TOXICOLOGICAL SCIENCES **77,** 206–218 (2004)
DOI: 10.1093/toxsci/kfh033
Advance Access publication December 2, 2003

A Physiologically Based Toxicokinetic Model for Dietary Uptake of Hydrophobic Organic Compounds by Fish

I. Feeding Studies with 2,2′,5,5′-Tetrachlorobiphenyl

John W. Nichols,*[,1] Patrick N. Fitzsimmons,* Frank W. Whiteman,* Timothy D. Dawson,†
Leo Babeu,† and Jamie Juenemann†

**Mid-Continent Ecology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Duluth, Minnesota, 55804, and †AScI Corporation, Duluth, Minnesota 55811*

Received on June 4, 2003; accepted on October 23, 2003

A physiologically based toxicokinetic (PBTK) model was developed to describe dietary uptake of hydrophobic organic compounds by fish. The gastrointestinal (GI) tract was modeled using four compartments corresponding to the stomach, pyloric ceca, upper intestine, and lower intestine, and the lumenal volume of each compartment was allowed to change in time as a function of bulk flow down the GI tract and (for the pyloric ceca and upper intestine) nutrient uptake. The model was developed using data from rainbow trout that were fed a single meal of 60-day-old fathead minnows contaminated with [UL-^{14}C] 2,2′,5,5′-tetrachlorobiphenyl ([^{14}C] PCB 52). Chemical partitioning coefficients for the gut contents and tissues were adjusted to account for changes in chemical affinity associated with uptake of dietary lipid. Permeability constants for the absorbing gut segments were then fitted by modeling to measured [^{14}C] PCB 52 concentrations in gut contents and tissues. The model accurately describes observed patterns of gastric evacuation and bulk flow of digesta, the concentration time course for [^{14}C] PCB 52 in contents and tissues of the GI tract, and [^{14}C] PCB 52 distribution to other major tissues. Most of the [^{14}C] PCB 52 was taken up in the pyloric ceca and upper intestine during the period of peak lipid absorption. It is concluded, however, that a kinetic limitation acting along the entire length of the GI tract resulted in a chemical disequilibrium between feces and tissues of the lower intestine.

Key Words: **physiologically based model; fish; dietary uptake.**

Dietary uptake is the primary route by which fish accumulate hydrophobic organic contaminants (log $K_{OW} > 5$; Bruggeman *et al*., 1984), including polychlorinated biphenyls (PCBs), dibenzo-p-dioxins, and diphenyl ethers, hexachlorobenzene, toxaphene, and various polyaromatic hydrocarbons (PAHs). Mathematical models have been developed to describe the trophic transfer of hydrophobic compounds in aquatic food chains, including dietary uptake by fish. Early models generally assumed that fish assimilate a constant fraction of chemical in the food that they ingest, and the kinetics of accumulation were calculated from a fixed uptake rate (ingestion rate times assimilation efficiency) and first-order elimination rate constant (Thomann, 1989; Thomann and Connolly, 1984). Assimilation efficiencies were typically obtained from feeding studies with fish, and the value of the elimination rate constant was fitted to simulate measured chemical concentrations. Later models calculated diffusive gradients in the gut as a function of chemical activity (or fugacity) in the gut contents and the fish (Barber *et al*., 1991; Gobas *et al*., 1988). Gobas *et al*. (1988) used a lumped transport parameter determined from exposure data to represent diffusive uptake throughout the gastrointestinal (GI) tract. Model parameters used by Barber *et al*. (1991) were developed independently of exposure data, but the model assumed that a chemical equilibrium was attained between the fish and the contents of its lower GI tract (a condition termed "fecal partitioning").

Despite this progress, a lack of detail in existing dietary uptake models for fish limits their application to specific questions of toxicological interest. All current models treat the contents of the GI tract as a single well-mixed compartment. This approach does not provide a means for describing kinetic phenomena that occur in specific gut regions. A description of the chemical time course in upper intestinal tissues may be important for understanding the effects of chemicals that undergo metabolic biotransformation, since these tissues act in concert with the liver to metabolically transform ingested compounds before they reach the general circulation (James and Kleinow, 1994; Kleinow and James, 2001; Van Veld, 1990). The GI tract also represents a potential site for toxic effects (James and Kleinow, 1994; Kleinow and James, 2001). A

[1] To whom correspondence should be addressed at U.S. Environmental Protection Agency, Mid-Continent Ecology Division, 6201 Congdon Boulevard, Duluth, MN 55804. Fax: (218) 529-5003. E-mail: nichols.john@epa.gov.

complete understanding of these effects requires knowledge of chemical kinetics in the tissues where they occur.

The goal of the present study was to incorporate a physiological description of dietary uptake into an existing physiologically based toxicokinetic (PBTK) model for fish (Nichols *et al*., 1990). The gut portion of this model consists of four compartments corresponding to the stomach, pyloric ceca, upper intestine (distal to the pyloric ceca), and lower intestine. This description accounts for the progressive decline in meal volume associated with nutrient uptake and changes in chemical affinity for digesta with changes in lipid content. The model was developed using data from dosing studies in which rainbow trout were fed a single meal of fathead minnows (*Pimephales promelas*) that had been exposed previously to [UL-^{14}C] 2,2′,5,5′-tetrachlorobiphenyl ([^{14}C] PCB 52; CAS no. 35693-99-3) in water (Nichols *et al*., 2001). In a companion report, the model was used to simulate hypothetical exposures to compounds of varying hydrophobicity under several exposure scenarios (Nichols *et al*., 2004).

MATERIALS AND METHODS

Fish PBTK model. A PBTK model for dietary uptake of hydrophobic compounds by fish was developed by incorporating a physiological description of the GI tract into an established inhalation (waterborne exposure) model (Nichols *et al*., 1990; Fig. 1). The model consists of a set of simultaneous mass-balance differential equations that describe the rate of change of the amount of chemical within each of the following nine tissue compartments: liver, kidney, fat, richly perfused (consisting of the heart, spleen, and gonads), poorly perfused (primarily white muscle), stomach, pyloric ceca (and associated upper intestine), upper intestine (distal to the pyloric ceca), and lower intestine. Additional equations describe the rate of change of the amount of chemical in contents of the stomach, pyloric ceca, upper intestine, and lower intestine, and the flow of digesta from one gut segment to another. Chemical flux between blood and tissues was assumed to be blood flow–limited, while chemical exchange at the gills was modeled as a countercurrent process regulated by flow and diffusion limitations (Erickson and McKim, 1990). Symbols and abbreviations used in the model are presented in Tables 1–3 and the appendix. The equations that comprise the dietary uptake description are presented in the appendix, while those that make up the systemic portion of the model are given elsewhere (Nichols *et al*., 1990). Solution sets for each point in time were obtained by numerical integration using a commercial software package (ACSL model, Aegis Technologies, Huntsville, AL).

Dietary uptake description: anatomy and physiology. In general, the morphology of the fish GI tract is simpler than that of mammals; however, marked species differences are known to exist (Kapoor *et al*., 1975; Kleinow and James, 2001; Smith, 1989). For example, in some species the surface area of the small intestine is greatly increased by the presence of blind diverticula, called pyloric ceca, while in others the small intestine is a short, simple tube (Buddington and Diamond, 1987). Similarly, some species possess a well-defined stomach while others do not. The GI tract of rainbow trout is characterized by a discrete stomach and numerous, well-developed ceca. The gut description developed in this study, therefore, is composed of four compartments corresponding to the stomach, pyloric ceca and associated upper intestine ("pyloric ceca"), upper intestine distal to the pyloric ceca ("upper intestine"), and lower intestine (Fig. 2). Functionally and morphologically, the pyloric ceca and upper intestine of trout share features of the mammalian jejunum and are the primary sites for uptake of lipid and amino acids. The lower intestine bears some resemblance to the mammalian colon but plays a more active role in the control of the salt/water balance and may also possess endocytotic activity.

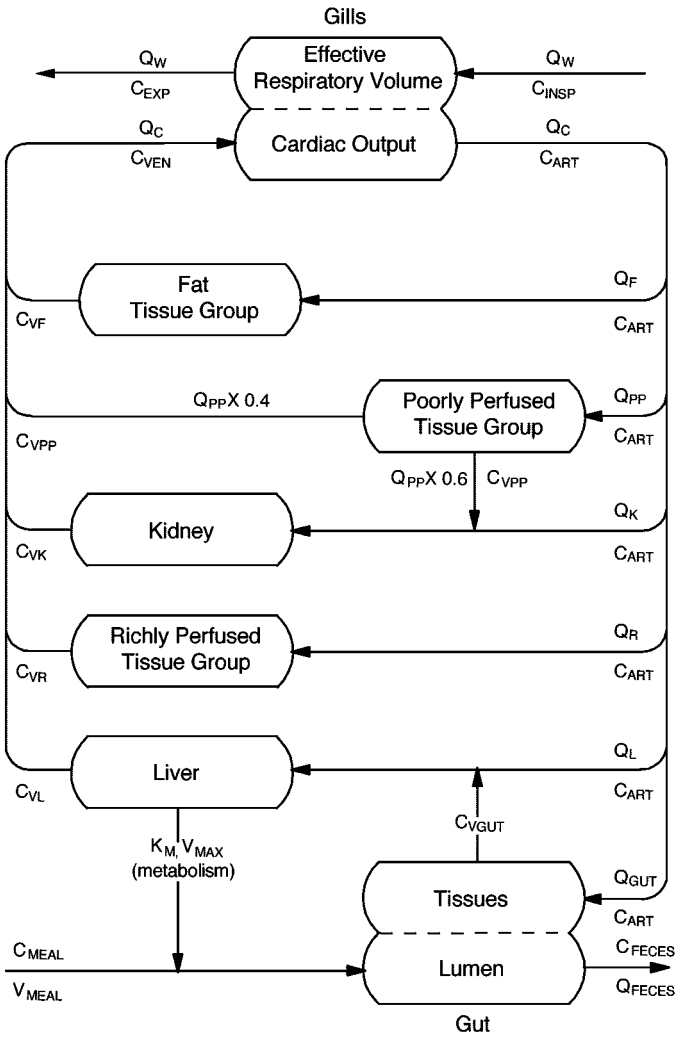

FIG. 1. Schematic representation of a PBTK model for fish incorporating both dietary and branchial routes of exposure. Symbols and abbreviations are described in the appendix and Table 1. The gut is shown here in a simplified form. The complete gut description is given in Figure 2.

An analysis of vascular corrosion casts from chinook and coho salmon suggests that, in salmonids, the coeliacomesenteric artery supplies most of the blood to the stomach, pyloric ceca, and intestines. Venous blood from all of these tissues collects in the hepatic portal vein before entering the liver (Smith and Bell, 1975; Thorarensen *et al*., 1991). Based on these observations, blood flow to the GI tract was modeled as a parallel arrangement of blood vessels, while the flow of digesta was described using a series arrangement of lumenal subcompartments.

Each gut compartment consists of two subcompartments corresponding to the gut tissues and lumenal contents. The volumes of all four lumenal subcompartments were allowed to change following the ingestion of a meal. Gastric emptying to the upper intestine was described using an exponential evacuation equation, as suggested for salmonids by several authors (Elliot, 1991; He and Wurtsbaugh, 1993; Persson, 1986). Integrating, an expression is obtained for the rate of change of stomach volume (V_{LST}) as well as the flow rate of partially digested material to the pyloric ceca as follows:

TABLE 1
Physiological and Anatomical Parameters Used in a PBTK Model for Dietary Uptake of [^{14}C] PCB 52 by Rainbow Trout

Parameter	Term	Parameter value
Body weight (kg)[a]	BW	0.104
Ventilation volume (L/h)[b]	Q_V	10.6
Effective respiratory volume (L/h)[b]	Q_W	7.4
Cardiac output (L/h)[b]	Q_C	2.1
Thickness of the gill diffusion path (μm)[c]	T_G	14.0
Branchial diffusivity of [^{14}C] PCB 52 (cm/h)[d]	G_{DIF}	0.008
Tissue group volumes (as fractions of BW)[e, f]		
Fat	V_F	0.040
Kidney	V_K	0.008
Richly perfused tissue	V_R	0.015
Liver	V_L	0.013
Poorly perfused tissue	V_{PP}	0.876
Stomach	V_{TST}	0.015
Pyloric ceca	V_{TPC}	0.021
Upper intestine	V_{TUI}	0.007
Lower intestine	V_{TLI}	0.005
Arterial blood flow to tissues (as fractions of Q_C)[e]		
Fat	Q_F	0.034
Kidney	Q_K	0.056
Richly perfused tissue	Q_R	0.055
Liver	Q_L	0.029
Poorly perfused tissue	Q_{PP}	0.652
Stomach	Q_{ST}	0.055
Pyloric ceca	Q_{PC}	0.076
Upper intestine	Q_{UI}	0.025
Lower intestine	Q_{LI}	0.018
Surface areas for absorbing gut segments (cm^2)[e]		
Pyloric ceca	A_{PC}	150
Upper intestine	A_{UI}	30
Lower intestine	A_{LI}	30

[a]The average size of fish used by Nichols *et al*. (2001) in dietary studies with [^{14}C] PCB 52.

[b]Average values reported for a 1 kg rainbow trout (Nichols *et al*., 1990). These values were then scaled to body weight as described in the text.

[c]Average branchial diffusion path length for rainbow trout given by Erickson and McKim (1990).

[d]Calculated as 0.5 times the aqueous diffusivity of [^{14}C] PCB 52, as recommended by Erickson and McKim (1990). The aqueous diffusivity of [^{14}C] PCB 52 at 12°C was estimated using the equation given by Wilke and Chang (1955), assuming a molar volume of 210 cm^3/mol.

[e]Sources given in the text.

[f]Calculated from body weight by assuming that all tissues have a specific gravity of 1.0.

$$dV_{LST}/dt = -K_{FST}V_{LST} \quad (1)$$

where K_{FST} is a fitted first-order constant.

Flow rates of digesta (Q_{Li}) from the pyloric ceca to the upper intestine, the upper intestine to the lower intestine, and the lower intestine to the environment were assumed to be first order with respect to the volume of each lumenal subcompartment exceeding a predetermined "baseline" value (V_{LLi}).

$$Q_{Li} = dV_{LLi}/dt = K_{Fi}V_{LLi} \quad (2)$$

The volume of lumenal contents into which chemical is diluted (V_{Li}) was then calculated as the sum of V_{LLi} and a positive real value representing the "empty" state of each intestinal segment (V_{LBi}).

$$V_{Li} = V_{LLi} + V_{LBi} \quad (3)$$

TABLE 2
[^{14}C] PCB 52 Blood:Water, Tissue:Blood, and Lumen:Tissue Partitioning Coefficients

Parameter	Term	Parameter value
Blood:water	$P_{B:W}$	4500
Fat:blood	$P_{F:B}$	140
Kidney:blood	$P_{K:B}$	1.3 (0.19)[a]
Liver:blood	$P_{L:B}$	2.0 (0.35)[a]
Richly perfused:blood	$P_{RP:B}$	2.0
Poorly perfused:blood	$P_{PP:B}$	0.52 (0.06)[a]
Stomach:blood	$P_{TST:B}$	0.97 (0.25)[a]
Pyloric ceca:blood	$P_{TPC:B}$	$6.4 - 0.083 \cdot T$[b]
Upper intestine:blood	$P_{TUI:B}$	$6.4 - 0.083 \cdot T$[b]
Lower intestine:blood	$P_{TLI:B}$	1.67 (0.61)[a]
Pyloric ceca lumen:tissue	$P_{LPC:TPC}$	0.2
Upper intestine lumen:tissue	$P_{LUI:TUI}$	0.2
Lower intestine lumen:tissue	$P_{LLI:TLI}$	0.5
Bile:liver[c]	$P_{B:L}$	0.7

Note. Unitless partitioning values were developed by assuming that blood, tissues, and intestinal contents have a specific gravity of approximately 1.0.

[a]Mean (SD) of values estimated from measured [^{14}C] PCB 52 concentrations under near steady-state conditions.

[b]From 0 to 48 h, repeating the pattern as necessary to simulate additional feedings.

[c]Referred to in the text as a concentration ratio because of the possibility that measured [^{14}C] PCB 52 concentrations in bile reflect both passive diffusion and active transport processes.

TABLE 3
Fitted Rate Constants for Bulk Flow of Digesta, Nutrient Uptake, Gastric Evacuation of [^{14}C] PCB 52, and [^{14}C] PCB 52 Permeability Across the Gastrointestinal Epithelium

Parameter	Term	Parameter value[a]
Bulk flow (1/h)		
Stomach → pyloric ceca	K_{FST}	0.12 (0.004)
Pyloric ceca → upper intestine	K_{FPC}	0.13 (0.003)
Upper intestine → lower intestine	K_{FUI}	0.19 (0.006)
Lower intestine → environment	K_{FLI}	0.89 (0.620)
Nutrient uptake (1/h)		
Pyloric ceca → fish	K_{NPC}	0.13 (0.004)
Upper intestine → fish	K_{NUI}	0.18 (0.006)
Gastric evacuation of [^{14}C] PCB 52 (1/h)		
Stomach → pyloric ceca	K_{STA}	0.21 (0.020)
[^{14}C] PCB 52 permeability coefficients (cm/h)		
Pyloric ceca lumen ↔ tissue	K_{PPC}	0.038 (0.004)
Upper intestine lumen ↔ tissue	K_{PUI}	0.008 (0.001)
Lower intestine lumen ↔ tissue	K_{PLI}	0.003 (0.001)

[a]The SD of the fitted value is given in parenthesis.

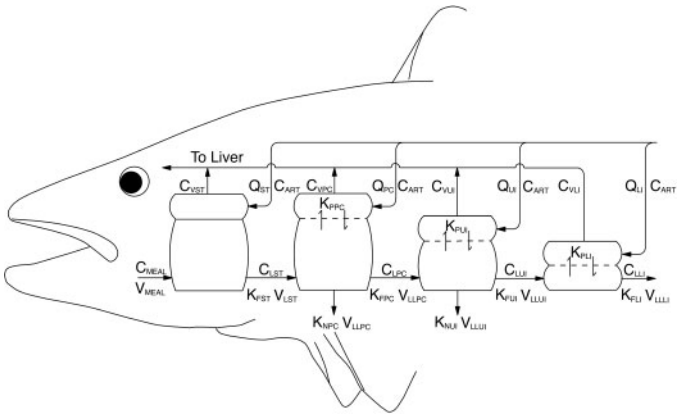

FIG. 2. Schematic representation of the gut description developed in this study. Symbols and abbreviations are given in the Appendix and Tables 1–4. The mathematical equations that correspond to this description are also given in the Appendix.

A second first-order term, $K_{Ni}\ V_{LLi}$, was incorporated into the description of lumenal volume in both the pyloric ceca and upper intestine to account for the reduction in meal volume due to nutrient uptake. Nutrient uptake was assumed to have a negligible impact on the volume of gut tissues or other tissue compartments that comprise the model. Mass-balance equations were then written to describe the rate of change of volume of these lumenal subcompartments (for the upper intestine, where *UI* replaces the subscript *i*).

$$dV_{LUI}/dt = K_{FPC}V_{LLPC} - K_{FUI}V_{LLUI} - K_{NI}V_{LLUI} \tag{4}$$

It was assumed that nutrient uptake does not occur in the lower intestine. Therefore, changes in lumenal volume were determined only by bulk flows in and out of the subcompartment.

Dietary uptake description: chemical flux. Chemical flux between tissues and contents of the pyloric ceca, upper intestine, and lower intestine was assumed to be limited by diffusion across the gastrointestinal epithelium and an associated unstirred water layer of unknown thickness. The direction and magnitude of the diffusion gradient is determined by the difference in chemical activity between gut tissues and contents, referenced to the chemical concentration in tissue using a lumen:tissue partitioning coefficient ($P_{Li:Ti}$). Diffusion rate constants for each gut segment were calculated as the product of surface area (A_i) and a permeability constant (K_{Pi}) representing the combined resistance of intervening diffusion barriers. This description assumes that chemical concentrations in gut contents and tissues can be represented as single, time-varying values. Chemical diffusion between adjacent lumenal subcompartments was considered to be negligible.

From mass-balance considerations, the rate of change of the mass of chemical within tissues of the pyloric ceca, upper intestine, and lower intestine equals the blood flow rate to the tissue (Q_i) times the arterial-to-venous difference in chemical concentration plus diffusive exchange with the corresponding lumenal subcompartment.

$$dM_{Ti}/dt = Q_i(C_A - C_{Vi}) + K_{Pi}A_i(C_{Li} - C_{Ti}P_{Li:Ti}) \tag{5}$$

where the chemical concentration in the tissue (C_{Ti}) is calculated as

$$C_{Ti} = M_{Ti}/V_{Ti} \tag{6}$$

and the concentration in venous blood exiting the tissue (C_{Vi}) is calculated as C_{Ti} divided by a tissue:blood equilibrium partitioning coefficient ($P_{Ti:B}$)

$$C_{Vi} = C_{Ti}/P_{Ti:B} \tag{7}$$

and the subscript i refers to the gut segment of interest.

Following Bungay *et al*. (1981), mass-balance equations used to calculate chemical mass in lumenal contents of the pyloric ceca, upper intestine, and lower intestine (M_{Li}) include terms for input from the upstream compartment (Li – 1), egestion to the downstream compartment or to the environment, and diffusive flux into or out of the corresponding tissue subcompartment.

$$dM_{Li}/dt = Q_{Li-1}C_{Li-1} - Q_{Li}C_{Li} - K_{Pi}A_i(C_{Ti}P_{Li:Ti} - C_{Li}) \tag{8}$$

where the chemical concentration in the lumen (C_{Li}) is calculated as

$$C_{Li} = M_{Li}/V_{Li} \tag{9}$$

An additional input term, $K_{BILE}C_{VL}$, was used to account for biliary elimination of chemical to contents of the pyloric ceca. The rate constant in this term was calculated as the product of bile flow rate, a bile:liver concentration ratio (representing the net result of all relevant transport processes), and a liver:blood partitioning coefficient. The assumption implied by this approach is that the rate of chemical elimination in bile is proportional to the chemical concentration in liver (Bungay *et al*., 1981).

For the stomach lumen, the input term $Q_{Li-1}\ C_{Li-1}$ was dropped and the mass of chemical in the meal was used as the initial condition. In feeding studies with [^{14}C] PCB 52, Nichols *et al*. (2001) found that chemical concentrations in the stomach contents declined sharply with time. The absence of a corresponding increase in [^{14}C] PCB 52 concentrations in blood and tissues during early time points suggested, however, that very little chemical was absorbed within the stomach. Instead, it is apparent that [^{14}C] PCB 52 was egested to the pyloric ceca at a faster rate than that of the meal itself (wet weight basis). Observed changes in the mass of [^{14}C] PCB 52 in the stomach contents were well described using a first-order equation.

$$dM_{LST}/dt = -K_{STA}M_{LST} \tag{10}$$

Biological parameters and scaling. Physiological and anatomical inputs to the model are summarized in Table 1. In mammals, blood flow to the gut increases after feeding (Fara, 1984). This increase tends to be localized to regions that contain food, and an increase in flow through the coeliac artery generally precedes a flow increase in the mesenteric arteries (Gallavan *et al*., 1980). Similar changes probably occur in fish (Axelsson *et al*., 2000); however, existing information is insufficient to permit the incorporation of time- or volume-dependent changes in blood flow to different gut segments. Blood flows to the stomach, pyloric ceca, and intestinal segments were, therefore, patterned after values obtained by Barron *et al*. (1987) and assumed to remain constant.

Blood flow to the liver was calculated as the sum of blood flowing from the GI tract (hepatic portal vein) and an arterial blood supply, estimated by Barron *et al*. (1987) to be 2.9% of cardiac output. The model also incorporates a portal blood supply to the kidney (caudal vein), which drains a large portion of the trunk musculature (Smith and Bell, 1975). Arterial and portal blood supplies to the liver and kidney were assumed to mix before entering each tissue. Volume-weighted blood flows to the fat, kidney, and richly perfused tissues were set equal to those determined by Nichols *et al*. (1990). Arterial blood flow to the poorly perfused tissues was calculated as the difference between cardiac output and the sum of all other blood flows.

Stomach, upper intestine, and lower intestine tissue volumes as fractions of body weight were patterned after values given by Barron *et al*. (1987), assuming a specific gravity of 1.0 for all tissues. In earlier models for trout (Nichols *et al*., 1990), these tissues were considered to be part of the richly perfused tissue compartment. Thus, the volume of the richly perfused compartment in the present model was calculated as the difference between the value (6.3%) reported by Nichols *et al*. (1990) and the summed volume (4.0%) of the GI tract. Liver and kidney volumes as fractions of body weight were set

equal to those reported by Nichols *et al*. (1990). The volume of the fat compartment (4.8%) was set equal to about one-half of that determined previously for adult rainbow trout (Nichols *et al*., 1990), reflecting a relative absence of adipose fat in subadult animals. The volume of the poorly perfused compartment was calculated as total body weight minus the summed weight of all other tissue compartments.

An examination of stomach content data suggested the need for a 2 h time lag between the time of feeding and the start of gastric evacuation. Gastric emptying was then allowed to proceed until the stomach was empty. In contrast to the stomach, the "empty" state of the upper and lower intestines between feedings was characterized by the retention of some digesta, corresponding to about 0.4% and 0.1%, respectively, of the fish's weight. Because of their number (typically 40–60 for trout; Buddington and Diamond, 1987) and small internal diameter, the pyloric ceca could not be sampled directly. The volume of digesta remaining in the pyloric ceca when "empty" was, therefore, set equal to 2.5 times that of the upper intestine, or somewhat less than the relative difference in volume of these two tissues.

Surface areas for diffusion within the pyloric ceca, upper intestine, and lower intestine were estimated from morphometric data reported by Buddington and Diamond (1987). Based on the relationship given for rainbow trout, the estimated postgastric gut area for a 1 kg fish was determined to be 210 cm^2 (not taking into account foldings, villi, and microvilli). The pyloric ceca account for approximately 70% of the total gut area in trout or about 150 cm^2 for a 1 kg animal. The remaining gut area was divided equally between the upper and lower intestinal segments.

Trout possess gall bladders and release bile as needed to assist in digestion of dietary lipid, but the timing and duration of bile release are poorly known. For the purposes of this study, the bile flow rate was set equal to that (0.05 ml/kg/h) determined by Schmidt and Weber (1973) in cannulation studies with rainbow trout and assumed to remain constant.

Cardiac output, ventilation volume, oxygen consumption rate, and the absorptive surface areas of the pyloric ceca, upper intestine, and lower intestine were scaled to body weight using the following relationship: $Y = \alpha W^{\beta}$, where α is the parameter value for a 1 kg rainbow trout, $\beta = 0.75$, and W is the fish's weight in kg. With respect to absorptive gut surfaces, an argument can be made for setting $\beta = 0.67$ (in accordance with the surface law); however, when data from rainbow trout were analyzed, the larger exponent was more predictive (total gut area; Buddington and Diamond, 1987). All compartment volumes were scaled directly to body weight.

Trout/fathead minnow experimental system. The dietary uptake description was calibrated using data from feeding studies with rainbow trout (Nichols *et al*., 2001). Subadult (103.8 ± 11.5 g) trout, which had been reared on a prepared diet (Silver Cup, Murray and Sons, Murray, UT), were converted to a diet of 60-day-old fathead minnows at a feeding rate of 4% of body weight once every 48 h. Typically, this involved 8 to 10 minnows per feeding, depending on the measured weight of each trout. The trout were then fed a single meal of minnows that had been exposed previously to [^{14}C] PCB 52 in water to achieve a "high" (mean of 1663 ng/g) or "low" (mean of 244 ng/g) residue concentration. This high concentration is comparable to total PCB concentrations in forage fish from the lower Great Lakes, while the low concentration is similar to summed levels of tetrachlorinated congeners (Oliver and Niimi, 1988). Trout were killed 6, 12, 24, 48, or 96 h after consuming the contaminated minnows. Those killed at 96 h were fed a meal of uncontaminated minnows at 48 h postdosing. The GI tract of each fish was partitioned into the stomach, upper intestine (excluding pyloric ceca), and lower intestine, and [^{14}C] PCB 52 concentrations were measured in gut contents and tissues as well as in other selected tissues. Using the same feeding protocol, Nichols *et al*. (2001) also described the pattern of gastric evacuation and changes in the wet weight, dry weight, and lipid content of digesta as it moved down the GI tract.

[^{14}C] PCB 52 partitioning coefficients. Equilibrium blood:water, tissue:blood, and lumenal contents:tissue partitioning coefficients for [^{14}C] PCB 52 are given in Table 2. The blood:water partitioning value ($P_{B:W}$) was set equal to that (4500) measured by Fitzsimmons *et al*. (2001) in branchial efflux studies with rainbow trout. A fat:water partitioning coefficient was calculated from an empirical relationship given by Bertelsen *et al*. (1998), ignoring the contribution of tissue water: $\log P_{F:W} = 0.9 \log K_{OW} + 0.31$. Using a $\log K_{OW}$ value of 6.1 (Shiu and Mackay, 1986), this relationship gives an estimated $P_{F:W}$ value of about 630,960. Fat:blood partitioning was then calculated by dividing $P_{F:W}$ by $P_{B:W}$. Tissue:blood partitioning coefficients for all other tissues except those of the GI tract were calculated from measured [^{14}C] PCB 52 concentrations in trout 96 h after they had ingested a meal of contaminated minnows (Nichols *et al*., 2001). This approach is supported by the observation that tissue:blood concentration ratios for well-perfused tissues did not change between 48 and 96 h.

Partitioning coefficients assigned to the GI tract were developed with the knowledge that digestion results in time-dependent changes in lipid content of both the digesta and tissues. The goal of this effort was to reproduce important trends in the direction and magnitude of chemical activity gradients without introducing unnecessary complexity. Tissue:blood partitioning coefficients for the upper intestine and pyloric ceca were calculated as declining linear functions of time, assuming a transient increase in the lipid content of these tissues. Slope and intercept terms were based on observed changes in the tissue:blood [^{14}C] PCB 52 concentration ratio in upper intestinal tissue from 0 to 48 h (Nichols *et al*., 2001). This pattern was then repeated as necessary to account for changes in partitioning associated with the consumption of additional meals.

Lumenal contents:tissue partitioning coefficients were based on observed (upper and lower intestines) and inferred (pyloric ceca) changes in the lipid content and wet weight of gut contents during digestion (Fig. 2 in Nichols *et al*., 2001) and on changes in the lipid content of tissues inferred from measured tissue:blood concentration ratios. Observed lumen:tissue [^{14}C] PCB 52 concentration ratios were not used to estimate lumen:tissue partitioning because of the possibility that these two subcompartments had not attained chemical equilibrium.

The pyloric ceca lumen:tissue partitioning coefficient was calculated as a linear function of time, starting at 1.0 and declining to 0.2 at 12 h after feeding. This approach assumes that the lipid content of both the digesta and tissue were high at early time points, followed by a rapid decline in the lipid content of digesta. Thereafter, the partitioning coefficient was set equal to 0.2, strongly favoring chemical uptake by tissue. The upper intestinal lumen:tissue partitioning coefficient was set equal to a constant value of 0.2, based on the low lipid content of chyme (1–1.5%) and the estimated lipid content of the tissue (>6% from 0 to 24 h). This latter estimate was based on observed tissue:blood chemical partitioning and the measured lipid content of rainbow trout blood (1.5%; Nichols *et al*., 1991). The lower intestinal lumen:tissue partitioning coefficient was set equal to 0.5, based on the average lipid content of feces (0.9%) and the estimated lipid content of the tissue (-2%; calculated in the same manner as for the upper intestine). The lipid content of feces increased somewhat between 12 and 36 h postfeeding, probably due to exfoliation of the gastrointestinal epithelium (Cotton, 1972). The need to develop a time-dependent lumen:tissue partitioning relationship for the lower intestine was obviated, however, by the relative insensitivity of the model to changes in this parameter value.

The bile:liver concentration ratio was based on [^{14}C] PCB 52–derived radioactivity in a pooled sample of bile collected 24 h postfeeding (Nichols *et al*., 2001). The gall bladders of fish sampled at 6 and 12 h were void of bile, presumably because fish were actively digesting the meal. The 24 h sample, therefore, represents bile that was formed between 12 and 24 h. The small size of the bile sample precluded further analysis. It is possible that some or all of the activity measured in this sample was present as a metabolite of [^{14}C] PCB 52. However, the absence of [^{14}C] PCB 52 metabolites in liver tissues or in contents of the upper intestine argues against this possibility (Nichols *et al*., 2001). A small amount of [^{14}C] 3-OH PCB 52 was detected in feces, but this material accounted for less than 1% of total radioactivity. Accordingly, metabolic rate and capacity parameters in the present modeling effort were set equal to zero.

Estimation of digestion parameters and gut permeability coefficients for [^{14}C] PCB 52. Calibration of the model was accomplished in three steps. In the first step, rate constants controlling gastric evacuation of digesta and [^{14}C] PCB 52 were determined by fitting model simulations to measured wet weights and [^{14}C] PCB 52 concentrations in stomach contents. The resulting equations were then treated as inputs to the remaining gut description.

Parameters controlling nutrient uptake and bulk flow of material down the GI tract were estimated by fitting model simulations to measured wet weights of digesta in the upper intestine and lower intestine. To constrain this effort, fecal egestion as a percentage of meal volume was set equal to 25% at 48 h. Expressed on a dry weight basis, digestibility in rainbow trout ranges from 50 to 90%, usually tending toward the upper end of this range when fish are fed a natural diet (Atkinson *et al*., 1984; Buddington, 1980; De La Noüe and Choubert, 1986; Tacon and Rodrigues, 1984). Because the water content of digesta determined by Nichols *et al*. (2001) changed very little with sampling time or location, digestibility expressed on a dry weight basis can be translated directly into an estimate of fecal egestion as a percentage of ingested meal volume.

In the third calibration step, permeability constants controlling [^{14}C] PCB 52 flux across the gastrointestinal epithelium were estimated by fitting model simulations to measured [^{14}C] PCB 52 concentrations in contents and tissues of the upper and lower intestines. This approach was considered to be valid because chemical absorbed within the gut was efficiently removed by blood, thereby maintaining a large inward gradient for uptake at all sampling times. Preliminary efforts using a more "global" approach to model calibration, including the simulation of [^{14}C] PCB 52 kinetics in blood and tissues, did not improve the fit of model simulations to data from the GI tract. Moreover, this approach transfers uncertainty in processes physically removed from the GI tract (e.g., blood flow rates to tissues) to estimates of chemical permeability. As indicated previously, it was not possible to obtain reliable data for the pyloric ceca. The role of the pyloric ceca in digestion and chemical uptake was inferred from changes in lipid content and chemical concentration in contents of the stomach and upper intestine.

The model was calibrated using ACSL Optimize (Aegis Technologies) in conjunction with the ACSL Model software package. Simulations were initially generated by setting the [^{14}C] PCB 52 concentration in food equal to that (1663 ng/g) of the high dose exposure group (Nichols *et al*., 2001). An examination of data presented by Nichols *et al*. (2001) suggested that the relative standard errors of mean [^{14}C] PCB 52 concentrations in gut contents and tissues were similar at all time points. Therefore, the simulations were optimized by minimizing relative differences between measured and predicted values using maximized likelihood estimation (Nelder-Mead algorithm). Starting parameter values were obtained by subjective evaluation of model performance. These starting values were then varied to ensure that fitted parameters provided a stable solution. Finally, the model calibrated using data from the high dose exposure group was evaluated by simulating [^{14}C] PCB 52 kinetics in the low (244 ng/g) dose group, changing only the [^{14}C] PCB 52 concentration in food.

Sensitivity analysis. A limited sensitivity analysis was conducted by independently changing the value of selected input parameters (*PAR*) by $\pm 1\%$ and examining the effects of these changes on important state variables (*SV*). Normalized sensitivity coefficients (*S*) were calculated as $(\Delta SV/\Delta PAR)(PAR/SV)$. This analysis was performed using the high [^{14}C] PCB 52 concentration in fathead minnows and parameter settings from Tables 1–3. Negative values of *S* indicate an inverse relationship between the state variable and the selected model parameter.

RESULTS

Gastrointestinal Physiology

Figure 3 shows the pattern of gastric evacuation and changes in wet weight of digesta in both the upper and lower intestines after feeding rainbow trout a single meal of 60-day-old minnows. First-order rate constants for bulk flow of digesta down the intestinal tract and nutrient uptake within the pyloric ceca and upper intestine were adjusted to simulate these patterns (Table 3). The principal constraints on this effort were the observed dampening of volume changes in both the upper and lower intestines and the need to simulate digestibility values measured in feeding studies with fish.

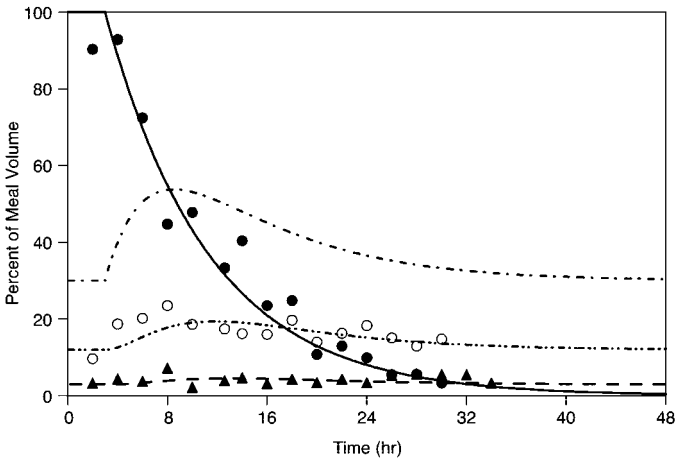

FIG. 3. Observed and predicted wet weight of contents from the stomach (●), upper intestine (○), and lower intestine (▲) after feeding rainbow trout a meal of 60-day-old fathead minnows (4% body weight). Model simulations, shown as solid (stomach), dot-dot-dashed (upper intestine), and dashed (lower intestine) lines, were obtained by fitting rate constants for nutrient uptake and bulk transport down the GI tract as described in the text. The simulation for the pyloric ceca (dot-dashed) is also given, although wet weights were not measured in this segment. Gastric evacuation data were given previously by Nichols *et al*. (2001).

Dietary Dosing Studies

Gut permeability coefficients for [^{14}C] PCB 52 were fitted by modeling to measured concentrations in gut contents and tissues from the high dose exposure group (Table 3). Figure 4 shows measured and predicted [^{14}C] PCB 52 concentrations in contents of the stomach, pyloric ceca (model simulation only), upper intestine, and lower intestine. Measured and predicted [^{14}C] PCB 52 concentrations in arterial blood and in the contents and tissues of the lower intestine are given in Figure 5. Overall, the optimized model predicted that trout would absorb more than 98% of [^{14}C] PCB 52 contained in the diet. Net assimilation efficiency, calculated as the mass of [^{14}C] PCB 52 in trout divided by the mass contained in the meal, reached a maximum value of 95% at about 48 h, declining slowly thereafter due to fecal elimination and branchial efflux.

Figure 6 shows simulated and observed [^{14}C] PCB 52 concentrations in tissues of the GI tract while Figure 7 shows simulated and measured values in fat, blood, liver, and muscle. [^{14}C] PCB 52 concentrations in tissues of the lower GI tract tended to follow the [^{14}C] PCB 52 concentration time course in

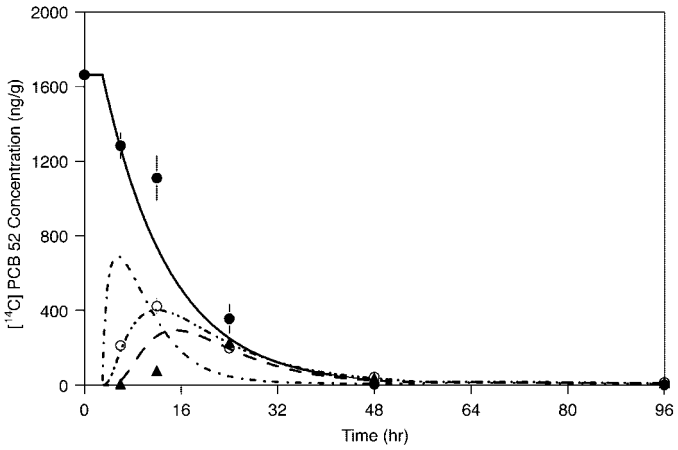

FIG. 4. Measured and predicted [^{14}C] PCB 52 concentrations in contents of the stomach (●), upper intestine (○), and lower intestine (▲). Model simulations are shown as solid (stomach), dot-dot-dashed (upper intestine), and dashed (lower intestine) lines. The simulation for the pyloric ceca (dot-dashed) is also given, although [^{14}C] PCB 52 concentrations were not measured in this segment. Measured values are given as individual points (mean ± SE). The data shown here and in Figures 5–7 are from the high dose group (1663 ng/g) tested by Nichols *et al*. (2001). Model simulations shown in Figures 4–8 were obtained after fitting rate constants for diffusive uptake from the GI tract as described in the text.

blood. A more complex pattern was predicted for tissues of the pyloric ceca and upper intestine due to the transient increase in tissue:blood partitioning associated with digestion of a second (uncontaminated) meal. The effect of this change in partitioning was to cause [^{14}C] PCB 52 to redistribute from other tissues to tissues of the upper GI tract. The model provided a good fit to measured [^{14}C] PCB 52 concentrations in all tissues, although observed uptake into gut tissues and chemical redistribution from lean tissues to fat occurred more slowly than predicted.

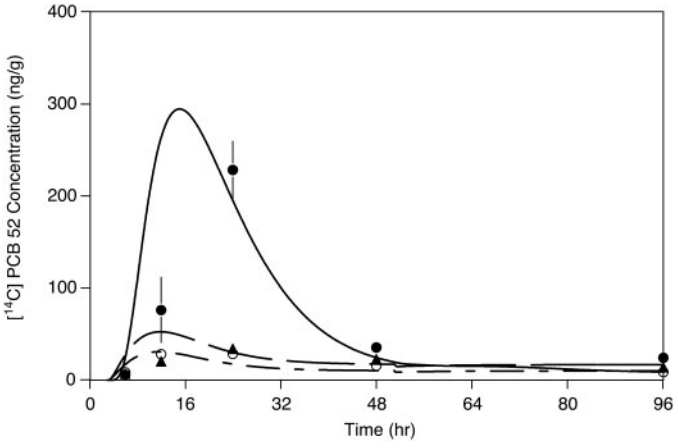

FIG. 5. Measured and predicted [^{14}C] PCB 52 concentrations in arterial blood (○) and in contents (●) and tissues (▲) of the lower intestine. Model simulations are shown as solid (contents), dashed (tissues), and dot-dashed (blood) lines.

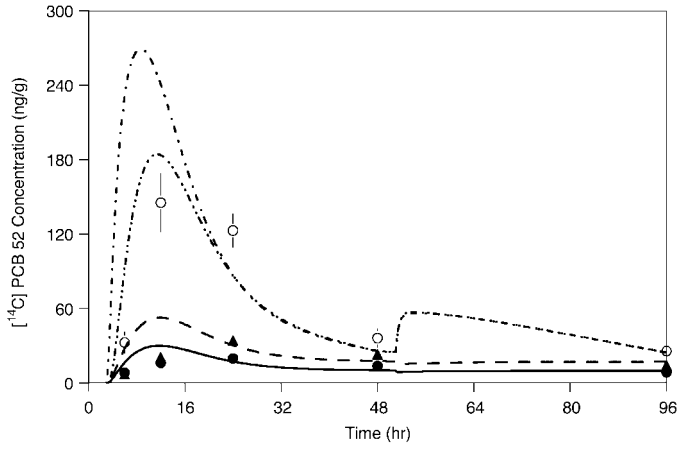

FIG. 6. Measured and predicted [^{14}C] PCB 52 concentrations in tissues of the stomach (●), upper intestine (○), and lower intestine (▲). Model simulations are shown as solid (stomach), dot-dot-dashed (upper intestine), and dashed (lower intestine) lines. The simulation for the pyloric ceca (dot-dashed) is also given, although [^{14}C] PCB 52 concentrations were not measured in this segment.

Fitted permeability coefficients for [^{14}C] PCB 52 were subsequently evaluated by simulating data from the low dose exposure group. The small number of samples collected from the low dose group limited this analysis to a qualitative assessment of model fit. Nevertheless, it is clear that the level of correspondence between simulated and measured values was comparable to that observed for the high dose exposure group (Fig. 8). This result was not surprising given the inherently linear nature of the model and the dose dependence of [^{14}C] PCB 52 kinetics noted previously in feeding studies with trout (Nichols *et al*., 2001).

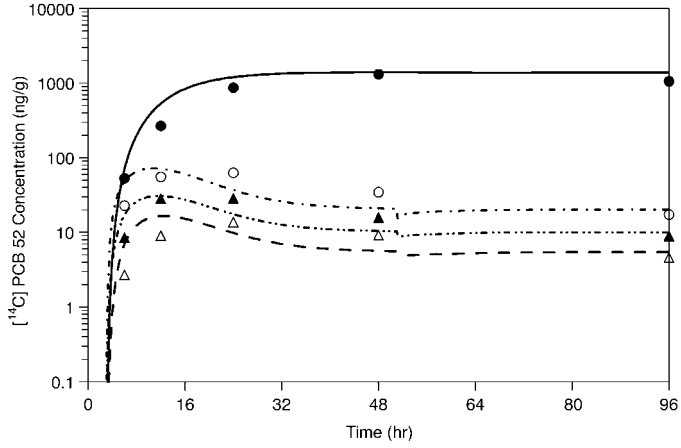

FIG. 7. Measured and predicted [^{14}C] PCB 52 concentrations in adipose fat (●), liver (○), arterial blood (▲), and white muscle (△) at the high dose (1663 ng/g) exposure level. Model simulations are shown as solid (fat), dot-dashed (liver), dot-dot-dashed (blood), and dashed (muscle) lines.

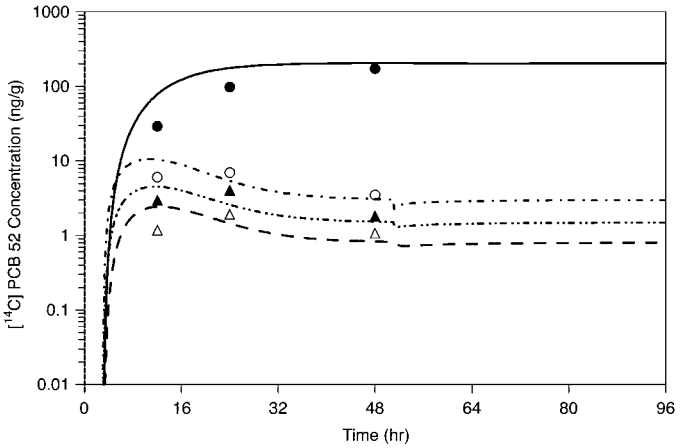

FIG. 8. Measured and predicted [^{14}C] PCB 52 concentrations in adipose fat (●), liver (○), arterial blood (▲), and white muscle (△) at the low dose (244 ng/g) exposure level. Model simulations are shown as solid (fat), dot-dashed (liver), dot-dot-dashed (blood), and dashed (muscle) lines.

Sensitivity Analysis

Table 4 shows the results of a restricted sensitivity analysis. Changes in digestive parameters pertaining to the pyloric ceca had a large impact on total fecal egestion (as a fraction of meal volume) and maximum [^{14}C] PCB 52 concentrations achieved within the gut contents, as indicated by normalized sensitivity coefficients close to 0.5. Changes in digestive parameters for the upper intestine had a large effect on fecal egestion but relatively little impact on maximum [^{14}C] PCB 52 concentrations within the gut. Changes in chemical permeability within each gut segment had a moderate (upper and lower intestines) to large (pyloric ceca) effect on maximum [^{14}C] PCB 52 concentrations, starting with the segment within which the adjustment was made.

In contrast, changes in blood flow to each gut segment had little impact on model simulations (data not shown). The model was also insensitive to changes in tissue:blood chemical partitioning coefficients and parameters that control biliary elimination of [^{14}C] PCB 52. Changes in pyloric ceca lumen:tissue partitioning had a small impact on predicted maximum [^{14}C] PCB 52 concentrations in the upper and lower intestines (positive coefficients of 0.05 to 0.07), while changes in lumen: tissue partitioning within the upper and lower intestines had little effect on model outputs. Because almost all of the [^{14}C] PCB 52 was taken up by trout, changes in gut model parameters had very little effect on net assimilation efficiency or the chemical concentration time course in blood.

Based on the results of the sensitivity analysis, an effort was made to determine how changes in gut physiology parameters could impact the fitting of gut permeability coefficients for [^{14}C] PCB 52. Special attention was given to rate constants for bulk flow out of the pyloric ceca and upper intestine, since both of these parameters were shown to have a large impact on [^{14}C] PCB 52 concentrations in the contents of downstream compartments. Generally, an increase in flow from either compartment could be offset by a comparable percentage-wise increase in the permeability coefficient for the compartment that was immediately downstream, resulting in similar overall [^{14}C] PCB 52 kinetics. These adjustments were constrained, however, by the fact that changes in bulk flow also impacted fecal egestion. The range of physiologically reasonable fecal egestion values is about 10–50% (see *Gastrointestinal physiology*). Given the sensitivity of the model to changes in bulk flow down the GI tract, this means that there is a relatively narrow range within which fitted flow rates, and by extension fitted permeability constants, could be adjusted to obtain good overall (physiology and chemical kinetics) model performance.

TABLE 4
Model Sensitivity to Changes in GI Tract Parameters

Model parameters	[^{14}C] PCB 52 concentration in blood at 48 h	Fecal egestion (fraction of meal volume)	Maximum [^{14}C] PCB 52 concentration in lumenal contents		
			P. ceca	U. intestine	L. intestine
Bulk flow rate (1/hr)					
Pyloric ceca → upper intestine	nc	+0.50	nc	+0.34	+0.50
Upper intestine → lower intestine	nc	+0.49	nc	nc	+0.08
Lower intestine → environment	nc	nc	nc	nc	nc
Nutrient uptake (1/h)					
Pyloric ceca → fish	nc	–0.49	nc	–0.07	–0.18
Upper intestine → fish	nc	–0.48	nc	+0.18	+0.07
[^{14}C] PCB 52 permeability (cm/h)					
Pyloric ceca lumen ↔ tissue	nc	nc	–0.53	–0.74	–0.74
Upper intestine lumen ↔ tissue	nc	nc	nc	–0.19	–0.21
Lower intestine lumen ↔ tissue	nc	nc	nc	nc	–0.18

Note. Model sensitivity is expressed using normalized sensitivity coefficients (S), calculated as described in the text. Coefficients calculated by changing each parameter by ±1% were identical to the second decimal place. No change (nc) refers to instances where: $-0.05 < S < 0.05$.

DISCUSSION

The purpose of this study was to develop a PBTK model for dietary uptake of hydrophobic organic chemicals by fish. This is the third in a series of PBTK models for fish that address the principal routes of environmental exposure. Previous models have been developed to describe both inhalation (Nichols *et al*., 1990) and dermal (Nichols *et al*., 1996) exposures.

Chemical uptake from the GI tract (oral gavage as well as dietary dosing) has been described in several PBTK models as a first-order absorptive process (Ramsey and Andersen, 1984). If the dose is modeled as the mass of the ingested compound, this description does not require the definition of a gut compartment. However, this approach does not provide for fecal elimination of unassimilated compound (i.e., oral bioavailability $<100\%$). A second approach is to define one or more gut compartments and to model ingestion and fecal egestion as bulk flow rates or periodic events (Bischoff *et al*., 1971; King *et al*., 1983; Lutz *et al*., 1977). A model structured in this manner can be used to describe biliary elimination and enterohepatic circulation as well as chemical accumulation in gut tissues.

A detailed PBTK model for dietary uptake of chlordecone in the rat was provided by Bungay *et al*. (1981). The gut portion of this model consists of six compartments corresponding to the stomach, small intestine (three compartments), cecum, and lower intestine. Lumenal volumes and rate constants for bulk transport of digesta down the GI tract were based on studies with nonabsorbable gut markers and were time-averaged for a specific feeding regime. Diffusion limitations on chemical flux were assumed to exist at both the tissue/blood and tissue/gut lumen interfaces. Diffusion rate constants (12) and equilibrium partitioning coefficients (12) were assumed to remain constant and were determined by modeling to measured chemical residues in tissues and gut contents.

The model developed in the present study includes four gut compartments corresponding to the stomach, pyloric ceca, upper intestine (distal to the pyloric ceca), and lower intestine. Factors that promote dietary uptake of hydrophobic compounds by fish were explicitly incorporated, including a reduction in meal volume and a decline in chemical affinity for gut contents associated with uptake of dietary lipid. In contrast to the model given by Bungay *et al*. (1981), the lumenal volume of each compartment was allowed to change in time with the passage of a meal. The resulting model structure is consistent with the relatively short length of the trout GI tract, reduces to a minimum the number of gut compartments required to simulate critical digestive events, and provides flexibility needed to model different dosing scenarios including changes in meal size and feeding frequency.

The model was calibrated using data from rainbow trout that were fed a single meal of [^{14}C] PCB 52–contaminated fathead minnows (Nichols *et al*., 2001). Generally, the model reproduced all major trends in the data and accurately predicted maximum [^{14}C] PCB 52 concentrations achieved within each tissue. The observed kinetics of uptake and distribution were somewhat slower than those predicted by the model, although these discrepancies were relatively minor. If chemical flux between blood and tissues was limited by diffusion instead of blood flow rate (as presently assumed), the overall kinetics of the system would slow down, depending on the location and extent of this diffusion limitation. This would not, however, explain the slower-than-predicted uptake of [^{14}C] PCB 52 by gut tissues, since the gut description already assumes that uptake across the gastrointestinal epithelium is diffusion-limited. Alternatively, digestive physiology parameters obtained by assuming that fecal egestion equals 25% of meal volume may not accurately reflect the physiology of fish employed in this effort. In particular, rate constants that control nutrient uptake within the GI tract influence the timing and extent of volume reduction within the gut lumen and the concentrating effect of this reduction on chemical concentration; generally, as these rate constants decrease, the rate of chemical uptake decreases and fecal egestion increases. Likely violations of one or more simplifying assumptions (e.g., constant blood flow to each gut segment during digestion) may have also contributed to differences between simulated and observed kinetics. This and other PBTK models for fish will improve as our understanding of fish anatomy, physiology, and biochemistry increases.

In feeding studies with trout, Nichols *et al*. (2001) described two major findings. Firstly, trout exposed to [^{14}C] PCB 52 in the form of a "naturally" contaminated diet absorbed greater than 95% of the chemical presented to them. Of this, two-thirds was absorbed within 12 h of feeding, probably in association with a liquid phase release of lipid from the stomach to the proximal intestine (Jobling, 1987). Secondly, peak [^{14}C] PCB 52 concentrations in trout feces were higher than those expected from lipid-based equilibrium partitioning between tissues and contents of the lower GI tract. At a glance, this second finding appears to contradict the first; the extent and timing of [^{14}C] PCB 52 uptake suggests that [^{14}C] PCB 52 and lipid move together, while the observed chemical disequilibrium in the lower GI tract suggests that they do not. Using the gut model it can be shown that this focus on lipid oversimplifies the process of dietary uptake for hydrophobic compounds by failing to recognize temporal and spatial features of the system. Thus, a chemical disequilibrium in the lower intestine may be produced by imposing a small diffusion limitation on uptake within each of the absorbing gut segments. This occurs partly because [^{14}C] PCB 52 is retained within the gut contents, but also because chemical absorbed across the gut is distributed to major storage tissues such as muscle and fat. By the time the pulse of digested material has reached the lower intestine, [^{14}C] PCB 52 concentrations in blood, and by extension those in tissues of the lower intestine, are extremely low.

Previously, Gobas *et al*. (1988) proposed that a diffusion limitation controls dietary uptake of hydrophobic compounds by fish and that the magnitude of this limitation increases with chemical log K_{OW}. In the model given by Gobas *et al*. (1988), the gut lumen is represented as a single well-mixed compartment, and a lumped transport parameter was used to describe chemical transfer between the gut contents and the chemical's final storage site in the fish. A resistance term was subsequently incorporated into an empirical model of digestion, and techniques were devised to estimate its value for different chemicals and species using experimental data (Gobas *et al*., 1988, 1993a,b). The current work is consistent with these earlier reports and extends them by providing an improved basis for estimating and interpreting these resistances.

An interesting outcome of the present study is that fitted permeability coefficients for all gut segments varied by less than a factor of 15. It must be pointed out, however, that surface areas ascribed to each gut segment do not take into account fine structures such as villi and microvilli. The actual surface areas for diffusion are, therefore, likely to be much larger than those given in Table 1. Because gut surface area and permeability are multiplied in Equation 4, an increase in surface area would result in a proportional decrease in the fitted permeability constant.

The true nature of the "diffusion" limitation within the gut is also unclear. Restricted diffusion of very hydrophobic compounds has been demonstrated in a variety of biological systems, but the possibility that other factors contribute to the observed kinetics cannot be ruled out. One way to address this question is to compare the magnitude of fitted gut permeability coefficients for [^{14}C] PCB 52 with the permeability coefficient for [^{14}C] PCB 52 flux at trout gills. Following the approach outlined by Erickson and McKim (1990), the gill permeability coefficient for [^{14}C] PCB 52 may be calculated as the aqueous diffusivity (0.017 cm^2/h) divided by the thickness of the gill diffusion path (14 μm). Application of an additional factor (0.5) to correct for reduced diffusion in the gill epithelium results in a final value of 12.1 cm/h.

Comparing values, the gill permeability coefficient for [^{14}C] PCB 52 is about 320 times greater than the fitted permeability coefficient for the pyloric ceca. Some of this difference may be due to a relatively greater diffusion distance across the gastrointestinal epithelium and associated unstirred water layer. Nevertheless, it seems likely that additional factors limit the rate of chemical uptake within the GI tract. Some of these factors, possibly including the rate of dissolution of lipid micelles and physical processes that stir the gut contents, might be expected to operate similarly on all compounds. Others, perhaps including the strength of chemical binding to undigested material as well as diffusivity within the gastrointestinal epithelium, may vary with chemical log K_{OW}. Whatever their nature, kinetic limitations on dietary uptake of [^{14}C] PCB 52, acting in concert with changes in meal volume and lipid content, appear to have maintained an inwardly directed activity gradient for diffusion of [^{14}C] PCB 52 along the entire length of the GI tract. As a result, the mass of [^{14}C] PCB 52 in feces depends on the extent of diffusive uptake in each gut compartment and on bulk flow of material down the GI tract.

A possible shortcoming of our study is that PCB 52 (unlabeled) does not accumulate in aquatic biota to the extent shown by more hydrophobic compounds. A compound is said to biomagnify when lipid-normalized chemical concentrations in a predator are greater than that of its prey. In field-collected fish, biomagnification is generally observed only for compounds with log K_{OW} values greater than 6.0. In the absence of metabolism, compounds with log K_{OW} values less that 6.0 tend to achieve concentrations that represent a thermodynamic equilibrium between the fish (predator or prey) and surrounding water. Evidence for biomagnification of PCB 52 was obtained by Russell *et al*. (1995, 1999) in two field sampling efforts. In the first study, lipid-normalized PCB 52 concentrations in Lake Erie white bass (*Morone chrysops*) were 2.7 times higher than those measured in their principal prey, the emerald shiner (*Notropis atherinoides*). In a second study, lipid-normalized PCB 52 concentrations in several fish species tended to increase with their trophic status. In both investigations, however, the extent of PCB 52 biomagnification was lower than that exhibited by several more hydrophobic PCB congeners. A need exists for additional studies using "naturally contaminated" diets and realistic feeding conditions to determine whether factors that control dietary uptake of [^{14}C] PCB 52 by trout can be generalized to higher log K_{OW} compounds and other fish species. It would also be instructive to determine whether a model with diffusion limitations on dietary uptake predicts the elimination of [^{14}C] PCB 52 by trout when contaminated fish are fed an uncontaminated meal.

With few exceptions, previous PBTK models incorporating a dietary uptake description have treated the gut as a single, well-mixed compartment. In the present study, the gut was divided into four functionally distinct segments. When combined with basic information on fish gastrointestinal physiology, this approach provides an improved understanding of the physical, chemical, and biological factors that control dietary uptake of hydrophobic organic compounds by fish. In contrast to other dietary uptake models for fish, our model can be used to simulate the chemical time course in specific tissues of interest, including those of the GI tract. A potentially important use of the model is to simulate the uptake of compounds that undergo substantial metabolic transformation. In studies with *in situ* gut preparations, the GI tract has been shown to play an important role in the metabolism of PAHs by fish, altering their form and limiting systemic bioavailability (Kleinow *et al*., 1998; Van Veld *et al*., 1988). Operating in series with first-pass metabolism in the liver, this activity can substantially reduce bioaccumulation of contaminant residues (James and Kleinow, 1994; Kleinow and James, 2001; Van Veld, 1990).

APPENDIX

Terms Used in the Dietary Uptake Description

V_{Ti} = volume of the tissue (L)
V_{Li} = volume of the lumenal contents (L)
V_{LBi} = "baseline" volume of the lumenal contents between feedings (L)
V_{LLi} = volume of the lumenal contents exceeding a predetermined "baseline" value (L)
M_{Ti} = mass of chemical in the tissue (μg)
M_{Li} = mass of chemical in the lumenal contents (μg)
C_A = concentration of chemical in arterial blood (μg/L, equivalent to ng/g)
C_{Ti} = concentration of chemical in the tissue (μg/L, equivalent to ng/g)
C_{Li} = concentration of chemical in the lumenal contents (μg/L, equivalent to ng/g)
C_{Vi} = concentration of chemical in venous blood draining the tissue (μg/L, equivalent to ng/g)
A_i = surface area for chemical diffusion (cm^2)
K_{Pi} = fitted first-order permeability coefficient for gut tissue/gut lumen chemical diffusion (cm/h)
K_{Ni} = fitted first-order constant for reduction in lumenal volume due to nutrient uptake (1/hr)
K_{Fi} = fitted first-order constant for reduction in lumenal volume due to bulk flow of digesta out of the (i) lumenal compartment (1/hr)
Q_{Li} = bulk flow of digesta out of a lumenal compartment (L/h)
Q_i = arterial blood flow to the tissue (L/h)

where the subscript i denotes the following: ST, stomach; PC, pyloric ceca; UI, upper intestine; LI, lower intestine; and L, liver.

A_{FOOD} = mass of chemical in the meal (μg)
V_{FOOD} = volume of the meal (L)
K_{STA} = fitted first-order constant for reduction in chemical mass in the stomach lumen (1/hr)
K_{BILE} = first-order constant for clearance of chemical in venous blood draining the liver (L/h)

Lumen:tissue ($P_{Li:Ti}$) and tissue:blood ($P_{Ti:B}$) equilibrium partitioning coefficients are given in Table 2.

Differential Equations

Stomach lumen equations are as follows:

$$dV_{LST}/dt = -K_{FST}V_{LST}$$

where $V_{LST} = V_{DOSE}$ when $T = 0$.

$$dM_{LST}/dt = -K_{STA}M_{LST}$$

where $M_{LST} = M_{DOSE}$ when $T = 0$.

$$C_{LST} = A_{LST}/V_{LST}$$

Stomach tissues equations are as follows:

$$dM_{TST}/dt = Q_{ST}(C_A - C_{STS}/P_{TST:B})$$

$$C_{TST} = M_{TST}/V_{TST}$$

$$C_{VST} = M_{TST}/(V_{TST}P_{TST:B})$$

Pyloric ceca lumen equations are as follows:

$$dV_{LLPC}/dt = K_{FST}V_{LST} - K_{FPC}V_{LLPC} - K_{NPC}V_{LLPC}$$

$$V_{LPC} = V_{LLPC} + V_{LBPC}$$

$$dM_{LPC}/dt = K_{STA}M_{LST} - K_{FPC}V_{LLPC}C_{LPC} - K_{PPC}A_{PC}(C_{LPC} - C_{TPC}P_{LPC:TPC}) + K_{BILE}C_{VL}$$

$$C_{LPC} = A_{LPC}/V_{LPC}$$

Pyloric ceca tissues equations are as follows:

$$dM_{TPC}/dt = Q_{PC}(C_A - C_{VPC}) + K_{PPC}A_{PC}(C_{LPC} - C_{TPC}P_{LPC:TPC})$$

$$C_{TPC} = M_{TPC}/V_{TPC}$$

$$C_{VPC} = M_{TPC}/(V_{TPC}P_{TPC:B})$$

Upper intestinal lumen equations are as follows:

$$dV_{LLUI}/dt = K_{FPC}V_{LLPC} - K_{FUI}V_{LLUI} - K_{NUI}V_{LLUI}$$

$$V_{LUI} = V_{LLUI} + V_{LBUI}$$

$$dM_{LUI}/dt = K_{FPC}V_{LLPC}C_{LPC} - K_{FUI}V_{LLUI}C_{LUI} - K_{PUI}A_{UI}(C_{LUI} - C_{TUI}P_{LUI:TUI})$$

$$C_{LUI} = M_{LUI}/V_{LUI}$$

Upper intestinal tissues equations are as follows:

$$dM_{TUI}/dt = Q_{UI}(C_A - C_{VUI}) + K_{PUI}A_{UI}(C_{LUI} - C_{TUI}P_{LUI:TUI})$$

$$C_{TUI} = M_{TUI}/V_{TUI}$$

$$C_{VUI} = M_{TUI}/(V_{TUI}P_{TUI:B})$$

Lower intestinal lumen equations are as follows:

$$dV_{LLLI}/dt = K_{FUI}V_{LLUI} - K_{FLI}V_{LLLI}$$

$$V_{LLI} = V_{LLLI} + V_{LBLI}$$

$$dM_{LLI}/dt = K_{FUI}V_{LLUI}C_{LUI} - K_{FLI}V_{LLLI}C_{LLI} - K_{PLI}A_{LI}(C_{LLI} - C_{TLI}P_{LLI:TLI})$$

$$C_{LLI} = M_{LLI}/V_{LLI}$$

Lower intestinal tissues equations are as follows:

$$dM_{TLI}/dt = Q_{LI}(C_A - C_{VLI}) + K_{PLI}A_{LI}(C_{LLI} - C_{TLI}P_{LLI:TLI})$$

$$C_{TLI} = M_{TLI}/V_{TLI}$$

$$C_{VLI} = M_{LT}/(V_{TLI}P_{TLI:B})$$

ACKNOWLEDGMENTS

The authors thank Dr. Russell Erickson and Dr. Kenneth Drouillard for reviewing this manuscript and Dr. James McKim for his help throughout the project. We also thank Sharon Batterman for managing the contract under which a portion of this work was performed; Roger LePage for preparing the figures; and Douglas Kuehl, Brian Butterworth, and Alex Hoffman for analytical support. The information in this document has been funded in part by the U.S. Environmental Protection Agency. It has been subjected to review by the National Health and Environmental Effects Research Laboratory and approved for publication. Approval does not signify that the contents reflect the views of the Agency and the mention of trade names or commercial products does not constitute endorsement or recommendation for use.

REFERENCES

Atkinson, J. L., Hilton, J. W., and Slinger, S. J. (1984). Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout (*Salmo gairdneri*). *Can. J. Fish. Aquat. Sci*. **41,** 1384–1386.

Axelsson, M., Thorarensen, H., Nilsson, S., and Farrell, A. P. (2000). Gastrointestinal blood flow in the red Irish lord, *Hemilepidotus hemilepidotus*: Long-term effects of feeding and adrenergic control. *J. Comp. Physiol*. *B* **170,** 145–152.

Barber, C. M., Suarez, L. A., and Lassiter, R. R. (1991). Modelling bioaccumulation of organic pollutants in fish with an application to PCBs in Lake Ontario salmonids. *Can. J. Fish. Aquat. Sci.* **48,** 318–337.

Barron, M. G., Tarr, B. D., and Hayton, W. L. (1987). Temperature-dependence of cardiac output and regional blood flow in rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol*. **31,** 735–744.

Bertelsen, S. L., Hoffman, A. D., Gallinat, C. A., Elonen, C. M., and Nichols, J. W. (1998). Evaluation of log K_{OW} and tissue lipid content as predictors of chemical partitioning to fish tissues. *Environ. Toxicol. Chem*. **17,** 1447–1455.

Bischoff, K. B., Dedrick, R. L., Zaharko, D. S., and Longstreth, J. A. (1971). Methotrexate pharmacokinetics. *J. Pharm. Sci.* **60,** 1128–1133.

Bruggeman, W. A., Opperhuizen, A., Wijbenga, A., and Hutzinger, O. (1984). Bioaccumulation of super-lipophilic chemicals in fish. *Toxicol. Environ. Chem*. **7,** 173–189.

Buddington, R. K. (1980). Hydrolysis-resistant organic matter as a reference for measurement of fish digestive efficiency. *Trans. Am. Fish. Soc*. **109,** 653–656.

Buddington, R. K., and Diamond, J. M. (1987). Pyloric ceca of fish: A "new" absorptive organ. *Am. J. Physiol.* **252,** G65–G76.

Bungay, P. M., Dedrick, R. L., and Matthews, H. B. (1981). Enteric transport of chlordecone (kepone®) in the rat. *J. Pharmacokinet. Biopharm*. **9,** 309–341.

Cotton, P. B. (1972). Non-dietary lipid in the intestinal lumen. *Gut* **13,** 675–681.

De La Noüe, J., and Choubert, G. (1986). Digestibility in rainbow trout: Comparison of the direct and indirect methods of measurement. *Prog. Fish Cult*. **48,** 190–195.

Elliot, J. M. (1991). Rates of gastric evacuation in piscivorous brown trout, *Salmo trutta*. *Fresh. Biol*. **25,** 297–305.

Erickson, R. J., and McKim, J. M. (1990). A model for exchange of organic chemicals at fish gills: Flow and diffusion limitations. *Aquat. Toxicol*. **18,** 175–198.

Fara, J. W. (1984). Postprandial mesenteric hyperemia. In *Physiology of the Intestinal Circulation* (A. P. Shepherd and D. N. Granger, Eds.), pp. 99–106. Raven Press, New York.

Fitzsimmons, P. N., Fernandez, J. D., Hoffman, A. D., Butterworth, B. C., and Nichols, J. W. (2001). Branchial elimination of superhydrophobic organic compounds by rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol*. **55,** 23–34.

Gallavan, R. H., Chou, C. C., Kvietys, P. R., and Sit, S. P. (1980). Regional blood flow during digestion in the conscious dog. *Am. J. Physiol*. **238,** H220–H225.

Gobas, F. A. P. C., McCorquodale, J. R., and Haffner, G. D. (1993a). Intestinal absorption and biomagnification of organochlorines. *Environ. Toxicol. Chem.* **12,** 567–576.

Gobas, F. A. P. C, Muir, D. C. G., and Mackay, D. (1988). Dynamics of dietary bioaccumulation and faecal elimination of hydrophobic organic chemicals in fish. *Chemosphere* **17,** 943–962.

Gobas, F. A. P. C., Zhang, X., and Wells, R. (1993b). Gastrointestinal magnification: The mechanism of biomagnification and food chain accumulation of organic chemicals. *Environ. Sci. Technol.* **27,** 2855–2863.

He, E., and Wurtsbaugh, W. A. (1993). An empirical model of gastric evacuation rates for fish and an analysis of digestion in piscivorous brown trout. *Trans. Am. Fish. Soc.* **122,** 717–730.

James, M. O., and Kleinow, K. M. (1994). Trophic transfer of chemicals in the aquatic environment. In *Aquatic Toxicology. Molecular, Biochemical, and Cellular Perspectives*. (D. C. Malins and G. K. Ostrander, Eds.), pp. 1–35. Lewis, Boca Raton, FL.

Jobling, M. (1987). Influences of food particle size and dietary energy content on patterns of gastric evacuation in fish: Test of a physiological model of gastric evacuation. *J. Fish. Biol.* **30,** 299–314.

Kapoor, B. G., Smit, H., and Verighina, I. A. (1975). The alimentary canal and digestion in teleosts. In *Advances in Marine Biology* (F. S. Russell and C. M. Yonge, Eds.), Vol. 13, pp. 109–239. Academic Press, London.

King, F. G., Dedrick, R. L., Collins, J. M., Matthews, H. B., and Birnbaum, L. S. (1983). Physiological model for the pharmacokinetics of 2,3,7,8-tetrachlorodibenzodioxin in several species. *Toxicol. Appl. Pharmacol*. **67,** 390–400.

Kleinow, K. M., and James, M. O. (2001). Response of the teleost gastrointestinal system to xenobiotics. In *Target Organ Toxicity in Marine and Freshwater Teleosts* (D. Schlenk and W. H. Benson, Eds.), Vol. 1, pp. 269–362. Taylor and Francis, London.

Kleinow, K. M., James, M. O., Tong, Z., Venugopalan, C. S. (1998). Bioavailability and biotransformation of benzo(a)pyrene in an isolated perfused *in situ* catfish intestinal preparation. *Environ. Health Perspec*. **106,** ISS 3, 155–166.

Lutz, R. J., Dedrick, R. L., Matthews, H. B., Eling, T. E., and Anderson, M. W. (1977). A preliminary pharmacokinetic model for several chlorinated biphenyls in the rat. *Drug Metab. Disposit*. **5,** 386–396.

Nichols, J. W., Fitzsimmons, P. N., and Whiteman, F. W. (2004). A physiologically based toxicokinetic model for dietary uptake of hydrophobic organic compounds by fish. II. Simulation of chronic exposure scenarios. *Toxicol. Sci*. **77,** 219–230.

Nichols, J. W., Fitzsimmons, P. N., Whiteman, F. W., Kuehl, D. W., Butterworth, B. C., and Jenson, C. T. (2001). Dietary uptake kinetics of 2,2′,5,5′-tetrachlorobiphenyl in rainbow trout. *Drug Metab. Disposit*. **29,** 1013–1022.

Nichols, J. W., McKim, J. M., Andersen, M. E., Gargas, M. L., Clewell, H. J., III, and Erickson, R. J. (1990). A physiologically based toxicokinetic model for the uptake and disposition of waterborne organic chemicals in fish. *Toxicol. Appl. Pharmacol*. **106,** 433–447.

Nichols, J. W., McKim, J. M., Lien, G. J., Hoffman, A. D., and Bertelsen, S.H. (1991). Physiologically based toxicokinetic modeling of three chlorinated ethanes in rainbow trout (*Oncorhynchus mykiss*). *Toxicol. Appl. Pharm.* **110,** 374–389.

Nichols, J. W., McKim, J. M., Lien, G. J., Hoffman, A. D., Bertelsen, S. L., and Elonen, C. M. (1996). A physiologically based toxicokinetic model for dermal absorption of organic chemicals by fish. *Fundam. Appl. Toxicol*. **31,** 229–242.

Oliver, B. G., and Niimi, A. J. (1988) Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. *Environ. Sci. Technol*. **22,** 388–397.

Persson, L. (1986). Patterns of food evacuation in fishes: A critical review. *Environ. Biol. Fish.* **16,** 51–58.

Ramsey, J. C., and Andersen, M. E. (1984). A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol. Appl. Pharmacol*. **73,** 159–175.

Russell, R. W., Gobas, F. A. P. C., and Haffner, G. D. (1999). Role of chemical and ecological factors in trophic transfer of organic chemicals in aquatic food webs. *Environ. Toxicol. Chem*. **18,** 1250–1257.

Russell, R. W., Lazar, R., and Haffner, G. D. (1995). Biomagnification of

organochlorines in Lake Erie white bass. *Environ. Toxicol. Chem*. **14,** 719–724.

Schmidt, D. C., and Weber, L. J. (1973). Metabolism and biliary excretion of sulfobromophthalein by rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Board Can*. **30,** 1301–1308.

Shiu, W. Y., and Mackay, D. (1986). Critical review of aqueous solubilities, vapour pressures, Henry's constants, and octanol-water partition coefficients of polychlorinated biphenyls. *J. Phys. Chem. Ref. Data* **15,** 911–929.

Smith, L. S. (1989). Digestive functions in teleost fishes. In *Fish Nutrition*, 2nd ed. (J. E. Halver, Ed.), pp. 331–421. Academic Press, San Diego.

Smith, L. S., and Bell, G. R. (1975). *A Practical Guide to the Anatomy and Physiology of Pacific Salmon*. Fisheries and Marine Service Special Publication 27, Department of the Environment, Ottawa, Canada.

Tacon, A. G. J., and Rodrigues, A. M. P. (1984). Comparison of chromic oxide, crude fibre, polyethylene, and acid-insoluble ash as dietary markers for the estimation of apparent digestibility coefficients in rainbow trout. *Aquacult*. **43,** 391–399.

Thomann, R. V. (1989). Bioaccumulation model of organic chemical distribution in aquatic food chains. *Environ. Sci. Technol*. **23,** 699–707.

Thomann, R. V., and Connolly, J. P. (1984). Model of PCB in the Lake Michigan lake trout food chain. *Environ. Sci. Technol*. **18,** 65–71.

Thorarensen, H., McLean, E., Donaldson, E. M., and Farrell, A. P. (1991). The blood vasculature of the gastrointestinal tract in chinook, *Oncorhynchus tshawytscha* (Walbaum), and coho, *O. kisutch* (Walbaum), salmon. *J. Fish Biol*. **38,** 525–531.

Van Veld, P. A. (1990). Absorption and metabolism of dietary xenobiotics by the intestine of fish. *Rev. Aquat. Sci.* **2,** 185–203.

Van Veld, P. A, Patton, J. S., and Lee, R. F. (1988). Effect of preexposure to dietary benzo[a]pyrene (BP) on the first-pass metabolism of BP by the intestine of toadfish (*Opsanus tau*): *In vivo* studies using portal vein–catheterized fish. *Toxicol. Appl. Pharmacol*. **92,** 255–265.

Wilke, C. R., and Chang, P. (1955). Correlation of diffusion coefficients in dilute solutions. *Am. Inst. Chem. Eng. J.* **1,** 264–270.