AN ABSTRACT OF THE THESIS OF

Dennis D. Dauble for the degree of Doctor of Philosophy in Fisheries presented on April 25, 1988.

Title: Mechanisms Affecting the Bioaccumulation of Dietary Quinoline by Rainbow Trout (Salmo gairdneri)

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Lawrence R. Curtis

Factors that influenced the uptake, storage, and elimination of dietary quinoline by rainbow trout (Salmo gairdneri) were studied to obtain an understanding of the mechanisms affecting the bioaccumulation of dietary contaminants in teleosts. Rainbow trout readily absorbed ^{14}C-quinoline from pelleted food (1% ration at 138 ug quinoline/g food) and most tissues reached apparent steady-state after 10 days feeding. Maximum whole-body concentrations of quinoline plus metabolites were only 30 ng/g after 7 days depuration. Uptake rate constants ranged from 0.00006/day for muscle to 0.1455/day for gallbladder plus bile. Mean elimination half-life for quinoline-derived radioactivity ranged from 0.4 days in gills to 8.7 days for muscle. Depending on tissue, 58-83% of the stored radioactivity was present as metabolites. About 14% of the radioactivity in the bile was present as glucuronide conjugates.

Quinoline was absorbed from the stomach by rainbow trout and peak serum levels occurred 4-8 hr after a single feeding. Pharmacokinetics were described using a two-compartment body model

with first-order absorption and disposition; estimated half-lives for the a and B phase were 4.1 and 54.1 hr, respectively. Depending on dose, 71 to 83% of the ingested radioactivity was excreted during the first 24 hr after feeding. Branchial excretion was the primary route of excretion, all other routes (fecal, biliary, urinary, dermal) contributing <5% of the eliminated dose within 48 hr after ingestion. There was evidence for saturation of minor excretory pathways as the dose was increased from 1 to 100 mg quinoline/kg body weight. Apparent spillover into the branchial route occurred at the 100 mg/kg dose. Quinoline was eliminated across the gills as parent compound; no peaks representing individual polar metabolites were detected.

Physiological processes associated with feeding and digestion influenced the absorption and fate of dietary quinoline. The pH of the gut environment affected the availability of quinoline and potential for transfer across tissue membranes. Acidic gastric secretions were initially buffered by the food bolus, affecting the degree of ionization of parent compound. In the more alkaline intestine, >99% of quinoline was available for absorption. About 60% of the residual body burden was stored in the gallbladder bile, but bile was retained only in starved fish. There was no evidence for enterohepatic circulation of quinoline or its metabolites following ejection of gallbladder bile. Increased feeding rates enhanced the movement of the food bolus and associated radioactivity through the intestine, but did not affect patterns of tissue disposition.

MECHANISMS AFFECTING THE BIOACCUMULATION OF DIETARY QUINOLINE BY RAINBOW TROUT (*SALMO GAIRDNERI*)

by

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PREFACE

The research described in Chapter 1 was part of a larger multi-investigator study conducted for the U.S. Department of Energy. The co-authors, R.M. Bean and D.W. Carlile were part of that investigative team and contributed to the final product. Dr. R.M. Bean provided expertise in analytical chemistry and Mr. D.W. Carlile advanced my knowledge of considerations related to statistics and modelling.

MECHANISMS AFFECTING THE BIOACCUMULATION OF DIETARY QUINOLINE BY RAINBOW TROUT (*SALMO GAIRDNERI*)

INTRODUCTION

The potential for bioaccumulation of xenobiotics is generally believed to be much less through dietary sources than through aqueous routes (Macek et al., 1979). Thus, the relative importance of dietary absorption of organic compounds by fish has received little attention despite evidence of accumulation for hydrophobic compounds such as polychlorinated biphenyls (PCBs) and certain insecticides (Chadwick and Brocksen, 1969; Pizza and Conner, 1983; Rubenstein et al., 1984). However, a direct comparison between aqueous and dietary routes of contaminant uptake is not possible because differences in absorption sites (branchial versus gastrointestinal epithelium) can affect key processes that influence transport, clearance, biotransformation, and excretion. These interactive processes ultimately determine threshold tissue concentrations.

It is now evident that the diet can be a major source of body residues in aquatic organisms, mainly for compounds that persist in biological or sediment compartments of aquatic systems. However, there is a lack of information on specific mechanisms affecting dietary accumulation of organic contaminants by fish, in particular for studies where normal physiological processes associated with feeding are not altered.

The potential for dietary accumulation of xenobiotics by fish can be linked to several factors that affect transfer across absorptive membranes. These factors may include physical or ecological conditions that limit compound availability in the environment, in addition to xenobiotic concentration and feeding rate (Rogers and Beamish, 1982; Shubat and Curtis, 1986). Another important variable for dietary accumulation is post-absorptive processes that influence the amount and form of chemical transported to storage or excretory tissues (Klaassan, 1980). Pioneering studies by Roubal et al., (1977), Gruger et al., (1981), Varanasis et al., (1979; 1981), and others, addressed concerns related to the potential hazard of common organic pollutants in fish by characterizing chemical fate and metabolism following uptake through oral routes. While these types of studies provided information on chemical form and fate, the various techniques employed (i.e., gavage, force-feeding) limited the application of results. Basic physiological processes that occur during feeding and digestion also need to be considered when assessing the hazard of xenobiotics that may be ingested via food or sediments.

The objective of this research was to obtain a general understanding of the processes that influence the bioaccumulation of dietary contaminants by teleost fish. Specific studies were directed towards identifying the physiological and biochemical mechanisms that affected the disposition and fate of the nitrogen heterocycle, quinoline, after its uptake via the diet. Quinoline is a common constituent of many fossil fuel materials, including shale oil, coal liquids, and petroleum products (Weisberger and Williams, 1980;

Wright et al., 1985). Specific mechanisms of bioaccumulation of nitrogen-containing polycyclic aromatic compounds have received little study despite evidence that some members of this chemical class are mutagenic (Guerin et al., 1980; Later et al., 1982). Quinoline itself is a hepatocarcinogen in mice (Hirao et al., 1976). Studies of the fate of nitrogen-containing compounds are important because recent studies link the presence of free radical derivatives of nitrogen heterocycles to hepatic lesions in bottom fish (Roubal and Malins, 1985).

The research in this thesis is described in three chapters that correspond to phases in the evolution of the research: a descriptive phase, then, specific problem-solving studies, and finally, development towards achieving a broader understanding of general processes. Initial studies (Chapter I) described where dietary quinoline was stored in the organism, what chemical forms were present, the steady-state concentrations, and the potential for food chain transfer to higher organisms. This initial phase of research provided the necessary background characterization for more detailed studies. The second phase of research (Chapter II) was designed to be more mechanistic in nature and provided insight into specific processes that affected the fate of dietary quinoline. Each of the potential excretory pathways for rainbow trout were studied (i.e., branchial, biliary, urinary, dermal, fecal) and relative contribution of each pathway to total excretion of quinoline was assessed. The importance of metabolism and dose on whole-body elimination of quinoline was also addressed in these studies. Finally, Chapter III describes a series of experiments that examined the interactions

between digestive processes and absorption and storage of quinoline by rainbow trout. This phase of research was important because it provided insight into general principles applicable to the bioaccumulation of other dietary contaminants by fish. Collectively, this research demonstrated that there are many variables affecting the uptake and fate of dietary quinoline. Each of these variables must be considered in hazard assessment because of the dynamic nature of mechanisms that affect uptake and storage of contaminants from the environment.

CHAPTER I. UPTAKE, DISTRIBUTION, AND ELIMINATION OF DIETARY QUINOLINE BY RAINBOW TROUT (*SALMO GAIRDNERI*)[1]

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ABSTRACT

Rainbow trout (Salmo gairdneri) readily absorbed ^{14}C-quinoline from pelleted food and whole body concentrations reached apparent equilibrium after 10 days feeding. Maximum whole body concentrations of ^{14}C-quinoline were only 30 ng/g of quinoline plus metabolites after 7 days depuration. Initial rates of uptake and elimination varied widely among tissues. The uptake constants ranged from 0.0006/day for muscle to 0.1455/day for gallbladder plus bile. Mean elimination half-life for quinoline and its metabolites ranged from 0.4 days in gills to 8.7 days for muscle. Depending on tissue, 58-83% of the stored radioactivity was present as metabolites. About 14% of the radioactivity in the bile was present as glucuronide conjugates. Rainbow trout eliminated 66% of the ingested dose 24 hr after feeding. Biliary and fecal excretion were minor routes of elimination.

INTRODUCTION

Dietary absorption of organic compounds by fish has received little study despite evidence that diet is a major source of body residues for hydrophobic compounds (Chadwick and Brocksen, 1969; Pizza and O'Conner, 1983; Rubenstein et al., 1984). Evidence for potential pollutant accumulation by fish through food chain transfer was recently reported by Hilton et al., (1983). They found that rainbow trout (Salmo gairdneri) accumulated tissue concentrations that exceeded limits prescribed to protect human health when fed fish-meal diets naturally contaminated with PCBs and mirex. While the potential for bioaccumulation of some toxic materials may generally be greater throught aqueous routes rather than through dietary sources (Macek et al., 1979), food chain transfer could be a major route of bioaccumulation for lipophilic compounds that partition into biological compartments of aqueous systems.

Many studies have been conducted to characterize the fate and metabolism of common organic pollutants by fish following uptake through oral routes (Roubal et al., 1977; Lu et al., 1978; Gruger et al., 1981; Varanasi et al., 1979, 1981; and others). While these studies described specific metabolic pathways that function in teleost fish, many involved force-feeding or gavage techniques known to affect absorption. Thus, interactions with normal digestive processes associated with feeding may have affected the disposition and fate of the compounds.

Our studies were designed to determine the potential for food chain transfer of the basic nitrogen heterocycle quinoline following

ingestion at low contaminant concentrations. Specific mechanisms of bioaccumulation of nitorgen-containing polycyclic aromatic compounds have received little study despite evidence that some members of this chemical class are mutagenic (Guerin et al., 1980; Later et al., 1982). Quinoline is found in many petroleum products and liquid fuel subsitutes and is a known hepatocarcinogen in mammalian systmes (Hirao et al., 1976). Most two-ring heterocycles, including quinoline, are relatively water-soluble and are readily taken up from the water by fish prey organisms, including small fish (Southworth et al., 1980; Bean et al., 1985) and crustacean zooplankton (Dauble et al., 1985).

We describe bioaccumulation of quinoline and its metabolites for rainbow trout fed known amounts of radiolabeled comound. Apparant assimilation rates and *in vivo* metabolism of quinoline are also presented.

METHODS

Juvenile rainbow trout, Kamloops strain, were used for all experiments. Fish were obtained from stocks reared and maintained at our laboratory, and place in the aquaria 1 week prior to testing to facilitate acceptance of food pellets. Fish averaged 99 ± 4 mm fork length and 10.5 ± 1.7 g wet wt. Exposure temperatures were maintained at 12°C by a water bath. The photoperiod was 12 hr light: 12 hr dark, and all exposures were static. Aquaria water was continuously recirculated through an air-lift carbon filter to remove soluble organics excreted to the water. The bottom of each aquarium was screened to restrict ingestion of feces.

EXPERIMENTAL

Three separate experiments were conducted: 1) initial studies described tissue disposition and uptake and elimination kinetics; 2) metabolism of stored quinoline was described at elevated levels; 3) temporal pattern of excretion for quinoline and metabolites was determined after a single feeding.

Bioaccumulation. Two fish each were housed in 30 L glass aquaria and separated by screens of 6 mm Vexar netting (N = 32). Four fish were removed from randomly selected aquaria after 1, 2, 4, 7, and 10 days feeding of ^{14}C-labeled quinoline (13.8 ug/day) for determination of uptake rates in tissues. Remaining fish were then fed a noncontaminated diet and sampled at 1, 4, and 7 days post-exposure to determine rates of elimination. Duplicate 1 mL water samples were

taken from each aquarium daily to measure levels of residual radioactivity. All fish were removed 24 hr after their last feeding for tissue analysis. Tissues sampled included gallbladder plus bile, liver, gills, muscle, gut, eye, brain, and carcass (remaining tissue and bone). Subsamples (0.5 g) were taken for analysis of muscle, eyes, and carcass as described by Bean et al., (1985). Gut contents were also analyses for total radioactivity. All tissue weights are reported as wet wt. Mean dry wt conversions as % wet wt (APHA, 1980) were determined as: eyes, 14.9%; brain, 19.2%; kidney, 20.0%, liver, 25.3%; gills, 30.1%; gut, 28.2%; muscle, 22.5%; carcass, 30.4%; gut contents, 21.1% (N = 5).

Metabolism- Individual fish were isolated and fed 170 ug quinoline/day for 2 days to determine the extent of metabolism of parent compound. Each fish (N = 16) was killed 24 hr after the second feeding. The gallbladder plus bile, liver, gut, gut contents, and muscle tissue were removed and stored at -50°C until chemical analysis.

Excretion- Two rainbow trout were separately housed in 30 L capacity glass aquaria under static conditions for short-term excretion studies. Duplicate 1 mL water samples were taken at 1, 2, 4, 8, and 24 hr after feeding of 1 uCi (1.5 mg quinoline/kg dose) of ^{14}C-quinoline each for determination of total radioactivity.

DIET AND FEEDING

The defined composition of the food used (BioProducts, Inc.) as % dry wt., was crude protein, 40.0% (min); fat 14.5% (min); ash, 11.5% (mas); and fiber, 2.7% (max). Food was mixed with radiolabeled

solution and water to achieve a moisture content of approximately 40% and reformed by syringe into pellets weighing about 100 mg each. Pellets were placed into preweighed glass vials and stored at -70°C until use. New food was mixed at days 0, 4, and 7 of the uptake phase and subsamples retained for chemical analysis.

Measured concentrations were 138 ug quinoline/g food (as ^{14}C quinoline) during the bioaccumulation and excretion studies and 1706 ug quinoline/g food (^{14}C plus nonlabeled quinoline) for the metabolism study. All feeding was at 1% body wt/day.

ANALYTICAL CHEMISTRY

The ^{14}C-quinoline (uniformly labeled in the benzo ring) was synthesized by Pathfinder Labs, Inc. St. Louis, MO, and purified (>99%) in our laboratory by liquid chromatography prior to use. Specific activity was 8.83 uCi/mM. Nonradioactive quinoline (ultra-pure grade Aldrich) was disolved in methanol for mixing in the food pellets. NCS tissue solubilizer and PCS scintillation mixture were purchsed from Amersham/Searle. All solvents were Burdick and Jackson "distilled in glass" grade. Beta-glucuronidase (type 3 from Helix pomatia) was obtained from Sigma Chemical Company.

Tissue samples were weighed and digested with 1.0 mL aqueous NaOH at 90°C for 1 hr. The digestate was subsampled for radiocounting by pipetting a 200 uL aliquot into tared scintillation vials. Weighed subsamples were then treated with 6 drops concentrated glacial acetic acid and 5 mL distilled water plus 12 mL PCS cocktail. Remaining digestate samples were stored at -70°C for chemical analysis.

Analysis of tissue samples for quinoline and quinoline metabolite hydrolysis products was conducted as described by Bean et al., (1985). Samples were digested with alkali, quinoline removed by solvent extraction and any hydroxyquinolines or quinolinethiols resulting from the alkaline hydrolysis were derivatized with acetic anhydride to form the corresponding acetates. Quinoline and derivatized metabolite hydroysis products were analyzed by gas chromatography and gas chromatography/mass spectrometry (GC/MS, Bean et al., 1985).

Additional analyses were performed on methanol extracts. Tissue samples were extracted three times with 1 mL methanol, the combined methanol extracts reducted to 1 mL and aliquots taken for determination of presence of glucuronide conjugates by enzyme hydrolysis and thin-layer chromatography as described by Bean et al (1985). The increase in quantity of mobile material after enzyme hydrolysis (as determined by scraping and counting absorbent at half-centimeter intervals) was taken as the quantity of quinoline-derived metabolites conjugated with glucuronide.

KINETIC MODEL ESTIMATES

The kinetic modeling was based on a two-compartment closed system (food-fish tissue), where the concentration of ^{14}C-quinoline for each tissue is expressed by the model:

$$\text{tissue concentration} = K_1/K_2 C_f\ (1\text{-}e^{-K_2 \text{days}}), \qquad (1)$$

where K_1= uptake rate constant, K_2= elimination rate constant and C_f= concentration of ^{14}C-quinoline in food.

Elimination of ^{14}C-quinoline plus metabolites based on the elimination phase of the experiment is expressed as:

$$\text{tissue concentration} = K_1 e^{-K2days} \qquad (2)$$

where K_1= concentration in the tissue at the beginning of the elimination phase.

Bioconcentration factors (BCF) are expressed as the ratio K_1/K_2. Van Veld et al., (1984) have referred to this ratio as the dietary accumulation factor. Values of BCFs were calculated based on elimination rate constants from both uptake and elimination phases of the experiment.

Elimination half-lives for individual organs were calculated with the equation:

$$D = \ln (C_{0.5}/K_1)/-K_2 \qquad (3)$$

where D = elimination half life and $C_{0.5}$ = 0.5 (concentration at end of uptake phase). This equation is based on equation (2) for the uptake phase.

Models were tested for lack of fit (Draper and Smith, 1981) following the estimation of uptake and elimination rate constants. The NLIN procedures in the Statistical Analysis System (SAS) was used to estimate the uptake and elimination rate constants of the models

using the approach outline by Ralston and Jennrich (1978) for parameter estimation via nonlinear least squares.

RESULTS

BIOACCUMULATION

Quinoline was taken up rapidly from the diet by juvenile rainbow trout and was distributed throughout all tissues. Although most tissues exhibited similar patterns of mobilization, initial rates of uptake and elimination varied widely among tissues (Figure I.1). Observed uptake rates for the gallbladder plus bile, gut, and liver were greater than those observed for whole body levels. Whole body concentrations of parent compound plus metabolites appeared to be near equilibrium after day 7 of the 10-day feeding period. Total body burden was relatively low; maximum whole body concentrations as ^{14}C-quinoline were 0.07 ng/g equivalent. Residual tissue levels declined to less than 0.03 ng/g equivalent quinoline plus metabolites after 7 days depuration.

The uptake rate constants for rainbow trout tissues ranged from 0.0006/day for muscle to 0.1455/day for gallbladder plus bile (Table I.1). Elimination rate constants (K_2) based on uptake were lowest for the carcass, liver, and gut tissue (0.2213-0.2519/day) and were consistent with the apparent non-equilibrium uptake profiles. When K_2 was estimated from the elimination data alone (Table I.1), predicted elimination rate constants showed the greatest changes for tissues that exhibited either the highest (gallbladder, gill) or lowest (eye, muscle) rate of elimination. Mean elimination half-life of quinoline and metabolites ranges from 0.3 days for material stored in the gallbladder to 8.7 days in muscle. The elimination rate

Figure I.1 Bioaccumulation profiles of ^{14}C-quinoline in gallbladder, gut, muscle, and whole body of rainbow trout during dietary exposures of 13.6 ug/day. Error bars represent 1 S.D. about the mean, N=4.

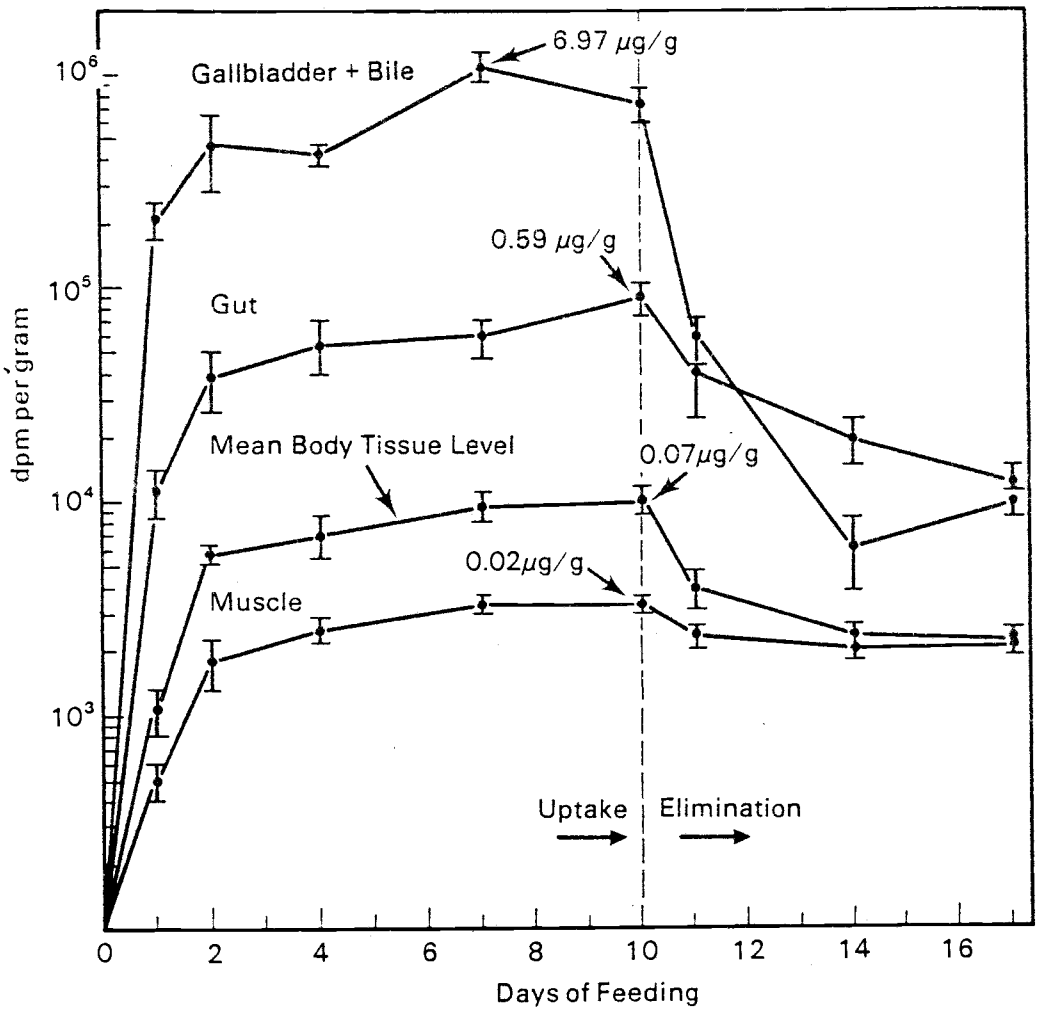


Table I.1 Rate constant estimates, bioconcentration factors and elimination half-lives for ^{14}C-labelled quinoline kinetics in rainbow trout

Organ	Rate Constant Estimates			Bioconcentration Factors Based on:		
	Based on Uptake		Based on Elimination			
	Uptake (K_1)	Elimination(K_2)	Elimination(K_2)	Uptake Phase	Elimination Phase	Mean Elimination Half Life (Days)
Brain	0.009 (0.006)D	0.4156 (0.3582)	0.1972 (0.1170)	0.0022	0.0046	3.3 (1.6)
Carcass	0.007 (0.003)	0.2519 (0.1740)	0.3299 (0.1660)	0.0028	0.0021	2.1 (0.9)
Eye	0.0011 (0.0002)	0.4910 (0.1372)	0.0957 (0.0285	0.0022	0.0115	6.0 (1.0)
Gall Bladder	0.1455 (0.0577)	0.3888 (0.7578)	2.3630 (2.3688)	0.3742	0.0615	0.3 (0.2)
Gill	0.0054 (0.0063)	0.5416 (0.7578)	2.5321 (2.3688)	0.0100	0.0021	0.4 (0.2)
Gut	0.0095 (0.0032)	0.2213 (0.1245)	0.4805 (0.1635)	0.0429	0.0198	1.4 (0.3)
Kidney	0.0022 (0.0006)	0.3022 (0.1219)	0.2145 (0.0646)	0.0073	0.0103	3.0 (1.0)
Liver	0.0032 (0.0009)	0.2316 (0.1127)	0.2542 (0.0774)	0.0138	0.0126	2.4 (0.8)
Muscle	0.0006 (0.0001)	0.3354 0.0830)	0.0662 (0.0219)	0.0018	0.0091	8.7 (0.9)

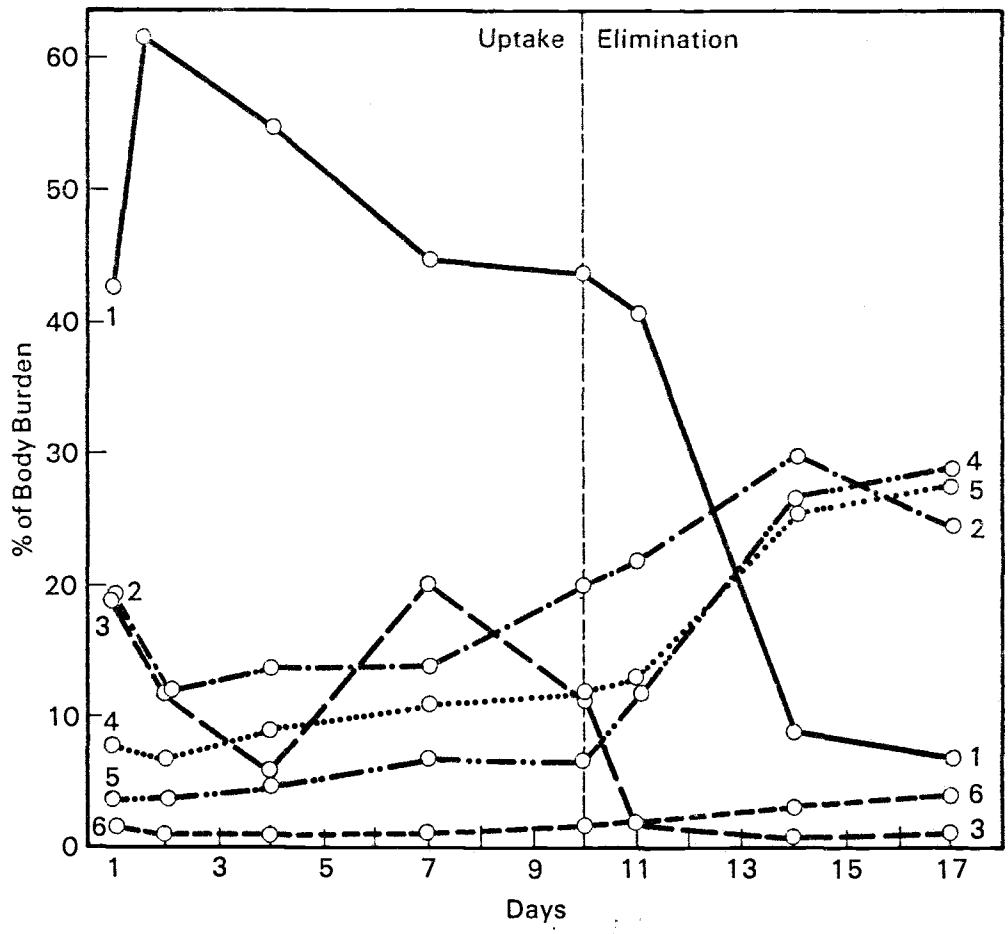
[a]Value in parentheses is asymptotic standard error, N=4

constants differed markedly for certain tissues (e.g., eye) and resulted in a marked difference in estimates of bioconcentration factors. K_2, based on the elimination phase may be a better value because it yields an estimate that is independent of K_1.

Calculated bioconcentration factors or dietary accumulation factors (DAF, Van Veld et al., 1984) based on total radioactivity were less than 1.0 for all tissues. Highest tissue DAFs were similar to observed values after 10 days feeding, model parameters for the uptake phase in gallbladder and the elimination phase for the eye yielded models with significant lack of fit ($P < 0.05$). In addition, residual analyses for all tissues in both the uptake and elimination phases suggested minor lack of fit to the two-compartment model employed.

Relative distribution of ^{14}C-quinoline changed little during the 10-day uptake period, but varied over the 7-day elimination period (Figure I.2). Collectively, the gallbladder and gut comprised an average of 48 to 64% of the total body burden for any day. The liver and kidney contained less than 7% of the total body burden (excluding gut contents) at all sampling intervals. Both the muscle tissue and carcass exhibited a slight increase in proportion of total radioactivity stored with duration of feeding. For example, relative amounts of quinoline plus metabolites increased from 6.4% to 11.6% and 16.0% to 21.1% in the muscle and carcass, respectively, at 1 and 10 days feeding. The greatest changes observed in relative distribution of ^{14}C-quinoline following depuration occurred in the gallbladder, carcass, and muscle tissue. Relative distribution of

Figure I.2 Relative distribution of radioactivity in rainbow trout tissues and gut contents as percent body burden. Key: 1, gut contents; 2, gut; 3, gallbladder plus bile; 4, carcass; 5, muscle; 6, liver. Values represent means of four fish.



quinoline plus metabolites in other tissues remained consistent with the pattern observed after the 10-day uptake period.

Total radioactivity after 10 days feeding, as percent of the body burden (excluding gut contents), was ranked as gut tissue> gallbladder plus bile> carcass> muscle> gill> liver> kidney> eye> brain (Figure I.3). Relative proportions of residual radioactivity found in the muscle and carcass increased after 7 days depuration, while material retained in the gut tissue and gallbladder plus bile decreased markedly. The amount of quinoline plus metabolites in the gut contents ranged from 80-203% of the total stored in all tissues. Maximum concentrations of radioactivity in gut contents were attained after the second feeding. Relative proportions declined after 4 days of feeding and were only 8% of the total tissue burden following 7 days of elimination.

Absolute concentrations (ug equivalent quinoline/g tissue) were always greatest in the gallbladder plus bile during the uptake period. These concentrations were similar to those measured in the gut contents at days 2, 4, and 10 (Table I.2). Maximum concentrations of ^{14}C-quinoline in the bile (6.97 ug/g) occurred after 7 days feeding. Total radioactivity in the stored bile at that time represented 0.4% of the daily ingested dose. Concentrations of ^{14}C-quinoline in the muscle, brain, and kidney never exceeded 0.10 ug/g. Maximum concentrations of parent compound plus metabolites in the gut tissue (0.59 ug/g) and liver (0.19 ug/g) were found after 10 days feeding and appeared to be increasing.

Quinoline and its metabolites were incorporated into rainbow trout tissues at rates ranging from 0.5 to 1.4% of the cumulative

Figure I.3 Distribution of quinoline and metabolites in tissues of juvenile rainbow trout at equilibrium whole body levels and after depuration. Error bars shown as mean ± S.E., N=4.

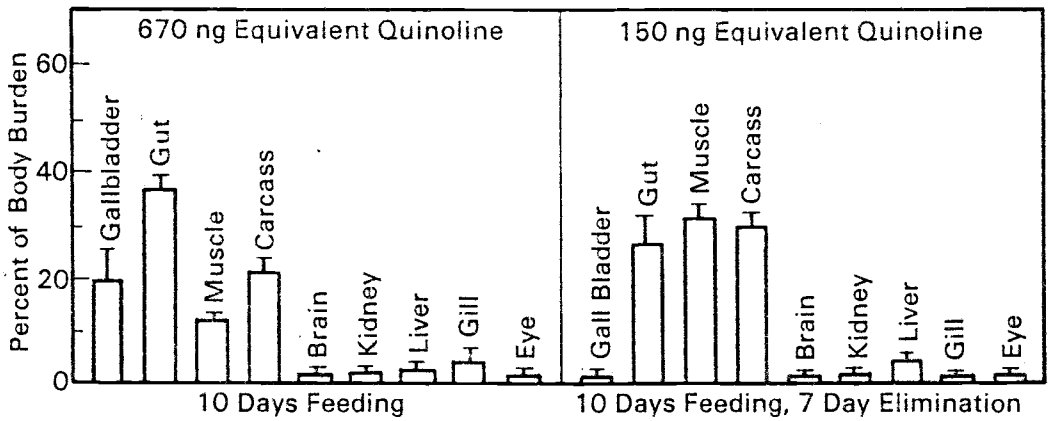


Table I.2 Concentration of ^{14}C-quinoline (µg/g ± SE) in juvenile rainbow trout tissues (N=4)

	Uptake (Days)					Clearance (Days)		
	1	2	4	7	10	11	14	17
gall bladder	1.36±0.25	3.87±0.99	2.66±0.27	6.97±0.74	3.96±0.80	0.37±0.10	0.04±0.02	0.06±0.01
gut	0.08±0.02	0.32±0.05	0.35±0.10	0.39±0.08	0.59±0.10	0.26±0.08	0.13±0.04	0.09±0.03
muscle	<0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.01	0.01
brain	<0.01	0.01	0.04±0.02	0.02±0.01	0.03±0.01	0.01	<0.01	0.01
kidney	0.02±0.01	0.06±0.01	0.07±0.02	0.09±0.01	0.10±0.02	0.05±0.01	0.03±0.01	0.03±0.01
liver	0.03±0.01	0.10±0.02	0.10±0.03	0.13±0.02	0.12±0.04	0.09±0.01	0.05±0.01	0.06±0.01
gill	0.04±0.03	0.03±0.01	0.24±0.12	0.06±0.03	0.15±0.06	0.01	<0.01	<0.01
eye	<0.01	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01	0.02±0.01	0.01	0.01±0.01
carcass	<0.01	0.02±0.01	0.02±0.01	0.03±0.01	0.04±0.01	0.01	0.01	<0.01
gut contents	0.77±0.22	3.82±0.34	2.55±0.74	3.34±0.37	3.74±0.40	1.03±0.24	0.10±0.07	0.06±0.01

ingested dose (Figure I.4) Maximum values occurred after the second day of feeding and declined slightly thereafter. Assimilation rates of ^{14}C-quinoline declined after the second feeding when based on the mean daily ingested dose. Total radioactivity stored in rainbow trout tissues and gut contents after 10 days feeding was 4.9% of the mean daily ingested dose (Figure I.4).

METABOLISM

Concentrations of accumulated parent compound and/or metabolite in fish tissue after 10 days feeding at 13.7 ug/day were too low for direct analysis of metabolites by gas chromotography (<0.1 ug per individual compound). Fish examined after 2 days ingestion of 170.6 ug of quinoline stored the majority of material as metabolites (Table I.3). The gallbladder plus bile contained the highest concentrations of both parent compound and total metabolites; mean levels were 12.5 and 50.8 ug/g, respectively. The gut tissue retained 42% of total radioactivity as quinoline, while only 17% of the total radioactivity in muscle tissue was parent compound.

Neither hydroxyquinolines nor quinolinethiols were detected in any of the high-dose tissues analysed using the base-digestion/derivatization methodology described by Bean et al., (1985) for rainbow trout exposed quinoline in water. Attempts to identify the metabolic products in methanol extracts from gallbladder bile (both before and after enzyme hydrolysis) and in alkaline digestates of bile were also made using derivatization techniques. Use of reagents that form methyl, acetyl, and silyl derivatives from

Figure I.4 Assimilation of dietary ^{14}C-quinoline into rainbow trout tissues and relationship to total (cumulative) and mean daily dose. Values shown as mean ± S.D., N=4.

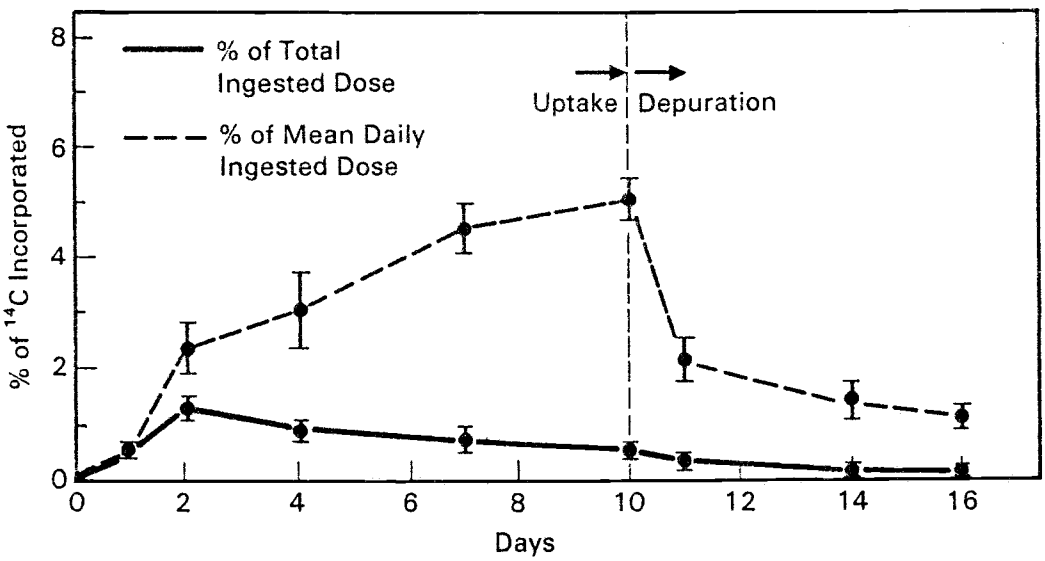


TABLE I.3. Concentration of quinoline plus metabolites (total radioactivity) in tissues of rainbow trout after 2 days feeding at 1706 ug/g 14C quinoline and relative amount stored as parent compound. All values mean ± S.D., N=3.

Tissue	ug/g equivalent 14C-quinoline	% as quinoline
liver	2.5 ± 0.9	32 ± 6
gallbladder	63.5 ± 20.5	20 ± 7
muscle	0.3 ± 0.2	17 ± 4
gut	7.6 ± 2.9	42 ± 1
gut contents	38.2 ± 9.9	32 ± 17

hydroxylated substrates did not result in products identifiable by GC/MS.

Methanol extracted 90% of the radiolabel from bile samples. Comparison of thin-layer mobility indivated that about 14% of the radiolabel was present as glucuronid conjugates. Methanol extraction removed about 80% of the radiolabel from samples of liver, gut, and gut contents, and about 50% from muscle tissue, but none of these samples gave evidence of glucuronide conjugation.

EXCRETION

Rainbow trout eliminated most of the ingested radioactivity within the first 12 hr after feeding. The greatest rate of excretion occurred in the first 6 hr following ingestion when approximately 31% of the parent compound and/or metabolites were eliminated to the water. Recovered radioactivity in the water accounted for 66% of the ingested dose after 24 hr. The fraction in fecal pellets was not measured, but only 0.7% of the total ingested dose remained in the digestive system 24 hr after the single feeding.

DISCUSSION

Juvenile rainbow trout were approaching an apparent equilibrium residue value based on whole body measurements after only 10 days feeding with quinoline. This may be partially due to the low ingested dose. We fed fish at concentrations potentially found in fish food organisms (Dauble et al., 1985; Bean et al., 1985); however, time to steady-state and absolute tissue burdens can vary according to both length of exposure and to ingested dose (Hamelink and Spacie, 1977). Tissue burdens may not exhibit a linear relationship with dose since the effeciency of elimination can change with contaminant level in the diet (Chadwick and Brocksen, 1969; Shubat and Curtis, 1986).

The greatest concentrations of quinoline plus metabolites were found in organs associated with the digestive process. Concentrations in the gut tissue and liver steadily increased during dietary exposure and, in contrast to other tissues, did not appear to be at steady-state after 10 days feeding. About 50% of the total body burden during the uptake period was in gut contents. Although relative contribution declined to 7% after 7 days depuration, it indicates the marked influence of gut contents on whole-body concentrations of this xenobiotic. In addition, it shows that the use of whole body measurements of organisms to assess the bioaccumulation of dietary contaminants can be misleading. For example, aquatic insects have been shown to contain up to 86% of the whole-body burden of metals in their gut, depending on feeding habits (Smock, 1983). Relative concentrations of material in the gut will

likely affect concentration in the gut tissue, in addition to potential for absorption and transport to other tissues. The high levels of quinoline and metabolites in the gut contents during the uptake phase may be indicative of incomplete absorption of dietary quinoline or of high hepatic clearance rates.

Because 14 to 36% of the tissue burden was stored in the gallbladder during feeding it appeared that biliary excretion was the major route of elimination. High concentrations of chemical in the bile are indicative of a high degree of hepatic uptake because the gallbladder stores exocrine secretions of the liver (Fange and Grove, 1979). However, discharge of bile into the gut is an active process that is stimulated by the presence of food in the stomach (Smith, 1973). Near maximum levels of radioactivity would have been present when we sampled the gallbladders (24 hr post-feeding) because they were full and because the bile tends to become concentrated during storage (Smith, 1974; Fange and Grove, 1979). However, despite the high concentrations relative to other tissues, the gallbladder never contained more than 0.36% of the cumulative ingested dose.

Principal storage of quinoline-derived radioactivity occurred in the muscle and carcass after depuration. This is not surprising since these tissues constitued approximately 72% of the total body mass. The apparent persistence of quinoline metabolites in these tissues suggests, however, that material remains highly bound and is available for food chain transfer.

That less than 10% of the total body burden was found in gills and kidney is partly the result of their small mass in addition to their primary role as excretory rather than storage organs. The

relatively high concentration of radioactivity found in the gills of fish after 4 and 10 days feeding may be indicative of the role of gills in clearance of quinoline. Contamination by food particles should have been minimal since most of the ration had moved out of the stomach at 24 hr post-feeding. Relative amounts or concentrations of quinoline are best examined with respect to temporal patterns because of functional patterns of storage. Indeed, patterns of bioaccumulation by tissue may not even be evident unless examined at the cellular level of organization. For example, Balk et al., (1984) showed that benzo(a)pyrene was heterogeneously mobilized in the kidney of northern pike (Esox lucius) and that the pattern was likely related to urine production.

Based on the results of tissue analysis for quinoline metabolite products, it is clear that the metabolic pathway of quinoline in rainbow trout after ingestion is different from that after aqueous exposures. None of the products observed after absorption of quinoline at 1 mg/L in water (Bean et al., 1985) could be detected in fish that were fed quinoline. It is possible that significant transformation of quinoline occurred in the gut prior to absorption since analysis of gut contents indicated that 58% of the radiolabel was in a form other than quinoline. However, altered quinoline material in the gut may have also originated from hepatic metabolism, biliary excretion, and subsequent discharge into the gut.

Equilibrium tissue residues of quinoline and metabolites were much lower for our dietary exposures than reported previously during aqueous exposures (Bean et al., 1985). However, this may be related to a dose-response phenomena rather than greater efficiency of uptake

by gills from water. Shubat and Curtis (1986) reported that proportionally higher body burdens were found in fed versus water-exposed rainbow trout using dieldrin (a chlorinated hyrocarbon stored and excreted primarily as parent compound), when based on relative toxicity. Thus, direct comparisons of tissue burden as a percent of the toxic dose may be misleading because differences in routes of absorption may affect metabolism and storage.

Differences between tissue distribution for aqueous and dietary sources of quinoline can be largely explained by routes of entry and absorptive surface available for exchange. For example, xenobiotics taken up in the diet are absorbed from the gastric and intestinal mucosa and transported to the liver via portal veins (Fange and Grove, 1979). Xenobiotics absorbed across the gill membranes enter the general systemic circulation before passing through the liver. Storage of quinoline metabolites is also related to physiological processes associated with feeding and digestion. Unfed fish exposed to water-borne quinoline retained high concentrations of metabolite residuals in stored bile during depuration (Bean et al., 1985). In contrast, fed fish that were depurated during dietary exposures apparently ejected gallbladder bile and retained minimal amounts of quinoline metabolites after only 1 day.

The hatchery diet used in our study was higher in total fat content, digestible organic matter, and protein than natural invertebrate foods (Phillips et al., 1954; Windell et al., 1969), but comparable in protein content to forage fish prey. The biochemical composition of the diet is important since it determines digestibility and gastric evacuation rates (Windell et al., 1969).

Additionally, interactons between the diet and toxic effects of chemicals occur that may be attributed to effects on the activities of specific, xenobiotic metabolizing enzymes (Gingerich, 1982). Because of these modulatory effects, chemicals presented by intraperitoneal (IP) or force-feeding routes can be expected to behave differently in organisms with voluntary feeding. For example, Roubel et al., (1977) reported that mode of exposure (IP vs. pelleted dose) affected assimilation rates or rates of incorporation of hydrocarbons into certain tissues of coho salmon (Oncorhynchus kisutch). In studies with naphthalene, Varanasi et al., (1979) found that the extent of biotransformation via IP dose was less than when starry flounder and rock sole were force-fed naphthalene in salmon oil. Force-feeding usually decreases the rate of evacuation of a meal from the gut when compared to fish that take the food voluntarily (Fange and Grove, 1979).

Our approach treated individual tissues as the second compartment in a series of two-compartment systems. This allowed for estimates of uptake and elimination rate constants, DAFs, and elimination half-lives to be based on a first-order kinetic model. This approach is a simplification of the food-fish tissue system since the kinetically described curve may, in reality, serve as a generalization of a family of curves, which represent uptake and elimination of parent comound and metabolites from several tissue pools. However, the model provides a means of evaluating the extent to which a comound and metabolites are accumulated within various tissues. The model employed was mainly useful from a comparative perspective (i.e, between tissues) rather than as a predictor of

bioaccumulation. The rate constants, DAFs, and elimination half-times describe, in part, the nature of the accumulation process for a particular tissue, even though confounding variables of intermediate metabolism and inter-tissue transport are known to contribute. Utility of this approach is suggested by the high degree of fit for individual tissues and the first-order kinetic model. These estimates also allow comparison of the effects of differing exposure rates on bioaccumulation, when similar estimates are obtained for fish tissues exposed via different routes of exposure.

Collectively, these studies indicated that uptake and storage of dietary ^{14}C-quinoline by rainbow trout is a dynamic process that is influenced by a wide variety of physiological and biochemical processes. There is little potential for quinoline to transfer to higher trophic levels as parent compound because most material is retained as metabolites. However, the nature of these metabolites should be elucidated because of apparent persistence in certain tissues.

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CHAPTER II. RAPID BRANCHIAL EXCRETION OF DIETARY QUINOLINE BY RAINBOW TROUT (*SALMO GAIRDNERI*)[1]

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[1] submitted to Toxicology and Applied Pharmacology

ABSTRACT

Excretion of the nitrogen heterocycle ^{14}C-quinoline was characterized in rainbow trout following its ingestion with food. Quinoline-derived radioactivity was readily absorbed from the stomach; peak serum levels occurred 4 to 8 hr after the single feeding. Pharmacokinetics were described using a two-compartment body model with first-order absorption and disposition; estimated half-lives for the α and β phase were 4.1 and 54.1 hr, respectively. About 98% of the ingested dose was absorbed by gut epithelium before fecal excretion. Depending on dose, 71-83% of the ingested radioactivity was excreted during the first 24 hr after feeding. Branchial excretion was the primary route of excretion since all other routes (fecal, biliary, urinary, dermal) contributed <5% of the eliminated dose within 48 hr after ingestion. There was evidence for saturation of minor excretory pathways as the dose was increased from 1 to 100 mg quinoline/kg body weight with apparant spillover into the branchial route at the 100 mg/kg dose. Quinoline was eliminated across the gills as parent compound.

INTRODUCTION

Most studies of the fate of nitrogen heterocycles in the aquatic environment have focused on tissue distribution and metabolism of stored materials because of the obvious links to human health. Few have examined specific mechanisms of excretion for contaminants potentially ingested by fish. Previous studies show that quinoline is rapidly eliminated by rainbow trout (Salmo gairdneri) following both aqueous (Bean et al., 1985) and dietary (Dauble et al., 1987) exposures. Although most stored material was present as quinoline metabolites, the role of biotransformation in elimination of quinoline is unknown.

Quinoline is a basic nitrogen-containing heterocyclic compound that occurs in many industrial products, petroleum, shale oil, and coal liquefaction materials (Weisberger and Williams, 1980; Wright and Later, 1985). It is a known hepatocarcinogen in mice and rats (Hirao et al., 1976; Shinohara et al., 1977) and mutagenic in Ames assays (Nagao et al., 1977; Hollstein et al., 1978). Quinoline is relatively soluble in water and rapidly absorbed by fish and some aquatic invertebrates (Southworth et al.; 1980, Bean et al., 1985; Dauble et al., 1985). That nitrogen-containing aromatic compounds persist in the aquatic environment is evident by their presence in sediments from polluted estuaries (Krone et al., 1986).

The objective of our studies was to determine the relative importance of major excretory pathways in the elimination of dietary quinoline by juvenile rainbow trout. Pharmacokinetics, absorption, and rates of gastrointestinal movement of quinoline were determined

following ingestion, and the chemical form of excreted radioactivity was characterized. The effect of dose on elimination was also examined.

METHODS

ANIMALS

Juvenile rainbow trout, Kamloops strain, were used for all experiments. Fish obtained from stocks reared at Pacific Northwest Laboratory (PNL) were placed in individual 20-L glass aquaria for 7 to 10 days before testing to facilitate voluntary feeding. Exposure temperatures were maintained at 12°C by a water bath, and fish were held on a 12-hr light-dark cycle.

CHEMICALS

(UL-^{14}C) Quinoline (8.83 mCi/mM) was synthesized by Pathfinder Labs, Inc. (St. Louis, Mo) and purified (>99%) at PNL by liquid chromatography before use. Quinoline, 5-hydroxyquinoline, 4-hydroxyquinoline, and 8-hydroxyquinoline glucuronide standards were obtained from Aldrich. The NCS tissue solubilizer and PCS scintillation mixture were purchased from Amersham/Searle. All solvents were Burdic and Jackson "distilled in glass" grade.

DIET AND FEEDING

Food pellets were prepared for each test according to methods described in Dauble *et al.* (1987). Individual doses were calculated by liquid scintillation counting of a subsample to obtain mg-equivalent quinoline and then dividing food weight by post-exposure fish weight. All feeding was at 1% body weight/day. Fish that did not consume their total daily ration within 10 min of feeding were not used in the analysis; most ate all food within 15 sec.

EXCRETORY ROUTE STUDIES

Juvenile trout (24-49 g) were individually isolated in separate aquaria, starved for 48 hr, then fed a single dose of radiolabelled quinoline in a reformed food pellet. At 1, 2, 4, 8, 16, 24, 36, and 48 hr after feeding, fish (N = 3-5) were removed and immobilized in an ice bath. Independent urine samples were taken by pressing the bladder and collecting urine in calibrated glass capillary tubes. Blood was collected by severing the caudal peduncle; duplicate 100-mL serum samples were obtained after centrifugation at 2000 g for 10 min. Epidermal mucus was scraped from the dorsal portion of the lateral line and placed in preweighed glass scintillation vials. The liver, gallbladder plus bile, anterior and posterior kidney, and gill filaments were dissected, rinsed in distilled water, blotted, and placed in pre-weighed vials for liquid scintillation counting. Digestive tract contents were taken from the stomach, pyloric caeca, upper intestine, mid-intestine, and rectum. Concentrations of radioactivity in the food and digestive tract were equalized based on dry weight determinations (APHA, 1980) of gut contents from other fish. Tissue samples were analyzed for total radioactivity as described in Dauble *et al.* (1987). Aquaria water was continuously recirculated through an air-lift carbon filter during the static exposures. Duplicate 1-mL water samples were taken at each tissue sampling interval to monitor for residual radioactivity.

Separate experiments were conducted to clarify the importance of urinary excretion. Repeated measurements of total radioactivity in trout urine were made from individual fish (N = 4) at 4-hr

intervals for 24 hr following feeding. Total excretion of the ingested dose was estimated based on mean measured urine concentrations and estimates of urine flow reported for freshwater-adapted rainbow trout (Hickman and Trump, 1969).

INJECTION STUDIES

Fish (45-72 g) were injected ip with 14-C quinoline to monitor flux across the gills independent of potential contamination from oral dosing. Each fish received a 0.8-mg/kg dose in 0.1-mL saline; water and gill concentrations were determined as described before at 0, 4, 8, and 24 hr post-injection. Tissue and water measurements (N = 2) were taken from paired exposures: aerated only and with recirculating carbon filters.

DOSE-DISPOSITION STUDIES

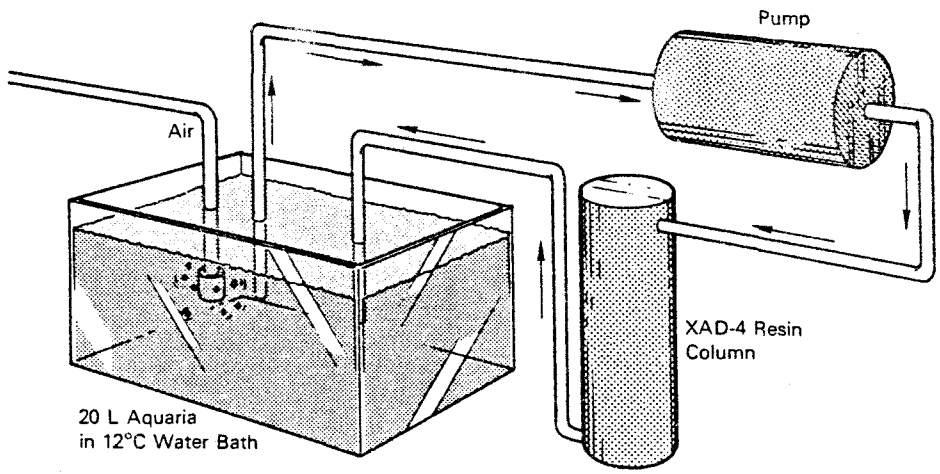
Fish (18-28 g) were separated into 3 treatment groups for feeding: 1 mg/kg, 10 mg/kg, and 100 mg/kg. Whole-body elimination to aerated aquaria was measured based on water concentrations at 0, 1, 2, 4, 6, 8, 12, 16, and 24 hr post-feeding (N = 3). Relative body burden at 24-hr post-feeding was determined for a separate group of fish that were maintained in carbon-filtered aquaria (N = 5). Samples taken included contents of stomach and intestine, liver, gallbladder plus bile, kidney, muscle, brain, mesenteric fat, and carcass (remaining tissue). Muscle and carcass samples were homogenized and subsampled to determine total radioactivity.

METABOLISM STUDIES

Fish (20-28 g) were isolated and fed a single dose of quinoline at 1 mg/kg, 10 mg/kg, and 100 mg/kg (N = 2), maintained in aquaria water from 0-8 hr after feeding, then removed. A recirculating chemical extraction system (Fig. II.1) was set up immediately after feeding to determine the chemical form of excreted radioactivity. Water was pumped through a pre-cleaned XAD-4 resin column (Junk et al., 1974) at 25 mL/min. Water samples were periodically collected from the aquaria water and after column passage to determine trapping efficiency. Aquaria water was filtered until 12 hr after feeding. The resin column was stored overnight at 4°C before extraction with three successive 10-mL washes of distilled ether. The water:ether ratio eluted from the column was adjusted to 1:3 and made acidic (pH 2) by addition of 1 N H_2SO_4. The solution was shaken vigorously for 5 min in a 50-mL glass-stoppered vial, and 100-mL aliquots were removed from both aqueous and ether fractions to determine extraction efficiency.

A 2-mL subsample from the aqueous fraction was neutralized with 0.5 N NaOH, sparged with N_2 at 10 lb/in^2 for 5 min, then analyzed for parent compound and metabolites using reverse-phase high pressure liquid chromatography (HPLC). Analysis by HPLC was conducted using a solvent delivery system of 60:40, methanol:water at 1 mL/min and a Beckman 5-mM ultrasphere C8, 25-cm X 4.6-mm column. Concentration of parent compound was determined by calculating relative peak area at 254-mM absorbance for duplicate 15 to 50-mL injections. Fractions

Figure II.1 Flow-through system used to collect excreted radioactivity from rainbow trout



were collected at 30-sec intervals, and recovered radioactivity compared with retention time of known standards.

STATISTICAL ANALYSIS

Kinetic parameters were determined using a two-compartment body model with first-order absorption and disposition; serum clearance data were resolved into distribution (α) and elimination (β) phases by curve stripping (O'Flaherty, 1981). Significance testing was conducted using analysis of variance (ANOVA) and the t-test for paired comparisons (Sokal and Rolf, 1981). Estimates of whole-body elimination rates were based on the mean percent of the administered dose excreted to the water.

RESULTS

ABSORPTION, TRANSPORT, AND CLEARANCE

Quinoline-derived radioactivity was readily absorbed by rainbow trout after dietary exposure; maximum serum concentrations occurred from 2-8 hr after ingestion (Fig. II.2). Levels of radioactivity declined rapidly after they peaked, and mean serum concentrations at 36 hr were <2% of those at 8 hr. The elimination portion of the curve (8 to 48 hr post-ingestion) was characterized by a distribution (α) phase with an estimated serum half-life of 4.1 hr and an elimination (β) phase with a half-life of 54.1 hr.

Only 8.0-12.7% of the ingested dose was recovered in the digestive tract 2 hr after feeding (N = 2). We found that 3.5-9.0% and 1.1-2.2% of the ingested dose remained at 16 hr and 24 hr, respectively. Based on differences observed between maximum concentrations observed in the stomach and those in the intestine of individual fish, 56-74% of the quinoline was absorbed from the stomach. About 87% of the total ingested dose was absorbed by the time the food bolus reached the mid-intestine at 24 hr.

Quinoline associated with the food was transported from the stomach to the upper intestine at 8-16 hr following ingestion (Fig. II.3). Less than 8% of the ingested dose remained in the stomach at 8 hr. Maximum concentrations of quinoline plus metabolites occcured at 24 hr in the upper region of the intestine. Quinoline-derived radioactivity in the rectum was highest at 48 hr after feeding (Fig. II.4). Equalized concentrations of radioactivity were determined as anterior intestine > mid-intestine = rectum.

Figure II.2 Pharmacokinetics of quinoline plus metabolites in the serum of rainbow trout following a single feeding at 1.6 mg quinoline/kg body weight. Values shown are mean ± S.E. from four to five fish

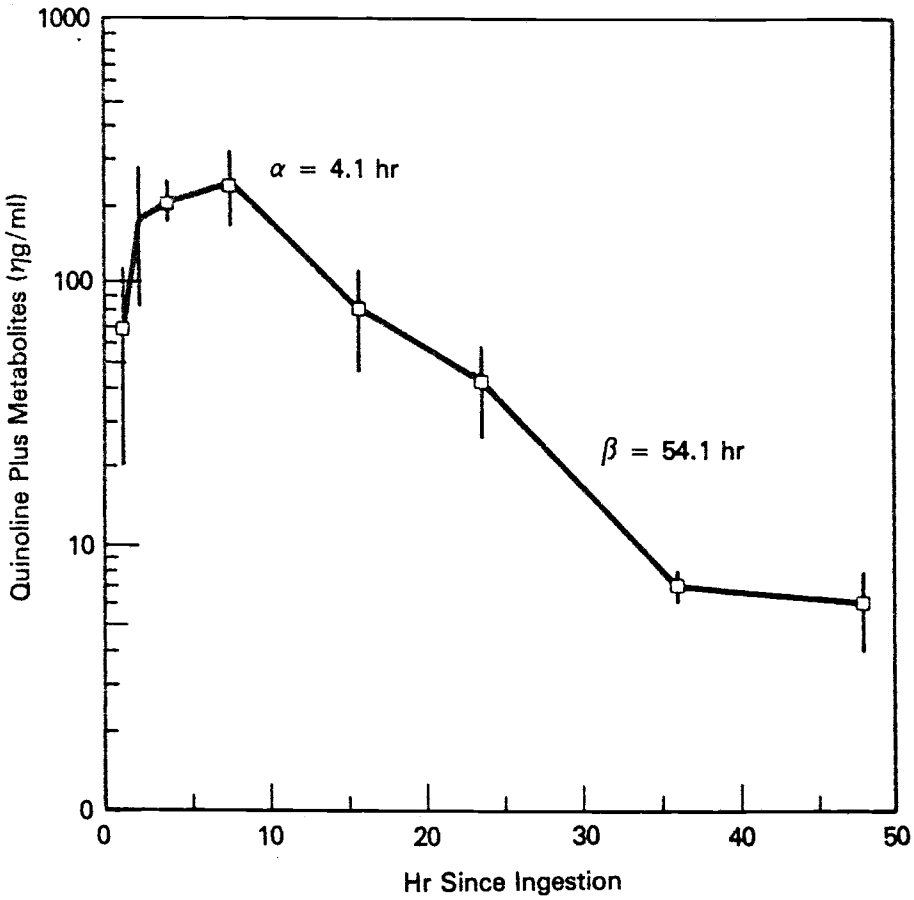


Figure II.3 Concentration of quinoline plus metabolites as the food bolus moved from the stomach into the intestine of rainbow trout. Trout were fed once at 1.6 up quinoline/kg body weight. Values shown are mean ± S.E. from four to five individual fish

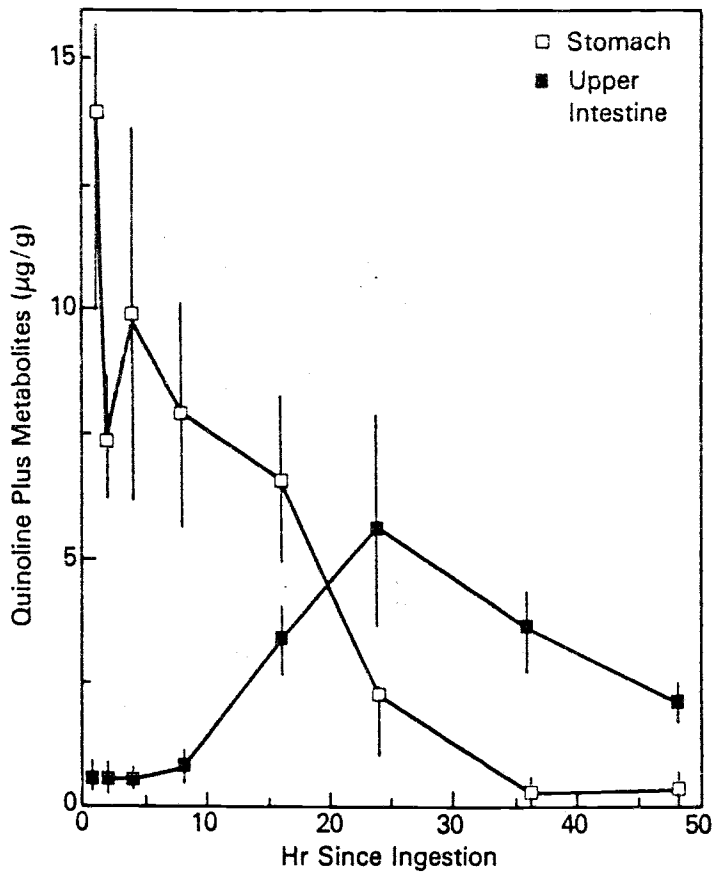
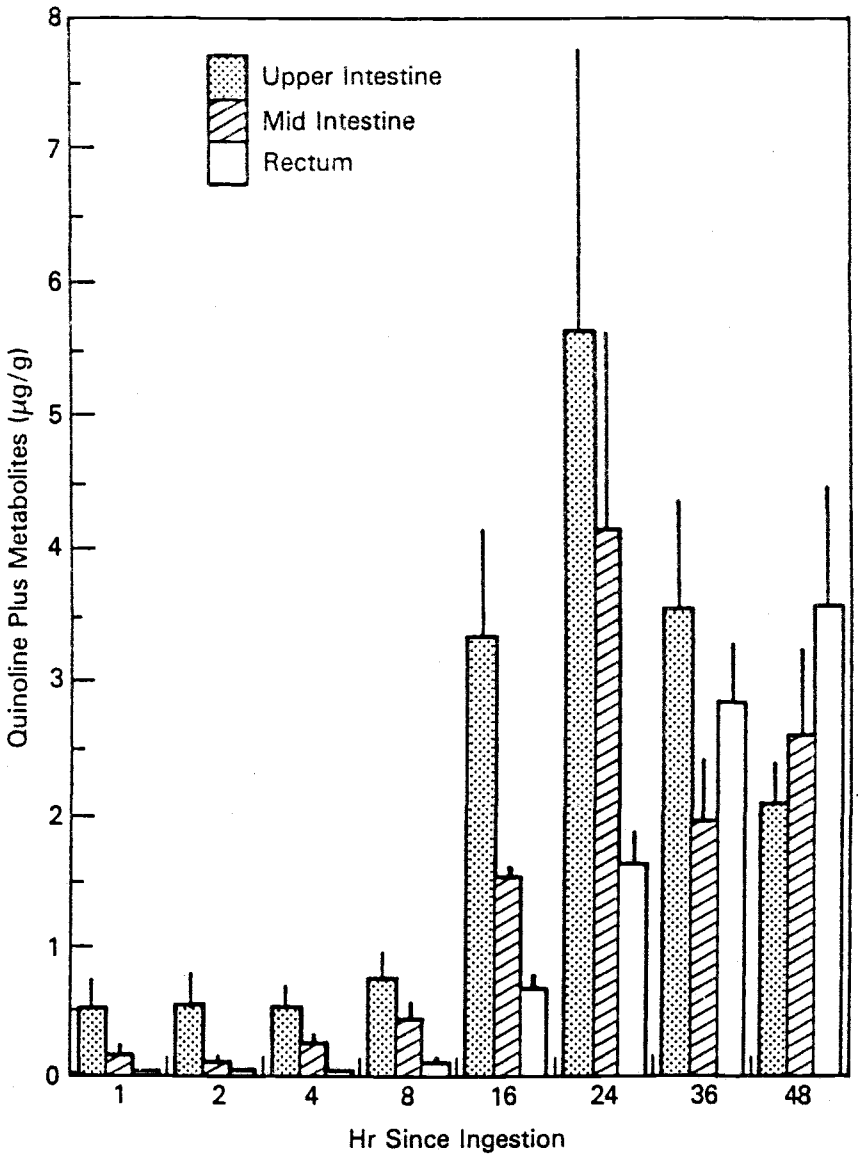


Figure II.4 Changes in mean concentration of quinoline-derived radioactivity during movement of food through the lower digestive tracts of rainbow trout. Vertical bars are 1 S.E.M. based on measurements from four to five fish



Temporal patterns of quinoline plus metabolites present in excretory tissues are summarized in Table II.1. Rapid disposition of radioactivity to the gills, liver, and kidney was evident. Maximum concentrations were observed in the gill at 1 hr, and levels remained higher than liver and kidney tissue for the first 8 hr following ingestion. Uptake patterns for the liver were similar to those observed in the serum. Although there was high variation for the anterior and posterior kidney samples, significant differences were found between the two regions in patterns of stored quinoline residues (ANOVA, $p = 0.001$). Mean maximum concentrations of radioactivity in the posterior kidney were only 42% of those detected in the anterior kidney (Fig. II.5).

CHARACTERIZATION OF EXCRETORY ROUTES

Maximum concentrations of quinoline and metabolites were found in the urine at 4-16 hr after ingestion; mean concentrations declined to 15% of peak values by 48 hr. Mean urine-to-serum ratios (U/S) were near unity for the first 8 hr following ingestion. However, mean U/S values increased to 6.3 at 16 hr and 24 hr, 9.8 at 36 hr, and 20.5 at 48 hr. Urinary excretion accounted for an estimated 0.8-1.6% of the ingested dose for the first 24 hr following ingestion (Table II.2).

Gallbladder bile volume decreased for the first 8 hr following feeding, then increased at 48 hr to about 73% of pre-experimental volume. Amounts of quinoline-derived radioactivity present in the gallbladder generally paralleled these trends (Fig. II.6). Mean values for total radioactivity found in the gallbladder bile ranged

Table II.1 Concentration of quinoline plus metabolites (ng/g) in excretory tissues and serum of rainbow trout following ingestion of ^{14}C-quinoline. Values as mean ± S.D., N=3.

Tissue	Time Since Ingestion (hr)							
	1	2	4	8	16	24	36	48
Gill	847±369[a]	673±233	545±93	431±133	135±5	24±7	8±1	14±7
Liver	324±114	410±93	420±161	331±102	204±40	79±18	35±4	33±10
Gallbladder plus bile	227±96	466+57	561±206	929±332	5129±1054	4574±456	5236±179	5165±1212
Anterior kidney	442±158	496±116	338±80	244±50	58±9	53±8	24±5	29±10
Posterior kidney	207±84	93±17	154±33	136±26	176±59	96±12	45±11	65±19
Serum	68±48	189±102	210±46	248±90	80±37	42±16	7±1	6±2

[a] Value as mean ± S.E, N = 4-5; concentrations are in ng/g.

Figure II.5 Patterns of quinoline-derived radioactivity in the anterior (K1) and posterior (K2) section of the trout kidney following ingestion of 14-C quinoline. Values are mean ± S.E. and based on measurements from four to five fish

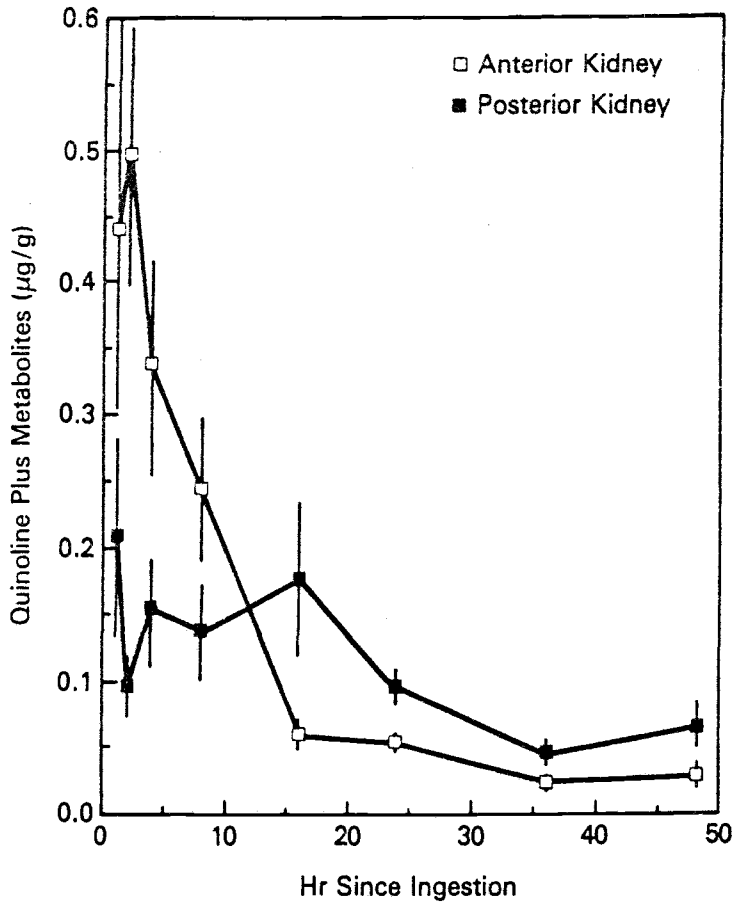


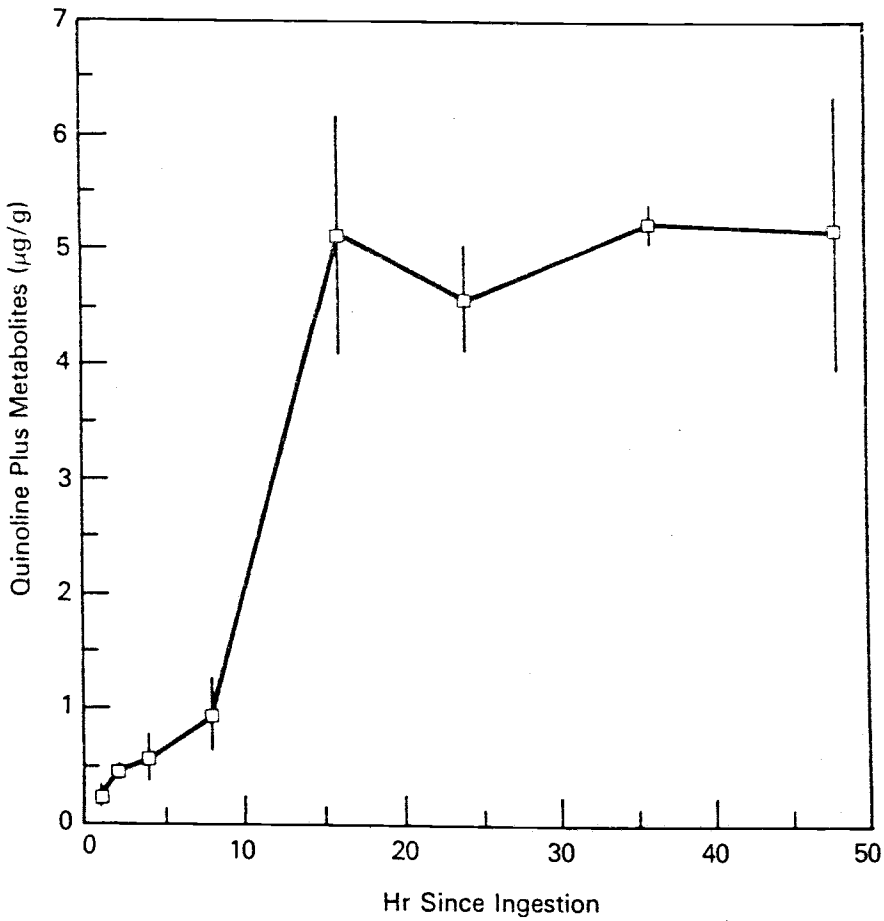
Table II.2 Urinary excretion of quinoline plus metabolites following ingestion and ip injection of ^{14}C-quinoline

Method[a]	Feeding Dose (mg/g)	Peak excretion		% of dose excreted[b] (hr)	
		Interval (hr)	Concentration (mg/mL)	0-24	24-48
Independent	1.6	8-16	0.56	1.6	0.5
Repeated	1.0	4-8	0.17	0.8	NA

[a] For treatment of fish see Methods. N = 3-5.

[b] Dose excreted based on 75 mL/hr/kb fish urine flow rate and measurements of mean urine concentrations.

Figure II.6 Accumulation of quinoline plus metabolites in the gallbladder bile of rainbow trout following ingestion of a single meal containing ^{14}C-quinoline. Values are mean + S.E. from four to six fish



from 0.02% of the dose at 4 hr post-ingestion to 1.1% of the dose at 48 hr. Maximum observed values represented 1.9% of the ingested dose.

Total radioactivity present in the lower digestive tract (pyloric caeca to rectum) ranged from 0.13% of the ingested dose at 1 hr post-feeding to 5.28% of the dose at 16 hr post-feeding. Maximum amounts measured in the rectum did not exceed 2.2% of the dose. Mean values obtained for individuals sampled from 24-48 hr (N = 6) were <0.8% of the ingested dose and represented the portion available for fecal excretion.

There was no evidence for secretion of quinoline or its metabolites in skin mucus. Levels of radioactivity from mucus did not exceed residual levels measured in aquaria water.

Although branchial excretion was not measured, indirect evidence (i.e., measurement of all other potential pathways) indicated that this route accounted for about 95% of the total excretion of quinoline-derived radioactivity by rainbow trout. The injection experiments, designed to measure flux of radioactivity across the gills, demonstrated that movement of quinoline occurred readily from the blood across gill epithelium. A nearly constant concentration gradient was maintained between concentrations of radioactivity in the serum and the gill at water concentrations from 0.05 to 1.64 ng/mL, following ip injection (Table II.3). Further evidence for a high rate of gill flux was shown where gill:water concentration ratios of fish held in carbon-filtered aquaria (low residual radioactivity) were nearly four times greater than fish allowed to depurate into non-filtered aquaria.

Table II.3 Apparent flux of quinoline across trout gills. Average Values from two fish as ng/mL or ng/g radioactivity

Sample Treatment	Interval (hr)	Tissue/water concentration		
		Water	Gill	Serum
Filtered aquaria	4	0.22[a]	8.24	14.24
	8	0.17	5.06	8.85
	24	0.05	1.13	2.20
Nonfiltered aquaria	4	1.32	9.50	15.23
	8	1.42	9.26	16.45
	24	1.52	16.48	8.10

[a]Average values from two fish as ng/mL or ng/g radioactivity.

EFFECTS OF DOSE ON DISPOSITON AND CLEARANCE

In general, tissue concentrations increased in direct proportion to the dose administered in food (Table II.4). However, differences in this trend were observed in gills at the higher doses; concentrations were about 1.6 and 2.7 times those predicted for a linear response at the 10-mg/kg and 100-mg/kg doses, respectively. At the 100-mg/L dose, gallbladder concentrations were about 34% of those predicted (Table II.4).

Distribution of quinoline-derived radioactivity was not significantly different at the range of doses tested (Table II.5). About 80% of the body burden remaining at 24 hr post-feeding was contained in the gallbladder bile, and an average of 11% of the dose remained unabsorbed in the digestive tract. Relative body burden for remaining tissues was carcass> muscle> gills≥ liver≥ kidney= brain> fat (Table II.5). The percentage of the total ingested dose retained by fish fed 100 mg quinoline/kg was about half that for fish fed the two lower doses.

Whole-body elimination, based on percent of total dose excreted, was similar for the 1-mg/kg and 10-mg/kg doses at all intervals from 0-24 hr post-ingestion. Rates of excretion were greatest during the first 8 hr after ingestion when >50% of the dose was eliminated (Table II.6). However, the rate of excretion for the 100-mg/kg dose was nearly twice that of the two lower doses for 0-6 hr post-ingestion. Variability of organism response appeared to be related to dose; coefficient of variation was highest for the

Table II.4 Tissue concentrations at three oral doses. Values shown as mean ±S.E., N=5.

Tissue	Dose (mg/kg)	Concentration (ng/g)[a]	Tissue Ratio[b]
Serum	1	5 ± 1 [a]	1
	10	69 ± 12	14
	100	370 ± 220	74
Brain	1	7 ± 2	1
	10	66 ±11	9
	100	630 ± 350	90
Fat	1	10 ± 4	1
	10	100 ± 20	10
	100	1150 ± 630	115
Liver	1	25 ± 6	1
	10	287 ± 39	11
	100	1870 ± 910	75
Gallbladder	1	2103 ± 450	1
	10	23213 ± 3664	11
	100	78470 ± 35600	34
Gill	1	13 ± 4	1
	10	207 ± 120	16
	100	3490 ± 2580	268
Kidney	1	52 ± 9	1
	10	576 ± 51	11
	100	3130 ± 1720	60
Muscle	1	3 ± 1	1
	10	40 ± 6	13
	100	170 ± 90	57
Carcass	1	7 ± 2	1
	10	78 ± 7	11
	100	750 ± 450	107

[a] Mean ± SE; N = 5.

[b] Based on tissue concentrations relative to 1 mg/kg dose.

Table II.5 Distribution of quinoline plus metabolites in tissues of rainbow trout. Values as percent body burden retained, 24 hr after feeding, mean ±S.D., N=5

Tissue	Ingested dose		
	1 mg/kg	10 mg/kg	100 mg/kg
Kidney	0.4 ± 0.1 [a,b]	0.3 ± 0.1	0.3 ± 0.1
Gill	0.3 ± 0.3	0.5 ± 0.8	1.0 ± 1.3
Liver	0.4 ± 0.2	0.2 ± 0.1	0.8 ± 0.9
Gallbladder	80.9 ± 5.1	80.3 ± 8.2	73.8 ±14.0
Fat	<0.1	<0.1	<0.1
Brain	0.3 ± 0.1	0.3 ± 0.2	0.4 ± 0.2
Muscle	0.9 ± 0.1	1.0 ± 0.2	0.9 ± 0.4
Carcass	5.9 ± 1.1	6.5 ± 3.1	10.3 ± 7.5
Stomach contents	2.6 ± 4.6	1.3 ± 2.3	1.7 ± 3.9
Intestinal contents	8.4 ± 1.8	9.6 ± 5.8	11.1 ± 9.1
% of ingested dose stored	11.0 ± 4.7	12.3 ± 3.9	5.4 ± 5.9

[a] Mean ± SD; N = 5.

[b] Values as percent body burden retained, 24 hr after feeding.

Table II.6 Cumulative rate of excretion (as percent of the dose) for quinoline following ingestion at 1, 10, 100 mg/kg dose. Values as mean ±S.D., N=3.

Hr since Feeding	Percent excretion at ingested dose[a] 1 mg/kg	10 mg/kg	100 mg/kg
1	4 ± 1 [b]	4 ± 2	7 ± 2
2	9 ± 2	5 ± 1	35 ± 18
4	19 ± 3	14 ± 2	63 ± 27
6	35 ± 2	28 ± 3	63 ± 24
8	53 ± 6	55 ± 15	65 ± 18
12	68 ± 2	58 ± 1	73 ± 14
16	63 ± 7	73 ± 3	80 ± 11
24	71 ± 2	75 ± 3	83 ± 3

[a] Values are cumulative excretion as percent of the ingested dose.
[b] Mean ± SD; N = 3.

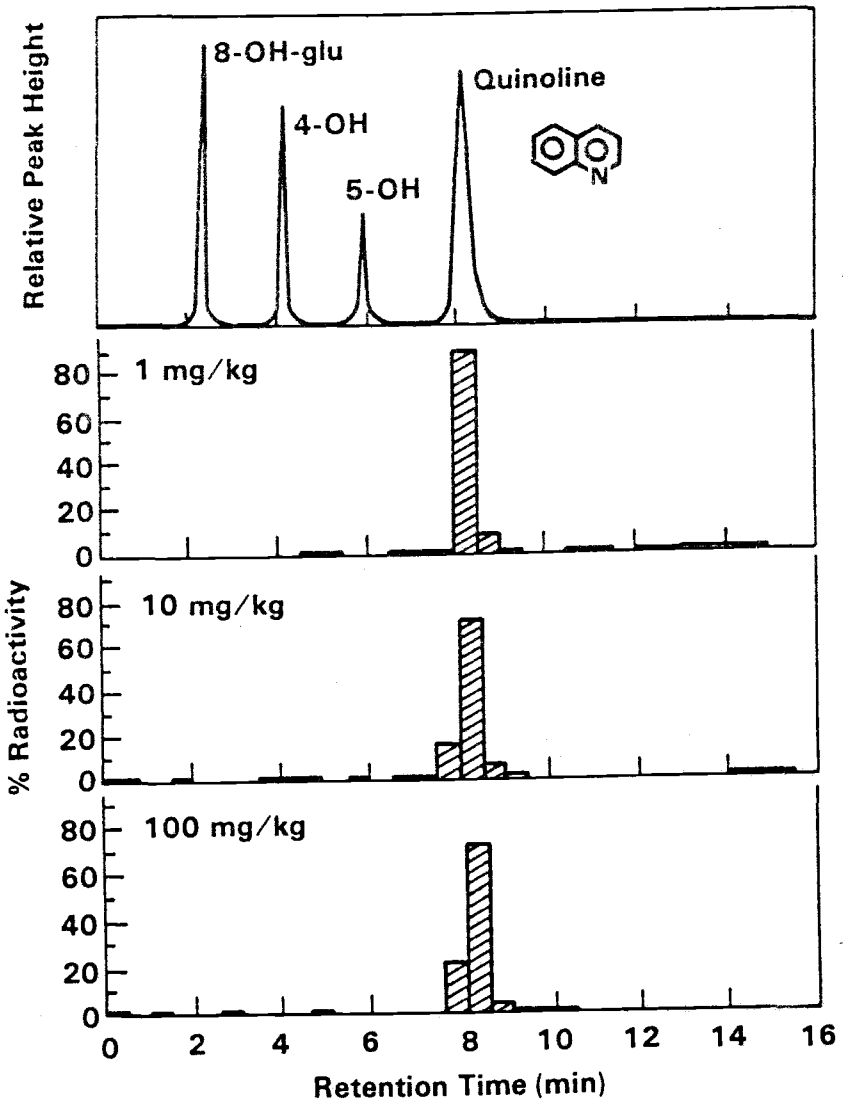
100 mg/kg dose. Total excretion at 24 hr post-ingestion was similar for each treatment and ranged from 71-83% of the ingested dose.

CHEMICAL FORM OF EXCRETED RADIOACTIVITY

Trout excreted 53-65% of the ingested dose during the 8-hr metabolism studies. We extracted about 18 L of water during the 12-hr extraction interval to obtain an average of 17% of the individual ingested dose from the aquaria water on the XAD column. Overall extraction efficiency from the water was >95%. Recovery of radioactivity from the XAD column using the ether extraction method was >96%. An average of 95% of the radioactivity remained in the aqueous fraction that was subsequently analyzed for parent compound and metabolites.

Retention time for quinoline ranged from 7.86 to 8.18 min for the six fish (N = 2 each treatment). The percent of radioactivity associated with the quinoline peak ranged from 94.3 to 97.8% of the injected radioactivity. Overall recovery of radioactivity during HPLC analysis was >99%. No peaks representing individual metabolites were detected (Fig. II.7). The sample fraction containing compounds more polar than quinoline ranged from only 0.8-1.7% of the total recovered radioactivity.

Figure II.7 Representative HPLC chromatogram of quinoline and metabolite standards and profiles of radioactivity at three oral doses. HPLC fractions were collected at 30 sec intervals and recovered radioactivity was calculated based on total radioactivity injected into the column



DISCUSSION

Peak serum concentrations of quinoline-derived radioactivity in serum occurred before most of the ingested dose reached the alkaline environment of the intestine. This indicated that quinoline is readily absorbed in the stomach of rainbow trout when not highly bound in a food matrix. Different kinetics for gastrointestinal uptake may occur for quinoline bound to sediment or to food organisms. However, our studies represent basic disposition of ingested quinoline by rainbow trout. Studies with mammals have shown drug uptake across absorptive epithelia is governed mainly by physical processes and can be predicted from the dissociation constant and lipid-solubility of the undissociated drug moity (Hogben et al., 1957; 1959). The gastric mucosa of both humans and rats is known to be selectively permeable to the undissociated form of drugs (Schanker et al., 1957; Hogben et al., 1957). That quinoline was absorbed in the stomach is evidence that it existed in the unionized form and diffused though the stomach epithelium into the blood. Gastric emptying time was likely delayed because of the single feeding, and this probably also influenced the absorption rate of quinoline. Passage time of a drug in a particular segment of the digestive system, rather than the absorptive capacity of that segment, is thought to be the crucial factor in determining the amount of compound absorbed (Levine, 1970).

We found that trout efficiently absorbed quinoline from the digestive tract. Of the total ingested dose, only about 2% was not absorbed by gut epithelium and available to be excreted via the

feces. A significant contribution of biliary excretion to fecal excretion was unlikely given time courses for gallbladder accumulation of radioactivity and bile retention (Fig. 6). Additionally, measurements of radioactivity in the digestive tract over time support the hypothesis that the unabsorbed dose was quite low. Thus, lack of significant bioaccumulation of dietary quinoline by rainbow trout (Dauble et al., 1987) is related to rapid excretion, rather than to inefficient absorption.

Branchial excretion was implicated as the primary route of excretion because all other possible routes of elimination contributed to <5% of the total eliminated dose during the first 48 hr after ingestion. Rapid excretion of a xenobiotic by fish can occur across the gills because this organ has a high surface area, receives the entire cardiac output, and is ventilated by large volumes of water. Other teleost excretory routes (i.e., renal, biliary) are more limited in their ability to rapidly excrete xenobiotics because they are dependent upon active processes. That absorbed quinoline was efficiently eliminated as parent compound across the gills is consistent with its physical and chemical characteristics (MW, 129; log P, 2.03). McKim et al., (1985) found that chemicals with log octanol/water coefficients <3 were rapidly eliminated across the gills. Branchial excretion of quinoline is also in accordance with studies conducted by Hunn and Allen (1974a) using quinaldine (3-methylquinoline). Thomas and Rice (1982) found that the size of aromatic hydrocarbons was a more important factor than partition coeffient in branchial excretion of xenobiotics fed to Dolly Varden char (Salvelinus malma). Lipid solubility is another

factor important for branchial excretion of chemicals (Maren et al., 1968). However, passive diffusion can occur across any barrier that is permeable to the chemical and across which a concentration gradient exists.

At higher doses, the minor excretory pathways (i.e., renal, biliary) may have become saturated. Evidence that saturation occurred was suggested by the decreased concentrations of radioactivity in kidney and bile, relative to dose, as the dose was increased from 1 to 100 mg/kg. Concomitant increases in gill concentrations occurred for the higher doses, indicating that as rates of urinary and biliary excretion were saturated, a spillover occurred into the branchial route.

Because quinoline is eliminated as parent compound, biotransformation only plays a minor role in excretion. Although metabolism may not be quantitatively important for compounds that rapidly diffuse through gill membranes, it influences both the form and persistence of stored quinoline derivatives. The role of metabolism in urinary excretion was suggested by temporal changes in the U/S ratio. Our observation of increased U/S ratios with time may indicate the presence of water-soluble metabolites, forms that would be less likely to be passively absorbed and more likely to be secreted by renal tubules (Pritchard and James, 1982). The slow rate of excretion of quinoline and metabolites via urine is similar to results obtained by Hunn and Allen (1974b) following exposure of channel catfish (Ictalurus punctatus) to quinaldine. A heterogenous pattern of radioactivity in kidney rainbow trout (See Table 1) was also reported by Gingerich (1986) following iv injection of the

piscicide rotenone and by Balk et al., (1984) who exposed northern pike (Esox lucius) to (3H)benzo(a)pyrene in food. Although the trout kidney does not appear morphologically distinct, there are differences: the head portion consists of lymphoid, hematapoietic, interrenal and chromaffin tissue, while modification and storage of urine occurs in the posterior end of the kidney (Hickman and Trump, 1969). Thus, storage and elimination of xenobiotics in the renal system of fish may be related to differences in both the structure and the functional role of kidney tissues.

That hepatic metabolism of quinoline is relatively slow was indicated by the appearance of relatively low concentrations of radioactivity in the bile until nearly 16 hr after ingestion. This indicates that quinoline may have a low hepatic extraction rate in rainbow trout, i.e., first-pass metabolism is insignificant. Biliary excretion would not be expected to be a major route of excretion for quinoline because of its molecular weight, polarity, or structural factors (Smith, 1973). Biliary excretion is a major route of excretion (50%) for benzo(f)quinoline in rats (Birnbaum and Johnson, 1985); benzo(f)quinoline is also extensively metabolized before urinary excretion. However, interspecies comparisons of excretion are difficult because ventilatory routes are often different. Previous studies have shown that metabolism is a prerequisite for elimination of quinoline via the bile by rainbow trout (Bean et al., 1985; Dauble et al., 1987). Although not quantitatively important in excretion, metabolism of quinoline by hepatic tissue can lead to more biologically active forms (LaVoie et al., 1983), including free radicals associated with hepatic neoplasms (Roubal and Malins, 1985).

Dietary contaminants that are excreted slowly are likely to be more important because of their persistence and increased potential for binding to cellular constituents. Because quinoline-derived radioactivity is retained in tissues mainly as biotransformation products (Bean et al., 1985; Dauble et al., 1987), biological activity would likely be limited to metabolites. This concept is consistent with the hypothesis posed by LaVoie et al., (1983) that the formation of 5-6-epoxy-5-6 dihyroquinoline is associated with the activation of quinoline to a mutagen/carcinogen in other organisms.

Bioaccumulation potential for chemicals taken up in food depends both on the efficiency of uptake and the clearance rate of the compound. Although biotransformation processes may be rapid enough to detoxify some compounds (Lech and Statham, 1975), other absorbed compounds (like quinoline) may have characteristics that allow elimination through passive processes.

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CHAPTER III. INFLUENCE OF DIGESTIVE PROCESSES ON THE ABSORPTION AND FATE OF QUINOLINE INGESTED BY RAINBOW TROUT (*SALMO GAIRDNERI*)

Dennis D. Dauble and Lawrence R. Curtis

ABSTRACT

Rainbow trout were fed pelleted food containing ^{14}C-quinoline to study interactions between digestive processes and xenobiotic pharmacokinetics. The pH of material in the stomach of rainbow trout ranged from 2.7 to 5.2 units and was highly correlated ($r = 0.89$) with the size of the food bolus remaining. At 2 hr after feeding, 67% of the quinoline was estimated to be unionized and available for absorption across gastric epithelium. Quinoline was >99% unionized in the alkaline environment of the intestine, however relative concentrations in the intestine were only 8% of those measured in the stomach. Rates of gallbladder emptying appeared to exceed rates of hepatic bile secretion until about 8 hr after a single feeding. Time since feeding influenced both the amount and concentration of quinoline-derived radioactivity in the bile. There was no evidence for enterohepatic circulation of quinoline or its metabolites following ejection of gallbladder bile. Consecutive feedings enhanced the movement of the food bolus and associated radioactivity from the upper and midgut regions, but had no effect on disposition of quinoline.

INTRODUCTION

Basic physiological processes that occur during consumption and digestion of food have not been described in relation to the fate of xenobiotic chemicals in fish. Indeed, many studies designed to predict the fate of xenobiotics in fish have used techniques (i.e., starvation, ip injection, force-feeding) which can alter *in vivo* processes that occur during consumption of food organisms (Higgs and Eales, 1979; Western, 1971; Roubal et al., 1977; Fange and Grove, 1979; Varanasi et al., 1979; Colloidi et al., 1984; Doull, 1980). While toxicological studies that employ these techniques provide necessary descriptive information, they also present a simplistic interpretation of compound fate.

The fate of dietary contaminants in fish may be influenced by several variables before the xenobiotic or its metabolites enter the general systemic circulation and are available for distribution to storage or excretory tissues. Specific variables that can affect the absorption and/or clearance of xenobiotics ingested by fish include: 1) characteristics of the diet that affect contact time with absorptive epithelium, (i.e., food type, meal frequency, and daily ration) (Windell and Norris, 1969; Grove et al., 1978; Rogers and Beamish, 1982; Pizza and Conner, 1983; Hilton et al., 1983; Shubat and Curtis, 1986), 2) characteristics of the gut environment that affect compound availability (Hogben et al., 1959; Levine, 1970), and 3) post-absorptive processes related to feeding that influence the amount and form of chemical that is transported to tissues, (i.e.,

hepatic clearance, metabolism, and biliary secretion) (Klaassan, 1980; Klaassan and Watkins, 1987). Other important variables, such as duration of exposure, environmental concentration and temperature, are not unique to dietary exposures.

Previous studies that described the uptake, elimination, and storage of dietary quinoline by rainbow trout (Salmo gairdneri) showed that this potential carcinogen was not readily bioaccumulated and that most absorbed material was stored as quinoline metabolites (Dauble et al., 1987). Lack of bioaccumulation was later explained by rapid branchial excretion of parent compound (Dauble and Curtis, 1988). While seeking a simple, mechanistic explanation for quinoline behavior in rainbow trout, it became apparant that general digestive processes were important variables affecting the fate of quinoline following its uptake in food. We conducted a series of experiments to address the importance of digestive processes in the pharmocokinetics of dietary quinoline in rainbow trout. Our results are broadly applicable to studies of xenobiotic bioaccumulation and toxicity in fish and will allow development of better predictive models for xenobiotic fate and effects.

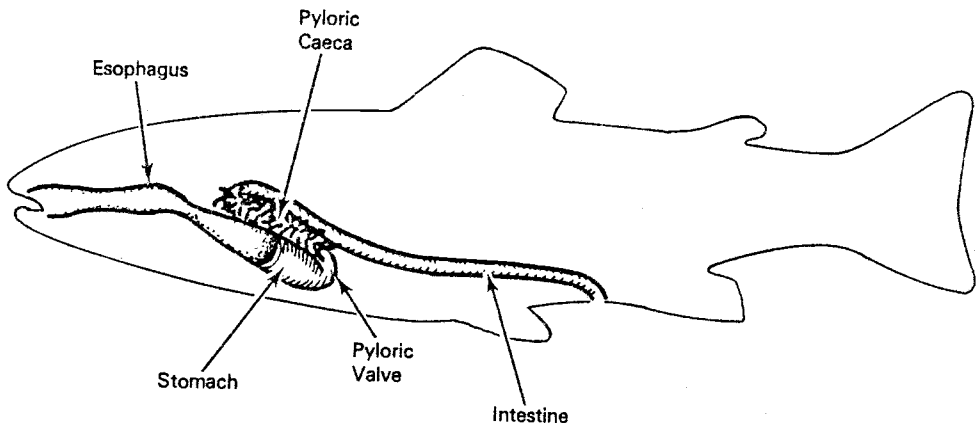
METHODS

A Kamloops strain of rainbow trout, ranging from 20-80 g, were used for all feeding studies. Juvenile fish were obtained from stocks raised in our laboratory and acclimated to test aquaria containing Columbia River water for 7 to 14 days before experimentation. All tests were conducted at 12°C with a 16 hr light:8 hr dark cycle. Feeding procedures and methods for preparation of food pellets were described by Dauble et al (1987).

DESCRIPTION OF THE SALMONID DIGESTIVE SYSTEM

The digestive system of the rainbow trout (family Salmonidae) contrasts with many other teleosts (i.e., family Cyprinidae, Catostomidae, Cyporinidontidae) in that the stomach is well differentiated from the intestine (Weinrab and Bilstad, 1955; Barrington, 1957). The trout digestive system has three general regions that correspond to respective roles in storage, absorption, and excretion of food: 1) the esophagus and stomach, 2) the pyloric caeca and upper intestine, and 3) the lower intestine or rectum (Figure III.1). These same three regions have been described as the upper gut, the midgut, and hindgut, respectively (Barrington, 1957). For trout, ingested material passes down the esophagus into the stomach where proteolytic gastric secretions break down the food and prepare it for further digestion. The partially digested material is then transported past the pyloric valve to the pyloric caeca region, where additional digestive secretions are added. The pyloric caeca are not common to all fish groups; these tissues

Figure III.1 Principal features of the salmonid digestive system



increase the effective surface area available for absorption and are thought to function in absorption of fats (Green, 1911). Finally, the undigested and unabsorbed material is moved down the intestine to the rectum and expulsed as feces.

EXPERIMENTATION

Three separate studies were conducted to examine interactions among digestive processes and the absorption and disposition of dietary quinoline in rainbow trout. For each study, rainbow trout were isolated in glass aquaria and fed preformed hatchery pellets at a daily ration of 1% body weight/day.

Digestive System pH- The pH of the food bolus (chyme) and fluid in the digestive system was determined using fish fed hatchery pellets containing unlabelled quinoline at 1 mg/kg dose. Fish (50-70 g) were starved in flow-through aquaria for 72 hr, fed once, then sacrificed at 0, 1, 2, 4, 8, 16, 24, and 48 hr after feeding for pH measurements of the stomach, midgut at the pyloric caeca, lower gut (rectum), and gallbladder bile. Measurements (N = 3) were made immediately after sacrifice using pH paper (colorpHast^{TM}). The pH paper was checked against known standards (American Scientific Products, Inc.) and shown to be accurate within 0.2 pH unit. Separate experiments were conducted to determine rates of movement for quinoline-derived radioactivity in the digestive system. The Henderson-Hasselbach equation for weak bases was used to determine the fraction of toxicant present in the non-ionized, lipid-soluble form.

Bile Storage and Excretion- Juvenile trout (24-49 g) were fasted for 48 hr and fed a 0.6 mg/kg dose of ^{14}C-quinoline (S.A., 8.83 uCi/mM;

>99% pure) contained in a reformed pellet. Immediately following sacrifice, the gallbladder plus bile was excised from the liver and weighed at 0, 1, 2, 4, 8, 16, 24, and 48 hr after feeding. Relative concentrations of quinoline-derived radioactivity were determined by liquid scintillation counting. Contents of the stomach and intestine were removed and weighed at the same intervals to determine the relationship between movement of food through the digestive system and gallbladder volume.

Frequency of Feeding- The effect of meal frequency on storage and elimination of dietary ^{14}C-quinoline was determined in two groups of rainbow trout. Individual fish (40 g) were isolated in 20 L aquaria and fed a single meal. At 48 hr following this feeding, one group of fish (N = 12) were fed a daily ration of unlabelled food; the remaining fish (N = 12) were starved. Six fish from each group were sacrificed at 52 and 56 hr (4 and 8 hr after the second feeding) and concentrations of radioactivity determined in selected tissues and excretory products. These samples intervals were designed to include the period of maximum biliary excretion. Procedures for tissue/fluid collection and analysis were described in Dauble and Curtis (1988).

STATISTICAL ANALYSIS

Comparison of differences among mean tissue/fluid and digestive material concentrations were analyzed by ANOVA using Statview 512+ for the Macintosh (Brainpower, 1986). Differences between individual treatment pairs were then tested at $\alpha = 0.05$ by t-tests. Relationships between test parameters were described by Spearmans Rank Correlation

RESULTS

INFLUENCE OF PH ON ABSORPTION OF QUINOLNE

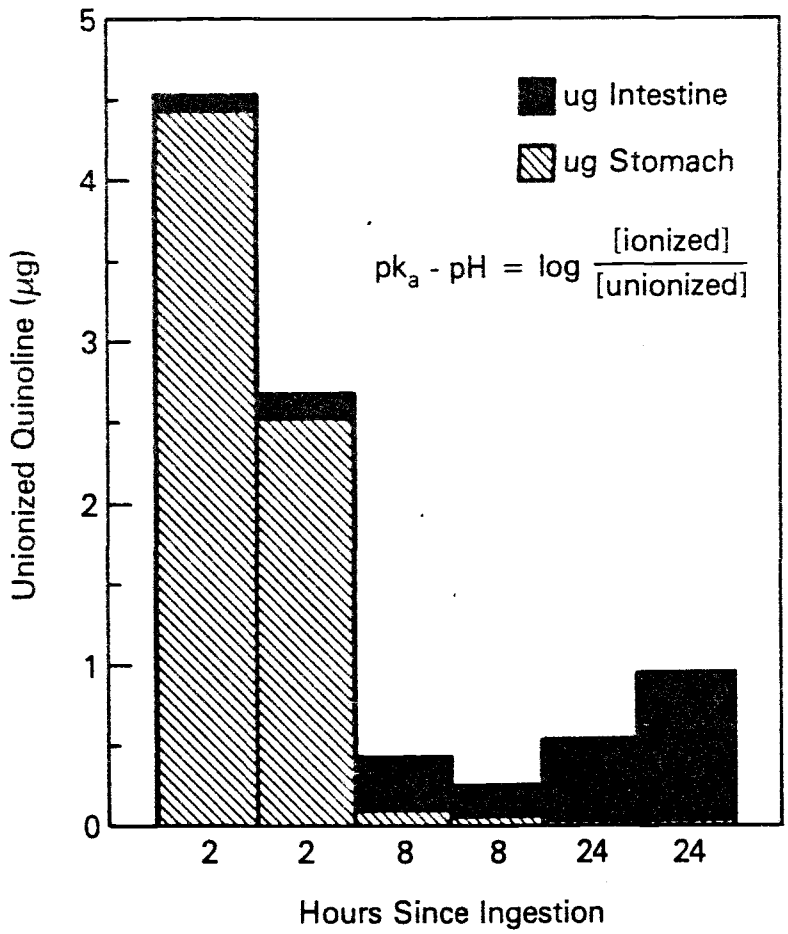
The mean pH of material in the stomach of rainbow trout ranged from 2.7 to 5.2 units over the 48 hr period following a single feeding (Table III.1). The pH for individual fish was highly correlated with the size of the food bolus in the stomach ($r = 0.89$, $N = 22$). In contrast, the pH of the intestinal contents was alkaline (pH range, 7.6-9.5) and appeared independent of the presence of food. No variations in intestinal content pH could be attributed to ejection of gallbladder bile. Since mean pH of gallbladder bile ranged from 7.3 to 7.7, additional digestive secretions (e.g., pancreatic) that are discharged into the midgut also must influence the more basic environment of the rainbow trout intestine. No significant difference was noted between the pH of digestive fluid in the midgut and in the rectum or hindgut.

The amount of unionized quinoline or that portion of the ingested dose available for absorption in the digestive tract was estimated from the pH measurements and concentrations of quinoline found in the digestive system (Figure III.2). At 2 hr after feeding, 67% of the quinoline was estimated to be unionized (pH 5.7). The pH in the stomach dropped to 3.3 and 2.7 at 8 hr and 24 hr post-ingestion, respectively. The low pH resulted in a shift in equilibrium to the protonated form of quinoline and theoretically limited absorption across the gastric epithelium. Quinoline was >99% unionized in the intestinal environment (pH 8.1-8.4). However,

Table III.1 Effects of feeding on the pH of the digestive contents and bile of rainbow trout. Fish were fed a single ration at 1% of body weight. Values are mean ± S.D., N = 3.

Interval Since Feeding (hr)	pH of Digestive Fluid			
	Stomach	Bile	Upper Intestine	Rectum
0	3.0±1.8	7.3±0.1	9.1±0.7	8.1±0.7
1	5.2±0.4	7.5±0.1	8.5±0.1	8.7±0.7
2	5.2±0.4	7.6	8.5±0.4	8.3±0.3
4	4.5±0.2	7.6	8.4±0.3	8.5±0.1
8	3.6±0.2	7.7±0.6	8.1	8.8±0.2
16	3.0±0.7	7.7±0.5	8.4±0.3	8.2±0.4
24	2.7±0.7	7.7±0.1	8.3±0.2	8.5±0.4
48	4.7±0.4	7.7±0.7	8.3±0.6	8.2±0.4

Figure III.2 Estimates of the relative amount of quinoline (pKa 4.9) available for absorption in the digestive tract of rainbow trout. Each value is a measurement from an individual fish. The sample times corresponded to known passage rates for the food matrix (Dauble and Curtis 1988)



relative concentrations were reduced by the time the food bolus passed into the intestine from the stomach. The maximum concentrations present in the stomach (2 hr after feeding) were up to 12 times higher that the maximum concentrations measured in the intestine (8 hr after feeding).

BILE STORAGE AND BILIARY EXCRETION OF QUINOLINE

Bile was ejected from the gallbladder soon after each fish ingested the pelleted food (Figure III.3). Rates of gallbladder emptying appeared to exceed rates of hepatic bile secretion until about 8 hr after the single feeding. The gallbladder then began to fill with bile as the food bolus moved from the stomach into the midgut. The relative volume of bile stored at 48 hr was about 74% of that present before feeding (starved for 72 hr). A different pattern would be expected for fish that received multiple feedings or a larger ration.

The interaction between the biliary secretion of quinoline plus metabolites and the process of bile storage and excretion that occurs following ingestion and absorption is shown in Figure III.4. Bile storage was limited during the first 8 hr after feeding because of ejection associated with feeding. From 8 to 48 hr after the single feeding, rates of biliary excretion of quinoline and metabolites appeared similar to bile flow into the gallbladder. These data indicate that time since feeding influences both the amount and concentration of a xenobiotic present in the bile.

Figure III.3 Relationship of ingestion and movement of food to biliary excretion and bile storage. Values are mean ± S.E., N=3.

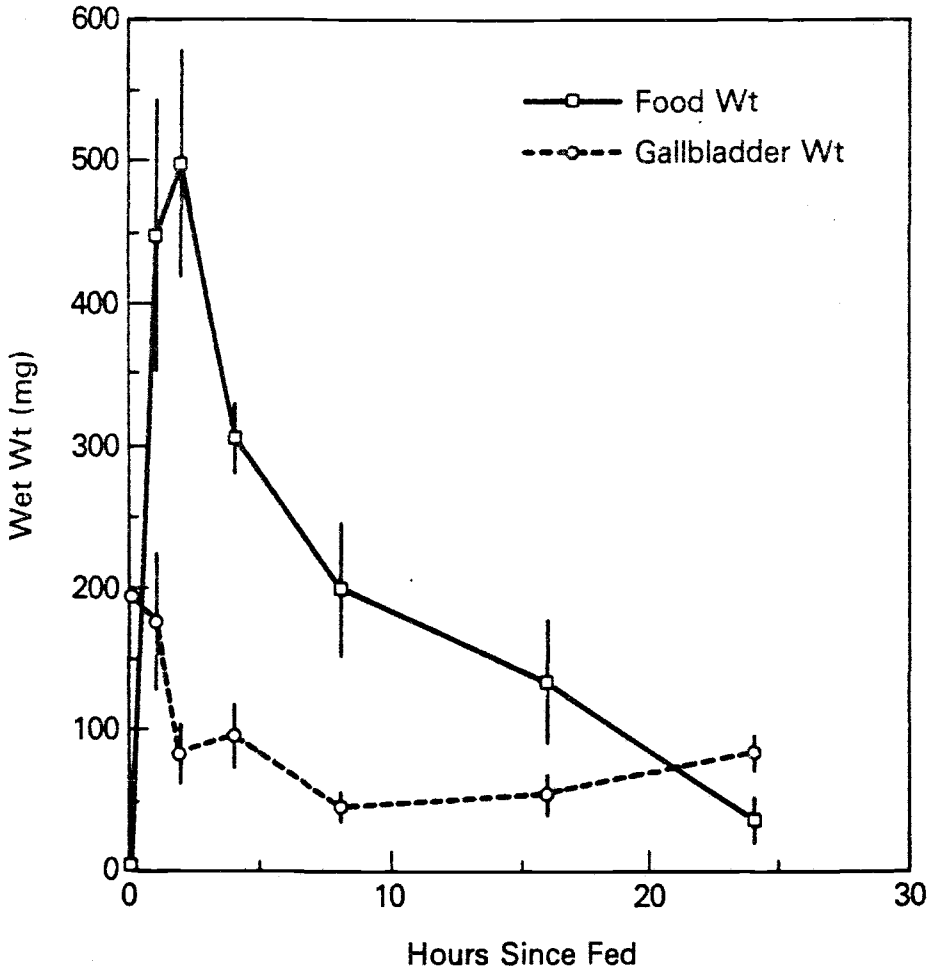
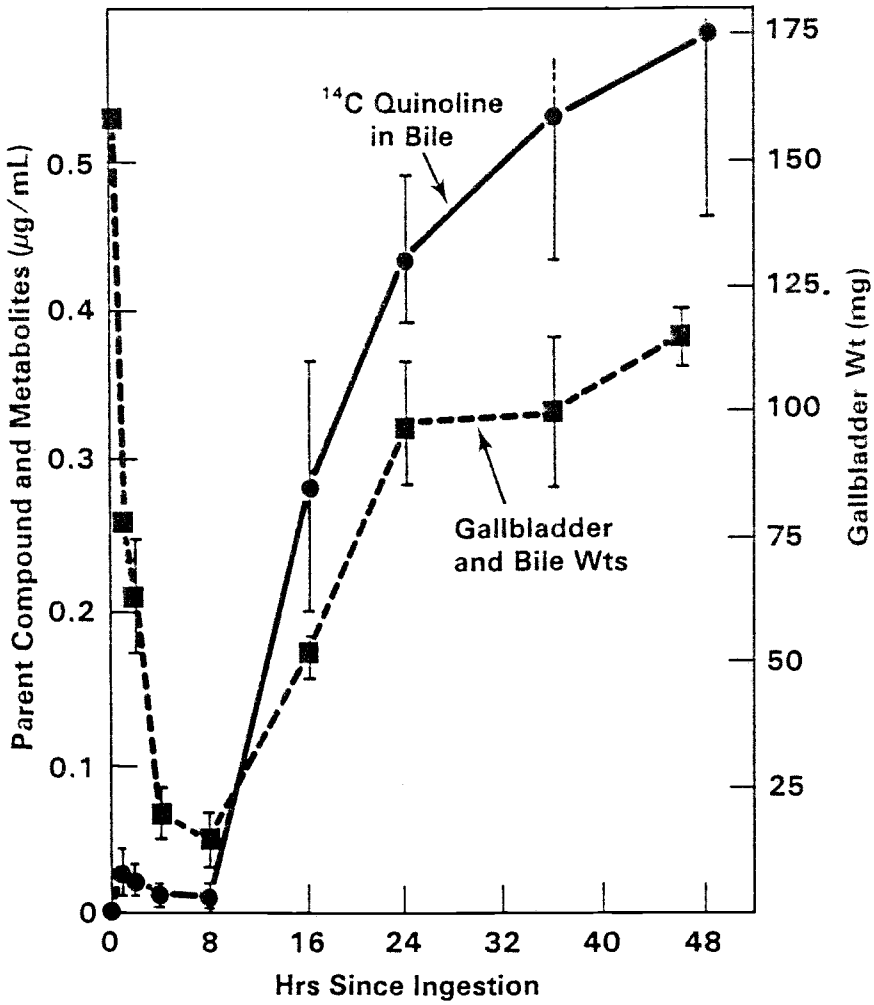


Figure III.4 Temporal pattern of gallbladder bile volume and accumulation of quinoline plus metabolites following ingestion of a single meal containing ^{14}C-quinoline. Values are mean ± S.D., N=3.5.



MEAL FREQUENCY AND DISPOSITON

Few differences were noted in the disposition of quinoline for rainbow trout fed at two different rates (Table III.2). Significant differences in tissue concentrations were noted only for radioactivity in the gallbladder bile (ANOVA, $\alpha = 0.01$). Since gallbladder bile volume was also reduced, differences can be attributed mainly to additional biliary excretion that occurred following the second feeding. There was no evidence for enterohepatic recirculation of quinoline or its metabolites following ejection of gallbladder bile. However, concentrations excreted in the bile may have been too low to result in measureable increases in serum levels, even if significant reabsorption occurred during the selected sample intervals.

Concentrations of quinoline-derived metabolites in the upper intestine were significantly different between once-fed treatments and twice-fed treatments (ANOVA, $\alpha = 0.01$). No difference was noted for concentrations in either the mid-intestine or rectum (Table III.2). This suggests that the second feeding enhanced the movement of the food bolus and associated radioactivity from the upper and midgut regions, but that insufficient time elapsed to result in fecal excretion and a decrease in concentrations for the lower gut region.

Table III.2 Effect of second feeding on the disposition of quinoline plus metabolites in tissues of rainbow trout. All values are concentrations as ug/g equivalent quinoline for bile and digestive system contents; serum, kidney, and gills are ng/g, mean ± S.E., N=6.

Treatment[a]	Sample Interval	Serum	Kidney	Bile	Gill	Liver	Upper Intestine	Mid-Intestine	Rectum
twice fed	28 hr	2.0±0.6	1.5±0.6	1.7±0.5	1.8±0.7	19.7±6.6	1.3±0.8	1.6±0.4	2.0±0.8
once fed	28 hr	1.7±0.4	1.3±0.6	2.8±0.6	1.8±0.6	14.3±3.3	1.3±0.4	1.6±0.9	3.7±2.7
twice fed	32 hr	1.4±0.4	1.3±0.5	0.2±0.2[b]	1.9±0.5	10.1±0.8	0.6±0.1[b]	0.7±0.1	1.9±0.5
once fed	32 hr	1.6±0.5	1.4±0.5	3.4±0.6	4.5±1.2	21.0±6.4	2.4±0.3	1.4±0.3	2.5±0.8

[a]Twice-fed fish received a second feeding of unlabelled food 24 hr after feeding a single radiolabelled dose. Once fed fish received a single radiolabelled dose at 0 hr.

[b]Significantly different from paired treatment at $\alpha = 0.05$.

DISCUSSION

High concentrations of quinoline were present in the stomachs of rainbow trout in an unionized form and thus available for absorption 2 hr after feeding. Evidence that quinoline was absorbed from the food matrix by the gastric epithelium is supported by previous studies that showed peak serum levels occurred within the first few hours after ingestion of dietary quinoline (Dauble and Curtis, 1988). The availability and subsequent gastric absorption of quinoline would not have been predicted based on textbook physiology. Since quinoline is a weak base (pKa = 4.9 or 50% ionized at a pH of 4.9) and the gastric mucosa of fish is known to produce acidic secretions (Barrington, 1957), equilibrium conditions in the stomach after ingestion would be predicted to be shifted toward the protonated (ionized) form. Thus, absorption of quinoline would be predicted to occur mainly in the alkaline environment of the intestine, a region where maximum absorption of weak bases is assumed to occur (Hogben et al., 1959; Klaassan, 1980).

The pH of the gastric contents was dependent upon the volume of material present. This suggests that the secreted acid was absorbed and buffered by the food bolus. Other investigators reported changes in gastric pH are affected by the food mass and type (Norris et al., 1973; Western, 1971). Because gastric secretions also influence time to digestion and absorption effeciency (Barrington, 1957), the volume of food probably influenced the pharmacokinetics of quinoline absorption.

Although equilibrium conditions for absorption of quinoline are not known, passive absorption across gastric epithelium appeared possible because of the high concentration gradient. This gradient would drive diffusion as long as the gastric pH remained near the pKa of quinoline. Gastric absorption would reduce the amount available for absorption in the intestine, independent of intestinal pH. Occurrence of gastric absorption was consistent with criteria reported by Hogben et al., (1957), i.e., ability of the drug to dissolve in the gastric contents and duration of time in the stomach. However, different kinetics of absorption may occur for quinoline under different exposure conditions or if protein-bound in a natural food organism.

Blood flow to particular regions of the gastrointestinal tract could be an important variable limiting the absorption of dietary contaminants, because blood is the major vehicle for removal of absorbed materials (Levine, 1970). Recent studies by Barron et al., (1987) indicate that blood flow past the gastric epithelium of rainbow trout is about half that for the intestinal region. Based on their values for tissue mass and perfusion rate, the stomach could have 74% of the absorptive potential of the intestine at 12°C. This suggests that availability rather than transport processes would limit gastric absorption. However, the large surface area of the intestine would favor absorption under similar conditons of availability.

We previously showed that dietary quinoline was present in rainbow trout serum at maximum concentrations within 2 to 8 hrs of ingestion (Dauble and Curtis, 1988). However, only low

concentrations of quinoline and metabolites are found in the gallbladder bile during this same interval. This may be explained in part by a low rate of hepatic clearance and/or first-pass metabolism. Levels of quinoline-derived radioactivity in the gallbladder remain low, even if bile is secreted from the liver into the gallbladder. This occurs because stored bile is also excreted into the intestine in response to the presence of the food bolus in the stomach.

Biliary secretion does not always result in elimination of a xenobiotic through fecal excretion. Enterohepatic recirculation can conserve endogenous substances such as bile salts and vitamins and increase duration of action for many drugs if their conjugates are hydrolyzed and subsequently reabsorbed (Levine, 1978; Klaassan and Watkins, 1987). However, since dietary quinoline is rapidly excreted by rainbow trout (Dauble and Curtis, 1988), enterohepatic circulation would not markely delay its elimination from the body. Because some glucuronide metabolites are present in the bile of rainbow trout fed quinoline (Dauble et al., 1987), a possibility exists for enterohepatic transformation of quinoline metabolites. Recycling may have little effect on peripheral plasma levels of a drug (Smith, 1973), even though extensive enterohepatic circulation is known to occur.

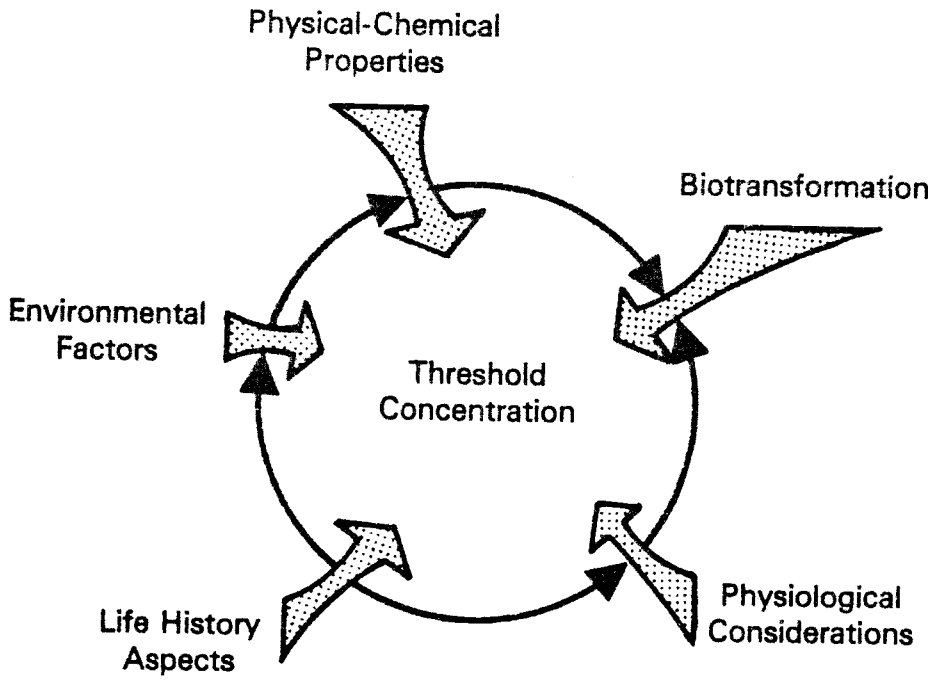
The relative importance of biliary excretion in the elimination of xenobiotics is easily overestimated because high concentrations of stored xenobiotics and/or metabolites can often be found in fish bile, relative to tissues. Although concentrations of xenobiotics in fish bile has been proposed as a useful indicator of xenobiotic exposure (Statham et al., 1976), the role of bile in digestion makes

this only of qualitative importance. This was apparant for our studies where absolute amounts of quinoline plus metabolites in the bile differed by up to 1800 times, depending on feeding rate and time since feeding. Thus, it is not possible to extrapolate back to environmental dose, based on a single point-in-time sample.

Our studies also demonstrated that feeding rates can influence the rate of passage and availability of a xenobiotic for absorption from the gastrointestinal tract of rainbow trout. Feeding rates are important variable that can affect the dietary accumulation of lipophilic compounds by fish (Pizza and Conner, 1983). However, laboratory-derived values for dietary accumulation of xenobiotics cannot be extrapolated to the field unless variables such as meal size and feeding habits are known. These types of ecological variables will differ by species, life-stage, and environmental requirements. For example, sculpins (Cottus spp.) can ingest up to 50% of their body weight with a single feeding (Fange and Grove, 1979). In contrast, bottom-feeding species that graze on benthos may ingest small smounts of food nearly continuously or may feed on a strict diel cycle. Since the rate of gastric motility depends on the volume of gastric contents (Grove et al., 1978; Windell et al., 1969), these variables will influence the transit time for food and the rate of absorption of dietary contaminats by the gut. Long periods of food deprivation may induce general changes in hepatobiliary function, including decreased plasma clearance and reduced biliary excretion (Eales, 1979). Thus, feeding behavior is also closely linked to patterns of storage and excretion of dietary contaminants.

Physiological processes associated with feeding and digestion are but one set of variables that can affect the uptake and fate of environmental contaminants by fish (Figure III.5). Other general factors that may influence relative tissue burdens and chemical form include potential for biotransformation, physical-chemical properties of a xenobiotic that affect availability, environmental factors such as temperature, and life history characteristics of an organism that may limit exposure. Only by considering the potential for each of these factors to influence contaminant fate in fish can we expect to improve our understanding and hence predictive capability of the hazard assessment process.

Figure III.5 Generalized hazard assessment model indicating parameters influencing the fate of contaminants in teleost fish.



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SUMMARY

Juvenile rainbow trout approached whole-body residue levels near apparent steady-state after only 10 days feeding of ^{14}C-quinoline in the laboratory. The highest concentrations of quinoline plus metabolites were found in digestive organs. About 70% of the body burden was stored in the gallbladder and gut contents 24 hr after feeding. Principal storage of quinoline-derived radioactivity occurred in the muscle and carcass after depuration. There is little potential for quinoline to transfer to higher trophic levels because the majority of residual radioactivity was present as metabolites. The metabolic pathway for quinoline after ingestion appears different from that following aqeous exposures. This may be due to different routes for uptake, absorption, and transport.

Quinoline was readily absorbed by the gastric mucosa of rainbow trout when it was ingested within a hatchery food pellet. Lack of significant dietary bioaccumulation was related to rapid excretion, rather than to ineffecient absorption. Branchial excretion was implicated as the primary route of excretion because all other possible routes of elimination (i.e., fecal, biliary, renal, dermal) contributed to < 5% of the total ingested dose. Branchial elimination by passive diffusion is consistent with chemical and physical characteristics of the compound and with the presence of a strong concentration gradient from the blood to the water following absorption. There was evidence for saturation of minor excretory routes as dietary dose was increased, with spillover into the branchial route. Because quinoline was readily eliminated across the

gills as parent compound, biotransformation plays only a minor role in excretion.

Collectively, these studies showed that the uptake and storage of dietary quinoline is a dynamic process. Pharmacokinetic parameters were influenced by a wide variety of physiological and biochemical processes. For example, gastric absorption of quinoline was influenced by the volume of food material present in the gut and by the secretion of digestive enzymes. Although gallbladder bile contained the highest concentrations of quinoline plus metabolites, its role in excretion was limited. Levels of quinoline-derived radioactivity remained low in the gallbladder because stored bile was excreted into the intestine in response to the food bolus in the stomach. Thus, the fate of dietary quinoline was also influenced by feeding rate and amount. Physiological variables associated with feeding and digestion can interact with environmental, ecological, behavioral, and biochemical factors to influence xenobiotic fate in an organism. Thus, considerations of cellular-, organism-, and community-level response can further our understanding of how teleosts respond to dietary contaminants in the environment.

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