PHARMACOKINETIC PROFILES OF OXYTETRACYCLINE IN YELLOW PERCH (*PERCA FLAVESCENS*) AS DETERMINED BY PLASMA CONCENTRATION FOLLOWING DIFFERENT ROUTES OF ADMINISTRATION

by

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Pharmacokinetic Profiles of Oxytetracycline in Yellow Perch (*Perca flavescens*) as Determined by Plasma Concentration Following Different Routes of Administration

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(ABSTRACT)

Oxytetracycline (OTC) is one of two antibiotics currently available and approved by the U.S. Food and Drug Administration for use as a chemotherapeutic agent in food fish and is widely used in the aquaculture industry. Previous pharmacokinetic studies of OTC have been conducted in cold water and warm water species of fish. However, no pharmacokinetic studies have been conducted on a cool water species such as yellow perch (*Perca flavescens*). The yellow perch is a cool water game and commercial species with high aquaculture potential. The pharmacokinetic profiles of oxytetracycline (OTC) was determined by measuring plasma concentrations in yellow perch following intraperitoneal (i.p.), intramuscular (i.m.), per os (p.o.), and intracardiac (i.c.) administration at a single dose of 50 mg/kg body weight. Using a modification of a high-performance-liquid-chromatographic (HPLC) technique, the plasma OTC concentrations were determined for each of the four routes of administration. Plasma concentrations were also evaluated in yellow perch exposed to a static 48-hour OTC water bath (100 mg/l). The terminal half-lives ($t_{1/2}$) of OTC in yellow perch for i.p., i.m., p.o., and i.c. administrations were 112, 124, 50, and 28 h, respectively. The $t_{1/2}$ for the i.m. route of administration was significantly longer than in any of the published i.m. OTC fish studies

to date. However, the times of maximum OTC concentration (t_{max}) for the i.p., i.m. and p.o. administrations (2, 4, and 15 h, respectively) occurred relatively early in the plasma concentration-time curves. This suggests, that in yellow perch, OTC is initially absorbed very rapidly. The area under the plasma concentration-time curves ($AUC_{0\rightarrow\infty}$) for the i.p., i.m., p.o., and i.c. routes of administration were 1718, 2659, 383, and 134 $\mu g \cdot h/ml$, respectively. No OTC was detected in the plasma of yellow perch following the water bath route of exposure. Finally, in yellow perch, effective therapy (plasma OTC concentrations above *MIC* values for most bacteria pathogenic to fish – 4 μg/ml) would be achieved for up to 168 hours following a single i.p. or i.m. injection of 50 mg/kg and for up to 15 hours following a single p.o dose of 50 mg/kg.

This thesis is dedicated to my parents,

Robert and Gail Bowden, with love and appreciation.

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CHAPTER 1

Review of Yellow Perch Culture, Oxytetracycline, and the Applied Plasma Pharmacokinetic Properties of Oxytetracycline in Freshwater Fish

INTRODUCTION

Oxytetracycline (OTC) is one of two antibiotics currently available and approved by the U.S. Food and Drug Administration for use as a chemotherapeutic agent in food fish. This broad-spectrum antibiotic is widely used in the aquaculture industry not only because of the limited availability of other approved drugs but also because oxytetracycline has a lower order of toxicity and a high ability to readily disperse into blood and most tissues (Ory, 1980; Barragry, 1994; Kapusnik-Uner et al., 1996). Previous pharmacokinetic studies of oxytetracycline have been conducted in the cold water species of rainbow trout (Salte and Liestol, 1983; Nordlander et al., 1987; Grondel et al., 1989; Jacobsen, 1989; Bjorklund et al., 1990; Bjorklund and Bylund, 1990; Black et al., 1991; Bjorklund and Bylund, 1991; Rogstad et al., 1991; Abedini et al., 1998), Atlantic salmon (Bruno, 1989; Elema et al., 1996; Namdari et al., 1998), chinook salmon (Abedini et al., 1998; Namdari et al., 1998), and tench (Reja et al., 1996), and the warm water species of African catfish (Grondel et al., 1989), carp (Grondel et al., 1987) and pacu (Doi et al., 1998). However, no pharmacokinetic studies have been conducted on a cool water species such as yellow perch (*Perca flavescens*). The yellow perch is a cool water game and commercial species with high aquaculture potential (Malison and Held, 1992; Heidinger and Kayes, 1993; Stickney, 1994). The objective of this research was to determine the plasma pharmacokinetic profiles of OTC in yellow perch following intraperitoneal (i.p.), intramuscular (i.m.), per os (p.o.), intracardiac (i.c.) and static water bath administrations using a modification of a high performance liquid chromatographic technique by Meinertz et al (1998).

LITERATURE REVIEW

Yellow Perch Culture

Classification

The yellow perch (*Perca flavescens*) is classified in the order Perciformes, suborder Percoidei, and family Percidae (Nelson, 1994). The family Percidae is quite diverse and includes the sand pike (*Stizostedion canadense*), walleyed pike (*Stizostedion vitreum*) and American darters. However, yellow perch can be distinguished from the other members of the family Percidae by the following characteristics: anal fin with two spines and six to eight soft rays; absence of canine teeth; and a large mouth with the maxilla extending to the midpoint of the eye (Heidinger and Kayes, 1993). In addition, although the North American yellow perch (*Perca flavescens*) and the Eurasian *perch (Perca fluviatilis*) were historically considered distinct species, Thorpe (1977) concluded that they are biologically equivalent.

Distribution and Life History

Yellow perch are a cool water species (temperature optima between 20-25°C) found in a variety of freshwater habitats including ponds, lakes, and slowing moving streams and rivers. Presently the yellow perch occurs naturally in the Hudson Bay drainage basin down to South Dakota, across the northern Midwest states from Missouri to Pennsylvania, in the upper levels of the Great Lakes, and in coastal streams from New Brunswick to Florida (Craig, 1987; Heidinger and Kayes, 1993).

Yellow perch are active during daylight hours and relatively inactive during the night. They are considered intermediate-sized percids reaching an average adult weight of 500 grams and length of 35 centimeters. Yellow perch are shoaling predators feeding almost exclusively during the day with dusk and dawn being peak times of feeding activity. During their larval stage, yellow perch are planktivores feeding upon immature copepods, cladocerans, and rotifers. As they continue to grow, their diet changes to include benthic prey and larger zooplankton. Finally when the yellow perch reach six months of age they become piscavorous, cannibalizing the young of other fish and their siblings (Craig, 1987).

Once a year in the spring, based upon photoperiod and water temperature, yellow perch spawn. Spawning usually lasts for approximately two weeks with a single female pairing off with two to five males. No investment is made by either the female or male to protect the eggs or the young (Heidinger and Kayes, 1993).

Game and Commercial Value

Yellow perch throughout their range in North America are valuable as a game and sport fish. Moreover in the upper Midwest of the United States, yellow perch in addition to being valuable as a game and sport fish are also economically valuable as a commercial foodfish. Historically since the beginning of the 20th century, the yellow perch fishery in the Great Lakes was able to adequately supply the market for yellow perch. However, over the past two decades, the commercial fisheries' harvest of yellow perch has declined so dramatically that at the present time it cannot keep up with the demand (Calbert and Huh, 1976; Brown et al., 1996; Kelly, 2000). Thus, based upon the

diminishing supply, the high market demand and the possible bioaccumulation of industrial pollutants in Great Lakes fish (Hopkins, 1999), the Industry Advisory Council of the North Central Regional Aquaculture Center has mandated yellow perch to be a species with a high and increasing aquaculture potential (Malison and Held, 1992).

Yellow Perch Aquaculture

Currently pond production is the primary and most economical method of yellow perch culture in the United States (Kelly, 2000). However pond production has four distinct disadvantages. First of all, pond culture requires vast areas of land with an abundant supply of suitable quality water. Second, neither the photoperiod nor the water temperature can be controlled. Third, yellow perch reared in ponds take significantly longer to reach their marketable size of approximately 150 grams (Calbert and Huh, 1976; Brown et al., 1995). And finally, pond culture in yellow perch is quite seasonal and variable due to the unpredictable nature of zooplankton populations at the time of spawning which the newly hatched fry depend upon for survival until they can be weaned onto artificial feed (NCRAC, 1996). Thus, due to the above challenges, yellow perch pond culture cannot consistently meet the growing market demand (NCRAC, 1996; Hopkins, 1999) and commercial yellow perch producers have started to turn to other forms of culture. More specifically, yellow perch producers have begun to experiment with growing yellow perch in cages, net-pens, flow-through systems, and indoor recirculating systems (Kelly, 2000). However, due to its potential for reducing seasonality and variability and its ability to control both temperature and photoperiod, indoor recirculating aquaculture systems have become the culture method of choice.

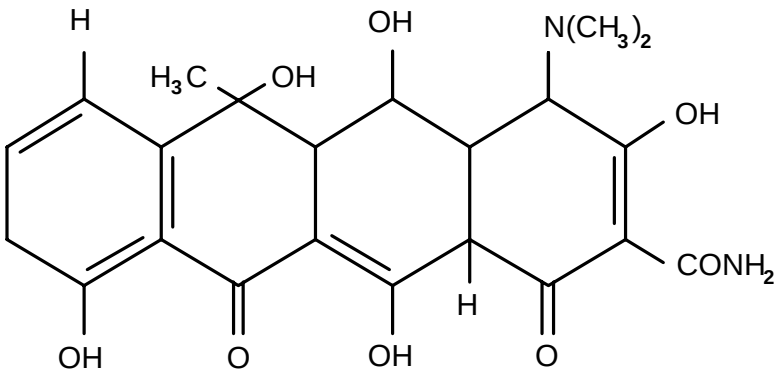
Disease Outbreaks and Treatment

Yet, despite the outstanding advantages of indoor recirculating aquaculture systems, in order for them to be economically feasible yellow perch have to be stocked at a very high density and consequently with such high-density, disease outbreaks can readily occur (Kelly, 2000). But unfortunately, the treatment of disease outbreaks in yellow perch is both complicated and compromised because there are presently no U.S. Food and Drug Administration approved chemotherapeutic agents for the treatment of this foodfish.

Oxytetracycline

Structure and solubility

Oxytetracycline (OTC) is one of the oldest antibiotics still in use in medicine. It is a yellow amphoteric crystalline compound with a molecular weight of 460.44. It has both a low solubility in water and a low octanol/water partition coefficient. It is stable as a powder but unstable in solution and therefore injections of oxytetracycline are often formulated as hydrochlorides (Treves-Brown, 2000). The chemical structure of oxytetracycline is given below.



Mechanism of Activity

Oxytetracycline possess antimicrobial activity by binding to the 30S ribosomal subunit of susceptible organisms. Upon binding, the oxytetracycline interferes with transfer RNA's ability to bind with messenger RNA, thereby preventing bacterial protein synthesis (Riviere and Spoo, 1995).

Spectrum of Activity

Oxytetracycline is a broad spectrum antibiotic. It has been approved by the U.S. Food and Drug Administration to treat only bacterial hemorrhagic septicemia and pseudomonas infection in catfish and salmonids by in-feed administration (Carpenter, 1994). In addition, although not approved by the U.S. Food and Drug Administration, it has been administered in feed to treat the following infections: flavobacteriosis in common carp (*Cypinus carpio*) and grass carp (*Ctenopharyngodon idella*); furunculosis in coho salmon (*Oncorhynchus kisutch*); and columnaris and streptococcosis in rainbow trout (*Oncorhynchus mykiss*) (Treves-Brown, 2000).

Toxicity, Distribution, and Absorption

Oxytetracycline has a low order of toxicity and a high ability to readily disperse into blood and most tissues (Ory, 1980; Barragry, 1994; Kapusnik-Uner et al., 1996). However despite these merits, oxytetracycline has a rather limited bioavailability (the total amount of the drug absorbed) because it chelates or forms complexes with polyvalent cations such as Ca^{++}, Fe^{++}, Al^{+++}, and Mg^{++} (Riviere and Spoo, 1995). These electrically charged complexes, which are microbiologically inert, are not able to easily traverse the lipid-rich biological membranes thereby causing a several fold decrease in

the absorption of oxytetracycline (Riviere and Spoo, 1995; Treves-Brown, 2000). In addition, oxytetracycline also will form complexes with organic material and clay. In the fish, it has been documented by Grondel et al. (1987) that oxytetracycline accumulates in bone tissue, the pronephros and the scales of carp.

Metabolism and Excretion

Oxytetracycline is not metabolized or biotransformed to a significant extent by fish. Thus virtually all of the administered dose is excreted or defecated into the environment (Cravedi et al., 1987; Treves-Brown, 2000). More specifically, it is thought that approximately 60% of the oxytetracycline is eliminated in the urine via glomerular filtration with the remaining 40% being eliminated in the feces (Rivere and Spoo, 1995). It is noteworthy to add that environmental temperature plays an important role in affecting the rate of oxytetracycline excretion. Salte and Liestol (1983), Jacobsen (1989) and Bjorklund and Bylund (1990) reported temperature dependency in relation to the excretion of oxytetracycline with the elimination of oxytetracycline being significantly slower at lower water temperatures. Thus cold water fish like trout and tench, excrete oxytetracycline more slowly than warm water fish like catfish, carp, and pacu, while cool water fish like yellow perch are probably intermediate to them.

Dose Regimens

Gavage/injection

Gavage (a form of oral administration) and injection are administration methods that are used almost exclusively for experimental purposes. They are rarely used in routine

fish management because they are both labor-intensive and stressful to the fish (Treves-Brown, 2000). For gavage or per os (p.o.) administration, injection is made into the stomach via a curved stainless steel gavage needle. For injection, there are five routes of delivery: intraperitoneal (i.p.), intramuscular (i.m.), intravenous (i.v.), intraaterial (i.a.), and intracardiac (i.c). For intraperitoneal administration, injection is made into the peritoneal cavity between the pelvic and anal fins to the right of the ventral midline. For intramuscular administration, injection is made into the epaxial musculature below the dorsal fin. For intravenous administration, injection is made into the caudal sinus. For intraaterial administration, injection is made into dorsal aorta via a cannula. For intracardiac administration, injection is made into the heart. In addition, with each of the above methods of administration it is less stressful on the fish and makes handling easier if the fish are sedated or anaesthetized. This is usually accomplished by immersion in anesthetic-medicated water. As previously mentioned, oxytetracycline is unstable in solution and as such injections of oxytetracycline are often formulated as hydrochlorides (Treves-Brown, 2000). Finally, it should be noted that although gavage and injection are not used in routine fish management, they are however quite useful methods for treating inherently valuable fish such as koi or brood stock which are not feeding because of their poor health and thus cannot be treated using in-feed medication (Treves-Brown, 2000). In the published pharmacokinetic fish literature, oxytetracycline doses range from 5 to 100 mg/kg body weight (Grondel et al., 1987; Grondel et al., 1989; Bjorklund and Bylund, 1991; Reja et al., 1996; Doi et al., 1998).

In-Feed

In-feed medication is the standard treatment regimen for foodfish. Even though oxytetracycline has only been approved by the U.S. Food and Drug Administration to treat bacterial hemorrhagic septicemia and pseudomonas in catfish and salmonids (Carpenter, 1994), it is often used to treat numerous other diseases in a variety of fish. However, in order for in-feed medication to be effective, the fish must consume the food. This can be a problem in that diseased fish often cease eating or cannot compete with other individuals for the food. Recommended oxytetracycline doses for foodfish range from 55 to 83 mg/kg body weight per day for 10 days (Noga, 1996; Treves-Brown, 2000).

Water Treatment

Water medication is by far the simplest of the dosing regimens. With water medication not only are you treating the fish itself but you are also potentially killing or reducing any potential bacterial pathogens that happen to be in the water. Since fish in freshwater do not drink, absorption occurs primarily across the epithelia of the gills and skin. As previously mentioned, oxytetracycline has a low solubility in water and for water treatment, oxytetracycline is often formulated as a water soluble powder (Treves-Brown, 2000). In addition, although the absorption of oxytetracycline by water bath is thought to be fish species specific, it is generally believed that oxytetracycline is not absorbed to any great extent by freshwater fish (Treves-Brown, 2000). Although administration of oxytetracycline by water bath is not a U.S. Food and Drug Administratoin approved route of exposure in foodfish, it is noteworthy in that producers of non-foodfish and tropical species often use this method of therapy. Recommended oxytetracycline doses for non

foodfish range from 5 to 120 mg/l for 1 to 4 hours (Stoskopf, 1988; Treves-Brown, 2000). Finally, it should be noted that since OTC accumulates and becomes incorporated into the otoliths of fish, hatchery managers routinely use OTC immersion (20-500 mg/l for 24 hours) as an effective marking technique. However, marking by OTC immersion has only been published in studies conducted on larval and juvenile fish (Choate, 1964; Lorson and Mudrak, 1987; Secor et al., 1991; Kayle, 1992; Brooks et al., 1994; Unkenholz et al., 1997).

Detection Assays

As mentioned above, oxytetracycline is one of two antibiotics currently available and approved by the U.S. Food and Drug Administration for use as a chemotherapeutic agent in foodfish and is widely used in the aquaculture industry. However in order for oxytetracycline to be useful to the aquaculture industry, the pharmacokinetic properties of oxytetracycline in various fish species need to be studied. Various assays have been developed to determine the concentration of oxytetracycline in blood and tissues, however because of its specificity, reliability, and sensitivity, high-performance-liquid-chromatography (HPLC) is now considered the present standard.

Applied Plasma Pharmacokinetic Properties of Oxytetracycline in Freshwater Fish

Cold Water Species

Rainbow Trout

In rainbow trout (*Oncorhynchus mykiss*), the plasma pharmacokinetic profile of oxytetracycline was studied following intramuscular administration at a dose of 60 mg/kg

body weight. The terminal half-life of OTC was 94.7 h and the time of maximum OTC concentration (56.9 μg/ml) was 4 hours post injection. The plasma concentration-time curve was described by a three-compartment model. The rainbow trout in this study had a mean body weight of 323 g and were maintained at 24°C (Grondel et al., 1989).

The plasma pharmacokinetic profile of oxytetracycline was also determined in rainbow trout (*Oncorhynchus mykiss*) following per os administration at a dose of 75 mg/kg body weight. The terminal half-life and the area under the plasma concentration-time curve from zero to infinity of OTC were 74.9 h and 258 μg · h/ml, respectively. The time of maximum OTC concentration (2.0 μg/ml) was 12 hours post-injection. The plasma concentration-time curve was described by a two-compartment model. The rainbow trout in this study had a mean body weight of 546 g and were maintained at 16°C (Bjorklund and Bylund, 1991).

Tench

In tench (*Tinca tinca* L.), the plasma pharmacokinetic profile of oxytetracycline was studied following intramuscular administration at a dose of 100 mg/kg body weight. The terminal half-life and the area under the plasma concentration-time curve from zero to infinity of OTC were 21.2 h and 6093.0 μg · h/ml, respectively. The time of maximum OTC concentration (99.7 μg/ml) was 6.4 hours post-injection. The plasma concentration-time curve was described by a one-compartment model. The tench in this study had a mean body weight of 133 g and were maintained at 12°C (Reja et al., 1996).

Warm Water Species

African Catfish

The plasma pharmacokinetic profile of oxytetracycline was determined in African catfish (*Clarias gariepinus*) following intramuscular administration at a dose of 60 mg/kg body weight. The terminal half-life of OTC was 74.4 h and the time of maximum OTC concentration (43.4 μg/ml) was 7 hours post injection. The plasma concentration-time curve was described by a two-compartment model. The African catfish in this study had a mean body weight of 293 g and were maintained at 25°C (Grondel et al., 1989).

Carp

In carp (*Cyprinus carpio* L.), the plasma pharmacokinetic profile of oxytetraycline was studied following intramuscular administration at a dose of 60 mg/kg body weight. The terminal half-life of OTC was 78.6 h and the time of maximum OTC concentration (56.8 μg/ml) was 14 hours post injection. The plasma concentration-time curve was described by a three-compartment model. The carp in this study had a mean body weight of 336 g and were maintained at 20°C (Grondel et al., 1987).

Red Pacu

The plasma pharmacokinetic profile of oxytetracycline was determined in red pacu (*Colossoma brachypomum*) following intramuscular administration at a dose of 5 mg/kg body weight. The terminal half-life and the area under the plasma concentration-time curve from zero to infinity of OTC were 62.7 h and 343.0 μg · h/ml, respectively. The plasma concentration-time curve was described by a non-compartmental model. The

pacu in this study had an approximate mean body weight of 200 g and were maintained at 23°C (Doi et al., 1998).

A summary of the applied plasma pharmacokinetic principles of oxytetracycline in freshwater fish following intramuscular or per os administration is outlined below. No oxytetracycline pharmacokinetic studies in freshwater fish have been published following i.p. or i.c. injection. In addition, no plasma pharmacokinetic studies in freshwater fish following oxytetracycline water bath exposure have been published.

		Intramuscular					Per os
		Cold water		Warm water			Cold water
Values*	Units	rainbow trout	tench	African catfish	carp	pacu	rainbow trout
Dose	mg/kg	60	100	60	60	5	75
$t_{1/2}$	h	94.7	21.2	74.4	78.6	62.4	74.9
$AUC_{0\rightarrow\infty}$	μg · h/ml	ND	6093	ND	ND	343	258
t_{max}	h	4	6.4	7	14	ND	12
C_{max}	μg/ml	56.9	99.7	43.4	56.8	ND	2.0

*Pharmacokinetic value abbreviations; $t_{1/2}$: Elimination half-life of OTC; $AUC_{0\rightarrow\infty}$: Area under the plasma concentration-time curve from zero to infinity; t_{max}: Time of maximum OTC concentration; C_{max}: Maximum OTC concentration (ND = Not determined)

Hypothesis:

The plasma pharmacokinetic profiles of oxytetracycline in yellow perch will be intermediate to the plasma pharmacokinetic profiles of oxytetracycline reported for warm water and cold water species of fish.

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CHAPTER 2

Modification of a Plasma and Tissue Preparation Technique for Determining Oxytetracycline Levels in Yellow Perch (*Perca flavescens*) using High-Performance-Liquid-Chromatography

(*Submitted to *Veterinary Therapeutics: Research in Applied Veterinary Medicine*)

Modification of a Plasma and Tissue Preparation Technique for Determining Oxytetracycline Levels in Yellow Perch (*Perca flavescens*) using High-Performance-Liquid-Chromatography

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ABSTRACT

A modification of a plasma and tissue preparation technique for determining oxytetracycline levels in yellow perch (*Perca flavescens*) using high-performance-liquid-chromatography is presented. More specifically, plasma and tissue samples of yellow perch were filtered with a disposable ultrafiltration membrane disc (30,000 molecular weight cutoff) and the filtrate was injected directly onto a high-performance-liquid-chromatography column. Oxytetracycline was separated on an ultrasphere octadecyldimethylsilyl analytical reversed phase C-18 column with the ultraviolet detection set to 355 nm. Overall recovery of oxytetracycline from the plasma and tissues was greater than 94%.

INTRODUCTION

Oxytetracycline (OTC) is one of two antibiotics currently available and approved by the U.S. Food and Drug Administration for use as a chemotherapeutic agent in foodfish. This broad-spectrum antibiotic is widely used in the aquaculture industry not only because of the limited availability of other approved drugs but also because OTC has a low order of toxicity and a high ability to readily disperse into blood and most tissues.[1, 2, 3] However in order for OTC to be useful to the aquaculture industry, the pharmacokinetic properties of OTC in various fish species need to be studied. Various assays have been developed to determine the concentration of OTC in blood and tissues, and because of its specificity, reliability, and sensitivity, high-performance-liquid-chromatography (HPLC) is now considered the present standard. Pharmacokinetic studies

utilizing HPLC to determine concentrations of OTC in blood and tissues have been conducted in many species of fish including channel catfish,[4-6] Atlantic salmon,[6-8] salmon,[9] Pacific salmon,[10] yellowtail,[11] coho salmon,[12] chinook salmon,[8,12-15] rainbow trout,[6,14-22] tench,[23] pacu,[24] walleye, striped bass and white sturgeon.[6] Unfortunately, each of these studies required a rather lengthy and complex pretreatment of the plasma or tissue sample before it could be injected onto the HPLC column. This paper describes a modification of a high performance liquid chromatographic technique by Meinertz et al.[6] for determining OTC concentrations in the plasma and tissues of yellow perch (*Perca flavescens*) using a micropartition filtering device as the only pretreatment step.

MATERIALS AND METHODS

Reagents and Chemicals

Acetonitrile (Burdick and Jackson, Muskegon, MI, USA) was of HPLC grade; Octane sulfonic acid sodium salt and oxalic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Fisher Scientific (Pittsburgh, PA, USA), respectively.

HPLC Apparatus

The analytical reversed phase column used for the OTC assays was an ultrasphere octadecyldimethylsilyl C-18, 75 mm x 4.6 mm ID with a 80 A pore (Beckman Instruments, Inc., Fullerton, CA, USA). The HPLC consisted of a Beckman 340 chromatography system equipped with a manual sample injector (Beckman Model 344) and 114M solvent delivery module (Beckman). The HPLC effluents were analyzed with a Beckman 165 variable wavelength detector set to 355 nm. The mobile phase (pH 3.3)

was composed of a 70:30 mixture of an aqueous mobile phase (0.01M oxalic acid and 0.030 M octane sulfonic acid sodium salt) and an organic mobile phase (acetonitrile).[6] The mobile phase was de-gassed and passed through a 0.22 μm filter (Millipore, Bedford, MA, USA) before use. The flow rate of the mobile phase was set to 1.5 ml/min with each sample run taking approximately 20 minutes. Data were digitized by a Beckman 406 analog interface and processed by a Beckman System Gold software.

Calibration, Detection Limit, and Recovery

Known standard solutions of OTC ranging from 1.0-10.0 μg/ml were prepared to determine a regression line to calculate the unknown OTC concentrations. In order to verify that the HPLC system was operating within normal parameters, known standard solutions of OTC was periodically injected onto the HPLC column. The detection limit of OTC for the HPLC system (l00 μl loop) was previously determined to be 0.5 μg/ml using known concentrations of plasma spiked with OTC. The percentage recovery of OTC from the plasma was determined by comparing analyzed concentrations of filtered plasma spiked at 5 μg/ml with analyzed concentrations of a 5 μg/ml standard of OTC injected directly (unfiltered) onto the HPLC column. The percentage recovery of OTC from the tissue (muscle and liver) was determined by a similar method.

Experimental Validation of Chromatographic Technique

Animals

Yellow perch (*Perca flavescens*) (n = 12), weighing 182.6 ± 5.6 g (mean ± SEM) were maintained at the Aquatic Medicine Laboratory of the Virginia-Maryland Regional

College of Veterinary Medicine (Blacksburg, VA, USA) in a 570 liter aerated recirculating aquaculture system. Fish were hand fed daily with a commercial floating pelleted feed (PMI Nutrition International St. Louis, MO, USA) at 2-3% body weight. Water quality parameters including ammonia, nitrite, and pH were checked daily (Hach Co., Loveland, CO, USA) with water changes done as needed. The ammonia, nitrite, and pH ranged from 0.1-0.5 mg/l, 1.0-3.0 mg/l, and 7.1-7.5, respectively. The photoperiod was set to a cycle of 14 hours light and 10 hours dark. All care and experimental use of fish in this study were approved by the Virginia Tech Animal Care Committee.

Percentage recovery study

Yellow perch (n = 6) were anesthetized with tricaine methanesulfonate (MS-222, 100 mg/l water, Sigma Chemical Co., St. Louis, MO, USA) and bled. Once bled, three yellow perch were euthanized with MS-222 and muscle (epaxial musculature below the dorsal fin) and liver tissues were harvested.

Antibiotic dosing study

Yellow perch (n = 6) were anesthetized and injected intraperitoneally (i.p.) with oxytetracycline hydrochloride (Oxybiotic-100, Butler, Columbus, OH, USA) at a dose of 50 mg/kg. At 4 hours post injection, these six fish were anesthetized, bled, and euthanized. The remaining three yellow perch were anestheized and injected i.p. with oxytetracycline (Oxybiotic-100) at a dose of 100 mg/kg. At 4 hours post injection, these three fish were euthanized with MS-222 and muscle and liver tissues were harvested.

Sample collection and storage

For blood collection, approximately 0.5 ml of blood was withdrawn from the caudal blood vessels with a 23 gauge needle on a 3 cc syringe. The blood was placed into a plasma separator tube containing lithium heparin (Microtainer, Becton Dickinson, Fisher Scientific, Pittsburgh, PA, USA) and kept on ice until the sample could be centrifuged (Fisher Scientific Microcentrifuge Model 235C) at 2500 x ***g*** for five minutes. The plasma was then pipetted into a 1.5 ml microcentrifuge tube and stored at -80°C until analysis. For tissue collection, approximately 1 g of weighed tissue was placed into individual 50 ml centrifuge tubes containing 4 ml of mobile phase (buffer) and homogenized (Polytron Homogenizer, Brinkman Instruments, Westbury, NY, USA). The homogenate was sonicated (Model W-225R, Heat Systems Ultrasonics, Inc, Plainview, NY, USA) at 20 kHz and centrifuged (International Equipment Co., Needham Heights, MA, USA) at 2000 x ***g*** for 15 minutes. The filtrate was then pipetted into a 1.5 ml microcentrifuge tube and stored at -80°C until analysis.

Plasma and tissue preparation

Frozen plasma and tissue filtrate samples were thawed at room temperature and pipetted into a micropartition device (MPS, Millipore, Bedford, MA USA) equipped with a disposable ultrafiltration membrane disc (YMT, 30,000 molecular weight cutoff, Amicon, Inc., Beverly, MA, USA) and centrifuged (Model MF-C 421, Bioanalytical Systems Inc., Lafayette, IN, USA) at 1500 x ***g*** for 40 minutes. Then 100 µl of this filtrate was injected directly onto the HPLC column.

RESULTS AND DISCUSSION

For the percentage recovery study, the recovery rate of OTC from the spiked (5 μg/ml) yellow perch plasma (n = 6) was 94.7 ± 0.12% (mean ± SEM). This rate of recovery is higher than or equal to any of the published OTC blood recovery studies.[14,18,25] The recovery rate of OTC from the yellow perch muscle (n = 3) and liver tissue (n = 3) was 96.4 ± 0.42% and 92.3 ± 0.73% (mean ± SEM), respectively. Again, these rates of recovery are equal to or higher than almost all of the published OTC muscle and liver recovery studies.[4-6,8-10,13,17-18]

In the antibiotic dosing study, OTC was detected in each of the plasma, muscle, and liver samples. More specifically, the concentration of OTC from the plasma (n = 6), muscle (n = 3), and liver (n = 3) was 18.89 ± 2.1, 0.69 ± 0.13, and 1.50 ± 0.48 μg/ml (mean ± SEM), respectively.

The retention times for oxytetracycline varied slightly with this variation in elution time being attributed to the settling and evaporation of the acetonitrile in the mobile phase. All calibration curves were linear over a range of 1.0-10 μg/ml with a correlation coefficient of 0.98 or higher. Typical chromatograms of an untreated yellow perch plasma sample, a 5 μg/ml OTC standard solution, a plasma sample from a yellow perch injected with a single i.p. dose of 50 mg/kg of OTC, and a muscle sample from a yellow perch injected with a single i.p. dose of 100 mg/kg of OTC are shown in Figures 1-4. No extraneous, interfering or additional peaks were observed throughout this study. The disposable ultrafiltration membrane discs used throughout this study and in subsequent studies (600+ samples filtered and centrifuged) of yellow perch[26] and summer flounder

(*Paralichthys dentatus*)[27] failed only once but the sample was easily retrieved from the centrifuge tube. In conclusion, this technique is a reliable, sensitive, simple, specific method for recovering OTC in the plasma and tissues of yellow perch and other fish species.

ACKNOWLEDGMENTS

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Figures

Figure 1. Typical chromatogram of an untreated yellow perch (*Perca flavescens*) plasma sample.

Figure 2. Typical chromatogram of a 5 µg/ml oxytetracycline (OTC) standard solution.

Figure 3. Typical chromatogram of a plasma sample from a yellow perch (*Perca flavescens*) injected with a single intraperitoneal (i.p.) dose of 50 mg/kg of OTC.

Figure 4. Typical chromatogram of a muscle sample from a yellow perch (*Perca flavescens*) injected with a single intraperitoneal (i.p.) dose of 100 mg/kg of OTC.

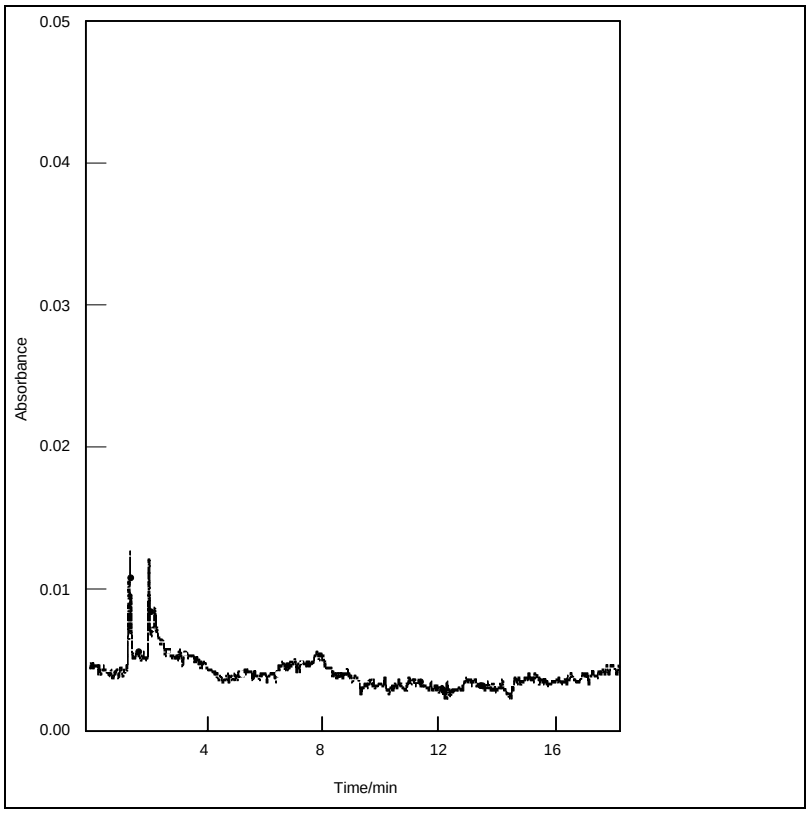

Figure 1. Typical chromatogram of an untreated yellow perch (*Perca flavescens*) plasma sample.

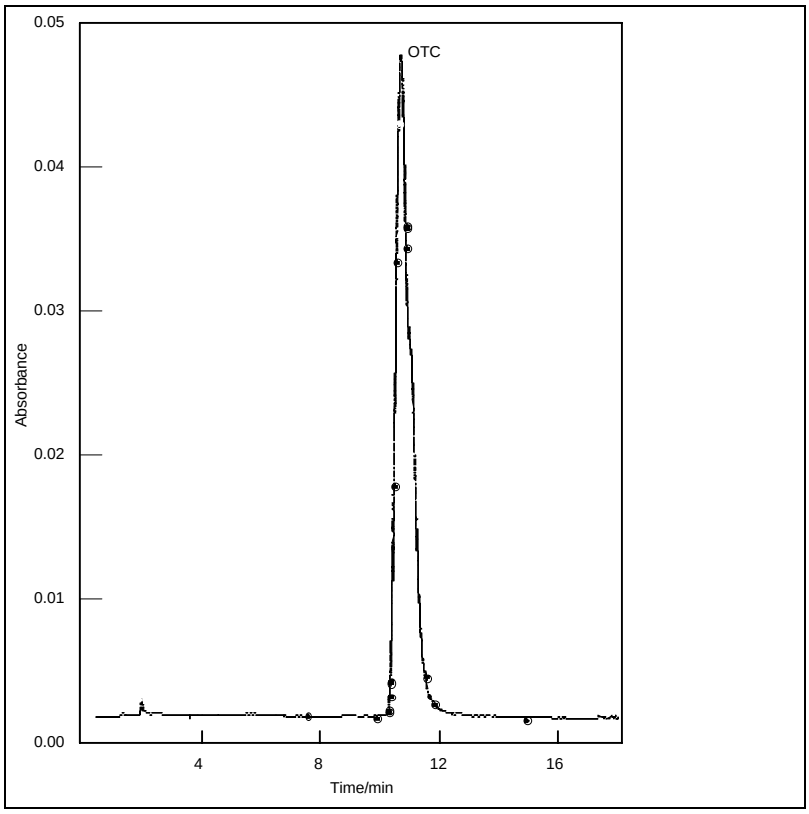

Figure 2. Typical chromatogram of a 5 μg/ml oxytetracycline (OTC) standard solution.

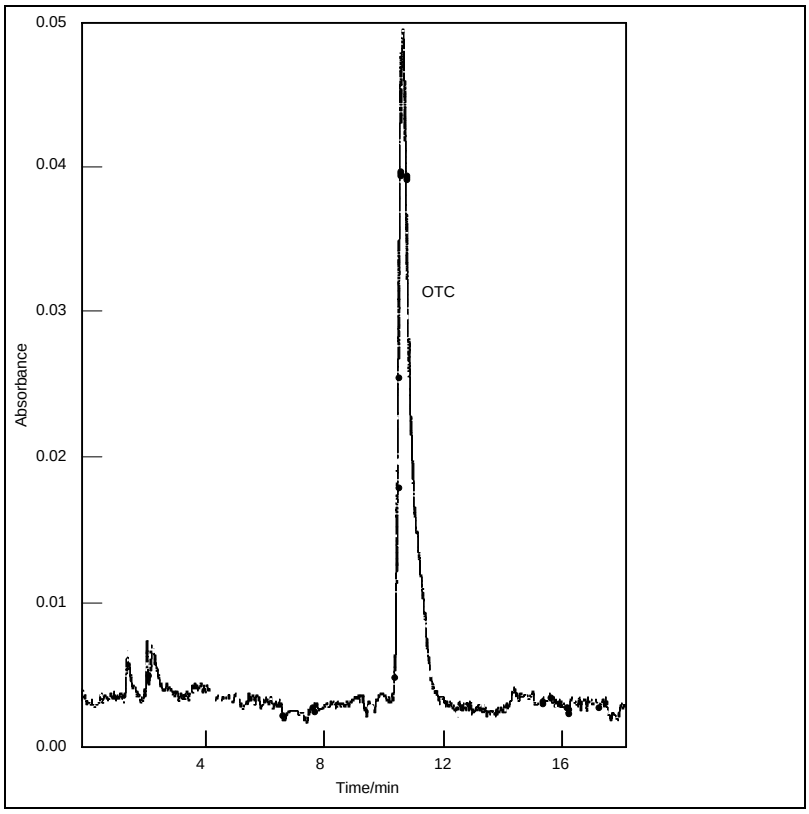

Figure 3. Typical chromatogram of a plasma sample from a yellow perch (*Perca flavescens*) injected with a single intraperitoneal (i.p.) dose of 50 mg/kg of OTC.

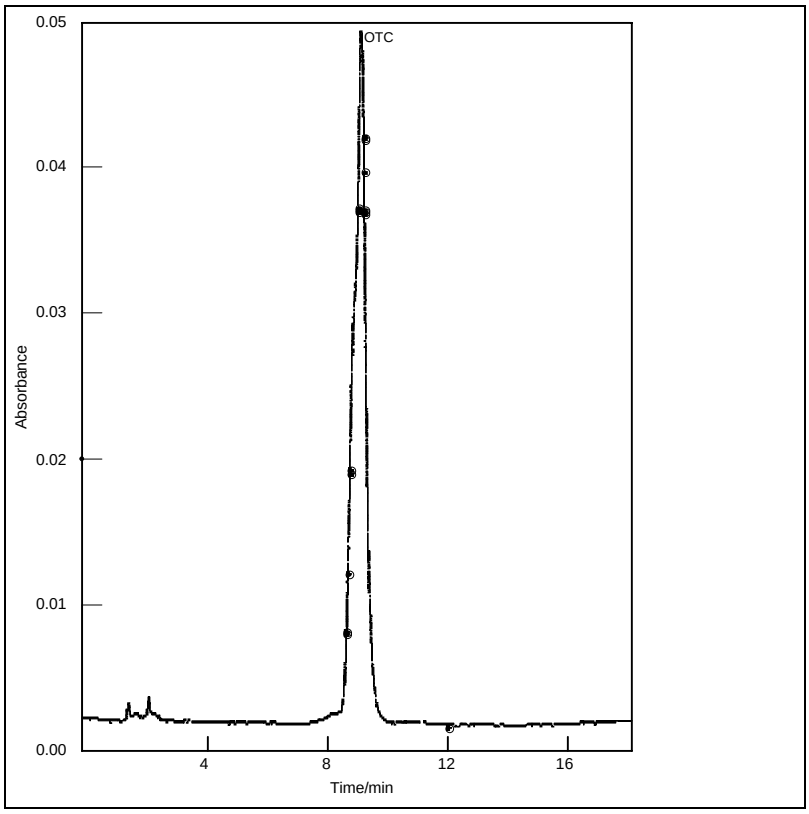

Figure 4. Typical chromatogram of a muscle sample from a yellow perch (*Perca flavescens*) injected with a single intraperitoneal (i.p.) dose of 100 mg/kg of OTC.

CHAPTER 3

Pharmacokinetic Profiles of Oxytetracycline in Yellow Perch (*Perca flavescens*) as Determined by Plasma Concentration Following Different Routes of Administration

(*Submitted to *Journal of Veterinary Pharmacology and Therapeutics*)

Pharmacokinetic Profiles of Oxytetracycline in Yellow Perch (*Perca flavescens*) as Determined by Plasma Concentration Following Different Routes of Administration

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ABSTRACT

The pharmacokinetic profiles of oxytetracycline (OTC) were determined by measuring plasma concentrations in yellow perch (*Perca flavescens*) following intraperitoneal (i.p.), intramuscular (i.m.), per os (p.o.), and intracardiac (i.c.) administration at a single dose of 50 mg/kg body weight. Using high-performance-liquid-chromatography, the plasma OTC concentrations were determined for each of the four routes of administration. Plasma concentrations were also evaluated in yellow perch exposed to a static 48-hour OTC water bath (100 mg/l). The terminal half-lives ($t_{1/2}$) of OTC in yellow perch for i.p., i.m., p.o., and i.c. administrations were 112, 124, 50, and 28 h, respectively. The $t_{1/2}$ for the i.m. route of administration was significantly longer than in any of the published i.m. OTC fish studies to date. However, the times of maximum OTC concentration (t_{max}) for the i.p., i.m. and p.o. administrations (2, 4, and 15 h, respectively) occurred relatively early in the plasma concentration-time curves. This suggests, that in yellow perch, OTC is initially absorbed very rapidly. The area under the plasma concentration-time curves ($AUC_{0\rightarrow\infty}$) for the i.p., i.m., p.o., and i.c. routes of administration were 1718, 2659, 383, and 134 $\mu g \cdot h/ml$, respectively. No OTC was detected in the plasma of yellow perch following the water bath route of exposure. In yellow perch, effective therapy would be achieved for up to 168 hours following a single i.p. or i.m. injection of 50 mg/kg and for up to 15 hours following a single p.o dose of 50 mg/kg.

INTRODUCTION

Oxytetracycline (OTC) is one of two antibiotics currently available and approved by the U.S. Food and Drug Administration for use as a chemotherapeutic agent in foodfish. This broad-spectrum antibiotic is widely used in the aquaculture industry not only because of the limited availability of other approved drugs but also because OTC has a low order of toxicity and a high ability to readily disperse into blood and most tissues (Ory, 1980; Barragry, 1994; Kapusnik-Uner et al., 1996). Previous pharmacokinetic studies of OTC have been conducted in the cold water species of rainbow trout (Salte and Liestol, 1983; Nordlander et al., 1987; Grondel et al., 1989; Jacobsen, 1989; Bjorklund et al., 1990; Bjorklund and Bylund, 1990; Black et al., 1991; Bjorklund and Bylund, 1991; Rogstad et al., 1991; Abedini et al., 1998), Atlantic salmon (Bruno, 1989; Elema et al., 1996; Namdari et al., 1998), chinook salmon (Abedini et al., 1998; Namdari et al., 1998), and tench (Reja et al., 1996), and the warm water species of African catfish (Grondel et al., 1989), carp (Grondel et al., 1987) and pacu (Doi et al., 1998). However, no pharmacokinetic studies have been conducted on a cool water species such as yellow perch (*Perca flavescens*). The yellow perch is a cool water game and commercial species with high aquaculture potential (Malison and Held, 1992; Heidinger and Kayes, 1993; Stickney, 1994). The purpose of this study was to determine the plasma pharmacokinetic profiles of OTC in yellow perch following intraperitoneal (i.p.), intramuscular (i.m.), per os (p.o.), intracardiac (i.c.) and static water bath administrations.

MATERIALS AND METHODS

Animals, care and husbandry

Approximately 600 commercially-reared female yellow perch were maintained at the Aquaculture Center of the College of Natural Resources at Virginia Polytechnic Institute & State University (Blacksburg, VA , USA) in an aerated 8360 liter recirculating aquaculture system until needed for the experimental studies. Prior to the start of each of the three (i.p., i.m., and p.o.) experimental studies, approximately 160 fish were placed into a separate aerated 8360 liter recirculating aquaculture system and allowed to acclimate for two weeks. Water quality analyses were conducted weekly during the acclimation period and daily for the duration of each experimental study. These analyses included monitoring levels of ammonia, nitrite, pH (Hach Co., Loveland, CO, USA) and water temperature. Water temperature was maintained at 16°C (± 1) with approximately five percent of the tank volume being replaced each day due to solid waste removal, evaporation and splash out. The yellow perch were fed daily using a commercial non-medicated floating pelleted feed (Rangen Feeds, Inc., Buhl, ID, USA) at 2-3% body weight. Additionally, 42 yellow perch from the 8360 liter holding aquaculture system were acclimated and maintained at the Aquatic Medicine Laboratory of the Virginia-Maryland Regional College of Veterinary Medicine (Blacksburg, VA, USA) for the i.c. experimental study. The fish were divided evenly among two 475 liter Living Streams (Frigid Units, Toledo, OH, USA) equipped with chillers in order to maintain the water temperature (18°C ± 2). Water quality parameters (ammonia, nitrite, pH) were checked weekly with water changes done as needed. These fish were also fed a similar

commercial non-medicated floating pellet (PMI Nutrition International, St. Louis, MO, USA) at 2-3% body weight. And finally, 59 yellow perch from the 8360 liter holding aquaculture system were placed into a 570 liter static tank containing water from the holding system and allowed to acclimate for an hour before OTC water bath exposure. For the duration of the 48 hour OTC water bath exposure, ammonia, pH and water temperature (16.5°C ± 0.5) were monitored daily.

During both the acclimation period and the experimental trials of the i.p., i.m., p.o., i.c., and water bath studies, the photoperiod was set to a cycle of 16 hours light and 8 hours dark. All care and experimental use of fish in these studies were approved by the Virginia Tech Animal Care Committee.

Antibiotic dosing

For antibiotic administration, the yellow perch were anesthetized with tricaine methanesulfonate (MS-222, Sigma Chemical Co., St. Louis, MO, USA, 100 mg/l water). Fish were then injected with oxytetracycline hydrochloride (Oxybiotic-100, Butler, Columbus, OH, USA) at a single dose of 50 mg/kg in each of the four experimental studies: i.p. into the peritoneal cavity between the pelvic and anal fins to the right of the ventral midline via a 26 gauge 1/2″ needle; i.m. into the epaxial musculature below the dorsal fin via a 23 gauge 1″ needle; p.o. into the stomach via a curved stainless steel gavage needle (24 hours prior to administration, the fish were kept off feed); and i.c. into the heart via a 26 gauge 1/2″ needle. The water for the static water bath route of exposure

was adjusted as close to 100 mg/l oxytetracycline hydrochloride (Pfizer, Animal Health, Exton, PA, USA) as possible.

Experimental design: a single individual-single sample approach

At the start of each of the i.p., i.m. and p.o. experiments, all fish were temporarily moved into several static holding tanks where they could easily be netted, anesthetized and weighed. The first 108 fish injected after being weighed were placed directly back into the recirculating aquaculture system to be maintained until bleeding at time points of nine hours and longer. Of the remaining 42 (36 p.o.) fish, six were bled as controls and 36 (30 p.o.) were injected as individual groups of no more than six and placed into separate static holding tanks. Fish were injected in smaller groups to ensure that the time interval between dosing and sampling was as accurate as possible. For the i.p. and i.m. studies, blood samples from six different fish were taken at each of the following 23 time points: 0, 0.25, 0.5, 1, 2, 4, 6, 9, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, 336 h. For the p.o. study, blood samples from six fish were taken at the above time points with the exception of the 12 h sample being obtained at 15 h and the 0.25 h time point being omitted.

For the i.c. study, fish were netted, anesthetized, weighed and injected in small groups again to ensure that the time interval between dosing and blood sampling would be as accurate as possible. Blood samples from six different fish were taken at each of the following time points: 0.083, 0.25, 0.5, 1, 2, 4, and 12 h (due to a limited number of fish, the i.c. study was only carried out to 12 hours). For the water bath study, fish were netted, anesthetized, weighed and placed into the static tank. After exposure, blood samples

from nine different fish were taken at each of the following time points: 0, 1, 4, 9, 12, 24, 48 hours with the exception of the 0 and 48 h time points in which only six and eight samples were collected, respectively. Samples of the water were collected at 0, 1, 2, 6, 12, 24, and 48 hours to evaluate the OTC concentration over the exposure time.

Blood collection and storage

Fish were anesthetized with MS-222 (100 mg/l water) at the specified times indicated by the experimental design, and approximately 0.5 ml of blood was withdrawn from the caudal blood vessels with a 23 gauge needle on a 3 cc syringe. The blood was placed into a plasma separator tube containing lithium heparin (Microtainer, Becton Dickinson, Fisher Scientific, Pittsburgh, PA, USA) and kept on ice until the sample could be centrifuged (Fisher Scientific Microcentrifuge Model 235C) at 2500 x ***g*** for five minutes. The plasma was then pipetted into a 1.5 ml microcentrifuge tube which was stored at –80°C until analysis could be completed. Sampled fish were removed from the study.

Plasma preparation, HPLC and data analysis

Plasma samples were thawed at room temperature and pipetted into a MPS micropartition device (Millipore, Bedford, MA USA) equipped with a disposable YMT ultrafiltration membrane disc (30,000 molecular weight cutoff, Amicon, Inc., Beverly MA, USA) and centrifuged (Model MF-C 421 Bioanalytical Systems Inc., Lafayette, IN, USA) at 1500 x ***g*** for 40 minutes. The filtrate (100 µl) was then injected directly onto the high-performance-liquid-chromatography (HPLC) column.

The analytical reversed phase column used for all of the OTC assays was an ultrasphere octadecyldimethylsilyl C-18, 75 mm x 4.6 mm ID with a 80 A pore (Beckman Instruments, Inc., Fullerton, CA, USA). The HPLC consisted of a Beckman 340 chromatography system equipped with a manual sample injector (Beckman Model 344) and 114M solvent delivery module (Beckman). HPLC effluents were analyzed with a Beckman 165 variable wavelength detector set to 355 nm. The mobile phase (pH 3.3) was composed of a 70:30 mixture of an aqueous mobile phase (0.01M oxalic acid and 0.030 M octane sulfonic acid sodium salt) and an organic mobile phase (acetonitrile) (Meinertz et al., 1998). The mobile phase was de-gassed and passed through a 0.22 μm filter (Millipore, Bedford, MA, USA) before use. The flow rate was set to 1.5 ml/min with each sample run taking approximately 20 minutes. Data were digitized by a Beckman 406 analog interface and processed by a Beckman System Gold software.

Known standard solutions of OTC ranging from 1.0-10.0 μg/ml were prepared to determine a regression line to calculate the unknown OTC concentrations. The detection limit of OTC for the HPLC system (100 μl loop) was determined to be 0.5 μg/ml. In order to verify that the HPLC system was operating within normal parameters, known standard solutions of OTC were periodically injected onto the HPLC column.

Statistical and Pharmacokinetic Analysis

All standard errors of the mean (SEM) were calculated using SAS (SAS Institute Inc., version 7.0, Cary, NC, USA). The mean plasma OTC concentrations for the pharmacokinetic studies following i.p., i.m., p.o., and i.c. administration were analyzed using the pharmacokinetic modeling software WinNonLin (PharSight, version 1.5, Palo

Alto, CA, USA). More specifically, each plasma concentration-time curve (i.p., i.m., p.o., and i.c.) was best described by a non-compartmental pharmacokinetic model with no lag time. In addition, all pharmacokinetic values listed in Table 1 were estimated by WinNonLin.

RESULTS

The plasma concentration-time curves of the i.p., i.m., p.o., and i.c. administrations are shown in Figs. 1-4 with their respective pharmacokinetic values listed in Table 1. The terminal half-lives ($t_{1/2}$) of the i.p., i.m., p.o., and i.c. administrations were 112.4, 123.8, 50.2, and 27.6 h, respectively. The area under the plasma concentration-time curve from zero to infinity ($AUC_{0\rightarrow\infty}$) for each of the four administrations (i.p., i.m., p.o., and i.c.) was 1718.3, 2658.5, 383.4, and 133.9 $\mu g \cdot h/ml$, respectively. The times of maximum OTC concentration (t_{max}) for the i.p., i.m., p.o., and i.c. administrations were 2, 4, 15, and 0.083 h, respectively. The maximum OTC concentrations (C_{max}) of the i.p., i.m., p.o., and i.c. administrations were 32 ± 8.0, 29 ± 2.6, 6.0 ± 0.7, and 32 ± 9.2 μg/ml (mean ± SEM), respectively. No OTC was detected in the plasma of yellow perch following the water bath route of exposure.

DISCUSSION

The terminal half-life ($t_{1/2}$) of OTC in yellow perch for the i.m. route of administration (123.8 h) was significantly longer than in any of the published OTC fish studies to date

(Grondel et al., 1987; Grondel et al., 1989; Reja et al., 1996; Doi et al., 1998). This prolonged terminal half-life in yellow perch in comparison to other fish species such as rainbow trout, tench, African catfish, carp, and pacu (Grondel et al., 1987; Grondel et al., 1989; Reja et al., 1996; Doi et al., 1998) could be explained primarily by interspecies metabolic and vascular variation in muscular composition, tissue membrane permeability and blood perfusion (Boddeke et al., 1959; Mosse, 1978; Akster, 1981). In addition, Boddeke et al. (1959) found that the muscles of perch (*Perca fluviatilis*) were composed almost entirely of broad white fibers whereas the muscles of rainbow trout and carp were largely composed of narrow red fibers. The broad white muscle fibers are considered less vascularized than the narrow red fibers (Duff et al., 1987) which may have contributed to a prolonged elimination of OTC. Also, the prolonged terminal half-life could be the result of both a much slower rate of passive diffusion at the gill and the glomerular sites and/or a gradual back diffusion from various tissues and organs in which the OTC had accumulated (Grondel et al., 1989). Grondel et al. (1987) documented that OTC accumulates in bone tissue, the pronephros and the scales of carp. For the p.o. route of administration, the terminal half-life of OTC in yellow perch (50.2 h) was somewhat smaller than that of rainbow trout (Bjorklund and Bylund, 1991). No OTC pharmacokinetic studies in freshwater fish have been published following i.p. or i.c. injection.

The area under the plasma concentration-time curve from zero to infinity ($AUC_{0\rightarrow\infty}$) in yellow perch for the i.m. route of administration (2658.5 $\mu g \cdot h/ml$) was intermediate to the values reported in the i.m. OTC studies of tench and pacu. (Reja et

al., 1996; Doi et al., 1998). For the p.o. route of administration, the area under the plasma concentration-time curve in yellow perch was similar to that of rainbow trout (Bjorklund and Bylund, 1991). It is important to note that OTC in the p.o. study was administered without feed since OTC tends to bind and form complexes with substances in the ailmentary tract causing incomplete and delayed absorption and may cause a small decrease in the bioavailabililty of the OTC (Grondel et. al, 1987). It should also be noted that due to the difficulty and variability associated with the intracardiac injection technique, the area under the plasma concentration-time curve for the i.c. study was quite low.

In addition, it should be noted that the times of maximum OTC concentration (t_{max}) for the i.p., i.m., and p.o. administrations (2, 4, and 15 h, respectively) occurred relatively early in the plasma concentration-time curves. This suggests, that in yellow perch, OTC is initially absorbed very rapidly.

Environmental temperature plays an important role in affecting the rate of OTC excretion. Salte and Liestol (1983), Jacobsen (1989) and Bjorklund and Bylund (1990) reported temperature dependency in relation to the excretion of OTC with the elimination of OTC being significantly slower at lower water temperatures. Thus cold water fish like trout, excrete oxytetracycline more slowly than warm water fish like catfish, while cool water fish like yellow perch are probably intermediate to the previous two species. Interestingly, as most of the yellow perch in this study were female, the absorption of OTC into the developing ovary perhaps via an intraovarian injection may have contributed to the diminished terminal half-life and area under the plasma concentration-time curve in the i.p. study.

Although the plasma concentration-time curves for the four administrations (i.p. i.m., p.o., and i.c.) were best described mathematically by a non-compartmental pharmacokinetic model, the curves nonetheless appear biologically to have three different compartments. In 1998 Abedini et al. described a three compartment model in rainbow trout and chinook salmon. In this model, the initial rapid decline of the OTC plasma concentration was attributed to the distribution of the OTC from the central compartment to the shallow and deep compartments; the intermediate decline phase reflecting not only the continuing distribution of the OTC into the shallow and deep compartments but also the elimination of the OTC by the fish; and the final decline phase being related to the slow elimination of OTC from the deep compartment. This pharmacokinetic study of yellow perch roughly parallels this three compartment model.

In the fish exposed to the OTC in the static 48-hour water bath, no OTC was detected in the plasma at any of the sampling times. Periodic analysis of the OTC concentration of the water bath showed that the OTC did not decrease significantly from the initial 100 mg/l dose over the 48-hour exposure period. Thus in yellow perch, the OTC molecule, due to undetermined factors would appear either to not be able to cross the gill and/or skin membranes altogether or only be able to cross them in very low concentrations when administered in a freshwater bath. Therefore, it should be noted that in yellow perch and perhaps in other freshwater fish that OTC in the water does not appear to be absorbed systemically and may only serve to treat external bacterial infections. Although administration of an antimicrobial chemotherapeutic agent by water bath is not a FDA-approved route of exposure in foodfish, it is common practice among producers of non-

foodfish and tropical species. In addition, it should also be noted that since OTC accumulates and becomes incorporated into the otoliths of fish, hatchery managers routinely use OTC immersion as an effective marking technique. However, marking by OTC immersion has only been published in studies conducted on larval and juvenile fish (Choate, 1964; Lorson and Mudrak, 1987; Secor et al., 1991; Kayle, 1992; Brooks et al., 1994; Unkenholz et al., 1997).

Finally, the plasma concentrations of OTC in yellow perch following i.p. i.m., and p.o administration need to be examined in relation to their therapeutic efficacy. According to Doi et. al (1998), effective therapy of OTC should be obtained when plasma concentrations are above the Minimum Inhibitory Concentration (*MIC)* for most bacteria pathogenic to fish (4 μg/ml). Therefore, in yellow perch, effective therapy would be achieved for up to 168 hours following a single i.p. or i.m. injection of 50 mg/kg and for up to 15 hours following a single p.o dose of 50 mg/kg.

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FIGURES

Figure 1. Semi-log plot of the mean plasma concentrations of oxytetracycline (OTC) in yellow perch (*Perca flavescens*) following a single intraperitoneal (i.p.) injection of 50 mg/kg. (Error bars = SEM, *n* = 6)

Figure 2. Semi-log plot of the mean plasma concentrations of oxytetracycline (OTC) in yellow perch (*Perca flavescens*) following a single intramuscular (i.m.) injection of 50 mg/kg. (Error bars = SEM, *n* = 6)

Figure 3. Semi-log plot of the mean plasma concentrations of oxytetracycline (OTC) in yellow perch (*Perca flavescens*) following a single per os (p.o.) dose of 50 mg/kg. (Error bars = SEM, *n* = 6)

Figure 4. Semi-log plot of the mean plasma concentrations of oxytetracycline (OTC) in yellow perch (*Perca flavescens*) following a single intracardiac (i.c.) injection of 50 mg/kg. (Error bars = SEM, *n* = 6)

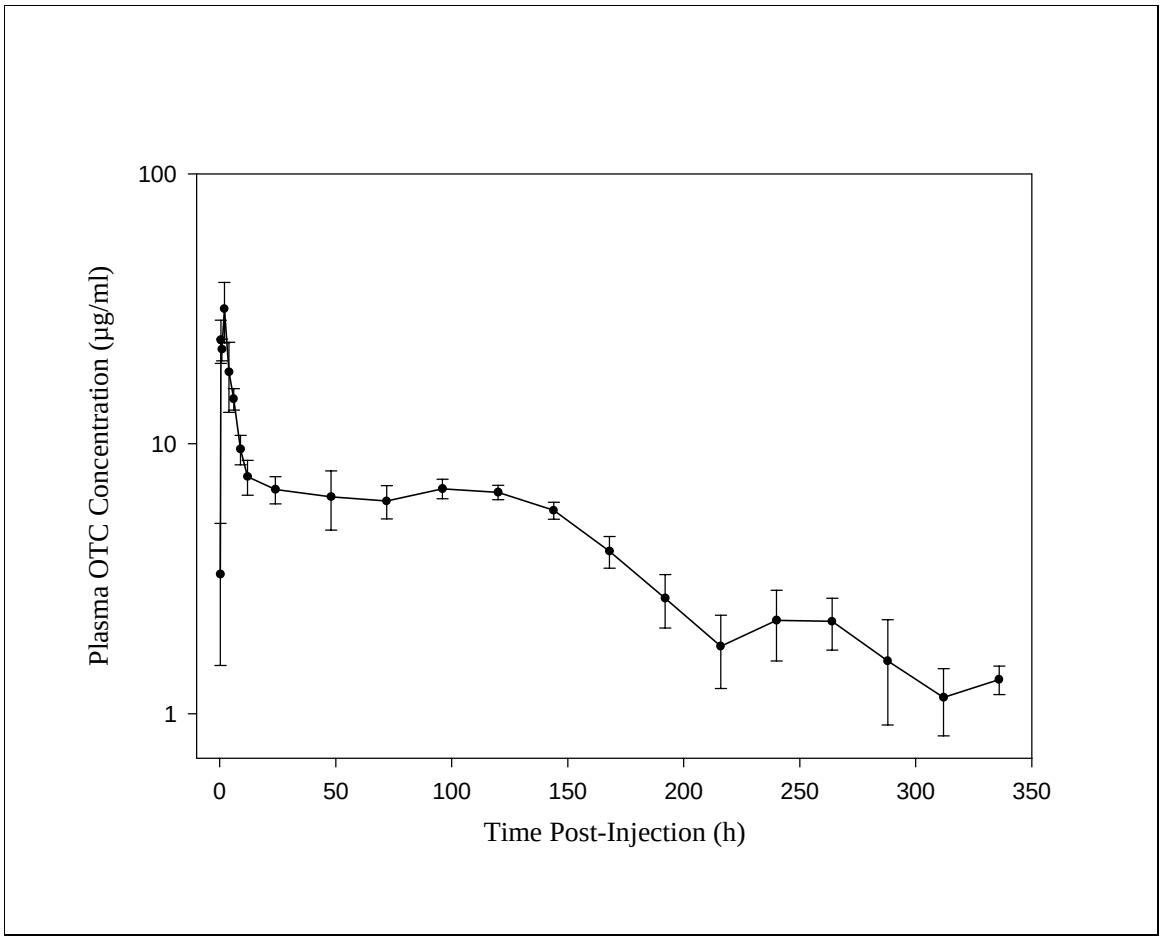

Figure 1. Semi-log plot of the mean plasma concentration of oxytetracycline (OTC) in yellow perch (*Perca flavescens*) following a single intraperitoneal (i.p.) injection of 50 mg/kg. (Error bars = SEM, *n* = 6)

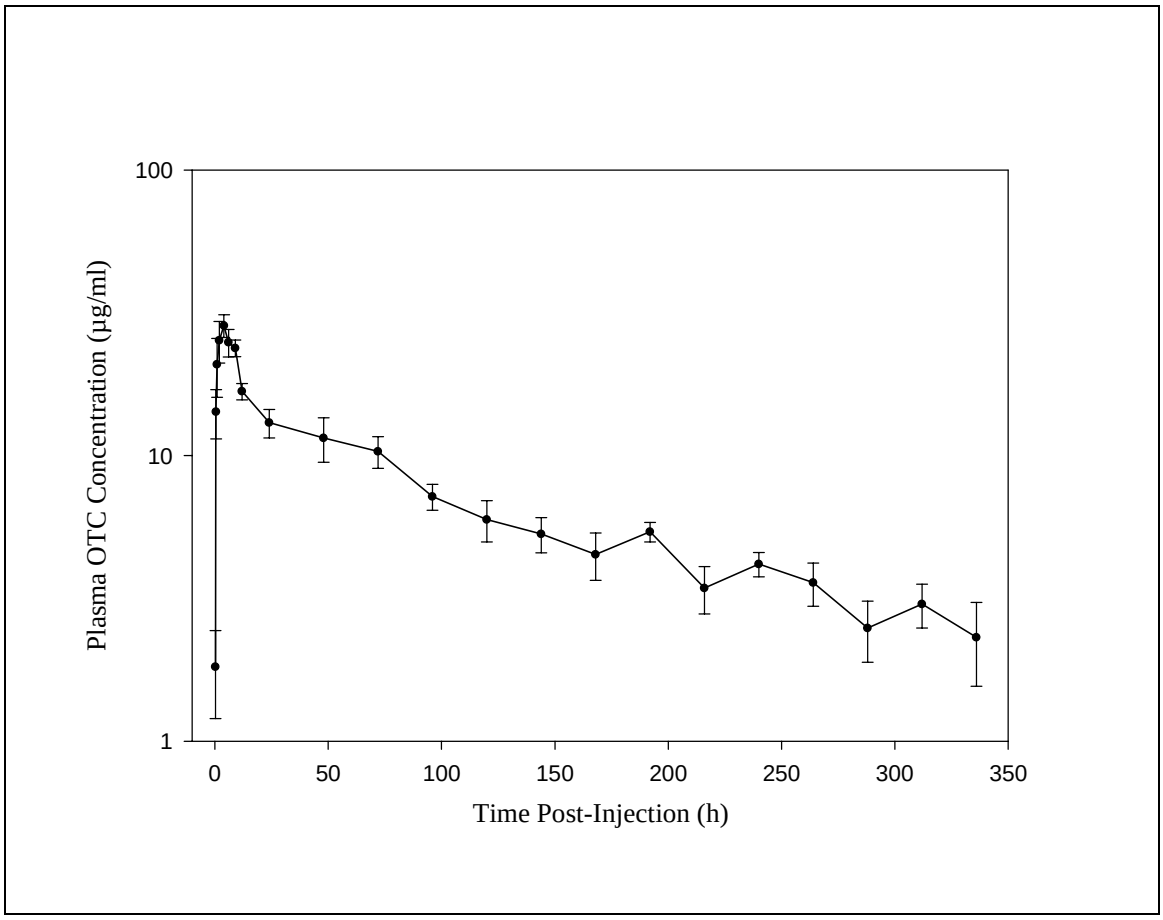

Figure 2. Semi-log plot of the mean plasma concentration of oxytetracycline (OTC) in yellow perch (*Perca flavescens*) following a single intramuscular (i.m.) injection of 50 mg/kg. (Error bars = SEM, *n* = 6)

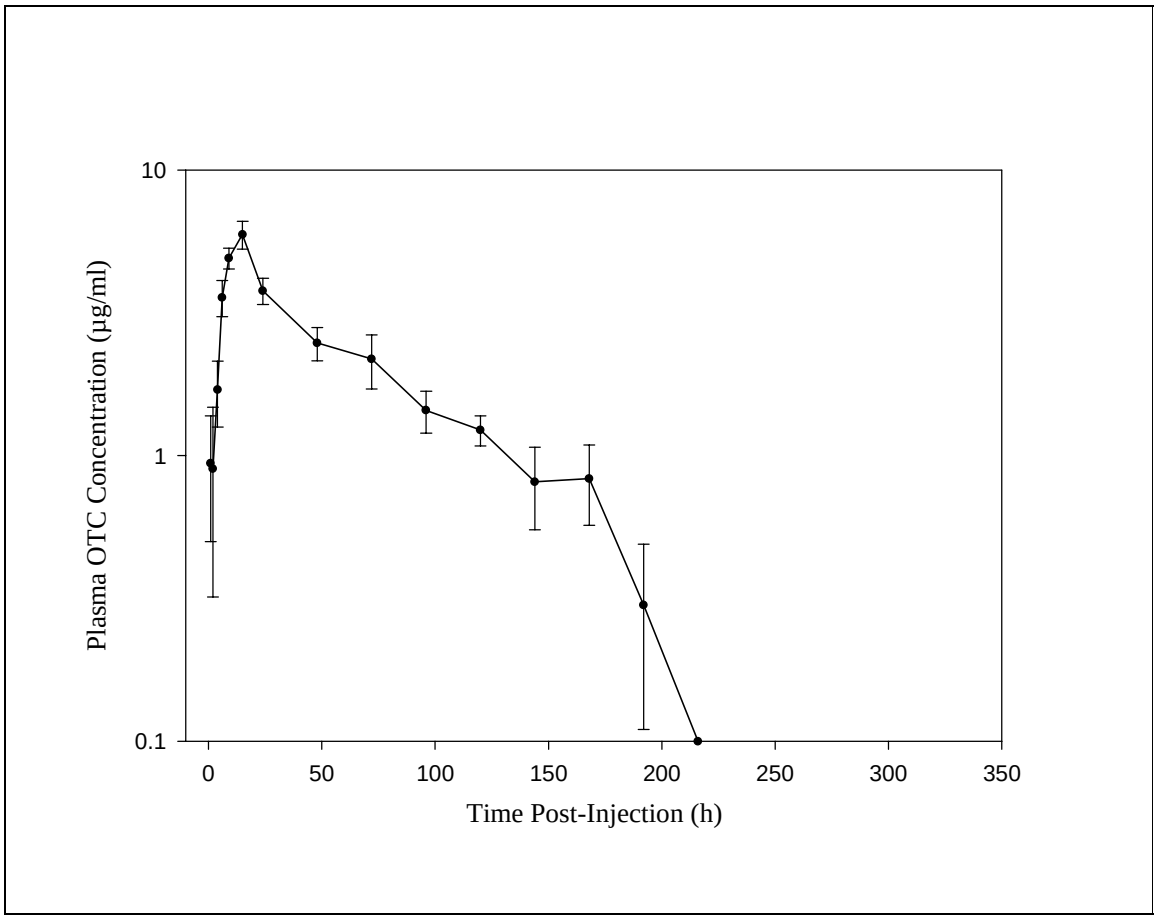

Figure 3. Semi-log plot of the mean plasma concentration of oxytetracycline (OTC) in yellow perch (*Perca flavescens*) following a single per os (p.o.) dose of 50 mg/kg. (Error bars = SEM, $n = 6$)

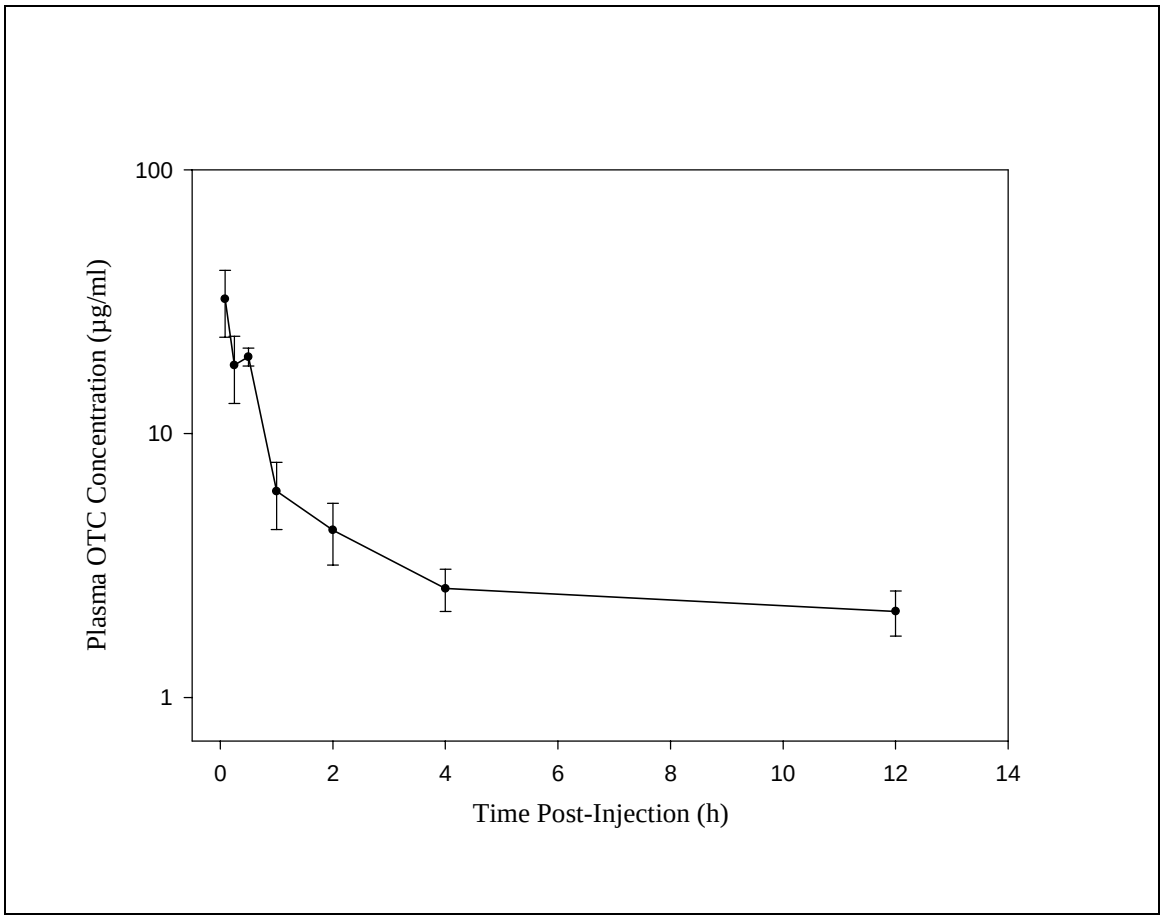

Figure 4. Semi-log plot of the mean plasma concentration of oxytetracycline (OTC) in yellow perch (*Perca flavescens*) following a single intracardiac (i.c.) injection of 50 mg/kg. (Error bars = SEM, *n* = 6)

Table 1. Oxytetracycline (OTC) pharmacokinetic values in yellow perch (*Perca flavescens*) following a single intraperitoneal (i.p.), intramuscular (i.m.), per os (p.o.) or intracardiac (i.c.) administration of 50 mg/kg or a static 48 hour water bath exposure of 100 mg/l.

Values*	Units	i.p.	i.m.	p.o.	i.c.	water bath
Weight of fish (mean ± SEM)	g	96 ± 1.3	93 ± 1.2	99 ± 1.3	94 ± 3.5	123 ± 5.3
Dose	mg/kg	50	50	50	50	
	mg/l					100
$t_{1/2}$	h	112.4	123.8	50.2	27.6	Not Applicable
$AUC_{0\rightarrow\infty}$	μg · h/ml	1718.3	2658.5	383.8	134.0	Not Applicable
t_{max}	h	2	4	15	0.083	Not Applicable
C_{max} (mean ± SEM)	μg/ml	32 ± 8.0	29 ± 2.6	6.0 ± 0.7	32 ± 9.2	Not Applicable

*Pharmacokinetic value abbreviations; $t_{1/2}$: Elimination half-life of OTC; $AUC_{0\rightarrow\infty}$: Area under the plasma concentration-time curve from zero to infinity; t_{max}: Time of maximum OTC concentration; C_{max}: Maximum OTC concentration

CHAPTER 4

Summary

SUMMARY

The modification of a high performance liquid chromatographic technique for determining OTC concentrations in the plasma and tissues of yellow perch using a micropartition filtering device as the only pretreatment was reliable, sensitive, simple, and specific with an overall recovery of greater than 94%.

The terminal half-lives ($t_{1/2}$) of OTC in yellow perch for i.p., i.m., p.o., and i.c. administrations were 112.4, 123.8, 50.2, and 27.6 h, respectively. The terminal half-life for the i.m. route of administration was significantly longer than in any of the published i.m. OTC fish studies to date. For the p.o. route of administration, the terminal half-life of OTC was somewhat smaller than that of rainbow trout. However, the times of maximum OTC concentration (t_{max}) for the i.p., i.m. and p.o. administrations (2, 4, and 15 h, respectively) occurred relatively early in the plasma concentration-time curves. This suggests, that in yellow perch, oxytetracycline is initially absorbed very rapidly. The area under the plasma concentration-time curves ($AUC_{0\rightarrow\infty}$) for the i.p., i.m., p.o., and i.c. administrations was 1718.3, 2658.5, 383.4, and 133.9 $\mu g \cdot h/ml$, respectively. The area under the plasma concentration-time curve for the i.m. route of administration was intermediate to the values reported in the i.m. OTC studies of tench and pacu. For the p.o. route of administration, the area under the plasma concentration-time curve was similar to that of rainbow trout. Therefore at this point based upon comparison of the i.m. and p.o. studies in yellow perch and other fish species, it appears that yellow perch are more like cold water fish than warm water fish in terms of the absorption and excretion of OTC.

Although the plasma concentration-time curves for the four administrations (i.p. i.m., p.o., and i.c.) were best described mathematically by a non-compartmental pharmacokinetic model, the curves nonetheless appear biologically to have three different compartments.

For the fish exposed to the OTC in the static 48-hour water bath, no OTC was detected in the plasma at any of the sampling times. Thus in yellow perch, OTC in the water does not appear to be absorbed systemically and may only serve to treat external bacterial infections.

Finally in yellow perch, effective therapy (plasma OTC concentrations above *MIC* values for most bacteria pathogenic to fish – 4 μg/ml) would be achieved for up to 168 hours following a single i.p. or i.m. injection of 50 mg/kg and for up to 15 hours following a single p.o dose of 50 mg/kg.

APPENDIX A

Table 2. Mean plasma oxytetracycline (OTC) concentration (μg/ml) in yellow perch (*Perca flavescens*) following a single intraperitoneal (i.p.), intramuscular (i.m.), or per os (p.o.) injection of 50 mg/kg. ND = not determined UD = undetected (± = SEM, *n* = 6)

Time post injection (h)	i.p.	i.m.	p.o.
0 (control)	UD	UD	UD
0.25	3.29 ± 1.78	1.82 ± 0.62	ND
0.5	24.27 ± 4.43	14.23 ± 2.79	UD
1	22.38 ± 2.1	20.88 ± 4.87	0.94 ± 0.44
2	31.67 ± 8.0	25.32 ± 4.21	0.90 ± 0.58
4	18.43 ± 5.33	28.53 ± 2.58	1.7 ± 0.44
6	14.68 ± 1.34	23.56 ± 2.74	3.58 ± 0.52
9	9.56 ± 1.2	23.78 ± 1.61	4.92 ± 0.41
12	7.56 ± 1.11	16.8 ± 1.1	ND
15	ND	ND	5.95 ± 0.67
24	6.78 ± 0.78	13.03 ± 1.5	3.78 ± 0.4
48	6.36 ± 1.57	11.53 ± 2.05	2.48 ± 0.33
72	6.14 ± 0.87	10.34 ± 1.31	2.18 ± 0.47
96	6.82 ± 0.57	7.19 ± 0.75	1.44 ± 0.24
120	6.62 ± 0.40	5.97 ± 0.99	1.23 ± 0.15
144	5.67 ± 0.41	5.32 ± 0.75	0.81 ± 0.26
168	4.00 ± 0.54	4.51 ± 0.85	0.83 ± 0.26
192	2.68 ± 0.60	5.41 ± 0.43	0.3 ± 0.19
216	1.78 ± 0.54	3.44 ± 0.65	UD
240	2.22 ± 0.65	4.17 ± 0.41	UD
264	2.20 ± 0.48	3.59 ± 0.62	UD
288	1.57 ± 0.66	2.49 ± 0.6	UD
312	1.15 ± 0.32	3.02 ± 0.53	UD
336	1.34 ± 0.16	2.31 ± 0.75	UD

Table 3. Mean plasma oxytetracycline (OTC) concentrations (μg/ml) in yellow perch (*Perca flavescens*) following a single intracardiac (i.c.) injection of 50 mg/kg. (± = SEM, *n* = 6)

Time post injection (h)	i.c.
0.083	32.41 ± 9.23
0.25	18.186 ± 5.19
0.5	19.56 ± 1.53
1	6.05 ± 1.72
2	4.31 ± 1.13
4	2.59 ± 0.47
12	2.12 ± 0.41

VITA

Brent Christopher Bowden, son of Dr. Robert and Gail Bowden, was born on August 24, 1972 in Radford, Virginia. He grew up in Blacksburg and graduated from North Cross High School in Roanoke, Virginia in June of 1990. In May of 1994, after receiving his Bachelor of Science in biology from Davidson College in Davidson, North Carolina, he returned to Blacksburg and enrolled at Virginia Tech in order to pursue a second Bachelor of Science degree. While at Virginia Tech, he majored in wildlife science and began working for Dr. Stephen Smith at the Virginia-Maryland Regional College of Veterinary Medicine. In August of 1997, after receiving his Bachelor of Science *magna cum laude* from Virginia Tech in May, he enrolled in a Master of Science program at the Virginia-Maryland Regional College of Veterinary Medicine with Dr. Stephen Smith as his graduate advisor. His research focused on the plasma pharmacokinetic profiles of oxytetracycline in yellow perch. He presently is employed by the Virginia-Maryland Regional College of Veterinary Medicine as an aquatic animal husbandry specialist.