

A Physiologically Based Pharmacokinetic Model of Oxytetracycline for Salmonids

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INTRODUCTION

As a result of an increased use of oxytetracycline (OTC) in the treatment and prevention of fish diseases, OTC residues in farmed fish have received increasing attention in recent years. Presently, the withdrawal periods of OTC-treated fish vary from country to country. For example, the withdrawal periods of OTC in Finland and Canada are 80 days when the water temperature is below 9° C and 40 days at higher temperatures. In the United States, the withdrawal period is 21 days for water temperatures above 9° C. OTC is prohibited from use on fish at water temperatures below 9° C.

Previous OTC tissue distribution studies have indicated that OTC bioavailability in fish varies with the route of drug administration and/or fish species (Grondel et al., 1987, Bjorklund and Bylund, 1991; Cravedi et al., 1987). The apparent oral bioavailability of OTC in trout (*Oncorhynchus mykiss*) was approximately 5.6% (Bjorklund and Bylund, 1991) and 7-9% (Cravedi et al., 1987). In contrast, when OTC was given to carp (*Cyprinus carpio L.*), the bioavailabilities were 80% and 0.6%, respectively, for the IM and oral routes (Grondel et al., 1987). The elimination of OTC from trout, however, was found to depend on water temperature (McCracken et al., 1976; Herman et al., 1969; Salte and Liestol, 1983; Bjorklund and Bylund, 1991). Grondel et al., (1987) also showed that a classical three-compartment open pharmacokinetic model described adequately the plasma OTC concentration-time curves of carp (*Cyprinus carpio L.*) following a bolus IV injection of OTC.

Current legislation on the withdrawal periods of OTC-treated fish does not take into consideration the various doses, dosing schedules, and fish weights in fish farms. These variables may significantly affect OTC distribution and residues in fish tissues. In this paper, I describe a physiologically based pharmacokinetic (PBPK) model of OTC for trout (*Oncorhynchus mykiss*) and chinook salmon (*Oncorhynchus kisutch*), which incorporates all these variables in the simulation. A comparison of the model-predicted results with actual laboratory salmonid data verifies that the model closely describes the empirical data.

MATERIALS AND METHODS

Drugs and OTC-Medicated Feed

OTC and tetracycline were obtained from Pfizer Canada Inc., Calgary, Alta. OTC-medicated feed was purchased from Moore-Clark Co (Canada) Inc., Vancouver, B.C.

Fish

Rainbow trout weighing about 275 gm were obtained from Spring Valley Trout Farms, Langely, B.C. The trout were kept in flowing, dechlorinated, fresh water at 9 °C in holding tanks. Chinook salmon with body weights of 55 gm and 275 gm were purchased from United Hatchery, Rosewell Creek, B.C. and Aquarius Seafarm, Seashelt, B.C., respectively. The salmon were kept in holding tanks with flowing sea-water at 9° C or 15° C temperature.

Single Oral Dose

Trout and chinook salmon weighing about 275 gm were administered a single oral dose of OTC (5 mg/kg or 50 mg/kg) in gelatin capsules which were prepared by adding a methanolic solution of OTC into pulverized fish feed. Following oral administration of the gelatin capsules, three fish were randomly selected at each sample time, humanely sacrificed, and frozen at -20° C for subsequent analysis.

Multiple Oral Dosing

Chinook salmon (*Oncorhynchus kisutch*) weighing about 55 gm were offered OTC-medicated feed at a dosage of 75 mg/kg/day for 21 days. The fish were fed twice daily to ensure the total amount of medicated feed was consumed. Following the last day of medicated feed, a non-medicated withdrawal diet was offered. The fish were fed for maximum growth throughout the withdrawal period. At the conclusion of the 21-day OTC treatment, six fish were randomly selected at each sample time, sacrificed and frozen at -20° C for subsequent analysis.

Determination of OTC Concentration in Tissues

After the fish were thawed, the gut, liver, kidney, bone, muscle, skin, and gills were removed from the carcass. Fat was trimmed from the gut which included the esophagus, stomach, and large and small intestines. Gut contents were also discarded. Scales were scrapped off from the skin. About 0.5 g of the tissue was weighed and homogenized in 10 mL McIlvaine Buffer/EDTA solution¹ containing tetracycline (1 µg/mL) to serve as an internal standard. The bone was digested with pepsin before being homogenized with McIlvaine buffer/EDTA solution. The tissue homogenate was centrifuged at 1,300 x g for 20 min to

¹McIlvaine Buffer: Dissolve 28.41 g dibasic sodium phosphate in distilled water in a 1 L flask. Dissolve 21.01 g citric acid monohydrate in distilled water in a 1 L flask. Combine 1 L of the citric acid solution with 625 ml of the sodium phosphate solution in a 2 L flask and adjust the pH of this solution to 4.0. McIlvaine Buffer/EDTA Solution: Add 37.22 g disodium ethylenediamine tetraacetate (EDTA) to litre of McIlvaine buffer.

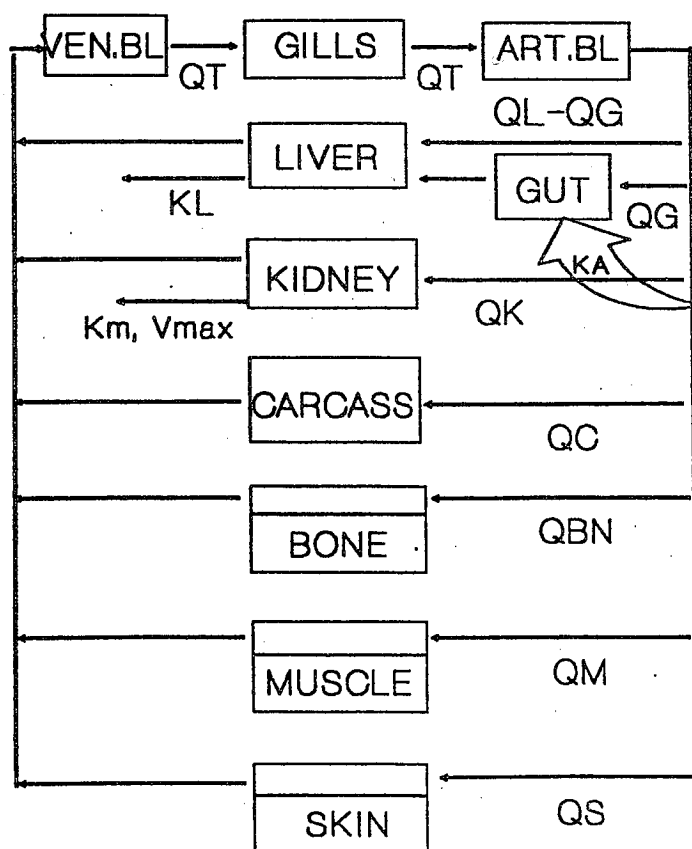
separate the layers. The supernatant layer was removed to a clean test tube. The remaining tissue pellets were extracted twice more with McIlvaine Buffer/EDTA solution (10 mL and 5 mL). The supernatants were combined and passed through a 3-ml Bond Elute extraction column. OTC was eluted from the column with 4 ml of methanol.

Three μL of the methanolic eluent was injected into an HPLC equipped with a microbore column (Hewlett-Packard ODS Hypersil 5 μm 100 x 2.1 mm) and a UV detector. The mobile phase was prepared from acetonitrile (190 mL), diammoniumhydrophosphate (5 g), diethanolamine (5 mL) and water (810 mL). The pH of the solution was adjusted to 2.5 using orthophosphoric acid. The flow rate of the mobile phase was 0.17 mL/min and the wavelength of the UV detector was set at 350 nm. The system was operated at 40 $^{\circ}\text{C}$.

PBPK Model

The PBPK model of OTC (Figure 1 and Table I) contained nine compartments: blood, gills, liver, kidney, gut, bone, muscle, skin and carcass. The gut compartment included highly perfused organs such as the esophagus, stomach, intestine, spleen, and heart. The carcass compartment was comprised of the remaining tissues such as the fins and eyes. The gills, liver, gut, kidney and carcass were depicted as blood-flow-limited compartments. The bone, muscle, and skin were membrane-limited compartments. Elimination of OTC was modeled by hepatic and renal clearances. Hepatic and renal clearances were first-order and zero-order rate processes, respectively.

Figure 1. Diagram of the pharmacokinetic model used to simulate the time course of oxytetracycline concentrations in the tissues of salmonids.



Model Parameters

The physiological parameters of trout were obtained from the literature and are summarized in Table I. The physiological parameters of chinook salmon were determined experimentally using the radio-labelled microspheres technique and the wet weights of the organs (data not shown). They were found to be very close to those of trout. The tissue/blood partition coefficients were estimated according to the methods of Dedrick et al. (1973). The mass transfer coefficients were estimated according to Dedrick and Bischoff (1968). Hepatic and renal clearances were estimated by trial and error. During the model development stage, these estimates were adjusted after comparing the experimental data with the computer simulation.

Table I. Physiological parameters used in the physiologically based pharmacological model for oxytetracycline

Tissue Compartment	Volume (% body wt)	Blood Flow (% C.O.)*
Blood	4.11	
Gill	3.90	100.0
Liver	1.16	21.2
Gut	8.52	15.4
Kidney	0.80	10.2
Carcass	26.50	1.6
W. Muscle	46.50	46.0
ECF	0.15	
ICF	46.4	
Bone	3.84	13.0
ECF	0.36	
ICF	3.48	
Skin	4.66	8.0
ECF	0.06	
ICF	4.60	

* C.O. = $3.95 \times \text{Temp} - 12.9$

Computer Simulation

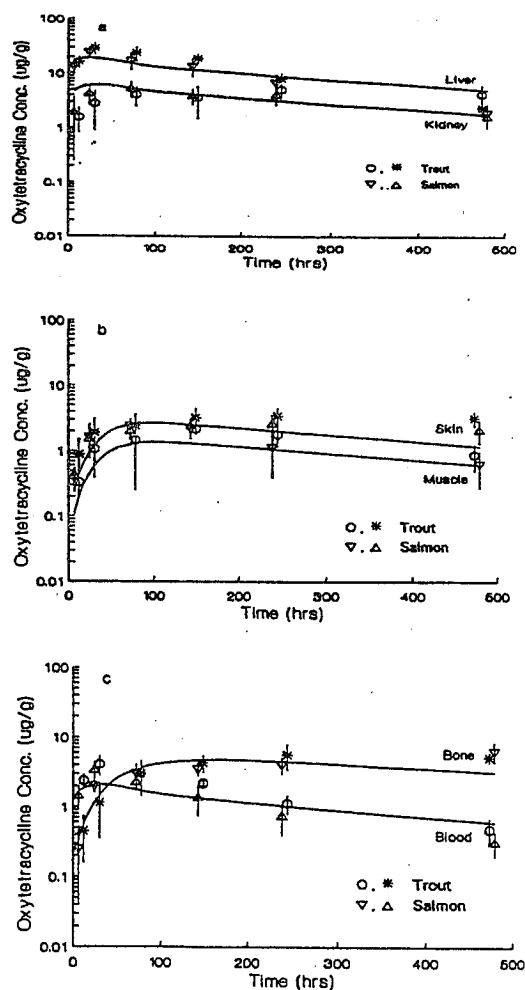
The differential and algebraic equations describing the movement of OTC through the salmonids were formulated as a computer program (data not shown). The set of differential and algebraic equations was solved numerically with the aid of ACSL (Advanced Continuous Simulation Language). The resultant solutions gave the predicted time course of OTC concentrations in the tissues.

RESULTS AND DISCUSSION

The extraction procedure was quite efficient in removing OTC from fish tissues. Percent recoveries reported as mean \pm SD of trout (salmon) tissues are: Liver, 76.2 ± 0.7 (78.1 ± 1.5); kidney, 69.5 ± 2.1 (69.4 ± 3.5); gill, 76.0 ± 1.8 (75.5 ± 1.1); gut, 77.1 ± 1.9 (79.8 ± 0.4); skin, 69.8 ± 1.2 (67.9 ± 1.3); muscle, 80.0 ± 2.4 (80.32 ± 2.4); blood, 80.1 ± 0.9 (81.0 ± 3.0). The HPLC assay was very sensitive for OTC detection; the detection limit was about 0.05-0.1 $\mu\text{g/g}$ tissue. The response of the UV detector to OTC was related to OTC concentration linearly to at least 50 $\mu\text{g/mL}$.

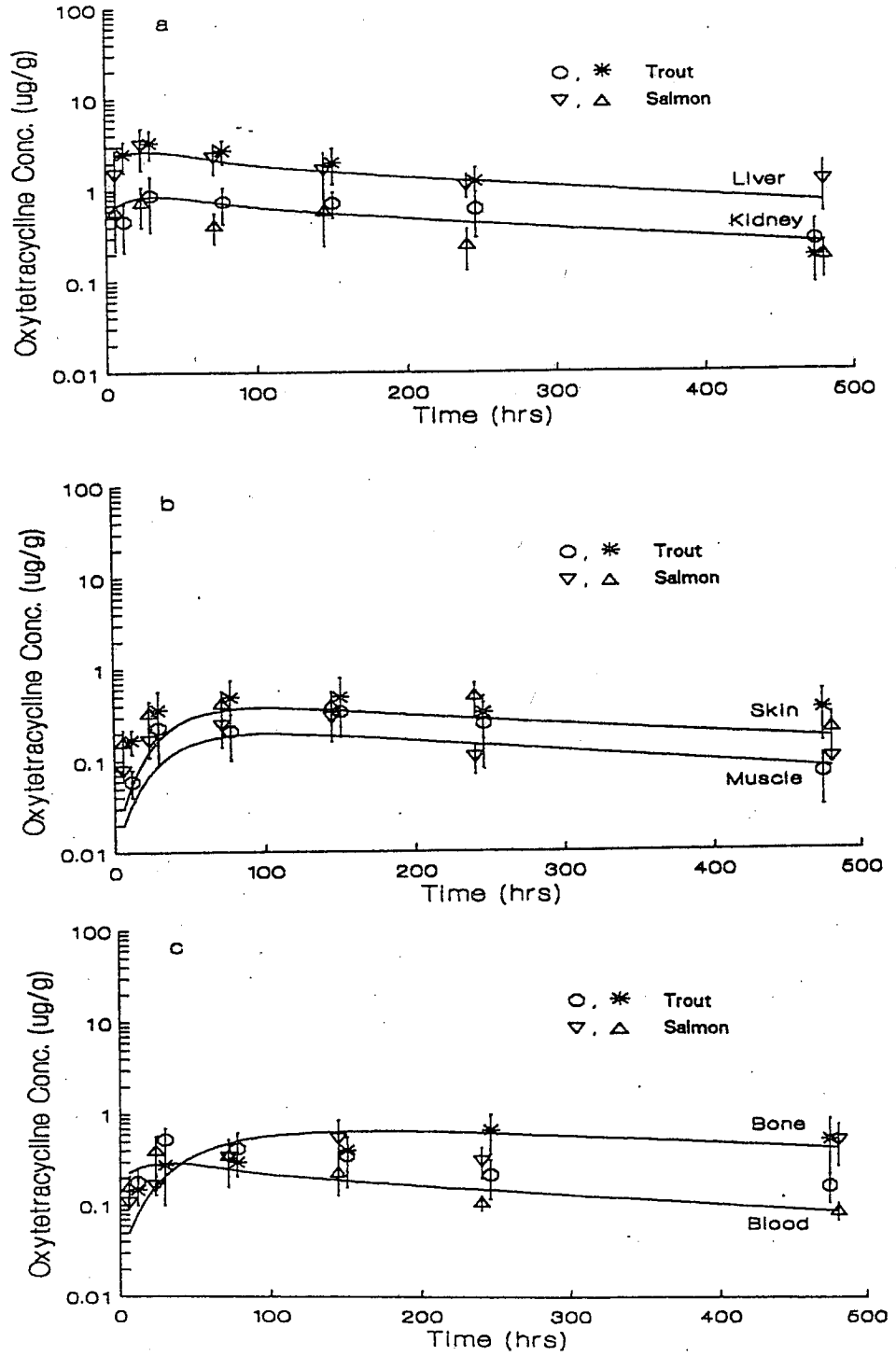
The PBPK model (Figure 1) was used to predict the time course of OTC tissue concentrations in trout and chinook salmon. The physiological and biochemical parameters used for modelling are shown in Table I. Oral OTC bioavailability was assumed to be 6%. Figures 2a-2c show the predicted and observed OTC concentrations in the liver, kidney, skin, muscle, bone and blood of the salmonids following a single oral dose of 5 mg/kg. Model-predicted results closely describe the empirical data.

Figure 2. Time course of tissue oxytetracycline concentrations in trout and salmon after a single oral dose of 5 mg/kg. Each data point represents the mean \pm SD of 3 fish. Solid curves represent simulation using the model. a. liver and kidney; b. skin and muscle; c. bone and blood.



Figures 3a-3c depict the observed and model-simulated OTC concentrations in the tissues of salmonids following a single oral dose of 50 mg/kg. The model also simulates most of the experimental data closely.

Figure 3. Time course of tissue oxytetracycline concentrations in trout and salmon after a single oral dose of 50 mg/kg. Each data point represents the mean \pm SD of 3 fish. Solid curves represent simulation using the model. a. liver and kidney; b. skin and muscle; c. bone and blood.



A comparison of the empirical data between trout and chinook salmon (Figures 2 and 3) shows that OTC tissue concentrations over time are exceedingly similar in these salmonids. This supports the finding of the radio-labelled microsphere study (see Methods and Materials) that the physiology of trout and chinook salmon is very similar. In other words, a single PBPK could be used to describe the distribution of OTC in these salmonids.

Figures 4 and 5 show the predicted and observed OTC concentrations in bone liver, kidney, skin and muscle of chinook salmon after feeding the fish with OTC-medicated feed at a dosage of 75 mg/kg/day for 21 days. OTC distribution in salmon tissues was examined at 9° C and 15° C water temperatures. Each data point represents the mean \pm SD of 6 fish. The curves are the simulation based on the model illustrated in Figure 1. After adjusting for body weight and water temperature, the physiological and biochemical parameters used in modelling the multiple dosing experiments are also the same as those of Table I. The model describes closely the empirical data (Figures 4 and 5).

Figure 4. Time course of tissue oxytetracycline concentrations in salmon after multiple dosing at 9° C water temperature. Each point represents the mean \pm SD of 6 fish. Solid curves represent simulation using the model. a. bone, liver and kidney; b. skin and muscle.

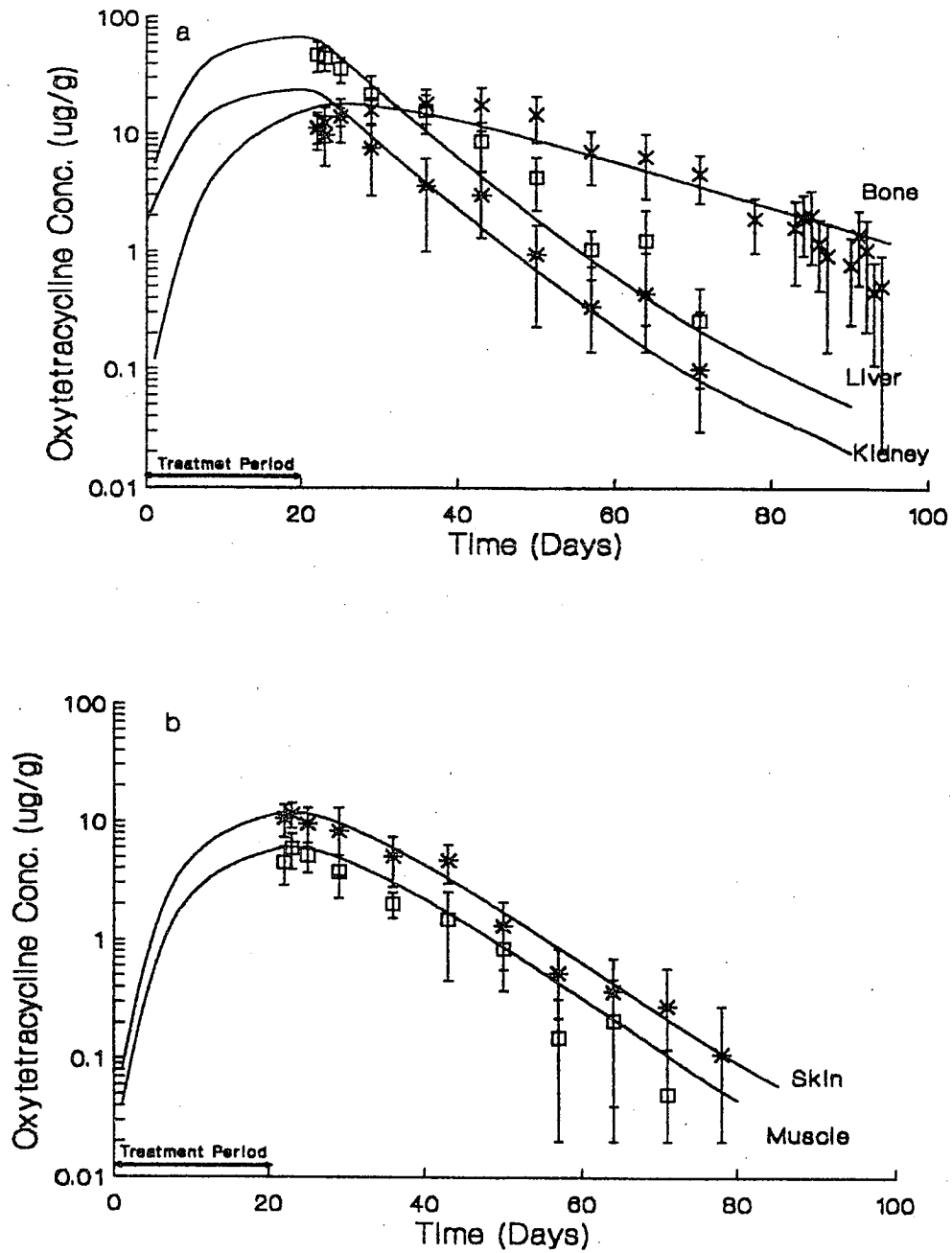
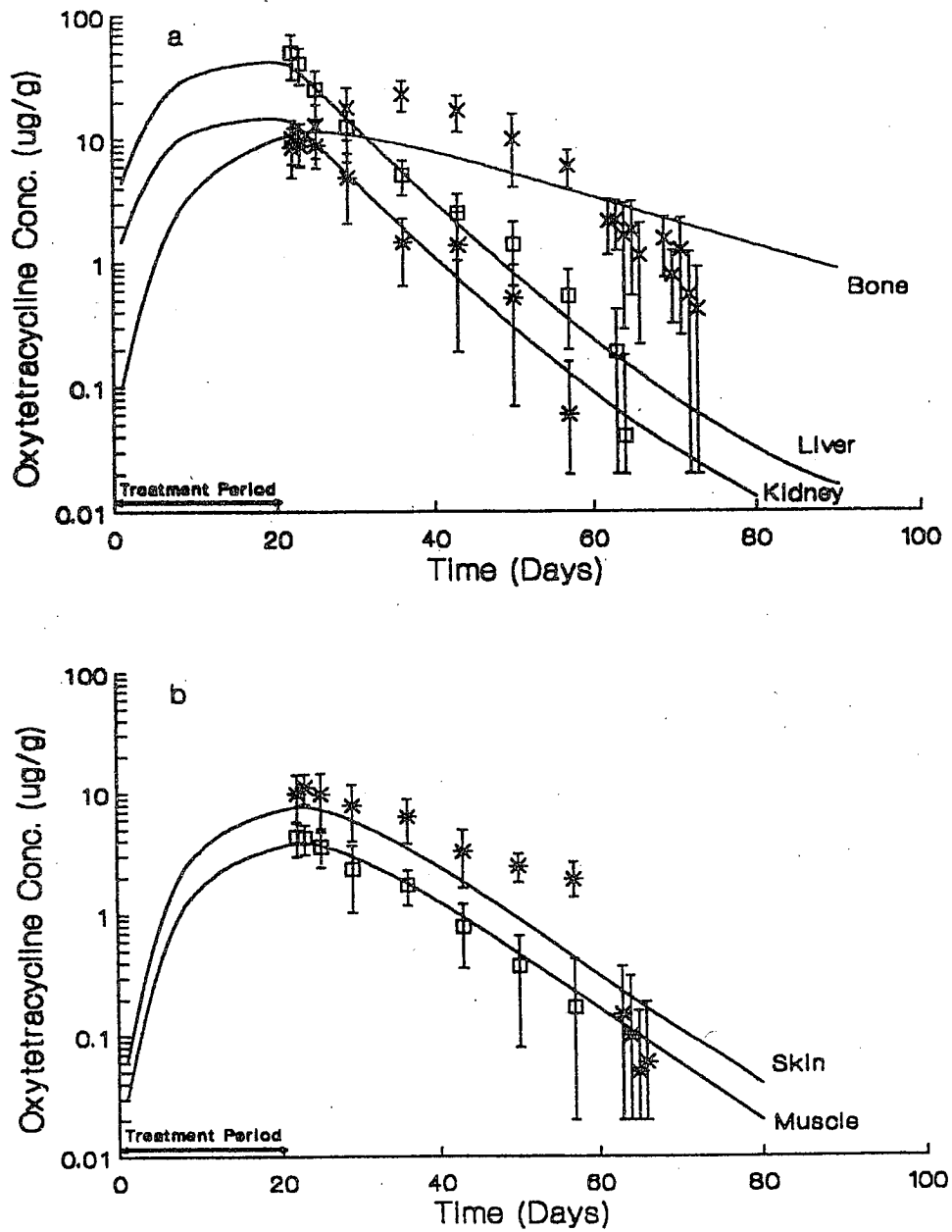


Figure 5. Time course of tissue oxytetracycline concentrations in salmon after multiple dosing at 15° C water temperature. Each point represents the mean \pm SD of 6 fish. Solid curves represent simulation using the model. a. bone, liver and kidney; b. skin and muscle.



In addition to predicting OTC withdrawal periods for farmed fish, there are other applications of the PBPK model. The model can be used to interpret experimental data in drug formulation research, to develop optimal dosage regimens for clinical applications, and to validate OTC assay results; especially those which are close to the detection limit of the current HPLC method.

CONCLUSIONS

In summary, a single PBPK model and the same set of physiological parameters have been used to simulate OTC tissue concentrations and residues in both trout (*Oncorhynchus mykiss*) and chinook salmon (*Oncorhynchus kisutch*) maintained at 9° C or 15° C water temperature. The model described closely the empirical data of the salmonids treated with different doses of OTC.

ACKNOWLEDGMENTS

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