ABSTRACT

THE DEVELOPMENT, EVALUATION, AND APPLICATION OF A PHYSIOLOGICALLY BASED TOXICOKINETIC MODEL FOR FLUORANTHENE IN RAINBOW TROUT (Onchorhyncus mykiss)

by Carrie Anne Smith

The objective of this research was to develop, evaluate and apply a physiologically based toxicokinetic model for the uptake of waterborne fluoranthene in rainbow trout. The model was parameterized and solved to predict the time course of fluoranthene in the liver, kidney, fat, poorly and richly perfused tissues, gonads, and brain of trout. Tissue concentrations observed after exposing trout to fluoranthene were compared with predicted tissue concentrations to evaluate the model. The model poorly characterized fluoranthene distribution during the initial uptake phase and accurately predicted uptake at later times. A suspected deviation from one of the model’s primary assumptions (flow-limited exchange) explained the discrepancies between observed and predicted fluoranthene concentrations at early times. The model was applied to predict fluoranthene uptake across the biological temperature range of trout and throughout the reproductive cycle of female trout. In general, uptake increased with temperature and decreased in the ovaries as spawning approached.
THE DEVELOPMENT, EVALUATION AND APPLICATION OF A
PHYSIOLOGICALLY BASED TOXICOKINETIC MODEL FOR FLUORANTHENE
IN RAINBOW TROUT (Onchorhyncus mykiss)

A Thesis

Submitted to the Faculty of Miami University
in partial fulfillment of
the requirements for the degree of
Master of Science
Department of Zoology

by

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Oxford, Ohio
2003

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ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Dr. James T. Oris, and my committee, Dr. John Bailar, Dr. Mike Vanni, and Dr. Steve Wright, for their guidance and patience over the last two years. I would also like to thank my lab-mates, Jennifer Hoffmann, Aaron Roberts, Paul Drevnick and Scott McClain for their willingness to take time out of their busy schedules to offer valuable technical advice and support. The timely completion of this research would not have been possible without the help of Megan Tylka and Elizabeth Arnold, two undergraduates in the Oris lab. Finally, I would like to extend my deepest appreciation to my family and friends, especially Brian Claytor, for their unwavering encouragement and understanding.
INTRODUCTION

1. Rationale

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous in the aquatic environment (Harvey, 1997). Their presence in waters around the world is the result of both natural and anthropogenic sources (Neff, 1979). Many studies have shown that PAH are both chronically and acutely toxic to aquatic organisms. Consequently, risk managers seek to understand the relationships among PAH contamination, bioaccumulation and toxic response. Toxicologists can elucidate these relationships through toxicokinetics: the mathematical characterization of chemical absorption, distribution, metabolism and elimination as a function of dose and time (Gibaldi and Perrier, 1982).

Physiologically based toxicokinetic models (PBTK) describe the movement of a chemical throughout biologically meaningful compartments, such as kinetically related tissues and organs (Landrum et al., 1992). Accordingly, they have been called the most realistic toxicokinetic approach (Barron et al., 1990). Typical compartments included in PBTK models are the liver, kidney, fat, poorly perfused tissues and richly perfused tissues, while organs such as the brain and gonads are very rarely considered. The exclusion of the brain and gonads may be due to the fact that these organs occupy a very small volume in fish, and these organs are often included in other compartments. For example, the gonads are frequently modeled as being part of the richly perfused tissues.

To date, the only PBTK model developed for PAH in fish did not include a brain or a gonads compartment (Law et al., 1991). Since it has been suggested that these organs are the targets of PAH-induced reproductive toxicity, it is critical that they be considered in a complete toxicokinetic evaluation of PAH. Therefore, the objective of this research was to develop, evaluate and apply a PBTK model for the uptake of waterborne fluoranthene, a widespread PAH, in the liver, kidney, fat, poorly perfused tissues, richly perfused tissues, gonads, and brain of rainbow trout, a common freshwater fish.

2. Polycyclic Aromatic Hydrocarbons

2.1 Sources of PAH

PAH are composed of two or more benzene rings, fused by a shared pair of carbon atoms lying in a single plane (Neff, 1979). A vast number of possible PAH exist; however, only a
small fraction of these derivatives will ever be synthesized (Harvey, 1997). PAH are formed naturally by high temperature pyrolysis of organic materials, fossil fuel production, and biosynthesis by plants and microbes (Neff, 1979). In addition, pyrolysis of kerosene and wood, oil refinery, coke, gas and alcohol production, forest and grass fires, and fossil fuel combustion are among the many human influenced activities responsible for PAH loading to the environment (Neff, 1979, Harvey, 1997). In particular, municipal effluent contamination, surface runoff from land, oil spills, and deposition of airborne particulates are the major routes of entry for the estimated 230,000 metric tons of PAH entering aquatic systems annually (Neff, 1979, Harvey, 1997).

2.2 Environmental Fate of PAH

Owing to their non-polar, hydrophobic nature, PAH have relatively low water solubilities, which tend to decrease as the number of aromatic rings or molecular weight increase (Neff, 1979). For example, at 25-27° C, the aqueous solubility of naphthalene, a 2-ring PAH, ranges from 12,500-34,000 µg/L, whereas at the same temperatures, water solubility values for benzo[a]pyrene, a 5-ring PAH, range from 1.48-2.16 µg/L (Neff, 1979, de Maagd et al., 1998). The octanol:water partition coefficient (K_{ow}) of PAH increases with the number of aromatic rings or molecular weight. The log K_{ow} for naphthalene at 25° C is 3.33, while the log K_{ow} for benzo[a]pyrene at 25° C is 6.13 (de Maagd et al., 1998).

Due to these chemical and physical characteristics, PAH are quickly adsorbed to particulate matter in the water column. However, once particulate PAH settle out in the sediments, biological activity and leaching (Neff, 1979), storm events (Ireland et al., 1996), boating activity (Mastran et al., 1994) and bioturbation (Clements et al., 1994) can restore a substantial amount of PAH to the water column where they are rapidly transferred across biological membranes and accumulate in organisms. Fish, in particular, may absorb lipophillic PAH across the gills and/or ingest PAH contaminated sediments and food.

2.3 PAH Degradation and Toxicity

PAH are degraded through many processes in the aquatic environment. Among the most important are metabolic biotransformation and photo-oxidation, as these reactions underlie the chronic and acute toxicity associated with PAH, respectively.
Metabolism of PAH occurs in two phases. In phase I, PAH undergo oxidative, hydrolytic or reductive processes via the cytochrome P-450-dependent mixed-function oxidase (MFO) enzyme system. Phase I metabolites are then combined with an endogenous polar compound in phase II. While this system is effective in transforming PAH into more water-soluble products available for elimination, phase I metabolites of PAH are often more toxic than their parent compound (Neff, 1979).

PAH metabolites include active epoxides and dihydrodiols, which can bind to macromolecules such as DNA, eliciting mutagenic and carcinogenic responses (Dipple, 1983). In the laboratory, DNA adducts have been measured in the intestine, liver, gills and brain of northern pike exposed to 25 mmol benzo[a]pyrene, benzo[k]fluoranthene and 7H-dibenzo[c, g]carbazol per kilogram body weight (Ericson et al., 1999) and in the blood, liver, kidney and spleen of mummichog exposed to 12 mg benzo[a]pyrene per kilogram body weight (Rose et al., 2001). Hepatic DNA adducts have also been found in feral perch from a creosote contaminated river in Sweden (Ericson et al., 1999) and in Atlantic cod and corkwing wrasse from the PAH contaminated Karmsund strait in western Norway (Aas et al., 2001).

2.3.1 Reproductive Toxicity

PAH-induced reproductive toxicity in fish has also been well documented. Several studies have reported abnormal reproductive hormone levels in feral fish from PAH-contaminated areas, as well as in fish exposed to PAH in the laboratory. Decreases in 17-estradiol have been observed in female dolly varden from areas impacted by the Exxon Valdez oil spill (Sol et al., 2000), English sole from sites heavily contaminated with aromatic hydrocarbons (Johnson et al., 1988), flounder exposed to 0.5, 2.5, or 12.5 nmol phenanthrene/g food and 0.4 nmol chrysene/g food (Thomas and Budiantara, 1995), and Atlantic croaker exposed to benzo[a]pyrene (Thomas, 1988). Furthermore, increases in testosterone production have been measured in goldfish and rainbow trout exposed to 7-naphthoflavone and naphthalene, respectively.

Effects on the gonads, gametogenesis and reproductive success have also been shown in both feral fish populations and laboratory studies. Atlantic croaker exposed to 0.5 ppm naphthalene failed to undergo sexual maturation and showed widespread oocyte atresia (Thomas and Budiantara, 1995). Dolly varden collected from areas affected by the Exxon Valdez oil spill
exhibited a low gonadosomatic index (GSI) (Sol et al., 2000). Spawning success in rock sole from areas with high concentrations of aromatic hydrocarbons was significantly impaired (Johnson et al., 1998). Finally, fathead minnows exposed to 6-12 μg anthracene/L exhibited a 52% decrease in reproductive potential (number of eggs laid) (Hall and Oris, 1991).

Although the mechanisms governing reproductive toxicity in fish remain unclear, it has been hypothesized that PAH may activate aryl hydrocarbon hydrolase, usually depressed during spawning, causing reproductive dysfunction due to altered hormone levels (Collier et al., 1986, Truscott et al., 1983). It has also been suggested that PAH may interfere with the binding of reproductive hormones to receptor sites and the production and activation of reproductive hormones (Bui et al., 1986, Kang and West, 1980). Since reproductive hormones are regulated by the pituitary gland and hypothalamus of the brain, these structures, along with the gonads, may be targets for PAH-induced reproductive toxicity. Since the magnitude of toxic response is a direct function of the amount of chemical at the site of toxic action (McKim and Nichols, 1994), an estimate of PAH distribution to these tissues may be highly useful in elucidating PAH-induced reproductive toxicity mechanisms.

2.3.2 Phototoxic Effects

Photo-oxidation is another method of PAH degradation in aquatic environments. Energy from the ultraviolet (UV) spectrum of light is transferred to PAH during photo-oxidation, resulting in the production of excited singlet and triplet state molecules (Neff, 1979, Larson and Berenbaum, 1988, Oris and Giesy, 1987). These excited state molecules give rise to reactive oxygen species (ROS), such as superoxide anions and hydroxyl radicals, which may attack biological membranes and DNA (Larson and Berenbaum, 1988).

A myriad of PAH-induced phototoxic effects have been reported. Reviews by Arfsten et al. (1996) and Larson and Berenbaum (1988) describe the UV-induced acute toxicity of PAH in protozoans, cladocerans, insects, benthic invertebrates, aquatic vertebrates, plants and mammals. Fluoranthene is one of the most frequently studied PAH. The phototoxicity of fluoranthene has been examined in zooplankton (Oris et al., 1998, Spehar et al., 1999), amphibians (Monson et al., 1999), oligochaetes, polychaetes, snails, shrimp, lobster, sea urchin, amphipods (Spehar et al., 1999), mussels (Weinstein et al., 2001), and several fish species, including fathead minnows (Weinstein et al., 1997, Oris et al., 1998, Spehar et al., 1999), bluegill, sheepshead minnows,
inland silversides, winter flounder and rainbow trout (Speahar et al., 1999). Several of these studies found that PAH can be phototoxic at concentrations well below their water solubility limits.

The interactions between fluoranthene phototoxicity and algae, dissolved humic materials (DHM) and methyl tert-butyl ether (MTBE) have also been studied. The presence of DHM and algae were both found to decrease the phototoxicity of fluoranthene in fathead minnows (Weinstein and Oris, 1999, Cho, 2000, respectively). In contrast, an increase in the mortality of fathead minnows exposed to MTBE and fluoranthene was observed, when compared to fluoranthene only treatments (Cho et al., 2003). The proposed mode of fluoranthene phototoxicity in fish is a disruption of membrane function and integrity due to lipid peroxidation (Weinstein et al., 1997). Furthermore, fluoranthene phototoxicity has been suggested to be a direct function of the length and intensity of the UV dose as well as the concentration of PAH in the tissues (Oris and Giesy, 1985).

The effects of fluoranthene in the absence of UV radiation have also been studied. Schlueter et al. (2000) found a genetically related difference in the survival of fathead minnows exposed to fluoranthene-contaminated sediments. Farr et al. (1995) observed a behavioral avoidance of fluoranthene by fathead minnows exposed to concentrations greater than 8.6 μg/L. Additionally, Weinstein and Oris (1997) found that in the absence of UV light, fluoranthene is not acutely toxic to fathead minnows below its water solubility limit at 24°C (125 μg/L).

Environmental concentrations of fluoranthene range from 928 mg/L in heavily contaminated areas like the Chesapeake Bay (Hardy et al., 1987), to 4.12 μg/L in mid-western reservoirs during peak boating season (Mastran et al., 1994), to 5-70 ng/L in, the generally considered pristine, Lake Tahoe, CA/NV (Oris et al., 1998). Additionally, fluoranthene is consistently one of the largest fractions of total PAH found in freshwaters around the world (Baker et al., 1990, Oris et al., 1998). Therefore, since fluoranthene is a very common, well-characterized toxin it was selected as the model PAH for this toxicokinetic study. The physical and chemical properties of fluoranthene are provided in Figure 1.

3. Rainbow Trout

Because rainbow trout are standard organisms in biological research, the physiology of these fish is very well understood. The average cardiac output (Barron et al., 1987), effective
respiratory volume (Erickson and McKim, 1990), and arterial blood flow to and volumes of the liver, kidney, fat, poorly perfused tissues, richly perfused tissues, gonads and brain (Barron et al., 1987, Cameron, 1974) have all been measured in rainbow trout. Since these are all parameters necessary for the development of a PBTK model, rainbow trout were chosen as the model organism for this research. Furthermore, rainbow trout are common organisms found in cold-water lakes, rivers and streams all around the world. Since PAH are ubiquitous in aquatic environments, this model will be useful in many real-world applications.

4. PBTK Models

PBTK models are powerful tools for predicting the fate of a chemical throughout the body of an exposed organism. PBTK models use physiological and anatomical data about an organism and physico-chemical information about the compound of interest, to deduce the time course of a chemical at pre-selected target tissues and organs where the chemical is thought to act upon, is eliminated, and is easily sampled (Gibaldi and Perrier, 1982). In addition to describing the concentration of a chemical in specific tissues, PBTK models can predict the effect of a changed parameter on chemical accumulation (Gibaldi and Perrier, 1982, Barron et al., 1990). For example, as the size of reproductive organs change during sexual maturation and gonadal development, distribution of a chemical at those tissues may also change and can be predicted by a PBTK model to estimate the risk of an organism during its breeding season. Furthermore, since PBTK models are based on physiological and mechanistic parameters, allometric relationships may permit the models to be scaled to other species, body sizes and environmental conditions by simply changing the appropriate variables in the model (Gibaldi and Perrier, 1982, Landrum et al., 1992, Barron et al., 1990). This allows one to extrapolate a PBTK model to a threatened organism, whose scarcity may render it inappropriate for use in exposure experiments.

PBTK models have a long background in human health risk assessment and pharmacological applications (recently reviewed by Andersen, 2003). However, PBTK models have recently gained considerable attention from ecological risk assessors who require a tool for linking environmental concentration to target tissue dose in exposure assessment (Andersen, 1995). A risk assessor can measure the environmental concentration of chemical in an area and,
using that concentration to develop a PBTK model, predict the dose of that chemical delivered to the tissues of organisms occupying that area (Andersen, 1995, Leung and Paustenbach, 1995).

According to a National Academy of Sciences/National Research Council (NAS/NRC) panel of scientists familiar with PBTK models, well-developed PBTK models may be the most precise and reliable method for assessing dosimetric-based risk, should be used over the current default exposure analysis methods (e.g. whole organism toxicokinetics), and have a great deal of potential for improving the quality of risk assessments (Frederick, 1995). This group also noted that, while care is required in employing such techniques, Monte Carlo simulation methods and sensitivity analyses may improve the quality of PBTK models by accounting for the natural variability in biological systems and more honestly depicting uncertainties.
OBJECTIVES AND HYPOTHESES

The primary objective of this research was to develop, evaluate and apply a PBTK model for the uptake of waterborne fluoranthene in rainbow trout. This objective was accomplished in three phases.

Phase I - Development: The model was:

A.) Parameterized with physiological, morphological and physico-chemical data either taken from the literature or experimentally gathered, and

B.) Solved to predict the time course of fluoranthene in the liver, kidney, fat, poorly perfused tissues, richly perfused tissues, gonads and brain of rainbow trout.

Phase II - Evaluation: Model performance was assessed by exposing rainbow trout to fluoranthene and:

A.) Comparing the observed tissue concentrations with predictions from the model, and

B.) Comparing the variability in the observed tissue concentrations with the variability in the model.

Phase III - Application: The model was used to predict fluoranthene uptake in two different changing environmental scenarios.

A.) Across the physiological temperature range of rainbow trout (4-20°C)

B.) Throughout the annual reproductive cycle of female rainbow trout, simulated by changes in the GSI (volume of the gonads).

Corresponding to these three phases, the following hypotheses were proposed:

Hypothesis I A: Due to the inclusion of gonads in the model, significant differences between male and female morphological parameters will be observed. Consequently, the model will have to be developed separately for both sexes.

Hypothesis I B: Based on results from other PBTK models for organic chemicals in fish, and the physico-chemical properties of PAH, the model will predict fluoranthene uptake to be highest in
compartments with high lipid content, followed by compartments with the ability to metabolize fluoranthene and the poorly perfused tissue compartment.

Hypothesis II A: The model will accurately predict the uptake of fluoranthene in all compartments, at all time points.
Hypothesis II B: The variability in the observed fluoranthene tissue concentrations will be explained by the variability in the model predictions.

Hypothesis III A: Since cardiac output is directly related to temperature, increasing temperature will result in higher blood flow to each compartment, which will subsequently increase uptake in all compartments.
Hypothesis III B: Increasing the volume of the gonads will result in a decrease in the outflow of fluoranthene from the ovaries at each time point. Consequently, the rate of fluoranthene uptake in the gonads will be slow when the GSI is high and fast when the GSI is low.
MATERIALS AND METHODS

1. Model Development
1.1 Model Structure

The model (Figure 2) was designed after Nichols et al. (1990) PBTK model for the uptake of waterborne organic chemicals in fish. The model predicted the time course of fluoranthene in eight “compartments”, which corresponded to the organs and kinetically related tissue groups of rainbow trout. These compartments included the gills, liver, kidney, fat, poorly perfused tissues (PPT), richly perfused tissues (RPT), gonads and brain. The PPT compartment was comprised of white muscle, skin, bones and fins. The RPT compartment included the esophagus, stomach, pyloric ceca, spleen, and large and small intestines.

The eight compartments were connected by the flow of blood and water, through which fluoranthene was distributed. At the gill:water interface, venous blood and inspired water flowed afferent to, or towards, the gills in opposite directions, and arterial blood and expired water flowed efferent to, or away from, the gills in opposite directions. Arterial blood flow was afferent and venous blood flow was efferent to all other compartments. In rainbow trout, the liver and kidney are supplied by both arterial and portal blood flow. Consequently, the model assumed that sixty percent of the blood leaving the PPT was diverted to the kidney via the caudal vein and all of the blood leaving the RPT was delivered to the liver via the hepatic portal vein (Nichols et al., 1990).

Although PAH metabolism probably occurs to a limited extent in all tissues, extrabranchial elimination was localized to the liver, kidney and RPT, since these are considered to be primary sites of fluoranthene loss due to metabolic biotransformation. In all three compartments, metabolism was described by a combination of Michaelis-Menton and first order kinetics, which corresponded to phase I and II metabolic pathways, respectively.

Compartmental parameters were abbreviated with a capital letter, followed by a subscript. The blood flow rate, volume, concentration of fluoranthene, amount of fluoranthene, and partition coefficients for each tissue, was abbreviated with a $Q$, $V$, $C$, $A$, and $P$, respectively. When referring generally to all compartments, the subscript $i$ was used. When referring to compartments individually, the subscripts $l$, $k$, $f$, $p$, $r$, $g$, and $b$ were used and corresponded to the liver, kidney, fat, PPT, RPT, gonads, and brain, respectively.
1.2 Branchial Uptake

The transfer of waterborne fluoranthene across the gills was assumed to be the primary route of exposure. Branchial uptake was modeled as a countercurrent exchange process where venous blood flowing through gill lamellae accumulated fluoranthene as inspired water passed through lamellar channels in the opposite direction (Figure 3). This process can be limited by either the equilibration of fluoranthene in inspired water with arterial blood (blood-flow limited) or the equilibration of fluoranthene in venous blood with expired water (ventilation-limited). Limitations on chemical exchange imposed by diffusion were not included in the model because for chemicals with a log $K_{ow}$ >3.0 and <6.0 [fluoranthene log $K_{ow}$ = 5.23 (deMaagd et al., 1998)], uptake has been shown to be almost entirely dependent on water flow (McKim and Nichols, 1994).

In ventilation-limited systems, the effective respiratory volume ($Q_w$, L water/hr) is the flow term that is limiting, whereas in blood-flow limited systems, the cardiac output ($Q_c$, L blood/hr) and fluoranthene blood:water partition coefficient ($P_{bw}$) are the limiting factors on chemical exchange. Although it was hypothesized that fluoranthene uptake would be limited solely by the flow of water, fluoranthene flux at the gills ($F_g$, mg/hr) was calculated using the following general expression derived by Erickson and McKim (1990):

$$F_g = \min(Q_w, Q_c \cdot P_{bw}) \cdot \frac{C_{insp}}{P_{pw}} \cdot \frac{C_{ven}}{C_{insp} - C_{ven} P_{bw}}$$

where $C_{insp}$ (mg/L) is the concentration of fluoranthene in inspired water and $C_{ven}$ (mg/L) is the concentration of fluoranthene in venous blood flowing though the gill lamellae.

$C_{ven}$ was calculated as the sum of the products of fluoranthene concentration in venous blood efferent to each compartment ($C_{vi}$, mg/L) and the rate of total blood flow to each compartment ($Q_i$, L blood/hr), divided by the cardiac output ($Q_c$, L blood/hr):

$$C_{ven} = \frac{Q_c \cdot C_{vi}}{Q_c} = \frac{(Q_i \cdot C_{vi}) + (Q_p \cdot C_{vf}) + [(Q_f \cdot C_{vf}) + (Q_g \cdot C_{vg})] + (Q_g \cdot C_{vg}) + (Q_b \cdot C_{vb})}{Q_c}$$

Eq. 1
Eq. 2
where \[ k = 0.6 \] is the proportion of venous blood diverted from the poorly perfused tissues to the kidney, and \[ l = 1.0 \] is the proportion of venous blood diverted from the richly perfused tissues to the liver.

Fluoranthene concentration in arterial blood \((C_{\text{art}}, \text{mg/L})\) was calculated as the chemical flux diluted into the blood flow using the following equation (Lien et al., 2001):

\[
\frac{dC_{\text{art}}}{dt} = C_{\text{ven}} + \frac{F_g}{Q_c}
\]

Eq. 3

### 1.3 Mass-Balance Tissue Uptake

The model assumes fluoranthene exchange at the tissues to be flow-limited. That is, fluoranthene concentration equilibrium was considered to exist between tissues in each compartment and venous blood flowing out of the compartment, due to the rapid transfer of fluoranthene across capillary walls and cell membranes. Given this assumption was true, the fluoranthene concentration in venous blood efferent to each compartment \((C_{\text{vi}})\) was calculated as the quotient of the fluoranthene concentration in the tissue \((C_i, \text{mg/L})\) and the fluoranthene tissue:blood partition coefficient \((P_i, \text{mg/L in tissue/\text{mg/L in blood}})\):

\[
C_{\text{vi}} = \frac{C_i}{P_i}
\]

Eq. 4

where \(C_i\) is the mass, or amount, of fluoranthene in the compartment \((A_i, \text{mg})\) divided by the volume of the compartment \((V_i, \text{L})\):

\[
C_i = \frac{A_i}{V_i}
\]

such that: \(C_{\text{vi}} = \frac{A_i}{P_i * V_i}\)

Eq. 5

To calculate the rate of change of the amount of fluoranthene in each compartment, a series of mass-balance differential equations were simultaneously solved using MATLAB® 6.5 technical computing software (Appendix). The general equation for non-eliminating compartments receiving arterial blood only (fat, PPT, gonads and brain) was:

\[
\frac{dA_i}{dt} = Q_i * (C_{\text{art}} * C_{\text{vi}})
\]

\[
= (Q_i * C_{\text{art}}) * \left(\frac{Q_i * A_i}{P_i * V_i}\right)
\]

Eq. 6
This general equation was expanded for the liver and kidney to reflect the fact that these compartments are supplied by both arterial and portal blood, which were assumed to mix prior to entering the compartment.

\[
\frac{dA_i}{dt} = Q_{mi} (C_{mi} - C_{vi}) \quad \text{Eq. 7}
\]

\[
Q_{mi} = Q_l + Q_{pi} \quad \text{Eq. 8}
\]

\[
C_{mi} = \frac{Q_{mi} C_{art} + Q_{pi} C_{pi}}{Q_{mi} + Q_{pi}} \quad \text{Eq. 9}
\]

where \( Q_{pi} \) (L blood/hr) is equal to the rate of portal blood flow and \( C_{pi} \) (mg/L) is equal to the concentration in the portal blood. After substituting and rearranging, the differential equations used to describe fluoranthene uptake from arterial and portal blood in the liver and kidney were:

\[
\frac{dA_l}{dt} = (Q_l * C_l) + (Q_r * C_{vr}) - \frac{dA_{met}}{dt} \quad \text{Eq. 10}
\]

\[
\frac{dA_k}{dt} = (Q_k * C_k) + (Q_p * C_{vp}) - \frac{dA_{met}}{dt} \quad \text{Eq. 11}
\]

Differential equations for the liver, kidney and RPT were then further expanded to account for fluoranthene loss due to metabolism:

\[
\frac{dA_{met}}{dt} = \frac{dA_{saturable \ metabolism}}{dt} + \frac{dA_{linear \ metabolism}}{dt} \quad \text{Eq. 12}
\]

\[
\frac{dA_l}{dt} = Q_{mi} (C_{mi} - C_{vl}) \frac{dA_{met}}{dt} \quad \text{Eq. 13}
\]

\[
\frac{dA_k}{dt} = Q_{mi} (C_{mi} - C_{vk}) \frac{dA_{met}}{dt} \quad \text{Eq. 14}
\]

\[
\frac{dA_{met}}{dt} = \frac{dA_{saturable \ metabolism}}{dt} + \frac{dA_{linear \ metabolism}}{dt} \quad \text{Eq. 15}
\]

where \( V_{max} \) (mg/hr/kg tissue) is the maximum enzymatic reaction rate per kilogram of metabolic tissue (liver, kidney or RPT), \( V_i \) is the volume (or weight, in kilograms) of the metabolic tissue, \( K_m \) (mg/L blood) is the Michaelis-Menton constant and \( K_f \) is the first order elimination rate constant (L/hr).
1.4 Model Parameterization

The model is characterized by several input parameters (Table 1) that describe the physiology and morphology of rainbow trout, the physico-chemical properties of fluoranthene, and the exposure conditions to be simulated. Parameter estimates were either obtained from the literature or experimentally measured as described below.

1.4.1 Physiological Parameters

**Effective Respiratory Volume**

The effective respiratory volume \( Q_w \) is the flow rate of inspired water that comes into equilibrium with the blood in perfused gill lamellae (Erickson and McKim, 1990). \( Q_w \) is generally less than the total gill ventilation volume, because some gill lamellae are not perfused with blood, and inspired water does not pass through all lamellar channels. Erickson and McKim (1990) calculated the mean \( Q_w \) for a 1 kg rainbow trout as the ratio of the oxygen consumption rate to the drop in oxygen concentration in water flowing through the secondary lamellar channels. Using Erickson and McKim’s estimate, \( Q_w \) was scaled to body weight with the following allometric equation, as suggested by Nichols et al. (1991):

\[
Q_w = a \cdot BW^{0.75}
\]

where \( a \) is equal to the \( Q_w \) for a one kg rainbow trout and \( BW \) is the fish’s weight in kilograms (Adolph, 1949).

**Cardiac Output**

The cardiac output \( Q_c \) is the total volume of blood (L) pumped by the heart per unit time (hr) (Eckert, 1997). \( Q_c \) (L/hr/kg) was estimated by the following linear relationship between temperature \( T, ^\circ C \) and cardiac output established by Barron et al. (1987). (The temperature range over which this equation was developed was 6-18 °C.)

\[
Q_c = (0.237 \cdot T - 0.774)
\]

\( Q_c \) (L/hr/kg) was then scaled linearly to body weight (kg) as suggested by Barron et al. (1987) for large (100-1000 g) rainbow trout.
Arterial blood flow

Estimates of arterial blood flow to each compartment \( Q_i \) were obtained by multiplying blood flow as a proportion of cardiac output, by the total cardiac output, \( Q_c \). Proportional cardiac output estimates were selected from values found in the literature, which were either measured using radio-labeled microspheres (Cameron, 1974, Barron et al., 1987), or used in previously published PBTK models for rainbow trout (Law et al., 1991). Blood flow to the RPT was calculated as the sum of blood flow to the esophagus, stomach, pyloric ceca, spleen, and intestines. The proportion of cardiac output allocated to the PPT was equal to the average value estimated for white muscle by Barron et al. (1987), Neumann et al. (1983) and Randall and Daxboeck (1982), plus an additional 10% to account for arterial blood flow to the skin, bones and fins (Nichols, 1991). Proportional cardiac output of the fat compartment was estimated by subtracting the sum of the arterial blood flow already accounted for by all other compartments, from the total cardiac output.

1.4.2 Morphological Parameters

Morphological parameters were measured directly for 100-200 g, male and female, sub-adult rainbow trout. Trout were obtained from the State of Ohio Fish and Wildlife Hatchery in London, OH and transported to the laboratory of Dr. James T. Oris, Miami University, Oxford OH, where they were held at 12°C for several months prior to experimentation. Trout were maintained in 500 gallon, fiberglass, flow-through tanks with a circular current. The circular current was generated to replicate real-world conditions of well-exercised fish, rather than sedentary laboratory-cultured fish. Fish were subject to an 18:6 hour (light/dark) photoperiod and were fed daily with 3/4 cup of fresh commercial trout chow (Purina Foods, Richmond, IN). Trout used for morphological measurements were killed by anesthetic overdose with 0.5 g/L tricaine methanesulfonate (MS-222) (Wang et al., 1994).

Total body weight and compartmental volume

Following loss of equilibrium, fish were removed from the anesthetic treatment and the total wet body weight \( BW, \) g of each fish was measured on a Mettler PM2000 balance. The liver, kidney, RPT, gonads and brain were then quickly dissected and the wet weight (g) of each tissue/organ was measured on a Mettler-Toledo AE163 microbalance. The wet weight of each
tissue was then divided by the total body weight and multiplied by 100 to calculate the percentage of total body weight for each compartment.

Assuming that all tissues have an equal specific gravity of 1.0 g/ml, the percentage of total body weight was then expressed as the percentage of total body volume for each tissue. The volume of each compartment, in liters, was then calculated as the product of the percentage of total body volume and total body volume, expressed as the total body weight in kilograms, divided by 100:

\[
V_i (L) = \frac{\text{percentage total body volume} \times L}{\text{kg} \times \text{BW (kg)} \times 100}
\]  
Eq. 18

Fatty tissue is extremely diffuse with deposits found in between muscle layers, along the back, around the viscera, and in lipid stores throughout the body. Since it was impossible to grossly dissect all fatty tissues, the volume of the fat compartment was derived from the value estimated by Nichols et al. (1990). However, the fish used to parameterize this study were assumed to be well exercised due to the circular current generated in the holding tanks. Therefore, it was suspected that the volume of the fat compartment for these fish was less than the value reported in the literature (9.8% body volume). Consequently, the volume of the fat compartment was set equal to 25% less than the estimate reported by Nichols et al. (1991) and the exposure data were examined for evidence to the contrary. Volume of the PPT was estimated by subtracting the sum of the volume already accounted for by the other compartments from the total body volume.

**Compartmental water and lipid content**

Estimates of the water and lipid content of blood and tissues were required to calculate the fluoranthene tissue:blood and blood:water partition coefficients (1.4.3. Physico-chemical Parameters). The water and lipid content of blood were measured directly for the same stock of rainbow trout previously mentioned. Following anesthetic overdose, one to three ml of blood was drawn from the caudal vein using a 22-G needle and a 3-cc syringe. Blood samples were then placed in pre-weighed, polystyrene weigh boats, covered with aluminum foil to prevent evaporation, and the wet weight (g) was measured. To determine the water fraction of blood, (\(\frac{\text{blood}}{\text{blood}}\)) samples were dried for 96 hours at 60°C in a Fisher Isotemp® 300 Series (Model 318F)
drying oven (Lien et al. 2001). Once completely dry, the dry weight was measured (g) and water content was calculated as the difference between the wet and dry weights, divided by the wet weight:

$$
\frac{\Delta \text{blood}}{\text{wet weight}} = \frac{(\text{wet weight} - \text{dry weight})}{\text{wet weight}}
$$

Eq. 19

The total lipid fraction of blood ($L_{\text{blood}}$) was measured following Radin (1981). Lipids were extracted using a 3:2 v/v hexane-isopropanol mixture. All chemicals used were of High Performance Liquid Chromatography (HPLC) grade. Blood samples were added to a 15 ml borosilicate Ten-Broeck tissue homogenizer with 25 ml/g extraction solvent and mixed until well-dispersed. The mixture was filtered through a wetted, glass-fiber filter into a pre-weighed 50 ml glass beaker. The insoluble residue was then re-suspended in 4 ml/g extraction solvent, filtered again into the same 50 ml beaker, and the rinse was repeated. The solvent was allowed to evaporate overnight, and total lipids in the blood were measured by subtracting the pre-extraction weight of the 50 ml beaker from the final weight. The lipid content of blood was estimated by dividing the total lipids measured by the wet weight wet of the sample.

To determine the water and lipid content of the liver, kidney, fat, PPT, RPT, brain, and gonads, fish were killed and samples were prepared as previously described. The water and lipid content of the poorly perfused tissues were assumed to be equal to that of white muscle, which was removed just anterior to the dorsal fin, above the lateral line. Adipose fat was taken from deposits around the richly perfused tissues and kidney. Tissues were dried and the lipid assay was performed as described above except tissues were extracted with 18 ml/g and rinsed with 3 ml/g extraction solvent instead of 25 and 4 ml/g, as suggested by Radin (1981).

Due to the large, fibrous nature of the richly perfused tissues, these samples could not be homogenized in a Ten-Broeck tissue homogenizer. Therefore, the RPT were chemically digested prior to performing the lipid extraction assay. Tissues were placed in a 200 ml Erlenmeyer flask with 5 ml/g 4N potassium hydroxide (KOH) and allowed to digest overnight (Chien et al., 1995). The mixture was then poured into a 500 ml, glass separatory funnel and 5 ml of hexane was added. The funnel was then capped, shaken vigorously and the phases were allowed to separate. The lower phase was collected in the same 200 ml Erlenmeyer flask and the upper phase was collected in a pre-weighed 50 ml beaker. The procedure was repeated two
times, the extract was allowed to evaporate overnight, and lipid content of the RPT was estimated gravimetrically as described above.

To test the hypothesis that morphological parameters varied with trout sex, a t-test (Samuels and Witmer, 1999) was conducted using the “anova1” function in the MATLAB® 6.5 Statistics Toolbox. The mean volume, water and lipid content of each compartment were compared among males and females. If the p-value returned from the t-test was less than 0.05, a statistically significant difference was considered to exist between the two sexes. This procedure served as a diagnostic tool to determine the level of detail with which the PBTK model was to be developed (males and females individually, or males and females combined).

1.4.3 Physico-chemical Parameters

Partition coefficients

The blood:water and tissue:water partition coefficients ($P_{bw}$, $P_{tw}$) described the affinity of fluoranthene to move from water to blood/tissues and were estimated using a regression model developed by Bertelsen et al. (1998):

$$P_{bw,tw} = \frac{\bar{\theta} + (K_{ow}^a \times L_i^b \times 10^c)}{\bar{\theta}}$$  \hspace{1cm} \text{Eq. 20}

where $\bar{\theta}$ is the fraction of water in the tissue, $L_i$ is the fraction of lipids in the tissue, $K_{ow}$ is the fluoranthene octanol:water partition coefficient and $a$, $b$, and $c$ are the parameters of the model. The value for the log $K_{ow}$ of fluoranthene was taken from the literature (de Maagd et al. 1998), $\bar{\theta}$ and $L_i$ were experimentally measured as described above, and $a$, $b$, and $c$ are defined in Table 2. The fluoranthene tissue:blood partition coefficients ($P_i$) described the affinity of fluoranthene to move from blood to tissues. $P_i$ was calculated as the ratio of $P_{bw}$ and $P_{tw}$ for each compartment (Bertelsen et al., 1998).

The regression model (Eq. 20) used to predict partition coefficients was developed and tested with compounds whose log $K_{ow}$ ranged from 1.46 to 4.04 (Bertelsen et al., 1998). Since the fluoranthene log $K_{ow}$ is 5.23, confidence in the partition coefficient estimates were low. Therefore, $P_{bw}$ and $P_i$ were varied up to one order of magnitude in either direction and the effects on model predictions were examined.

Metabolic rate parameters
Recall from Eq. 15, the rate of change of the amount of fluoranthene metabolized in the liver, kidney and richly perfused tissues was solved using the following equation:

\[
\frac{dA_{\text{met}}}{dt} = \frac{V_{\text{max}} \cdot V_i \cdot C_{vi}}{(K_m + C_{vi})} + (K_f \cdot C_{vi})
\]

where \( V_{\text{max}} \) is the maximum rate of metabolic reaction per kilogram of metabolic tissue, \( K_m \) is the chemical concentration in venous blood leaving the compartment \((C_{vi})\) at one-half \( V_{\text{max}} \), and \( K_f \) is the first-order elimination rate constant. \( V_{\text{max}} \) and \( K_m \) describe the rate of fluoranthene elimination due to metabolic pathways that follow saturable kinetics, while \( K_f \) describes the rate of fluoranthene loss due to linear metabolism. It has been shown in mammals that phase I xenobiotic metabolizing enzymes tend to exhibit Michaelis-Menton type kinetics, while phase II enzyme activity can be described as a first-order rate process (Gargas et al., 1986). Since little information is available regarding xenobiotic metabolism kinetics in fish, it was assumed that mammalian and piscine enzyme systems would follow similar trends.

Estimates of fluoranthene \( V_{\text{max}}, K_m \) and \( K_f \) have not been published for any tissue, in any fish species. However, hepatic \( V_{\text{max}}, K_m \) and \( K_f \) have been measured in rainbow trout exposed to pyrene (Law et al., 1991). Studies have shown in rainbow trout that pyrene and fluoranthene are both known to be weak inducers of cytochrome P-4501A, as measured by the catalytic activity of phase I enzyme 7-ethoxyresorufin-O-deethylase (EROD) (Behrens et al., 2001, Bols et al., 1999). Therefore, because phase I enzyme activity levels in rainbow trout exposed to pyrene and fluoranthene have been shown to be very similar, pyrene hepatic \( V_{\text{max}} \) and \( K_m \) (Law et al., 1991) were used as surrogates for fluoranthene hepatic \( V_{\text{max}} \) and \( K_m \) in this study. Unfortunately, very little is known about the relationship between pyrene and fluoranthene linear metabolism. However, due to the well-documented parallel between pyrene and fluoranthene saturable kinetics, the fluoranthene hepatic \( K_f \) was initially set equal to the pyrene hepatic \( K_f \) measured by Law et al. (1991).

Renal \( V_{\text{max}}, K_m \) and \( K_f \) values were estimated from the relationship between phase I and II metabolic enzyme activity in the liver and kidney of brown bullhead exposed to PAH. Pangrekar and Sikka (1992) found that the renal activities of expoxide hydrase (EH), a phase I metabolic enzyme, and glutathione-S-transferase (GSH), a phase II metabolic enzyme, were 183% and 60% of the EH and GSH activity measured in the liver, respectively. Based on these findings, the
renal $V_{\text{max}}$ and $K_m$ were set equal to 1.83 times the hepatic $V_{\text{max}}$ and $K_m$, and the renal $K_f$ was set equal to 0.6 times the hepatic $K_f$.

Similarly, $V_{\text{max}}$, $K_m$ and $K_f$ for the RPT were estimated from the relationship between cytochrome P-450 activity in the liver and intestine of spot from a PAH contaminated environment. Van Veld et al. (1990) found that cytochrome P-450 activity in the gut was consistently half the activity measured in the liver of fish from seven different sites along a the Elizabeth River, a PAH contaminated tributary of the Chesapeake Bay. Consequently, $V_{\text{max}}$, $K_m$ and $K_f$ in the richly perfused tissues were set equal to one half the hepatic $V_{\text{max}}$, $K_m$ and $K_f$.

Although $K_m$ tends to remain constant, $K_f$ and $V_{\text{max}}$ were scaled to body weight using the following allometric equations developed by Travis (1987):

$$V_{\text{max}} = V_{\text{maxc}} \times BW^{(0.7)}$$  \hspace{1cm} \text{Eq. 21}

$$K_f = K_{fc} \times BW^{(-0.3)}$$  \hspace{1cm} \text{Eq. 22}

where $V_{\text{maxc}}$ and $K_{fc}$ are scaling coefficients determined by Law et al. (1991). $V_{\text{max}}$ was also scaled linearly to the weight of the metabolic tissue.

Due to the great deal of uncertainty associated with these metabolic rate parameters, initial estimates were varied up to one order of magnitude in either direction and the effect on model predictions was examined.

### 1.4.4 Exposure Conditions

Values chosen for temperature ($T$) and the fluoranthene concentration in inspired water ($C_{\text{insp}}$) were selected to reflect the exposure conditions to be simulated.

### 1.5 Model Solutions

Solutions to the model were generated in two ways. First, to predict the time course of fluoranthene in an average, individual rainbow trout, the model was solved using the mean value for each input parameter obtained from the literature or determined experimentally, hereafter referred to as the “deterministic model”. To account for the natural variability in biological systems, the model was also solved, using Monte Carlo simulation, to predict the time course of
fluoranthene across a wide range of rainbow trout, hereafter referred to as the “probabilistic model”.

The first step towards solving the probabilistic model was to classify model parameters as variable or constant. If a parameter was considered to be constant, its value remained fixed. Otherwise, a range of possible values for each variable parameter was defined with a probability distribution suitable for the conditions surrounding that parameter. Parameter distributions were selected following guidelines established by Lipton et al. (1995). If the distributions of variable parameters were known, parameters of those distributions (e.g. mean, standard deviation, maximum, minimum) were estimated for input into the Monte Carlo simulation. If the distributions of variable parameters were not known, parameters were allocated to classes of distributions based on their behavior and range of possible values, or else a uniform or default distribution found in the literature was used.

The concentration in inspired water, temperature, total body weight and volumes of the liver, kidney, RPT, gonads and brain were all assumed to follow a lognormal distribution. The lognormal distributions were defined with the mean and standard deviation of the empirical \( C_{\text{insp}}, T, BW \) and \( V_l, V_k, V_r, V_g, V_b \) data. A uniform distribution was assumed for the partition coefficients, metabolic rate parameters, compartmental arterial blood flows, and the volume of the fat and poorly perfused tissues. A uniform distribution was selected for the volume of the fat and PPT because these quantities were not empirically measured as were the volumes of the other compartments. The range of each distribution was set equal to 10% above to 10% below the estimated or literature-reported values of \( P_i, V_{\text{max}}, K_f, K_m, Q_i, Q_f, V_f \) and \( V_m \). Distributions for the effective respiratory volume and cardiac output were not available in the literature. However, the variability associated with the total body weight and temperature was translated into variability in \( Q_w \) and \( Q_e \), since these parameters were calculated as a function of \( T \) and/or \( BW \). The only parameter held constant in the Monte Carlo simulation was \( l \) (the proportion of venous blood diverted from the RPT to the liver) as this value is rigidly defined by the anatomy of rainbow trout.

The probabilistic model was solved by sampling the fixed value or values from the probability distributions of each input parameter 800 times, to generate a distribution of each model output (Table 3) for the following time points: 1, 2, 4, 6, 8, 12, 16, 32, 36, 48, 64, 96, 128, 152, 218, and 240 hours. Summary statistics for model outputs were then calculated and used to
plot curves describing the mean, the mean +1 SD and the mean −1 SD of the probabilistic model predictions.

2. Model Evaluation

To evaluate the model, rainbow trout were exposed to waterborne fluoranthene and the measured versus predicted tissue concentrations were compared. Exposures were done with the same population of rainbow trout used to parameterize the model (sub-adult 100-200 g fish) and the highest quality fluoranthene commercially available (Aldrich Chemical, Milwaukee, WI). In order to accommodate the constraints of a large fish exposure, the experiment was replicated 2 times. All procedures followed protocol approved by Miami University’s Institutional Animal Care and Use Committee (IACUC Protocol Number 581). The model was parameterized with exposure conditions equal to the average temperature (11.7 °C) and concentration in inspired water (20 μg/L) measured throughout the course of both exposure experiments.

2.1 Exposure Experiments

Experimental set-up

In the first experiment, 24 rainbow trout, approximately 100-200 g, were placed, three per tank, in one of eight 80 L flow-through glass aquaria. In the second experiment 32 fish were placed, four per tank, in eight aquaria. Randomly selected fish were systematically assigned to aquaria. Trout were added 1 per tank across all eight tanks, until all 24 or 32 fish were allocated. Aquaria contained 10 to 12°C, carbon-filtered, de-chlorinated tap water maintained at flow rates allowing at least six turnovers per day. Temperature, dissolved oxygen and pH were monitored in all eight tanks throughout the course of both experiments.

To measure the time-course of fluoranthene uptake, trout were exposed for 0, 6, 12, 36, 48, 96, 152 or 218 hours. In each experiment, at each time step, four fish were removed from the treatment, anesthetized in a solution of MS-222, killed by cervical separation, and held on ice until dissection. Body weight and standard length (cm) were measured and the liver, kidney, RPT, gonads, brain and samples of fat and white muscle were dissected, weighed and stored at -80°C.

The random selection of fish for removal from tanks was stratified to ensure that each tank would contain approximately the same number of fish throughout the course of the
experiment. Tanks were numbered from one to eight, then, a random permutation of the sequence of numbers between one and eight were repeatedly generated three (first experiment) or four (second experiment) times. The resulting sequence of 24 or 32 digits between one and eight corresponded to the order and number of tanks from which to sample the 24 or 32 fish used in the experiment.

**Dosing system**

Fluoranthene was delivered to aquaria by passing water through an elution column filled with fluoranthene-coated sand and then to a diluter system (Figure 4). The fluoranthene-coated sand was made by adding 2000 g of dry, acetone-washed silica sand and 600 ml of a solution of 20 g fluoranthene, dissolved in 1 L of acetone, to an uncapped 4 L bottle. The bottle was placed on a Rollacell rolling mixer (New Brunswick Scientific, New Brunswick, NJ) and rolled at a low speed until sand was completely coated and dry (Diamond, 1995). In a flask connected to each aquarium, water flowing through the fluoranthene column was diluted with water flowing through a control column, containing acetone washed silica sand, to maintain a desired concentration of 20 µg/L. The target water concentration of 20 µg/L was chosen to be well below the water solubility limit of fluoranthene (125 µg/L at 24°C). In the absence of UV radiation, fluoranthene is not acutely toxic at or below this concentration (Weinstein and Oris, 1997). In addition, 20 µg/L is a realistic, moderate concentration found in the environment (Hardy et al., 1994, Mastran et al., 1987).

**Fluoranthene water analysis**

To confirm the target concentration of 20 µg/L, fluoranthene concentration in each tank was measured daily by reverse-phase High Performance Liquid Chromatography (HPLC) (Table 6). 20 ml of water sample was injected directly into a 3.9 mm x 15 cm Bondpack C18 column at 30°C. An isocratic elution was performed using 90% acetonitrile: 10% water at 1.0 ml/min (Weinstein et al., 1997). A Waters 474 scanning fluorescence detector was used with excitation and emission detection wavelengths set at 360 and 460 nm, respectively (Weinstein et al., 1997). Peaks were recorded and quantified using a Waters Millennium Chromatography Manager.
Fluoranthene tissue analysis

Fluoranthene tissue concentrations were also measured using reverse-phase HPLC. Tissues were removed from the freezer, cut into small pieces and placed into a mortar. After adding 10 grams of Na₂SO₄ (drying agent) per gram of tissue, samples were ground into a fine, dry powder. Fluoranthene was extracted from the powder with 6 equal rinses adding up to a total of 45 ml acetonitrile/g tissue. The final volume of the extraction fluid was then recorded. Five ml of the extraction fluid was centrifuged for 10 minutes at 1,000 g at 4°C, to remove solid materials. 20 [ml] of the supernatant was then injected into an HPLC vial for quantification of fluoranthene concentration. Analysis of fluoranthene followed steps previously described for water samples. Fluoranthene concentration in the tissue was calculated using the following equation (Hoffman, 2001):

\[
Tissue\ conc.(\text{mg/kg}) = \frac{volume\ extract\ (L) \times conc.\ extract\ (\text{mg/L}) \times \frac{1}{fish\ weight\ (kg)}}{}
\]

Eq. 23

2.2 PBTK Model Assessment

The goodness of fit of the model was assessed graphically, by plotting the measured tissue concentrations from each time point sampled in both experiments, along with the predicted time-course of fluoranthene uptake. If the model-predicted curve was found to pass through most of the data points, the model fit was considered to be adequate.

The model was also evaluated by plotting the average (± 1 SD) measured tissue concentration from each time point sampled in both experiments, against the average and ± 1 SD model-predicted curves. If the standard deviation of the mean observed tissue concentration at each time point was within the standard deviation of the mean model predicted tissue concentration at each time point, the model fit was considered to be adequate.

2.3 Sensitivity Analysis

Sensitivity of the model to variation in each parameter was evaluated by comparing the results of two successive runs of the individual-based model, differing only in the value of one input parameter. The value of each parameter was changed ± 50% and sensitivity was quantified.
by calculating the percent change in the area under the concentration versus time curve (AUC) for each compartment. The AUC was calculated directly from the solutions to the model generated by Matlab® (Runge-Kutta integration). Parameters with the largest percent change in AUC were deemed most sensitive.

3. Model Application

To predict the change in fluoranthene toxicokinetics across the biological temperature range of rainbow trout (4-20°C), the model was repeatedly solved, changing the temperature 2°C with each successive run.

Data from Bon et al. (1999) was used to predict changes in the ovarian condition of rainbow trout throughout their annual reproductive cycle. These changes were used to simulate the reproductive cycle, from December to November, by changing the volume of the gonads to reflect the approximate monthly change in the gonadosomatic index (GSI = ovary weight/body weight * 100), measured by Bon et al., 1999. The volume of the ovaries was held constant at 0.5% BW from December through May, increased gradually from 1% in June to 5.5% in September, and further increased dramatically in October and November from 9.25% to a maximum of 15.5% at spawning.
RESULTS

1. Model Development

1.1 Model Parameterization

Physiological parameter estimates obtained from the literature are summarized in Table 4. Values shown reflect those calculated for a 150 g rainbow trout at 12° C. The morphological parameter estimates measured in males, females, and males plus females combined are provided in Table 5. In general, the PPT occupied the largest percentage of total body volume, followed by the fat, RPT, liver, kidney, brain, and gonads, respectively. The largest water content was found to be in the blood, kidney and RPT, followed by the PPT, gonads/brain (equal), liver, and fat, respectively. The largest fraction of total lipids was found in the fat, RPT, brain, kidney, liver, and gonads/blood/PPT (equal), respectively. Results (p-values) from the analysis of variance between male and female morphological parameters are also given in Table 5. The only significant differences were in the volume (p<0.001), water content (p=0.037) and lipid content (p=0.0002) of the gonads, and in the water content of the PPT (p=0.031).

Since the only consistent significant differences observed between male and female morphological parameters were in the gonads compartment, and the observed fluoranthene gonads concentrations were not statistically different between males and females (p=0.091) (Figure 5), the model was not developed individually for both sexes. Consequently, the blood:water and tissue:blood partition coefficients were calculated using the mean water and lipid content of males and females combined (Table 5). The calculated tissue:blood partition coefficients ranged from 0.78 (PPT) to 127.51 (fat) and were found to be fairly accurate. The only compartments whose partition coefficients required adjusting, to improve the model fit to the observed data, were the fat, PPT, RPT and brain. In most cases, only minor (15-25%) changes were made. Calculated and adjusted partition coefficients are provided in Table 2.

The estimated metabolic rate parameters for the liver, kidney and RPT required no adjusting, to increase goodness of fit, and are provided in Table 4. Values shown reflect those calculated for a 150 g rainbow trout at 12° C.

1.2 Model Solutions

The model predicted that fluoranthene uptake would be greatest in the fat, brain, RPT, liver, kidney, gonads, and PPT, respectively. Predictions indicated that fluoranthene uptake
would be rapid in the liver, kidney, poorly perfused tissues, richly perfused tissues, and gonads, while uptake would be more gradual in the fat and brain.

2. Model Evaluation

2.1 Exposure Experiments

In the first experiment, three of the 24 fish died. Two of these fish were found dead on the ground and were assumed to have jumped out of the aquaria. The third fish probably died due to handling stress as it was found dead only four hours after the start of the experiment. No mortality was observed in the second experiment.

Throughout the course of both experiments, temperature in the eight tanks ranged from 10.6 to 12.5°C with a mean (±SD) temperature of 11.7 (±0.49)°C. Dissolved oxygen (DO) and pH ranged from 8.7 to 12.5 mg/L and 6.86 to 7.76 with mean (±SD) DO and pH of 10.79 (±1.30) mg/L and 7.48 (±0.31), respectively. The results from the fluoranthene water concentration analyses are provided in Table 6. Fluoranthene concentrations were close to the target concentration of 20 µg/L and were fairly stable across all eight tanks, throughout both experiments. Although fluoranthene concentrations were generally higher in the first experiment than in the second, the average fluoranthene concentration across both experiments was exactly 20 µg/L.

A total of 28 females and 25 males were sampled during the two experiments. However, three of these females and four of these males were fully mature and, therefore, were not used in the analysis. Of the 25 females and 21 males that were used in the analysis, an approximate equal number of males and females were taken at each of the time points sampled. The distribution of males and females sampled at all seven time points is given in Table 7.

2.2 PBTK Model Assessment

The observed and predicted fluoranthene tissue concentrations were in agreement for some compartments at some time points, but not in/at others (Figure 5). In the liver, kidney, PPT and RPT, the model greatly over-predicted fluoranthene concentrations at early time points (6 and 12 hours). However, in the fat and gonads, the model only slightly over-predicted fluoranthene concentrations at early times, and in the brain, the model under-predicted fluoranthene concentrations at early times. At intermediate time points (36 and 48 hours), the
model overestimated fluoranthene concentration in the liver, kidney and fat compartments, while the model-uptake curve passed through some or all of the measured data points in the PPT, RPT, gonads and brain. For all compartments except the kidney, the model accurately predicted fluoranthene concentrations at all late time points (96, 152, and 218 hours). The model underpredicted fluoranthene concentration in the kidney at 218 hours.

In general, the variability in the measured fluoranthene tissue concentrations was within the variability in the model predictions, in the compartments/at the time points where the model was considered to be accurate (Figure 6 A-G). For example, at late time points, the standard deviation of the observed data was completely within the standard deviation of the model predictions for most compartments, while at intermediate and early times, the standard deviation of the observed data was only partially within the standard deviation of the model predictions, if at all, for most compartments.

2.3 Sensitivity Analysis

The input parameters found to have the most effect on model predictions were the effective respiratory volume, cardiac output, tissue:blood partition coefficients, body weight, temperature and the concentration in inspired water. Changes in the exposure conditions and physiological parameters had the largest effect on the concentration of fluoranthene in arterial blood, while changes in the partition coefficients affected the uptake of fluoranthene in all compartments. In general, any change made to the RPT affected uptake in the liver, and any change made to the PPT affected uptake in the kidney.

3. Model Application

Fluoranthene uptake across the biological temperature range of rainbow trout was found to increase with temperature in the liver, fat, RPT, and brain, whereas fluoranthene uptake decreased with increasing temperature in the kidney, PPT and gonads (Figure 7 A-G). A large change in fluoranthene uptake was observed after changing the temperature from 4 to 6 °C, especially in the PPT and gonads, while smaller changes were observed with each subsequent temperature change. With each change in temperature, the time at which fluoranthene concentration reached steady state was fairly stable in the liver, kidney, PPT, and gonads.
However, as temperature increased in the fat, RPT, and brain, the time at which fluoranthene concentration reached steady state decreased.

The only tissues affected by simulating the reproductive cycle in rainbow trout were the gonads. The rate of fluoranthene uptake in the ovaries decreased steadily throughout the year, reached a minimum at spawning and increased dramatically to pre-spawning rates shortly after spawning (Figure 8). Fluoranthene uptake was unchanged in all other compartments.
DISCUSSION

1. Model Development
1.1 Model Parameterization

The measured morphological parameters were consistent with values reported in the literature for rainbow trout. For example, the measured volume of the kidney was exactly the same as that reported by Nichols et al. (1990) and the measured volume of the brain (0.21 % $BW$) was only slightly higher than the value reported by Cameron (1974) (0.154% $BW$). The mean volumes of the liver, RPT and gonads (1.16, 5.65, and 0.15 % $BW$, respectively) were slightly less than the values reported by Nichols et al. (1990) and Barron et al. (1987) (1.27, 6.30 and 0.23 % $BW$, respectively). The difference between the measured and reported values for the volume of the RPT was expected, however, since the Nichols et al. (1990) estimate included the gonads in the richly perfused tissues and the gonads were modeled separately in this research. The difference between the measured and reported volume of the gonads compartment could be due to differences in the developmental/reproductive stage of measured fish.

The measured water and lipid content of tissues were also in agreement with values reported for rainbow trout in the literature. The mean water contents of the blood, liver, kidney, and PPT were within 3% of the values reported by Bertelsen et al. (1998). On average, the measured lipid content of the tissues was 25% less than the values reported by Bertelsen et al. (1998) and Nichols et al. (1990). This difference was expected, however, due to the fact that the fish used to parameterize this model were well exercised and were suspected to be leaner than those used in Bertelsen et al. (1998) and Nichols et al. (1990).

Hypothesis I A stated that “due to the inclusion of gonads in this model, significant differences between male and female morphological parameters will be observed and consequently the model will have to be developed individually for both sexes”. This hypothesis was partially supported because the volume, water, and lipid content of the gonads, and the water content of the PPT were found to be significantly different between males and females. However, since the gonads were the only tissues found to consistently differ between the two sexes, the model was not developed individually for males and females. This decision was further supported by the fact that the observed fluoranthene concentrations in the gonads were not statistically different between males and females ($p=0.091$) (Figure 5).
1.2 Model Solutions

As hypothesized, model predictions were driven largely by the lipid content and metabolic activity of tissues (Hypothesis I B). The two compartments predicted to accumulate the most fluoranthene were the fat and brain. These were also the tissues found to have the first and third largest fraction of total lipids. The RPT had the second largest lipid content; however, these tissues are metabolically active and will eliminate some of the fluoranthene entering the compartment. Therefore, it made sense that the model predicted the RPT would accumulate the next largest amount of fluoranthene, behind the fat and brain. Although the difference between the two was small, the kidney and liver were found to have the fourth and fifth largest lipid contents, respectively. Likewise, the model predicted that the liver and kidney would accumulate the next largest amounts of fluoranthene, behind the RPT, and the difference in uptake between the two would be small. Finally, the model predicted that the gonads and PPT would accumulate the least amount of fluoranthene. Correspondingly, the two smallest fractions of total lipids were found in the gonads and PPT, respectively.

2. Model Evaluation
2.1 PBTK Model Assessment

Hypothesis II A was not supported because the model did not accurately predict the uptake of fluoranthene in all compartments, at all time points. Although, the model was useful for predicting fluoranthene uptake at later time points, this system of equations and/or parameter estimates were not suitable for predicting the initial phase of fluoranthene uptake in most compartments. The discrepancies between the observed and predicted fluoranthene concentrations at early times may be explained by a deviation from one of the model’s primary assumptions.

Fluoranthene uptake at the tissues was assumed to be flow-limited, a frequently used default assumption in PBTK modeling (Nichols and McKim, 1994). Flow-limited chemical distribution implies that the rate-limiting step for mass transfer is the blood-perfusion rate of the tissue. Therefore, due to the rapid transfer of chemical across capillary walls and cell membranes, equilibrium exists between the chemical in the tissues and the blood flowing through those tissues. An alternative assumption, less frequently used in PBTK modeling, is that chemical exchange is diffusion-limited. Diffusion-limited chemical exchange occurs when the
transfer of chemical across the capillary wall is not rapid, but rather diffusion impedes the mass transfer of chemical to the rest of the tissue. Thus, the rate of chemical uptake in diffusion-limited systems is generally slower than in flow-limited systems.

Since the model predicted that the initial phase of fluoranthene uptake was too rapid in the liver, kidney, PPT, RPT and gonads, changing fluoranthene exchange from flow-limited to diffusion-limited may improve the fit of the model in these compartments. However, diffusion-limited models are solved with a more complex and structurally different set of equations and require the knowledge of both the intra and extracellular fluid spaces of each compartment assumed to undergo diffusion-limited chemical exchange. Since this information was not available for each compartment and re-programming the structure of the model was beyond the scope of this research, this topic should be explored in future research.

The Monte Carlo simulation was employed to predict how the variability in model parameters would affect the variability in model predictions. This was done in an attempt to apply the PBTK model to a population level, assuming that since there is natural variability in the biological and chemical systems that define the model, that variability should be reflected in the outputs of the model. It was hypothesized that the variability in the observed fluoranthene tissue concentrations would be accounted for in the variability in the probabilistic model’s predictions (Hypothesis II B). Because the variability in the measured data was within the variability in the model predictions, in compartments/at times where the model was considered to be accurate, Hypothesis II B was supported.

2.2 Sensitivity Analysis

The sensitivity analysis was performed to determine which parameters had the largest effects on model solutions and consequently, which parameters should be defined with the most care. The parameters found to be most critical were the effective respiratory volume, cardiac output, tissue:blood partition coefficients, body weight, temperature and the concentration in inspired water.

It is intuitive that the temperature, concentration in inspired water and total body weight were very important parameters since they regulate the exposure conditions to be simulated. Furthermore, temperature was used to define the total cardiac output, which, in turn, was used to define the rate of arterial and portal blood flow to each compartment, and several other
parameters in the model are scaled linearly, or allometrically, to body weight. In nature, the
temperature, environmental concentration of a contaminant, and body weight of the fish living in
a system are all highly variable. Therefore, when implementing a PBTK model, the most
realistic approach is to define $BW$, $T$ and $C_{insp}$ with a range of possible values and perform a
Monte Carlo simulation.

The sensitivity of the model to changes in the cardiac output and effective respiratory
volume was also expected. These parameters govern the flux of fluoranthene from inspired
water into arterial blood, which is then distributed to every compartment in the model. The
effective respiratory volume and cardiac output of rainbow trout have been very well studied,
and the relationships between these physiological parameters and temperature and body weight
have also been documented. Since these estimates have been widely used in other PBTK
models, confidence in the literature-reported values was high.

Tissue:blood partition coefficients were the driving force of the model. Even small
changes in partition coefficients affected the uptake of fluoranthene in all compartments.
Unfortunately, chemical partitioning information is extremely difficult to estimate. Several
authors have measured chemical partitioning in vivo from fish exposed to near steady state.
However, this defeats one of the ultimate objectives of toxicokinetic modeling by resorting to
prior exposure experiments to parameterize the model. Others have employed an in vitro gas
equilibration method (Hoffman et al., 1992), which performs headspace analysis using gas
chromatography. Unfortunately, this method is only useful for volatile compounds and was
beyond the scope of this research. By far, the easiest approach to estimating blood:water and
tissue:blood partition coefficients was the linear regression model developed by Bertelsen et al.
(1998). Although this model was developed with compounds whose log $K_{ow}$ was well below the
log $K_{ow}$ of fluoranthene, this set of equations was found to be fairly accurate. However,
expansion of this regression model to include compounds with a higher log $K_{ow}$ would possibly
eliminate the need to adjust some of these estimates and would provide much more confidence in
the model.

3. Model Application

The model was applied to predict fluoranthene uptake across the biological temperature
range of male and female rainbow trout, as well as throughout the annual reproductive cycle of
female rainbow trout. Applications such as these are one of the primary reasons for developing a PBTK model. The model can be modified an infinite number ways to predict chemical uptake in a variety of environmental circumstances, which may correspond to specific risk assessment applications.

Hypothesis III A stated that “since cardiac output is directly related to temperature, increasing the temperature will result in a higher blood flow to each compartment, which will subsequently increase uptake in all compartments”. This hypothesis was not supported because, while fluoranthene uptake did increase with temperature in most compartments, fluoranthene uptake decreased with increasing temperature in the gonads, PPT and kidney. While it is unclear why this trend was observed in the gonads and PPT, the decrease in fluoranthene uptake with increasing temperature in the kidney was probably due to the fact that the kidney is supplied with blood from the poorly perfused tissues, and any decrease in the concentration of fluoranthene in the poorly perfused tissues will cause a decrease in the concentration of fluoranthene in the kidney. The large change in fluoranthene concentrations predicted after changing temperature from 4 to 6°C was probably a result of the linear model used to estimate cardiac output (Barron et al., 1987), which gives values close to zero as temperature approaches 3.5°C.

Implications of these findings are that the liver, fat, RPT, and brain may be highly susceptible to PAH toxicity in the winter when temperatures are low and less susceptible in the summer when temperatures are high. The opposite will be true for the kidney, gonads, and PPT. Seasonal fluctuations in temperature and, consequently, in PAH uptake, may affect reproduction in female rainbow trout. During the very slow ovarian development phase (December-May), temperatures are at a minimum and, therefore, mobilization of PAH to the ovaries will also be at a minimum. Because, the onset of vitellogenesis occurs during this time (Bon et al., 1999), disruption of the mitotic division of oogonia and follicular previtellogenesis/early vitellogenesis will be unlikely given PAH contamination during these months. However, the majority of ovarian development occurs during the warmest months of the year (June-August), and vitellogenesis may be disrupted as PAH mobilization is at a maximum during this time. The rapid stage of ovarian development is marked by the completion of vitellogenesis and maturation events (Bon et al., 1999). Since this phase occurs when temperatures are generally decreasing (September-November), the risk associated with PAH mobilization to maturing eggs will be lowest in the fall.
Hypothesis III B was supported because the rate of fluoranthene uptake in the gonads was slow when the GSI (volume of the gonads) was high and fast when the GSI was slow, as predicted. Time to steady state and fluoranthene equilibrium concentrations were also found to decrease gradually as the female trout approached spawning. This suggests that as spawning season approaches, since PAH uptake in the female is decreasing, developing eggs may be less susceptible to the maternal transfer of PAH. That is, PAH mobilization to the eggs will be low, as is predicted based on temperature alone, the rate of fluoranthene uptake will be slow.

Model predictions such as these could be useful in a variety of risk assessment applications. For example, the combination of seasonal fluctuations in temperature and GSI could be examined to more accurately predict fluoranthene uptake throughout the reproductive cycle of female rainbow trout. Running the model with the initial conditions of each compartment set equal to steady-state concentrations could simulate repeated fluoranthene exposure. Incorporating additional routes of exposure, such as dermal and dietary uptake, may also be useful since fish often ingest PAH contaminated sediments and food and the skin may be an important site of chemical flux in very small fish and juvenile fish of larger species (McKim and Nichols, 1994). Additional applications of the model may include extrapolation to other species such as the threatened Lahontan cutthroat trout and other PAH whose physico-chemical properties are well characterized.

4. Conclusions and Future Directions

A physiologically based toxicokinetic model for the uptake of waterborne fluoranthene in rainbow trout was developed, evaluated, and applied in this research. The model was fairly accurate at predicting the uptake of fluoranthene in the liver, kidney, fat, poorly perfused tissues, richly perfused tissues, gonads and brain at 96, 152 and 218 hours. However, at 6, 12, 36, and 48 hours, the model did not predict the uptake of fluoranthene as well in all tissues except the brain. The rate of fluoranthene uptake was over-predicted at these early times and may be explained by diffusion limitations on fluoranthene exchange that were unaccounted for in the model. The variability in the measured fluoranthene tissue concentrations was explained by the variability in the deterministic model’s predictions in compartments/at times when the model was considered to be accurate. Application of the model revealed that, in general, fluoranthene uptake increases
with temperature, and the rate of fluoranthene uptake decreases as female rainbow trout approach spawning.

Possibilities for future research include:

1.) Re-programming and parameterizing model to include diffusion limitations on fluoranthene exchange in the liver, kidney, poorly perfused tissues, richly perfused tissues and gonads. Accounting for diffusion limitations may improve model fit during initial uptake phase.

2.) Refine the model, particularly, the tissue:blood partition coefficients and input parameter distributions for Monte Carlo simulation.

3.) Extrapolate the model to different environmental scenarios, such as those listed above, for use in exposure analysis of ecological risk assessments.
Figure 1. Physical and chemical properties of fluoranthene (deMaagd et al., 1998).

Name: Fluoranthene
Molecular Weight: 202.26
Water Solubility: 207 mg/L @ 25°C
log $K_{ow}$: 5.23
Henry’s Law Constant: 1.1 Pa/m$^3$/mol @ 20°C
Figure 2. Flow diagram of the PBTK model for fluoranthene in rainbow trout. Abbreviations used in the model are defined in Tables 1 and 3. Model adapted from Nichols et al. (1990).
Figure 3. Schematic of the counter-current exchange of fluoranthene in the gills of rainbow trout. Abbreviations are defined in Tables 1 and 3.
Table 1. Input parameters for PBTK model for fluoranthene in rainbow trout. PPT = poorly perfused tissues, RPT = richly perfused tissues.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Abbreviation</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EXPOSURE CONDITIONS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>$T$</td>
<td>°C</td>
</tr>
<tr>
<td>Fluoranthene concentration in inspired water</td>
<td>$C_{\text{insp}}$</td>
<td>g/L</td>
</tr>
<tr>
<td><strong>PHYSIOLOGICAL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac output</td>
<td>$Q_c$</td>
<td>L blood/hr</td>
</tr>
<tr>
<td>Effective respiratory volume</td>
<td>$Q_w$</td>
<td>L water/hr</td>
</tr>
<tr>
<td>Arterial blood flow to compartment</td>
<td>$Q_i$</td>
<td>L blood/hr</td>
</tr>
<tr>
<td>Proportion fluoranthene transferred via portal blood flow</td>
<td>$\theta$</td>
<td>-</td>
</tr>
<tr>
<td>From PPT to kidney</td>
<td>$\theta$</td>
<td>-</td>
</tr>
<tr>
<td>From RPT to liver</td>
<td>$\theta$</td>
<td>-</td>
</tr>
<tr>
<td><strong>MORPHOLOGICAL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight</td>
<td>$BW$</td>
<td>kg</td>
</tr>
<tr>
<td>Compartment volume</td>
<td>$V_i$</td>
<td>L</td>
</tr>
<tr>
<td>Compartment, blood fraction total lipid</td>
<td>$L_{i, L_{\text{blood}}}$</td>
<td>-</td>
</tr>
<tr>
<td>Compartment, blood fraction water</td>
<td>$g_{i, g_{\text{blood}}}$</td>
<td>-</td>
</tr>
<tr>
<td><strong>PHYSICO-CHEMICAL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compartment Michaelis-Menten constant</td>
<td>$K_m$</td>
<td>g/L blood</td>
</tr>
<tr>
<td>Compartment maximum enzymatic reaction rate constant</td>
<td>$V_{\text{max}}$</td>
<td>g/hr</td>
</tr>
<tr>
<td>Compartment first order elimination rate constant</td>
<td>$K_r$</td>
<td>L/hr</td>
</tr>
<tr>
<td>Fluoranthene octanol:water partition coefficient</td>
<td>$K_{ow}$</td>
<td>-</td>
</tr>
<tr>
<td>Fluoranthene blood:water partition coefficient</td>
<td>$P_{bw}$</td>
<td>g/L blood/g/L water</td>
</tr>
<tr>
<td>Fluoranthene tissue:water partition coefficient</td>
<td>$P_{tw}$</td>
<td>g/L tissue/g/L water</td>
</tr>
<tr>
<td>Fluoranthene tissue:blood partition coefficient</td>
<td>$P_i$</td>
<td>g/L tissue/g/L blood</td>
</tr>
</tbody>
</table>
Table 2. Estimated regression model coefficients used to predict partition coefficients (Bertelsen et al., 1998), and calculated and adjusted blood:water (P$_{bw}$) and tissue:blood (P$_i$) partition coefficients. Partition coefficients were adjusted to improve the model fit to the observed data. $g_i$ and $L_i$ estimates are provided in Table 5. PPT = poorly perfused tissues, RPT = richly perfused tissues.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>a (slope)</th>
<th>b (slope)</th>
<th>c (intercept)</th>
<th>Calculated P$_{bw}$</th>
<th>P$_i$</th>
<th>Adjusted P$_{bw}$</th>
<th>P$_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.65</td>
<td>1.72</td>
<td>2.49</td>
<td>665.22</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Liver</td>
<td>0.97</td>
<td>2.17</td>
<td>1.57</td>
<td>4.98</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.74</td>
<td>1</td>
<td>0.72</td>
<td>2.37</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fat</td>
<td>0.9</td>
<td>0.31</td>
<td>-</td>
<td>127.51</td>
<td>85.01</td>
<td>-</td>
<td>85.01</td>
</tr>
<tr>
<td>PPT</td>
<td>0.69</td>
<td>0.92</td>
<td>0.76</td>
<td>0.78</td>
<td>0.98</td>
<td>-</td>
<td>0.98</td>
</tr>
<tr>
<td>RPT</td>
<td>0.74</td>
<td>1</td>
<td>0.72</td>
<td>16.95</td>
<td>21.19</td>
<td>21.19</td>
<td>-</td>
</tr>
<tr>
<td>Gonads</td>
<td>0.74</td>
<td>1</td>
<td>0.72</td>
<td>1.78</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Brain</td>
<td>0.74</td>
<td>1</td>
<td>0.72</td>
<td>5.89</td>
<td>5.13</td>
<td>5.13</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3. Quantities solved by PBTK model for fluoranthene in rainbow trout.

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Abbreviation</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoranthene flux at the gills</td>
<td>$F_g$</td>
<td>g/hr</td>
</tr>
<tr>
<td>Fluoranthene concentration in arterial blood</td>
<td>$C_{art}$</td>
<td>g/L</td>
</tr>
<tr>
<td>Fluoranthene concentration in venous blood</td>
<td>$C_{ven}$</td>
<td>g/L</td>
</tr>
<tr>
<td>Amount of fluoranthene in compartment</td>
<td>$A_i$</td>
<td>g</td>
</tr>
<tr>
<td>Fluoranthene concentration in compartment</td>
<td>$C_i$</td>
<td>g/L</td>
</tr>
<tr>
<td>Fluoranthene concentration in venous blood leaving compartment</td>
<td>$C_{vi}$</td>
<td>g/L</td>
</tr>
</tbody>
</table>
Figure 4. Exposure system for model evaluation.
Table 4. Mean estimates and references for physiological and metabolic rate parameters obtained from the literature. Values given are for a 150 g rainbow trout at 12° C. PPT = poorly perfused tissues, RPT = richly perfused tissues.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output (L blood/hr)</td>
<td>0.414</td>
<td>Barron et al. (1987)</td>
</tr>
<tr>
<td>Effective respiratory volume (L water/hr)</td>
<td>2.153</td>
<td>Erickson and McKim (1990)</td>
</tr>
<tr>
<td>Arterial blood flow to compartment (L blood/hr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.0119</td>
<td>Barron et al. (1987)</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.0226</td>
<td>Barron et al. (1987)</td>
</tr>
<tr>
<td>Fat</td>
<td>0.0118</td>
<td>Nichols et al. (1990)</td>
</tr>
<tr>
<td>PPT</td>
<td>0.2741</td>
<td>Nichols et al. (1990)</td>
</tr>
<tr>
<td>RPT</td>
<td>0.0928</td>
<td>Barron et al. (1987)</td>
</tr>
<tr>
<td>Gonads</td>
<td>0.0008</td>
<td>Barron et al. (1987)</td>
</tr>
<tr>
<td>Brain</td>
<td>0.00002</td>
<td>Cameron et al. (1974)</td>
</tr>
<tr>
<td>( V_{\text{max}} ) (mg/hr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>12.34</td>
<td>Law et al. (1991)</td>
</tr>
<tr>
<td>Kidney</td>
<td>14.79</td>
<td>Pangrekar and Sikka (1992)</td>
</tr>
<tr>
<td>RPT</td>
<td>30.05</td>
<td>Van Veld et al. (1990)</td>
</tr>
<tr>
<td>( K_m ) (mg/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>3050</td>
<td>Law et al. (1991)</td>
</tr>
<tr>
<td>Kidney</td>
<td>5582</td>
<td>Pangrekar and Sikka (1992)</td>
</tr>
<tr>
<td>RPT</td>
<td>1525</td>
<td>Van Veld et al. (1990)</td>
</tr>
<tr>
<td>( K_l ) (L/hr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.037</td>
<td>Law et al. (1991)</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.023</td>
<td>Pangrekar and Sikka (1992)</td>
</tr>
<tr>
<td>RPT</td>
<td>0.019</td>
<td>Van Veld et al. (1990)</td>
</tr>
</tbody>
</table>
Table 5. Morphological parameter estimates for males, females, and males plus females combined. Asterisks (*) indicate statistically significant differences at \( \alpha = 0.05 \). PPT = poorly perfused tissues, RPT = richly perfused tissues.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Female</th>
<th></th>
<th></th>
<th>Male</th>
<th></th>
<th></th>
<th>Male and Female</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>N</td>
<td>Mean</td>
<td>SE</td>
<td>N</td>
<td>Mean</td>
<td>SE</td>
<td>N</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>0.14</td>
<td>0.005</td>
<td>34</td>
<td>0.143</td>
<td>0.007</td>
<td>29</td>
<td>0.712</td>
<td>0.141</td>
<td>0.004</td>
</tr>
<tr>
<td>Volume (% body volume)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1.138</td>
<td>0.041</td>
<td>34</td>
<td>1.181</td>
<td>0.058</td>
<td>29</td>
<td>0.544</td>
<td>1.16</td>
<td>0.03</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.753</td>
<td>0.023</td>
<td>31</td>
<td>0.775</td>
<td>0.031</td>
<td>26</td>
<td>0.556</td>
<td>0.76</td>
<td>0.02</td>
</tr>
<tr>
<td>Fat</td>
<td>7.35</td>
<td>-</td>
<td></td>
<td>7.35</td>
<td>-</td>
<td></td>
<td>7.35</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>PPT</td>
<td>84.593</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPT</td>
<td>5.72</td>
<td>0.138</td>
<td>34</td>
<td>5.567</td>
<td>0.131</td>
<td>29</td>
<td>0.427</td>
<td>5.65</td>
<td>0.1</td>
</tr>
<tr>
<td>Gonads</td>
<td>0.231</td>
<td>0.011</td>
<td>34</td>
<td>0.044</td>
<td>0.003</td>
<td>29</td>
<td>0.001*</td>
<td>0.15</td>
<td>0.01</td>
</tr>
<tr>
<td>Brain</td>
<td>0.215</td>
<td>0.009</td>
<td>34</td>
<td>0.208</td>
<td>0.011</td>
<td>29</td>
<td>0.615</td>
<td>0.21</td>
<td>0.01</td>
</tr>
<tr>
<td>Fraction water ((%))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>0.84</td>
<td>0.003</td>
<td>4</td>
<td>0.85</td>
<td>0.008</td>
<td>5</td>
<td>0.326</td>
<td>0.85</td>
<td>0.005</td>
</tr>
<tr>
<td>Liver</td>
<td>0.723</td>
<td>0.006</td>
<td>4</td>
<td>0.712</td>
<td>0.007</td>
<td>5</td>
<td>0.275</td>
<td>0.72</td>
<td>0.005</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.801</td>
<td>0.001</td>
<td>4</td>
<td>0.81</td>
<td>0.004</td>
<td>5</td>
<td>0.117</td>
<td>0.81</td>
<td>0.003</td>
</tr>
<tr>
<td>Fat</td>
<td>0.431</td>
<td>0.143</td>
<td>4</td>
<td>0.415</td>
<td>0.052</td>
<td>4</td>
<td>0.920</td>
<td>0.42</td>
<td>0.071</td>
</tr>
<tr>
<td>PPT</td>
<td>0.791</td>
<td>0.002</td>
<td>4</td>
<td>0.798</td>
<td>0.002</td>
<td>5</td>
<td>0.031*</td>
<td>0.79</td>
<td>0.002</td>
</tr>
<tr>
<td>RPT</td>
<td>0.797</td>
<td>0.011</td>
<td>4</td>
<td>0.802</td>
<td>0.008</td>
<td>5</td>
<td>0.714</td>
<td>0.8</td>
<td>0.006</td>
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<tr>
<td>Gonads</td>
<td>0.851</td>
<td>0.005</td>
<td>4</td>
<td>0.725</td>
<td>0.0938</td>
<td>4</td>
<td>0.037*</td>
<td>0.79</td>
<td>0.032</td>
</tr>
<tr>
<td>Brain</td>
<td>0.787</td>
<td>0.004</td>
<td>4</td>
<td>0.786</td>
<td>0.005</td>
<td>5</td>
<td>0.987</td>
<td>0.79</td>
<td>0.003</td>
</tr>
<tr>
<td>Fraction total lipid ((L_i))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>0.017</td>
<td>0.002</td>
<td>5</td>
<td>0.016</td>
<td>0.001</td>
<td>4</td>
<td>0.374</td>
<td>0.016</td>
<td>0.001</td>
</tr>
<tr>
<td>Liver</td>
<td>0.035</td>
<td>0.002</td>
<td>5</td>
<td>0.038</td>
<td>0.002</td>
<td>4</td>
<td>0.342</td>
<td>0.036</td>
<td>0.001</td>
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<tr>
<td>Kidney</td>
<td>0.036</td>
<td>0.001</td>
<td>4</td>
<td>0.044</td>
<td>0.005</td>
<td>4</td>
<td>0.150</td>
<td>0.04</td>
<td>0.003</td>
</tr>
<tr>
<td>Fat</td>
<td>0.607</td>
<td>0.05</td>
<td>5</td>
<td>0.679</td>
<td>0.032</td>
<td>4</td>
<td>0.289</td>
<td>0.639</td>
<td>0.032</td>
</tr>
<tr>
<td>PPT</td>
<td>0.016</td>
<td>0.002</td>
<td>5</td>
<td>0.016</td>
<td>0.002</td>
<td>4</td>
<td>0.819</td>
<td>0.016</td>
<td>0.001</td>
</tr>
<tr>
<td>RPT</td>
<td>0.33</td>
<td>0.06</td>
<td>4</td>
<td>0.249</td>
<td>0.037</td>
<td>4</td>
<td>0.112</td>
<td>0.29</td>
<td>0.036</td>
</tr>
<tr>
<td>Gonads</td>
<td>0.018</td>
<td>0.003</td>
<td>5</td>
<td>0.051</td>
<td>0.003</td>
<td>3</td>
<td>0.0002*</td>
<td>0.03</td>
<td>0.006</td>
</tr>
<tr>
<td>Brain</td>
<td>0.106</td>
<td>0.008</td>
<td>5</td>
<td>0.094</td>
<td>0.005</td>
<td>4</td>
<td>0.236</td>
<td>0.101</td>
<td>0.005</td>
</tr>
</tbody>
</table>
Table 6. Measured fluoranthene water concentrations in experiments 1, 2 and experiment 1 plus 2 combined. N=16 for experiment 1 and N=11 for experiment 2.

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1</th>
<th></th>
<th>Experiment 2</th>
<th></th>
<th>Experiment 1 and 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (±SD)</td>
<td>Range</td>
<td>Mean (±SD)</td>
<td>Range</td>
<td>Mean (±SD)</td>
<td>Range</td>
</tr>
<tr>
<td>Tank 1</td>
<td>22.6 (3.66)</td>
<td>14.7 - 29.2</td>
<td>17.2 (1.93)</td>
<td>12.9 - 19.8</td>
<td>20.4 (4.06)</td>
<td>12.9 - 29.2</td>
</tr>
<tr>
<td>Tank 2</td>
<td>22.7 (4.19)</td>
<td>14.5 - 28.6</td>
<td>17.0 (2.14)</td>
<td>13.7 - 22.0</td>
<td>20.4 (4.48)</td>
<td>13.7 - 28.6</td>
</tr>
<tr>
<td>Tank 3</td>
<td>21.3 (4.25)</td>
<td>13.6 - 25.3</td>
<td>17.0 (1.63)</td>
<td>12.8 - 19.2</td>
<td>19.5 (4.00)</td>
<td>12.8 - 25.3</td>
</tr>
<tr>
<td>Tank 4</td>
<td>22.8 (3.63)</td>
<td>15.3 - 26.7</td>
<td>16.3 (1.43)</td>
<td>14.2 - 18.4</td>
<td>20.2 (4.37)</td>
<td>14.2 - 26.7</td>
</tr>
<tr>
<td>Tank 5</td>
<td>22.5 (3.88)</td>
<td>15.1 - 26.1</td>
<td>17.1 (1.43)</td>
<td>13.6 - 18.9</td>
<td>20.3 (4.11)</td>
<td>13.6 - 26.1</td>
</tr>
<tr>
<td>Tank 6</td>
<td>21.7 (4.26)</td>
<td>13.6 - 26.5</td>
<td>15.4 (1.33)</td>
<td>13.2 - 16.9</td>
<td>19.1 (4.57)</td>
<td>13.2 - 26.4</td>
</tr>
<tr>
<td>Tank 7</td>
<td>22.6 (4.33)</td>
<td>13.7 - 26.7</td>
<td>16.2 (1.35)</td>
<td>14.0 - 18.2</td>
<td>20.0 (4.66)</td>
<td>13.7 - 26.7</td>
</tr>
<tr>
<td>Tank 8</td>
<td>22.3 (4.58)</td>
<td>13.8 - 26.2</td>
<td>16.2 (1.10)</td>
<td>14.7 - 17.6</td>
<td>19.8 (4.68)</td>
<td>13.8 - 26.2</td>
</tr>
<tr>
<td>All Tanks</td>
<td>22.3 (4.03)</td>
<td>13.6 - 29.2</td>
<td>16.6 (1.62)</td>
<td>12.8 - 22.0</td>
<td>20.0 (4.32)</td>
<td>12.8 - 29.2</td>
</tr>
</tbody>
</table>
Table 7. Distribution of males and females sampled throughout the course of both experiments at each time point.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Number of Females</th>
<th>Number of Males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sub-adult (Used)</td>
<td>Adult (Not-used)</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>36</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>48</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>96</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>152</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>218</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>3</td>
</tr>
</tbody>
</table>
Figure 5. Observed (points) and predicted (line) fluoranthene tissue concentrations in the liver, kidney, fat, poorly perfused tissues (PPT), richly perfused tissues (RPT), gonads, and brain.
Figure 5. (Continued)
Figure 5. (Continued)
Figure 5. (Continued)
Figure 6. Mean (±1 SD) observed fluoranthene concentrations (points) and the mean (solid lines) (±1 SD) (dashed lines) model-predicted curves of fluoranthene uptake in the liver, kidney, fat, poorly perfused tissues (PPT), richly perfused tissues (RPT), gonads, and brain.
Figure 6. (Continued)
Figure 6. (Continued)
Figure 7. Fluoranthene uptake in the liver, kidney, fat, poorly perfused tissues (PPT), richly perfused tissues (RPT), gonads, and brain, across the physiological temperature range of rainbow trout (4-20°C).
Figure 7. (Continued)
Figure 7. (Continued)
Figure 7. (Continued)
Figure 8. Fluoranthene uptake in the ovaries of female rainbow trout during their annual reproductive cycle.
APPENDIX

Matlab® code used to solve PBTK model for fluoranthene in rainbow trout. All code written by Dr. Steve Wright, Department of Mathematics and Statistics, Miami University and Carrie Smith, Department of Zoology, Miami University.

function pbtkflu(t,p)
% PBTK model for fluoranthene (FLU) in rainbow trout (RBT).
% The call pbtkflu(t,p) plots concentration trajectories and calculates AUC's for the time-interval [0,t].
% This a top-level function that calls 3 additional functions "params", "calcC", and "solveODE".
% The m-files for each of these functions must be located in the same path as pbtkflu and must be named params, calcC and solveODE.
% 't' is a required input argument which defines the time interval.
% 'p' is an optional input argument which may be used to change default parameter values outlined defined in params.m.

% if the number of arguments is less than 1 (i.e. t or p are not defined), let the user know (print) that he must specify the desired time interval before the file will run.
if nargin<1
    fprintf('Sorry! Correct usage is:  pbtkflu(t)');
    return
end

% if the number of arguments is less than two (i.e. p is not defined) there are no changes to the default parameter list. Get the default parameter structure/call the function "params".
if nargin<2
    p=Params;
end

% call the function "solveODE" to solve the set of differential equations.
sol=solveODE(t,p);
t=sol.x; % define 't' as the x vector (time) of ODE solution structure.
A=sol.y; % define 'A' as the y vector (amounts (ug)) of ODE solution structure.

% call the function "calcC" to calculate the concentration vector C (ug/L) from the amount vector A (ug), concentration of venous blood Cven (ug/L), and concentration of arterial blood Cart (ug/L).
[C,Cven,Cart]=calcC(A,p);

% calculate areas under concentration trajectories (AUC)
areaA=zeros(size(C,1),1);
for i=1:size(C,1)
    areaA(i)=quad(@(deval,0,t,[],[],sol,i); % quadrature, sol tells deval what to do.
end

[areaC,areaCven,areaCart]=calcC(areaA,p); % rescale AUC from amounts (ug) to concentrations (ug/L).

%% plot trajectories

Concen=[C;Cven;Cart]; % define 'Concen' as array of all concentration calculations (C vector, Cart, Cven).

AUC=[areaC;areaCven;areaCart]; % define 'AUC' as array of all AUC concentration calculations.

name={p.organs{:} 'venous blood' 'arterial blood'}; % define 'name' as array of organ vector (name) from params structure, venous blood, and arterial blood.

close all

for i=1:size(Concen,1)
    figure
    if min(Concen(i,:))>max(Concen(i,:))/10 % adjust axes
        axis([min(t) max(t) 0 1.1*max(Concen(i,:))])
    end
    hold on
    plot(t,Concen(i,:)) % plot t (time) on x axis and Concen on y axis
    title(sprintf('%s  (AUC=%g)',name{i},AUC(i))) % define title for plot as organ name, AUC calculation
    xlabel('Hours') % define label for x axis
    ylabel('FLU concentration (\mu g/kg)') % define label for y axis
    A=[t;Concen];
    B=rot90(A);
    C=flipud(B);
    dlmwrite('results',C,'\t');
end
function p=Params(varargin)
% Sets up structure of parameter values to pass to ODE solver, including:
%  
%  % BW    : body weight (kg)
%  % T     : temperature (degrees Celsius)
%  % Qc    : cardiac output (L blood/hr)
%  % Qw    : effective respiratory volume (L water/hr)
%  % Q     : vector of arterial blood flow rates to organs (L blood/hr)
%  % V     : vector of organ volumes (L)
%  % Pbw   : blood:water partition coefficient
%  % P     : vector of blood:organ partition coefficients
%  % KmL   : Michaelis-Menton constant (ug/L blood)
%  % VmaxL : maximum enzymatic reaction rate (ug/hr/kg liver)
%  % KL    : first order elimination rate constant (L/hr)
%  % KK    : first order elimination rate constant (L/hr)
%  % KmK   : Michaelis-Menton constant (ug/L blood)
%  % VmaxK : maximum enzymatic reaction rate (ug/hr/kg kidney)
%  % Cinsp : Concentration of toxin in inspired water (ug/L)
%  % kappa : proportion of blood exiting PPT which enters kidney via caudal vein
%  
% If no input is specified, all parameters are given default values. To specify other values, use a 
call of the form
%  
%    p=params('ParameterName',ParameterValue,...)
%  
% For example, the call
%  
%    p=params('Vmax',13.0,'Km',4.1)
%  
% returns p with the default values in all fields except Vmax and Km. If a parameter name 
occurs several times, the final value is used. This routine incorporates several parameter 
dependencies, namely,
%
%  % BW --> Qw, V
%  % BW, T --> Qc --> Q
%
% so that, for example, requesting a new value for BW changes the values of Qw, V, Qc, and Q. 
To override this effect, one may also specify values for the dependent quantities, as in the call
%
%    p=params('BW',0.29,'Qc',0.57)
%
% To change the value of an entry in a vector (Q, V, P), specify the value as 
{OrganName,OrganValue} as in the call
%
%    p=params('P',{'liver',41.0})
%
% Organ names must be one of 'liver', 'RPT', 'kidney', 'PPT', 'fat',
% 'gonads', or 'brain'.

p=struct; % Establish p as a structure such that each parameter (BW, T, V) can be represented as
worrying about order.

% Check input arguments for changes to default values.

if nargin>1
    names=varargin(1:2:end-1); % define 'names' as the 1st, 3rd, 5th, etc. items in the input
    values=varargin(2:2:end); % define 'values' as the 2nd, 4th, 6th, etc. items in the input
    argument list.
arg = length(names)-length(values);
    if arg
        error('Input arguments should occur in Name/Value pairs.')
    end
else
    names=cell(0);
    values=cell(0);
end

% declare default values for scalar parameters.
defaults=
    'BW'  '0.141'
    'KmL'  '3050'
    'VmaxL'  '(26757*(p.BW^0.7))'
    'KL'  '(0.021*(p.BW^-0.3))'
    'KmK'  '5582'
    'VmaxK'  '(48965*(p.BW^0.7))'
    'KK'  '(0.013*(p.BW^-0.3))'
    'KmR'  '1525'
    'VmaxR'  '(13379*(p.BW^0.7))'
    'KR'  '(0.0105*(p.BW^-0.3))'
    'T'  '11.7'
    'Qc'  '(0.237*p.T-0.774)*p.BW'
    'Qw'  '7.2*(p.BW^0.75)'
    'lambda'  '1'
    'kappa'  '0.6'
% process input arguments to replace default values for scalar parameters.

for j=1:size(defaults,1) % loop over the 1st dimension of 'defaults'.
    name=defaults{j,1}; % define 'name' as the 1st row of 'defaults'.
    i=max(find(strcmpi(name,names))); % identify any 'names' in the last returned input argument list and compare these with 'name'.
    % for any 'name' that has no corresponding 'names' in the input argument list, define 'val' for that 'name' as the 2nd row of 'defaults'.
    if isempty(i)
        val=eval(defaults{j,2});
    % for any 'name' that does have a corresponding 'names' in the input argument list, define 'val' for that 'name' as the 'values' entered.
    else
        val=values{i};
        % identify invalid value in input argument list. error-checking for non-real values.
        if ~isreal(val)
            error(['Invalid value specified for parameter ''' name '''.'])
        end
    end
    p=setfield(p,name,val); % return parameter structure with any changes requested.
end

% declare default organ-specific values.

p.organs={'Liver' 'Kidney' 'Fat' 'PPT' 'RPT' 'Gonads' 'Brain'}; % define 'organs' as vector of compartment names within params structure.
defaults={
    'Q' [0.0289 0.0546 0.0905 0.6 0.2241 0.0018 0.0001]*p.Qc % vector of arterial blood flows (as proportion Qc) * Qc field of params structure.
    'V' [0.0116 0.0076 0.0735 0.8472 0.0565 0.0015 0.0021]*p.BW % vector of compartment volumes (as %BW) * BW field of params structure. All tissues were assumed to have equal densities of 1.0 g/ml.
    'P' [4.98 2.37 127.51/1.5 0.78*1.25 6.95*1.25 1.78 5.89/1.15]
};

% perform some black magic to identify compartment by index vector of params structure without worrying about order.

p.index=inline(['find(strcmpi(name,{'...
    sprintf("%s",p.organs{1}) ... 
    sprintf("%s",p.organs{2:end}) '})'),name');

% process input arguments to replace default values for organ-specific parameters.
for j=1:size(defaults,1)
    name=defaults{j,1};
    try
        vals=cat(1,values{strcmpi(name,names)});
    catch
        error(['Invalid value(s) specified for parameter "' name '".'])
    end
    if ~isempty(vals)
        for k=1:length(p.organs)
            i=max(find(strcmpi(p.organs(k),vals(:,1))));
            if ~isempty(i)
                val=vals{i,2};
                if isreal(val)
                    defaults{j,2}(k)=val;
                else
                    error(['Invalid value specified for "' ...
                            p.organs(k) " entry of parameter "' name '".'])
                end
            end
        end
    end
    p=setfield(p,name,defaults{j,2});
end
function sol=solveODE(t,p)
% Calculates trajectories over time for amounts in organs using ODE model.
% Input: time horizon and parameter structure p (as prepared by params)
% Output: ODE solution structure suitable for use in 'deval'

sol=ode45(@model,[0 t],zeros(size(p.Q)),...
% input arguments = model (set of differential equations),
... % time domain, initial condition (with same size matrix (same number of zeros) as Q vector of params structure.
[],... % ODE solver options (in this case none)
p); % structure containing model parameters to be passed into model (from params)

function dA=model(t,A,p)
% autonomous DE system for compartmental model (ignore t)
% dAl/dt = (Ql*Cart) + lambda*(Qr*Ar)/(Pr*Vr) - (Ql*Al)/(Pl*Vl) -
% (Vmax*(Al/(Vl*Pl)))/(Km+(Al/(Vl*Pl)))*
% dAr/dt = (Qr*Cart) - (Qr*Ar)/(Pr*Vr)
% dAk/dt = (Qk*Cart) + kappa*(Qm*Am)/(Pm*Vm) - (Qk*Ak)/(Pk*Vk)
% dAm/dt = (Qm*Cart) - (Qm*Am)/(Pm*Vm)
% dAf/dt = (Qf*Cart) - (Qf*Af)/(Pf*Vf)
% dAg/dt = (Qg*Cart) - (Qg*Ag)/(Pg*Vg)
% dAb/dt = (Qb*Cart) - (Qb*Ab)/(Pb*Vb)
% with Cart determined from Ci=Ai/(Pi*Vi) via gill flux:
% Cven = (Ql*Cl + (1-lambda)*Qr*Cr + Qk*Ck + (1-kappa)*Qm*Cm + Qf*Cf + Qg*Cg +
% Qb*Cb)/Qc
% Cart = Cven + min(Qw/Qc,Pbw)*( Cinsp - Cven/Pbw )

% call the function "calcC" to convert amounts (ug) into concentrations (ug/L) AND calculate Cven and Cart for use in differential equations.
[C,Cven,Cart]=calcC(A,p);

% rescale concentrations by dividing by the P (partition coefficient) vector of the params structure for convenient use in formulas. represents concentration of venous blood leaving each compartment.
CdivP=C./p.P;

% differential equation for most compartments.
% general interaction given by [net rate of change in amount] = [blood flow rate]*[arterial concentration-venous concentration].

dA=p.Q.*(Cart-CdivP);

% special interactions for metabolizing compartment and compartments receiving arterial and portal blood flow.
liver=p.index('liver'); % variable 'liver' gives relative location of liver-specific quantities, as set in params.
rpt=p.index('rpt'); % variable 'rpt' gives relative location of rpt-specific quantities, as set in params.
kidney=p.index('kidney'); % variable 'kidney' gives relative location of kidney-specific quantities, as set in params.
ppt=p.index('ppt'); % variable 'ppt' gives relative location of ppt-specific quantities, as set in params.

% differential equation 1 for liver (gains via RPT).
dA(liver)=dA(liver)+(p.lambda*p.Q(rpt))*(CdivP(rpt)-CdivP(liver));

% differential equation 2 for liver (loses via metabolism).

% differential equation 3 for liver (loses via first order metabolism).
dA(liver)=dA(liver)-(p.KL*CdivP(liver));

% differential equation 1 for kidney (gains via PPT).

% differential equation 2 for kidney (loses via MM metabolism).

% differential equation 3 for kidney (loses via first order metabolism).
dA(kidney)=dA(kidney)-(p.KK*CdivP(kidney));

% differential equation 1 for rpt (loses via MM metabolism).

% differential equation 2 for rpt (loses via first order metabolism).
dA(rpt)=dA(rpt)-(p.KR*CdivP(rpt));
function [C,Cven,Cart]=calcC(A,p)
% Calculates concentration in organs (ug/L) from amounts (ug), concentration mixed venous blood (ug/L) and concentration arterial blood (ug/L).

% calculate 'C' (concentration) vector by dividing A (amounts) vector by diagonal V (volume) vector of params structure.
C=diag(1./p.V)*A;

% define 'flow' as a vector containing all ones that is the same size as the Q (arterial blood flow) vector of the params structure.
flow=ones(size(p.Q));

% define 'flow(p.index('rpt'))' as 1 - the value of the lambda field of the params structure.
flow(p.index('rpt'))=1-p.lambda;

% define 'flow(p.index('ppt'))' as 1 - the value of the kappa field of the params structure.
flow(p.index('ppt'))=1-p.kappa;

% special interactions for metabolizing compartment and compartments receiving arterial and portal blood flow.
liver=p.index('liver'); % variable 'liver' gives relative location of liver-specific quantities, as set in params.
rpt=p.index('rpt'); % variable 'rpt' gives relative location of rpt-specific quantities, as set in params.
kidney=p.index('kidney'); % variable 'kidney' gives relative location of kidney-specific quantities, as set in params.
ppt=p.index('ppt'); % variable 'ppt' gives relative location of ppt-specific quantities, as set in params.

% calculate 'Cven' (mixed venous blood concentration) using flow, the Q and P vectors of the params structure, % the Qc field of the params structure, and the C vector.

%calculate 'Fg' (chemical flux at the gills)

% calculate 'Cart' (arterial blood concentration) using Cven, and the Qw, Qc, Pbw and Cinsp fields of the params structure.
Cart=Cven+(Fg/p.Qc);
function mc(time,n)
% MC(time,n)
% Plots trajectories on [0,time] for n randomly chosen values of input parameters.

p=Params; % default parameter values
liver=p.index('liver'); % variable 'liver' gives relative location of liver-specific quantities, as set in params.
rpt=p.index('rpt'); % variable 'rpt' gives relative location of rpt-specific quantities, as set in params.
kidney=p.index('kidney'); % variable 'kidney' gives relative location of kidney-specific quantities, as set in params.
ppt=p.index('ppt'); % variable 'ppt' gives relative location of ppt-specific quantities, as set in params.
fat=p.index('fat');
gonads=p.index('gonads');
brain=p.index('brain');

MCBW=exp(normrnd(-1.99,0.26,[1 n]));
MCCinsp=exp(normrnd(2.97,0.22,[1 n]));
MCT=exp(normrnd(2.46,0.04,[1 n]));
MCVl=exp(normrnd(0.12,0.22,[1 n]));
MCVk=exp(normrnd(-0.29,0.17,[1 n]));
MCF=unifrnd(0.9*p.V(fat),1.1*p.V(fat),[1 n]);
MCVm=unifrnd(0.9*p.V(ppt),1.1*p.V(ppt),[1 n]);
MCVr=exp(normrnd(1.72,0.14,[1 n]));
MCVg=exp(normrnd(-2.30,0.96,[1 n]));
MCVb=exp(normrnd(-1.59,0.27,[1 n]));
MCPl=unifrnd(0.9*p.P(liver),1.1*p.P(liver),[1 n]);
MCPk=unifrnd(0.9*p.P(kidney),1.1*p.P(kidney),[1 n]);
MCPf=unifrnd(0.9*p.P(fat),1.1*p.P(fat),[1 n]);
MCPm=unifrnd(0.9*p.P(ppt),1.1*p.P(ppt),[1 n]);
MCP=unifrnd(0.9*p.P(rpt),1.1*p.P(rpt),[1 n],2.46)
MCPg=unifrnd(0.9*p.P(gonads),1.1*p.P(gonads),[1 n]);
MCPb=unifrnd(0.9*p.P(brain),1.1*p.P(brain),[1 n]);
MCPbw=unifrnd(0.9*p.Pbw,1.1*p.Pbw,[1 n]);
MCKmL=unifrnd(0.9*p.KmL,1.1*p.KmL,[1 n]);
MCVmaxL=unifrnd(0.9*p.VmaxL,1.1*p.VmaxL,[1 n]);
MCKL=unifrnd(0.9*p.KL,1.1*p.KL,[1 n]);
MCKmK=unifrnd(0.9*p.KmK,1.1*p.KmK,[1 n]);
MCVmaxK=unifrnd(0.9*p.VmaxK,1.1*p.VmaxK,[1 n]);
MCKK=unifrnd(0.9*p.KK,1.1*p.KK,[1 n]);
MCKmR=unifrnd(0.9*p.KmR,1.1*p.KmR,[1 n]);
MCVmaxR=unifrnd(0.9*p.VmaxR,1.1*p.VmaxR,[1 n]);
MCKR=unifrnd(0.9*p.KR,1.1*p.KR,[1 n]);
MCQl=unifrnd(0.9*p.Q(liver),1.1*p.Q(liver),[1 n]);
MCQk = unifrnd(0.9*p.Q(kidney), 1.1*p.Q(kidney), [1 n]);
MCQf = unifrnd(0.9*p.Q(fat), 1.1*p.Q(fat), [1 n]);
MCQm = unifrnd(0.9*p.Q(ppt), 1.1*p.Q(ppt), [1 n]);
MCQr = unifrnd(0.9*p.Q(rpt), 1.1*p.Q(rpt), [1 n]);
MCQg = unifrnd(0.9*p.Q(gonads), 1.1*p.Q(gonads), [1 n]);
MCQb = unifrnd(0.9*p.Q(brain), 1.1*p.Q(brain), [1 n]);
MCKappa = unifrnd(0.9*p.Kappa, 1.1*p.Kappa, [1 n]);
close all

t = [1, 2, 4, 6, 8, 12, 16, 32, 48, 64, 96, 128, 152, 218, 240];

fid = fopen('mcresults', 'w');

for j = 1:n
    % solve model
    sol = solveODE(time, Params('BW', MCBW(j), 'Cinsp', MCCinsp(j), 'T', MCT(j), 'V(liver)', MCVl(j), 'V(kidney)', MCVk(j), 'V(fat)', MCVf(j), 'V(ppt)', MCVm(j), 'V(rpt)', MCVr(j), 'V(gonads)', MCVg(j), 'V(brain)', MCVb(j), 'P(liver)', MCPl(j), 'P(kidney)', MCPk(j), 'P(fat)', MCPf(j), 'P(ppt)', MCPm(j), 'P(rpt)', MCPr(j), 'P(gonads)', MCPg(j), 'P(brain)', MCPb(j), 'Pbw', MCPbw(j), 'VmaxL', MCVMaxL(j), 'KmL', MCKmL(j), 'KL', MCKL(j), 'VmaxK', MCVMaxK(j), 'KmK', MCKmK(j), 'KK', MCKK(j), 'VmaxR', MCVMaxR(j), 'KmR', MCKmR(j), 'KR', MCKR(j), 'Q(liver)', MCQl(j), 'Q(kidney)', MCQk(j), 'Q(fat)', MCQf(j), 'Q(ppt)', MCQm(j), 'Q(rpt)', MCQr(j), 'Q(gonads)', MCQg(j), 'Q(brain)', MCQb(j), 'Kappa', MCKappa(j));

    % t = sol.x; % define 't' as the x vector (time) of ODE solution structure.
    A = deval(sol, t); % sol.y; % define 'A' as the y vector (amounts (ug)) of ODE solution structure.
    [C, Cven, Cart] = calcC(A, p);
    Concentration = [C; Cven; Cart];
    name = [p.organs{:}; 'venous blood'; 'arterial blood'];

    for it = 1:length(t)
        fprintf(fid,'%d	%d', j, t(it));
        fprintf(fid, '%g', Concentration(:, it));
        fprintf(fid, '%n');
    end
end

fclose(fid);
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