmacokinetic study of oxytetracycline in healthy and vibriosis-infected ayu (*Plecoglossus altivelis*). Aquaculture 143:33–42.

U.S. Environmental Protection Agency (EPA).1997. Ecological risk assessment guidance for superfund: Process for designing and conducting ecological risk assessments. Interim Final EPA-540-R-97-006. U.S. Environmental Protection Agency, Environmental Response Team, Edison, New Jersey. [Full copies may be found in the report entitled "Use of Chloramine-T in Intensive Fresh-Water Aquaculture: An Evaluation of Potential Environmental Fate and Effects" (Appendix I) submitted to PMF #5637 or in the report entitled "Environmental Assessment for the Proposed Use of Hydrogen Peroxide in Aquaculture for Treating External Fungal, Bacterial, and Parasitic Diseases of Cultured Fish" (Appendix B) submitted to PMF # 5639.]

_____. 1998. Guidelines for ecological risk assessment. EPA/630/R-95/002F. U.S. Environmental Protection Agency, Washington, D.C. 114 pp. [Full copies may be found in the report entitled "Use of Chloramine-T in Intensive Fresh-Water Aquaculture: An Evaluation of Potential Environmental Fate and Effects" (Appendix I) submitted to PMF #5637 or in the report entitled "Environmental Assessment for the Proposed Use of Hydrogen Peroxide in Aquaculture for Treating External Fungal, Bacterial, and Parasitic Diseases of Cultured Fish" (Appendix B) submitted to PMF #5639.]

U.S. Pharmacopeia. 1995. The National Formulary. USP 23rd Edition, January 1995, Rockville, MD.

Vaughan, S., and P. Smith. 1996. Estimation of the influence of a river sediment on the biological activity of oxytetracycline hydrochloride. Aquaculture 141:67–76.

Wassef, M. K. 1983. Environmental assessment: Finalization for oxytetracycline water soluble and premix formulations for food producing animals. Environmental Impact Staff, Bureau of Veterinary Medicine, U.S. Food and Drug Administration, October 1983. 52 pp.

Weiss, P. J., M. L. Andrew, and W. W. Wright. 1956. Solubility of antibiotics in twenty-four solvents: Use in analysis. Antibiotics and Chemotherapy 7:374–377.

Wellborn, T. L., Jr. 1969. The toxicity of nine therapeutic and herbicidal compounds to striped bass. The Progressive Fish-Culturist 31: 27–32.

Weston, D. P., D. G. Capone, R. P. Herwig, and J. T. Staley. 1994. Environmental fate and effects of aquaculture antibacterials in Puget Sound: A report of the University of California pursuant to the National Oceanographic and Atmospheric Administration (Award No. NA26FD0109-01), University of California, Berkeley, California.

Williams, R.R., T.A. Bell, and D.V. Lightner. 1992. Shrimp Antimicrobial Testing. II. Toxicity Testing and Safety Determination for Twelve Antimicrobials with Penaeid Shrimp Larvae. Journal of Aquatic Animal Health 4: 262-270.

Wilson, C.J., Brain, R.A., Sanderson, H., Johnson, D.J., Bestari, K.T., Sibley, P.K., and K.R. Solomon. 2004. Structural and functional responses of plankton to a mixture of four tetracyclines in aquatic microcosms. Environmental Science and Technology 38: 6430-6439.

Windholz, M., S. Budavari, R. F. Blumetti, and E .S. Otterbein. 1983. Merck Index: Tenth Edition. Merck & Co., Rahway, New Jersey. 1463 pp.

Winsby, M., B. Sander, D. Archibald, M. Daykin, P. Nix, F. J. R. Taylor, and D. Munday. 1996. The environmental effects of salmon netcage culture in British Columbia. Hatfield Consultants Ltd., West Vancouver, British Columbia.

Wollenberger, L., B. Halling-Soerensen, and K. O. Kusk. 2000. Acute and chronic toxicity of veterinary antibiotics to *Daphnia magna*. Chemosphere 40:723–730.

Wool, T. A., R. B. Ambrose, J. L. Martin, and E. A. Comer. (n.d.) Water quality analysis simulation program (WASP), version 6.0. Draft Users Manual. U.S. Environmental Agency, Athens, Georgia. Available online at http://www.cee.odu.edu/mbin/wasp/win/wasp6_manual.pdf.; accessed January 2006.

13. Glossary of acronyms and abbreviations

3N HCl	hydrochloric acid in water a normality of 3
Al	aluminum
ASTM	American Society for Testing and Materials
BCF	bioconcentration factor
BW	(fish) body weight
C_8-C_{18}	methyl substituted at the octyl to octadecyl level.
Ca	calcium
$CaCO_3$	calcium carbonate
$CaCl_2$	calcium chloride
cm	centimeter
Co	cobalt
Cu	copper
CVM	Center for Veterinary Medicine
d	day
DOM	dissolved organic material
EA	environmental assessment
EC	effective concentration
EC_{10}	effective concentration (expected to produce the specified effect in 10% of the population within the specified time)
EC_{50}	effective concentration (expected to produce the specified effect in 50% of the population within the specified time)
EIC	environmental introduction concentration
EMEA	European Agency for the Evaluation of Medicinal Products
EPA	U.S. Environmental Protection Agency
FDA	U.S. Food and Drug Administration
Fe	iron
g	gram(s)
g/d	gram(s) per day
h	hour
HCl	hydrochloric acid
HPLC	high performance liquid chromatography

H_2O	water
INAD	Investigational New Animal Drug
IR	infrared
K_d	distribution coefficient
kg	kilogram(s)
$kg/m^3/s$	kilograms per cubic meter per second
L	liter
lb	pound
L/d	liters per day
L/m^3	liters per cubic meter
LC	lethal concentration
LC_{50}	lethal concentration (50% of the population within the specified time)
LC/MS	liquid chromatograph/mass spectrometer
LOEL or LOEC	lowest-observed-effect-level or lowest-observed-effect concentration
L/min	liters per minute
m	meter(s)
M	molar
m^3	cubic meter
m^3/s	cubic meters per second
mg	milligram
Mg	magnesium
mg/kg	milligrams per kilogram
mg/L	milligram per liter
mg/mL	milligram per milliliter
MIC	minimum inhibitory concentration
min	minute
min/d	minutes/day
mL	milliliter(s)
mM	millimolar
MSDS	material safety data sheet
MW	molecular weight

NOEC or NOEL	no-observed-effect-concentration or no-observed-effect level
OECD	Office of Economic Cooperation and Development
OTC	oxytetracycline
pH	- log $[H_3O^+]$
PNEC	predicted no-effect concentration
ppb	parts per billion
ppm	parts per million
ROI	receptor of interest
RQ	risk quotient
TC	tetracycline
UMESC	Upper Midwest Environmental Sciences Center
USGS	U.S. Geological Survey
μg	microgram(s)
μg/g	micrograms per gram
μg/kg	micrograms per kilogram
μg/L	micrograms per liter
μm	micrometer
μM	micromolar
W	watt
w/v	weight per volume
WASP-6	Water Quality Analysis Simulation Program, version 6.0
XRD	X-ray diffraction

Table 1. Draft oxytetracycline label including current disease claims (unbolded) and proposed expansions and extensions (bolded).

DRAFT LABEL

Tradename: Terramycin® (TM-100F)
Sponsor: Phibro Animal Health Corp.
Ingredients: dihydrate salt of oxytetracycline.
CAS #: 79-57-2 (anhydrous)

Species: Pacific salmon
Indication: Mark skeletal tissue
Dosage Regimen: 250 mg/kg BW/day for 4 days
Limitations/comments: For use on salmonids less than 30 g incorporated in the feed as the sole ration. Seven day withdrawal time after use. The hydrochloride salt of oxytetracycline may also be used.

Species: Lobster
Indication: Control mortalities resulting from gaffkemia (*Aerococcus viridans*)
Dosage Regimen: 1 g/lb medicated feed for 5 days
Limitations/comments: Use in feed as the sole ration. Withdrawal time is 30 days after treatment.

Species: **Catfish**
Indication: Control mortalities resulting from bacterial hemorrhagic septicemia (*Aeromonas liquifaciens*), **columnaris (*Flavobacterium columnare*)** and pseudomonas disease
Dosage Regimen: 2.5 to 3.75 g/100 lb BW/day for 10 days
Limitations/comments: In a mixed feed ration with the water temperature not less than 62 °F. Withdrawal time after treatment is 21 days.

Species: Salmonids
Indication: Control mortalities resulting from ulcer disease (*Hemophilus piscium*), furunculosis (*Aeromonas salmonicida*), bacterial hemorrhagic septicemia (*Aeromonas liqufaciens*) and pseudomonas disease (*Pseudomonas*)
Dosage Regimen: 2.5 to 3.75 g/100 lb BW/day for 10 days
Limitations/comments: In a mixed feed ration at all water temperatures used for culture. Withdrawal time after treatment is 21 days.

Species: **Salmonids**
Indication: **Control mortalities resulting from bacterial coldwater disease (*Flavobacterium psychrophilum*) and columnaris disease (*Flavobacterium columnare*).**
Dosage Regimen: 3.75 g/100 lb BW/day for 10 days
Limitations/comments: In a mixed feed ration at all water temperatures used for culture. Withdrawal time after treatment is 21 days.

Species: **Coolwater finfish**
Indication: **Control mortalities resulting from furunculosis (*Aeromonas salmonicida*), bacterial hemorrhagic septicemia (*Aeromonas liqufaciens*), pseudomonas disease (*Pseudomonas*), and columnaris disease (*Flavobacterium columnare*).**
Dosage Regimen: 2.5 to 3.75 g/100 lb BW/day for 10 days.
Limitations/comments: In a mixed feed ration at all water temperatures used for culture. Withdrawal time after treatment is 21 days.

Species: **Scaled warmwater finfish**
Indication: **Control mortalities resulting from bacterial hemorrhagic septicemia *(Aeromonas liqufaciens*), pseudomonas disease (*Pseudomonas*), and columnaris disease (*Flavobacterium columnare*).**
Dosage Regimen: 2.5 to 3.75 g/100 lb BW/day for 10 days
Limitations/comments: In a mixed feed ration at all water temperatures used for culture. Withdrawal time after treatment is 21 days.

DRAFT LABEL

Table 2. Oxytetracycline identification information**.**

Chemical name	2-naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-, [4S-(4 alpha,4a alpha,5 alpha,5a alpha,6 beta,12a alpha)]-,
Synonyms	glomycin, terrafungine, riomitsin, hydroxytetracycline (Budavari et al. 1989)
Common names	Agrimycin, Biomycin, Duramycin, Kelamycin, Liquamycin, Medamycin, Oxybiotic, OxyCure, Oxy-Mycin, Oxymycine, Oxyshot, Oxysol, Oxytet, Oxyvet, Promycin, Procure, Promycin, Terramycin, Terra-Vet, Tetra-Biotic, Tetraject, T-Vet (use of trade names does not imply government endorsement)
CAS registry number	6153-64-6 (dihydate), 79-57-2 (anhydrous)
Molecular weight	496.47 (dihydrate)
Molecular formula	$C_{22}\ H_{24}\ N_2\ O_9 \bullet 2H_2O$
General physical and chemical characteristics	OTC (USP) - Pale yellow to tan, odorless, crystalline powder. Stable in air, but exposure to strong sunlight causes the material to darken. Loses potency in solutions at pH <2 and is rapidly destroyed by alkaline solutions (Micromedex, Inc. 2003).

Table 3. Oxytetracycline physicochemical properties**.**

Parameter	Value	Reference
Boiling point (°C)	not available	
Melting point (°C)	179-182 (decomposes)	Lewis 1997
pH	6.115 (saturated solution)	Lewis 1997
Density (kg/m^3)	not known	
Flash point (°C)	not available	
Stability	Stable in air, light sensitive	Lewis 1997
Solubility	Very slightly soluble in water; freely soluble in 3 N HCl and in alkaline solutions; sparingly soluble in alcohol	U.S. Pharmacopeia 1995
solubility - monoalkyl (C_8-C_{18}) trimethyl ammonium salt	1.0 mg/mL in water	Pfizer, Inc., MSDS[a] #116
solubility - dihydrate	0.6 mg/mL in water	Wassef 1983, p. 28, Weiss et al. 1956
solubility - hydrochloride salt	6.9 mg/mL in water	Wassef 1983, p. 28, Weiss et al. 1956

[a] MSDS = material data safety sheet.

Table 4. Oxytetracycline therapeutic and pesticide use in the United States (1997-2003).

Target Fauna/Flora	1997 (lb)	2001 (lb)	2002 (lb)	2003 (lb)
Aquaculture	-	33,480[a]	17,600[a]	32,780[a]
Farm and companion animal	-	7,145,000[b] (6,700,000 in 2000)[(b)]	6,650,000[a]	6,578,000[a]
Fruit trees (fungicide)	26,700[c]	21,300[d]	-	16,400[e]
Marine paint additive (algaecide)		insignificant[f, g]		
Human		unknown, probably insignificant[f]		

[a]Industry representatives, PhiBro Animal Health Co., 2003, personal communication.

[b]All tetracyclines, 2001 Animal Health Institute Survey, available online at http://www.ahi.org/mediaCenter/pressReleases/surveyShowsDecline.asp.; accessed January 2006.

[c]Available online at http://www.apsnet.org/online/feature/Antibiotics/table_3.htm or http://usda.mannlib.cornell.edu/reports/nassr/other/pcu-bb/agch0798.pdf. Both accessed January 2006.

[d]2001 National Agricultural Statistics Service report, available at http://usda.mannlib.cornell.edu/reports/nassr/other/pcu-bb/agcf0802.pdf.; accessed January 2006.

[e]2003 National Agricultural Statistics Service report, available at http://usda.mannlib.cornell.edu/reports/nassr/other/pcu-bb/agcf0804.pdf.; accessed January 2006.

[f]<1,000 lb. annually.

[g]U.S. Environmental Protection Agency Pesticide Fact Sheet 12/88 (No. 188), available online at http://pmep.cce.cornell.edu/profiles/fung-nemat/febuconazole-sulfur/oxytetracycline/fung-prof-oxytetracycline.html. Accessed December 2005.

Table 5. Suggested chemical values (mg/L) for hatchery water supplies (Piper et al. 1982).

Variable	Salmonids	Warmwater species
Dissolved oxygen	>5	>5
Carbon dioxide	0–10	0–15
Total alkalinity (as $CaCO_3$ [a])	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

[a] $CaCO_3$ = calcium carbonate.

Table 6. Oxytetracycline n-octanol/water partition coefficient as a function of pH (Collaizzi and Klink 1969; Wassef 1983).

pH	Partition coefficient
2.1	0.0035
3.0	0.018
3.9	9.078
5.6	0.075
6.6	0.087
7.5	0.025
8.5	0.0086

Table 7. Gut transit time through the digestive tract of various fish (taken mostly from Hoar et al. 1979 pp. 201-203)**.**

Species, reference	Temperature (°C)	Gut transit time (h)
Salmonidae		
Oncorhynchus mykiss (rainbow trout), Grove et al. 1978 (f)[a]	8	49-51
	11	46
	13.5	35
	15	40
	18	30.5
Salvelinus namaycush (lake trout), Lane and Jackson 1969 [L&J 1969] (f)	12	60-108
Salmo salar (Atlantic salmon), Talbot et al. 1984 (f)	9-13	60 (max. starving) 18 (no starving)
Esocidae		
Esox lucius (northern pike), L&J 1969 (f)	12	72
Percidae		
Perca flavescens (yellow perch), L&J 1969 (f)	12	36-60
Stizostedion vitreum (walleye), L&J 1969 (f)	12	60
Macquaria ambigua (golden perch), Anderson and Braley 1993 (f)	20	96.6
Centrarchidae		
Micropterus salmoides (largemouth bass) L&J 1969 (f)	12	48-84
	20	60
Beamish 1972 (f)	20	36-48
Micropterus dolomieui (smallmouth bass), L&J 1969 (f)	12	48-72
Lepomis macrochirus (bluegill sunfish), L&J 1969 (f)	12	36-84
	17	48
	20	36-60
	22	36
	25	36
Cichlidae		
Sarotherodon niloticus (L.) x *S. aureus* (tilapia), Ross and Jauncey 1981[b] (f)	30	18.3

[a] (f) = in fresh water.

[b] Stomach evacuation times for tilapia were reported by these authors to be 8.5 h at 30 °C, 10.8 h at 25 °C, and 16.4 h at 20 °C. Increase in gastric evacuation rates with increasing temperature have also been reported by other authors (Jobling 1980).

Table 8. Bioavailability of oxytetracycline in various fresh and saltwater fish**.**

Species, reference	OTC bioavailability (%)	Temperature (°C)
Rainbow trout (*Oncorhynchus mykiss*)		
Abedini et al. 1998 (f)[a]	30.3	11
Cravedi et al. 1987 (f)	7-9	14
Rogstad et al. 1991(f)	2.6	7
Arctic charr (*Salvelinus aplinus* L), Haug and Hals 2000 (f)	3.2-7.3	6.3
Chinook salmon (*Oncorhynchus tshawytscha*), Abedini et al. 1998 (s)[b]	24.8	11
Atlanic salmon (*Salmo salar*)		
Elema et al. 1996 (s)	2	7-8
Pye-MacSwain et al. 1992 (s)	6.88	not given
Carp (*Cyprinus carpio* L), Grondel et al. 1987(f)	0.6	20
Ayu (*Plecoglossus altivelis*), Uno 1996 (f)	3.8 sick, 9.3 healthy	18
Sea bass (*Dicentrarchus labrax*), Rigos et al. 2004 (s)	22	22
Gilthead sea bream (*Sparus aurata*), Rigos et al. 2003 (s)	9	20

[a] (f) = in fresh water.
[b] (s) = in salt water.

Table 9. Half-life of oxytetracycline in marine sediment. Table transferred in part from Winsby et al. (1996).

Half-life (d)	Description	Source
70	anoxic sediments	Jacobsen and Berglind (1988)
32 64	low sedimentation from farm high sedimentation from farm	Samuelsen (1989)
9 419	bottom exposed to currents bottom stagnant	Björklund et al. (1990)
55	aquaria	Samuelsen (1992)
87 to 144	under three cages	Samuelsen et al. (1992)
125	artificial fish farm sediment	Hansen et al. (1992)
16	under one cage	Coyne et al. (1994a)
>180	artificial marine aquaculture sediment	Samuelsen et al. (1994)
36	marine sediment microcosm	Capone et al. (1996)

Table 10. Oxytetracycline concentration in marine sediment. Table transferred in part from Winsby et al. (1996).

Concentration in Sediment	**Source**
0.1 to 4.9 mg/kg dry matter	Jacobsen and Berglind (1988)
180 mg/kg, low sedimentation from farm	Samuelsen (1989)
260 mg/kg, high sedimentation from farm	Samuelsen et al. (1992)
Farm A, mean 0.1 mg/kg, bottom exposed to current	Björklund et al. (1990)
Farm B, mean 2.0 mg/kg, stagnant bottom	Björklund et al. (1991)
Farms A, B, and E, 2.4, 4.3, and 0.7 mg/kg	Björklund et al. (1991)
9.9 to 10.9 mg/kg	Coyne et al. (1994a)
0.5 to 4.0 mg/kg	Capone et al. (1996)
0.5 to 1.6 mg/kg	Weston et al. (1994)
0.65 to 4.2 mg/kg	Kerry et al. (1996)
4.6 ± 3.7 mg/kg	Kerry et al. (1995)

Table 11. Number of fish treated, total fish weight, hatchery water flow and fish loading density reported during oxytetracycline-medicated feed administrations at hatcheries participating in Investigational New Animal Drug Permit 9332 from 2000 through 2007. The daily mass of oxytetracycline and estimated oxytetracycline discharge concentrations were estimated based on the total fish mass and daily water flow reported by the hatchery at a hypothetical dose of 82.5 mg/kg bodyweight (BW)/day (the highest treatment rate expected to be on the oxytetracycline-medicated feed label). Data were categorized to represent all freshwater-reared finfish, all freshwater-reared salmonids, and all other freshwater-reared finfish.

	Facitlity Reported Data				**Estimates based on hypothetical dosing at the maximum label dose rate (82.5 mg/kg BW)**	
	Total number of fish	**Total fish weight (kg)**	**Hatchery flow (m^3/s)**	**Fish loading density (kg/m^3/s)**	**Daily oxytetracycline administered (g)**	**Estimated oxytetracycline discharge concentration (µg/L)[a]**
All freshwater-reared finfish (834 administrations)						
Mean	200,738	1,643	2.0	11,108	135.6	10.6
Median	81,502	367.7	0.3	1,258	30.3	1.2
Min	200	0.1	0.0003	1.1	0.0078	0.001
Max	5,098,029	36,211	1,001	1,312,583	2,987	1,254
Std Dev	410,033	3,672	34.7	76,771	302.9	73.3
25th %ile	33, 246	57.6	0.1	267.9	4.8	0.3
50th %ile	81,502	367.7	0.3	1,258	30.3	1.2
75th %ile	202,368	1,407	1.2	4,072	116.1	3.9
95th %ile	820,309	7,441	3.0	20,019	613.9	19.1
All freshwater-reared salmonids (642 administrations [including 126 marking treatments])						
Mean	228,116.4	1,820	2.4	11,293	150.2	10.8
Median	96,000	474	0.3	1,051	39.1	1.0
Min	788	0.1	0.0003	1.1	0.008	0.001
Max	5,098,029	36,211	1,001	1,312,583	2,987	1,254
Std Dev	445,773	3,913	39.5	76,539	322.8	73.0
25th %ile	45,000	65.6	0.2	217.7	5.4	0.2
50th %ile	96,000	474	0.3	1,051	39.1	1.0
75th %ile	211,891	1,582	1.2	3,358	130.5	3.2
95th %ile	948,333	7,525.1	3.2	20,928	620.8	20.0
All other freshwater-reared finfish (192 administrations)						
Mean	109,193	1,052	0.77	10,491	86.8	10.0
Median	19,890	216.7	0.13	3,117	17.9	3.0
Min	200	5.8	0.002	25.8	0.48	0.025
Max	2,201,753	26,891	31.4	1,076,144	2,219	1,028
Std Dev	235,402	2,638	2.4	77,742	217.6	74.3
25th %ile	750	50.4	0.015	759.7	4.2	0.7
50th %ile	19,890	216.7	0.13	3,117	17.9	3.0
75th %ile	139,004	612.7	0.75	6,461	50.5	6.2
95th %ile	385,715	5,109	2.7	14,147	421.5	13.5

[a] A simple estimate of facility 136 OTC discharge concentration (treatment date 1/7/00) was estimated from mass OTC (ug)/total flow L/d example: 325,470,000 µg OTC / (1.12 m^3/s x 1,000 L/m^3 x 86,400 s/d) = 325,470,000/96,768,000 L = 3.36 µg OTC/L.

Appendix A, INAD 9332 2000-2007 extracted data.xls

Table 12. Summary of acute and chronic toxicity studies of oxytetracycline in several freshwater species. For each species, the endpoint measured and the Lethal Concentration (LC) or Effective Concentration (EC) are given in mg/L for selected time points. Data points used for the risk assessment are bolded.

		EC_{50} or LC_{50} - (mg/L)				
Species tested	**Measured endpoint**	**24 h**	**48 h**	**96 h**	**Other - (mg/L)**	**Reference**
Activated sludge bacteria	growth inhibition	-	-	-	1.2 (4 to 6 h EC_{50})	Halling-Sørensen 2001
Activated sludge bacteria	nitrification	-	-	-	decreases nitrification rate	Halling-Sørensen 2001
Nitrosomonas europaea	growth inhibition, suspended culture	-	-	-	1.7 (10-d EC_{50})	Halling-Sørensen 2001
Activated sludge bacteria	growth inhibition, pour plate method	-	0.14	-	-	Halling-Sørensen 2001
Activated sludge bacteria	**growth inhibition**	-	**0.08**	-	-	**Halling-Sørensen et al. 2002**
Nitrosomonas europaea	**growth inhibition, pour plate method**	-	-	-	**0.32 (5 to 6-d EC_{50})**	**Halling-Sørensen 2001**
Cyanobacteria *Microcystis aeruginosa* (blue-green algae)	**growth inhibition**	-	-	-	**0.207 (7-d EC_{50})**	**Holten Lützhøft et al. 1999**
Green algae *Scenedesmus quadricauda*	cell multiplication/growth inhibition	-	-	-	36.70 (72-h EC_{50})	Dvořáková, et al. 1999
Green algae *Selenastrum capricornutum*	cell multiplication/growth inhibition	-	-	-	4.27 (72-h EC_{50})	Dvořáková, et al. 1999
Green algae *Raphidocelis subcapitata (Selenastrum capricornutum)*	**cell multiplication/growth inhibition**	-	-	-	**1.99**-4.49 **(72-h EC_{50})**	**Rojíčková, et al. 1998**
Green algae *Selenastrum capricornutum*	growth inhibition	-	-	-	4.18 (72-h EC_{50})	De Liguoro et al. (2003)
Green algae *Selenastrum capricornutum*	growth inhibition	-	-	-	4.5 (72-h EC_{50})	Holten Lützhøft et al. 1999
Lemma gibba (aquatic higher plant)	phytotoxicity: multiple growth and biochemical endpoints	-	-	-	0.87 (7-d EC_{10})	Brain et al. 2004
Daphnia magna	immobilization	-	>102	-	-	Bellantoni et al. 1991
D. magna	**immobilization**	-	-	-	**100 (48-h LOEC)[a]**	**Wollenberger et al. 2000**
D. magna	**reproduction rate, pH=7.5**	-	-	-	46.2 (21-d EC_{50}) **7.4 (21-d EC_{10})**	**Wollenberger et al. 2000**
Bluegill, juvenile *Lepomis macrochirus*	mortality	-	-	>94.9	-	Murphy and Peters 1991a
Rainbow trout, juvenile *Oncorhynchus mykiss*	mortality	-	-	>116	-	Murphy and Peters 1991b
Lake trout, fingerling *Salvelinus namaycush*	mortality	<200	-	<200	-	Marking et al. 1988
Striped bass, **fingerlings (60 mm)** *Morone saxatilis*	mortality	>250	>250	178	-	Wellborn 1969
Striped bass, fingerlings (35-51 mm)	**mortality**	150	125	**75**	50 (96-h LC_0)	**Hughes 1973**
Striped bass, larvae	mortality	-	-	-	**50 (24, 48, 96-h LC_0)** 75 (24, 48, 96-h LC_{100})	Hughes 1973

[a] LOEC = lowest observed effect concentration.

Table 13. Summary of acute and chronic toxicity studies of oxytetracycline in several brackish species. For each species, the endpoint measured and the Lethal Concentration (LC) or Effective Concentration (EC) are given in mg/L for selected time points. For toxicity studies on striped bass, see Table 12. Data points used for the risk assessment are bolded.

Species tested	Measured endpoint	EC_{50} or LC_{50} - (mg/L)			Other - (mg/L)	Reference
		24 h	48 h	96 h		
Rhodomonas salina	**growth inhibition**	-	-	-	**72-h EC_{50} = 1.6**	**Holten Lützhøft et al. 1999**
Whiteleg shrimp, *Penaeus vannamei*	intoxication, immobilization[a]	>160	-	-		Williams et al. 1992
Whiteleg shrimp, protozoea	intoxication, immobilization[a]	-	61.1	-		Williams et al. 1992
Whiteleg shrimp, protozoea III – mysis I interface	intoxication, immobilization[a]	-	214.1	-		Williams et al. 1992
Whiteleg shrimp, mysis I	intoxication, immobilization[a]	-	>160	-	48-h LOEC > 160.9, 48-h NOEC =160.9	Williams et al. 1992
Whiteleg shrimp, postlarva I	intoxication, immobilization[a]	-	>160	-	48-h LOEC > 160.9, 48-h NOEC =160.9	Williams et al. 1992
Whiteleg shrimp, nauplius I	intoxication, immobilization[a]	-	-	-	24-h LOEC > 160.9, 24-h NOEC = 160.9	Williams et al. 1992
Whiteleg shrimp, protozoea I	intoxication, immobilization[a]	-	-	-	48-h LOEC = 108.9, 48-h NOEC = 54.9	Williams et al. 1992
Whiteleg shrimp, protozoea III – mysis I interface	intoxication, immobilization[a]	-	-	-	48-h LOEC = 377.8	Williams et al. 1992
Whiteleg shrimp, protozoea III – mysis I interface	intoxication, immobilization[a]	-	-	-	48-h NOEC = 102.5	Williams et al. 1992
Whiteleg shrimp, nauplius I	mortality[a]	>160	-	-	-	Williams et al. 1992
Whiteleg shrimp, protozoea I	**mortality[a]**	-	**135.7**	-	-	**Williams et al. 1992**
Whiteleg shrimp, protozoea III – mysis I interface	mortality[a]	-	238.4	-	-	Williams et al. 1992
Whiteleg shrimp, mysis I	mortality[a]	-	>160	-	-	Williams et al. 1992
Whiteleg shrimp, postlarva I	mortality[a]	-	>160	-	-	Williams et al. 1992

[a] These tests were static.

Table 14. Acute toxicity of oxytetracycline (OTC) and its degradates to activated sludge bacteria. For each compound, the Effective Concentration (EC) to inhibit growth by 50% in 48 h is given. Data from Halling-Sørensen et al. (2002).

Test compound	**48-h EC_{50} (mg/L)**
OTC	0.08
4-Epi-OTC	0.27
α-Apo-OTC	1.14
β-Apo-OTC	0.77
Terrinolide	12.3

Table 15. 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	Interspecies variability; Acute to chronic ratio; Extrapolation to field/community level effects
Fish acute study	EC_{50}	1,000	
Tier B			
Algal growth inhibition (72 h)	NOEC	10	Extrapolation from lab/single species test to field/community level effects
Daphnia magna reproduction (fresh) / crustacean chronic study (brackish)	NOEC	10	
Fish early-life stage	NOEC	10	
Sediment invertebrate toxicity	NOEC	10	

Table 16. Oxytetracycline risk characterization based on the VICH Phase II Tier A and Tier B assessment factors.

Species	Assessment Endpoint and Value (mg/L)	VICH AF	PNEC (μg/L)	RQ (EIC/PNEC)			
				Mean EIC (10.6 µg/L)	Median EIC (1.2 µg/L)	75th Percentile EIC (3.9 µg/L)	95th Percentile EIC (19.1 µg/L)
FRESH							
Microcyrtis aeroginosa	7-d EC_{50} = 0.207	100	2.1	5.05	0.57	1.86	9.10
Selenastrum capricornutum	72-h EC_{50} = 1.99	100	19.9	0.53	0.06	0.20	0.96
Daphnia magna	48-h LOEC = 100	1,000	100	0.11	0.01	0.04	0.19
Daphnia magna	21-d EC_{10} = 7.4	10	740	0.01	<0.01	<0.01	0.03
Striped bass, fingerlings	96 h LC_{50} = 178	1,000	178	0.06	<0.01	0.02	0.11
Striped bass, fingerlings & larvae	96 h LC_{50} = 75	1,000	75	0.14	0.02	0.05	0.25
Bluegill sunfish, juvenile	96-h NOEC = 94.9	1,000	94.9	0.11	0.01	0.04	0.20
Rainbow trout, juvenile	96-h NOEC = 116	1,000	116	0.09	0.01	0.03	0.16
BRACKISH							
Rhodomonas salina	72-h EC_{50}=1.6	100	16	0.66	0.08	0.24	1.19
Whiteleg shrimp, *Penaeus vannamei*	48-h LC_{50} = 136	1000	136	0.08	<0.01	0.03	0.14
Striped bass, fingerlings & larvae	96-h LC_{50} = 75	1000	75	0.14	0.02	0.05	0.25

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

Table 17. Oxytetracycline acute effects risk characterization based on refined assessment factors.

Species	Assessment Endpoint and Value (mg/L)	VICH AF	PNEC (μg/L)	RQ (EIC/PNEC)			
				Mean EIC (10.6 µg/L)	Median EIC (1.2 µg/L)	75th Percentile EIC (3.9 µg/L)	95th Percentile EIC (19.1 µg/L)
FRESH							
Daphnia magna	48-h LOEC = 100	100[a]	1,000	0.01	<0.01	<0.01	0.02
Striped bass, fingerlings	96-h LC_{50} = 178	20[b]	8,900	<0.01	<0.01	<0.01	<0.01
Striped bass, fingerlings & larvae	96-h LC_{50} = 75	20[b]	3,750	<0.01	<0.01	<0.01	<0.01
Bluegill sunfish, juvenile	The 96-h NOEC = 94.9	10[c]	9,490	<0.01	<0.01	<0.01	<0.01
Rainbow trout, juvenile	The 96-h NOEC = 116	10[c]	11,600	<0.01	<0.01	<0.01	<0.01
BRACKISH							
Whiteleg shrimp, *Penaeus vannamei*	48-h LC_{50} = 136	100[d]	1,360	<0.01	<0.01	<0.01	0.01
Striped bass, fingerlings & larvae	96-h LC_{50} = 75	20[b]	3,750	<0.01	<0.01	<0.01	<0.01

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

[a] Value of 10 for inter- and intra-species variability (because data were available for only one invertebrate species) and for extrapolation from the LOEC to the PNEC; Value of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects).

[b] Value of 2 for extrapolation of the acute LC_{50} to acute PNEC (based on OTC fish toxicity data in Hughes, 1973, and Wellborn, 1969); Value of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects).

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

[d] Value of 10 for inter- and intra-species variability (because data were available for only one invertebrate species) and for extrapolation from the acute LC_{50} to the PNEC; Value of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects).

Table 18. Oxytetracycline chronic effects risk characterization based on refined assessment factors.

Species	Assessment Endpoint and Value (mg/L)	VICH AF	PNEC (μg/L)	RQ (EIC/PNEC)			
				Mean EIC (10.6 µg/L)	Median EIC (1.2 µg/L)	75th Percentile EIC (3.9 µg/L)	95th Percentile EIC (19.1 µg/L)
FRESH							
Nitrosomonas europaea (bacteria)	5 to 6-d EC_{50} = 0.32	100 [a]	3.2	3.31	0.38	1.22	5.97
Activated sludge bacteria	48 h EC_{50} = 0.08	10[b]	8	1.3	0.15	0.49	2.39
Microcyrtis aeroginosa	7-d EC_{50} = 0.207	100 [a]	2.1	5.05	0.57	1.86	9.10
Selenastrum capricornutum	72-h EC_{50} = 1.99	100 [a]	19.9	0.53	0.06	0.20	0.96
Lemma giba (duckweed)	7-d EC_{10} = 0.87	10[b]	87	0.12	0.14	0.04	0.22
Daphnia magna	21-d EC_{10} = 7.4	10 [b]	740	0.01	<0.01	<0.01	0.03
Striped bass, fingerlings	96 h LC_{50} = 178	100[c]	1,780	<0.01	<0.01	<0.01	0.01
Striped bass, fingerlings & larvae	96 h LC_{50} = 75	100 [c]	750	0.01	<0.01	<0.01	0.03
BRACKISH							
Rhodomonas salina	72-h EC_{50}=1.6	100[a]	16	0.66	0.08	0.24	1.19
Striped bass, fingerlings & larvae	96 h LC_{50} = 75	100[c]	750	0.01	<0.01	<0.01	0.03

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

[a] Value of 10 for extrapolation of laboratory data to the field (single species effects to multiple species / community level effects); Value of 10 for extrapolation of the chronic EC_{50} to the chronic PNEC (based on OTC data in Halling-Sørensen, 2001).

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

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

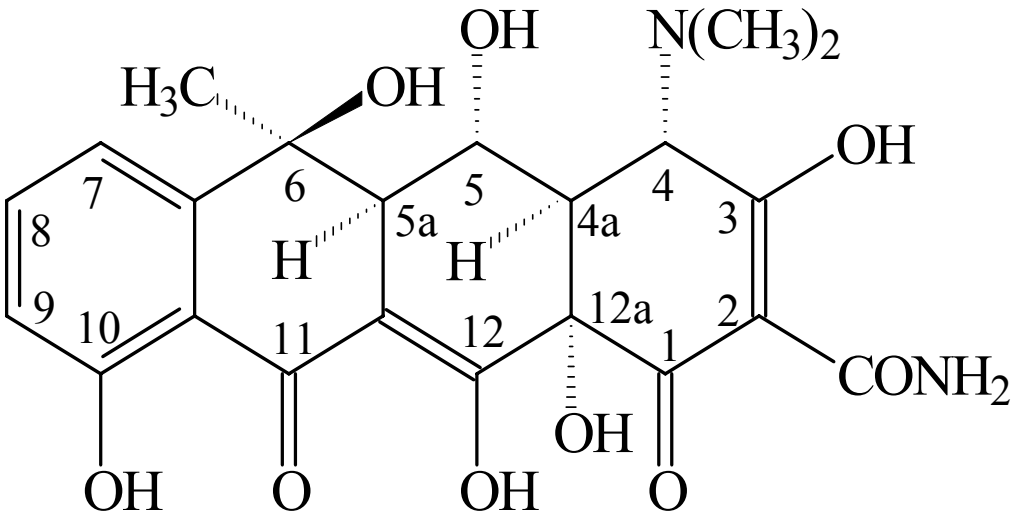

Figure 1. Chemical structure of oxytetracycline free base.

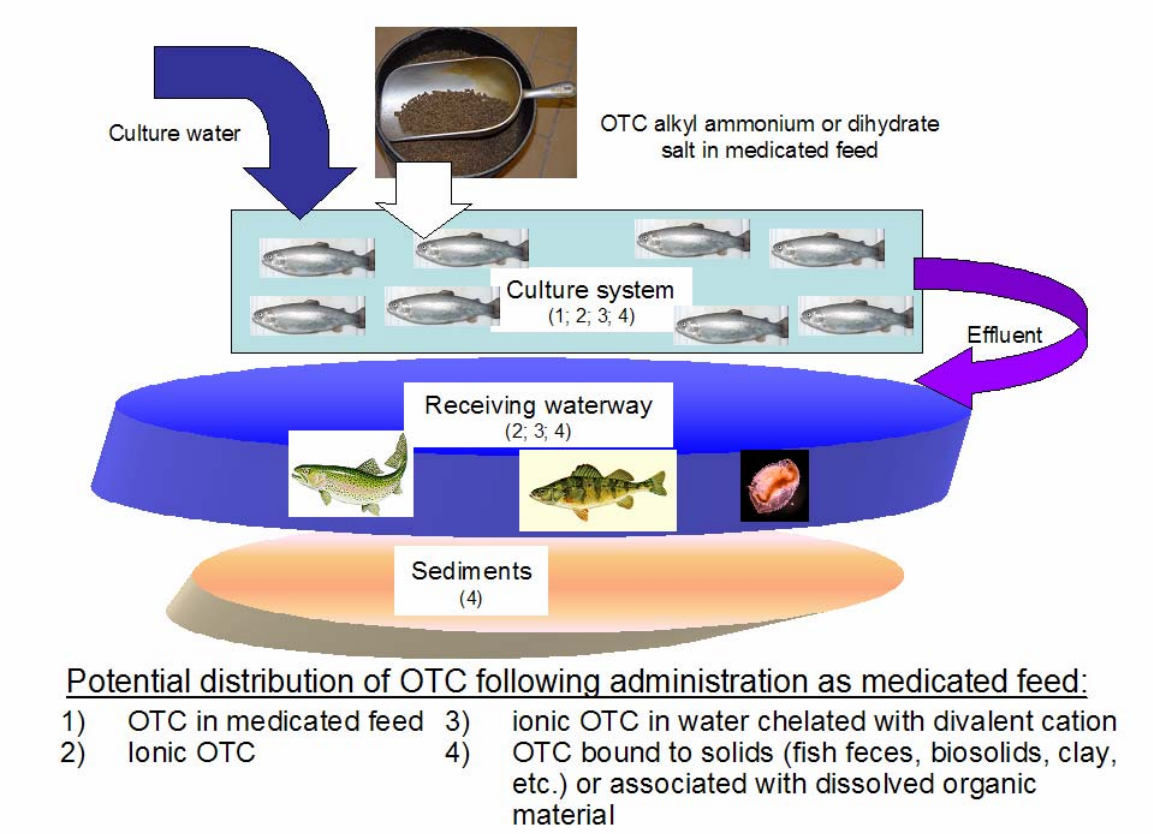

Figure 2. Conceptual model of the distribution of oxytetracycline following administration as medicated feed at an aquaculture facility.

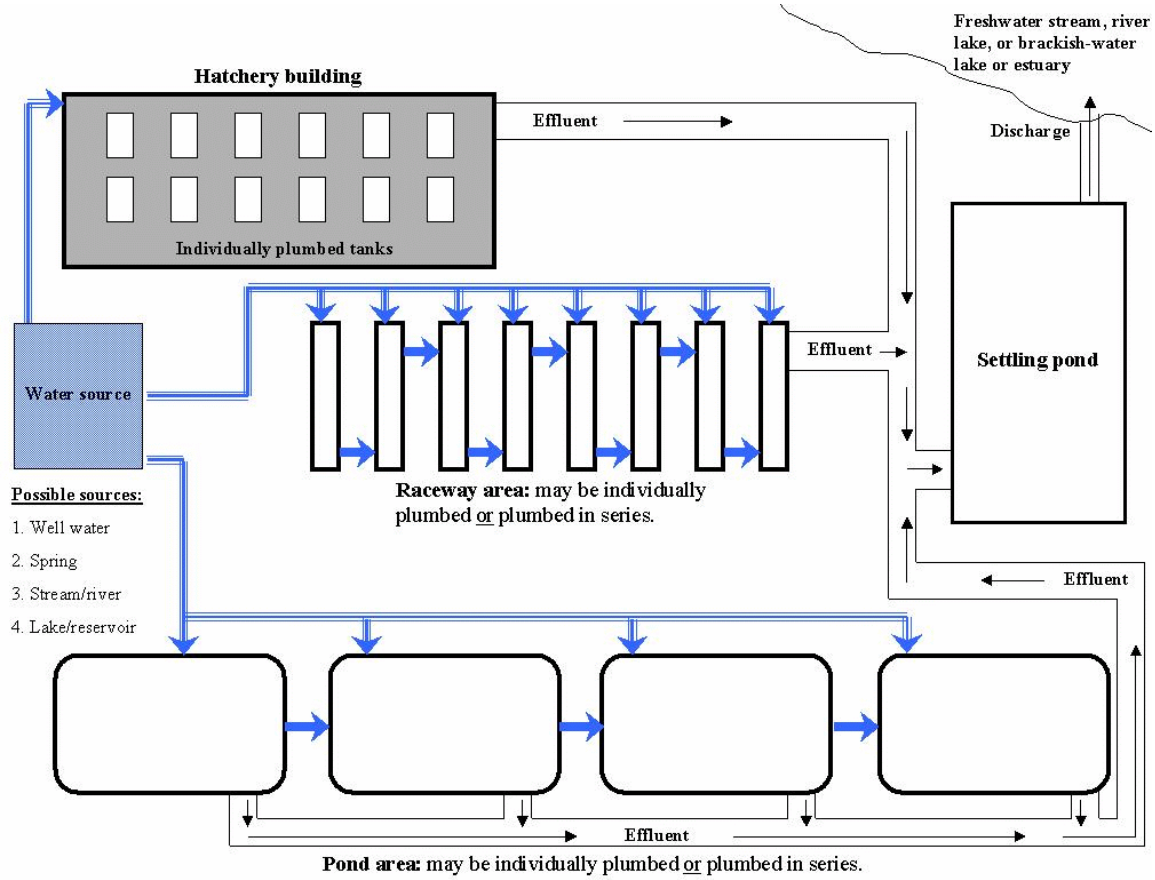

Figure 3. Conceptual diagram of a typical intensive aquaculture facility.

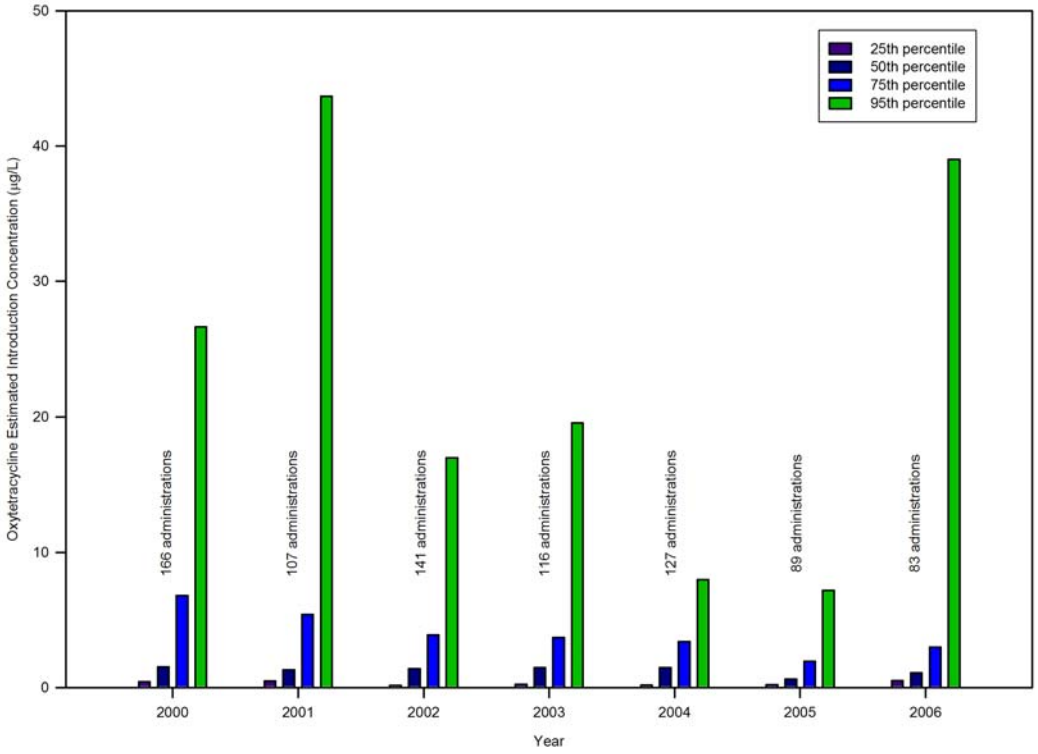

Figure 4. Annual 25th, 50th, 75th, and 95th percentile Environmental Introduction Concentration (EIC) estimates from 834 oxytetracycline-medicated feed administrations under Investigational New Animal Drug permit 9332 from 2000 through 2006. The EIC estimates include administrations to salmonid and nonsalmonid fish for a variety of diseases caused by gram-negative bacteria.

Appendix A. Data files on CD-ROM for INAD 9332, the WASP-6 model, and the hatchery survey (in electronic file only)

CD-ROM title: Electronic files for INAD 9332, WASP-6 and hatchery survey.

USGS Survey of Public and Private Aquaculture Facilities

HatchSURVEYforOTC_EA.doc – Questionaire submitted to gather facility information on water use/treatment, species cultured, and disease treatments administered.

USGS hatchery survey.otc ea.xls – extracted data from hatchery survey responses of pertinence to administration of oxytetracycline-medicated feed

INAD 9332 electronic file data

INAD 9332 2000-2007 extracted data.xls - Data describing oxytetracycline use at facilities participating in INAD 9332 during 2000-2007.

WASP-6 electronic file

WASP-6 output.xls - WASP-6 model output including application of 199 and 5,830 g/d, as well as phase distribution and sensitivity analysis output.

Appendix B. Semi-quantitative evaluation of the fate of oxytetracycline in medicated feed using the WASP-6 model

Semi-quantitative evaluation of the fate of oxytetracycline in medicated feed using the WASP-6 model.

The potential aquatic fate of OTC following administration at a fish hatchery was simulated using the Water Quality Analysis Simulation Program version 6.0 (WASP-6) software (Wool et al., no date). Rose and Pedersen (2005) describe the WASP-6 model, including input constants and variables relevant to feed-additive OTC therapies, and present select simulation results[1]. The WASP-6 simulations were run using representative hatchery and water quality data. Input data for constants in the model were derived from literature values or based on reasonable, conservative estimates. Total hatchery flow and river water flow were estimated from data in INAD 9332 and a USGS Upper Midwest Environmental Sciences Center (UMESC) survey of public and private fish hatcheries. The river flow rate was the average river flow reported in the UMESC survey (2.25 m^3/s; Rose and Pedersen [2005]). The hatchery flow rate used (0.75 m^3/s) was estimated from the ratio of hatchery flow to receiving water flow reported in the UMESC hatchery survey (about 1:3). A settling pond (about 379 m^3) was incorporated into the model hatchery simulated in WASP-6. The settling pond size met the guidelines issued by the State of Idaho (Idaho Division of Environmental Quality 1997) for the simulated hatchery flow. The WASP-6 input constants used by Rose and Pedersen (2005) are listed their Table 1.

Simulation input variables for total mass of OTC applied per day and hatchery total flow were interpolated from the data reported on OTC use in INAD 9332. A simulation with the WASP-6 model could not be done for each therapy reported in the INAD because of the time and expense involved; therefore, simulated treatments with OTC application rates of 238.8 and 6,996 g OTC/d for 10 d were used. These values represent total OTC masses of 2.9 and 85 times the median mass of OTC used (82 g/d; Table 11) for all fish treatments that reported discharge in INAD 9332. The 6,996 g/d treatment was twice the 95th percentile OTC mass applied per day (3,550 g/d; Table 11). Water flow through the simulated hatchery (0.75 m^3/s) was similar to the mean hatchery flow in INAD 9332 (Table 11). Oxytetracycline-laden fecal material may begin to be eliminated from the gut in as little as 8 h after medicated feed consumption (Smith et al. 1994), but gut transit times in fish are typically about 48 h (Table 7 summary). A gut passage time of 2 d was therefore added to the 10-d treatment, i.e., the mass of feed offered over the 10-d treatment was assumed to pass through the fish over 12 consecutive days. In effect, this reduced the daily OTC mass applied to the simulated hatchery by a factor of 10/12. Therefore, treatment at 238.8 g/d for 10 d was considered to be represented by an input of 199 g/d for 12 d. Similarly, treatment at 6,996 g/d for 10 d was modified to 5,830 g/d for 12 d. Total-dissolved concentration values for the settling pond were assumed to be the concentration of the settling pond discharge, i.e., the EIC.

Estimates for the settling pond water column OTC total and total-dissolved concentrations were made every 5 d after initiation of therapy using WASP-6, starting with day 5 (which occurred during therapy) in a 12-month period during and after the therapy. The total simulation period was 8 years and assumed one therapy per year beginning July 1, 2003. This is done as a precautionary practice to

[1] This EA was prepared assuming that total-dissolved OTC represented by the sum of freely dissolved, dissolved-chelated and dissolved-adsorbed to DOM is the only biologically available form of OTC. We therefore focused on the total-dissolved estimates developed by Rose and Pedersen (see Appendix A, WASP-6 electronic file) for a model hatchery settling pond and not on the bound OTC predicted to occur in hatchery settling ponds and downstream segments, as did Rose and Pedersen (2005).

evaluate possible influence from previous therapies, especially with regard to ambient residual concentrations.

Sensitivity data were collected for the 199 g/d therapy to estimate the impact of extreme variations in model parameters on predicted OTC concentrations. Sensitivity testing involved individually varying input constants by the extreme ranges of possibility to determine each parameter's influence on resulting estimates. Sensitivity analysis is of paramount importance when using WASP-6 and similar models because it clearly indicates those input parameters that are critical in producing large variations in results and those that are not. For a given input parameter, this variation depends on the segment in question (e.g., settling pond, receiving segment, first downstream segment), the compartment (e.g. water, sediment), the phase (e.g., total-dissolved, sorbed to settleable solids), and the OTC concentration of interest (e.g., peak, 95th percentile, median, see Rose and Pedersen [2005] for further details). The OTC concentration of interest (peak total-dissolved OTC in settling pond water) especially reacted to changes in the values of the various sensitivity parameters differently. Sensitivity analysis indicated that the most important factors regarding the fate of peak total-dissolved OTC in settling pond water were (1) OTC depuration kinetics from biosolids, (2) hatchery flow and (3) biosolids settling velocity. By contrast, biosolids load in the settling pond was not an important factor (Figure B-1). Data for OTC depuration rates from biosolids and for biosolids settling velocity under controlled conditions and by particle size ranges were not available. Rose and Pedersen (2005) discuss sensitivity results on only a single dose (199 g/d) but provide a more thorough account of the sensitivity analyses than presented here. Their sensitivity analyses predict that factors potentially affected by water temperature and pH (e.g., abiotic transformations such as hydrolysis and photolysis) are relatively unimportant in influencing predicted concentrations, although they do not include chelation in their model (nor do we in our assessment of risk), which is pH sensitive. Even so, whether chelation is an important factor in freshwater OTC fate before discharge is not known from the model exercise performed, although it probably would be important in seawater.

The WASP-6 input parameters listed in Table 1 in Rose and Pedersen (2005) were biased mostly toward coldwater aquaculture because it was initially assumed that coldwater hatcheries represented the worst-case use and discharge. Table B-1 was constructed to demonstrate that WASP-6 would predict lower total-dissolved OTC discharge estimates if input parameters for cool/warm water had been used instead. The table shows that, in most instances, either (1) the coldwater input constant would have resulted in the highest total-dissolved OTC concentration in the settling pond, or (2) there is no known systematic difference between cold and cool/warm water aquaculture for the issue/input constant involved, or (3) the WASP-6 sensitivity tests indicated that the input constant was not an important one for predicting total-dissolved OTC concentration in the settling pond. The table also indicates that using coldwater input constants, if anything, resulted in higher total-dissolved OTC concentrations in the settling ponds of cool- and warmwater hatcheries than would have been predicted if cool-/warmwater constants had instead been used for these hatcheries.

The simulation results provided estimates for the total OTC concentrations in the settling pond water column, as well as phase distribution estimates between total-dissolved OTC, OTC attached to solids, and OTC associated with DOM. The peak[2] settling pond concentrations predicted by the

[2] Absolute peak concentrations could not be obtained from the model. The “peak” concentrations are actually the year 8 day 10 concentrations estimated for the settling pond and are probably extremely close to the absolute peak concentration (Peter Rose, Department of Soil Science, University of Wisconsin-Madison, 2004, personal communication).

WASP-6 model occurred during therapy and were nearly the same for day 5 as for day 10 during each of the eight simulated annual therapies (Figure B-2; Table B-2). Settling pond concentrations dramatically decrease once therapy ceases, to the point where if another therapy began 10 d after the first ended, the peak concentrations of the second subsequent therapy would not be significantly affected[3]. The decay curve for the year 8 administration is presented in Figure B-2b.

The WASP-6 model predicts that considerable OTC will be lost to rapidly settling solids, and the remaining OTC in the settling pond water column during the treatment and peak discharge period will mostly not be in the total-dissolved form, but instead attached to suspended solids. The peak total-dissolved discharge concentration according to the WASP-6 simulation for administration of 5,830 g/d was 3.55 µg/L (Figure B-2b). The WASP-6 simulation predicted a peak total-dissolved concentration of 0.12 µg/L during administration of 199 g/d. Both simulations predict that the peak total-dissolved OTC concentration will occur during treatment, i.e., at some point during the 10 d that medicated feed is offered plus the 1-2 d that will typically be required for intestinal passage.

The WASP-6 model predicts that the total-dissolved peak concentrations in settling pond water will decline within 3 d post-treatment to about 1.7 % of the day 10 concentrations (Table B-2; Figures B-2a and b). Likewise, from Figure 2A in Rose and Pedersen (2005), OTC concentrations in settling pond sediment are predicted to rapidly decline after the treatment period, probably because of dilution and burial by settling of biosolids that are free of OTC. The present WASP-6 model suggests that peak total-dissolved OTC concentrations at discharge are not influenced by a previous treatment, probably because of the burial processes that essentially isolate previous OTC administrations in lower sediment strata. Total-dissolved OTC is predicted to have similar peak concentrations for days 2, 5, and 10 of treatment (Table B-2). Thus, the present model suggests that each day's application of OTC during the treatment period will not significantly alter the peak discharge concentrations.

When simulation results were compared to OTC concentrations predicted by simply dividing the total OTC applied to the hatchery per day by the total daily water flow, treatments of 199 g/d or 5,830 g/d yielded similar proportions (Table B-3). The simple calculation yielded predicted OTC total-dissolved peak concentrations about 25-fold higher than the WASP-6 simulation peak total-dissolved concentration because the simple calculation assumed that all administered OTC would be total-dissolved and remain dissolved in the water column. By contrast, the WASP-6 model predicts that, for the base simulation, 76% of OTC mass administered is lost to the settling pond sediment during treatment. It also predicts that the total peak OTC concentration in the settling pond water column will be 83% sorbed to settleable solids and presumably biologically unavailable, 17% total-dissolved. The total-dissolved OTC in the water column is comprised of freely-dissolved (chelated or unchelated, >95%) and DOM associated (<5%) OTC.

Although the 25-fold factor reduction from the simple hatchery calculation was based on conservative estimates for all hatchery model parameters, and thus represents a most probable total-dissolved OTC discharge concentration estimate, an attempt to derive a "worst-case" estimate was made. The most apparent way to do this is to examine extreme deviations from the most probable OTC concentration produced by the sensitivity analysis. The sensitivity analysis indicated that

---

[3] The predicted total-dissolved peak, median, and 95th percentile OTC concentrations in the settling pond are based on estimates before, during, and 50 weeks after treatment. The median and 95th percentile concentrations reported in Rose and Pedersen (2005) occur several weeks/months after treatment and are irrelevant to the estimations of peak water-soluble OTC concentrations at discharge.

substantially higher total-dissolved OTC concentrations (0.880 µg/L vs 0.119 µg/L, a 7.4-fold increase) would result from an OTC depuration rate from biosolids that is tenfold the base simulation rate (344 vs 34.4; Figure B-1). Given the apparently strong relation between the total-dissolved OTC concentration and the biosolids depuration rate (Figure B-1), we chose to estimate a worst-case total-dissolved OTC concentration based on the simulated tenfold increase in the biosolids depuration rate. The resultant worst-case reduction factor (25/7.4 or 3.4)[4] was then used to estimate the maximum likely total-dissolved OTC concentration resulting from each hatchery's OTC application described in INAD 9332. Given that the model simulations predict that the fate of more than 80% of the OTC mass administered is to be bound to settling pond sediment during therapy, with much of the remaining OTC during therapy being bound to suspended solids, the 3.4-fold worst-case reduction factor seems to be, if anything, overly conservative. These estimates are even more conservative if the OTC dihydrate is used in place of the ammonium salt because the depuration rate of the less soluble dihydrate is likely to be less than the ammonium salt (the depuration rate used in the WASP-6 model was for the ammonium salt; Fribourgh et al. 1969). Nonetheless, the 3.4-fold reduction factor was used to derive a candidate predicted environmental concentration (PEC) in Section 6.12.2 in the EA summary[5].

The consistent proportionality between predicted discharge from the simple calculation versus the model estimation means that the variability between discharge concentrations using the simple calculation should be very close to that if simulation results had been available for all OTC therapies. The relation between potential OTC discharge concentrations and total flow from hatcheries that treated cold-, cool- and warmwater fish in INAD 9332 are presented in Figure B-3. Potential discharge concentrations of >100 µg/L (based on the simple hatchery calculation) were restricted to facilities with a discharge of <0.5 m^3/s with one exception (Figure B-3). Potential total-dissolved OTC concentrations determined by dividing the simple hatchery concentration by either 25 (most probable) or 3.4 (maximum likely) provide substantially lower estimated OTC discharge concentrations (Figure B-3b and c, respectively) relative to the simple calculation.

---

[4] For this constant, OTC depuration rate from biosolids, the only constant for which higher OTC discharge estimates would result if the input constant for cool/warm water had been used, the model input constant used was based on cold water (Table 7). Higher temperatures could result in depuration rate constants 2.7-5-fold higher (P. Rose, Department of Soil Science, University of Wisconsin-Madison, 2004, personal communication), and this in turn could lead to total-dissolved OTC concentration estimate at discharge up to 3.7-fold higher, according to WASP-6 sensitivity data. This exception and the fact that the model is not calibrated justify our use of a 7.4 increase of the 25-fold reduction factor (to 3.4-fold) to account for maximum likely OTC use and discharge scenarios for all freshwater culture.

[5] The WASP-6 model was not validated at a hatchery to determine the departure of the model from actual measured OTC concentrations. Validation of the model is the next logical step to refine the model parameters and parameter estimates to ensure the best fit between the model and reality. Because the WASP-6 model has not been validated, we chose WASP-6 estimates that represent very conservative predictions of total-dissolved OTC concentrations at discharge.

Table B-1. Comparison of sensitivity results for cold- vs cool-warmwater total-dissolved oxytetracycline (OTC) settling pond concentrations from the input constants and other factors used for the WASP-6 model. Bold text in table denotes the hatchery model that produces the highest OTC discharge concentration**.** Highest OTC estimates were predicted for the coldwater model for all but two factors, OTC depuration rate from biosolids and hatchery flow rate.

Factor	Actual assumption used in WASP-6 model	Coldwater model (<15 °C)	Cool- warmwater model (>15 °C)	WASP-6 sensitivity testing result (Does the factor significantly affect est. of OTC conc.?)	Worst-case aquaculture model (higher expected OTC estimate)
Abiotic transformation reactions (photolysis, hydrolysis)	CW[a]	**highest estimated OTC concentration** (slower reaction)	lower estimated OTC concentration	not significant	not significant
Settling pond presence	CW	equivalent[b]	equivalent	not performed	equivalent
Biotic degradation	none known	-	-	not performed	-
Dissolved organic material (DOM)	CW	**highest estimated OTC concentration** (less DOM)	lower estimated OTC concentration	not performed for settling pond peak OTC concentrations	CW
Biosolids load	CWW[c]	**highest estimated OTC concentration** (lower load)	lower estimated OTC concentration	not significant	not significant
Other hatchery solids load (eutrophicity, surface water intake solids)	not used	**highest estimated OTC concentration** (lower load)	lower estimated OTC concentration	not done	CW
Biosolids settling velocity	CW	**highest estimated OTC concentration** (higher velocity)	lower estimated OTC concentration	yes, biosolids settling velocity is significant	CW
OTC depuration rate from biosolids	CW (13 °C)	lower estimated OTC concentration	**highest estimated OTC concentration** (higher rate)	yes, OTC depuration rate is significant	CWW
$K_{d, bio}$	CWW (20 °C)	equivalent	equivalent	not significant	equivalent
Hatchery flow rate	overall mean from survey	lower estimated OTC concentration	**highest estimated OTC concentration** (lower flow)	yes, flow rate is significant, but only if hatcheries are of similar design[d]	CWW
Ponds vs tanks vs raceways (different biosolids loads and biosolids fates)	CW (raceway)	**highest estimated OTC concentration**[d]	lower estimated OTC concentration[e]	yes, ponds vs tanks vs raceways is significant	CW

[a] Coldwater aquaculture
[b] No systematic difference between cold and cool/warmwater aquaculture
[c] Cool/warmwater aquaculture
[d] Mostly raceways, least ponds
[e] Most ponds, least raceways

Table B-2. Total-dissolved peak oxytetracycline (OTC) concentrations in settling pond water predicted by the WASP-6 simulation for the eighth annual application of 199 g and 5,830 g OTC/day for 12 days. Day 10 was used in both applications to represent the peak settling pond concentration.

Day after start of treatment	**OTC *total-dissolved* peak concentration in the settling pond, µg/L, 199 g/day administration**	**OTC *total-dissolved* peak concentration in the settling pond, µg/L, 5,830 g/day administration**
-1	0	0
2	estimated at 0.112 from data from first annual administration (0.93)[a]	estimated at 3.29 from data from first annual administration (0.94)
5	0.118404 (0.99)	3.469731(0.99)
10	0.119939 (1.00)	3.514718 (1.00)
15	0.002032 (0.017)	0.059535 (0.017)
20	0.001507 (0.013)	0.044148 (0.013)
25	0.001072 (0.0089)	0.03139 (0.0089)
30	0.000773 (0.0064)	0.022628 (0.0064)
35	0.000567 (0.0047)	0.016603 (0.0047)
40	0.000412 (0.0034)	0.012072 (0.0034)
45	0.000295 (0.0025)	0.00864 (0.0025)
50	0.000215 (0.0018)	0.00628 (0.0018)

[a] The numbers in parentheses are ratios of the stated concentration to the concentrations at day 10 after start of treatment.

Table B-3. Maximum oxytetracycline (OTC) concentration in settling pond water predicted by WASP-6 simulation of eight annual applications of 199 g or 5,830 g OTC/d for 12 d. The ratio of a simple estimate of total-dissolved OTC to a WASP-6 estimate of total-dissolved OTC was used to predict total-dissolved OTC concentrations resulting from the therapies listed in Investigational New Animal Drug permit 9332 if a dose of 82.5 mg/kg BW was administered.

Mass of OTC applied (g/day)	Total OTC (µg/L)[a]	WASP-6 estimate of Total-dissolved OTC (µg/L)[a]	Simple estimate of Total-dissolved OTC (µg/L)[b]	Ratio of simple estimate to Wasp-6 estimate
199	0.72	0.12	3.07	25.6
5,830	21.05	3.55	89.97	25.3

[a] Data from the WASP-6 simulation (Rose and Pedersen 2005).

[b] The simple OTC concentration estimate was determined by dividing the OTC applied/d by total hatchery flow (e.g., 199 g/d / 64, 800,000 L/d = 3.07 µg/L).

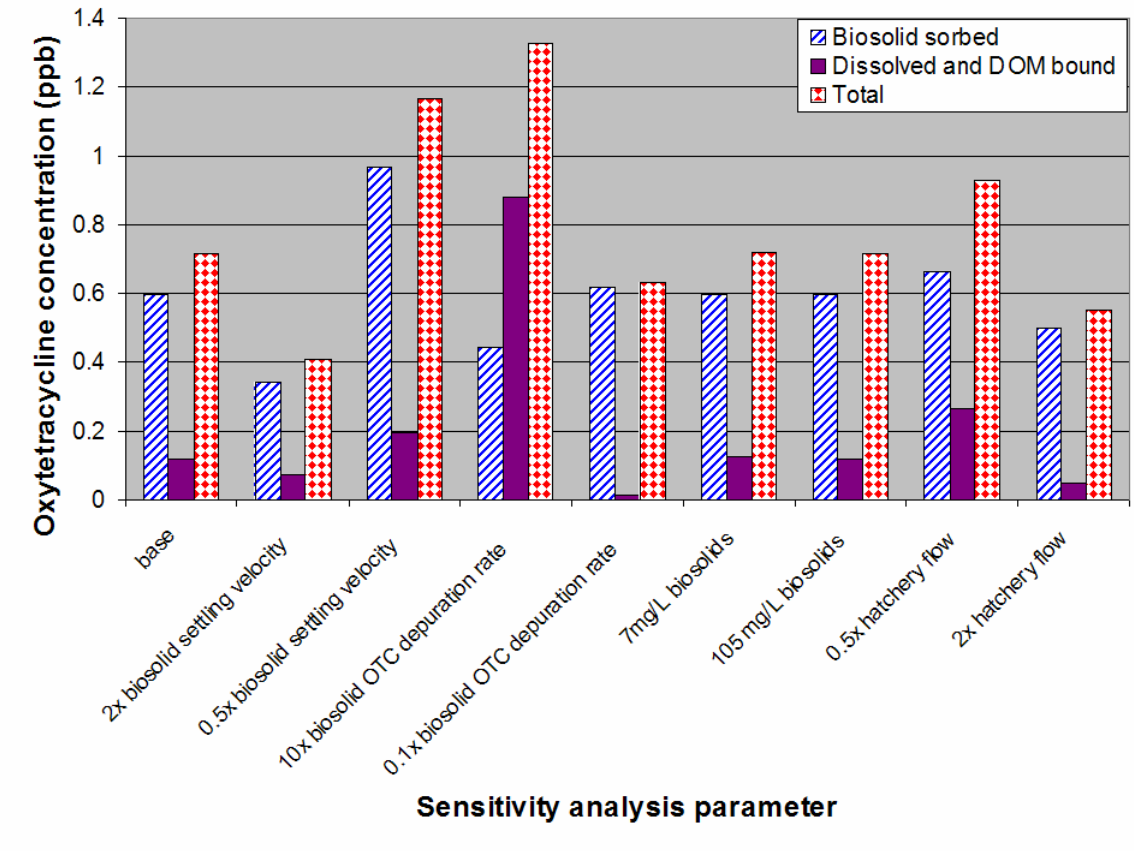

Figure B-1. Summary of the sensitivity analysis performed with Water Quality Analysis Simulation Program version 6.0 (WASP-6) to simulate oxytetracycline (OTC) concentration in the settling pond water column. Conservative estimates of 16 different input constants were used in the WASP-6 base model (Table 1 of Rose and Pedersen 2005) to simulate OTC concentration in the settling pond water column. The nine input parameters expected to produce the greatest variance in simulated OTC concentration for the settling pond were varied in the sensitivity analyses (Rose and Pedersen 2005). The sensitivity analyses were completed by individually varying the selected nine input parameters at the extreme range of possibility to determine each parameter's influence on the resulting OTC estimates. The sensitivity analyses were performed using the WASP-6 model for a simulated treatment at 199 g OTC/d for 12 d (at day 10 of the eighth annual treatment). The above figure shows variation for the three most sensitive parameters and a relatively insensitive one (7-105 mg/L biosolids).

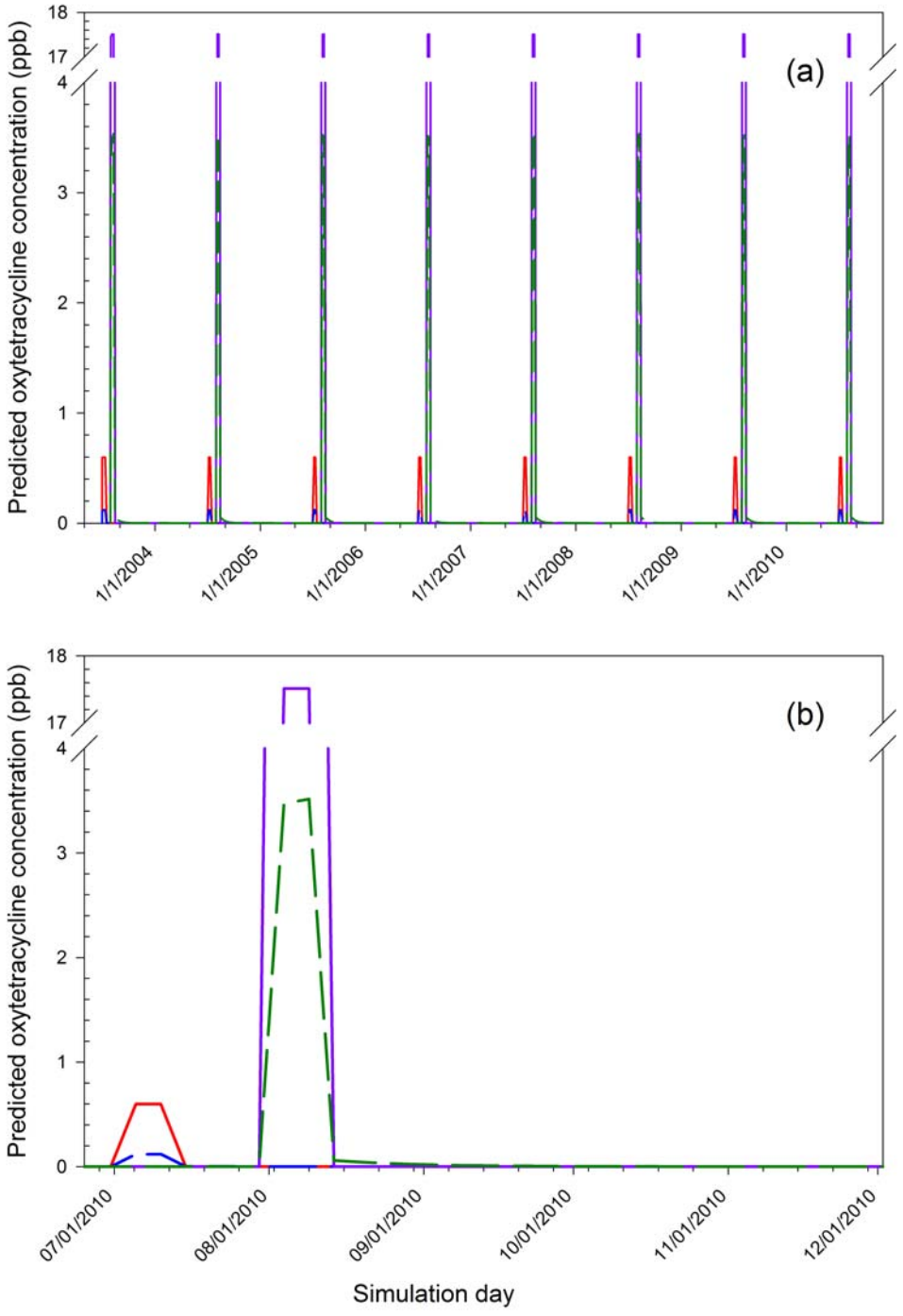

Figure B-2. Predicted oxytetracycline concentration in the settling pond following WASP-6 simulated applications of annual therapies of 199 (bound [red line]; total-dissolved [dashed blue line]) or 5,830 g/d (bound [purple line]; total-dissolved [dashed green line]) for 12 d. The 5,830 g/d application data are off-set by 30 d from the actual simulation day to improve readability. (a) presents the eight annual applications, whereas (b) extracts the eighth application data from (a).

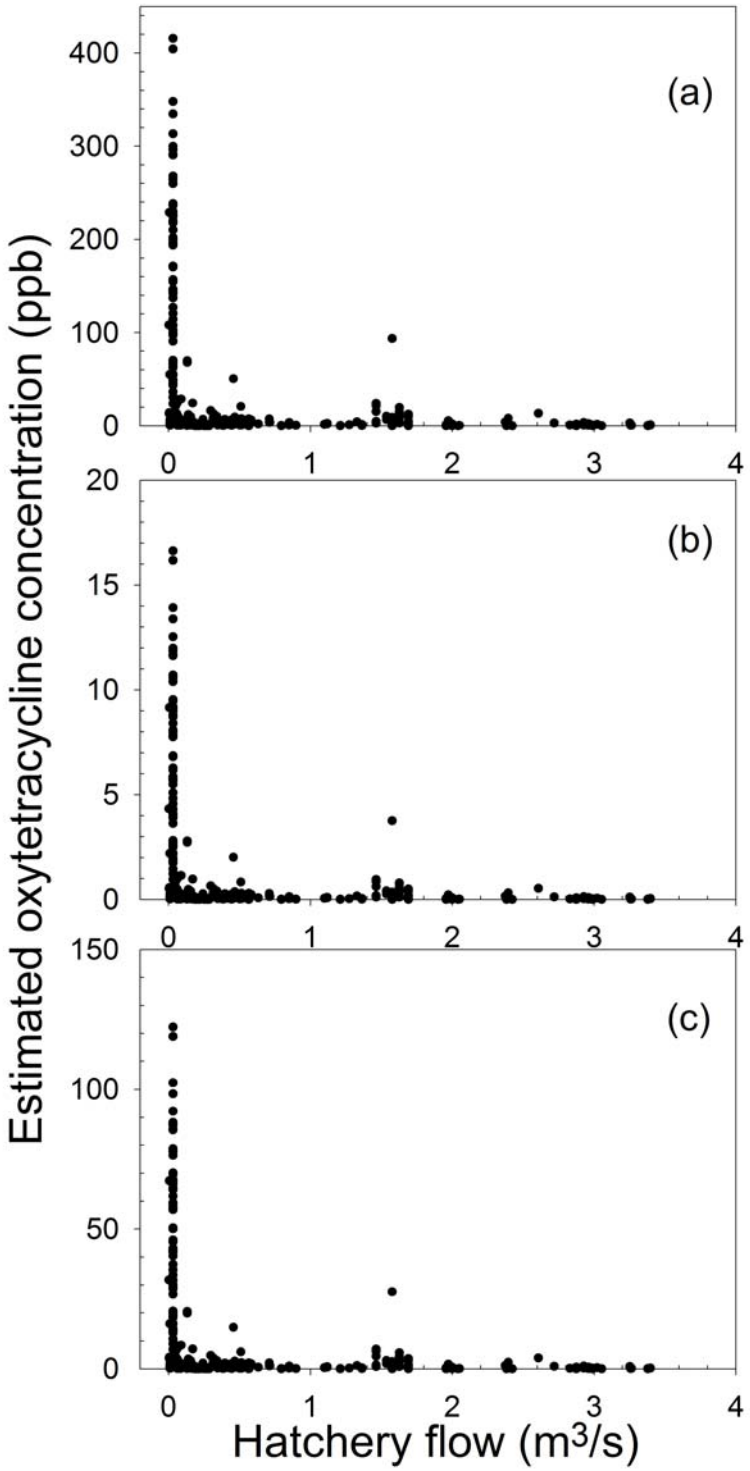

Figure B-3. Estimated oxytetracycline (OTC) total-dissolved discharge concentration as a function of hatchery flow.

Appendix C. Summaries of key toxicity studies used for the total-dissolved oxytetracycline risk assessment

---

Bellantoni, D. C., C. M. Holmes, and G. T. Peters. 1991. Oxytetracycline hydrochloride: A 48-hour static acute toxicity test with the cladoceran (*Daphnia magna*). Laboratory Study No. 260A-101. Prepared by Wildlife International, Ltd., Easton, Maryland. Submitted by Pfizer, Inc., New York. EPA MRID 417832-03. Data Evaluation Record (DER) provided by EPA under EFED Document Number E9001100.

This summary information was obtained from EPA's DER and not from the original study. Bellantoni et al. (1991) studied the acute toxicity of OTC to *Daphnia magna*. The study was conducted in accordance with FIFRA Good Laboratory Practices set forth in 40 CFR Part 160. The daphnids were obtained from in-house cultures. The daphnids in the cultures were in good health and showed no signs of disease or stress. Neonates were obtained for testing by transferring individual adult daphnids to dilution waters prior to test initiation. First instar larvae (< 24 h old) from at least 3 different adults were chosen for the test. The test chambers were 250-mL glass beakers containing 150 mL of the test solution. The test chambers were maintained in a water bath at 20 ± 1 °C. Lighting was a 16-h daylight photoperiod (25-50 foot candles) with 30-min dawn and dusk simulations. Aerated and filtered medium-hard well water was used as dilution water. Water quality characteristics (conductivity, hardness, alkalinity, and pH) were measured for a 4-week period immediately before the study. Five nominal concentrations 13, 22, 36, 60, and 100 mg active ingredient (ai)/L and a dilution water control were used.

Daphnids were impartially distributed to each test beaker by twos for a total of 10 individuals per concentration (5 replicates). Observations of mortality and immobility were made every 24 h. The daphnids were not fed during the tests. Dissolved oxygen and pH were measured in all concentrations and the control at the beginning and end of the test. Dissolved oxygen ranged from 7.0 to 9.0 mg/L. The pH values ranged from 7.5 to 8.4. The temperature was 19.8 - 21.4 °C throughout the test. Water used in the study had a hardness of 132 mg/L as $CaCO_3$. The temperature of the dilution water control was monitored continuously and each test chamber measured at the beginning and end of the test. Samples were taken to verify the concentration of test material in the water, using microbial zone inhibition. No statistical analysis was conducted by the authors.

The measured OTC concentrations were 12.1, 19.7, 35.6, 60.1, and 102 mg (ai)/L. The 48-h EC_{50} was >102 mg ai/L. The NOEC was 60.1 mg ai/L. This study provided strong supportive data for the 48-h LOEC (100 mg/L) by Wollenberger et al. (2000).

EPA reviewer's comments: "Although an inadequate number of organisms was used at each test level, this study is acceptable in demonstrating low toxicity to *Daphnia magna.* The NOEC was determined to be 60.1 mg ai/L, based on signs of toxicity, mortality, and immobilization at the 102 mg ai/L test level."

Halling-Sørensen, B. 2001. Inhibition of aerobic growth and nitrification of bacteria in sewage sludge by antibacterial agents. Archives of Environmental Contamination and Toxicology 40:451–460.

Halling-Sørensen (2001) investigated the toxicity of OTC and other antibacterial agents on environmentally relevant bacteria using activated sludge. The growth inhibitory effect of antibacterial agents on sewage microorganisms was performed in accordance with the ISO 15522 (ISO 1999) guideline. All tests were made in accordance with the test protocol. A test consisted of the following samples: 6 control replicates, 6 concentration levels of OTC in duplicate, and 5 concentration levels of the positive control (3,5-dichloropheniol) in duplicate. The test was ended when exponential growth ceased in the controls (typically 4-6 h).

Tests were performed with an activated sludge from the primary aeration tank at a pilot-scale activated sludge sewage treatment plant receiving municipal wastewater (Institute of Environmental Science and Technology, Lyungby, Denmark). Precondition (aeration) of the sludge began within 1 h of collection and took 20 h at room temperature. The bacterial biomass was quantified as turbidity in both control and test vessels spectrophotometrically at OD_{350}nm. Results were reported as the effect concentration to 50% inhibition. If no effects were observed, a no-effect concentration level (NOEC) was reported as the maximum test concentration in the test design. Initial pH of all test solutions was between 8.1 and 8.3. Concentrations of the antibacterial agent were not not determined by analytical chemical methods. The respective nominal concentrations were used as exposure concentrations in the calculation of EC_{50} values. The 10-d EC_{50} for OTC for inhibition of activated sludge bacteria was 1.2 mg/L.

In addition, a viable plate counting (pour plate method) of active sludge bacteria was conducted. A 10-mL final test solution was transferred to a Petri plate and incubated at 21 ± 1 °C for 48 h in a temperate box. Control plates were produced identically, only instead of antimicrobial solution additional buffer solution was added. Identical number of controls, concentration levels of OTC and reference compound were used as in the growth inhibition test. In addition, a blank reference testing the bacterial contamination of water was applied. After 48 h all plates were counted and the inhibitions were calculated as EC_{50} values. The 48 h EC_{50} was 0.14 mg/L for OTC for the pour plate method for the inhibition of the activated slude bacteria.

A pure culture of nitrifying bacteria (*Nitrosomonas europaea)* was obtained from the Department of Ecology, Section of Genetics and Microbiology, Royal University and Agricultural University, Copenhagen, Denmark. The effect of antibacterial agents on the nitrification rate of *N. europaea* was assessed using the ISO 9509 (ISO 1989) guideline. *N. europaea* was inoculated in the growth media and shaken during the entire test period. The test period was expanded to 10 days because of the slower growth rate compared to the heterotrophs in the sludge. Exponential growth was obtained in the controls during the entire test period. At the end of the 10 days, the ammonium and nitrite content was measured spectrophotometrically in accordance with ISO 7150 (ISO 1984a) and ISO 6777 (ISO 1984b), respectively. The inhibition of nitrite production in the test vessels compared to the controls was used as a measure for calculation. Initial pH of all test solutions was between 8.1 and 8.3. Concentrations of the antibacterial agent were not not determined by analytical chemical methods. The respective nominal concentrations were used as exposure concentrations in the calculation of EC_{50} values. The 10-d EC_{50} for the inhibition of nitrifying bacteria was 1.7 mg/L.

A viable plate counting (pour plate method) of *N. Europa*ea was also conducted. The same procedure and quantification as for the sludge bacteria was used. The pure culture (between 20 and 200 bacteria) of *N. Europa*ea was inoculated in the plates and viable colonies were calculated after 5 or 6 days. The 5 to 6-d EC_{50} was 0.32 mg/L OTC for the pour plate method for growth inhinition with *N. Europa*.

This study on *Nitrosomonas europaea* produced a key data point for our risk assessment.

ISO. 1999 (15522). Determination of the inhibitory effect of water constituents on the growth of activated sludge micro-organisms. ISO, Switzerland.

ISO. 1989 (9509). Method for assessing the inhibition of nitrification of activated sludge micro-organisms by chemicals and waste waters. ISO, Switzerland.

ISO. 1984a (7150/1). Water quality – determination of ammonium – Part 1: Manual spectrophotometric method. ISO, Switzerland.

ISO. 1984b (6777). Water quality – determination of nitrit – molecular absorption spectrophotometric method. ISO, Switzerland.

Halling-Sørensen, B., G. Sengeløv, and J. Tjørnelund. 2002. Toxicity of tetracyclines and tetracycline degradation products to environmentally relevant bacteria, including selected tetracycline-resistant bacteria. Archives of Environmental Contamination and Toxicology 42:263–271.

Halling-Sørensen et al. (2002) investigated the toxicity of tetracyclines to activated sludge bacteria. Tests were performed with an activated sludge from the primary aeration tank at a pilot-scale activated sludge sewage treatment plant receiving municipal wastewater (Institute of Environmental Science and Technology, Lyungby, Denmark). Preconditioning (aeration) of the sludge began within 1 h of collection and took 20-24 h at 20 °C.

Viable plate counting (pour plate method) of aerobic sludge bacteria was used in all the growth inhibition toxicity tests and applied exactly as described in detail in Halling-Sørensen (2001, see study summary below) and ISO 15522 (1999). Because the bacteria were immobilized in the agar, only well-defined, easily countable colonies were detected. After 48 h all plates were counted and the inhibition (I) (%) was calculated as I = ((D-E) /D) x 100, where D = mean number of counted colonies on the nonexposed agar plates at the end of the incubation period (48 h) and E = mean number of counted colonies on the antimicrobial-treated agar plates at the end of the incubation period (48 h). From these data EC_{50} (mg/L) values were calculated.

A 48-h EC_{50} of 0.08 mg/L was reported for OTC. This study provided a key data point used in our risk assessment.

ISO. 1999 (15522). Determination of the inhibitory effect of water constituents on the growth of activated sludge micro-organisms. ISO, Switzerland.

Holten Lützhøft, H. C., B. Halling-Sørensen, and S. E. Jørgensen. 1999. Algal toxicity of antibacterial agents applied in Danish fish farming. Archives of Environmental Contamination and Toxicology 36:1–6.

Holten Lützhøft et al. (1999) investigated the toxicity of OTC and other antibacterial agents to algae. The growth-inhibiting effect of OTC on *Microcystis aeruginosa* (freshwater cyanobacteria), *Selenastrum capricornutum* (freshwater algae) and *Rhodomonas salina* (saltwater algae) was performed in accordance with the ISO 8692 (ISO 1989) guideline. Nonaxenic unicultures of the test organisms were obtained from the Scandinavian Culture Centre for Algae and Protazoa, U. of Copenhagen (*M. aeruginosa*), Laboratory of Marine Biology, Helsingør, Denmark (*R. salina*), and Norwegian Institute of Water Research Culture collection, Oslo (*S. capricornutum*). To prevent contamination, the two freshwater algae were only cultured one at a time. Occurrence of contamination of other algal species was controlled monthly with the aid an Olympus BHM microscope.

The duration of the tests with *M. aeruginosa* was set from the recommended 3 days in ISO 1989 to 7 days in order to obtain at least a 16 doubling in cell numbers, which is prescribed in the ISO protocol. All solutions were prepared from water purified with a Millipore system. OTC was tested at 3 concentration levels without replicates, but the controls were grown in triplicates. The number of tests were ≥ 2. No analytical confirmation of OTC concentrations was done. The initial pH was 7.9 ± 0.2, 7.3 ± 0.2 and 7.9 ± 0.4 for *M. aeruginosa, R. salina* and *S.capricornutum*, respectively. The pH was measured throughout the test and did not increase beyond 1.5 pH unit. Tests were performed at 21 ± 1, 21 ± 1, and 23 ± 1 °C for *M. aeruginosa, R. salina* and *S.capricornutum*, respectively. No other water quality parameters were measured. The light intensity in the tests with *M. aeruginosa*, R. salina, and *S.capricornutum* were 3.1 ± 0.2, 3.3 ± 0.3, and 6.8 ± 0.4 Klux, respectively. The results of the algal toxicity tests were quantified in terms of growth rates calculated from

pooled measurements of chlorophyll content in 3 subsequent tests. Algal chlorophyll was quantified with a luminescence spectrometer using a modified version of the method described by Mayer et. al (1997).

The 7-d EC_{50} for ***M. aeruginosa*** for OTC was 0.207 mg/L (95% confidence interval 0.175-0.246 mg/L). The 72-h EC_{50} for ***S.capricornutum*** for OTC was 4.5 mg/L. This study on ***M. aeroginosa*** produced a key data point for our risk assessment.

Mayer, P., R. Cuhel, and N. Nyholm. 1997. A simple *in vitro* fluorescence method for biomass measurement in algal growth inhibition tests. Water Research 31: 2525-2531.

Hughes, J. S. 1973. Acute toxicity of thirty chemicals to striped bass (*Morone saxatilis*). Presented at the Western Association of State Game and Fish Commissioners, Salt Lake City, Utah, July 1973. 15 pp.

Hughes (1973) studied the acute effects of OTC and 29 other chemicals to larvae and fingerling striped bass. The striped bass were obtained either as larvae from the South Carolina Wildlife Resources Department or from eggs taken in Louisiana. Bioassay techniques used were modifications of those described by Doudoroff et al. (1951). Reconstituted water (Hughes 1971) maintained at 70 °F (21.1 ºC) was used for dilution. One liter of water was used for larva bioassays and 2 liters were used for fingerling. Ten larvae were used per test; 2 fingerlings were used per test for the bioassays of one month old fish. The fingerlings were obtained from a hatchery pond. The fingerlings, with some water, were dipped from the net with a 1 gallon plastic bucket and poured into a 3 gallon plastic tub. The fish were tempered in the tub for 30 min and then removed by hand and placed in the bioassay containers. Fingerlings were acclimated for 2-4 h before the test chemical was added. At least one control for every 10 bioassay containers was used. Replicate tests were conducted for each concentration. A range (unspecified) of concentrations was used. No confirmation of dose was mentioned. The live and dead fish were checked and the numbers were recorded at the end of each 24-h period for 96 h. Toxicity results are reported as LC_0, LC_{50}, and LC_{100}. Measurement of water quality parameters other than temperature was not mentioned.

The 96-h LC_{50} for OTC for striped bass fingerlings was 75 mg/L. This study on striped bass produced a key data point for our risk assessment.

Doudoroff, P., B.G. Anderson, G.E. Burdick, P.S. Galtsoff, W.B. Hart, R. Patrick, E.R. Stron, E.W. Surber, and W.M. Van Horn. 1951. Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish. Sewage and Industrial Wastes 23: 1380-1397.

Hughes, J. S.. 1971. Tolerance of striped bass, *Morone saxatilis* (Walbaum), larvae and fingerlings to nine chemicals used in pond culture. Proceedings of the 24th annual conference Southeastern Association of Game and Fish Commissioners (1970). pp. 431-438.

Murphy, D., and G. T. Peters. 1991a. Oxytetracycline hydrochloride: A 96-hour static acute toxicity test with the bluegill (*Lepomis macrochirus*). Project No. 260A-102. Performed by Wildlife International, Ltd., Easton, Maryland. Submitted by Pfizer, Inc., New York, EPA MRID 417832-01. Data Evaluation Record (DER) provided by EPA under EFED Document Number E9001098.

This summary information was obtained from EPA's DER and not from the original study. Murphy and Peters (1991a) performed a 96-h static acute toxicity test with juvenile bluegill sunfish. The study was conducted in accordance with FIFRA Good Laboratory Practices set forth in 40 CFR Part 160. All fish used during the test were from the same source and year class. Fish were acclimated to test conditions for approximately 9 days prior to test initiation. Fish were not fed for 48-h prior to the test or during the test. Nominal OTC

concentrations were 11.8, 19.6, 32.7, 54.5, and 90.9 mg/L active ingredient (ai)/L. A control using test dilution water was also included in the test. Bluegill were impartially distributed in groups of 2, to each test chamber for a total of 10 per concentration. Recordings of mortality and sublethal effects were made every 24 h after the test initiation. Dissolved oxygen concentration and pH were measured every 24 h in each concentration and the control. Temperature was measured at test initiation and conclusion in each concentration and the control. Temperature was also measured continuously in the dilution water control. The dissolved oxygen concentration ranged from 6.3 to 8.6 mg/L. The pH ranged from 6.2 to 7.2. The continuous temperature of the dilution water control ranged from 21.4 to 22.4 °C and the temperature measured at the beginning and end of the test ranged from 21.4 to 22.8 °C. The dilution water had a hardness of 40 mg/L as $CaCO_3$ and an alkalinity of 40 mg/L as $CaCO_3$. The concentration of OTC hydrochloride present in each chamber was determined at test initiation and test termination by using the microbial zone inhibition method (AOAC 1990).

Mean measured concentrations were 10.9, 19.5, 33.7, 44.1, and 94.9 mg ai/L. There was no mortality or sublethal responses at any of the tested concentrations. The 24-, 48-, 72-, and 96-h LC_{50} values obtained for OTC hydrochloride were >94.9 mg ai/L. The 96-h NOEC was 94.9 mg ai/L. This study on juvenile bluegill sunfish produced a key data point for our risk assessment.

EPA reviewer's comments: "This study is scientifically sound and meets the guideline requirements[6] for a static acute freshwater fish toxicity study. In this test, the dosage levels tested were less than 100 mg/L but not high enough to produce a precise LC_{50} or NOEC. In order to determine that a chemical will have an LC_{50} greater than 100 mg/L, the test should have been conducted by exposing 30 individuals to a concentration of 100 mg/L or greater. Since no mortality or sublethal effects occurred at any test concentration (up to 94.9 mg ai/L mean measured concentration), the LC_{50} was probably >100 mg/L."

Exact citation for AOAC (1990) not given in the Murphy and Peters (1991a) article.

Murphy, D., and G. T. Peters. 1991b. Oxytetracycline hydrochloride: A 96-hour static acute toxicity test with the rainbow trout (*Oncorhynchus mykiss*). Laboratory Study No. 260A-103. Prepared by Wildlife International, Ltd., Easton, Maryland. Submitted by Pfizer, Inc., New York, EPA MRID 417832-02. Data Evaluation Record (DER) provided by EPA under EFED Document Number E9001099.

This summary information was obtained from EPA's DER and not from the original study. Murphy and Peters (1991b) performed a 96-h static acute toxicity test with juvenile rainbow trout. The study was conducted in accordance with FIFRA Good Laboratory Practices set forth in 40 CFR Part 160. Fish were acclimated to test conditions for 48 hours prior to test initiation. Fish were not fed for 48-h prior to the test (or during the test). Nominal OTC concentrations were 14.1, 23.6, 39.3, 65.5, and 109 mg active ingredient (ai)/L. A contol using test dilution water was also included in the test. The rainbow trout were impartially distributed in groups of 2, to each test chamber for a total of 10 per concentration. Recordings of mortality and sublethal effects were made every 24 h after the test initiation. Dissolved oxygen concentration and pH were measured every 24 h in each concentration and the control. Temperature was measured at test initiation and conclusion in each concentration and continuously in the control. The concentration of OTC hydrochloride present in each chamber was determined at test initiation and test termination by using the microbial zone inhibition method (AOAC 1990).

[6]Acute Toxicity Test for Freshwater Fish, OPP Guideline 72-1, Office of Pesticides Programs, EPA 540/09-82-024.

The hardness and alkalinity of the test dilution water were 40 and 37.5 mg/L as $CaCO_3$, respectively. Disssolved oxygen ranged from 8.4 to 9.8 mg/L. The pH ranged from 6.2 to 7.5. The temperature was 11.5 - 12.5 °C. throughout the test. The laboratory environment was maintained on a 16-h daylight photoperiod with 30-min dawn and dusk simulations.

Mean measured concentrations were 15.8, 24.1, 39.7, 69.4, and 116 mg ai/L. There was no mortality or sublethal responses at any of the tested concentrations. The 96-h LC_{50} value based on measured concentration was >116 mg ai/L. The 96-h NOEC was 116 mg ai/L.

EPA reviewer's comments: "This study is scientifically sound and meets the guideline requirements6 for a static acute freshwater fish toxicity study."

Rojíčková, R., D. Dvořáková, and B. Maršálek. 1998. The use of miniaturized algal bioassays in comparison to the standard flask assay. Environmental Toxicology and Water Quality 13:235–241.

Rojíčková et al. (1998) conducted an 72-h toxicity study of OTC and other chemicals to the green algae *Raphidocelis subc*apitata (*Selenastrum capricornutum*), using cell multiplication/growth inhibition as an endpoint and 3 different bioassay techniques: flask (standard), tube, and a plate algal assay. An algal culture that was 3 days old was used for the inoculation of tested concentrations and controls. The tested substance, ISO medium, and algal inoculum were mixed to obtain initial algal concentration of 120,000 cells/mL that could be detected by all types of spectrophotometers. The suspensions with algae were incubated at 27 ± 1 °C under cool continuous white luminescent light (6000 lux) for 72 h. The control pH was checked after 72 h to ensure that it did not vary by more than 1.5 units. No other water quality parameters were mentioned. The final algal biomass was inspected to confirm that it had increased by at least a factor of 16 in 72 h.

For the flask bioassay, sterile 100 mL glass flasks were used and there were 3 replicates for each concentration or control tested. Twenty milliliters of test solution were used. Flasks were closed with a foil permeable to CO2. The suspensions were shaken manually 3 times per day. Density of algal culture was measured at 680 nm in 5 cm glass cuvettes after 72 h.

For the tube assay, 15 mL polystyrene test tubes permeable to CO_2 were sterilized and filled with 2.5 mL of test solution. Three replicates were used for each test concentration or control. The definitive dilution series of tested substances were prepared according to results of range–finding tests. Verification of dose was not mentioned. Three replicates were used for each concentration or control tested. The tubes were closed with plastic caps and were continuously shaken on a tilting plate. Samples of the 72-h test were measured at 690 nm on a spectrophotometer for test tubes.

For the plate assay, 96-well polystyrene FB microplates with a well volume of 0.3 mL were sterilized and the peripheral wells were filled with 0.25 mL of distilled water. Ten control wells were located in the 4^{th} row and 5 replicates per test solution were located from the lowest to the highest concentration in the columns (2-11, respectively, no confirmation of dose was mentioned). The bioassay volume was 0.25 mL. The well content was resuspended with a multichannel pipette several times before measurement. The samples were read at 680 nm on a spectrophotometer for microplates.

The percent growth inhibition in relation to the controls was calculated from the 72-h optical density values for each test concentration. The EC_{50}s were determined from the polynomial curve fitted to the data from the least-squares method.

For OTC, the 72-h EC_{50} was 1.99 for the flask (standard) bioassay, 3.55 for the tube bioassay, and 4.49 for the plate bioassay. This study on *Selenastrum capricornutum* produced a key data point for our risk assessment.

Wellborn, T. L., Jr. 1969. The toxicity of nine therapeutic and herbicidal compounds to striped bass. The Progressive Fish-Culturist 31:27–32.

Wellborn, Jr. (1969) conducted an acute toxicity study of OTC and 8 other chemicals to striped bass fingerlings at 21 °C following the standard procedure of Doudoroff et al. (1951). Striped bass fingerlings were obtained from the Edenton National Fish Hatchery, North Carolina. The fingerlings used in the bioassay test averaged 2.7 g in weight and 60 mm in total length. The fish were held in troughs for 1 week to acclimate them before the start of the bioassays. Feeding was stopped 48 h before test initiation. The bioassays were done in 55-L aquaria containing 40 L of dechlorinated tap water. There was no aeration of the water during the tests. Each aquaria was stocked with 10 fish (max. 0.75 g fish /L). Five or six concentrations of OTC were used (exact number or concentration range for a given chemical was not stated), with three replications at each concentration. There were two control aquaria. Fish were allowed to acclimate in the aquaria for 24-h before test initiation. Mortalities were recorded 24-, 48-, and 96-h. Test water composition consisted of oxygen (7.8 mg/L), total hardness (35.0 mg/L as $CaCO_3$), total alkalinity (64.0 mg/L as $CaCO_3$), pH (8.2) and temperature (21.0 °C). Confirmation of dose was not mentioned. The data were analyzed according to the method of Litchfield and Wilcoxon (1949) to determine the LC_{50}, and the 95% confidence interval (ci) was also determined.

For OTC, the 96-h LC_{50} was determined to be 178 mg/L (95% ci = 144-221 mg/L). Oxytetracycline was the least toxic of the 9 chemicals tested. This study on striped bass fingerlings produced a key data point for our risk assessment.

Doudoroff, P., B.G. Anderson, G.E. Burdick, P.S. Galtsoff, W.B. Hart, R. Patrick, E.R. Stron, E.W. Surber, and W.M. Van Horn. 1951. Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish. Sewage and Industrial Wastes 23: 1380-1397.

Litchfield, J.T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. Journal of Pharmacology and Experimental Therapeutics 96: 99-113.

Williams, R.R., T.A. Bell, and D.V. Lightner. 1992. Shrimp Antimicrobial Testing. II. Toxicity Testing and Safety Determination for Twelve Antimicrobials with Penaeid Shrimp Larvae. Journal of Aquatic Animal Health 4: 262-270.

Williams et al. (1992) investigated the toxicity of OTC and other antibacterial agents to penaeid shrimp larvae. All toxicity trials reported were conducted at commercial or commercial-scale research hatcheries in Sonora, Mexico or in Ohau, Hawaii. Animals were obtained from the facility at which the trials were conducted and reared to the required life stage. Test containers consisted of 1.0 L plastic Imhof cones (Nalge Co.) individually supplied with aeration via pasteur pipettes and maintained at ambient room temperature (range 25-29 °C). No other water quality conditions were mentioned.

The test usually comprised 12 cones for OTC concentrations and 6 cones for the controls. There were 3 replicates of each OTC concentration tested and for the control sets. Each cone contained 900 mL of seawater and was stocked with 30 larvae. The control set consisted of untreated sea water. Concentrations tested were based on preliminary tests and were logarithmically spaced over the selected range or selected based on FDA-recommended 1,3,5, and 10 times the estimated efficacious level (FDA 1986). The tests on individual larval

stages were begun as close to the transitions into that stage as logistically possible. Verification of test concentrations was not mentioned.

The placement of the animals was randomized. Larvae were initially counted into a 250-500 mL beaker containing seawater and then were transferred to the cone. Larvae were fed. Experiments were conducted for 24 h for the nauplii and 48 h for larvae of all other life stages. At the end of the test, animals were examined under a dissection microscope, and their status (dead, morbid, or active) was recorded. The trimmed Spearman-Karber method (Hamilton et al. 1977) was used to estimate an LC_{50} for OTC from the survival results for the test and calculate an EC_{50}. To estimate the margin of safety, values for the highest NOEC and LOEC were calculated from the data on total toxic effect. This study on whiteleg shrimp larvae produced a key data point for our risk assessment.

Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environmental Science and Technology 11: 714-719.

U.S. Food and Drug Administration (FDA). 1986. Guidelines for the preparation of data to satisfy the requirements of section 512 of the act regarding minor use of animal drugs. FDA, Center of Veterinary Medicine, Rockville, Maryland.

Wollenberger, L., B. Halling-Sørensen, and K. O. Kusk. 2000. Acute and chronic toxicity of veterinary antibiotics to *Daphnia magna*. Chemosphere 40:723–730.

Wollenberger et al. (2000) studied the acute and chronic toxicity of OTC and other veterinary antibiotics to *Daphnia magna. D. magna* were cultured in a fully defined medium M7 (OECD 1996). The growth medium for the food algae *Raphidocelis subcapitata* was prepared as described in the protocol for the algal growth inhibition test (ISO, 1989a). Both media were made up with distilled water filtered through a Millipore unit. For the chronic reproduction test, the pH of the medium was adjusted to 7.5. *D. magna* cultures consisted of 1 L glass beakers containing 800 mL of medium and 20 daphnids. The acute test was performed in accordance with ISO, 1989b. Approximately 20 neonates aged less than 24 h, divided into 4 groups, were exposed to each concentration for 48 h in a static test. The controls consisted of about 30 daphnids divided into 6 groups. The test containers used were 100-mL glass beakers filled with 25 mL of test solution. The test was performed at 21 ± 0.5 °C. under dark. The number of mobile and immobile specimens was registered after 24 and 48 h. Oxygen and pH were measured in the controls and at the highest test concentrations. There was no other mention of water quality measurement. Five OTC concentrations of 1.0 to 100 mg/L were used. No confirmation of dose was mentioned. The LC_{50} values were determined by probit analysis.

For the chronic assessment, a semi-static reproduction test according to (OECD, 1996) was conducted. Daphnia aged less than 24 h at the start of the test were exposed for a period of 21-d to OTC at 5 concentrations in a geometric concentration series with a factor of 2 between the concentrations. Results of the acute test were used to determine the concentration range to be used in the chronic test. Each treatment consisted of ten 100-mL beakers each containing 80 mL of test solution and a single test organism. Daphnids were fed daily. The feeding rate was 3×10^7 cells per animal per day. Test solutions were renewed 3 times weekly. Survival and offspring production were assessed whenever solutions were renewed; pH and oxygen were measured. Test beakers were covered under glass lids and maintained at 21 ± 0.5 °C under a 12-h light, 12-h dark photoperiod. For calculation of EC values and confidence limits for the inhibition of offspring production in the *D. Magna* chronic test, a program assuming continuous response data and a logarithmic normal distribution (Andersen 1994) was used.

In the acute tests with neonates no control mortality occurred. The oxygen content was not reduced and the pH did not change during the tests. The 48-h LOEC was 100 mg/L, the highest OTC concentration tested (since no

EC_{10} was calculated, we assume that less than 10% of the daphnids were affected at 48 h). For the chronic test, the 21-d EC_{10} was 7.4 mg/L (95% confidence interval = 0.35-153 mg/L) and the 21-d EC_{50} was 46.2 mg/L (95% confidence interval = 20.6-104 mg/L).

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

Andersen, H. 1994. Statistical methods fot the evaluation of toxicity of wastewater. M.Sc. thesis. Department of Mathematical Modelling. Technical University of Denmark, Lyngby, Denmark (in Danish).

International Organisation for Standardization (ISO). 1989. Water quality – Fresh water algal growth inhibition test with *Scenedesmus subspicaticus* and *Selenastrum capricornutum*. ISO 8692. Geneva, Switzerland.

This page intentionally left blank.