A PHARMACOKINETIC STUDY OF THE EFFECTS OF STRESS AND EXERCISE ON CHEMICAL EXPOSURE

THESIS

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AFIT/GEE/ENV/01M-23

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AFIT/GEE/ENV/01M-23

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Presented to the Faculty

Department of Systems and Engineering Management

Graduate School of Engineering and Management

Air Force Institute of Technology

Air University

Air Education and Training Command

In Partial Fulfillment of the Requirements for the

Degree of Master of Science in Engineering and Environmental Management

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March 2001

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Acknowledgements

I would like to express my sincere appreciation to my thesis advisor, Dr. Michael L. Shelley. His patience and faith in my abilities was key to the completion of this thesis effort. Without his support and constant reasoning, I would not have learned as much as I did about the system dynamics process, and how to approach this specific model.

I would also like to thank Dr. Robert Grubbs, Dr. James Olson, Major Peter LaPuma, and Dr. Peter Robinson for their support throughout my entire thesis effort. Their insight and experience helped to make my experience both educational and enjoyable. Their continued patience and understanding was greatly appreciated.

I would like to thank my family and friends for their understanding and support throughout my experience at AFIT. Their love and encouragement was an essential factor in keeping my motivation level high.

Karen M. Watson

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Abstract

Several concerns about the effects of the combination of human chemical exposure with the stressful conditions of the Gulf War have been raised. The wartime stress experienced by soldiers can vary from physical to mental and emotional stress. Each type of stress causes changes in the human body, including blood flow, hormonal, and ventilation changes, and may increase the permeability of the blood-brain barrier. Each change influences the chemical uptake, distribution, and accumulation in the body.

The purpose of this thesis was to model and predict the changes that occur when stress and exercise are combined with chemical exposure. A Physiologically-based pharmacokinetic (PBPK) model was used as a tool to visualize, predict, and generate a hypothesis about chemical exposures. The PBPK model developed simulated human tissue compartments during chemical exposure under varying stress and exercise conditions.

As a result of the system dynamics process, the PBPK model developed may be a valid tool for helping to explain and predict the fate and transport of a chemical on an individual under the physical stress of exercise, and other less-defined stressors which directly affect the blood-brain barrier transport mechanisms in the model. The results suggest that the chemical concentrations in the brain are highly dependent on the transport mechanisms involved. The transport mechanisms and their respective strengths have been identified as key parameters for further study. The model developed is a simple tool that can be applied to future exploration.

A PHARMACOKINETIC STUDY OF THE EFFECTS OF STRESS AND EXERCISE ON CHEMICAL EXPOSURE

I. Introduction

Background

Although a credible threat of chemical warfare existed during the Persian Gulf War, there is some doubt as to whether chemical agents were actually used (Dunn and others, 1991:693). Of the 697,000 troops deployed to the Persian Gulf during the Gulf War, most of the veterans who complained of illnesses had diagnosable conditions. Despite the apparent lack of chemical warfare, the symptoms of several thousand veterans, such as headaches, forgetfulness, and chronic fatigue, have not been easily explained (Persian Gulf Veterans Coordinating Board, 1995:262). Several theories have been proposed as to the cause of the Gulf War Syndrome, but the true mechanisms behind the illness have not yet been determined.

The U.S. Department of Defense (DoD) formed a Presidential Advisory Committee (PAC) on Gulf War Veterans' Illnesses that conducted an $80 million evaluation of the long-term health problems observed in Persian Gulf War Veterans (Pennisi, 1996:479). Although a specific cause behind the disease symptoms was not found by the PAC, a variety of ailments were attributed to wartime stress, based on several studies of post-traumatic stress disorder (PTSD) in Gulf War Veterans (Haley, 1997:695). The stress experienced by the Gulf War veterans includes a unique combination of physical and psychological stressors. Stressors are defined as

perturbations that disrupt homeostasis in the body (Sapolsky, 1992:3). This disruption may include changes in blood flow, breathing, and responses to enzymes and hormones that may influence the body's response to chemical exposure (Suhajda, 2000:2). Suhajda demonstrated that exercise (physical stress) can have a large influence on chemical distributions in the body.

Some veterans and researchers are hesitant to link stress to the cause of the Gulf War Syndrome. The soldiers were exposed to a variety of unique health risks such as their living conditions, the chemical and biological warfare threat, immunizations, infectious diseases, and environmental hazards (Persian Gulf Veterans Coordinating Board, 1995:262). A common factor among many veterans was use of pyridostigmine bromide (PB) tablets as a prophylactic pretreatment for nerve agent exposure. PB, coupled with post nerve agent exposure administration of atropine and pralidoxime chloride, significantly increases survival after lethal nerve agent exposures (Dunn and others, 1991:693). PB is also used in the treatment of myasthenia gravis (MG), a disease causing muscle weakness, difficulties in limb movement, and respiratory or swallowing impairment (Shen, 1998:235). Shen (1998) noted that although patients with MG required much higher doses of PB, and showed few side effects, that was not sufficient evidence to discount the theory that PB is a main cause of some of the symptoms the veterans suffer from.

PB was taken by the soldiers to counter the lethal action of organophosphate nerve agents, which are inhibitors of acetylcholinesterase (AChE), such as sarin and tabun (Dunn and others, 1991:693). PB acts as a reversible inhibitor of AChE that competitively inhibits nerve agent binding, allowing the reactivation of AChE activity

and the return of normal nerve cell synapse operation over time (Cook and others, 1992:250).

Nerve agent inhibition of AChE, the enzyme responsible for acetylcholine (ACh) hydrolysis, leads to accumulation of ACh at the synapse of nerve cells, which may result in death due to hyperstimulation of cholinergic receptors (Costa and others, 1988:48). Hyperstimulation of cholinergic receptors may cause central nervous system effects such as loss of consciousness, convulsions, and respiratory depression (Cook and others, 1992:250).

Evidence of central nervous system effects is associated with significant penetration of chemicals across the blood-brain barrier (BBB). Significant penetration of a chemical is likely to cause neurotoxic effects. Under normal conditions, PB does not significantly penetrate the BBB due to its hydrophilic quaternary amine structure (Cook and others, 1992:250). However, there has been speculation that PB may penetrate the BBB under stressed conditions.

The BBB segregates blood and brain interstitial fluid, and is characterized by three properties: endothelial tight junctions in the brain capillary bed, minimal pinocytosis, and astrocyte foot processes (Pardridge, 1998:1782). The endothelial tight junctions provide the high resistance that eliminates pores in the brain capillary walls, which are present in the capillary beds of other body tissues (Pardridge, 1998:1781). This elimination of pores prevents charged molecules from leaking across the BBB. Minimal pinocytosis removes the trans-cellular route for free solute movement into the brain. The properties of endothelial junctions and minimal pinocytosis help to form the protective mechanism for the brain, prohibiting most unwanted molecules from crossing

the BBB in notable quantities in normal conditions. Molecules able to penetrate the BBB have the dual properties of lipid solubility and molecular size under 400-600 Da (Pardridge, 1998:1782).

In addition, astrocytes are believed to secrete trophic, or 'food' factors, to the brain endothelium (Pardridge, 1998:1782). These trophic factors are involved in the nourishment of the brain. Astrocytes form endfeet along endothelial cells, which may be associated with vessel formation and maintenance of the BBB (Baba and others, 2000:122). In addition to tightening intercellular junctions, astrocytic contact with endothelial cells may lessen L-glucose permeability (Asai and others, 1999:164). The abundance of astrocytes around the brain capillary endothelial cells is believed to assist in maintaining potassium homeostasis in the brain (U.S. Department of Health and Human Services, 1992:63). Other astrocytic influences on cerebral endothelial cells include the induction of tight junctions, stimulation of glucose uptake, and increase of acetylcholinesterase activity (Joo, 1996:267).

Several studies have been performed to help determine the possible role of PB in producing the Gulf War Syndrome, but due to differing conclusions, a broadly accepted theory has not yet emerged. Suhajda hypothesized that BBB permeability increased under exercise conditions (Suhajda, 2000:88). This increased permeability may allow significant passage of PB under the physical stressors encountered by Gulf War veterans experiencing unexplained illnesses. Significant passage of PB into the CNS may have produced potentially damaging long-term consequences in the soldiers (Suhajda, 2000:4). These long-term consequences may have been manifested as the unexplained illnesses of the Gulf War Syndrome.

A new field of study called deployment toxicology comprises protection of military personnel from toxic and hazardous substances, practices in preventative medicine, and identification of environmental and occupational stressors (Suhajda, 2000:4). This effort requires an advanced look at possible exposures and hazards, both individually and collectively, under a variety of conditions. The conditions could be a range of stress levels experienced by the soldiers, or differing tolerances to varying exposure levels.

Problem Statement

Further understanding of the Gulf War Syndrome will assist in determining the mechanisms behind the unexplained illness experienced by the Gulf War veterans. The observation of the behavior of chemicals such as PB in the body under various conditions of stress could provide the insight necessary to prevent future occurrences of similar syndromes. Stress conditions such as physical exertion, anticipated combat, sleep deprivation, and field conditions should be taken into account when considering the veterans' exposure conditions. Stress involves the possible combinations of cardiovascular and endocrine related responses.

Purpose Statement

The purpose of this thesis is to model and predict changes in the body when stress is combined with chemical exposure. A physiologically-based pharmacokinetic (PBPK) model will be used to visualize, predict, and test a hypothesis about this exposure. This thesis effort will be directed toward expanding on Suhajda's recent effort of

understanding the concepts behind stress and chemical exposure. The extended effort will concentrate on the behavior of chemicals with similar BBB characteristics to PB. The model will increase the understanding of the mechanisms of chemical transport across the BBB under stressed and nonstressed conditions.

Understanding the mechanisms of BBB transport under various stress conditions will prove critical in determining any potential adverse effects of these chemicals. The transport mechanisms that dominate in the BBB will determine the possible chemical penetration across the BBB. This model will be a tool in developing procedures to prevent future adverse effects and aid in the physical performance of an individual (Suhajda, 2000:5).

Research Objectives

In order to expand on Suhajda's general model for the prediction of stress and chemical distributions in the human body as they relate to stress, there are four research questions that are pursued:

1. Characterize the mechanisms by which stress alters chemical uptake/distribution in the brain. This includes the transport mechanisms that dominate in BBB penetration. This will provide further detail to Suhajda's hypothesized mechanisms in the BBB.
2. Use the principles of system dynamics modeling to better understand the influences of stress on the uptake and distribution of chemicals with similar properties to PB.
3. Through use of a PBPK model, test the hypothesis that exercise and other stressors will affect chemical distribution in the human body, specifically the brain.
4. Establish a framework for ongoing investigation of chemical exposures as modified by deployment and stress (Suhajda, 2000:6).

II. Literature Review

The purpose of this literature review is to present relevant literature background on the subjects of stress and chemical exposure in relation to BBB transport. The first section contains a brief overview of cholinesterase and its inhibition. The second section discusses the literature and theories behind the Gulf War Syndrome. Third, the literature on the BBB and theories related to its transport mechanisms and various stressors will be presented. Next, literature on chemical exposure and distribution will be presented. Finally, a review of system dynamics and PBPK modeling in relation to the study of chemical exposure will be discussed.

The Nervous System and Cholinesterase

Since a primary emphasis of this research effort is on the BBB, a brief overview of the relevant properties of the nervous system will be discussed. The nervous system is comprised of the central and peripheral nervous systems. The brain and spinal cord are part of the central nervous system (CNS), while the rest of the body falls under the peripheral nervous system. The basic structural and functional units of the nervous system are neurons, which can be classified as motor or sensory neurons (Fox, 1999:150). Motor neurons send impulses from the CNS to effector organs such as glands and muscle, while sensory neurons send impulses from sensory receptors to the CNS (Fox, 1999:150). The motor neurons can be further subdivided into somatic neurons, which control skeletal muscle, and autonomic neurons, which control smooth muscle, cardiac muscle, and glands (Fox, 1999:150). The autonomic motor neurons are divided into the

sympathetic and parasympathetic systems, which also comprise the cholinergic system. While the sympathetic system is characterized by the 'fight or flight' response of preparing the body for intense physical activity, the parasympathetic system has the opposite reaction of relaxing the body (LaPuma, 2000).

The principal transmitter in the neural-neural connections, or synapses of the cholinergic system is acetylcholine (ACh), which is hydrolyzed by the acetylcholinesterase (AChE) enzyme. The cholinergic effects of ACh are excitatory or stimulating in nature. AChE is able to inactivate ACh by its almost immediate presence after ACh is released (Fox, 1999:170). ACh can then be reactivated and released upon further nerve stimulation. When AChE is blocked, it cannot participate in the hydrolysis of ACh, causing the neurotransmitter to accumulate and its action becomes enhanced (Lotti, 1995:1814). If this type of response remained uncontrolled, the stimulatory effects such as a muscle remaining contracted too long, may cause physiological damage. Hence, an inhibition of AChE would cause an overstimulation of nerve transmissions, due to a buildup of ACh, in the cholinergic system.

Cholinesterases such as AChE are often measured to assess exposures to or effects of organophosphates (OPs), which are irreversible inhibitors, and carbamates, which are reversible inhibitors (Lotti, 1995:1814). AChE is often measured because the actual measurement of OPs and carbamates in the tissue of interest, specifically the brain, is more difficult. Although AChE is involved in synaptic transmission, it is also present in red blood cells, or erythrocyte outer membranes, and to a lesser extent in plasma (Lotti, 1995:1814). However, because plasma AChE has no recognized functions, the inhibition of plasma cholinesterase indicates exposure, while the inhibition of erythrocyte or brain

AChE indicates toxicity (Lotti, 1995:1816). In addition, the correlation between AChE inhibition in erythrocytes and in the brain or nervous system depends on the pharmacokinetics of the chemical of exposure such as how effectively it crosses the BBB.

Gulf War Syndrome

The Gulf War Syndrome is associated with soldiers who served in the Southwest Asia theater of operations during the Persian Gulf War. This syndrome includes the unexplained complaints of symptoms that have not been localized to any one organ system, and the lack of consistent physical signs or abnormality in laboratory testing that indicates a single specific disease (Persian Gulf Veterans Coordinating Board, 1995:262).

The Persian Gulf War troops were exposed to several potentially harmful environmental hazards such as organophosphates, carbamates, pesticides, low-level nerve agents, synthetic chemical compounds, environmental pollutants, diseases endemic to the region, and condensed multiple vaccines (United States Congress, 1999:1840). Organophosphates such as diazinon and malathion are considered cholinesterase inhibitors, while carbamates such as physostigmine and pyridostigmine bromide (PB) are considered reversible cholinesterase inhibitors. The possible Gulf War exposures to organophosphates such as diazinon and malathion were in the form of pesticides, while exposure to PB was in the form of a pill to counteract nerve agent exposure.

Low-levels of nerve agents such as sarin and tabun may have been in released during the Gulf War as well as mustard agents, volatile organic compounds, and solvents. Organophosphate nerve agents are able to readily cross the BBB and irreversibly inhibit brain cholinesterase, producing behavior deficits and rapidy disturbing brain functions

(Blick and others, 1994:311). Diesel heater fumes and sand particles are examples of possible environmental pollutants, while sand fly fever and leishmaniasis are included in the possible diseases of exposure (United States Congress, 1999:1851). The condensed administration of multiple vaccines administered to the soldiers may be a factor in the GWS because they were given within a short time period, creating the possibility of enhanced side effects.

The behavior of a number of the chemicals the soldiers were exposed to may have been determined under experimental laboratory conditions. Some of these chemicals were tested individually under ideal conditions. However, the reactions to multiple chemical exposures under stressful wartime conditions may provoke different reactions than those seen in laboratory conditions. For example, the physical tasks performed by combat infantry soldiers may have resulted in significant physical and chemical changes in the body. The time course of a chemical in the body is influenced by exercise dynamics, which was certainly relevant in the Gulf War veterans who underwent heavy military duty and were exposed to PB (Somani and others, 2000:327). This combination of physical stress and PB exposure is hypothesized to enhance the delayed toxic effects of PB.

Pyridostigmine Bromide

The US Armed Forces used PB as a pretreatment of anticipated nerve agent injuries. PB has also been used in the treatment of patients with myasthenia gravis, a disease associated with muscle weakness, causing difficulty in eye, face, or limb movement, and respiratory impairments (Shen, 1998:235). In the case of myasthenia

gravis, PB is used to inhibit AChE so that nerve firing can increase to the areas of muscle weakness (Grubbs, 2000). The PB dosage for the soldiers was prescribed as one 30-mg tablet every eight hours, and the duration of tablet intake was depended on each unit commander (Keeler and others, 1991:694). This dosage should not substantially alter brain AChE activity, and corresponds to a 20% to 30% inhibition of peripheral cholinesterase activity (Servatius and others, 1998:1020). Although side effects related to gastrointestinal and urinary tract were common, PB was deemed safe because it does not readily cross the BBB or interfere with cognitive or psychomotor function (Keeler and others, 1991:694). However, it must be kept in mind that there may exist a population variability in the different tolerance levels for PB. People who do not carry the normal AChE gene may not be able to cope with PB even at a safe and low dosage, exposing them to potential damage from AChE inhibitor attacks (Shen, 1998:236).

Pyridostigmine Bromide Characteristics

Because PB is a carbamate, it is able to form reversible bonds with AChE, which detach over time and allow AChE activity to return to its normal function. PB can competitively inhibit nerve agent binding, preventing organophosphates such as sarin from irreversibly attaching to AChE. Its quaternary amine structure prevents significant penetration across the BBB, allowing unaltered CNS neurotransmission (Cook and Kolka, 1992:250). This insignificant access to the brain is through areas that lack a BBB (Petrali and others, 1991:337). PB has been estimated to have a low bioavailability, which may suggest that this very hydrophilic compound has a low degree of absorption. However, systemic hydrolysis in the blood and liver metabolism must also be considered (Aquilonius and Hartvig, 1986:239).

Urinary filtration is also an important elimination mechanism for PB. It is excreted in the urine virtually unchanged. After oral administration, the urinary excretion of the unchanged drug is reported to range from 5 to 15%, providing a measure of the degree of absorption in the body (Aquilonius and Hartvig, 1986:242). PB kinetics have been studied with and without renal function. The chemical's kinetics remained the same even after a renal transplantation (Aquilonius and Hartvig, 1986:244). However, abnormal renal function has a profound effect of PB excretion, and renal function declines with age. Deterioration of excretion capability has been found to occur approximately 1% per year after middle age (Stone and others, 1995:775). Reduced excretion capability would allow more chemical to be available to the body, increasing the chances of possible toxic effects. This observation points to the fact that soldiers with abnormal renal function may have had abnormally high PB concentrations in their system. Table 1 below shows PB characteristics for humans found during the literature review. The characteristics for animals may vary depending on the species under study. As with most chemical parameters, the values found had slight variations from the mean.

Table 1. Pyridostigmine Bromide Characteristics for Humans

Onset of action after oral administration[a]	30 – 45 min
Onset (time to initial detection in blood)[a]	30 min
Duration of action after oral administration[a]	3 – 6 hr
Bioavailability[a]	14%
Mean concentration at 50% red blood cell AChE activity inhibition[a]	31.8 ng/ml
Time at which 95% of drug is eliminated[a]	8 – 10 hr
Urinary excretion after oral administration[b]	5 – 15%
Liver/plasma partition coefficient[a]	8
Kidney/plasma partition coefficient[a]	15
Octanol/water partition coefficient[c]	0.002

a. Golomb (2000)
b. Aquilonius and Hartvig (1986)
c. McLauchlan (2000)

PB Related Research

Several studies have been performed to determine the effects of PB and the ability of animals and humans to exercise in the heat. Gallo and Lawryk reported that one measurement of erythrocyte AChE before exposure must detect a minimum of 15% inhibition to be statistically significant. A study involving an intravenous PB pretreatment in rats in association with exercise resulted in a 64% inhibition of circulating cholinesterase activity that corresponded to a reduced endurance capacity of exercise in heat (Francesconi and others, 1984:894). The results of this study demonstrated that hyperthermic exhaustion was reached in a shorter time when rats were exercised in the heat, following PB pretreatment vs. the rats not given PB (Francesconi and others, 1984:894). A later study administered PB to rats in drinking water to simulate the oral dosages taken by soldiers. However, this study did not show a link between prolonged PB consumption and heat endurance (Francesconi and others, 1986:1073). An average of 30% cholinesterase inhibition was achieved in the rats'

circulating cholinesterase (Francesconi and others, 1986:1074). These studies indicate that the physiological and protective effects of carbamates such as PB may depend on a narrow range of cholinesterase inhibition.

Blood-Brain Barrier

Ehrlich performed the first studies that led to the concept of the BBB in 1885, during which he showed that many intravenously injected dyes stained the body tissues except for the brain (Abbot, 1992:371). Goldman's later experiments in 1909 and 1913 demonstrated the distinctive existence of the BBB by showing that the brain remained unstained by an intravenous trypan blue dye injection, and the dye was not found in the cerebrospinal fluid (Davson, 1989:27).

BBB Function and Properties

The BBB acts as a protective mechanism for the brain and maintains the homeostasis required for normal functions. The barrier separates the two major compartments of the CNS, the brain and cerebrospinal fluid (CSF), from the blood. This regulatory interface both controls and sometimes hinders metabolism between the blood and the brain, while unwanted metabolic products and other substances that diffuse into the brain from the blood are excreted from the CSF by the barrier (Rapoport, 1976:43). The BBB can be characterized as somewhat of an impermeable wall. It is only somewhat impermeable because it must facilitate the exchange of selected solutes; for example, oxygen and carbon dioxide waste products encounter little difficulty crossing the BBB (Betz, 1992:56). Because some of the neural tissue is in direct contact with extracellular

fluid, access to proteins, including viruses that are normally excluded from the BBB, may be provided (Rapoport, 1976:77).

Several aspects of the BBB are relevant in relation to its permeability. The BBB is characterized by its microvascular endothelial wall, which has epithelial-like, high-resistance tight junctions that eliminate pores in the walls of brain capillaries (Pardridge, 1998:1781). These tight junctions exist to prevent free diffusion between the brain capillaries, almost certainly representing the most important feature of the BBB. The brain capillaries have the following special features: high-resistance, epithelial-like tight junctions, astrocyte foot processes that encompass more than 99% of the endothelium brain surface, and minimal endothelial pinocytosis (Pardridge, 1998:1781). The cerebral endothelial cells also have high membrane resistance, indicating low ion permeability (Joo, 1996:262). Transport through the BBB results in the movement through two membranes in series; the luminal, or blood membrane, and the abluminal, or brain tissue membrane of the endothelium (Pardridge, 1997:714). Several studies have been performed to determine the degree of movement across the BBB. Robinson (1987) performed a study involving the osmotic opening of the BBB, which resulted in an average bulk water flow from the blood to brain of 1.6×10^{-4} cm^3/s per g of brain during the first ten minutes of opening, which possibly resulted from the osmotically induced shrinkage of endothelial cells and consequent widening of tight junctions.

Astrocyte foot processes adhere to the common basement membrane that surrounds the cerebral vessels (Yamagata and others, 1997:710). The astrocyte foot processes are thought to be important in the nourishment of the brain, and are believed to possibly reduce the permeability of the BBB to circulating molecules (Joo, 1996:266).

Studies have been performed that demonstrate that factors produced by astrocytes play an important role in making endothelial tight junctions. Yamagata and others (1997) performed a study involving astrocyte-conditioned medium that resulted in the production of intercellular tight junctions and the reduction of vesicular transport that blocked the permeation of soluble substances in the blood through endothelial cells. Schroeter and others (1999) also tested the theory of the astrocytes' role in BBB permeability, and found that the astrocytes protected the structure and functions of the endothelial cells against pathological situations with oxidative stress, such as inflammation. However, the astrocyte foot processes do not constitute a significant permeability barrier to solute diffusion once the solute has crossed the endothelial tight junctions (Pardridge, 1997:714).

The minimal pinocytosis feature prevents free solute movement into the brain. However, recent evidence has pointed to the possibility of a transcytotic pathway that allows the passage of some substances through the endothelial cell layer, involving the movement of endothelial vesicles within the cell and a series of fusions of the vesicular and cellular membranes (Stewart, 1998:149). Because BBB capillaries are capable of transcytosing specific proteins such as insulin, they may play a role in endothelial permeability of other substances by fusing to form continuous channels that cross the capillary wall (Stewart, 1998:150). This potential transcytosis of blood-borne molecules through the endothelial cells indicates that the BBB can be circumvented (Broadwell, 1993:137).

Molecules able to penetrate the BBB have the properties of size under 400 to 600 Daltons, and lipid solubility (Pardridge, 1998:1781). Mayhan and Heistad (1985)

performed a study to determine the permeability of a disrupted BBB to various sized molecules, and concluded that the primary mechanism of transport may be the separation of endothelial tight junctions, which in effect form functional pores in the membrane. Their study included the disruption of the BBB by acute hypertension and a hyperosmolar solution (Mayhan and Heistad, 1985:H712). The lipid solubility requirement of BBB passage would prevent chemicals such as PB, which is highly hydrophilic, from readily crossing the BBB under normal conditions. BBB permeability is also expected to be approximately proportional to the chemical's octanol/water partition coefficient (Robinson and Rapoport, 1992:279). This again points to a low permeability of PB and similar chemicals across the BBB.

Stress and BBB Permeability

Stress has the ability to impact every part of the body, and its effects may cause differing impacts to individuals. For example, a physical stress such as swimming, may impact individuals of varying fitness levels differently. Stress is known to affect the cardiovascular, endocrine, immune, and central nervous systems (Gherman, 1981:150). For example, physical stress in the form of exercise affects the cardiovascular system by increasing the blood flow rate in the body. Although the body may be able to readily react to various stressors over a short period of time, sustained stress may have numerous pathological effects. Molecules that may cause adverse effects upon sustained secretion include human glucocorticoid (GC) hydrocortisone, which is essential for surviving acute physical stress (Sapolsky, 1996:749). Sapolsky (1996) noted that excessive exposure to GCs has adverse effects in the rodent brain, and GC overexposure endangers brain

neurons, decreasing their ability to survive seizures. This phenomenon provides an example of why stress is unhealthy for the brain.

The concept of stress in direct relation to the BBB permeability has been studied in various animal models, including acute immobilization stress, cold or isolation exposure, and prolonged heat exposure. While some of these studies used dye penetration to measure BBB permeability, others related AChE activity to BBB penetration. Sapolsky (1998) noted that synaptic concentrations of ACh could be increased by both AChE inhibitors and by stress, thus increasing neuronal excitability. Many of these studies resulted in the increased BBB penetration of chemicals, neurotransmitters, and viruses that are normally excluded (Hanin, 1996:1308). However, the results of some recent studies, which are discussed below, contradict these findings.

Belova and Johnson (1982) performed a study using trypan blue dye, involving BBB permeability and immobilization stress in the rat brain. Upon comparison between normal and stressed conditions, dramatic changes of BBB permeability in certain parts of the brain were observed following stress (Belova and Johnson, 1982:26). Belova and Johnson (1982) further noted that the signs of BBB permeability were most evident after short time periods, which may denote a more pronounced BBB and reversible permeability than what their analysis could demonstrate. Sharma and others (1991) performed a study using Evans blue albumin (EBA) staining to indicate BBB permeability in rats under forced swims. Forced swimming, a severely stressful condition, has been well documented in the literature to have the effect of cerebral circulation alteration, vertebral artery injury, cerebellar stroke, and abnormal neurological function (Sharma and others, 1991:212). Sharma and others (1991) found that forced

swimming increased BBB to EBA in specific regions of the brain associated with the swimming mechanism, and was time dependent and reversible in nature. Their observations may indicate a receptor-mediated increase in BBB permeability caused by serotonin, an active neurochemical mediator that is involved in various stressful situations and neurological disease (Sharma and others, 1991:219).

Friedman and others (1996) studied pyridostigmine brain penetration in mice under a forced swim, which resulted in increased BBB permeability, reducing the intravenous pyridostigmine dose required to inhibit brain AChE activity by 50%. This study points to the fact that compounds that are normally excluded from brain entry, such as PB, might become centrally active under stressed conditions. This study also demonstrates a significant correlation between stress and BBB penetration of PB, which may be relevant in soldiers experiencing neurological effects of the Gulf War Syndrome. Friedman and others' (1996) study was used as a basis of comparison for future studies involving increased BBB permeability under chemical exposures.

Sinton and others (2000) studied pyridostigmine brain penetration in rats under a restraint, forced swim, and combination of restraint/forced swim, which resulted in a slight reduction of BBB permeability. The BBB permeability was measured by AChE inhibition and the stressors' effect was independent of the protocol used. This study, which was performed to relicate the previous work of Friedman and others (1996), indicated that the data reported in the previous study might not extend to other species (Sinton and others, 2000:100). Lallement and others (1998) studied pyridostigmine brain penetration in guinea pigs under a high ambient temperature, in order to simulate the physiological stress encountered by soldiers wearing protective gear during the Gulf War.

The intravenous PB administration did not cause an inhibition of central AChE activity, which may have resulted from the varying behaviors of different animal species and stressors in previous experiments that had contradictory results (Lallement and others, 1998:759). Grauer and others (2000) studied pyridostigmine brain penetration in mice under both a forced swim and cold stress, which resulted in unchanged brain ChE activity after an intramuscular or intraperitoneal pyridostigmine administration.

These conflicting results in the literature demonstrate the difficulty in the interpretation and comparison of experimental findings. Comparisons across different species and ages may affect results. In addition, the various stressors and their intensities may also affect experimental results. Lallement and others (1998) observed that before drawing any conclusions about the possible passage of pyridostigmine into the brain, the nature and/or intensity of the stress experienced by the Gulf War veterans, needs to be well defined.

Chemical Exposure and Distribution

An understanding of the chemical exposure processes and distribution in the human body is essential in this thesis effort. Environmental toxicology includes the study of the hazardous effects that foreign substances have on human health (Suhajda, 2000). One method of studying toxicology is by physiologically-based pharmacokinetic (PBPK) modeling. PBPK modeling is an example of toxicokinetics, which is concerned with the exposure, absorption, distribution, storage, biotransformation, and elimination of toxicants in an organism (Hughes, 1996:21). An understanding of both the time-dependent processes and specific organism responses is imperative. The time-dependent

processes relate to the final effect that a toxicant has on an organism's system, as well as the possible behavior and interactions leading up to the end effects. The specific response relates to sub-lethal to lethal, acute to chronic, or immediate to delayed effects (Suhajda, 2000).

Absorption is the process during which a toxic substance crosses into the extracellular spaces of an organism, and has three primary routes of: skin, respiratory, and digestive system absorption. Distribution may occur through the lymph or blood circulatory systems. Distribution of a toxicant to body tissues includes the combination of exposure duration, dose, and toxicant characteristics. The concentration gradient, volume of blood flow, volume of target tissue, cardiac output ratios to each tissue, and the toxicant's affinity for a particular tissue also prove to be important (Hughes, 1996:49-64). Once distributed through the body, storage, biotransformation, and elimination occur. Storage, which corresponds to a buildup of toxic substance in a specific tissue, occurs mostly in the bone, kidneys, liver, and fat (Hughes, 1996:67). Biotransformation involves the change of a toxic substance to an alternate form that may facilitate easier elimination from the body, and may also cause a more or less toxic substance to be formed. Elimination occurs through several routes to include: urine, feces, sweat, saliva, exhaled air, and milk (Suhajda 2000).

Modeling

PBPK modeling provides an accurate tool for tissue exposure assessments as it uses the physiological mechanisms of the human body to visualize, validate, and predict the susceptibility of the human body to chemical exposures (Brown, 1994:Ch.2,10). This

tool allows the modeler to observe and predict chemical concentrations in specific tissue compartments, and therefore estimate possible health hazards. These hazards in combination with stress would provide a basis of studies involving chemical uptake during stressed conditions, as in the Gulf War Syndrome. PBPK models can also reduce the cost and time that is normally devoted to animal studies. In addition, if the human model parameters are well defined, more accurate predictions of chemical exposures can be accomplished, eliminating the often inaccurate extrapolation of animal to human exposures.

The behavior of a substance within various tissue groups in PBPK models, which are divided into separate compartments on the basis of their physical and biochemical parameters, are represented by mass-balance differential equations (Brown, 1994:Ch2.,11). The Ramsey and Andersen model (1984) provided the foundation of modeling in the extrapolation of animal data to human chemical hazards. Their model, which simulated the behavior of inhaled styrene in rats and humans, contained the following tissue groups: highly perfused tissue, moderately perfused tissue, slowly perfused tissue, and tissues that metabolized a large amount of styrene, i.e. liver. The rat model, which accurately compared to actual rat exposure data, became the foundation of PBPK research (Brown, 1994:CH.2,13). Figure 1 below represents an example of a generic PBPK model (Suhajda, 2000). While the alveolar space and lung blood

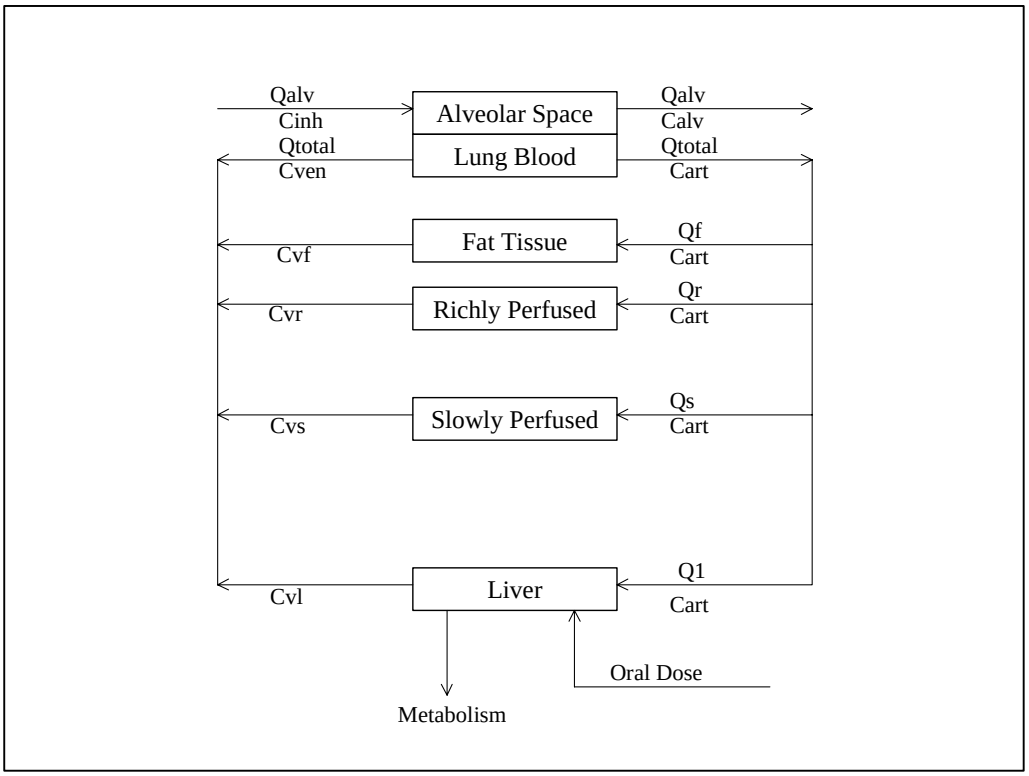


Figure 1. Generic Physiologically Based Pharmacokinetic Model

compartments represent initial chemical uptake through inhalation, the liver represents the first-pass effect of chemical uptake by oral dosage. The fat tissue compartment would show the chemical's affinity for fat, while the slowly perfused compartment represents muscle and lean tissues. The richly perfused compartment represents organs such as the kidney, heart, and brain, where blood flow is abundant. The liver tissue represents metabolic activity undergone by the chemical. These compartments are linked by arterial and venous blood flow (Q variables) and venous and arterial chemical concentrations (Cv and Cart variables) into each compartment. This generic model can be altered to include other tissue compartments to address specific chemical exposures, and to observe the behaviors of the chemical in these different areas. With the use of

numerical integration of a system of differential mass balance equations, rates of input and output, and metabolic activity through each compartment in the model can be simulated (Brown, 1994:Ch.2,11).

The addition of two brain compartments to the model developed by Ramsey and Andersen was performed to represent the BBB transport mechanisms to be used in this research effort. The BBB is hypothesized to play a significant role in the effects of stress on chemical exposure. Figure 2 represents the PBPK model that will be used in this study. The brain was divided into two sub-compartments (brain blood and brain tissue) to show a more accurate representation of the transport that occurs in the human brain. This model addresses the interactions of stress and chemical exposure, and can now explore the system behavior, helping to predict possible causes of the Gulf War Syndrome. The developed PBPK model will help determine important variables and their changes in relation to the model behavior.

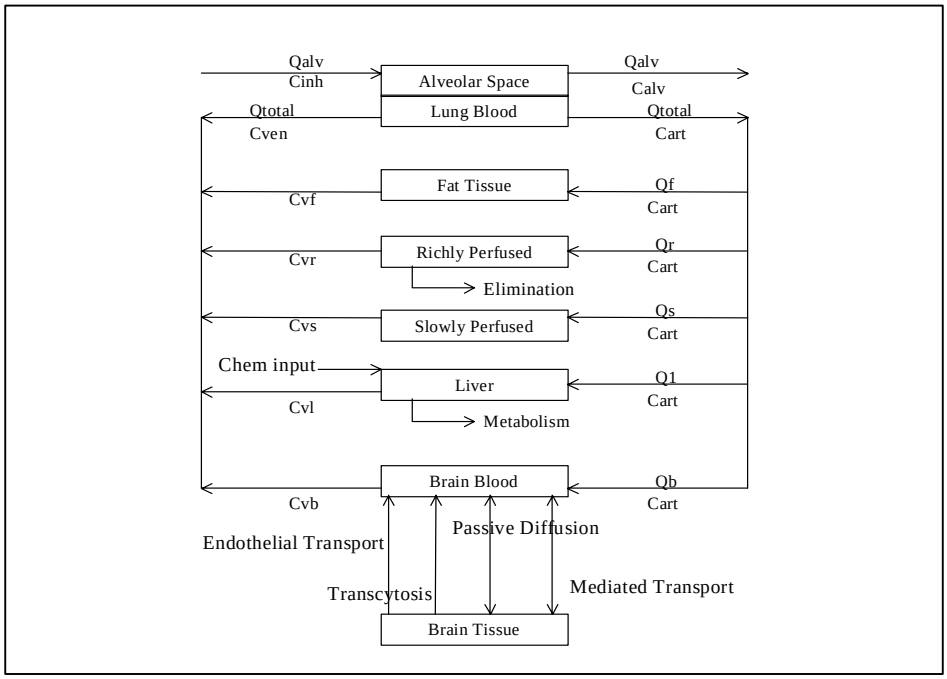


Figure 2. PBPK Model

Model Parameters

Parameters such as partition coefficients, metabolic values, and other physiological values are necessary to develop a PBPK model. Physiological values such as ventilation and cardiac output can be used with different chemical compounds. Chemical-specific parameters in this model will be tested over a range of different values, as discussed in Chapter III. This was done due to the lack of data on specific chemical characteristics of PB and other similar chemicals.

Difficulties encountered when attempting to define stress in this model stemmed from the lack of specific literature data concerning the specific causes and effects of a variety of stressors. The majority of literature containing stressors failed to differentiate between the intensities of different stresses and also failed to indicate the mechanisms,

specifically BBB transport, associated with that stress. A classification system that has been used to identify various stressors is shown in Table 2 below.

Table 2. Classification of Stressors (Elliot and Eisdorfer, 1982)

1. Acute, time-limited stressor	e.g. brief exposure to a natural predator
2. Stressor sequence	e.g. changes set in motion by a single precipitating event, such as a loss of a family member or job
3. Chronic, intermittent stressor	e.g. recurring stressor such as preparing monthly reports
4. Chronic stressor	e.g. a permanent or persistent physical or emotional disability

Physical exercise, which is a well-known stress effect in the human body, will be one kind of stressor characterized in this model. This type of physical stress results in an increase in ventilation and cardiac output, and change in the distribution of cardiac output. The flow to skeletal muscle or the slowly perfused tissue compartment increases due to redistribution of blood away from the fat and liver compartments, while the richly perfused tissue compartments (including the brain) will maintain the same amount of blood flow. This redistribution allows the body to maintain homeostasis for vital organs such as the brain and kidneys (Suhajda, 2000). Table 3 lists the physiological and biochemical parameters found in the literature for both rest and physical activity for a generic chemical. Since some of the chemical-specific parameters for PB and similar chemicals were unavailable in the literature, they will be tested over a range of values, to be discussed in Chapter III. The dashed boxes indicate the values that will be tested.

Table 3. Physiological and Biochemical Parameters for a Human Simulation

	PARAMETER	AT REST	HEAVY PHYSICAL ACTIVITY
Body Weight (kg)[a]	BW	70	70
Alveolar Ventilation (l/hr)[a]	QP	300	2100
Cardiac Output (l/hr)[a]	QC	312	594
Blood Flow rates (l/hr)			
Fat	QF	15.6[b]	9.36[c]
Slowly Perfused	QS	78[b]	124.8[c]
Richly Perfused	QR	137.28[b]	137.28[c]
Liver	QL	81.12[b]	40.56[c]
Fractional Distribution of Blood Flow (%)			
Fat	FF	0.05[b]	0.03[c]
Slowly Perfused	FS	0.25[b]	0.4[c]
Richly Perfused	FR	0.44[b]	0.44[c]
Liver	FL	0.26[b]	0.13[c]
Tissue Group Volume (l)[b]			
Fat	VF	13.3	13.3
Slowly Perfused	VS	43.4	43.4
Richly Perfused	VR	3.5	3.5
Liver	VL	1.82	1.82
Partition Coefficients			
Liver/blood [(mg chem in liv/L liv)/(mg chem bld/L bld)	PL	-	-
Fat/blood [(mg chem in fat/L fat)/(mg chem bld/L bld)	PF	-	-
Slowly Perfused/blood [(mg chem slow/Lslow)/(mg chem bld/Lbld)	PS	-	-
Richly Perfused/blood [(mg chem rich/L rich)/(mg chem bld/L bld)	PR	-	-
Blood/air [(mg chem bld/L bld)/(mg chem air/L air)	PBloodAir		
Metabolic Parameters			
Michaelis-Menten Constant (mg/l)	LKm	-	-
Max. Velocity of Metabolism (mg/hr)	LVmax	-	-
Kidney elimat. fract.	elim fract	-	-

a. Physiological Parameter Values for PBPK Models (1994)
b. Allen and Fisher (1993)
c. Rowell (1986)

These unknown values will be defined either numerically or graphically. Table 4 shows the physiological and biochemical parameters found in the literature for both rest and physical activity in the brain.

Table 4. Brain Physiological and Biochemical Parameters

	PARAMETER	AT REST	HEAVY PHYSICAL ACTIVITY
Blood Flow rates (l/hr)			
Brain	QB	37.44	37.44
Fractional Distribution of Blood Flow (%)			
Brain	FB	0.12	0.12
Tissue Group Volume (l)			
Brain Blood Compartment[a]	VBB	3.5	-
Brain Tissue	VBT	1.4	1.4
Partition Coefficients			
Brain Tissue/Blood	PB	-	-
Transport Parameters			
Endothelial Transport (mg/hr)			
Baseline blood flow fraction	QB junct fract	-	-
Transcytosis (mg/hr)			
Baseline blood flow fraction	QB trans fract	-	-
Passive Diffusion (mg/hr)			
Transfer Rate	TR	-	-
Mediated Transport (mg/hr)			
Max Transport (blood to brain)	Max Transport	-	-
Max Transport2 (brain to blood)	Max Transport2	-	-
Transport Constant (blood to brain)	Transport Constant	-	-
Transport Constant2 (brain to blood)	Transport Constant2	-	-

- Physiological Parameter Values for PBPK Models (1994)
a. Suhajda (2000)

Suhajda (2000) noted that the volume of the brain blood sub-compartment needed to be increased to an unrealistically large volume in order to effectively study the behaviors

that would be produced. However, the resulting inaccurate accumulation of chemical in the blood of this sub-compartment, which is still relatively small in comparison to other tissues, is not considered significant in characterizing the overall system behavior. In addition to physical exercise being simulated in the model, a form of stress that directly influences the brain will also be added. Once again, there was a lack of specific literature data concerning the specific causes and effects of brain stressors. Specific stressors that would fall under this category include but are not limited to heat, cold, immobilization, and forced swimming stressors. These types of stressors are prevalent in BBB permeability studies, but have not been well quantified. The majority of literature containing stressors also failed to differentiate between the intensities of different stresses and also failed to indicate the BBB transport mechanisms relevant to its permeability. Graphical relationships of stress and the various BBB transport mechanisms will be formed to provide an algorithmic tool to adjust the strengths of their influences, as will be discussed in Chapter III. The roles of these transport mechanisms in chemical exposure may be simulated and tested through the use of the PBPK model developed in this study.

Scaling

The development of scaling relationships can provide insight during the testing of a PBPK model. Suhajda's (2000) previous effort incorporated the use of mathematical equations to allow modelers to scale the model parameters to a specific body weight. The recommended reference human body weights of 70 kg for males and 58 kg for females can be used initially in a model, but scaling allows the model to be simulated over a variety of ranges. Although the testing of different body weights will not be performed in

this research effort, the scaling equations to test these changes will remain in the model for purposes of future research. The scaling factors for tissue group volumes, ventilation, cardiac output, and liver metabolic parameters that can be tested are listed below in Table 5 (Allen and Fisher, 1993:72). The brain tissue is the only scaled parameter in the brain compartment. The impacts of exercise on alveolar ventilation and cardiac output will be discussed below in the "exercise" sub-heading. The impacts of stress on the BBB transport mechanisms will be discussed in Chapter III.

Table 5. Scaling Parameters

	SCALING PARAMETER	VALUE	EQUATION
Alveolar Ventilation (l/hr)[a]	QP_c	*See Table 6	QP_c*BW^.74
Cardiac Output (l/hr)[a]	QC_c	*See Table 6	QC_c*BW^.74
Tissue Group Volume (l)			
Fat[a]	VF_c	0.19	VF_c*BW
Slowly Perfused[a]	VS_c	0.62	VS_c*BW
Richly Perfused[a]	VR_c	0.05	(VR_c*BW)-VBT
Liver[a]	VL_c	0.026	VL_c*BW
Brain[a]	VBT_c	0.02	VBT_c*BW
Liver Metabolic Parameters[a]			
Max. Velocity of Metabolism (mg/hr)	$LVmax_c$	14.9	$Lvmax_c$*BW^.7

a. Allen and Fisher (1993)

Exercise

This PBPK model will test various intensities of exercise. These different intensities will be considered due to their possible effects on chemical exposure. The International Life Sciences Institute compiled a report that included ventilation and

cardiac output based on their linear relationship with exercise (Physiological Parameter Values for PBPK Models, 1994:44,79). These exercise values, based on a 100% scale, were used to define the scaling parameters needed for cardiac output and ventilation. The 100% value corresponds to the maximum cardiac output in the model. Suhajda (2000) used the scaling equation that was listed for both ventilation and cardiac output, to calculate varying scaling factors (QCc and QPc) based on the standard male bodyweight of 70 kg (Fisher and Allen, 1993:75). If the individual's weight and exercise level is known, the cardiac output and ventilation rate can be calculated using the scaling factors listed below in Table 6. This value and the determined individual bodyweight would then be input into the scaling equations listed previously in Table 5.

Table 6. Exercise Parameters

Level of Exercise[a]	**Percentage-based value (0-100% scale)**	**QCc**	**QPc**
Rest	0	15[b]	12.9[b]
Moderate Physical Activity	15	25.61	64.68
Heavy Physical Activity	25	38.81	90.56
Strenuous Physical Activity	55	54.33	168.18
Maximal Physical Activity	100	77.62	232.86

a. Physiological Parameter Values for PBPK Models (1994)
b. Allen and Fisher (1993)

Analysis and Summary

The troops involved in the Gulf War underwent a variety of exposures. Research still continues to determine the possible causes of the GWS. Although there are conflicting theories in the literature, the continued efforts of researchers to solve this mystery behind the GWS indicates the seriousness of the problem. Much of the research involving the Gulf War Syndrome includes the stress of the soldiers' environment in combination with a variety of exposures. This stress has been hypothesized to have an effect on BBB permeability. Although several theories were addressed in this chapter, the following main assumptions will be the focus of this research effort as the PBPK model is further developed.

1. Exposure to stress and chemical exposure has the undesirable effect of increased neurotoxicity (Sapolsky, 1998).
2. Stress increases permeability in the BBB (Friedman and others, 1996, Belova and Jonsson, 1982, and Sharma and others, 1991). The increased permeability may be due to changes in four types of transport across the blood-brain barrier (passive diffusion, mediated transport, transcytosis, and endothelial transport).
3. Exercise causes changes in cardiac output, alveolar ventilation, and fractional distributions, while brain stress causes changes in the BBB transport mechanisms.

III. Methodology

This section defines the concepts and elements of the system dynamics approach that will be used to develop the PBPK model in this thesis effort. First, the proper modeling approach will be explored. Then the four developmental stages of system dynamics models will be discussed. These stages include conceptualization, formulation, testing, and implementation. A discussion of these four stages in direct relation to this research also will be included as the concepts are defined. A detailed discussion of the model scenarios simulated also will be provided.

Model Approach

Each modeling approach is dependent upon its unique underlying assumptions, which determines how the model should be approached (Meadows, 1980:23). In this thesis a kinetic model will be developed to assist in developing a theory of physiological changes during chemical exposure, exercise, and other stressful conditions. Although the major tissue groups of the body will be represented, the primary focus of this model is on the brain tissue compartment. The questions that will be addressed include the following: How does the addition of stress and exercise increase the chemical exposure to tissue groups in the body? Which BBB mechanism is likely to be most important in terms of permeability? Do exercise and stress play a role in the effects experienced after chemical exposure?

System dynamic models represent one of many approaches used to explore various system behaviors. The system dynamics paradigm consists of a dynamic

perspective, an endogenous viewpoint, a closed loop perspective, and mechanistic thinking, which will be discussed in further detail in this chapter (Shelley, 2000). This mechanistic mode of thinking is especially suitable for the human physiological system modeled here.

The dynamic perspective is particularly appropriate for providing insight into the changing behaviors of the human body. The tissue compartments that clearly have real world counterparts will be interconnected, which will help to display the causal structures within the system. Although these tissue compartments are vastly complex in reality, the model will simplify these structures in order to focus on the general understanding of the system. This general-understanding model will be more process oriented than product oriented, in that as it is developed. Questions will be asked systematically which will improve the insight gained (Meadows, 1980:28).

Conceptualization

Conceptualization is the first developmental stage in system dynamics modeling. This stage consists of striving for a mental model to gain familiarity with the general problem area (Shelley, 2000). This general-understanding stage encompasses all of the problem's causes and consequences over the long-term (Meadows, 1980:28). The questions to be addressed in this thesis effort were presented in the research objectives in Chapter I. The reference mode and influence diagrams are also included in the conceptualization stage. The information found during the literature review for this thesis indicated possible mechanisms of stress in association with chemical exposure.

Reference Mode

The reference mode defines the time development of the model over a specific range of interest, which suggests the basic causal loop structure of the model (Shelley, 2000). The behavior of interest is the time development of the various tissue concentrations. The five main tissue groups in this system are the fat, slowly perfused tissue, richly perfused tissue, liver, and the brain. These compartments are associated with the following parameters: chemical intake, cardiac output, arterial concentrations, venous concentrations, partitioning coefficients, metabolism, elimination, transport, and the fractional blood distributions to each tissue group.

The basic causal loop structure or flow diagram should give rise to the behavior shown in the reference mode.. There are two reference modes of interest in this thesis effort. One includes the fat, slowly perfused tissue, richly perfused tissue, and the liver. The other reference mode is specific to the brain compartment. These reference modes were distinguished because although exercise influences each of the main tissue compartments, stressors beyond exercise will only affect the brain compartment in this model. The stress mechanisms directly influencing the BBB transport mechanisms will be addressed in the influence diagram discussion to follow. Under rest and stress-free conditions, the chemical concentrations in all of the tissues should increase and then level off to a steady state, for a continual dose. As shown in Figure 3 below, exercise and stressed conditions will theoretically cause a more drastic increase in the rate of chemical buildup, but eventually reach a steady state chemical concentration values in the following tissue groups: fat, slowly perfused tissue, richly perfused tissue, and liver. This more drastic increase in chemical concentration should be seen because of the increased

delivery of chemical to the tissue due to increased blood flow during exercise. However, once the tissue steady state chemical concentration is reached, the graph should level off.

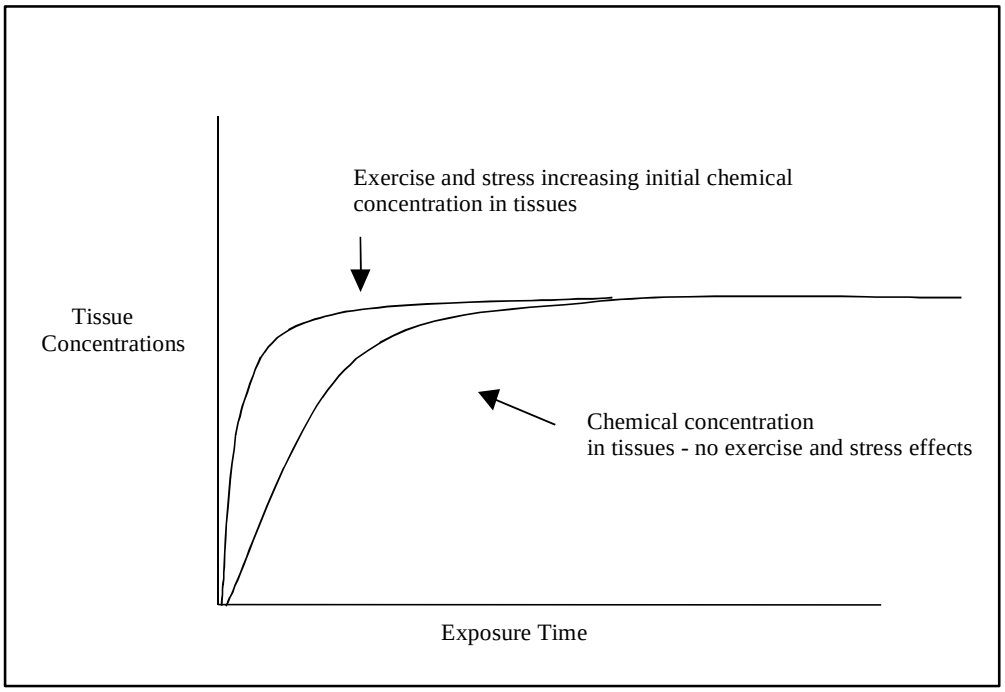


Figure 3. Reference Mode for Fat, Slowly Perfused, Richly Perfused, and Liver Tissue

The reference mode for the brain compartment is shown below in Figure 4. Under rest and stress-free conditions, the chemical concentration in the brain tissues should increase and then level off to a steady state. Exercise and stressed conditions will hypothetically cause a more drastic increase in the rate and eventually higher steady state of chemical concentration in the brain tissue. This increase in chemical concentration should be seen because of the increased delivery of chemical to the brain due to increased blood flow

during exercise. Once the tissue steady state chemical concentration is reached, the graph should level off. The steady state concentration is hypothesized to reach a higher value due to the direct influence of stress on the BBB transport mechanisms. The information found during the literature review for this thesis indicated possible mechanisms of stress in association with chemical exposure. Along with the possible mechanisms of stress in association with chemical exposure found in the literature, there were conflicting theories concerning whether the chemical concentrations in the brain in fact increased or decreased under stress. The reference mode shown in Figure 4 represents an increased chemical concentration in the brain after steady state has been reached, under exercise and stress. This figure simply represents an increase in brain concentration of the chemical, although the exact amount of the expected increase unknown

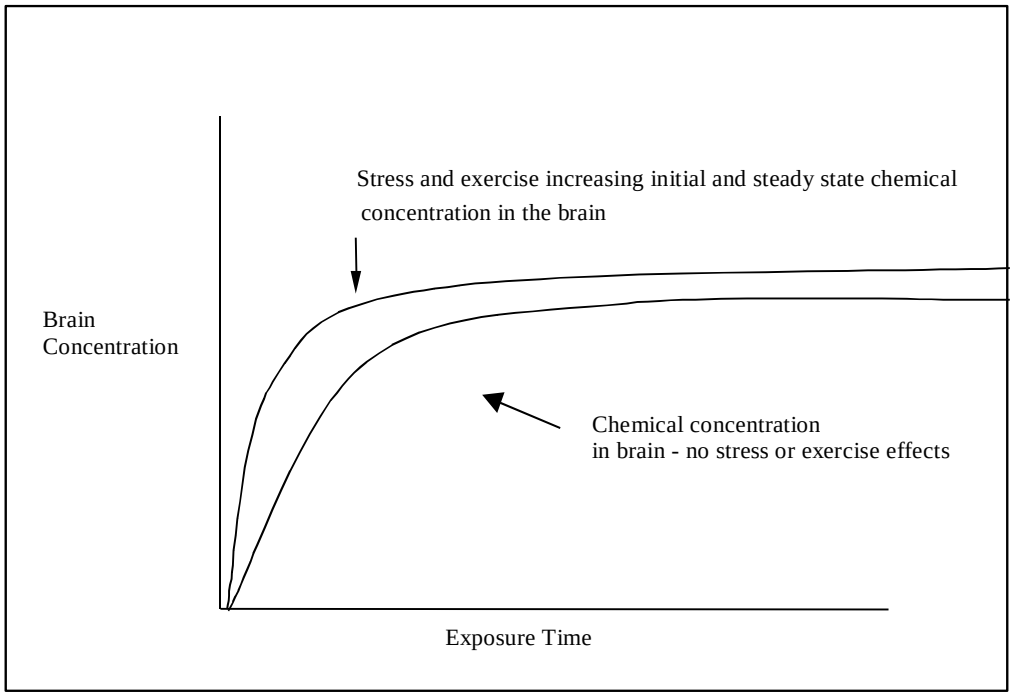


Figure 4. Reference Mode for Brain Tissue

Influence Diagram

The influence diagram demonstrates the most direct expression related to system behavior. It clearly denotes the various cause and effect relationships to be modeled. The loops within the influence diagram should give rise to the behavior represented in the reference mode. The closed feedback loops in the diagram are the key to establishing the system boundary (Shelley, 2000). This boundary indicates the system characteristics of concern. Without this closure, endless causal chains would prevent the determination of the cause and effect influences inherent in the system.

Figure 5 (Suhajda, 2000:44) is a simple representation of the cause and effect relationships between arterial and venous concentrations and the chemical concentration in a tissue.

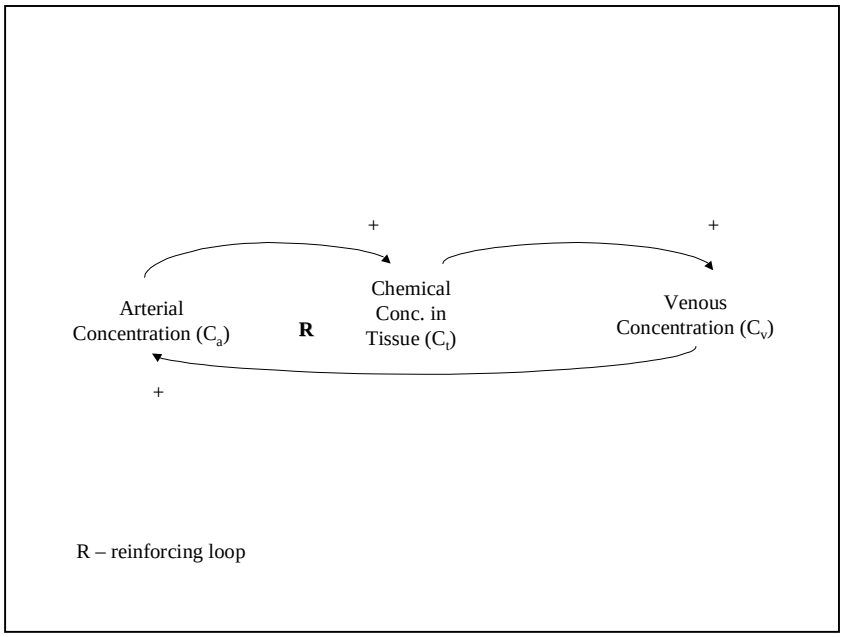


Figure 5. Simple Influence Diagram

The arrows represent the cause and effect relationships of the model. A positive symbol represents a reinforcing (R) or unstable behavior while a negative symbol represents compensating (C) or stable behavior. The behavior of multiple positive and negative loops depends on which loop is dominating, and the time specific system state (Shelley, 2000). The reinforcing loop, as seen in Figure 5, gives rise to an unstable behavior of continuously increasing chemical tissue concentration. In order to stabilize this behavior, a compensating loop would need to be inserted to cause the tissue

concentration to level to a steady state. Because the representation of the cause and effect loops in each of the tissue compartments would create an extremely complex influence diagram, only the fat and slowly perfused tissue compartments are shown in Figure 6 to illustrate the concept (Suhajda, 2000:56). The influence diagram shown in Figure 6 clearly demonstrates added complexity as compared to the simple diagram shown in the previous simple influence diagram. Figure 6 includes the compensating loops that stabilize the reinforcing behavior that would otherwise cause the chemical tissue concentrations to continually increase in Figure 5. Under a continuous chemical exposure, the arterial inflow would equal the venous outflow of the chemical, once steady state is reached.

The outside influence of exercise was also added to Figure 6. The addition of exercise to the influence diagram should affect the initial uptake and final steady state concentrations of chemical in the tissues. Exercise influences the cardiac output, ventilation rate, and fractional blood flow distributions. These conditions lead to an increased blood flow to all tissues, which allows more chemical to be delivered to the different tissue compartments. It should be kept in mind that although exercise influences the ventilation rate, the chemical exposure modeled in this thesis was through ingestion versus inhalation exposure.

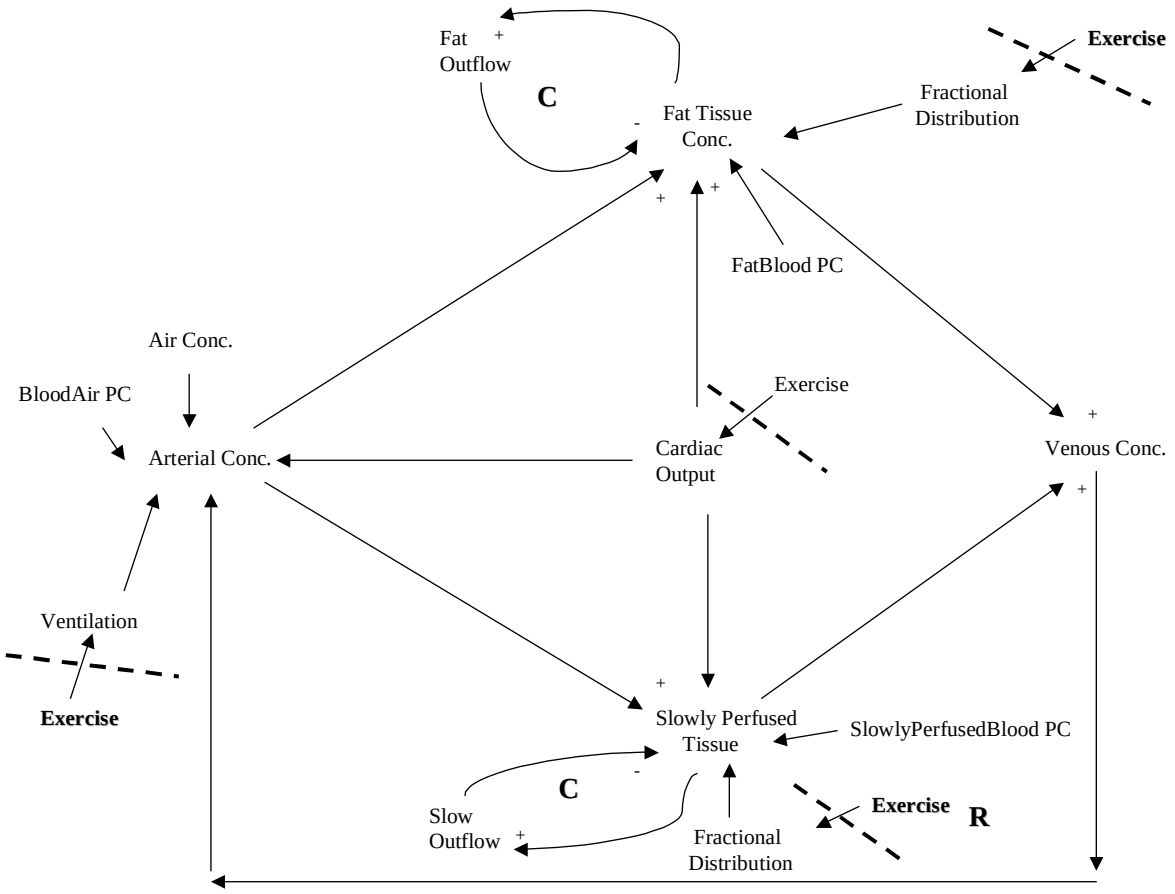


Figure 6. Influence Diagram of Fat and Slowly Perfused Tissues

The influence diagram of the brain compartment is shown in Figure 7 below. The brain was divided into two compartments: the brain blood and the brain tissue. The brain compartment will provide a mechanistic view of the processes taking place that composes the BBB. To realistically represent the transport processes that occur, the brain was divided into two sub-compartments.

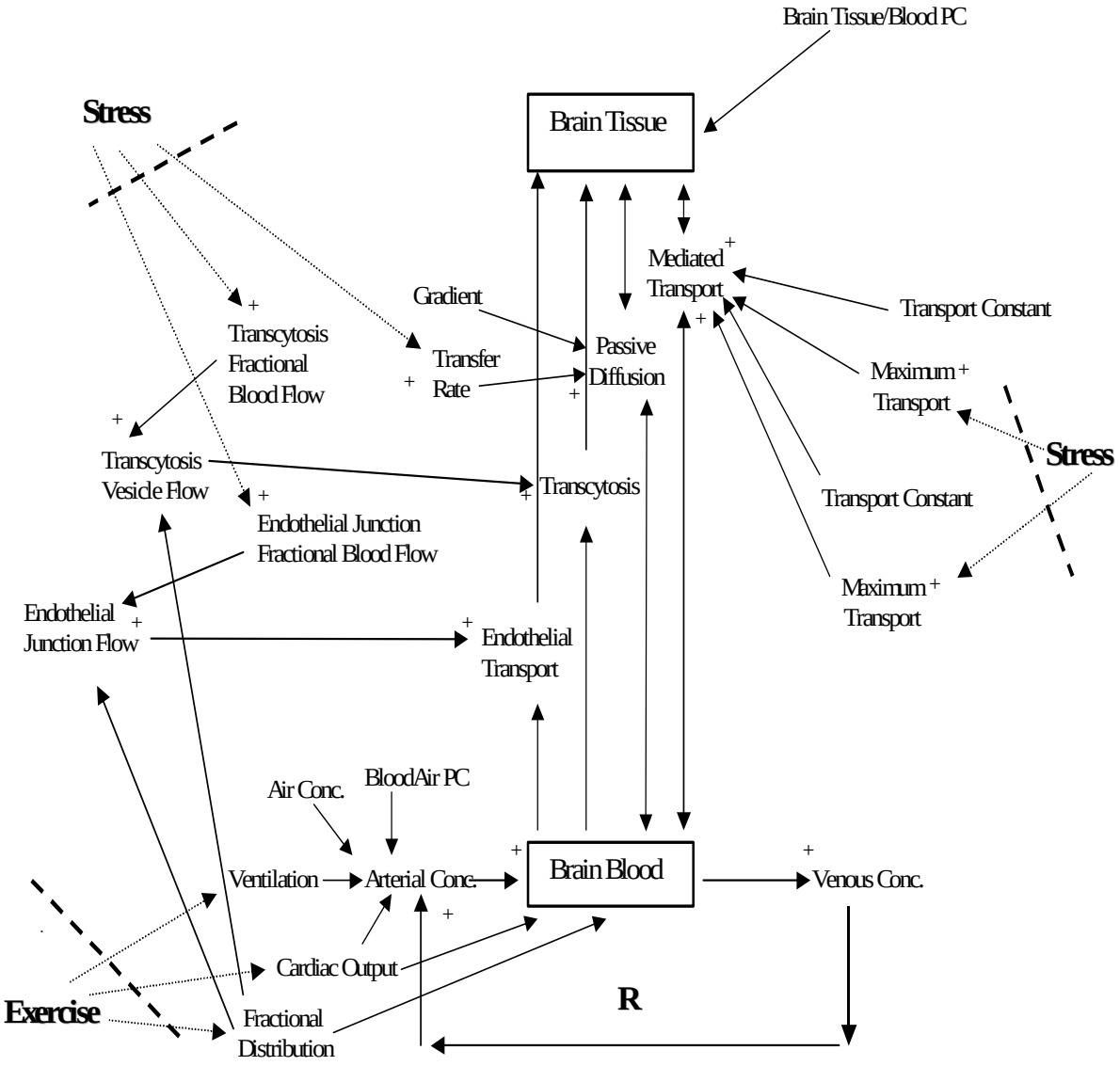


Figure 7. Influence Diagram of Brain Tissue

The transport processes prevalent in the literature include passive diffusion, mediated transport, transcytosis, and endothelial cell junction transport. Each of the transport processes is measured in mg of chemical per hour. Passive diffusion in the brain represents the net movement of chemical across the BBB down a concentration gradient,

that is not carrier mediated. The passive diffusion transfer rate represents the rate at which chemical flows into the brain per unit difference between tissue and blood concentrations:

$$PassiveDiffusion = CG \times TR$$

where

$$CG = Concentration\,Gradient = C_{BrainBlood} - \frac{C_{Brain\,Tissue}}{P_{BT/b}}$$

$P_{BT/b}$ = *"Brain Tissue/Blood Partition Coefficient"*

TR = transfer rate

Mediated transport is defined by the transport constant and maximum transport values:

$$MediatedTransport = \left(\left(\frac{Max\ Transport \times C_{BrainBlood}}{Transport\,Const + C_{BrainBlood}}\right) - \left(\frac{Max\,Transport\,2 \times C_{Brain\,Tissue}/P_{BT/b}}{Transport\,Const\,2 + C_{Brain\,Tissue}/P_{BT/b}}\right)\right)$$

Mediated transport represents an active type of transport in which the chemical flow works against a concentration gradient. Facilitated transport, which is saturable but not energy dependent, can also work against a concentration gradient. Mediated transport has two maximum transport and transport constant values to allow for a chemical that behaves differently while transported to and from the brain tissue.

Transcytosis of the chemical represents the amount that traverses the BBB's endothelial tight junctions via transcytotic vesicles:

$$Transcytosis = C_{BrainBlood} \times Q_{Transcytotic\,Vesicle}$$

where

$$Q_{Transcytotic\,Vesicle} = TranscytoticVesicleFlow = Q_{Brain\,Blood} \times Transcytotic\ Fraction\ of\ BrainBlood\ \ flow$$

Endothelial cell transport represents the amount of chemical that passes by bulk flow through the tight junctions:

$$Endothelial\,Transport = C_{BrainBlood} \times Q_{Endothelial\ Junction}$$

where

$$Q_{Endothelial\ Junction} = Q_{Brain\,Blood} \times Endothelial\ Fraction\ of\ BrainBlood\ \ flow$$

Both passive diffusion and mediated transport exist in the brain as bi-directional flows, while transcytosis and endothelial junction transport exist as a unidirectional flow.

As with the other tissues, exercise affects the brain compartment by increased blood flow. In addition to exercise, stress was added to the brain as a direct influence to each of the four BBB permeability mechanisms. These conditions would hypothetically lead increased to BBB 'permeability', which would allow more chemical to be delivered to the brain. As discussed in the literature review, events beyond physical stress (exercise) may cause the BBB to become more permeable. Therefore, the additional influence of stress was specifically added to affect the BBB transport mechanisms. Stress

may affect the passive diffusion transfer rate by increasing passive permeability, which would increase the passive diffusion between the brain blood and the brain. The maximum transport is also directly affected by stress in mediated transport, which would increase the amount actively transported into the brain.

The amount of material that can traverse or go through the endothelial cell junctions would be affected by stress in transcytosis and endothelial cell junction transport, respectively. This amount of material is dependent on the fraction of blood flow that is available to these transport processes.

Formulation

Formulation is the process of creating a detailed model structure where the actual parameter values are selected (Shelley, 2000). The cause and effect relationships of the influence diagram will be mathematically coded into a numerical integration modeling program. STELLA Research 5.0, by High Performance Systems will be used as the system dynamics modeling software for this thesis. The flow diagram in Figure 8 (Suhajda, 2000:44) below represents the mechanistic relationships shown in the simple influence diagram, Figure 5.

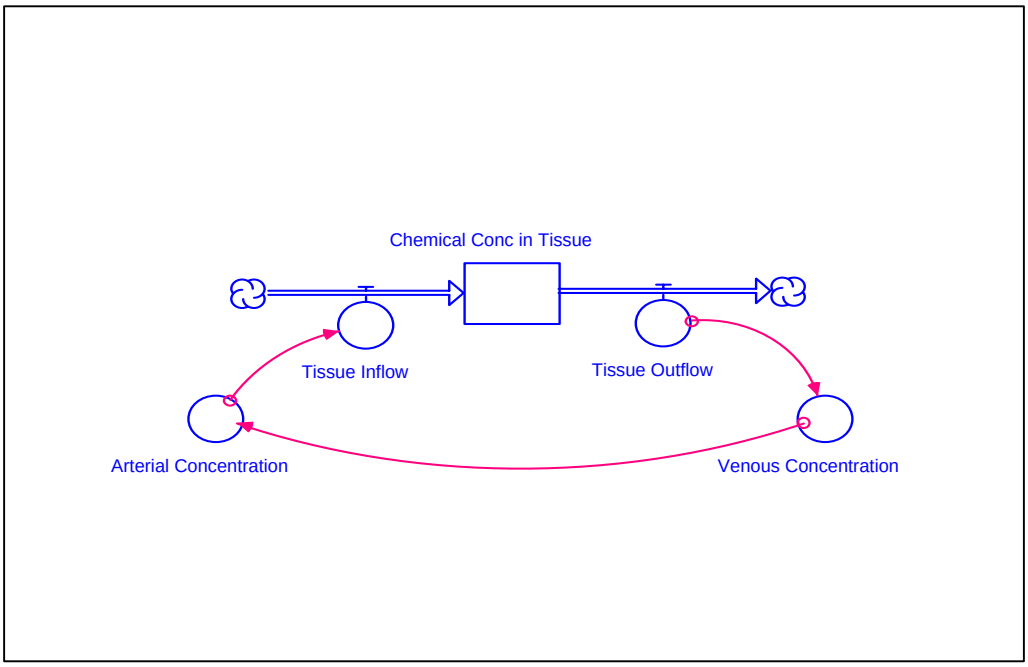


Figure 8. Simple Flow Diagram

The stocks, flow rates, concentrations, and other parameters in a flow diagram, which represent the real operating system, should always comply with the logic represented in the influence diagram (Shelley, 2000). It is important to exclude relationships that are not represented in the influence diagram, as this may lead to confusion, and unnecessary complication of the model. System dynamics models should be kept simple so that the general behavior of a system within the specific boundaries of interest can be observed.

The sequence of steps to be taken in the system dynamics process of taking the initial flow diagram to the proposed final diagram will now be discussed. Based on Suhajda's PBPK model, a set of tissue groups including the fat, liver, slowly perfused (skin and muscle), richly perfused (kidney), and brain will be enhanced. The model below in Figure 9 is the PBPK model that will be used in this study.

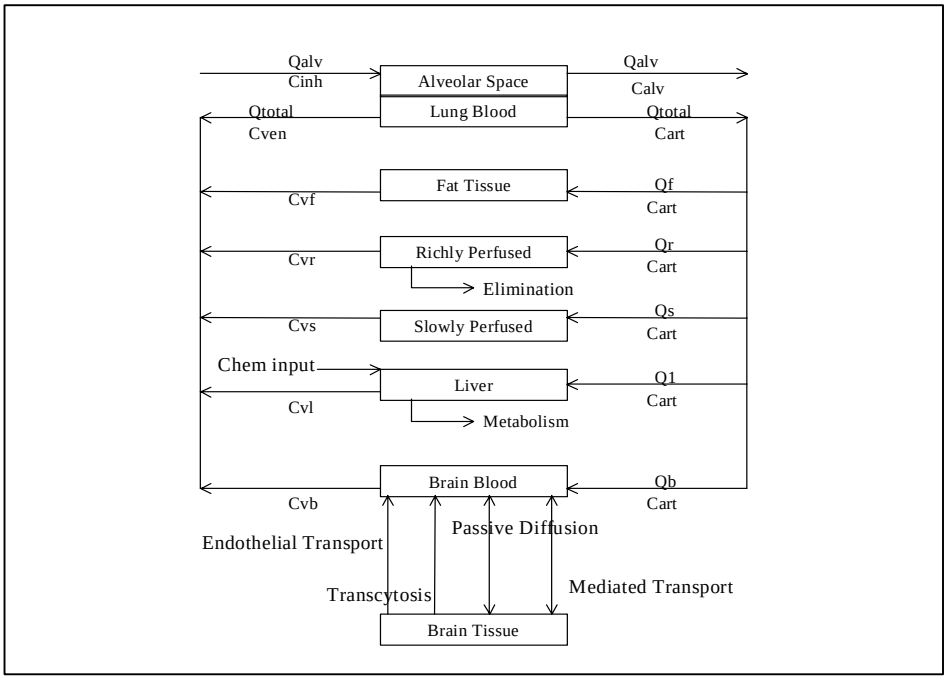


Figure 9. PBPK Model

The following is a general mass balance equation describing the accumulation of chemical in the system, assuming instantaneous equilibration between blood and tissue:

$$\frac{dA}{dt} = Q_t (C_a - C_t / P_{t/b}) - metabolism \ - elimination$$

where

dA/dt = Chemical Accumulation in Tissue over time

Q_t = *Fractional Blood Flow to Tissue*

C_a = *Arterial Blood Concentration of Chemical*

C_t = *Tissue Concentration of Chemical*

$$P_{t/b} = \text{“Tissue/Blood Partition Coefficient”}$$

$$metabolism = \frac{Liver\ Vmax \times C_{Liv}/P_{Liv/b}}{Liver\ Km + C_{Liv}/P_{Liv/b}}$$

$$elimination = C_a \times Q_R \times elimination\ fraction$$

A discussion of the model parameters was presented in Chapter II. The actual parameters used during the model simulations will be discussed under each simulation. The richly perfused compartment includes an elimination flow for PB-like chemicals, as discussed in the literature review. A realistic representation of the liver includes a metabolism flow and an oral uptake of the chemical. Although this model will include chemicals with a range of parameters, there will be scenarios included in the simulations that will attempt to capture the behavior of a chemical similar to PB, which are taken orally as opposed to chemicals taken intravenously or are airborne in nature. The chemical will only be ‘similar’ to PB because only certain parameter values of this chemical are available in the literature. The liver uptake is a dose of 30 mg of chemical every eight hours over each 24-h period of exposure. In an effort to maintain the desired simplicity of a system dynamics model, the chemical will be taken into the liver at a steady rate over the eight hours instead of being represented as a pulse input. Although the chemical uptake is represented as a constant, intravenous-like chemical flow, this will assist in interpreting the behavior of the model output. The bioavailability, amount of chemical actually available to the system upon oral intake, of the chemical in this model was assumed to be 10%, which stemmed from the PB results found in the literature review.

The brain compartment will provide a mechanistic view of the processes taking place that composes the BBB. To realistically represent the transport processes that occur, the brain was divided into two sub-compartments. The transport processes include passive diffusion, mediated transport, transcytosis, and endothelial cell junction transport. Stress was added to the model by the use of graphical relationships with each of the four transport mechanisms. Stress affects transcytosis and endothelial transport through the fraction of blood flow available to these processes. This approach entails treating transcytosis and endothelial transport as bulk flow processes. As stress increases, the amount of material able to penetrate the brain would increase. Although this is an oversimplification of the real system, it will prevent the model structure from being encumbered with too many details. Stress affects passive diffusion and mediated transport through the passive diffusion transfer rate and maximum transport, respectively. As stress increases, the amount of material able to penetrate the brain would again increase. The stress factor graphs represent the direct relationships between the transport mechanisms discussed above, and their associated levels of stress from 0 to 100%, the stress associated with maximum BBB permeability. The graphical relationships represented in this model include a linear, exponential, and concave up increase. Figure 10 demonstrates the curves to be included in the graphical relationships.

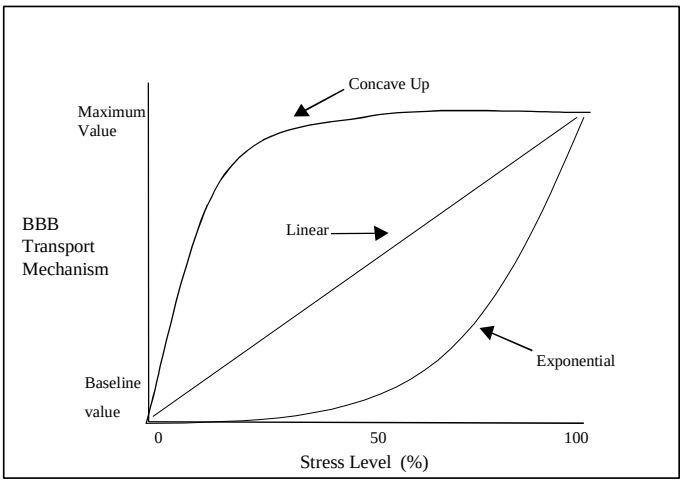


Figure 10. Stress Factor Graphical Relationships in BBB Transport

The final flow diagram developed in STELLA can be referenced in Appendix A. The equations and documentation for this flow diagram can be referenced in Appendix B. Seven scenarios will be represented using the PBPK model discussed. The known parameters in Table 3 of Chapter II will remain constant throughout the model simulations. The model structure objectives begin with observing chemical exposure under normal (exercise and stress-free) conditions. This exposure scenario is important in order to develop baseline conditions of chemicals with a range of different properties to compare future scenarios. This model will be distinctive in that the majority of chemical parameters were not available in the literature. Therefore, a unique opportunity is presented to examine a range of different chemical parameters to test their varying

effects. The model will then be simulated under exercise and stressed conditions. While exercise will affect the blood flow in the entire body, the stress will only affect the four BBB transport mechanisms. These differing exercise and stress effects will demonstrate the behavior of the system while under chemical exposure.

The second scenario will involve a PB-like chemical under exercise conditions in order to provide a means of comparison when stressed conditions are introduced. The third scenario individually changes the graphical relationships of the BBB mechanisms under rest conditions. The fourth scenario changes the maximum permeability values of each BBB mechanism to demonstrate the model's sensitivity under maximum stress. The fifth scenario changes the graphical relationships of the BBB mechanisms simultaneously under rest conditions to demonstrate the combination of mechanisms most important under these conditions. The sixth scenario incorporates exercise to the fifth scenario to demonstrate the combination of mechanisms important under exercise and stressed conditions. The last scenario involves an extreme conditions test of the parameters tested in the first scenario to provide further insight of the chemicals' behaviors under maximum exercise and stressed conditions.

The chief objectives of the model structure are to better understand the BBB mechanisms, how they are influenced by stress and exercise conditions, and to suggest the BBB mechanism that may be most important in terms of permeability. Before reviewing the different modeling scenarios, the different testing phases will be discussed.

Testing

The testing phase consists of the examination of the dynamic hypothesis, which questions whether the basic mechanisms actually create the reference mode (Shelley, 2000). Model testing is performed to determine if the model is a realistic representation of the system under study. Validation establishes confidence in the usefulness of a model for its specified purpose (Shelley, 2000). It is possible that a model be valid, but outside of the scope of its desired use.

An important principle in the validation process is to get a second opinion from others, such as experts in the field of study, to avoid bias and gain further insight into possible modeling errors. This type of validation should be performed throughout the modeling process, including during the construction of the flow diagram and definition of the parameters. Once a general understanding of the model behavior is established, further confidence in the model can be gained. A general understanding of the model behavior should be established during each of the scenarios performed.

Several tests can be used to build confidence in system dynamics models. Various tests of model structure, behavior, and policy implementation can be used to validate a system dynamics model, as there is no single test which can encompass each area (Forrester and Senge, 1980:209). The following tests will be performed on this PBPK model: structure-verification test, extreme-conditions test, behavior-anomaly test, surprise-behavior test, and behavior sensitivity test. The first two validation tests check the model structure, while the last three test the model behavior (Forrester and Senge, 1980:209).

Structure-Verification Test

Structure-verification tests compare the model structure with the real system represented (Forrester and Senge, 1980:212). Both available literature and review by experts should be used in this test. Although the human physiological system is quite complex in reality, it must be kept in mind that this model is only a simple representation of the system. However, the simple structures represented in the model should correspond to the general components of the real system.

Extreme-conditions test

The extreme-conditions test allows the model to be tested outside of its normal operating range. The minimum and maximum conditions would be examined to both test for flaws in the model structure, and to enhance the usefulness of the model during conditions outside of its normal operating range (Forrester and Senge: 1980:214). These extreme conditions will be tested during Scenarios 4 and 7.

Behavior-Anomaly Test

The behavior-anomaly test allows the model-builder to discover behavior that conflicts with real-system behavior (Forrester and Senge, 1980:220). This test can be used throughout the simulation process to account for flaws in the model structure or assumptions made. This test may also reveal behavior in the system that has not yet been observed.

Surprise-Behavior Test

The surprise-behavior test may reveal aspects of the system that were previously unrealized. The first step for the modeler is to understand the unexpected behavior and then to determine the cause by comparison to the real system (Forrester and Senge,

1980:221). If an insight of the real system is gained during this process, further confidence can be gained in the model.

Behavior-Sensitivity Test

The behavior-sensitivity test determines the degree of sensitivity of the model behavior to the model parameter values (Forrester and Senge, 1980:222). Shifts in model parameters may cause the model to behave abnormally. This test is especially important when model parameters of the real system are known, so that the model behavior can be verified. Sensitivity tests of this PBPK model were performed throughout the majority of the scenarios.

Model Scenarios

Scenario 1

The initial simulations will compare chemical concentrations in the fat, slowly and richly perfused tissue, liver, and brain compartments, under rest and stress-free conditions. Therefore, the stress factors in the brain compartment will remain at zero, and there will be no effect of exercise on blood flow. The purpose of these simulations will be to establish an understanding of baseline conditions for chemicals with varying properties. The partitioning coefficients, metabolic parameters, and transport parameters would change during these initial simulations. These parameters would change to explore a range of chemical properties and the model's sensitivity to parameter values, according to the values listed in Table 7 below. The above simulations will be accomplished with eighty (sixteen parameters changing five times) model runs. Each parameter will be tested while the other fifteen are held constant at their mid-range

(simulation 3) values. These initial simulations should show the behavior of chemicals with differing characteristics in various body compartments and demonstrate to what extent they can cross the BBB under rest and stress-free conditions.

Table 7. Physiological, Biochemical, and BBB Transport Parameters for Baseline Conditions in Scenario 1

	PARAMETER	SIMULATION VALUES				
		1	2	3	4	5
Partition Coefficients						
Liver/blood [(mg chem in liv/L liv)/ (mg chem bld/L bld)	PL	0.2	8	15	75	250
Fat/blood [(mg chem in fat/L fat)/ (mg chem bld/L bld)	PF	0.002	5	75	250	500
Slowly Perfused/blood [(mg chem in slow/L slow)/(mg chem bld/L bld)	PS	0.02	8	15	75	250
Richly Perfused/blood [(mg chem in rich/L rich)/(mg chem bld/L bld)	PR	0.2	8	15	75	250
Brain tissue/blood [(mg chem in brain/L brain)/(mg chem bld/L bld)	PB	0.002	8	15	75	250
Blood/air [(mg chem in blood/L blood)/(mg chem air/L air)	PBloodAir	5	25	75	200	300
Metabolic Parameters						
Liver Km (mg/L)	LKm	0.1	0.5	1.5	2.5	3.5
Liv Vmax (mg/hr)	LVmax	50	250	500	1000	2000
Kidney elimat. fract.	elim fract	0	0.15	0.35	0.5	0.75
Transport Parameters						
Endothelial Transport (mg/hr)						
Baseline bld flow fract.	QB junct fract	0	1E-12	1E-10	1E-08	1E-07
Transcytosis (mg/hr)						
Baseline bld flow fract.	QB trans fract	0	1E-13	1E-11	1E-09	1E-08
Passive Diffusion (mg/hr)						
Transfer Rate	TR	0	0.5	1	3	5
Mediated Transport (mg/hr)						
Max Transport	Max Transport	0.5	5	10	20	30
Max Transport2	Max Transport2	0	5	10	20	30
Transport Constant	Transport Constant	0.5	1.75	3.5	5	7.5
Transport Constant2	Transport Constant2	0.5	1.75	3.5	5	7.5

Scenario 2

The next set of simulations involves incorporating blood flow changes due to exercise. These simulations would also represent stress-free conditions in the brain compartment (stress factor graphs would be set to zero) for a specific PB-like chemical with the baseline and maximum values for the parameters listed in Table 8 below. The baseline values refer to stress-free conditions, while the maximum values refer to maximum stressed conditions in the brain compartment. Unless otherwise noted, the remainder of the simulations will be performed using these parameters.

Table 8. Parameters for PB-like Chemical for Scenario 2

	PARAMETER	BASELINE VALUE	MAX VALUE
Partition Coefficients			
Liver/blood[a] [(mg chem in liv/L liv)/ (mg chem bld/L bld)	PL	8	8
Fat/blood[b] [(mg chem in fat/L fat)/ (mg chem bld/L bld)	PF	0.002	0.002
Slowly Perfused/blood [(mg chem in slow/L slow)/(mg chem bld/L bld)	PS	8	8
Richly Perfused/blood[a] [(mg chem in rich/L rich)/(mg chem bld/L bld)	PR	15	15
Brain tissue/blood [(mg chem in brain/L brain)/(mg chem bld/L bld)	PB	0.002	0.002
Blood/air [(mg chem in blood/L blood)/(mg chem air/L air)	PBloodAir	200	200
Metabolic Parameters			
Liver Km (mg/L)	LKm	1.5	1.5
Liv Vmax (mg/hr–kg)	LVmax	500	500
Kidney elimat. fract.	elim fract	0.15	0.15
Transport Parameters			
Endothelial Transport (mg/hr)			
Baseline bld flow fract.	QB junct fract	0	1E-08
Transcytosis (mg/hr)			
Baseline bld flow fract.	QB trans fract	0	1E-09
Passive Diffusion (mg/hr)			
Transfer Rate	TR	0.5	1
Mediated Transport (mg/hr)			
Max Transport	Max Transport	5	30
Max Transport2	Max Transport2	0	0
Transport Constant	Transport Constant	1.75	1.75
Transport Constant2	Transport Constant2	0.5	0.5

a. Golumb (2000)
b. McLauchlan (2000)

As shown in Table 8, a uni-directional mediated transport with no brain efflux will be assumed for this chemical. Once again, the simulations will compare the chemical concentrations in the fat, slowly and richly perfused tissue, liver, and brain compartments. The compartment concentrations will be compared at rest, 15%, 25%, 55%, and 100% exercise. These simulations should demonstrate the behavior of the chemical under exercise conditions and provide a means of comparison of BBB passage under stress-free/exercise and stressed/exercise conditions. Five simulations will be performed to include each change in exercise under baseline conditions.

Scenario 3

The next set of simulations would add stress relationships with the BBB transport mechanisms into the brain compartment. The initial model runs here would represent the body at rest, so that there can be a comparison between stress/rest and stress/exercise conditions. The stress factor graphs would change individually for each BBB mechanism so that the individual effects could be seen. These graphs would be changed individually three times each under the same parameters listed previously in Table 8. The three changes of stress factor graphs within each BBB mechanism include a linear increase, exponential increase, and a concave up representation, respectively. The maximum stress values for the brain transport parameters are shown above in Table 8. The stress factor levels would be changed from stress-free to 15%, 25%, 55%, and 100%. While each of the BBB mechanisms is changing, the remaining three will be held constant at their associated baseline values from Table 8. Sixty simulations (5 stress levels with 3 graphical relationships for each of the 4 mechanisms) would accomplish the exercise-free

conditions in the brain compartment. These simulations should demonstrate the behavior of the chemical under rest/stress conditions and provide a means of comparison of BBB passage when exercise conditions are introduced.

Scenario 4

The next simulations would include changing the maximum permeability values of each BBB mechanism five times under maximum stress conditions, as shown in Table 9 below. While each BBB mechanism is changing, the remaining three will be held constant at their associated maximum values from Table 8. This scenario involves twenty simulations (5 changes of max values with the 4 mechanisms). These simulations should demonstrate the sensitivity of the model to BBB maximum flows under maximum stress.

Table 9. Maximum Permeability Changes for BBB Compartment for Scenario 4

BBB Mechanism	**Simulation Changes**				
	1	2	3	4	5
Endothelial Transport Fraction	0	1E-10	1E-08	1E-06	1E-04
Transcytosis Transport Fraction	0	1E-11	1E-09	1E-07	1E-05
Passive Diffusion Transfer Rate	0	0.5	1	3	5
Mediated Maximum Transport	0.5	5	10	20	30

Scenario 5

The next simulations would include changing the graphical relationships of the various BBB mechanisms simultaneously, at stress factors from no stress to 15%, 25%, 55%, and 100%. The three changes of stress factor graphs within each BBB mechanism would again include a linear increase, exponential increase, and a concave up

representation, respectively. The body is still at rest during these simulations. The different combinations of simulations are shown in Table 10 below. While two of the BBB mechanisms are changing, the graphical relationships of the remaining two mechanisms would remain at their baseline values from Table 8. Future efforts may focus on other combinations of simultaneous changes for the BBB mechanisms. Ninety simulations (5 changes in stress factors with 3 graphical relationship for 6 combinations) would accomplish the combinations of BBB mechanisms under rest conditions in the brain compartment. The number of simulations for this scenario may be reduced pending output of the previous scenario. If two of the previously inserted graphical relationships provide similar output, the number of graphical changes in this scenario may be reduced. These simulations should demonstrate the combination of BBB mechanisms that is most important by the amount of chemical buildup in brain tissue, under rest conditions.

Table 10. BBB Mechanism Simulation Combinations for Scenario 5 and 6

BBB Mechanism	Combination Change
Mediated Transport	Passive Diffusion
Mediated Transport	Transcytosis
Mediated Transport	Endothelial Transport
Passive Diffusion	Transcytosis
Passive Diffusion	Endothelial Transport
Transcytosis	Endothelial Transport

Scenario 6

The next simulations would again include changing the graphical relationships of the various BBB mechanisms simultaneously, at stress factors from rest to 15%, 25%, 55%, and 100%. The three changes of stress factor graphs within each BBB mechanism

would again include a linear increase, exponential increase, and a concave up representation, respectively. The body will now be compared at rest, 15%, 25%, 55%, and 100% exercise. This scenario involves simulating the combinations listed in Table 10 an additional four times each for the various exercise levels. Ninety (6 combinations with 3 graphical relationships at 5 levels of stress and exercise) simulations would accomplish the combinations of BBB mechanisms under exercise conditions in the brain compartment. Once again, the number of simulations for this scenario may be reduced pending output of the previous scenarios. These simulations should demonstrate the BBB mechanism that is most important by the amount of chemical buildup in brain tissue, under exercise and stressed conditions.

Scenario 7

The next simulations would involve a sensitivity analysis for extreme ranges of a sampling of parameter values for the different chemicals simulated in the baseline scenario (Table 7). These simulations would assess the baseline sensitivity analysis under extreme exercise and stress conditions (100%). The parameters would change according to the values listed in Table 11 below. These parameter values represent the low, mid, and high range of parameter values that were tested in Scenario 1. Each parameter would change individually while the others are held at their mid-range (simulation 2) values. These simulations would provide further insight into the behavior of chemicals with these specific parameters under extreme conditions, and whether their sensitivity changes under this scenario. Forty-eight (16 parameters changing 3 times) simulations would accomplish this extreme-conditions scenario.

Table 11. Parameters Tested for Extreme Exercise/Stress Conditions in Scenario 7

	Parameter	Simulation Values		
		1	2	3
Partition Coefficients				
Liver/blood [(mg chem in liv/L liv)/ (mg chem bld/L bld)	PL	0.2	15	250
Fat/blood [(mg chem in fat/L fat)/ (mg chem bld/L bld)	PF	0.002	75	500
Slowly Perfused/blood [(mg chem in slow/L slow)/(mg chem bld/L bld)	PS	0.02	15	250
Richly Perfused/blood [(mg chem in rich/L rich)/(mg chem bld/L bld)	PR	0.2	15	250
Brain tissue/blood [(mg chem in brain/L brain)/(mg chem bld/L bld)	PB	0.002	15	250
Blood/air [(mg chem in blood/L blood)/(mg chem air/L air)	PBloodAir	5	75	300
Metabolic Parameters				
Liver Km (mg/L)	LKm	0.1	1.5	3.5
Liv Vmax (mg/hr)	LVmax	50	500	2000
Kidney elimat. fract.	elim fract	0	0.35	0.75
Transport Parameters				
Endothelial Transport (mg/hr)				
Baseline bld flow fract.	QB junct fract	0	1E-10	1E-07
Transcytosis (mg/hr)				
Baseline bld flow fract.	QB trans fract	0	1E-11	1E-08
Passive Diffusion (mg/hr)				
Transfer Rate	TR	0	1	5
Mediated Transport (mg/hr)				
Max Transport	Max Transport	0.5	10	30
Max Transport2	Max Transport2	0	10	30
Transport Constant	Transport Constant	0.5	3.5	7.5
Transport Constant2	Transport Constant2	0.5	3.5	7.5

Implementation

Implementation refers to the performance of simulations with a model, after successful confidence tests, which are designed to explore effective management scenarios to achieve a goal. (Shelley, 2000). This PBPK model may be used to provide further insight into the on-going investigation of the behavior of chemical exposures in the human body under stressed and exercise conditions.

In summary, this methodology provides an organized development of the steps taken in building and testing this PBPK model. Although this model is a simple representation of the human body, it should provide insight into physiological changes experienced during deployment situations. Effective management scenarios of exposure can be further explored once specific data is available concerning the chemicals of interest.

IV. Results and Analysis

The PBPK model was developed and tested according to the methodology outlined in Chapter III. This chapter reveals the results and conclusions gained through the first three stages of the system dynamics process: conceptualization, formulation, and testing. The insights gained from the seven modeling scenarios will be presented below. An interesting observation of the simulated reference mode behavior is found in Figure 11 below. Compared to rest and stress-free conditions, the brain tissue level increases

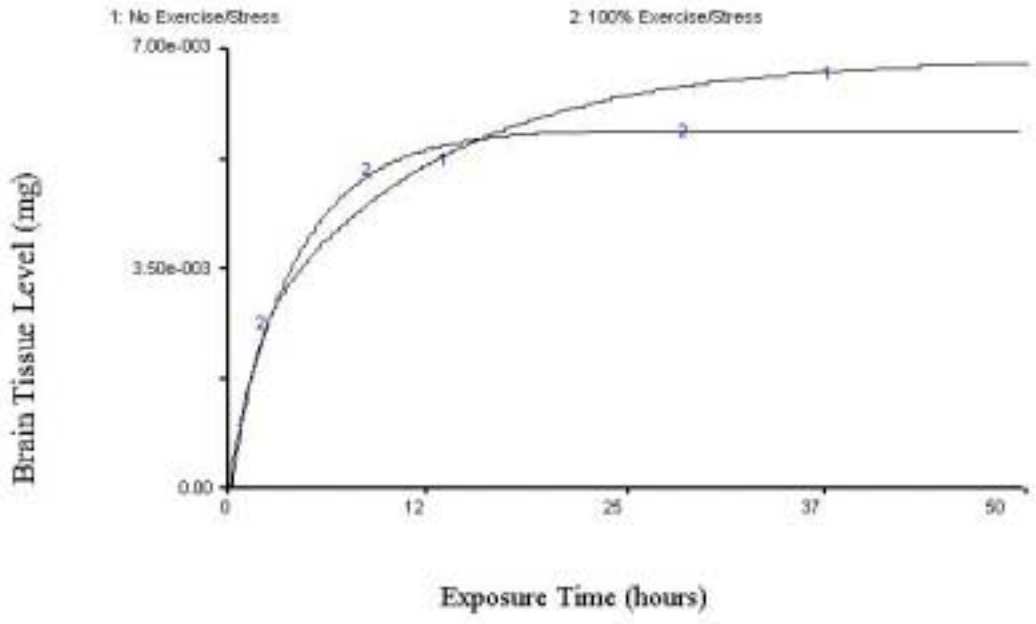


Figure 11. Simulated Reference Mode

at a faster rate, but reaches a lower steady state level at maximum stress and exercise. This does not match the predicted reference mode as shown in Figure 4. Upon assessment of the physiological changes occurring in the body during maximum stress and exercise, it was concluded that this model behavior is appropriate for this system. The strength of kidney elimination was not considered during the initial conceptualization of the reference mode. However, the fractional redistributions of blood to the other tissues in combination with kidney elimination would cause this behavior in the simulated reference mode. Further confidence in the model behavior was gained after this realization.

Scenario 1

The initial simulations compared chemical concentrations in each tissue compartment under rest and stress-free conditions, to establish an understanding of chemical behaviors over a range of varying parameters. These simulations provide the foundation for the behavior-sensitivity tests that were performed throughout the scenarios. The exposure scenario used throughout the simulations included a continuous dose of 30 mg of chemical for each 8-hour period. Figure 12 represents the liver tissue compartment. Each line corresponds to the liver tissue level of chemical with increasing liver partition coefficients.

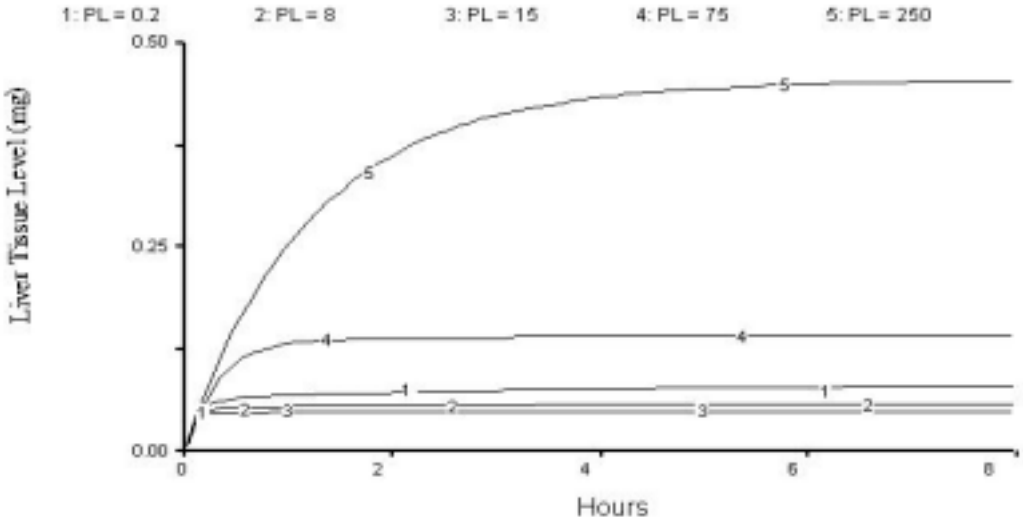


Figure 12. Scenario 1: Liver Tissue Level for Increasing Liver/Blood PC Values

The time was extended to eight hours in Figure 12 to see the general behavior over time. As expected, the liver tissue reaches steady state sooner at lower partition coefficients. With less chemical allowed to partition into the tissue, the liver is able to equilibrate faster. It may be initially expected that as a tissue partitioning coefficient increases, the tissue level would increase. However, other factors acting in the body must also be considered. A direct influence on liver tissue level arises from metabolism, as depicted in Figure 13 below. At partition coefficients above 15, the liver tissue level increases. This indicates that the maximum rate of metabolism has been reached, and the liver cannot degrade the chemical any faster, thereby causing an increase in liver tissue level. Figure 13 represents the metabolism flow with increasing liver partition

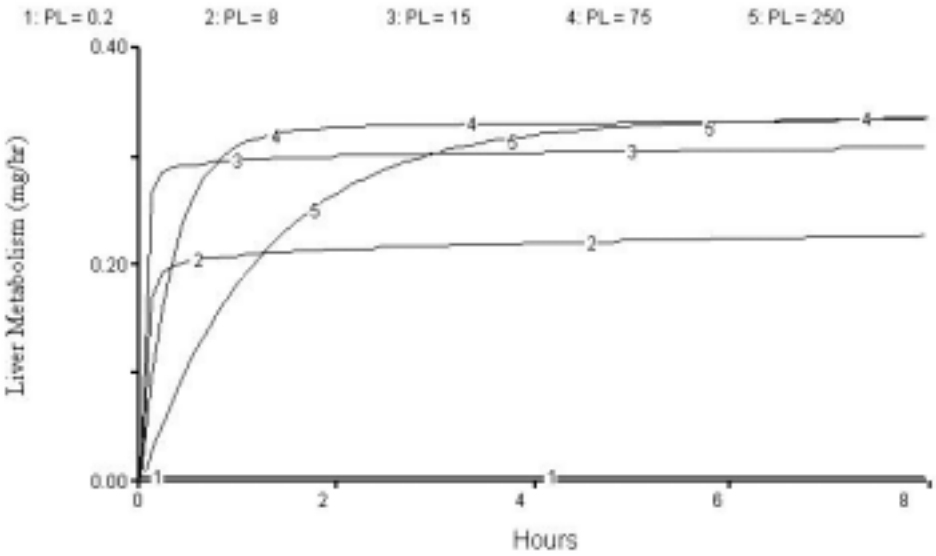


Figure 13. Scenario 1: Liver Metabolism for Increasing Liver/Blood PC Values

coefficients. Although the first liver partition coefficient is not zero, it is small enough that metabolisms cannot be distinguished on this scale. Metabolism continues to increase until it levels at liver partition coefficients above 15. This corresponds to the liver tissue levels previously discussed in Figure 12. Since the metabolism rate does not increase, the liver tissue level increases. The steady state levels of metabolism are clearly reflected in the final liver tissue levels, and the initial systemic effects provided further insight into the tissue behaviors. The metabolism rate with the initial liver partition coefficients reach steady state almost instantaneously, which is reflected in the initial liver tissue levels,

which also immediately reach steady state. At higher partition coefficients, the metabolism reaches steady state at a slower rate, which is reflected in the liver tissue levels with high liver partition coefficients, which also take longer to reach steady state. Another explanation of the initial systemic effects of metabolism may arise from one of the liver tissue compartment's inputs of arterial blood concentration, shown below in Figure 14. While

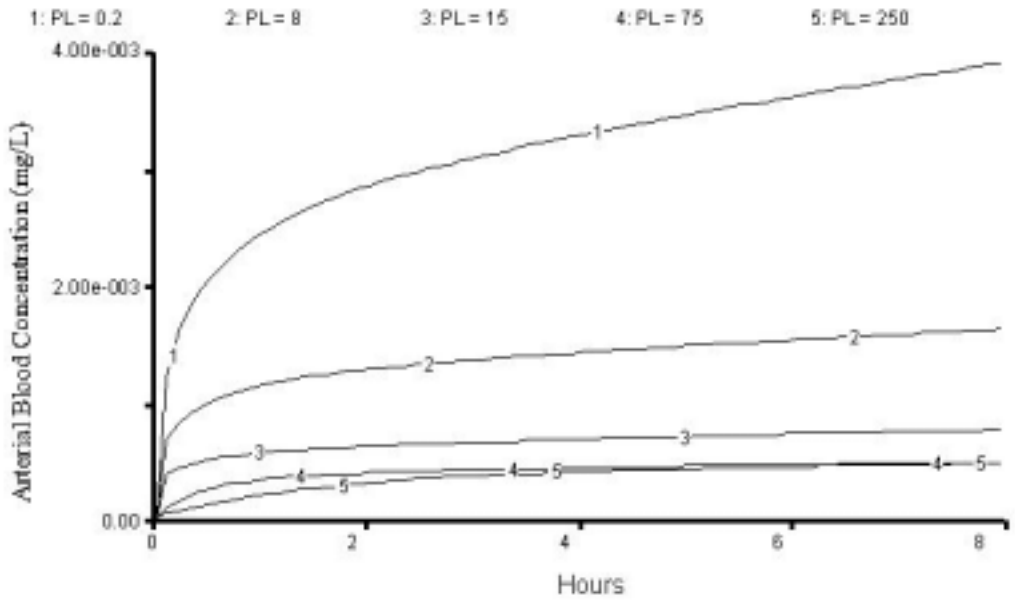


Figure 14. Scenario 1: Arterial Blood Concentration for Increasing Liver/Blood PCs

the oral uptake of chemical and fractional blood flow available to the liver remain constant, the arterial blood concentration decreases. The arterial concentration decreases

by a smaller amount with increasing liver partition coefficients. As the liver tissue level increases and metabolism levels off, the arterial blood concentration also begins to level off due to the greater amount of chemical available to the liver.

Figure 15 represents the brain tissue compartment with each line corresponding to the brain tissue level of chemical with increasing endothelial flow fractions. The model

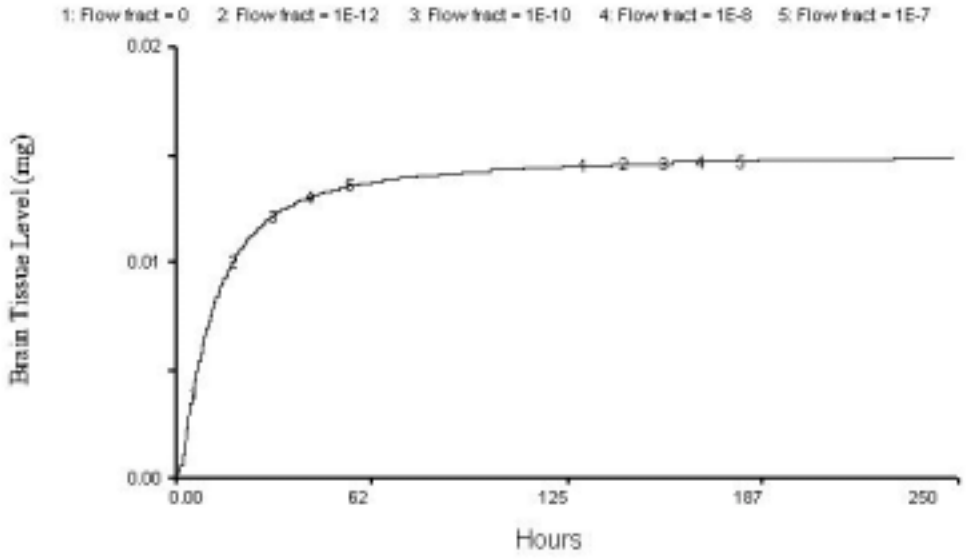


Figure 15. Scenario 1: Brain Tissue Level for Increasing Endothelial Flow Fractions

is insensitive to this range of fractions as the brain concentration differences cannot be seen on this scale. It must be noted that although the endothelial flow fractions are increasing, the other BBB transport parameters are acting at their mid-range simulation

values. Therefore, these increasing endothelial flow fractions have no significant effect on the brain tissue level with the combination of other BBB transport values and other physiological parameters in effect. The physiological relevance of the flow fractions may change under a different set of parameters. Although the model is insensitive to the above range of endothelial flow fractions, Figure 16 below demonstrates that higher brain tissue levels can be achieved with increased values. These values represent several

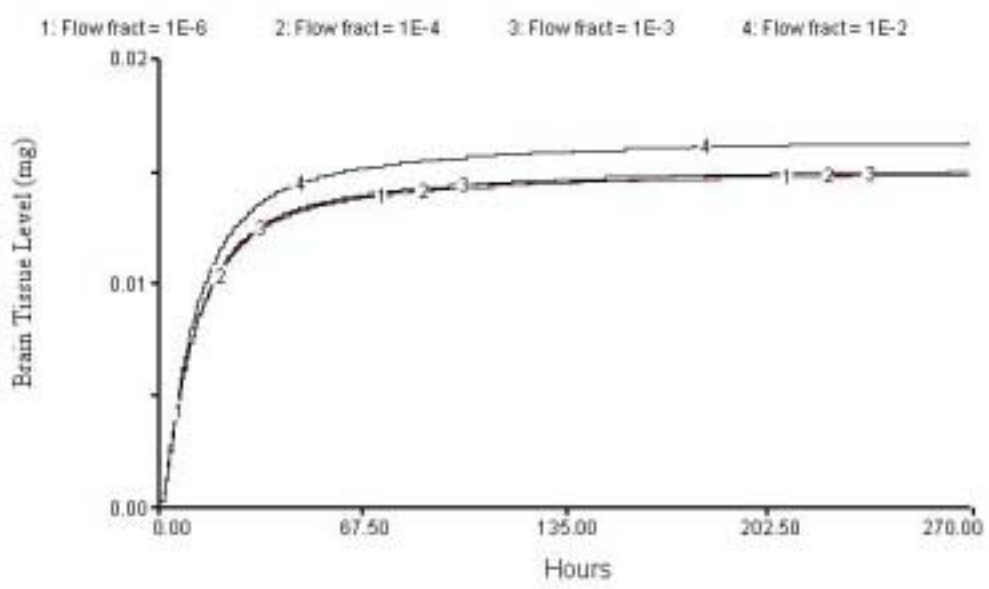


Figure 16. Scenario 1: Brain Tissue Level for Higher Endothelial Flow Fractions

flow fractions at orders of magnitude higher than were previously tested. A significant change in brain tissue level cannot be seen until the flow fraction rises above 1E-3.

However, there are questions as to whether these values of endothelial flow fractions are unrealistically high. This level of endothelial flow into the brain may represent a total BBB breakdown.

Figure 17 below represents brain tissue levels at increasing passive diffusion transfer rates. As the transfer rate increases, the steady state chemical level in the

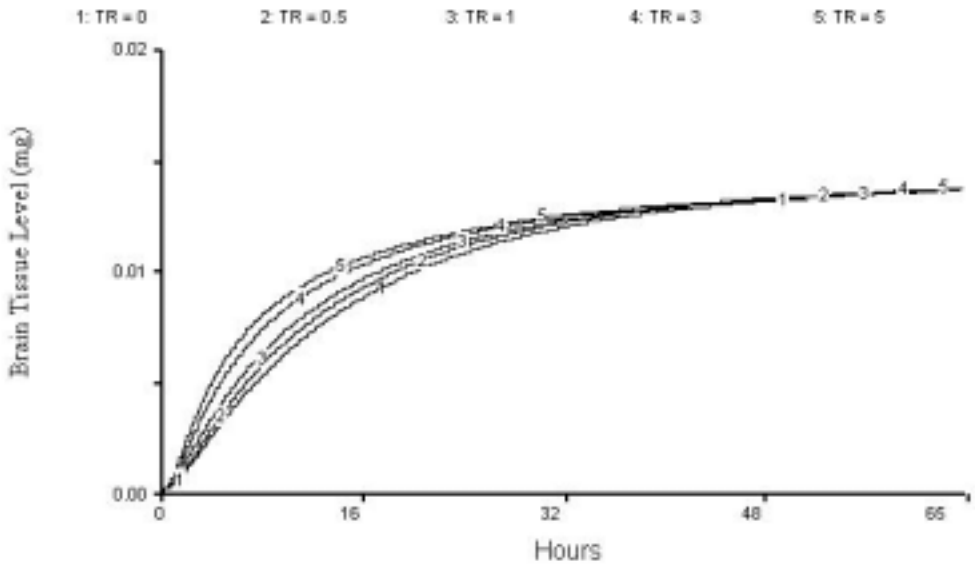


Figure 17. Scenario 1: Brain Tissue Level for Increasing Passive Diffusion Transfer Rate

brain tissue reaches steady state at a faster rate. Yet again, as the passive diffusion transfer rates increase, endothelial transport, transcytosis, and bi-directional mediated

transport are acting at their mid-range simulation values. The range of transfer rates tested here are significant enough to increase the rate that steady state is reached with the present BBB transport mechanism values.

This scenario showed the behavior of chemicals with varying characteristics under rest and stress-free conditions. The model behavior of the increasing partition coefficients caused an overall increase in chemical tissue levels, as expected. The relative strengths of these parameters will be discussed in future scenarios. As discussed in Chapter III, eighty simulations were performed in this scenario. Appendix C contains the simulation results for the remaining parameters tested in Scenario 1.

Scenario 2

This scenario was simulated under exercise and stress-free conditions for a specific PB-like chemical to demonstrate its behavior and provide a means of comparison of BBB passage under stress-free/exercise and stressed/exercise conditions. Figure 18 below shows the brain tissue level with increasing exercise. As discussed in Chapter III,

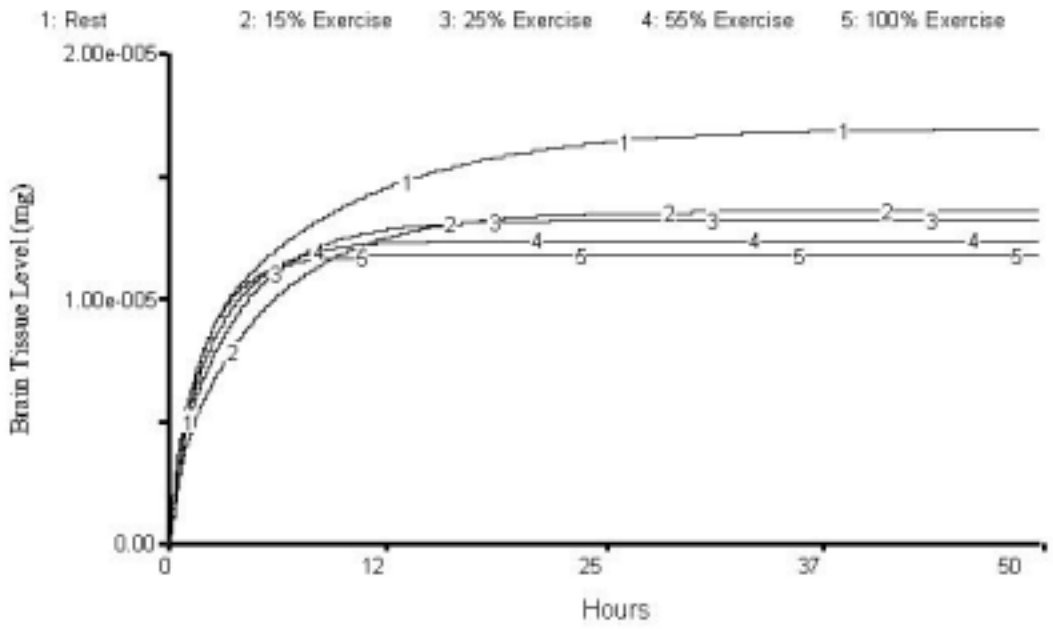


Figure 18. Scenario 2: Brain Tissue Level with Increasing Exercise

there is unidirectional mediated transport to the brain for this specific chemical, and under stress-free conditions, there is no endothelial transport or transcytosis. As exercise increases, the brain tissue chemical level decreases, also reaching a lower steady state value than the rest and stress-free conditions of Scenario 1. This behavior was expected because although the fractional blood flow to the brain remains unchanged under rest and exercise conditions, the increased overall blood flow in the body allows more chemical to be both metabolized and eliminated. The initial kinetics of the brain tissue level were unexpected. The largest effect on brain tissue level occurs after 15% exercise. It appears as if the brain tissue level begins to equilibrate above this exercise level. The

physiological adjustments being made in the body may account for the increased time to reach steady state at 15% exercise. At this point, the fractional distributions of blood flow are being adjusted, as shown in Table 3 of Chapter II. As discussed in Chapter II, the fractional distributions change according to whether exercise exists in the model, not according to the level of exercise. Therefore the initial kinetics of the model behavior after this initial increase in exercise should be expected. Figure 19 below represents the kidney elimination with increasing exercise. The kidney elimination flow clearly

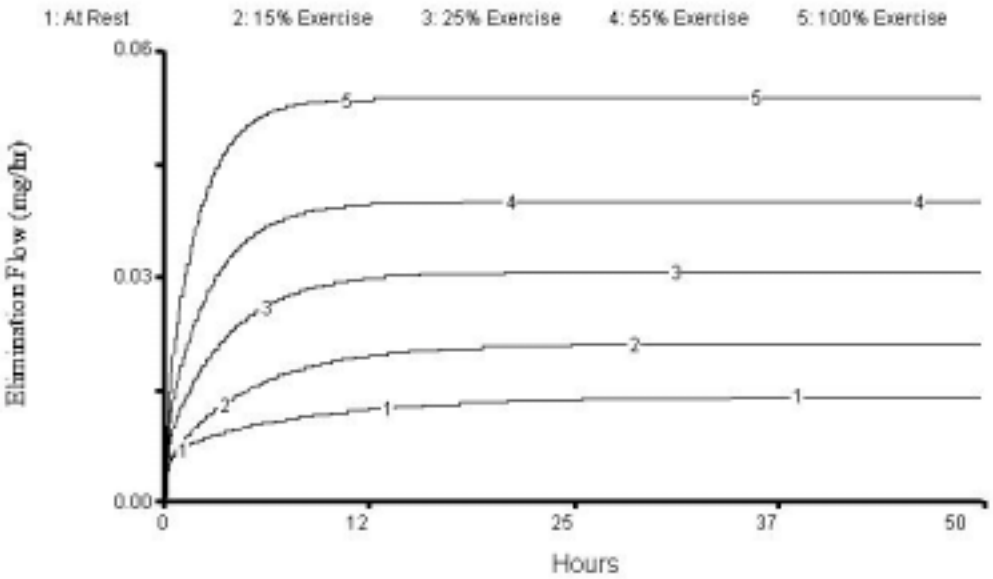


Figure 19. Scenario 2: Kidney Elimination with Increasing Exercise

increases with increasing exercise. This behavior can be explained by the model inputs to the richly perfused tissue. Because the cardiac output is higher with increased exercise, the overall blood flow available to the tissue increases, causing an increased elimination flow.

This scenario showed the behavior of a specific chemical under exercise and stress-free conditions. The model behavior of the increasing exercise caused an overall decrease in chemical tissue levels, and caused the tissues to reach steady state at a faster rate as compared to Scenario 1. As discussed in Chapter III, five simulations were performed in this scenario. Appendix D contains the simulation results for the remaining exercise changes tested in Scenario 2.

Scenario 3

These simulations added stress into the brain compartment by individually changing the stress factor graphs under rest conditions, so that there could be a comparison between stress/rest and stress/exercise conditions. Figure 20 below shows the brain tissue level for linearly increasing passive diffusion transfer rate with increasing brain stress. The model is sensitive to this range of transfer rates. An instantaneous

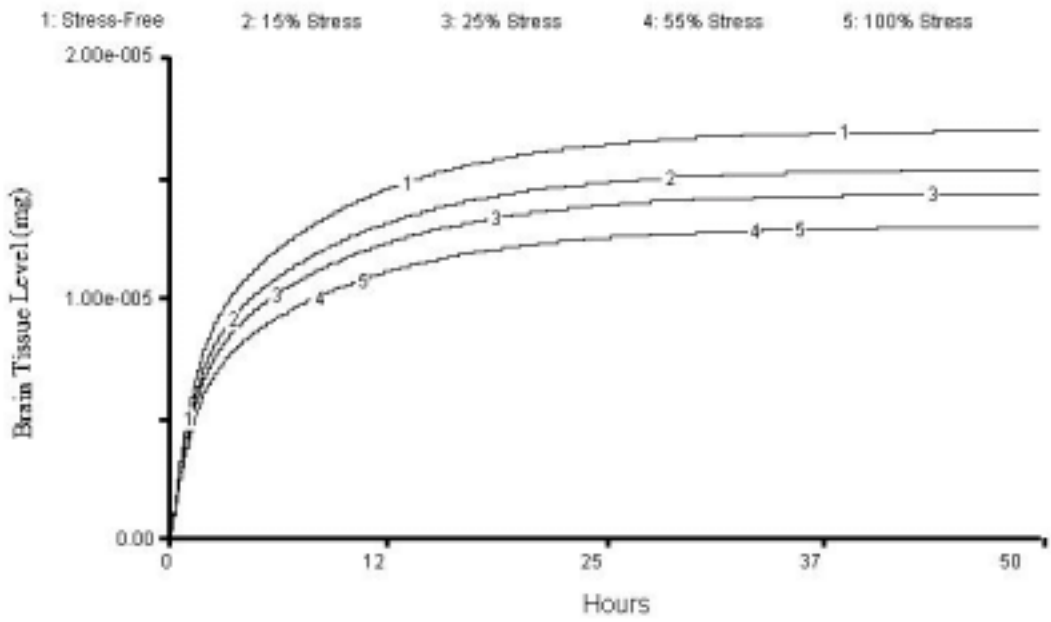


Figure 20. Scenario 3: Brain Tissue Level for Linearly Increasing Passive Diffusion Transfer Rate (0.5 to 1) with Increasing Stress

equilibrium is not reached until stress values approach 55%, where the brain tissue level reaches the same steady state values. The instantaneous equilibrium above 55% stress signifies that there is no diffusion limitation. The decreased brain tissue levels with increasing transfer rates yielded a surprise behavior. The effects of mediated transport that is still acting in this scenario must be discussed. Mediated transport allows the brain tissue levels to begin at higher values, but because more chemical buildup exists in the brain than the blood, the chemical had to exit the brain compartment. The chemical was allowed to leave the brain compartment through the passive diffusion process, therefore decreasing the brain tissue level until instantaneous equilibrium was reached. This figure

can be compared to Figure 17 of Scenario 1 with an increasing transfer rate under rest and stress-free conditions. This scenario yields a decreased brain tissue level under stressed conditions. Unlike the conditions in Scenario 1, this simulation has lower mediated transport values to the brain, which may account for a decrease in brain tissue levels. This implies that mediated transport may play an important role in increasing brain tissue levels.

Figure 21 below represents the brain tissue level for linearly increasing transcytotic flow fractions. The model is insensitive to this range of flow fractions.

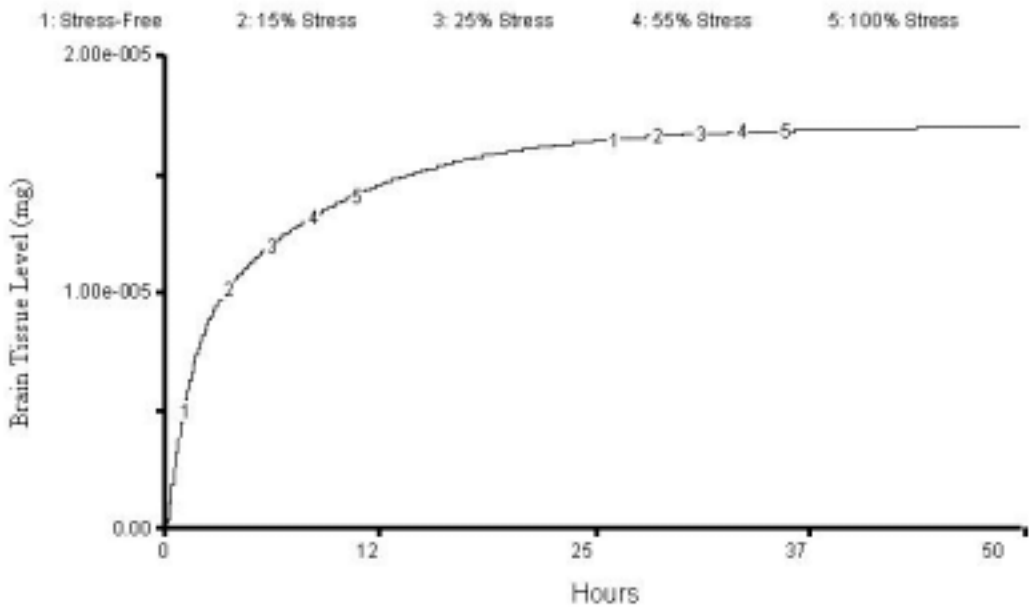


Figure 21. Scenario 3: Brain Tissue Level for Linearly Increasing Transcytosis Flow Fractions (0 to 1E-9) with Increasing Stress

The brain tissue reached the same level as the passive diffusion transfer rate under stress-free conditions as shown in Figure 20. Identical results were found with increasing endothelial flow fractions, as shown in Appendix E. This may indicate that while the values of the transcytosis flow fractions are not significant to produce noticeable changes in the model, the passive diffusion transfer rate acting in this scenario is not significant enough to reduce the brain tissue level. The sensitivity of the model to varying flow fractions is shown in Figure 21 below. The model is sensitive at each of the increased

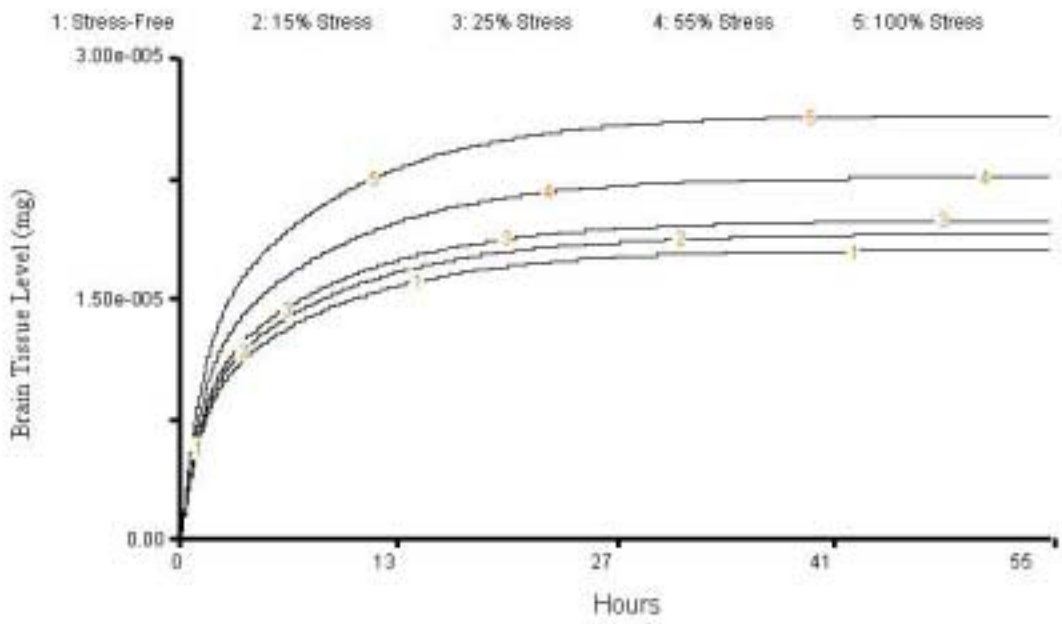


Figure 22. Scenario 3: Brain Tissue Level for Linearly Transcytosis Flow Fractions (0.05 to 0.005) with Increasing Stress

flow fraction values. Because the passive diffusion transfer rate remains at the same value in this simulation, the amount of chemical available to the brain tissue is able to increase. However, transcytotic flow fractions at this level may be unrealistic.

Figure 23 below shows linearly increasing maximum transport under increasing stress conditions. The changes in brain tissue level are most responsive to this transport

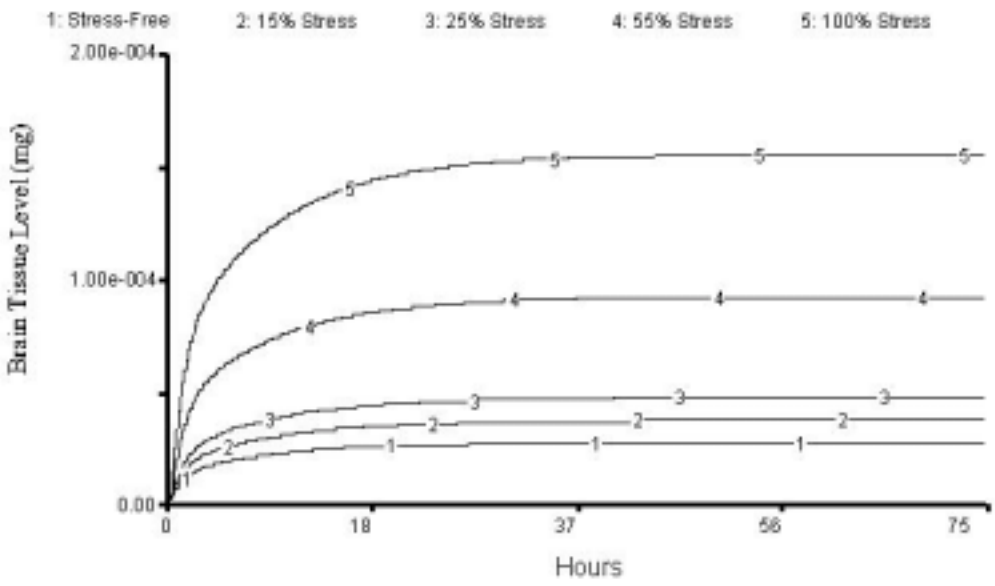


Figure 23. Scenario 3: Brain Tissue Level for Linearly Increasing Mediated Maximum Transport (5 to 30) with Increasing Stress

parameter. The model is most sensitive to higher maximum transport values. This behavior points to the fact that mediated transport has a substantial impact on increased

BBB transport. This observation proves to be important in the later examinations of combinations of BBB transport mechanisms.

The stress factor graphical relationships for each BBB mechanism were changed three times so that the individual effects of each transport mechanism could be seen. The behavior-sensitivity test was applicable to these simulations. Upon changing the graphical relationships from linear to an exponential increase and concave up increase, there was similar behavior in the model outputs. Mediated transport was the only BBB transport parameter that showed some degree of sensitivity to the changing graphs. However, the stress-free and maximum stress brain tissue levels that were reached during each graphical change remained the same. Future model scenarios only included linear graphical relationships within the BBB transport mechanisms. These simulations demonstrated the behavior of the chemical under rest/stress conditions and provide a means of comparison of BBB passage when exercise conditions were introduced. As discussed in Chapter III, sixty simulations were performed in this scenario. Appendix E contains the simulation results for the remaining parameters tested in Scenario 3.

Scenario 4

The next simulations changed the maximum permeability values of each BBB mechanism five times under maximum stress conditions, to demonstrate the model's sensitivity to these BBB maximum flows. These changes are represented in Table 9 of Chapter III. The extreme-conditions test is relevant in this scenario. Figure 24 below shows brain tissue levels with increasing maximum endothelial flow fractions.

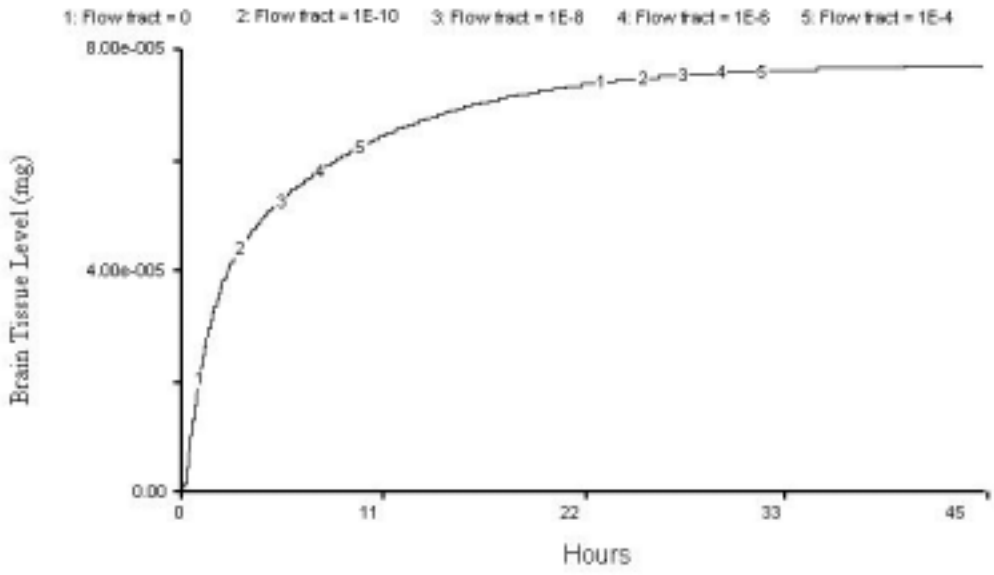


Figure 24. Scenario 4: Brain Tissue Level with Increasing Maximum Endothelial Flow Fractions at Maximum Stress

The model is insensitive to the increases in the endothelial transport mechanism under maximum stress. However, the steady state brain tissue level reached under these conditions is slightly higher than in Scenario 3. This phenomenon is due to the remaining BBB transport mechanisms acting at their associated maximum values. Although the higher values of endothelial flow fractions may be outside of their normal operating range, they were tested to determine the model sensitivity to this extreme of values. Mediated transport and passive diffusion are still acting as in previous scenarios.

Figure 25 below shows the brain tissue level for increasing passive diffusion transfer rates under maximum stress. The brain tissue level is able to equilibrate at values

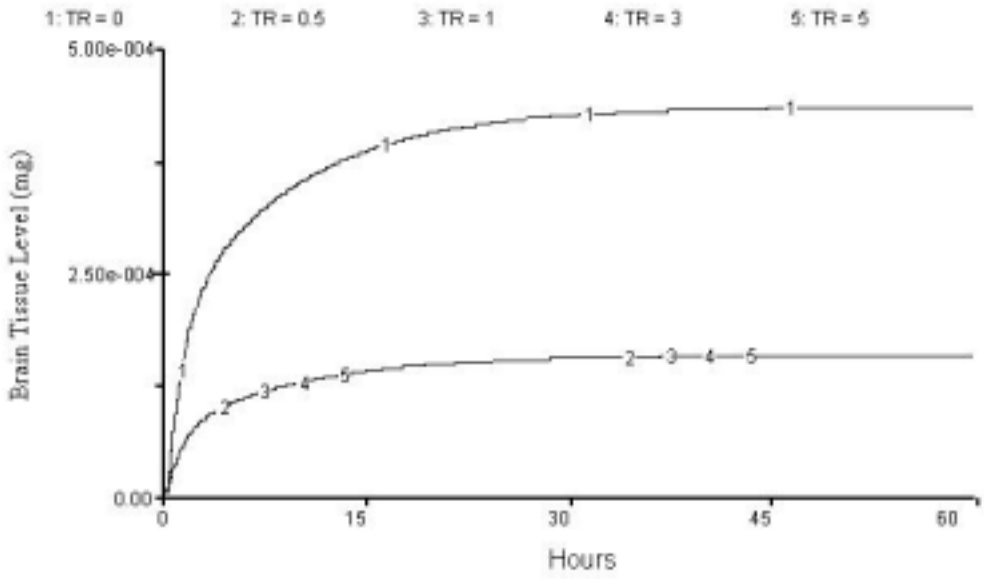


Figure 25. Scenario 4: Brain Tissue Level with Increasing Maximum Passive Diffusion Transfer Rates at Maximum Stress

from 0.5 and higher. In this case, the model is sensitive to lower values of the transfer rate. As in previous scenarios, the increasing passive diffusion transfer rate allows the chemical to leave the brain due to the increased amount of chemical in the brain as compared to the brain blood. Again, the steady state brain tissue level reached under these conditions is slightly higher than in Scenario 3. This phenomenon is due to the remaining BBB transport mechanisms acting at their associated maximum values.

These simulations demonstrate that there is indeed an effect of changing the maximum permeability values of each BBB transport mechanism. Although the model may be insensitive to certain ranges of the maximum permeability values, the brain tissue

level does increase under these conditions. The model behavior is similar to previous scenarios, as to which mechanism is most sensitive to the ranges tested. As discussed in Chapter III, twenty simulations were performed in this scenario. Appendix F contains the simulation results for the remaining maximum permeability values tested in Scenario 4.

Scenario 5

The next simulations include changing the graphical relationships of the four BBB mechanisms simultaneously under rest and stress, to demonstrate the combination of BBB mechanisms that is most important by the amount of chemical buildup in brain tissue, under rest conditions. Figure 26 below shows the linear combination of mediated and endothelial transport under increasing stress conditions.

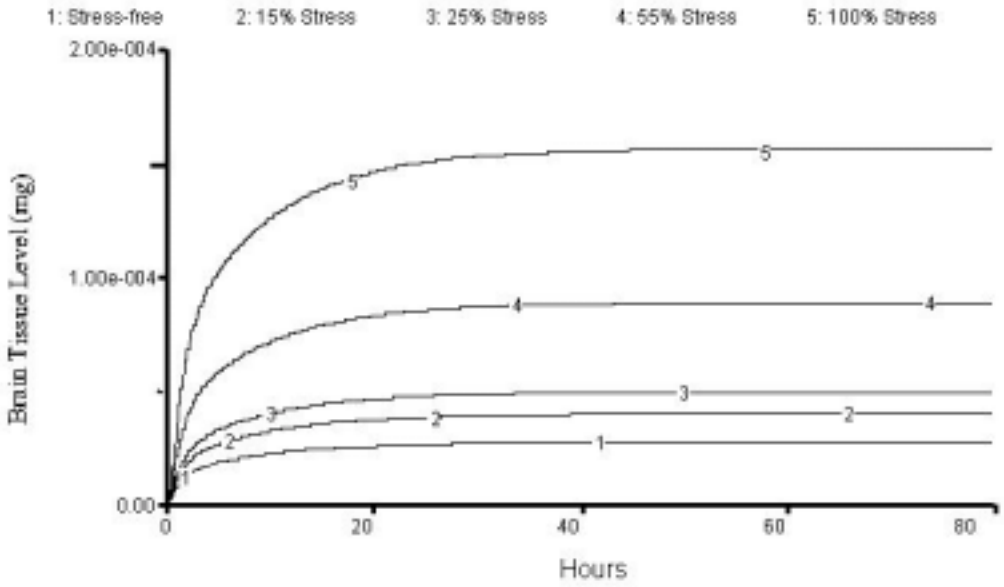


Figure 26. Scenario 5: Brain Tissue Level for Linearly Increasing Mediated Maximum Transport/ Endothelial Transport Combination for Increasing Stress

The behavior-sensitivity test is relevant in this scenario. The model output from this simulation was identical to Figure 23 of Scenario 3's mediated transport under increasing stress. This behavior points to the fact that endothelial transport has an insignificant effect on brain tissue level when combined with mediated transport. Figure 27 below shows the linear combination of passive diffusion and transcytosis under increasing

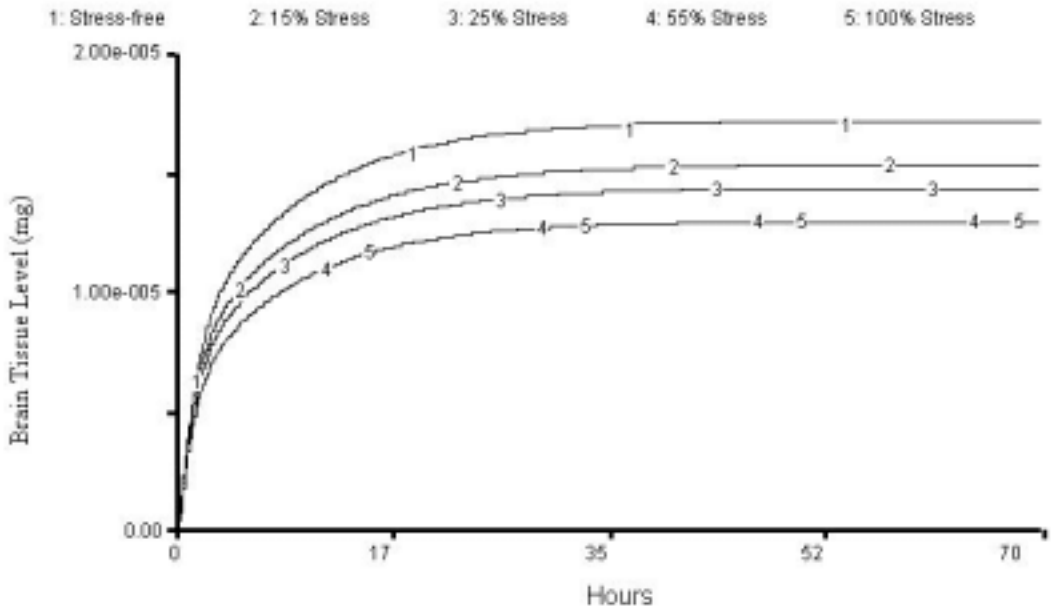


Figure 27. Scenario 5: Brain Tissue Level for Linearly Increasing Passive Diffusion Transfer Rate/ Transcytosis Combination for Increasing Stress

stress. The combination of these transport mechanisms yields an identical output compared to Figure 20 in Scenario 3. The model, which was insensitive to the ranges tested for the transcytotic flow fractions when tested individually, seems to have no distinguishable effect on passive diffusion. The combination again yields decreasing brain tissue levels up to 55% stress, upon which the brain tissue equilibrates. Because mediated transport is still acting in this scenario, the strength of the passive diffusion yet again prevents the brain tissue level from increasing.

This scenario was key in determining the strengths of the combinations of BBB transport mechanisms. The brain tissue levels increase with each combination involving

increasing mediated transport, while the brain tissue levels decrease with each combination involving increased passive diffusion and an unchanged mediated transport. The model was insensitive to the combination of transcytosis and endothelial transport. The number of simulations for this scenario was reduced to thirty from the original ninety that were anticipated, due to the lack of change in model output of three graphical relationships of stress factors tested in Scenario 3. Appendix G contains the simulation results for the remaining combinations tested in Scenario 5.

Scenario 6

The next simulations would again include changing the graphical relationships of the four BBB mechanisms simultaneously, under exercise and stressed conditions, to demonstrate the BBB mechanism that is most important by the amount of chemical buildup in brain tissue, under exercise and stressed conditions. Figure 28 below shows the linear combination of mediated and endothelial transport under increasing stress and

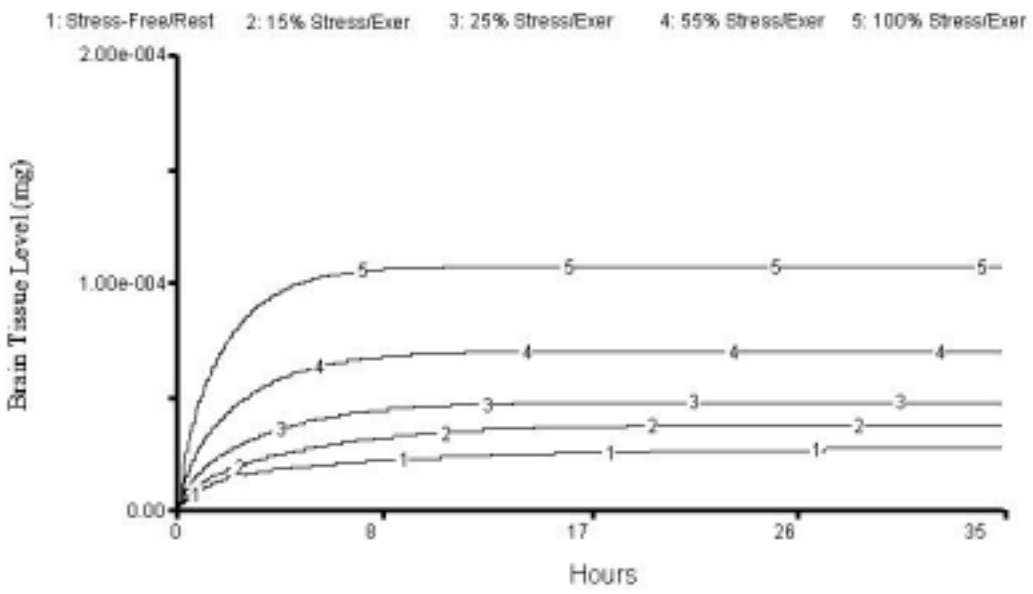


Figure 28. Scenario 6: Brain Tissue Level for Linearly Increasing Mediated Maximum Transport/ Endothelial Transport Combination for Increasing Stress and Exercise

exercise conditions. As in Scenario 5, the model is sensitive to this combination of BBB transport mechanisms. The model behavior is also sensitive to the increased blood flow due to exercise in combination with brain stress. The addition of exercise in the model causes the brain tissue level to reach steady state at lesser values, again resulting from increased metabolism and elimination.

Figure 29 below shows the linear combination of passive diffusion and transcytosis under increasing stress and exercise. As in Scenario 5, the model is sensitive

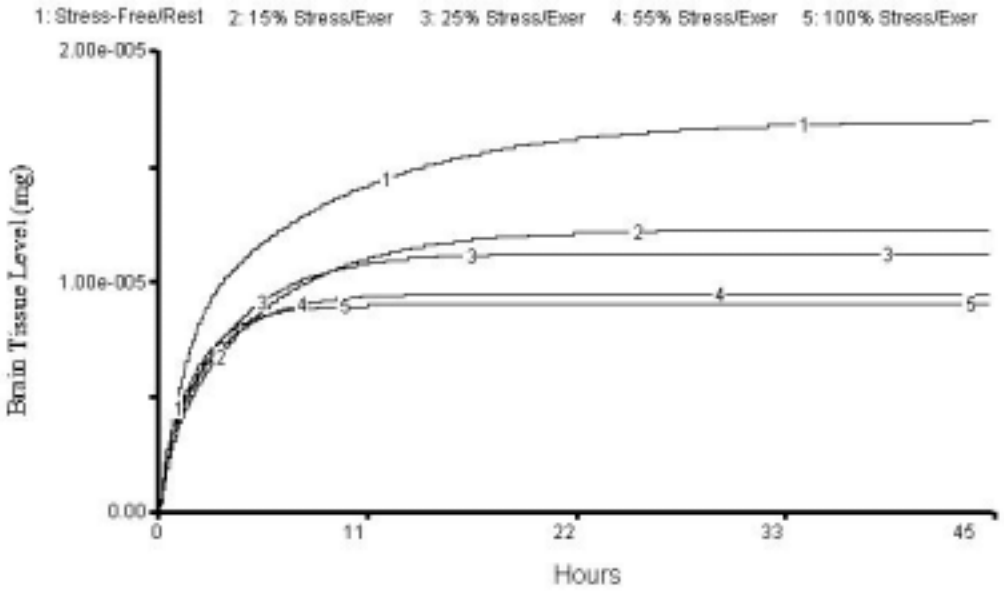


Figure 29. Scenario 6: Brain Tissue Level for Linearly Increasing Passive Diffusion Transfer Rate/ Transcytosis Combination for Increasing Stress and Exercise

to this combination of transport mechanisms. However, the model is now also sensitive to values above 55% stress and exercise. The initial kinetics of this model output are quite different when exercise is introduced. This model output is identical to Figure 18 in Scenario 2's brain tissue levels with increasing exercise under stress-free conditions, where there was no endothelial transport or transcytosis. The largest effect on brain tissue level occurs after 15% exercise. The brain tissue level begins to equilibrate above this exercise level. The physiological adjustments being made in the body may account for the increased time to reach steady state at 15% exercise. At this point, the fractional distributions of blood flow are being adjusted. At this point in the simulations where

both exercise and stressed conditions are introduced, it is extremely difficult to isolate the parameter that is causing this model behavior.

As in Scenario 5, it is still evident that any combinations involving increased mediated transport under both stressed and exercise conditions, cause the brain tissue levels to increase, while the remaining combinations in this scenario cause a decrease in brain tissue level. The number of simulations for this scenario was reduced to thirty from the original ninety that were anticipated, due to the lack of change in model output of three graphical relationships of stress factors tested in Scenario 3. Appendix H contains the simulation results for the remaining combinations tested in Scenario 6.

Scenario 7

The next simulations involved a sensitivity analysis for extreme ranges of a sampling of parameter values for the different chemicals simulated in the baseline scenario (Table 7), to assess the baseline sensitivity analysis under extreme exercise and stress conditions (100%). The three parameter values tested in this extreme-conditions scenario correspond to the first, third, and fifth simulation values tested in Scenario 1. Figure 30 below shows the liver tissue levels for increasing liver partition coefficients.

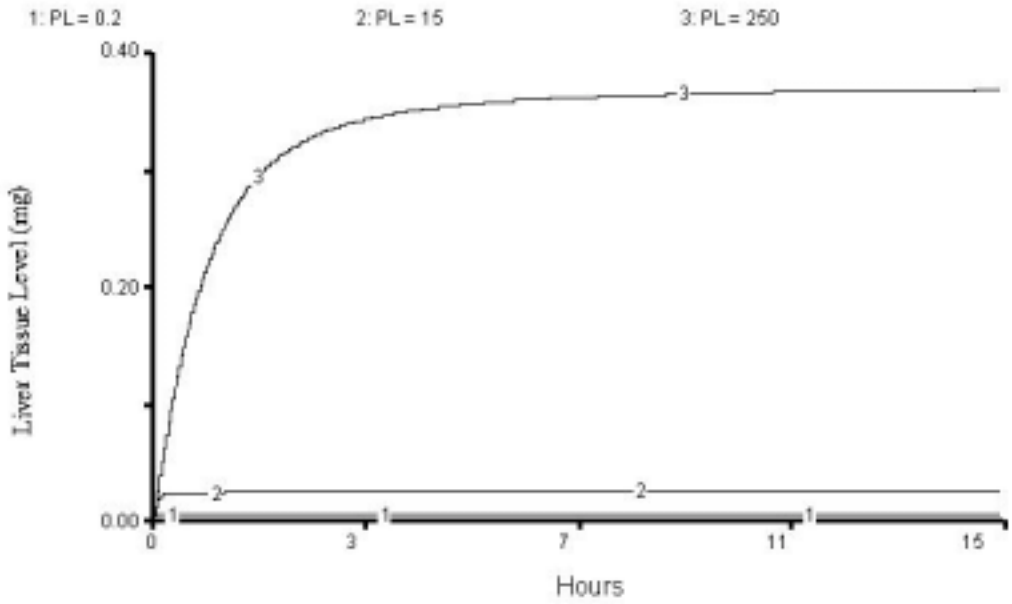


Figure 30. Scenario 7: Liver Tissue Level for Increasing Liver/Blood PC Values at Maximum Stress and Exercise

The dynamics of the system change under these extreme conditions. The final steady state values take longer to reach under these extreme conditions. Instead of the liver tissue level initially decreasing as in Scenario 1 when metabolism had not reached its maximum rate, the liver tissue in this case continually increases. This would indicate that the increased exercise and stress causes the liver metabolism to reach steady state almost instantaneously, as shown in Figure 31 below, which shows the liver metabolism with increasing liver partition coefficients. In addition, the liver tissue level reaches lower steady state values than in Figure 12 of Scenario 1. This may be a direct result of the decreased fractional blood distribution to the liver under exercise conditions.

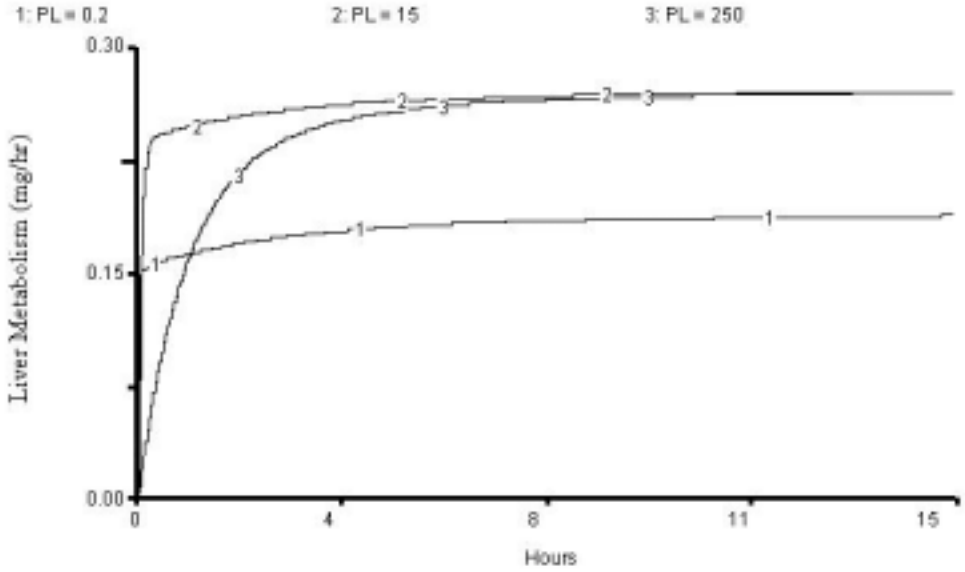


Figure 31. Scenario 7: Liver Metabolism for Increasing Liver/Blood PC Values at Maximum Stress and Exercise

The liver metabolizes chemical at a slower rate than shown in Figure 13 of Scenario 1. However the same general behavior is demonstrated here. At partition coefficients greater than 15, the metabolism no longer increases, indicating that its maximum rate has been reached. Because the partition coefficients increase from 15 to 250 in these simulations, the drastic increase in liver tissue levels of Figure 30 above can be expected.

Figure 32 below shows the brain tissue levels for increasing passive diffusion transfer rates. The behavior of an increased rate of reaching the final steady state

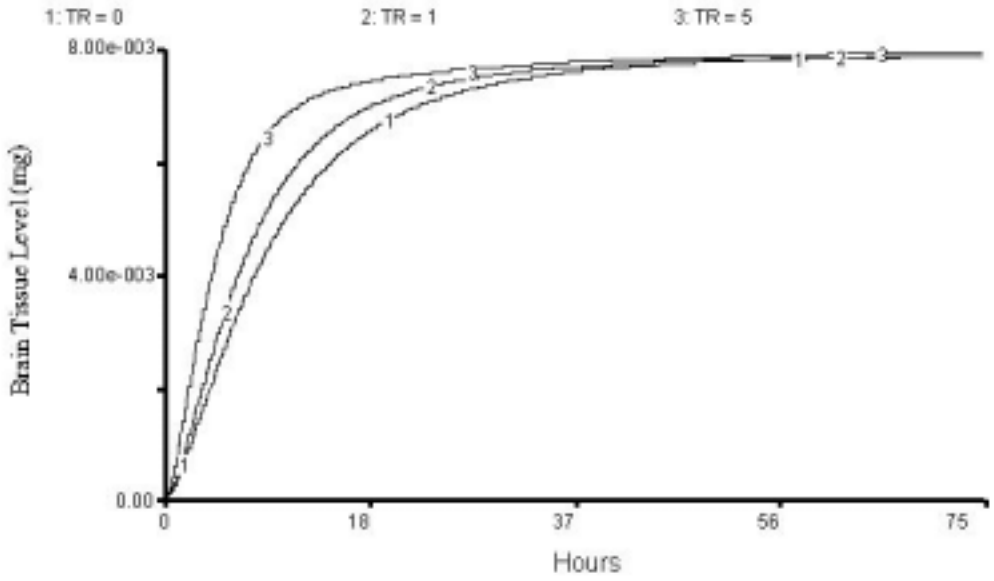


Figure 32. Scenario 7: Brain Tissue Level for Increasing Passive Diffusion Transfer Rate at Maximum Stress and Exercise

values in the brain tissue level is similar to Figure 17 in Scenario 1, except that lower final steady state values are achieved. In this scenario, mediated transport, endothelial transport, and transcytosis are also in effect, as well as the redistribution of blood flow to the other body tissues. It appears that the strengths of the combination of BBB transport parameters in this scenario are not significant enough to cause noticeable rises in brain tissue levels.

These simulations provided further insight into the behavior of chemicals with these specific parameters under extreme conditions, and whether their sensitivity changed. Forty-eight simulations accomplished this extreme-conditions scenario. Appendix I contains the simulation results for the remaining parameters tested in Scenario 7.

V. Conclusions and Recommendations

The purpose of this research was to explore the mechanisms of stress and chemical uptake and distribution in the brain. This research effort was based on the expansion of a model that predicts the changes that occur when stress and exercise are combined with chemical exposure. Physiologically-based pharmacokinetic (PBPK) models are excellent tools that can aid in the further understanding of stress and exercise when combined with chemical exposure. PBPK models can also be used to test hypotheses involved in the Gulf War Syndrome.

The PBPK model constructed in this thesis effort provides a foundation for the study of the physiological responses of the human body in response to stress and exercise, and whether these influences can enhance the effects of chemical exposure. This model has investigated the effects of the stressors of exercise and brain stress on the system behavior. A great amount of insight into this system has been gained throughout the system dynamics modeling stages of conceptualization, formulation, and testing. This chapter represents the final stage in the modeling process of implementation, which includes the translation of insights gained with respect to the research objectives. The strengths and weaknesses of the model in addition to possible future research will be addressed.

Research Objectives

The objectives of this research effort included the characterizations of the mechanisms by which stress alters the chemical uptake and distribution in the brain, and

investigate the overall internal interactions of the human body when affected by these conditions. The literature reviewed provided the basis of the BBB transport mechanisms that were tested in the model. Several studies have theorized the behaviors of the human body under numerous stressors affecting chemical uptake, specifically in the brain. Homeostasis is a critical aspect of the brain, which when influenced by these stressors, may be disrupted, causing harmful effects in the brain and other areas of the human body. A vast amount of literature was reviewed to learn more about the BBB transport mechanisms and the effects of stressors on chemical uptake.

This information was used to further develop a PBPK model that addressed the above topics. The quantitative description of this research was placed in the STELLA ® modeling program, using the system dynamics approach. This approach included the conceptualization, formulation, and testing stages. The quantitative description falls under the formulation stage. Reference modes and influence diagrams were developed in the conceptualization stages, while the model was tested over reasonable and extreme ranges during the testing phase. Confidence in the model and its resulting behaviors was gained throughout the various modeling scenarios. This confidence stemmed from an analysis of the various physiological mechanisms that changed simultaneously, when compared to the hypothesized changes in the real system.

A hypothesis can now be formed regarding the influences of stress and exercise on chemical exposure. This thesis effort demonstrates that exercise and stress do have an influence on the chemical concentrations in the body; and the direct influence of stress on the BBB transport mechanisms is an important component in buildup of chemical concentration in the brain. It has been demonstrated that mediated transport, passive

diffusion, endothelial transport, and transcytosis in the brain are all contributing causes to changing concentrations in the brain in this model. While endothelial transport and transcytosis contributed to changing brain concentrations, the values simulated to produce these changes may have been unrealistic. Once the actual contributors and strengths of BBB transport in the real system are determined, they may be modeled in a similar fashion. This research effort has demonstrated the complexity of the influences of stress and exercise on the different BBB transport mechanisms. The behavior of the model upon influence of these stressors was sensitive to the combination of parameters involved. Researchers and modelers must carefully consider each BBB mechanism that is in effect in the system when attempting to determine the actual causes of BBB chemical transport. Each chemical under study may have different transport mechanisms in effect during increased BBB permeability.

The relevant concern now is how to prevent possible harm to the brain, and how to predict the consequences of stressors, such as brain stress and exercise, on the human body. In order to predict the consequences of these stressors, the most important transport mechanisms involved must be understood.

Model Strengths and Limitations

Model Strengths

1. Provides insights and the groundwork for studying the effects of stress and exercise with chemical exposure.

2. Provides a simplified, but substantial basis for simulating the behavior of chemicals foreign to the human body, in the context of circulation throughout the system and buildup in brain tissue.

3. Demonstrates the complexity of model behavior upon the addition of stressors to the system, which allows modelers to observe the various associated systemic effects.

4. Suggests areas of future research that will prove important in the implementation phase of the model. The model can be used as an exploration tool of differing parameter sensitivities.

Model Limitations

1. Rests upon extensive generalities and assumptions of the physiological parameters and stress effects throughout the model. In addition to the unknown specific parameters for the chemical of interest, the various graphical stress relationships were assumed.

2. The stress response addressed the combination of stressors found in the literature review. The stress response was not separated into the various aspects of the stressors such as differing physical effects beyond exercise, and emotional effects. In addition, the causal links between stress and the BBB transport mechanisms was assumed.

3. The modeling software program had numerical integration limits which caused order of magnitude changes in tissue compartment chemical concentrations with varying time steps.

Areas of Further Research

1. Expand upon the brain compartment's transport processes in the model. Bi-directional transport for passive diffusion and unidirectional transport for mediated transport, endothelial transport, and transcytosis were assumed when testing the individual chemicals in the model. A different combination of directional flows may be more appropriate for other cases. The separation of the BBB transport mechanisms was a realistic but very simplified view of the real system. While endothelial transport and transcytosis were simplified as bulk flow processes, future modelers may be able to provide further detail on these mechanisms. The hormones as well as the BBB interactions on a cellular level involved in the various transport mechanisms when influenced by stress can be better characterized. In addition to the transport processes, different areas of the brain should be studied to determine the local concentration changes during chemical exposure.

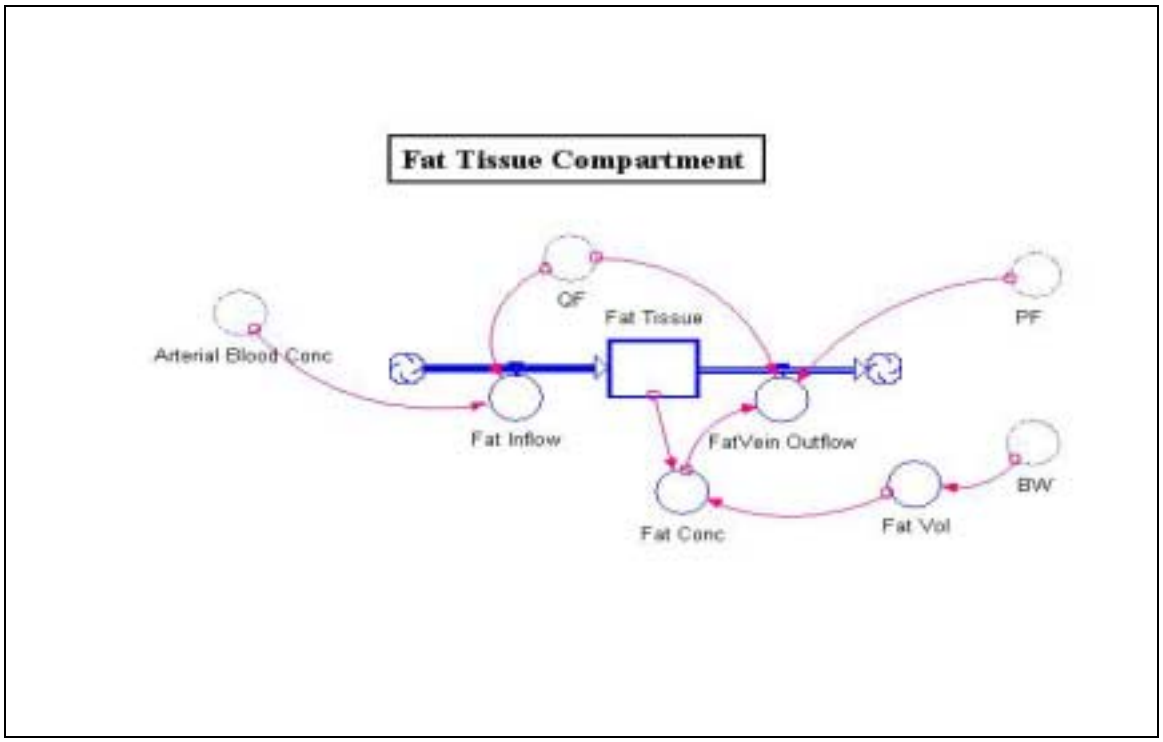
2. Explore the behavior of specific chemicals that are more relevant to deployment toxicology concerns. A chemical that was similar to PB was simulated in this model. However, when more detailed data is available in the literature, those specific chemical parameters should be used in the model to predict their effect in human tissue under the effects of various stressors. Varying chemical uptakes such as inhalation, oral, and intravenous, can also be studied using this model. The chemical behaviors in individuals with varying characteristics, such as body weight, may also provide further insight.

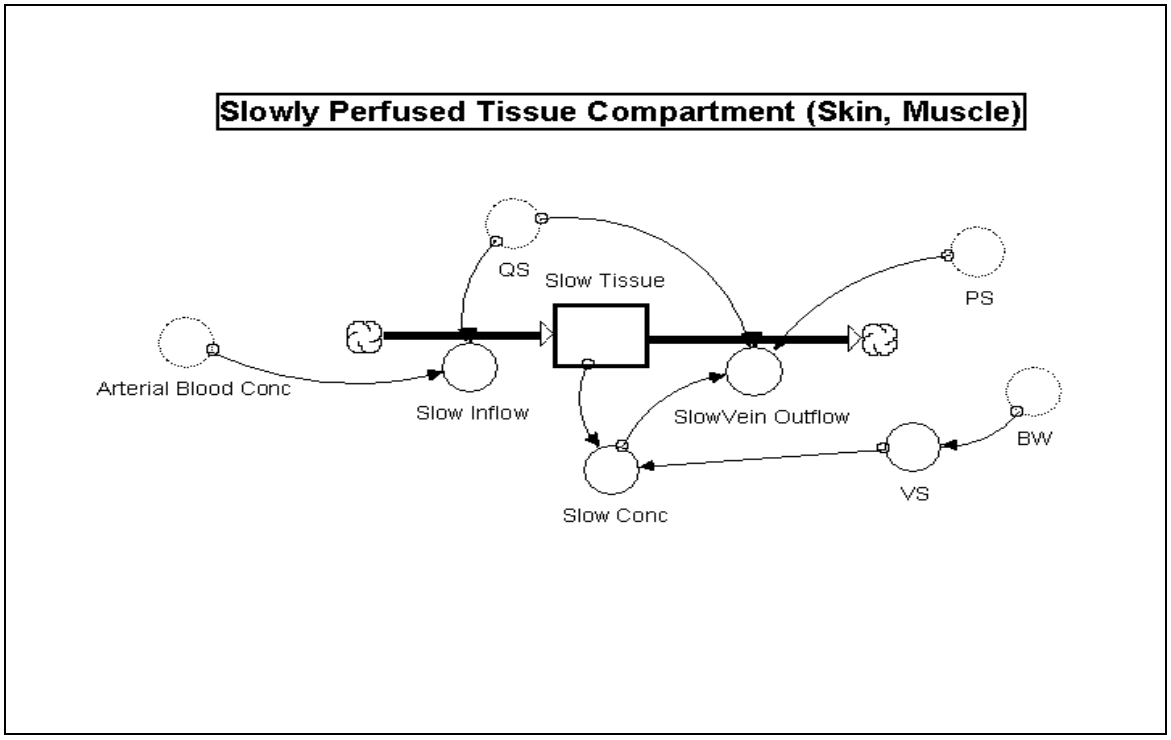
3. Add different aspects of stress such as mental, emotion, and other physical effects beyond exercise to the model. This model characterizes stress in a very general format. Differing stressors may have different physiological effects in different areas of the human body. These stressors may also have diverse hormones involved when activated. In addition, the various stress effects that directly apply to each BBB transport mechanism can be added.

Conclusions

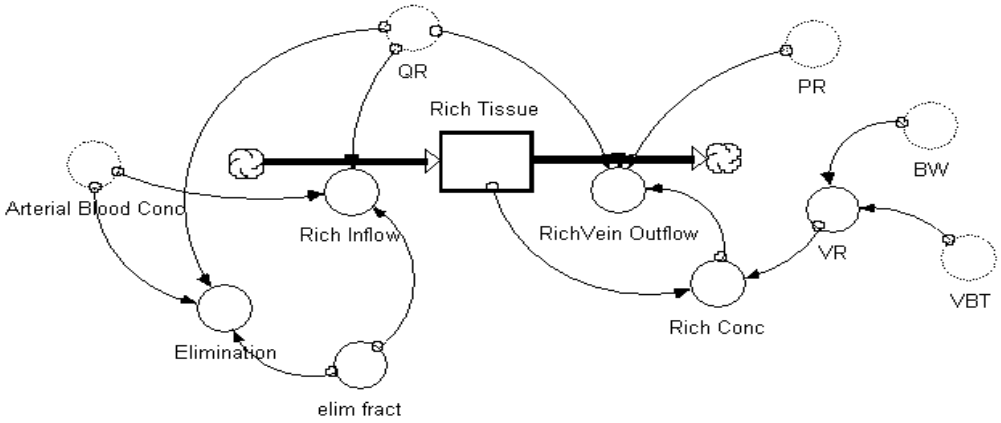
This model and the testing performed throughout the scenarios has laid the groundwork for future research and will further the ability of future modelers to determine the physiological effects of chemical exposure in combination with stress and exercise. The model developed is a valuable tool in demonstrating the complexity of the systemic behaviors upon addition of stressors. Although the main focus of the model scenarios was on the brain compartment, the different physiological behaviors produced in the other areas of the human body were readily observed. Insight into the strengths of the various BBB transport mechanisms was also gained. This information may assist the military in understanding the causes and effects of the Gulf War Syndrome and provide a visual demonstration of possible physiological behaviors associated with chemical exposure under a mixture of stressors.

Appendix A – Model Flow Diagram

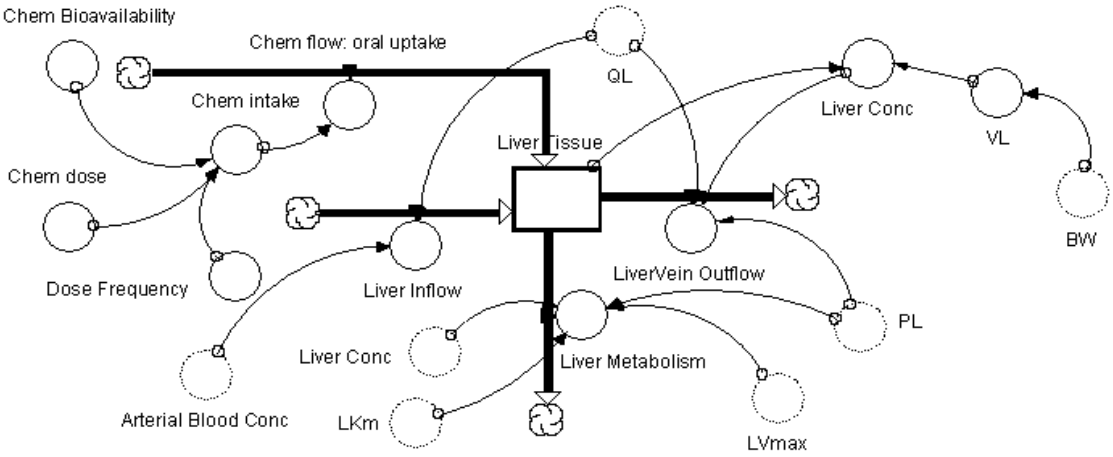


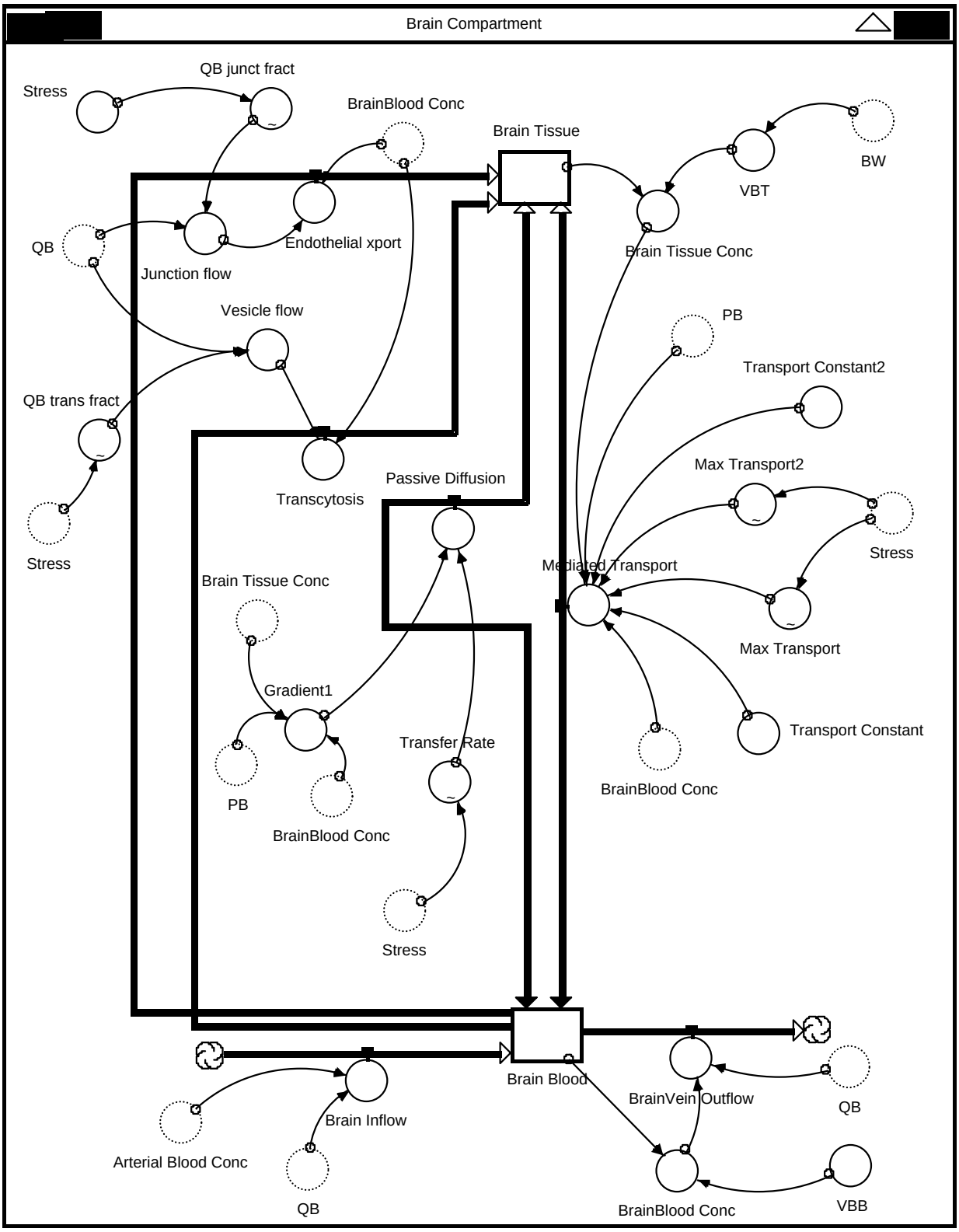


Richly Perfused Tissue Compartment (Kidneys)

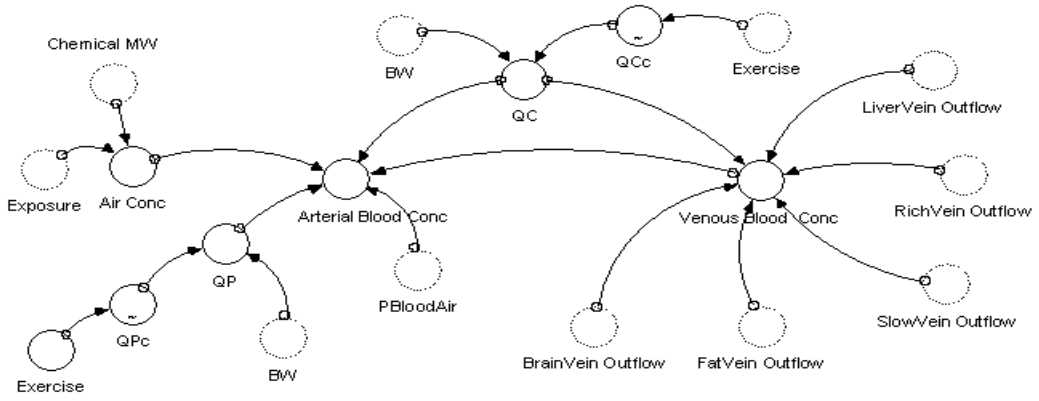


Liver (Metabolizing) Tissue Compartment

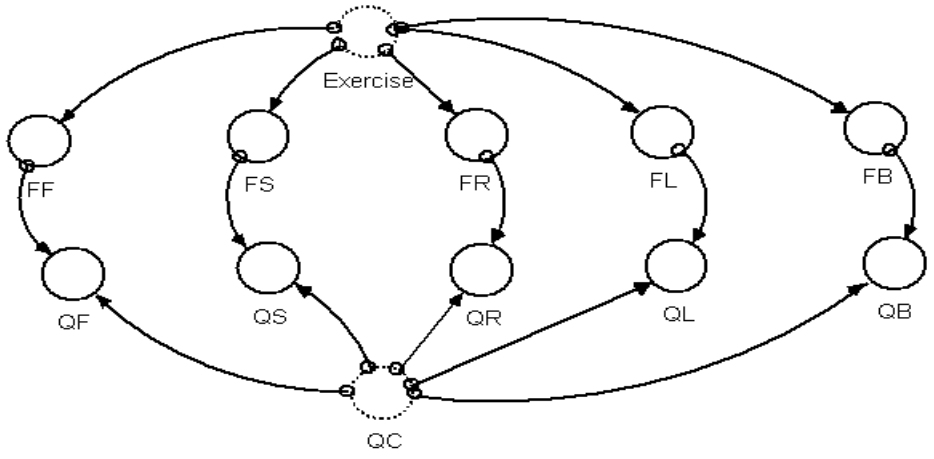


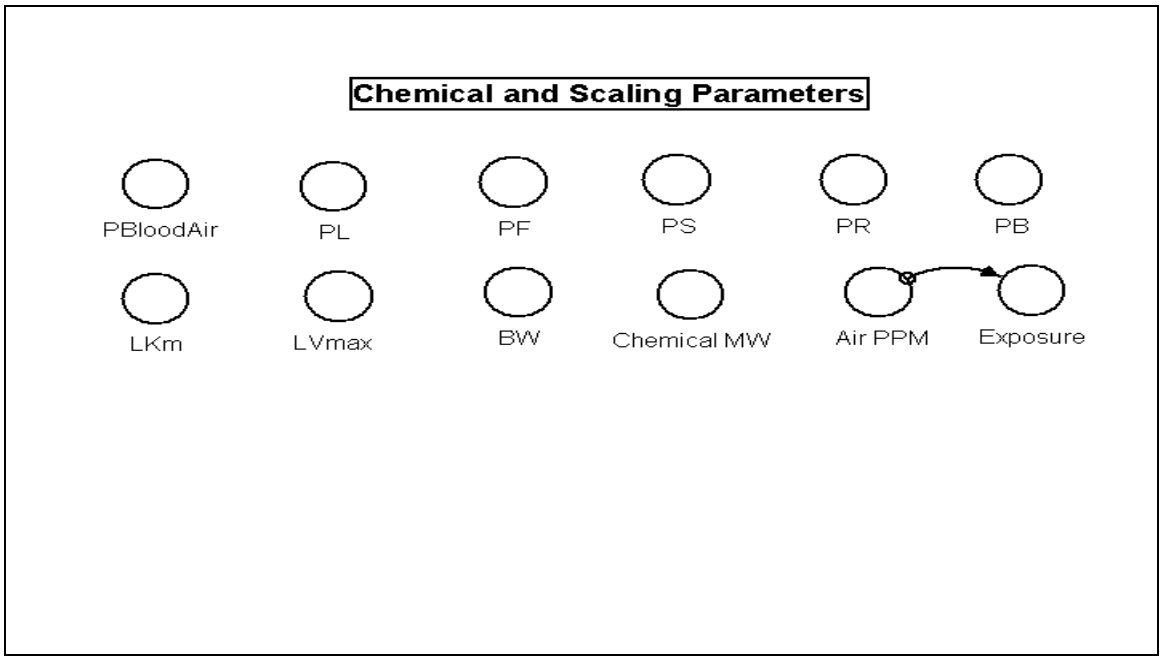

Brain Compartment
QB junct fract
Stress
BrainBlood Conc
Brain Tissue
BW
VBT
Endothelial xport
QB
Junction flow
Brain Tissue Conc
Vesicle flow
PB
Transport Constant2
QB trans fract
Max Transport2
Passive Diffusion
Transcytosis
Stress
Stress
Mediated Transport
Brain Tissue Conc
Max Transport
Gradient1
Transport Constant
Transfer Rate
BrainBlood Conc
PB
BrainBlood Conc
Stress
Brain Blood
BrainVein Outflow
QB
Brain Inflow
Arterial Blood Conc
QB
BrainBlood Conc
VBB

Blood Flow



Fractional Distributions




Chemical and Scaling Parameters
PBloodAir
PL
PF
PS
PR
PB
LKm
LVmax
BW
Chemical MW
Air PPM
Exposure

Appendix B – Model Equations and Documentation

Blood Flow

Parameters

Air_Conc = Exposure*Chemical_MW/24450
DOCUMENT: This is the air concentration that an individual is exposed to. The molecular weight of the chemical must be converted into parts per million (PPM).

Arterial_Blood_Conc =
(QP*Air_Conc+QC*Venous_Blood__Conc)/((QP/PBloodAir)+QC)
DOCUMENT: This represents the arterial blood flow, which connects each of the tissue groups or compartments in this particular PBPK model.

Exercise = 100
DOCUMENT: Exercise values will be assigned a percentage value based on the level of activity.
Rest = 0
Moderate Activity = 15%
Heavy Activity = 25%
Strenous Activity = 55%
Maximum = 100%

QC = QCc*(BW^.74)
DOCUMENT: QC=Cardiac Output L/hr) that has been scaled down to a particular bodyweight. 'Physiological Parameter Values for PBPK Models' (December 1994) - pg. 44. The following cardiac outputs are an example of the changing values for a 70 kg male (QCc = 13.45).
Resting Individuals = 5.2 L/min = 312 L/hr
Moderate Exercise = 9.9 L/min = 594 L/hr
Heavy Exercise = 15 L/min = 900 L/hr
Strenuous Exercise = 21 L/min = 1260 L/hr
Maximum = 30 L/min = 1800 L/hr

QP = QPc*(BW^.74)
DOCUMENT: QP=Alveolar Ventilation (L/hr) that has been scaled down to a particular bodyweight. 'Physiological Parameter Values for PBPK Models' (December 1994) - pg. 79 The following ventilation rates are an example of the changing values for a 70 kg male (QPc = 12.9).
Resting Individuals = 5 L/min = 300 L/hr
Moderate Exercise = 25 L/min = 1500 L/hr
Heavy Exercise = 35 L/min = 2100 L/hr
Strenuous Exercise = 65 L/min = 3900 L/hr
Maximum = 90 L/min = 5400 L/hr

Venous_Blood__Conc =
(FatVein_Outflow+LiverVein_Outflow+RichVein_Outflow+SlowVein_Outflow+Brain Vein_Outflow)/(QC)
DOCUMENT: This represents the venous blood flow, which also connects each of the tissue groups or compartments in this particular PBPK model.

Graphs
QCc = GRAPH(Exercise)
(0.00, 13.4), (5.00, 16.3), (10.0, 20.6), (15.0, 25.6), (20.0, 31.0), (25.0, 38.8), (30.0, 42.3), (35.0, 45.4), (40.0, 48.1), (45.0, 51.6), (50.0, 53.2), (55.0, 54.3), (60.0, 57.1), (65.0, 59.8), (70.0, 63.6), (75.0, 67.5), (80.0, 70.6), (85.0, 71.8), (90.0, 73.0), (95.0, 74.9), (100, 77.6)
DOCUMENT: QCc = Cardiac Output Scaling Factor. A linear relationship between exercise and the cardiac output scaling factors was formed based on the initial QCc (15) value found in 'Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans' by Allen and Fisher (1993)'. A graph was important in forming this linear relationship due to the fact that the scaling factor will change for different levels of exercise, which will then effect cardiac output differently for different bodyweights. The following are the scaling factors for each category of exercise.
When Exercise = 0 (Rest) QCc = 13.45
When Exercise = 15 (Moderate) QCc = 25.61
When Exercise = 25 (Heavy) QCc = 38.81
When Exercise = 55 (Strenuous) QCc = 54.33
When Exercise = 100 (Maximum) QCc = 77.62

QPc = GRAPH(Exercise)
(0.00, 12.9), (5.00, 24.5), (10.0, 48.9), (15.0, 64.7), (20.0, 80.3), (25.0, 90.6), (30.0, 104), (35.0, 113), (40.0, 122), (45.0, 141), (50.0, 156), (55.0, 168), (60.0, 175), (65.0, 182), (70.0, 187), (75.0, 194), (80.0, 200), (85.0, 208), (90.0, 217), (95.0, 222), (100, 233)
DOCUMENT: QPc = Alveolar Ventilation Scaling Factor. A linear relationship between exercise and the alveolar ventilation scaling factors was formed based on the initial QPc (12.9) value found in 'Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans' by Allen and Fisher (1993)'. A graph was important in forming this linear relationship due to the fact that the scaling factor will change for different levels of exercise, which will then effect the ventilation rate differently for different bodyweights. The following are the scaling factors for each category of exercise.
When Exercise = 0 (Rest) QPc = 12.9
When Exercise = 15 (Moderate) QPc = 64.68
When Exercise = 25 (Heavy) QPc = 90.56
When Exercise = 55 (Strenuous) QPc = 168.18
When Exercise = 100 (Maximum) QPc = 232.86

Brain Compartment

Stock
Brain_Blood(t) = Brain_Blood(t - dt) + (Brain_Inflow - BrainVein_Outflow - Passive_Diffusion - Endothelial_xport - Transcytosis - Mediated_Transport) * dt
INIT Brain_Blood = 0
DOCUMENT: Assumption - this brain blood reservoir is a homogenous well-mixed compartment, representing the average concentration of the arterial and venous concentrations. This reservoir represents the accumulation point in the brainblood where arterial blood flow enters and venous blood flow exits.

Inflows
Brain_Inflow = Arterial_Blood_Conc*QB
DOCUMENT: The fraction of the arterial blood flow which enters the brainblood portion of the brain tissue compartment.

Outflows
BrainVein_Outflow = QB*BrainBlood_Conc
DOCUMENT: This represents the fraction of venous blood flow which exits the brainblood portion of the brain tissue compartment.

Transport Mechanisms
Passive_Diffusion = Gradient1*Transfer_Rate
Endothelial_xport = BrainBlood_Conc * Junction_flow
Transcytosis = BrainBlood_Conc * Vesicle_flow
Mediated_Transport =
((Max_Transport*BrainBlood_Conc)/(Transport_Constant+BrainBlood_Conc))-
((Max_Transport2*Brain_Tissue_Conc/PB)/(Transport_Constant2+Brain_Tissue_Conc/
PB))

Stock
Brain_Tissue(t) = Brain_Tissue(t - dt) + (Mediated_Transport + Passive_Diffusion + Endothelial_xport + Transcytosis) * dt
INIT Brain_Tissue = 0
DOCUMENT: This reservoir represents the accumulation point in the brain tissue where there is a flow of material (passive diffusion, trannscytosis, endothelial transport, and mediated transport) passing between the brain tissue and the brain blood.

Parameters
BrainBlood_Conc = Brain_Blood/VBB
DOCUMENT: This connector represents the concentration of chemical in the brain blood.

Brain_Tissue_Conc = Brain_Tissue/VBT
DOCUMENT: This connector represents the concentration of chemical in the brain tissue stock.

Gradient1 = (BrainBlood_Conc-(Brain_Tissue_Conc/PB))

Junction_flow = QB * QB_junct_fract

Transport_Constant = 3.5

Transport_Constant2 = 3.5

VBB = 30

VBT = .02*BW
DOCUMENT: The brain tissue volume is scaled so that any bodyweight can be used to find the behavior of chemical concentrations in a particular sized individual.
The value of .02 was found in - 'Physiological Parameter Values for PBPK Models' (A report prepared by the International Life Sciences Institute Risk Science Institute. December 1994 - Pg. 25).

Vesicle_flow = QB * QB_trans_fract

DOCUMENT: Stress Relationships were simulated for each BBB transport mechanism as linearly increasing, exponentially increasing, or concave up. Refer to the specific testing scenario for exact ranges. These transport mechanisms were tested from the baseline values (0% stress) to the maximum values (100% stress)

Max_Transport = GRAPH(Stress)
(0.00, 0.5), (10.0, 0.5), (20.0, 0.5), (30.0, 0.5), (40.0, 0.5), (50.0, 0.5), (60.0, 0.5), (70.0, 0.5), (80.0, 0.5), (90.0, 0.5), (100, 0.5)

Max_Transport2 = GRAPH(Stress)
(0.00, 0.00), (10.0, 0.00), (20.0, 0.00), (30.0, 0.00), (40.0, 0.00), (50.0, 0.00), (60.0, 0.00), (70.0, 0.00), (80.0, 0.00), (90.0, 0.00), (100, 0.00)

QB_junct_fract = GRAPH(Stress)
(0.00, 1e-010), (10.0, 1e-010), (20.0, 1e-010), (30.0, 1e-010), (40.0, 1e-010), (50.0, 1e-010), (60.0, 1e-010), (70.0, 1e-010), (80.0, 1e-010), (90.0, 1e-010), (100, 1e-010)

QB_trans_fract = GRAPH(Stress)
(0.00, 1e-010), (10.0, 1e-010), (20.0, 1e-010), (30.0, 1e-010), (40.0, 1e-010), (50.0, 1e-010), (60.0, 1e-010), (70.0, 1e-010), (80.0, 1e-010), (90.0, 1e-010), (100, 1e-010)

Transfer_Rate = GRAPH(Stress)
(0.00, 1.00), (10.0, 1.00), (20.0, 1.00), (30.0, 1.00), (40.0, 1.00), (50.0, 1.00), (60.0, 1.00), (70.0, 1.00), (80.0, 1.00), (90.0, 1.00), (100, 1.00)

Chemical and Scaling Parameters
Air_PPM = 0
DOCUMENT: This value is the concentration of a particular chemical exposure in parts per million (PPM). For this model, PB and similar chemicals are not airborne.

BW = 70
DOCUMENT: The bodyweight of the individual used in the simulation of this model.

Chemical_MW = Not needed here unless simulating an airborne exposure

Exposure = Air_PPM-STEP(Air_PPM,0)
DOCUMENT: The step function used in this parameter defines the length of exposure to the chemical concentration in the air. In this case the individual is not exposed to the Air PPM.

LKm = 1.5
DOCUMENT: Liver Km - Michaelis constant (mg/L). The Vmax value for the liver can now be computed for any bodyweight.

LVmax = 500
DOCUMENT: The scaling parameter of 14.9 was used to scale the Liver Vmax (mg/hr) - maximum rate of enxymatic (saturable) metabolism. The Vmax value for the liver can now be computed for any bodyweight.

Formula: 14.9 * (BW^7) for changing body weights

PB = 15
DOCUMENT: Brain-Blood Partition Coefficient
This value is an assumption at this time - no documentation in the literature.
PBloodAir = 75
DOCUMENT: Blood-Air Partition Coefficient

PF = .002
DOCUMENT: Fat-Blood Partition Coefficient

PL = 15
DOCUMENT: Liver-Blood Partition Coefficient

Pyridostigmine Bromide (from Golomb - gulflink) = 8

PR = 15
DOCUMENT: Richly Perfused-Blood Partition Coefficient
Pyridostigmine Bromide (from Golomb - gulflink) = 15

PS = 15
DOCUMENT: Slowly Perfused-Blood Partition Coefficient

Fat Tissue Compartment

Stock
Fat_Tissue(t) = Fat_Tissue(t - dt) + (Fat_Inflow - FatVein_Outflow) * dt
INIT Fat_Tissue = 0
DOCUMENT: This stock represents the accumulation point in the fat tissue where arterial blood flow enters and venous blood flow exits.

Inflows
Fat_Inflow = Arterial_Blood_Conc*QF
DOCUMENT: The fraction of the arterial blood flow which enters the fat tissue compartment.

Outflows
FatVein_Outflow = QF*Fat_Conc/PF
DOCUMENT: This represents the fraction of venous blood flow which exits the fat tissue.

Parameters
Fat_Conc = Fat_Tissue/Fat_Vol
DOCUMENT: This connector represents the concentration of chemical in the fat stock or tissue.

Fat_Vol = .19*BW
DOCUMENT: The fat volume is scaled so that any bodyweight can be used to find the behavior of chemical concentrations in a particular sized individual.
Value of .19 found in - Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans' by Allen and Fisher (1993)

Fractional Distributions

FB = IF(Exercise=0)THEN(.12)ELSE(.12)
DOCUMENT: This is the fractional distribution of the blood flow rate to the brain tissue compartment. The distributions to the different tissues will change with the addition of stress or exercise.
'Physiological Parameter Values for PBPK Models' (December 1994) - Value found on page 51 (the human brain receives about 12% of the cardiac output in the human body and Distribution does not change during exercise)
Resting Individual = 12%
Exercise = 12%

FF = IF(Exercise=0)THEN(.05)ELSE(.03)
DOCUMENT: This is the fractional distribution of the blood flow rate to the fat tissue compartment. The distributions to the different tissues will change with the addition of stress or exercise.
'Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans' by Allen and Fisher (1993) - Resting value
'Human Circulation Regulation During Physical Stress' by Rowell (1986) - Exercise value found on page 235 (Distribution decreases by 40% during exercise)
Resting Individual = 5%
Exercise = 3%

FL = IF(Exercise=0)THEN(.26)ELSE(.13)
DOCUMENT: This is the fractional distribution of the blood flow rate to the liver tissue compartment. The distributions to the different tissues will change with the addition of stress or exercise.
'Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans' by Allen and Fisher (1993) - Resting value
'Human Circulation Regulation During Physical Stress' by Rowell (1986) - Exercise value found on page 240 (Distribution decreases by 50% during exercise)
Resting Individual = 26%
Exercise = 13%

FR = IF(Exercise=0)THEN(.32)ELSE(.32)
DOCUMENT: This is the fractional distribution of the blood flow rate to the richly perfused tissue compartment. The distributions to the different tissues will change with the addition of stress or exercise.
'Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans' by Allen and Fisher (1993) - Resting value
'Human Circulation Regulation During Physical Stress' by Rowell (1986) - Exercise value on page 240 (Distribution does not change during exercise)
Resting Individual = 32%
Exercise = 32%

FS = IF(Exercise=0)THEN(.25)ELSE(.40)
DOCUMENT: This is the fractional distribution of the blood flow rate to the slowly perfused tissue compartment. The distributions to the different tissues will change with the addition of stress or exercise.
'Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans' by Allen and Fisher (1993) - Resting value
'Human Circulation Regulation During Physical Stress' by Rowell (1986) - Distribution increases by 62.5% during exercise
Resting Individual = 25%
Exercise = 40%

QB = FB*QC
DOCUMENT: Blood flow to the brain tissue group. This is calculated by multiplying the total cardiac output by the particular fraction to each tissue.

QF = FF*QC
DOCUMENT: Blood flow rate to the fat tissue group. This is calculated by multiplying the total cardiac output by the particular fraction to each tissue.

QL = FL*QC
DOCUMENT: Blood flow to the liver tissue group. This is calculated by multiplying the total cardiac output by the particular fraction to each tissue.

QR = FR*QC
DOCUMENT: Blood flow to the richly perfused tissue group. This is calculated by multiplying the total cardiac output by the particular fraction to each tissue.

QS = FS*QC
DOCUMENT: Blood flow rate to the slowly perfused tissue group. This is calculated by multiplying the total cardiac output by the particular fraction to each tissue.

Liver (Metabolizing) Tissue Compartment

Stock
Liver_Tissue(t) = Liver_Tissue(t - dt) + (Liver_Inflow + Chem_flow:_oral_uptake - LiverVein_Outflow - Liver_Metabolism) * dt
INIT Liver_Tissue = 0
DOCUMENT: This stock represents the accumulation point in the liver tissue where arterial blood flow enters and venous blood flow exits.

Inflows

Liver_Inflow = Arterial_Blood_Conc*QL
DOCUMENT: The fraction of the arterial blood flow which enters the liver tissue compartment.

Chem_flow:_oral_uptake = Chem_intake

OUTFLOWS:
LiverVein_Outflow = QL*Liver_Conc/PL
DOCUMENT: This represents the fraction of venous blood flow which exits the liver tissue.

Liver_Metabolism = (LVmax*Liver_Conc/PL)/(LKm+Liver_Conc/PL)
DOCUMENT: Metabolism was assumed to occur in the liver tissue for this particular model. The saturable metabolic transformation of any particular chemical in the liver was defined using the Michaelis-Menten equation with the bio-chemical constants Vmax and Km.

Chem_Bioavailability = .1
DOCUMENT: A 10% bioavailabilty of the chemicals simulated was assumed

Chem_dose = 30
DOCUMENT: 30 mg PB tablets taken by soldiers in Gulf War – oral dosing

Chem_intake = Chem_Bioavailability * (Chem_dose / Dose_Frequency)
DOCUMENT: 30 mg doses 3 times per day equates to an intravenous dose of 3.75mg PB

Dose_Frequency = 8
DOCUMENT: PB tablets taken every 8 hours

Liver_Conc = Liver_Tissue/VL
DOCUMENT: This connector represents the concentration of chemical in the liver stock or tissue.

VL = .026*BW
DOCUMENT: The liver volume is scaled so that any bodyweight can be used to find the behavior of chemical concentrations in a particular sized individual.
Value of .026 found in - Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans' by Allen and Fisher (1993)

Richly Perfused Tissue Compartment (Kidneys)

Stock
Rich_Tissue(t) = Rich_Tissue(t - dt) + (Rich_Inflow - RichVein_Outflow) * dt
INIT Rich_Tissue = 0
DOCUMENT: This stock represents the accumulation point in the richly perfused tissue where arterial blood flow enters and venous blood flow exits.

Inflows
Rich_Inflow = Arterial_Blood_Conc*QR*(1-elim_fract)
DOCUMENT: The fraction of the arterial blood flow which enters the richly perfused tissue compartment.

Outflows
RichVein_Outflow = QR*Rich_Conc/PR
DOCUMENT: This represents the fraction of venous blood flow which exits the richly perfused tissue.

Elimination = Arterial_Blood_Conc*elim_fract*QR
DOCUMENT: Kidney elimination is an important excretion route of PB-like chemicals

elim_fract = .35

Rich_Conc = Rich_Tissue/VR
DOCUMENT: This connector represents the concentration of chemical in the richly perfused stock or tissue.

VR = (.05*BW)-VBT
DOCUMENT: The richly perfuse volume is scaled so that any bodyweight can be used to find the behavior of chemical concentrations in a particular sized individual. The brain tissue compartment volume is also subtracted from this value since the brain is a separate compartment in this model and should not be included in the richly perfused volume. Value of .05 found in - Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans' by Allen and Fisher (1993)

<u>Slowly Perfused Tissue Compartment (Skin, Muscle)</u>

Stock
Slow_Tissue(t) = Slow_Tissue(t - dt) + (Slow_Inflow - SlowVein_Outflow) * dt
INIT Slow_Tissue = 0
DOCUMENT: This stock represents the accumulation point in the slowly perfused tissue where arterial blood flow enters and venous blood flow exits.

Inflows

Slow_Inflow = Arterial_Blood_Conc*QS
DOCUMENT: The fraction of the arterial blood flow which enters the slowly perfused tissue compartment.

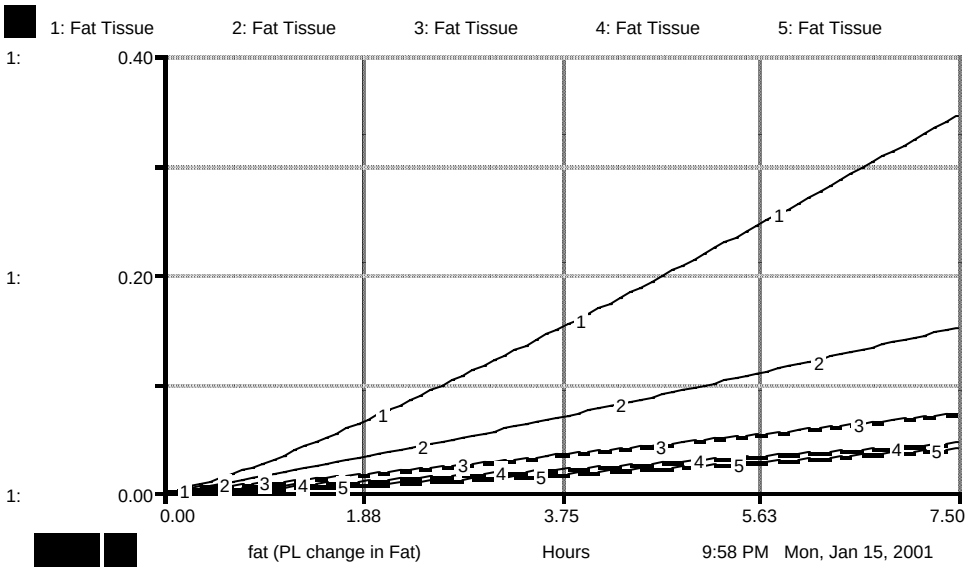
Outflows
SlowVein_Outflow = QS*Slow_Conc/PS
DOCUMENT: This represents the fraction of venous blood flow which exits the slowly perfused tissue.

Slow_Conc = Slow_Tissue/VS
DOCUMENT: This connector represents the concentration of chemical in the slowly perfused stock or tissue.

VS = .62*BW
DOCUMENT: The slowly perfused volume is scaled so that any bodyweight can be used to find the behavior of chemical concentrations in a particular sized individual.
Value of .62 found in - Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans' by Allen and Fisher (1993)

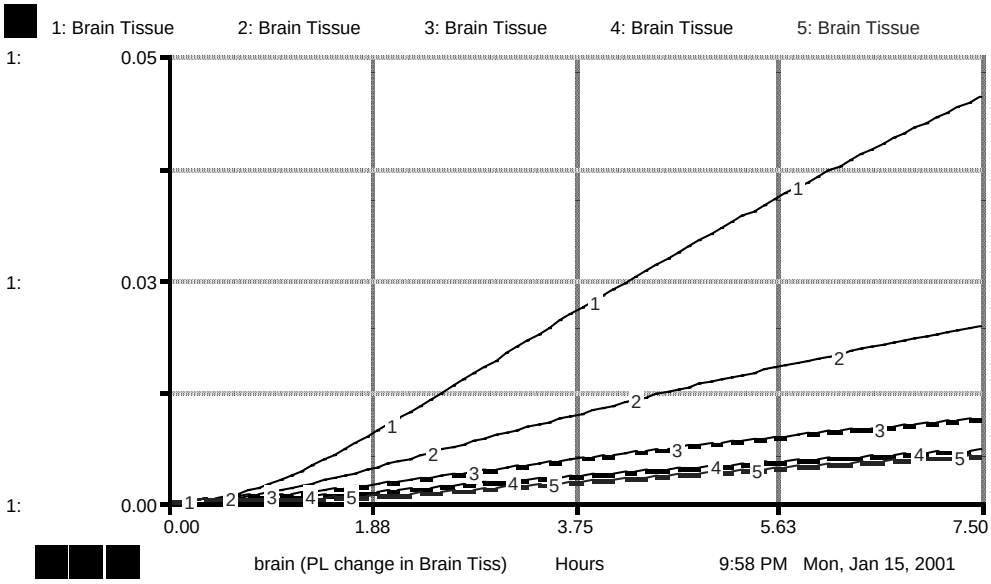
Appendix C – Scenario 1

1: PL=0.2 2: PL=8 3: PL=15 4: PL=75 5: PL=250



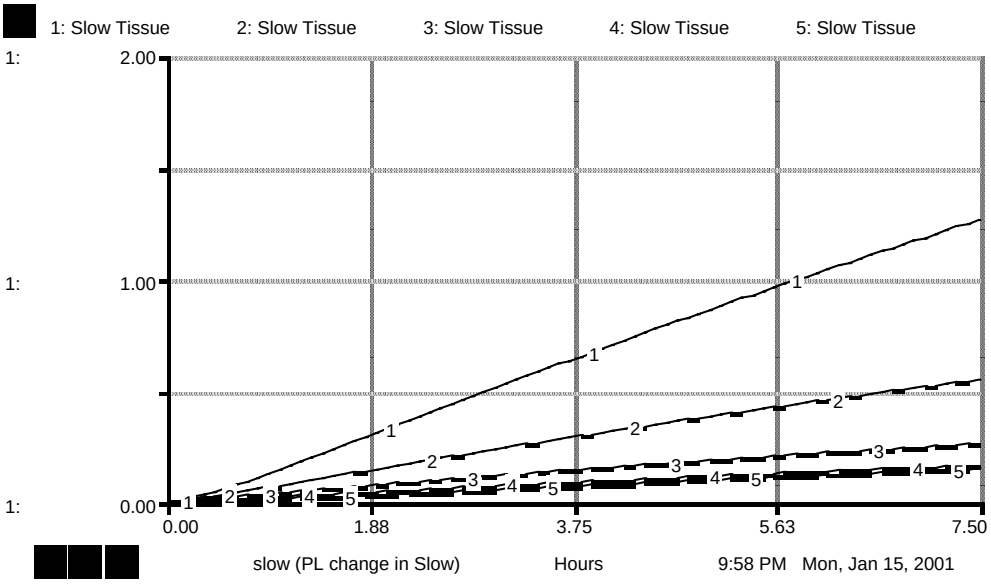
Fat Tissue Level (mg) for Increasing Liver/Blood PC Values

1: PL=0.2 2: PL=8 3: PL=15 4: PL=75 5: PL=250



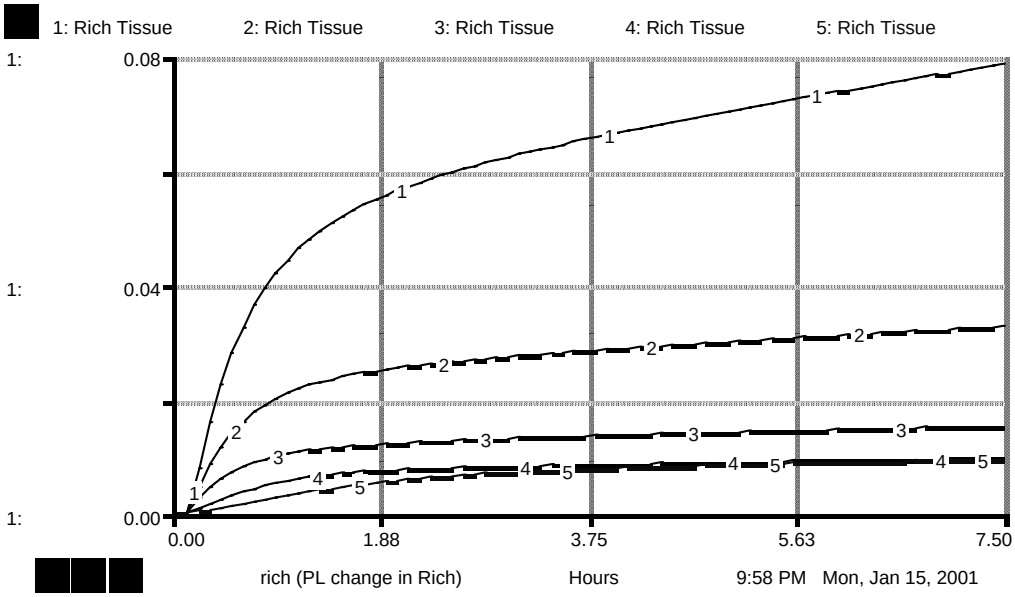
Brain Tissue Level (mg) for Increasing Liver/Blood PC Values

1: PL=0.2 2: PL=8 3: PL=15 4: PL=75 5: PL=250



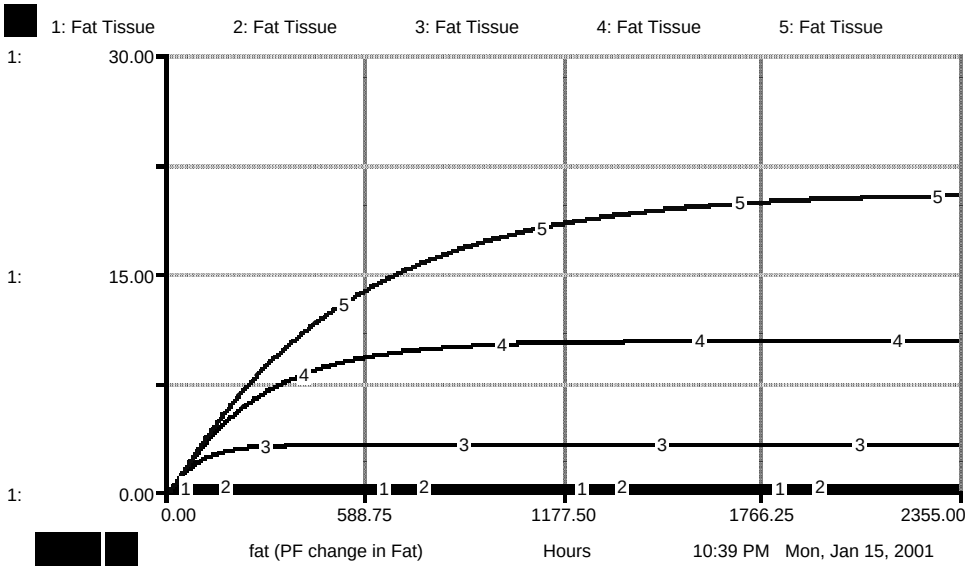
Slowly Perfused Tissue Level (mg) for Increasing Liver/Blood PC Values

1: PL=0.2 2: PL=8 3: PL=15 4: PL=75 5: PL=250



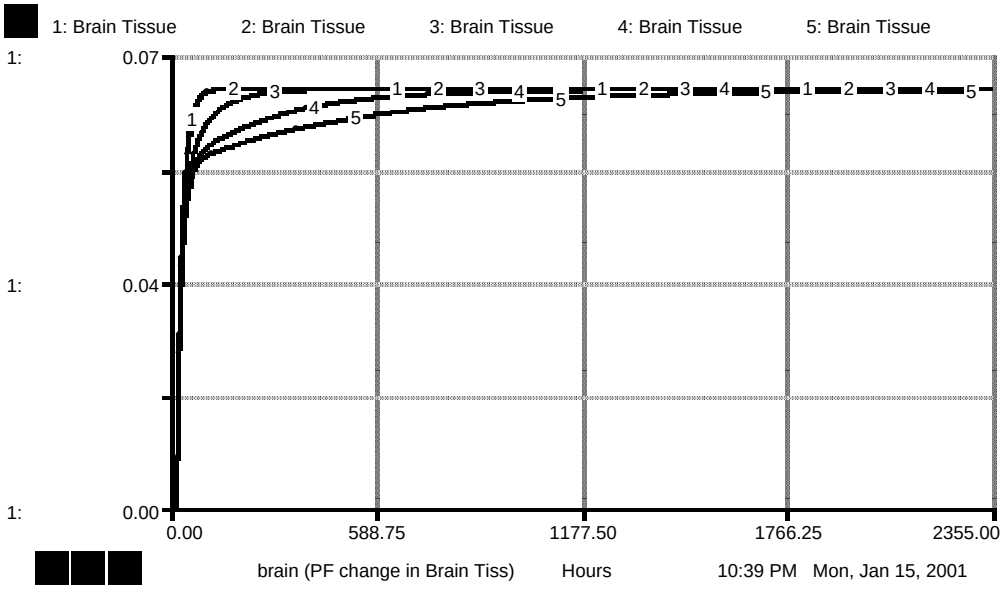
Richly Perfused Tissue Level (mg) for Increasing Liver/Blood PC Values

1: PF=0.002 2: PF=5 3: PF=75 4: PF=250 5: PF=500



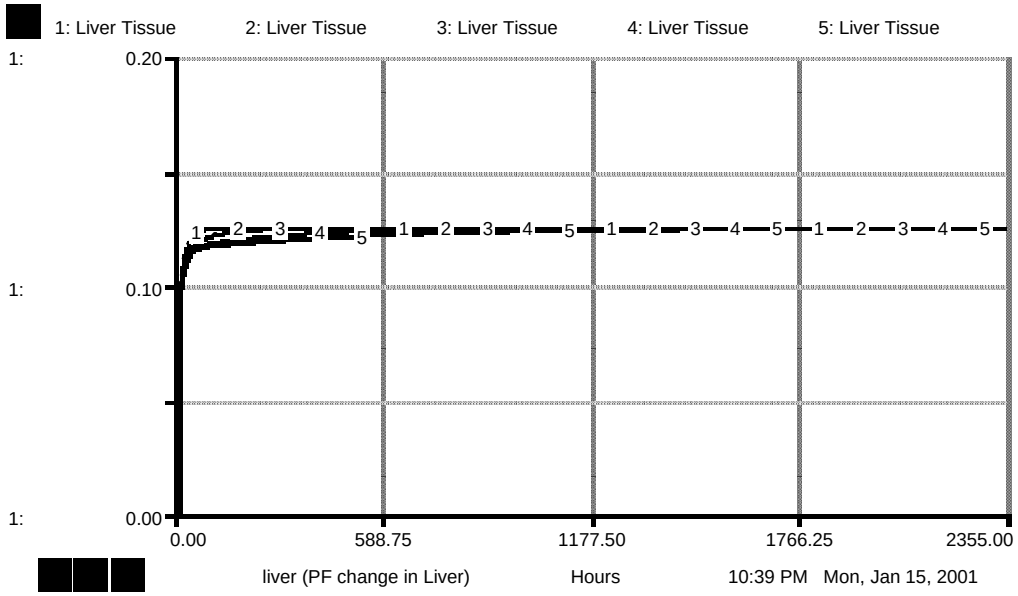
Fat Tissue Level (mg) for Increasing Fat/Blood PC Values

1: PF=0.002 2: PF=5 3: PF=75 4: PF=250 5: PF=500



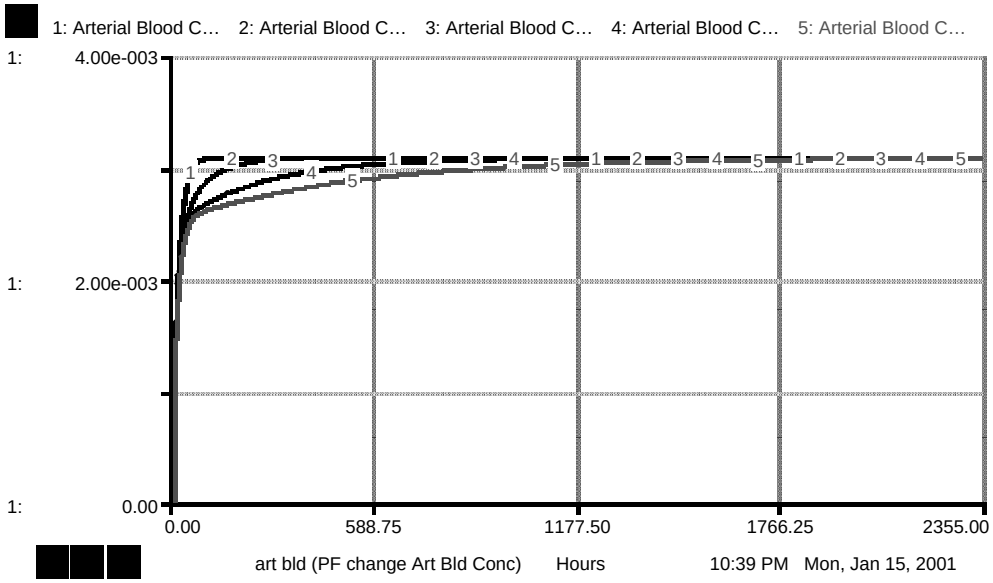
Brain Tissue Level (mg) for Increasing Fat/Blood PC Values

1: PF=0.002 2: PF=5 3: PF=75 4: PF=250 5: PF=500



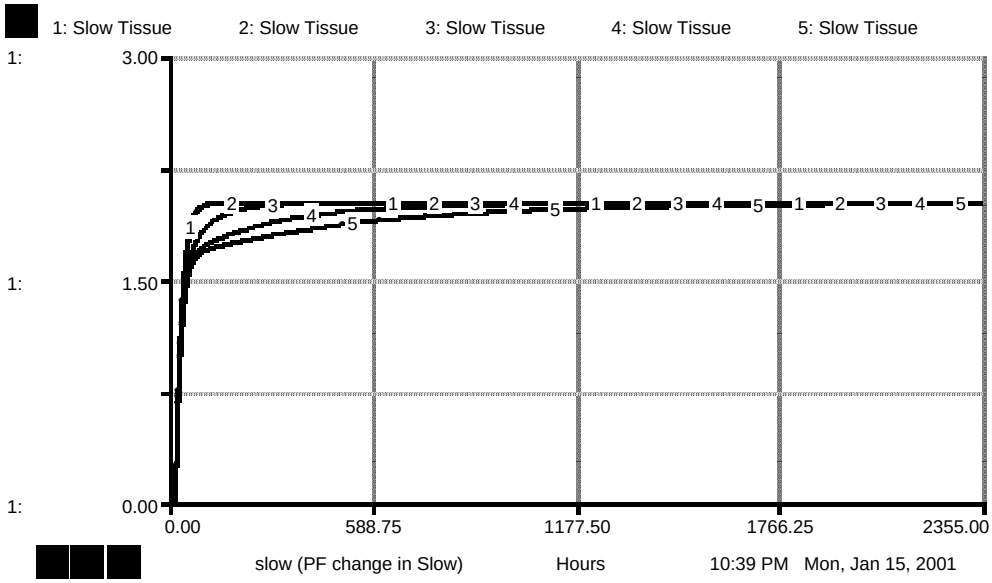
Liver Tissue Level (mg) for Increasing Fat/Blood PC Values

1: PF=0.002 2: PF=5 3: PF=75 4: PF=250 5: PF=500



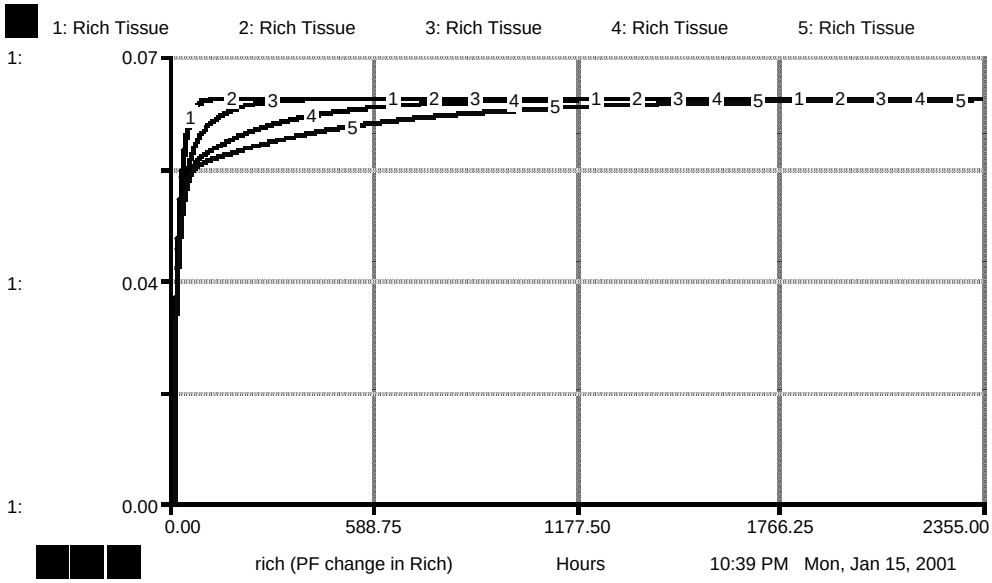
Arterial Blood Concentration (mg/L) for Increasing Fat/Blood PC Values

1: PF=0.002 2: PF=5 3: PF=75 4: PF=250 5: PF=500



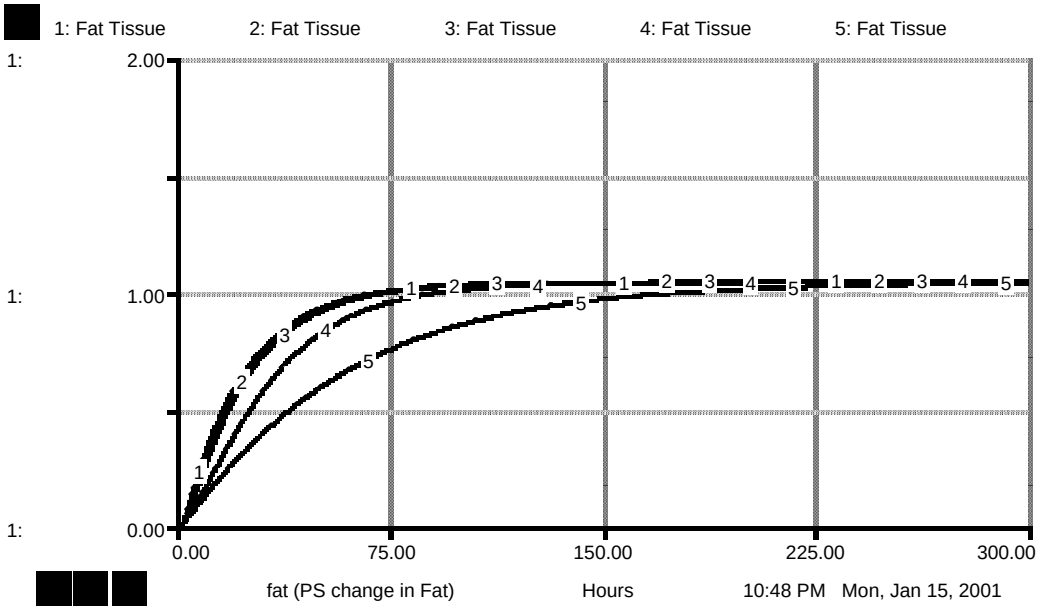
Slowly Perfused Tissue Level (mg) for Increasing Fat/Blood PC Values

1: PF=0.002 2: PF=5 3: PF=75 4: PF=250 5: PF=500



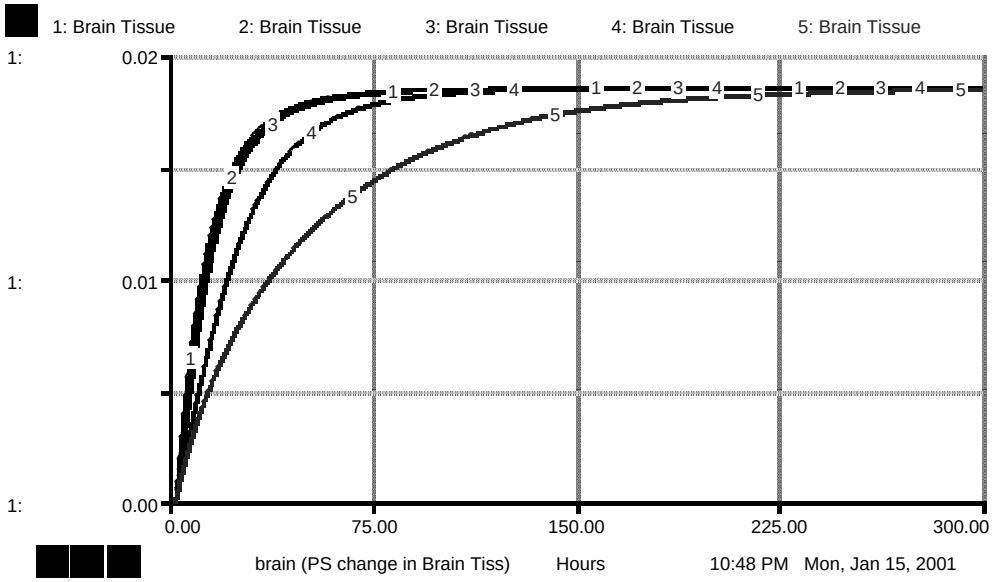
Richly Perfused Tissue Level (mg) for Increasing Fat/Blood PC Values

1: PS=0.02 2: PS=8 3: PS=15 4: PS=75 5: PS=250



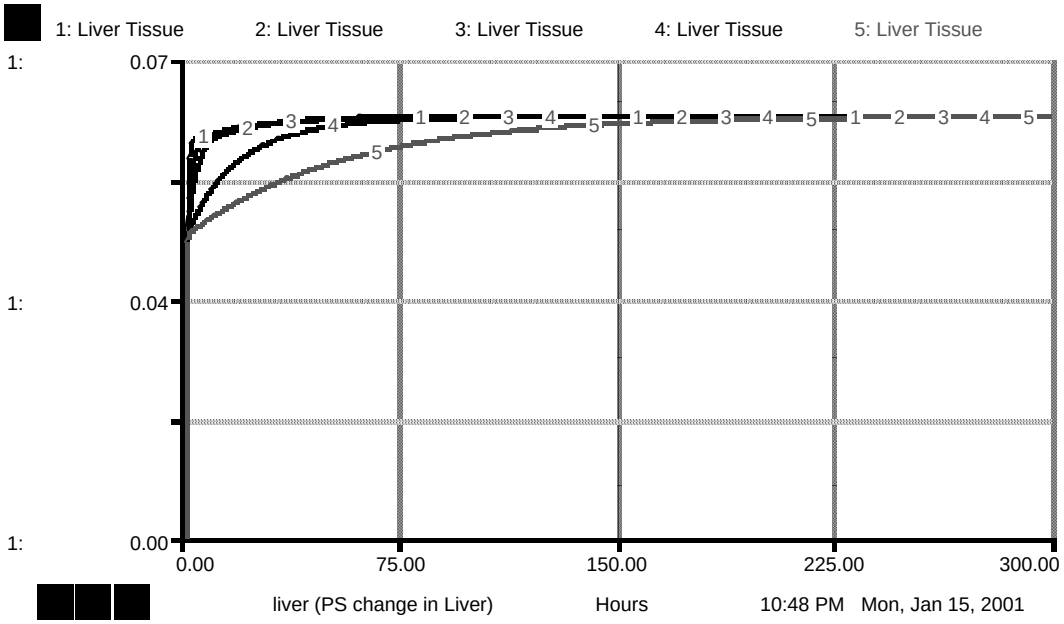
Fat Tissue Level (mg) for Increasing Slowly Perfused/Blood PC Values

1: PS=0.02 2: PS=8 3: PS=15 4: PS=75 5: PS=250



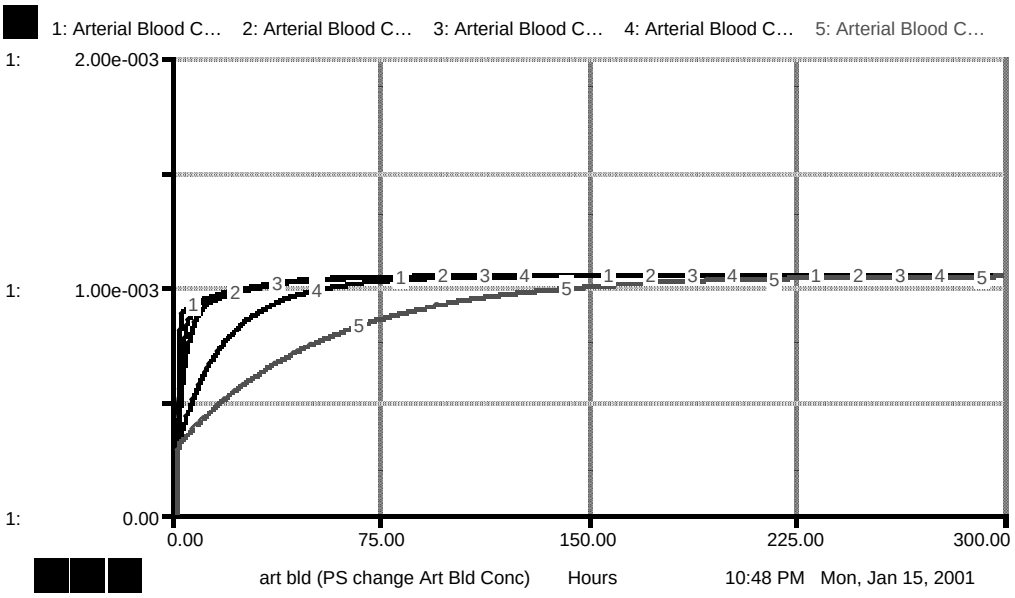
Brain Tissue Level (mg) for Increasing Slowly Perfused/Blood PC Values

1: PS=0.02 2: PS=8 3: PS=15 4: PS=75 5: PS=250



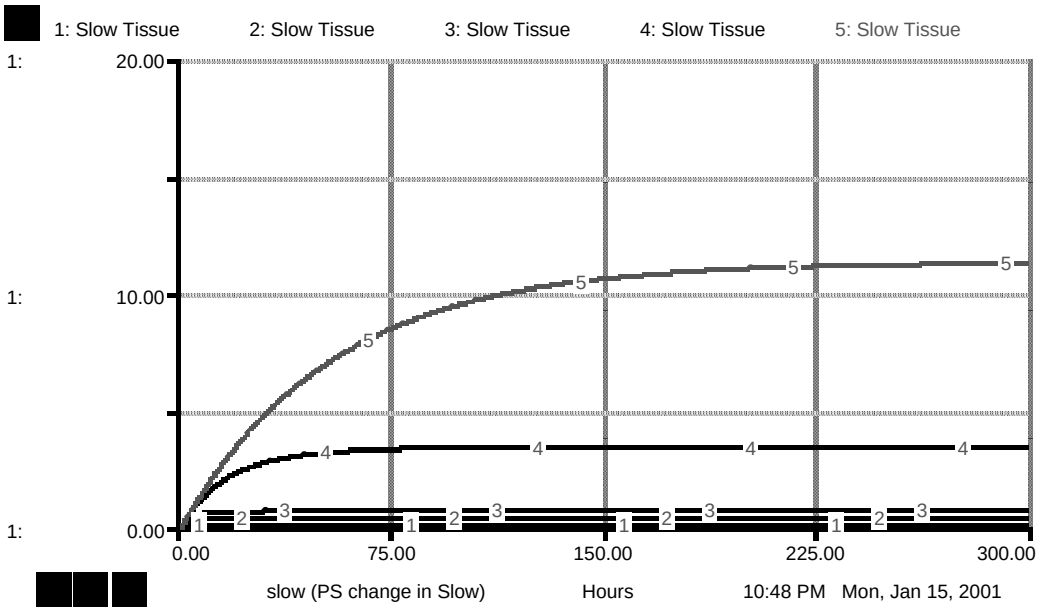
Liver Tissue Level (mg) for Increasing Slowly Perfused/Blood PC Values

1: PS=0.02 2: PS=8 3: PS=15 4: PS=75 5: PS=250



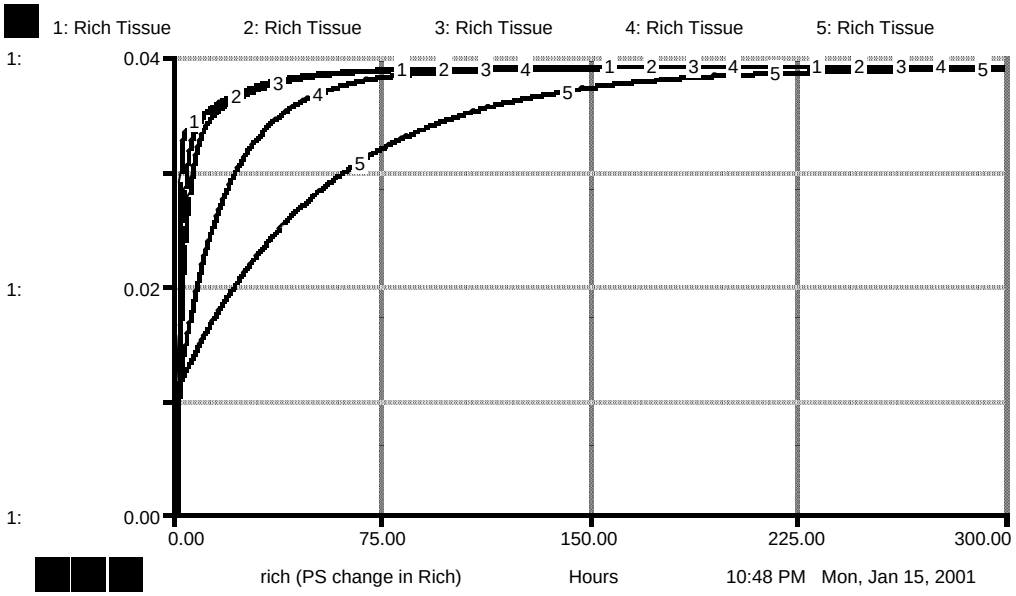
Arterial Blood Conc. (mg/L) for Increasing Slowly Perfused/Blood PC Values

1: PS=0.02 2: PS=8 3: PS=15 4: PS=75 5: PS=250



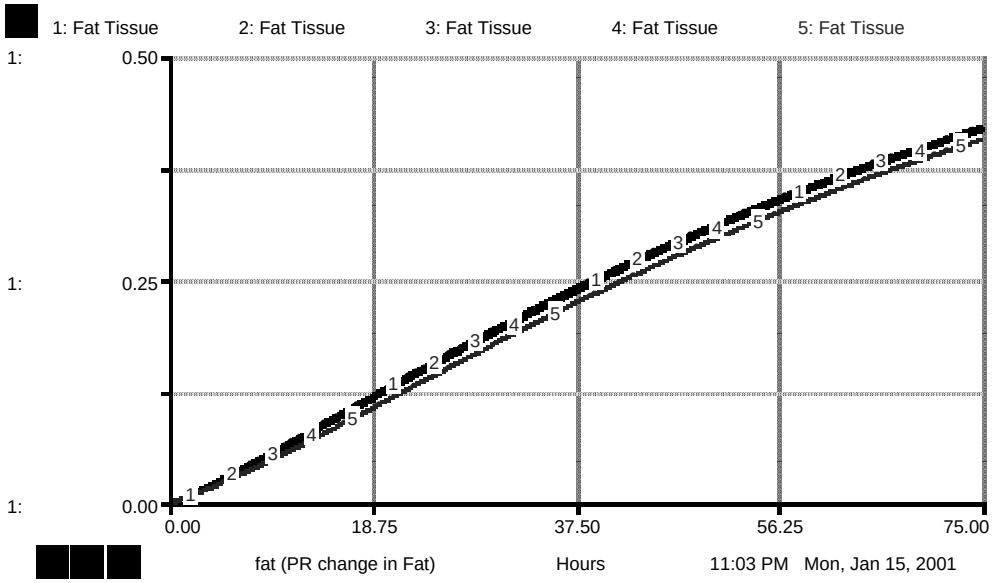
Slowly Perfused Tissue Level (mg) for Increasing Slowly Perf./Blood PC Values

1: PS=0.02 2: PS=8 3: PS=15 4: PS=75 5: PS=250



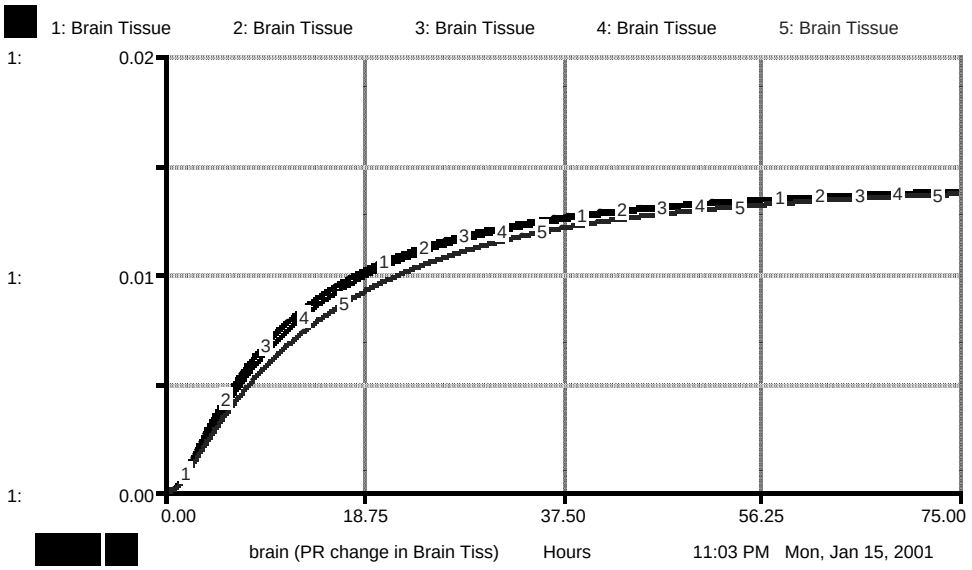
Richly Perfused Tissue Level (mg) for Increasing Slowly Perf./Blood PC Values

1: PR=0.2 2: PR=8 3: PR=15 4: PR=75 5: PR=250



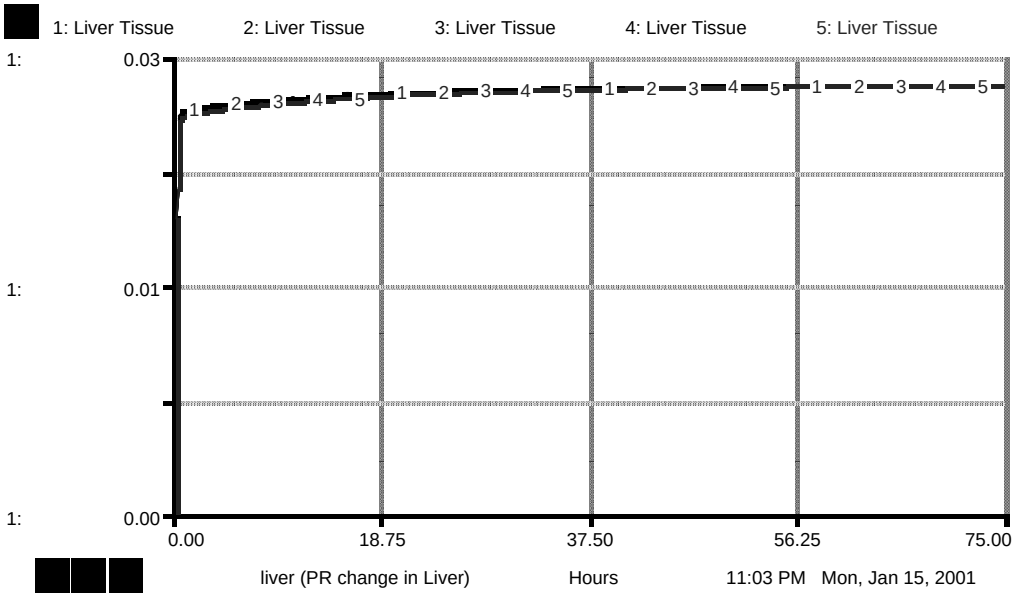
Fat Tissue Level (mg) for Increasing Richly Perf./Blood PC Values

1: PR=0.2 2: PR=8 3: PR=15 4: PR=75 5: PR=250



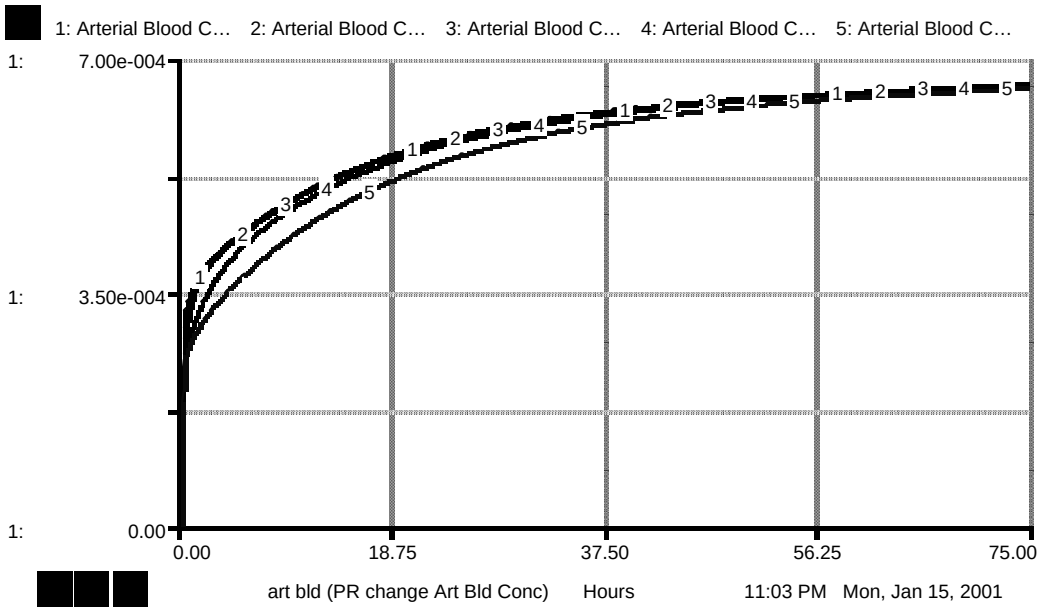
Brain Tissue Level (mg) for Increasing Richly Perf./Blood PC Values

1: PR=0.2 2: PR=8 3: PR=15 4: PR=75 5: PR=250



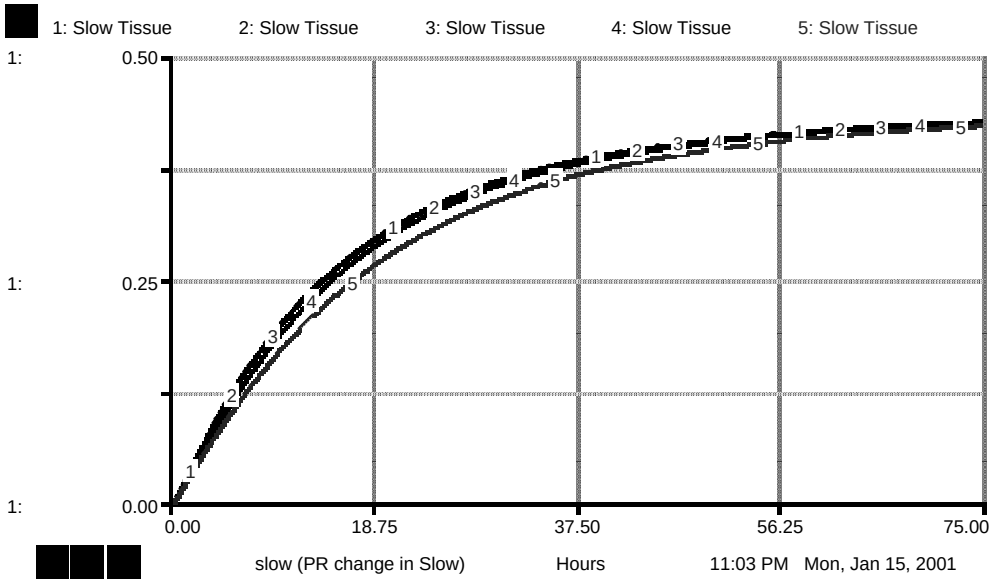
Liver Tissue Level (mg) for Increasing Richly Perf./Blood PC Values

1: PR=0.2 2: PR=8 3: PR=15 4: PR=75 5: PR=250



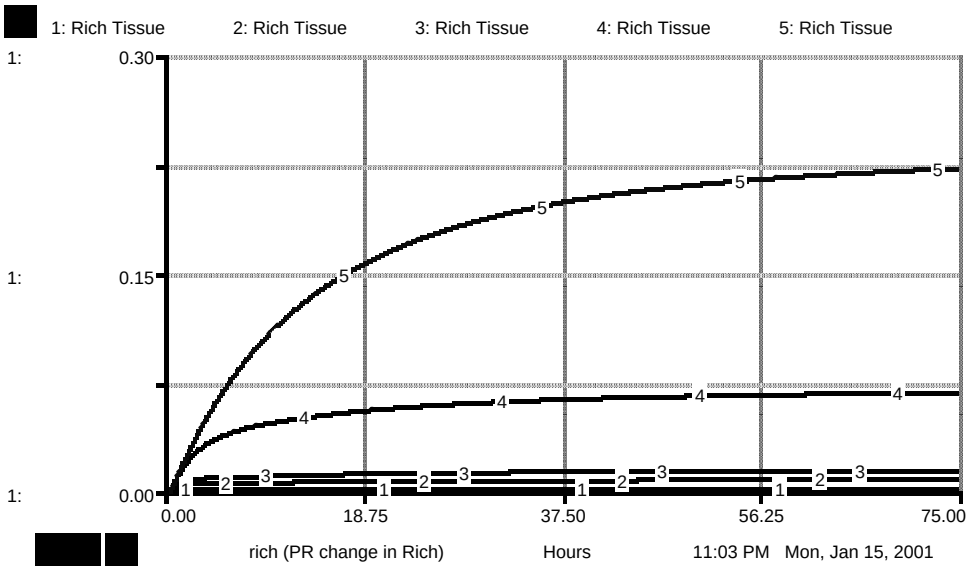
Arterial Blood Conc. (mg/L) for Increasing Richly Perf./Blood PC Values

1: PR=0.2 2: PR=8 3: PR=15 4: PR=75 5: PR=250



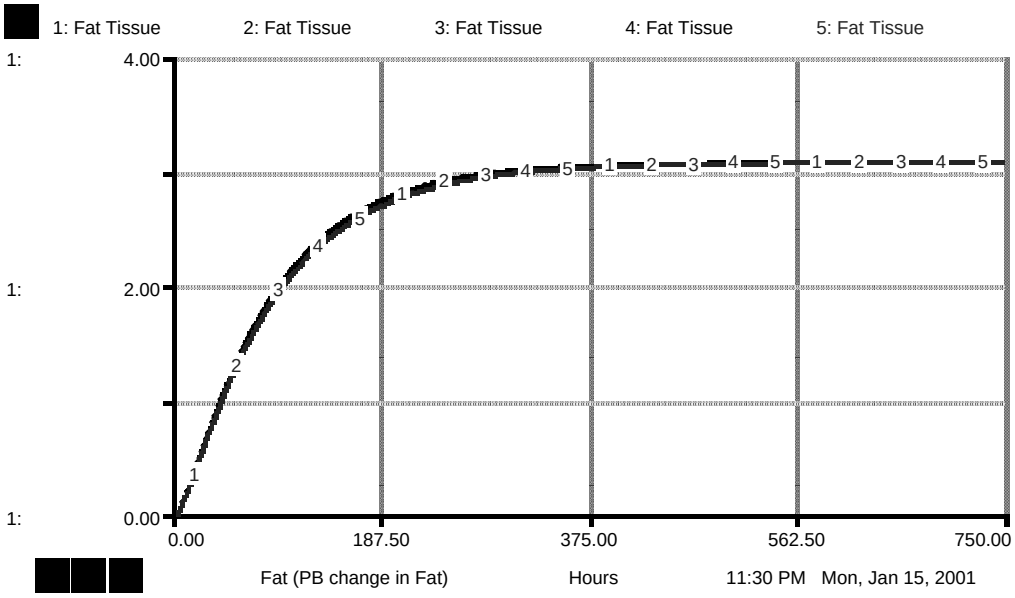
Slowly Perfused Tissue Level (mg) for Increasing Richly Perf./Blood PC Values

1: PR=0.2 2: PR=8 3: PR=15 4: PR=75 5: PR=250



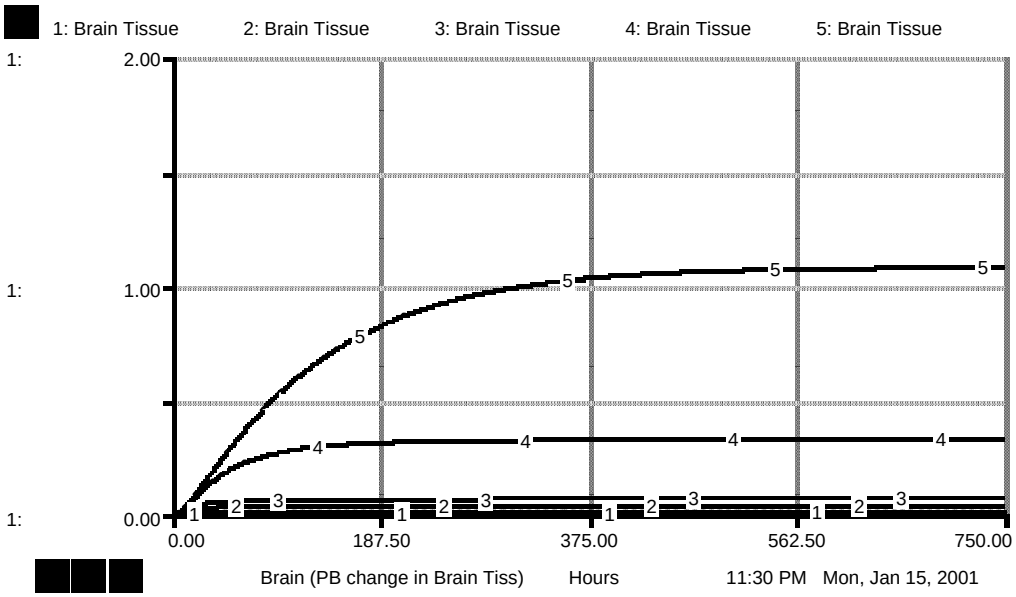
Richly Perfused Tissue Level (mg) for Increasing Richly Perf./Blood PC Values

1: PB=0.002 2: PB=8 3: PB=15 4: PB=75 5: PB=250



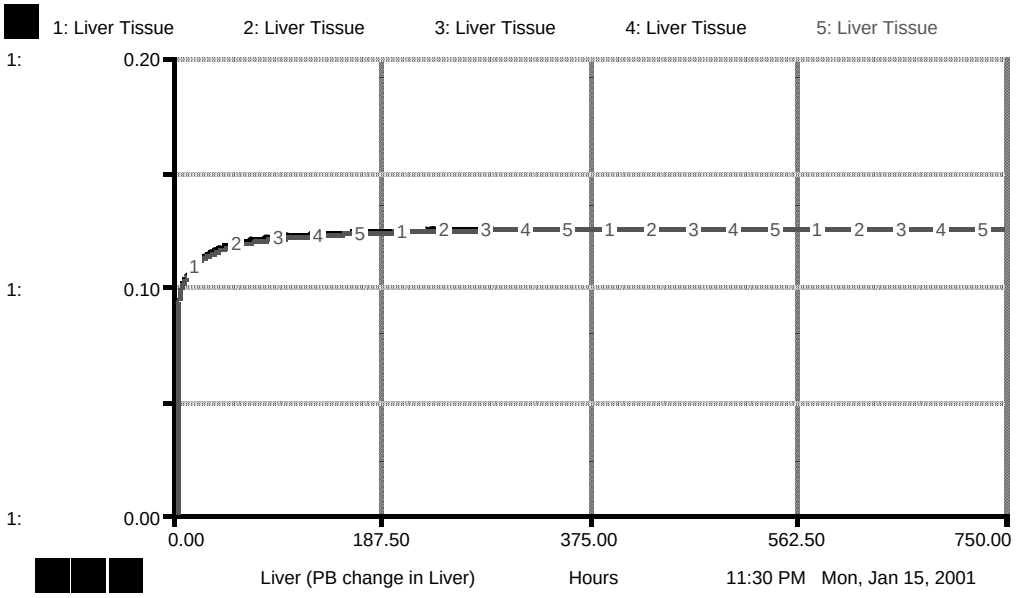
Fat Tissue Level (mg) for Increasing Brain/Blood PC Values

1: PB=0.002 2: PB=8 3: PB=15 4: PB=75 5: PB=250



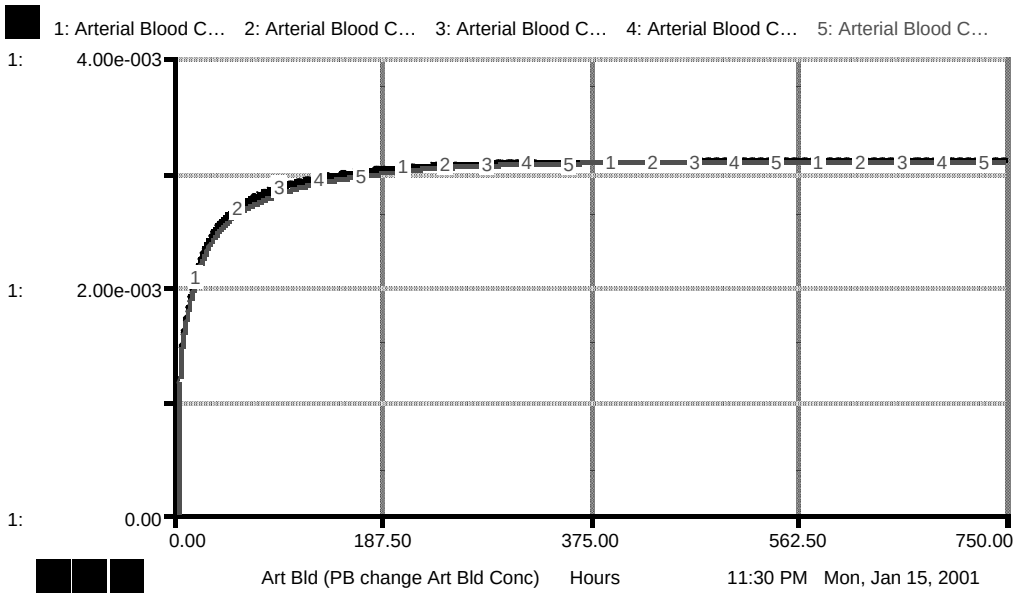
Brain Tissue Level (mg) for Increasing Brain/Blood PC Values

1: PB=0.002 2: PB=8 3: PB=15 4: PB=75 5: PB=250



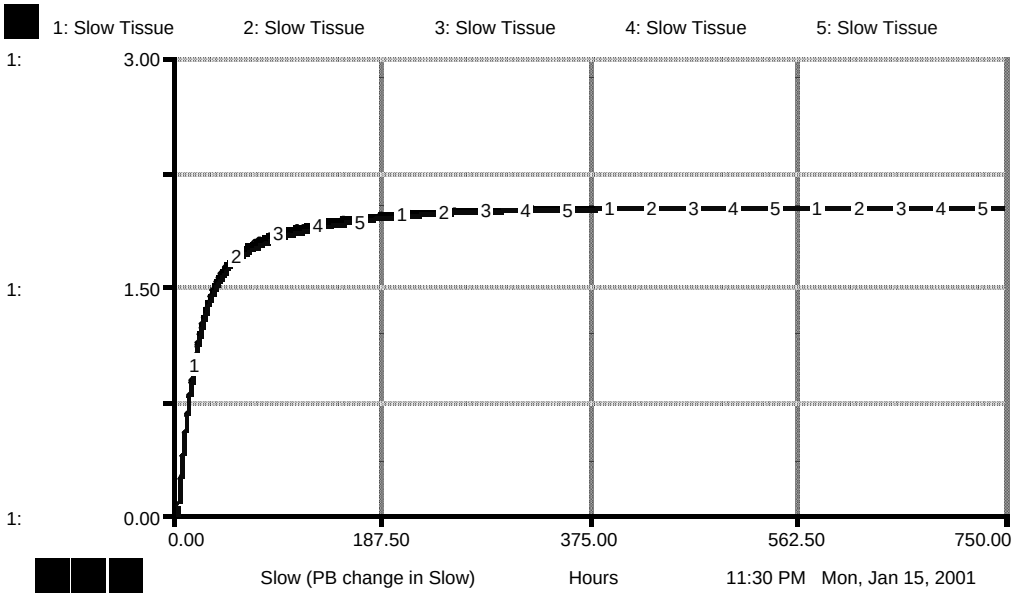
Brain Tissue Level (mg) for Increasing Brain/Blood PC Values

1: PB=0.002 2: PB=8 3: PB=15 4: PB=75 5: PB=250



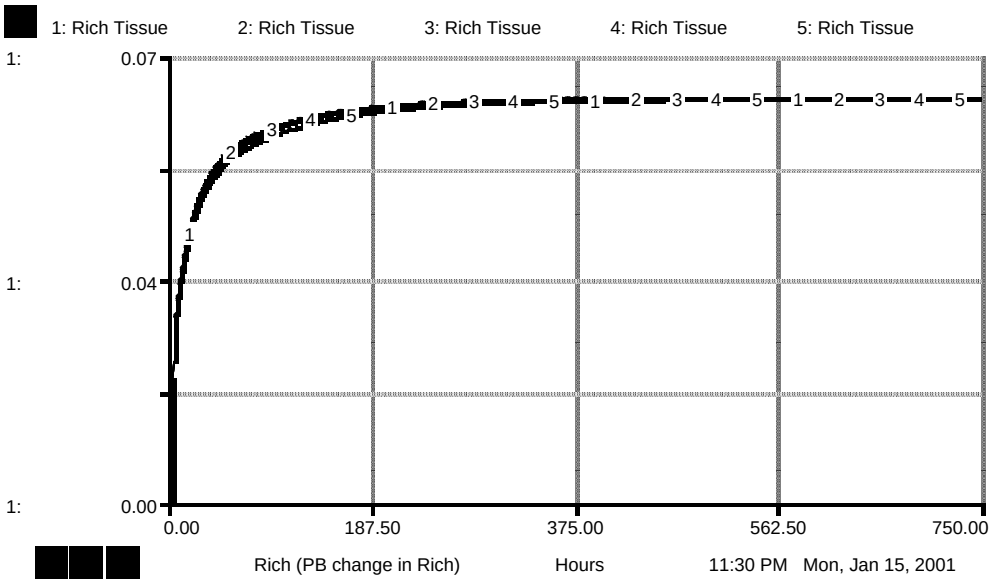
Arterial Blood Concentration (mg/L) for Increasing Brain/Blood PC Values

1: PB=0.002 2: PB=8 3: PB=15 4: PB=75 5: PB=250



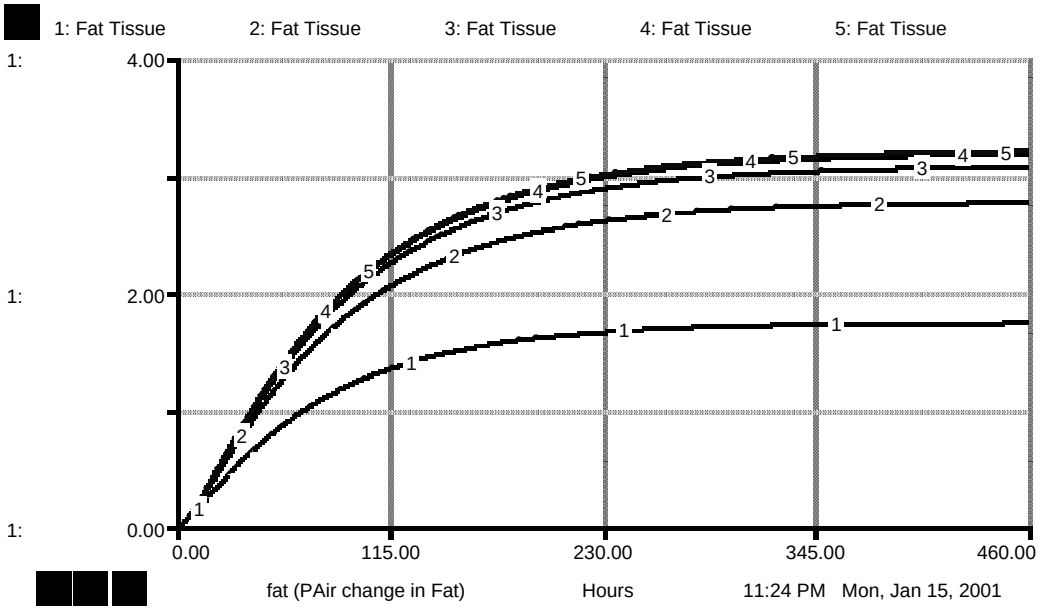
Slowly Perfused Tissue Level (mg) for Increasing Brain/Blood PC Values

1: PB=0.002 2: PB=8 3: PB=15 4: PB=75 5: PB=250



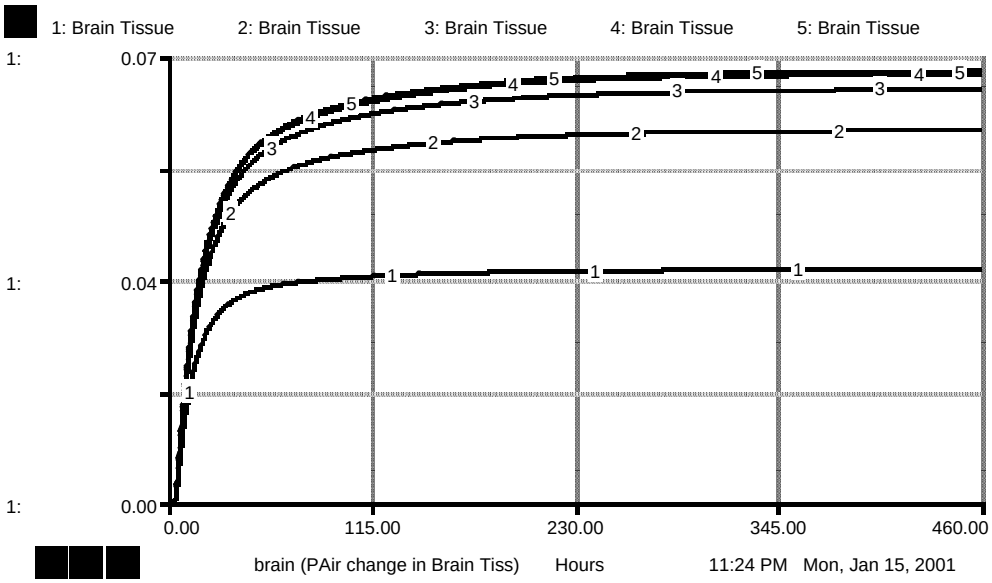
Richly Perfused Tissue Level (mg) for Increasing Brain/Blood PC Values

1: PB/A=5 2: PB/A=25 3: PB/A=75 4: PB/A=200 5: PB/A=300



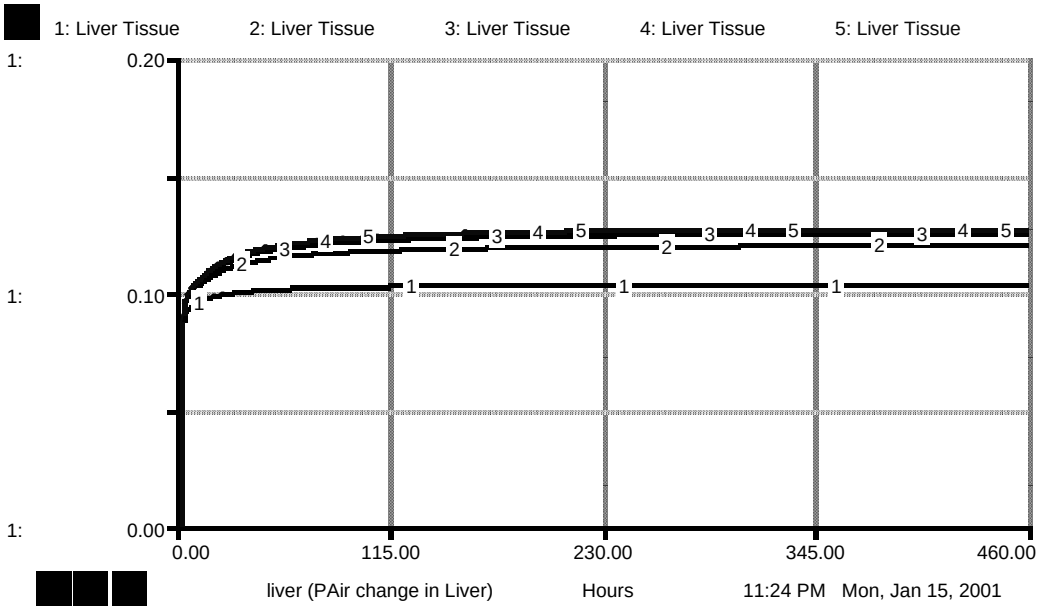
Fat Tissue Level (mg) for Increasing Blood/Air PC Values

1: PB/A=5 2: PB/A=25 3: PB/A=75 4: PB/A=200 5: PB/A=300



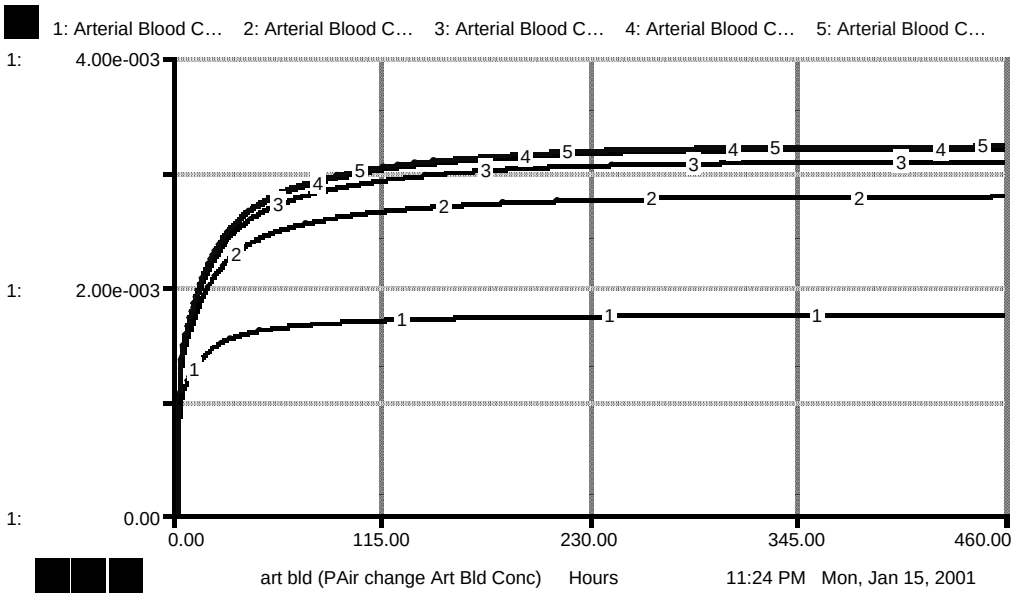
Fat Tissue Level (mg) for Increasing Blood/Air PC Values

1: PB/A=5 2: PB/A=25 3: PB/A=75 4: PB/A=200 5: PB/A=300



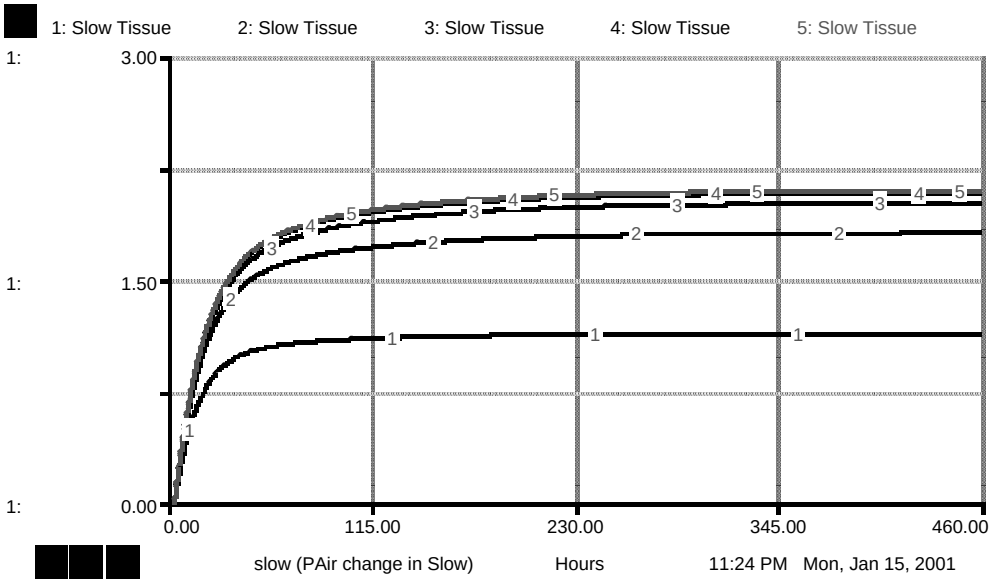
Liver Tissue Level (mg) for Increasing Blood/Air PC Values

1: PB/A=5 2: PB/A=25 3: PB/A=75 4: PB/A=200 5: PB/A=300



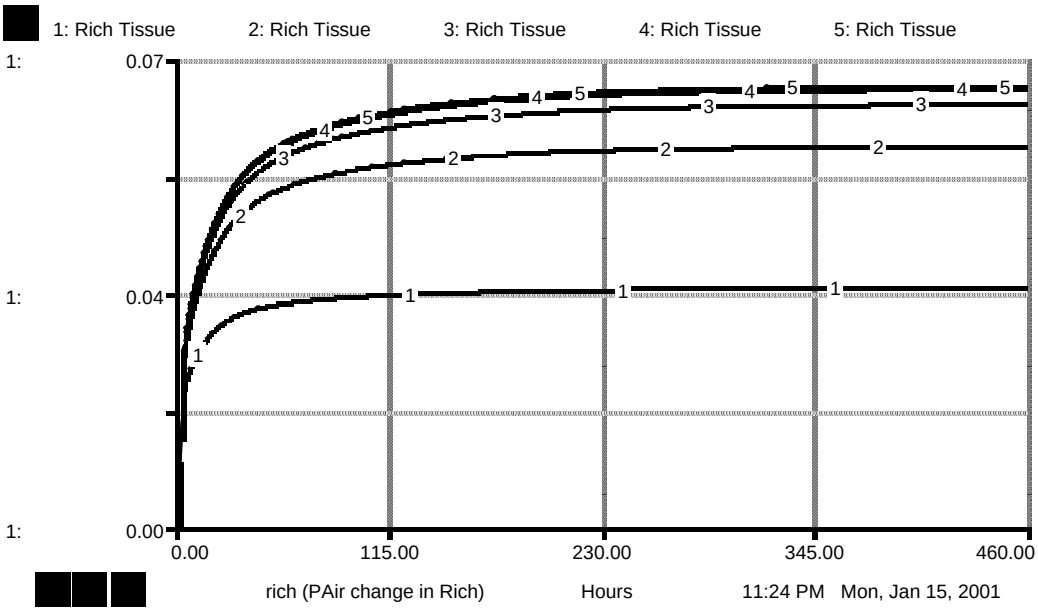
Arterial Blood Concentration (mg/L) for Increasing Blood/Air PC Values

1: PB/A=5 2: PB/A=25 3: PB/A=75 4: PB/A=200 5: PB/A=300



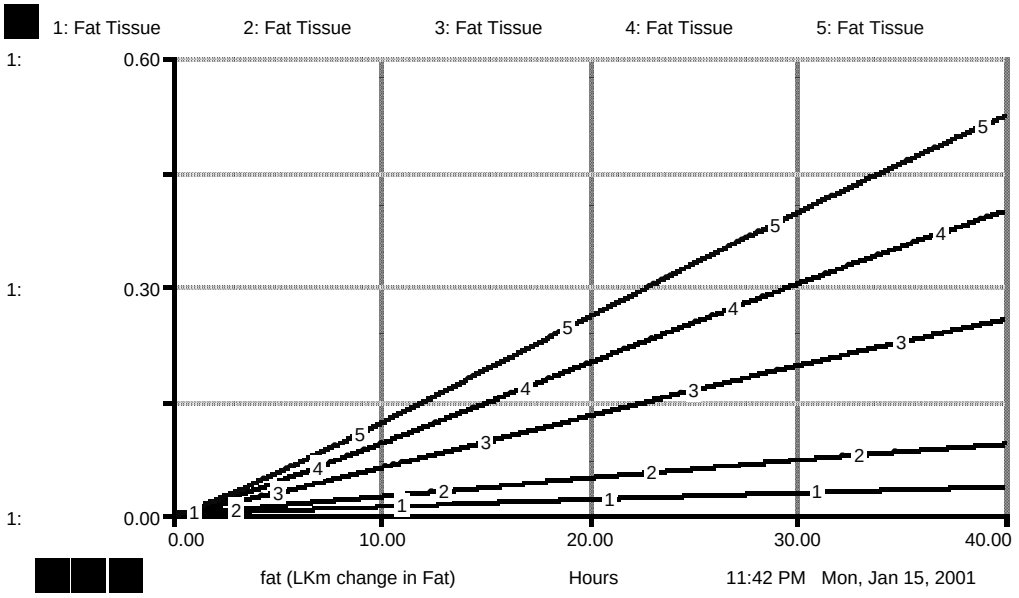
Slowly Perfused Tissue Level (mg) for Increasing Blood/Air PC Values

1: PB/A=5 2: PB/A=25 3: PB/A=75 4: PB/A=200 5: PB/A=300



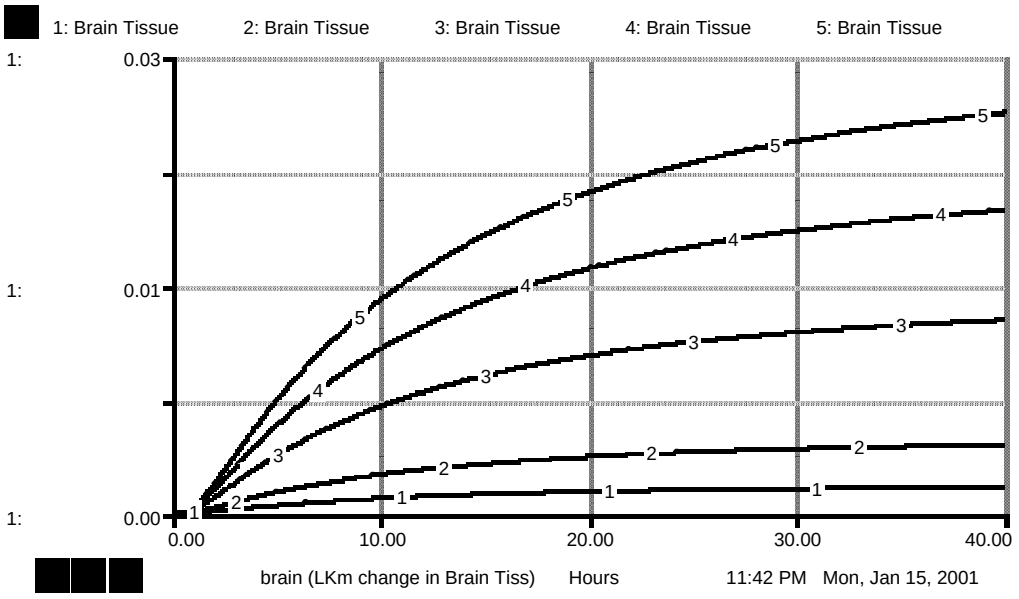
Richly Perfused Tissue Level (mg) for Increasing Blood/Air PC Values

1: LKm=0.1 2: LKm=0.5 3: LKm=1.5 4: LKm=2.5 5: LKm=3.5



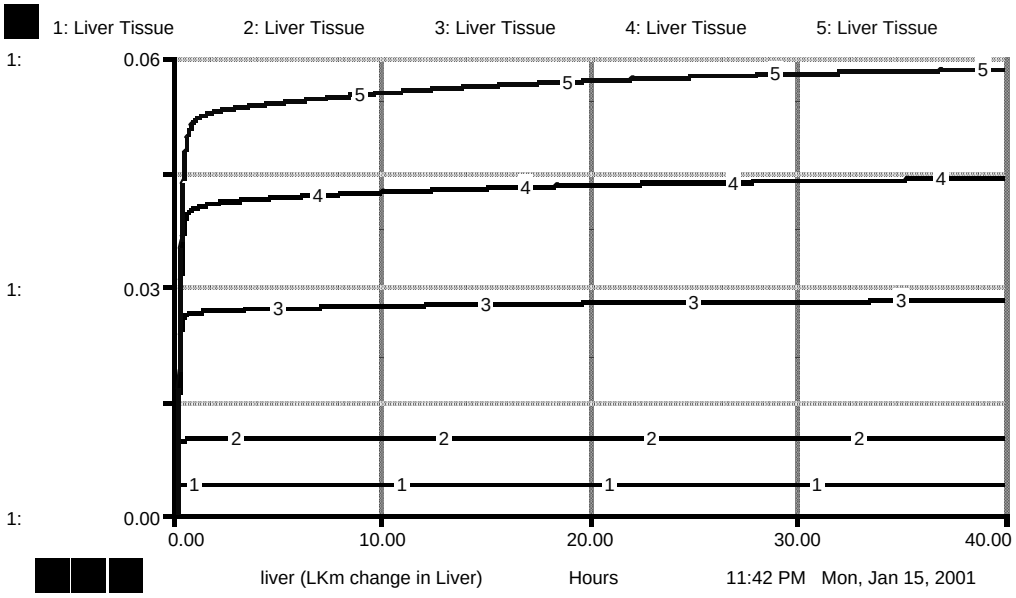
Fat Tissue Level (mg) for Increasing Liver Km Values

1: LKm=0.1 2: LKm=0.5 3: LKm=1.5 4: LKm=2.5 5: LKm=3.5



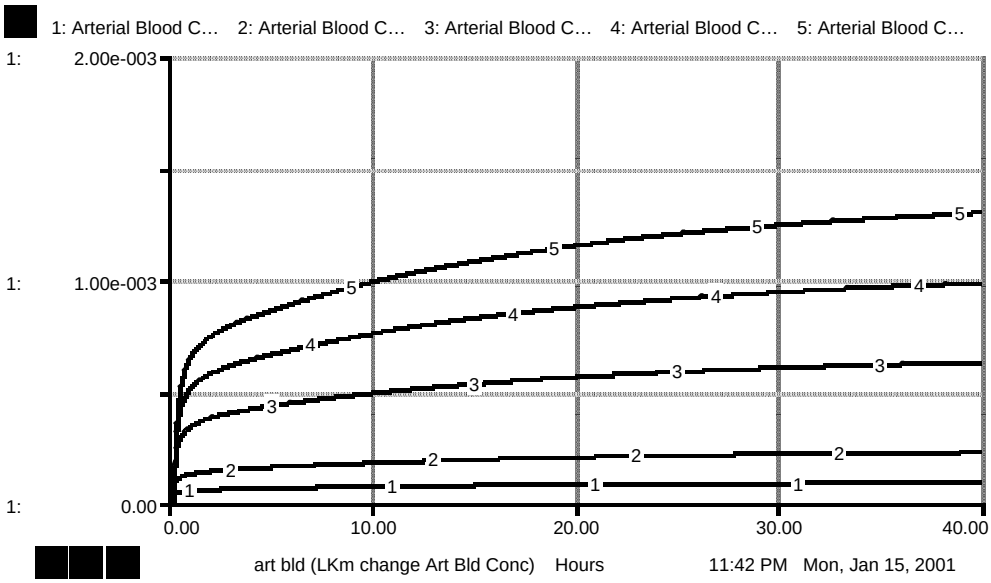
Brain Tissue Level (mg) for Increasing Liver Km Values

1: LKm=0.1 2: LKm=0.5 3: LKm=1.5 4: LKm=2.5 5: LKm=3.5



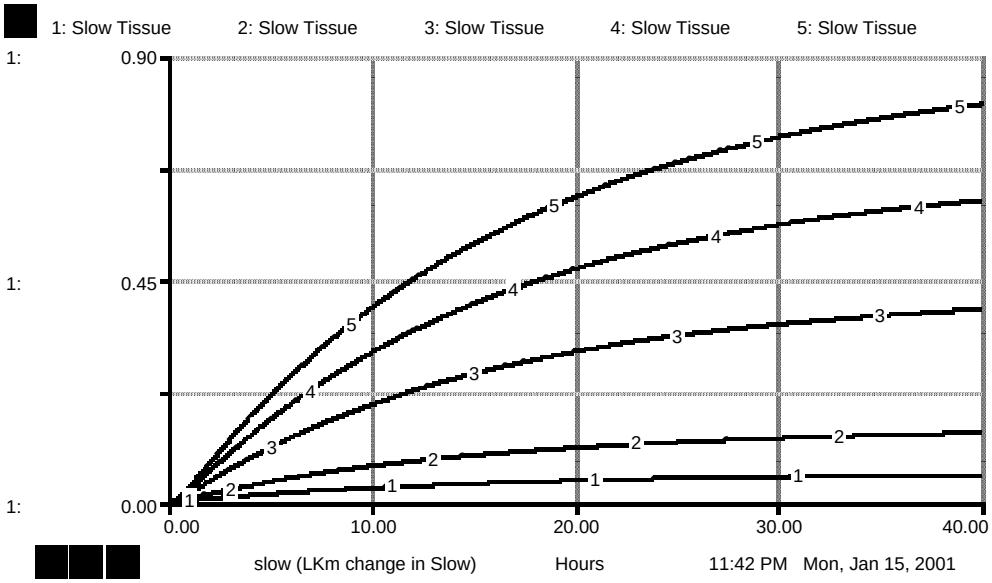
Liver Tissue Level (mg) for Increasing Liver Km Values

1: LKm=0.1 2: LKm=0.5 3: LKm=1.5 4: LKm=2.5 5: LKm=3.5



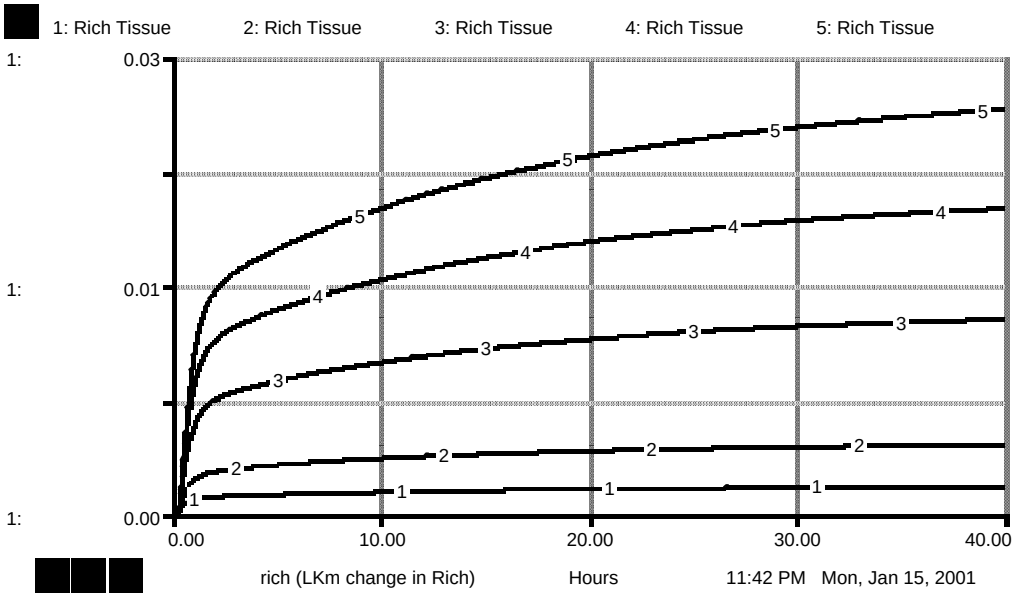
Arterial Blood Concentration (mg/L) for Increasing Liver Km Values

1: LKm=0.1 2: LKm=0.5 3: LKm=1.5 4: LKm=2.5 5: LKm=3.5



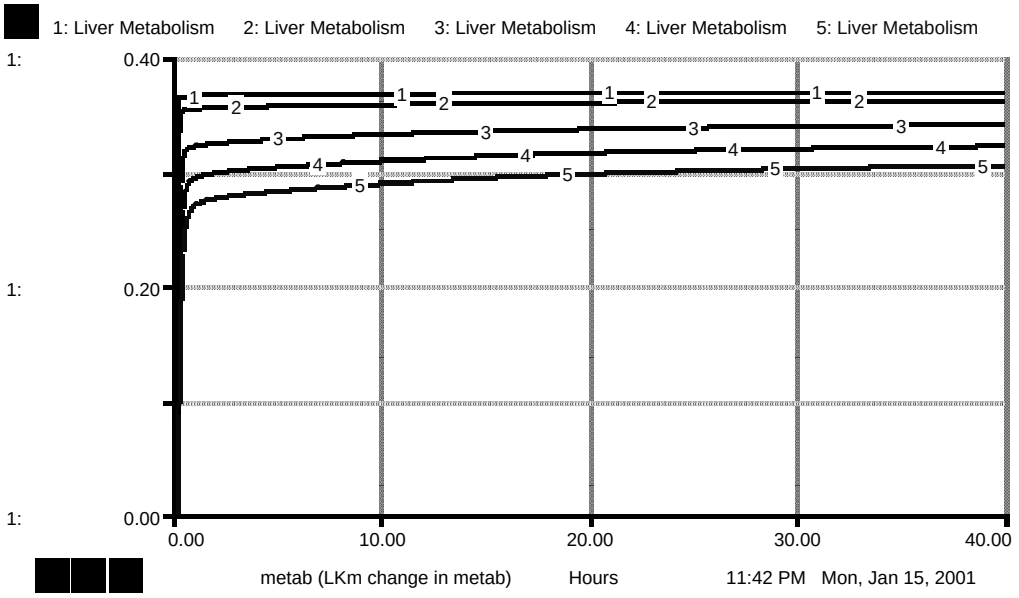
Slowly Perfused Tissue Level (mg) for Increasing Liver Km Values

1: LKm=0.1 2: LKm=0.5 3: LKm=1.5 4: LKm=2.5 5: LKm=3.5



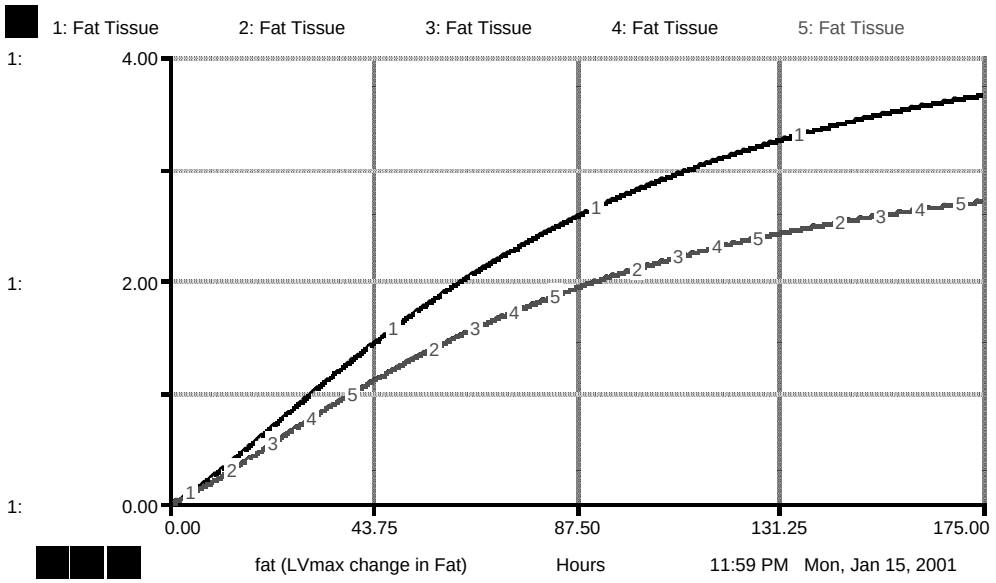
Richly Perfused Tissue Level (mg) for Increasing Liver Km Values

1: LKm=0.1 2: LKm=0.5 3: LKm=1.5 4: LKm=2.5 5: LKm=3.5



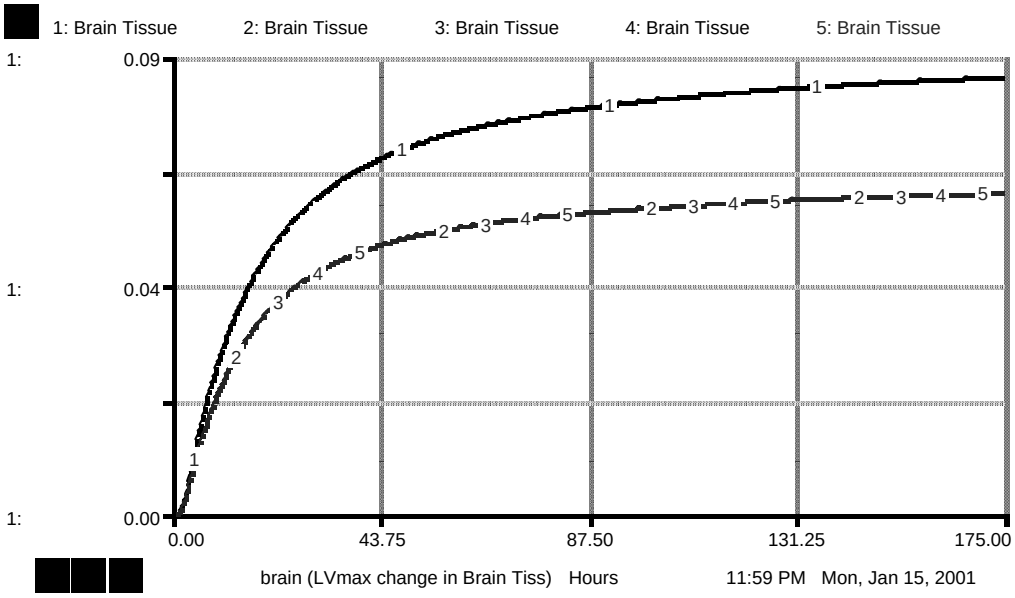
Richly Perfused Tissue Level (mg) for Increasing Liver Km Values

1: LVmax=50 2: LVmax=250 3: Lvmax=500 4: LVmax=1000 5: LVmax=2000



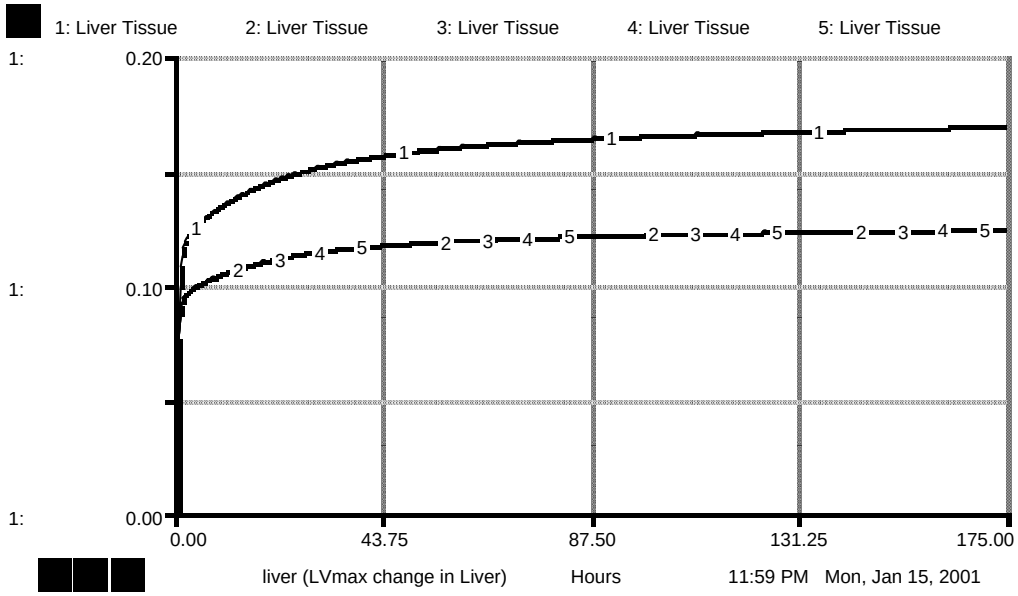
Fat Tissue Level (mg) for Increasing Liver Vmax Values

1: LVmax=50 2: LVmax=250 3: LVmax=500 4: LVmax=1000 5: LVmax=2000



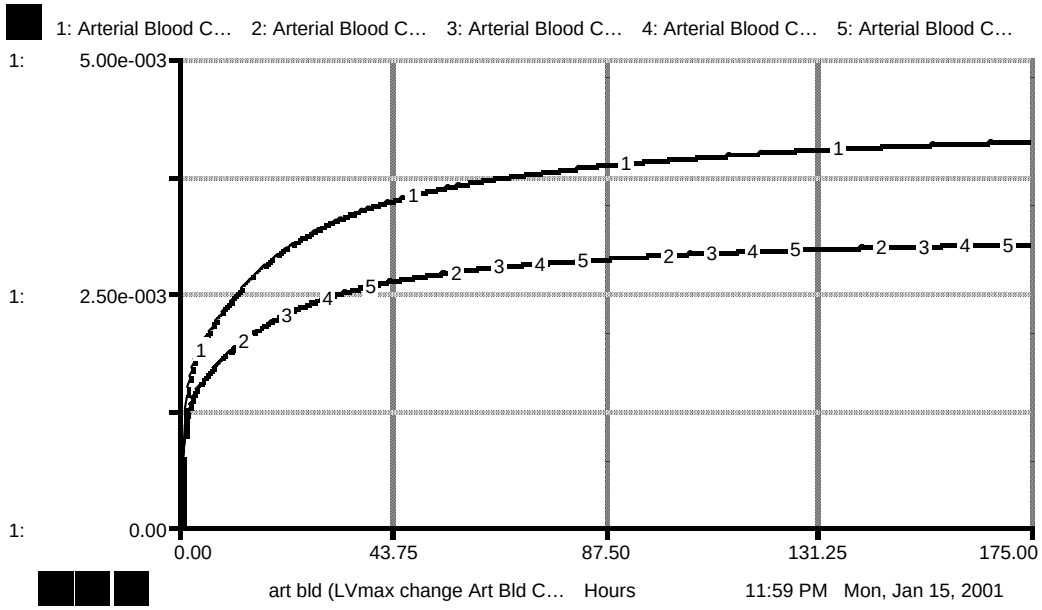
Brain Tissue Level (mg) for Increasing Liver Vmax Values

1: LVmax=50 2: LVmax=250 3: LVmax=500 4: LVmax=1000 5: LVmax=2000



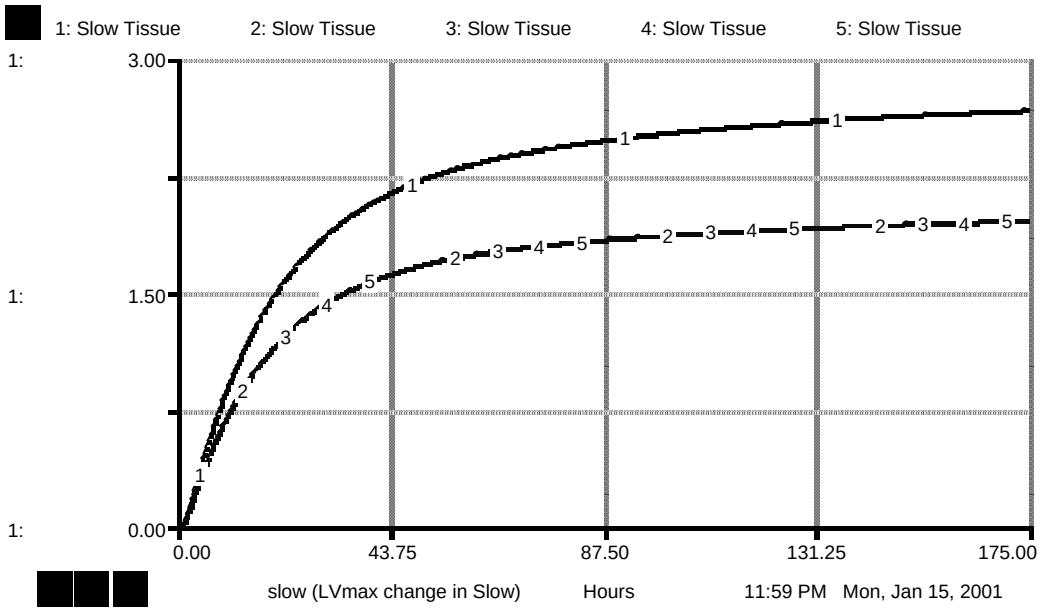
Liver Tissue Level (mg) for Increasing Liver Vmax Values

1: LVmax=50 2: LVmax=250 3: LVmax=500 4: LVmax=1000 5: LVmax=2000



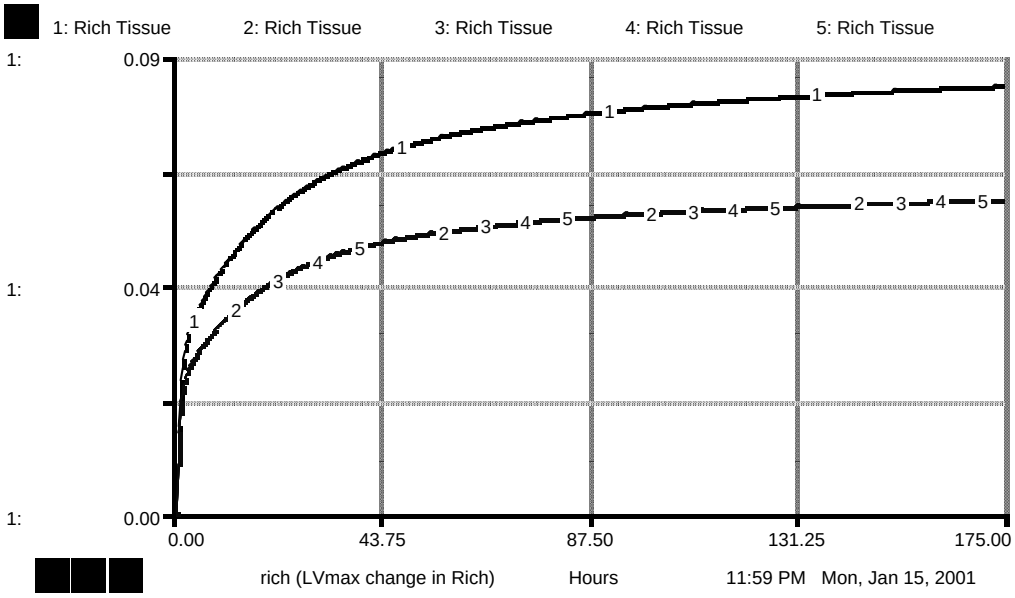
Arterial Blood Concentration (mg/L) for Increasing Liver Vmax Values

1: LVmax=50 2: LVmax=250 3: LVmax=500 4: LVmax=1000 5: LVmax=2000



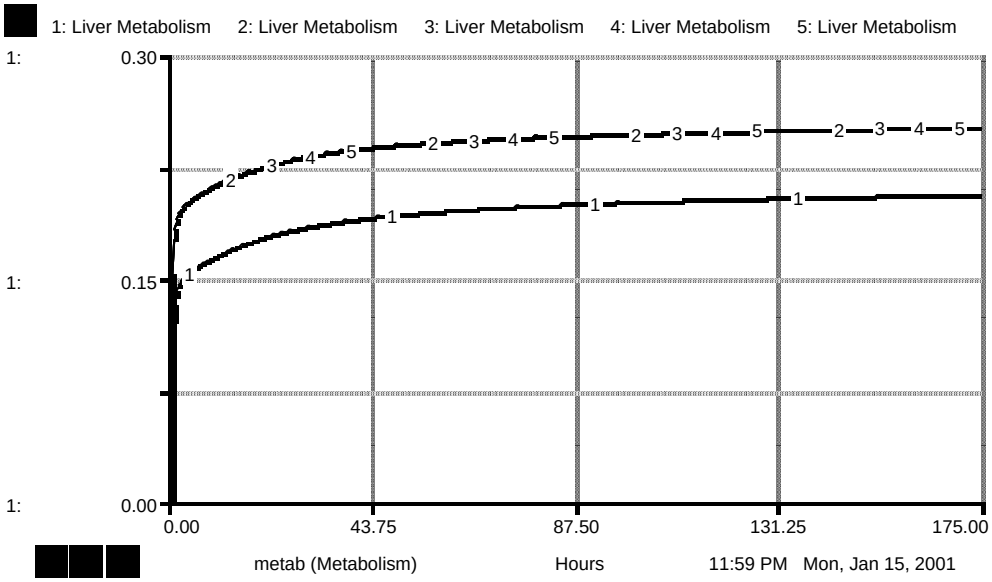
Slowly Perfused Tissue Level (mg) for Increasing Liver Vmax Values

1: LVmax=50 2: LVmax=250 3: LVmax=500 4: LVmax=1000 5: LVmax=2000



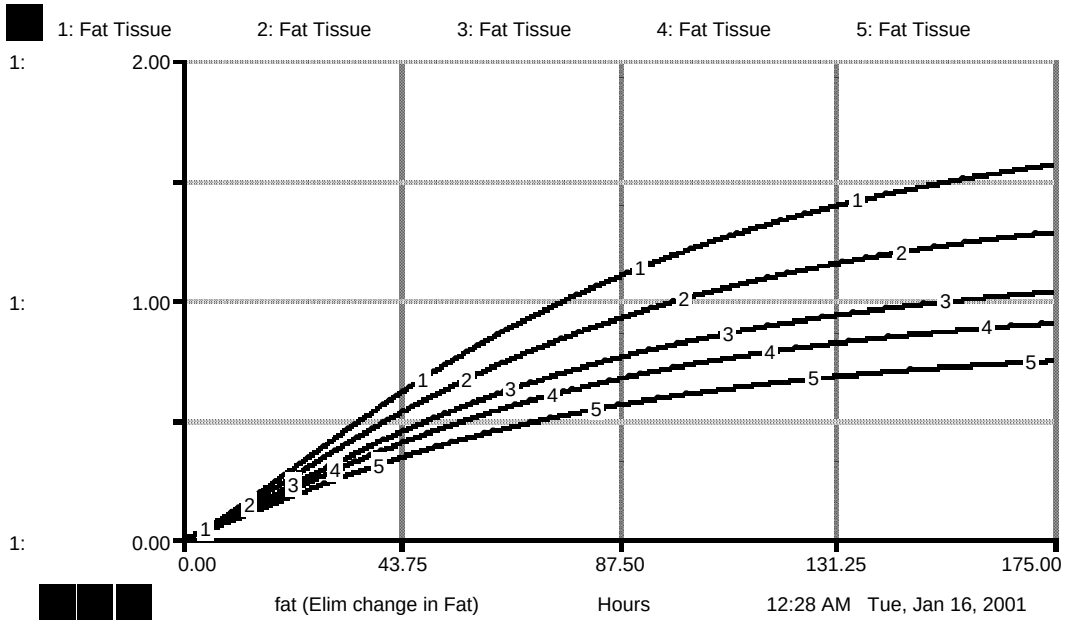
Richly Perfused Tissue Level (mg) for Increasing Liver Vmax Values

1: LVmax=50 2: LVmax=250 3: LVmax=500 4: LVmax=1000 5: LVmax=2000



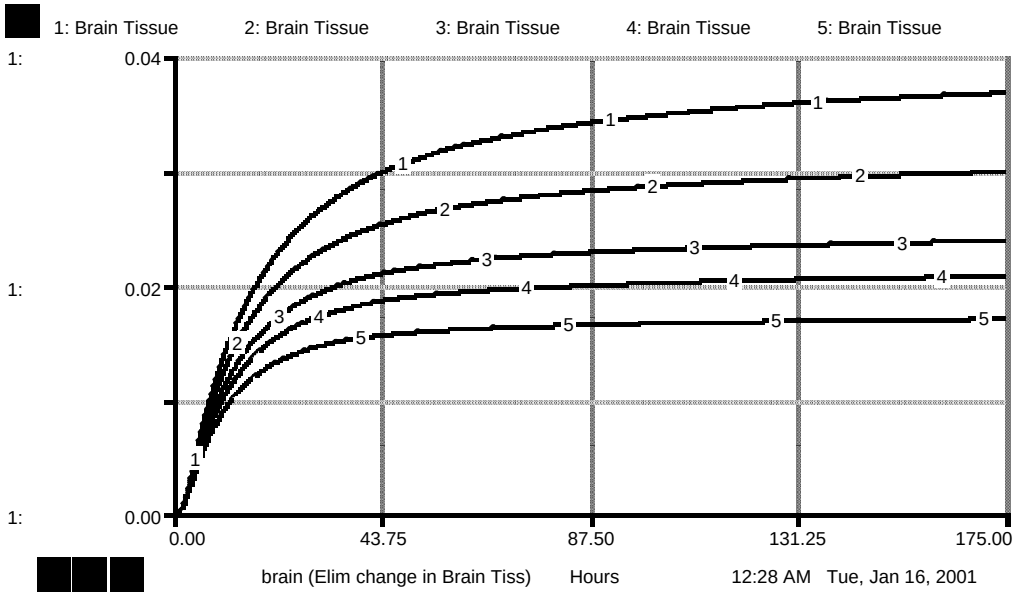
Liver Metabolism (mg/hr) for Increasing Liver Vmax Values

1: Fract=0 2: Fract=0.15 3: Fract=0.35 4: Fract=0.5 5: Fract=0.75



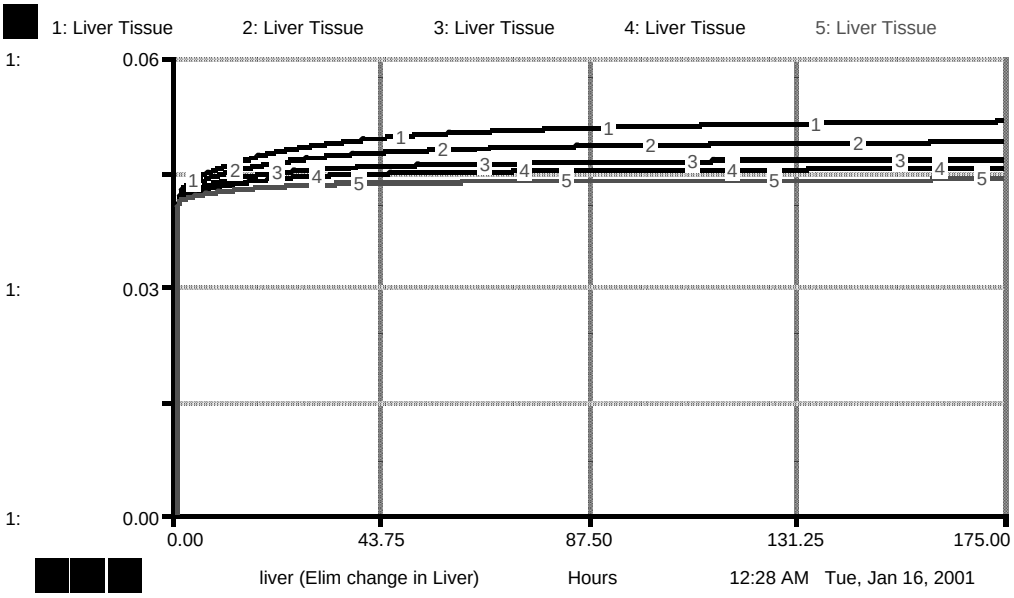
Fat Tissue Level (mg) for Increasing Kidney Elimination Fractions

1: Fract=0 2: Fract=0.15 3: Fract=0.35 4: Fract=0.5 5: Fract=0.75



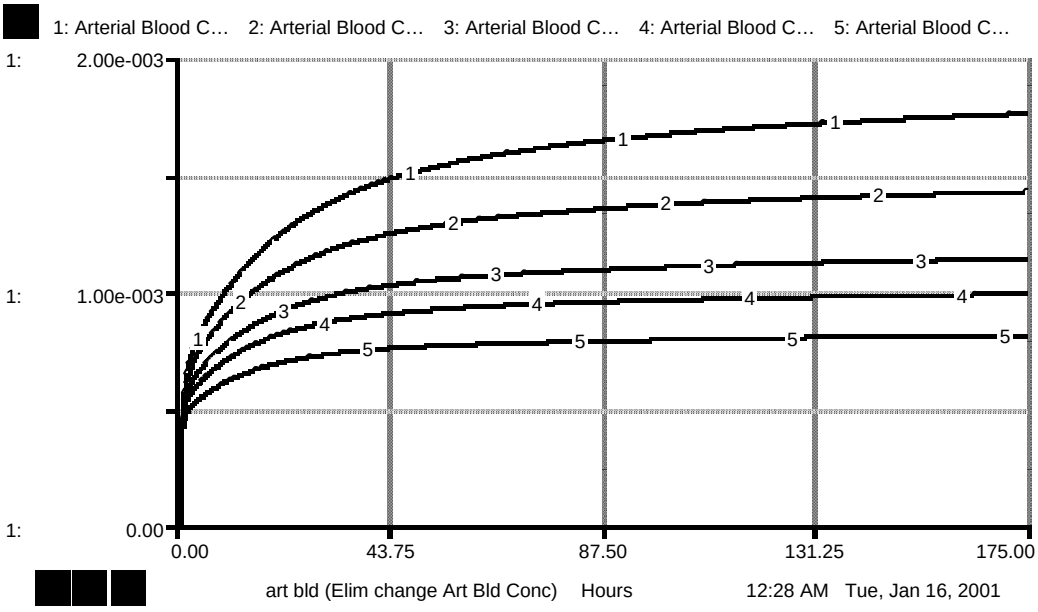
Brain Tissue Level (mg) for Increasing Kidney Elimination Fractions

1: Fract=0 2: Fract=0.15 3: Fract=0.35 4: Fract=0.5 5: Fract=0.75



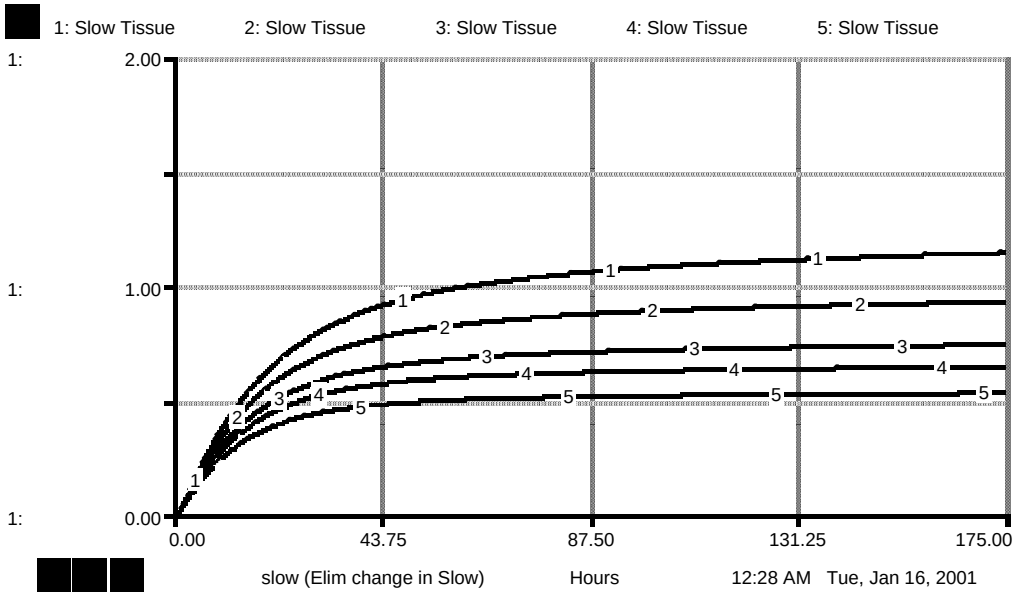
Liver Tissue Level (mg) for Increasing Kidney Elimination Fractions

1: Fract=0 2: Fract=0.15 3: Fract=0.35 4: Fract=0.5 5: Fract=0.75



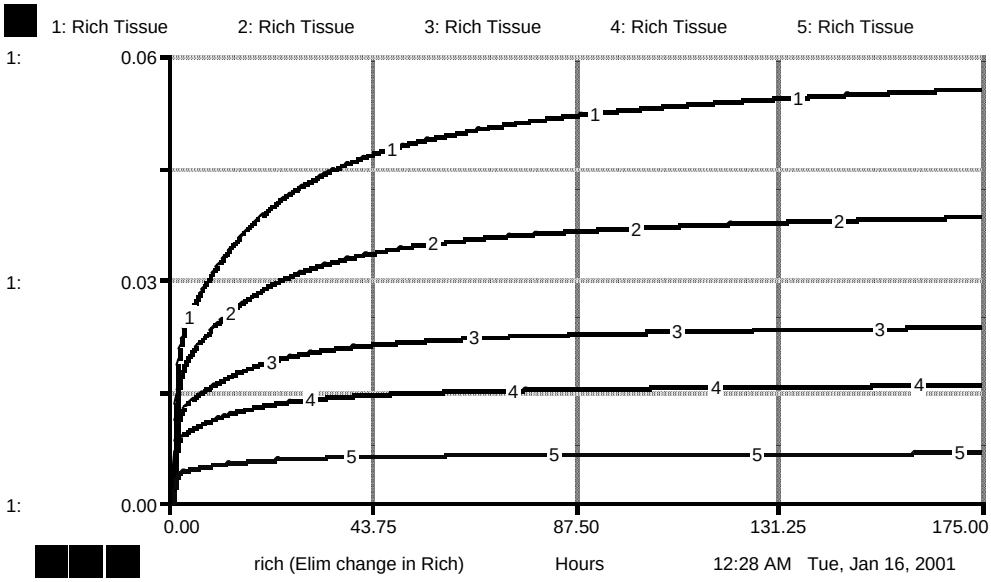
Arterial Blood Concentration (mg/L) for Increasing Kidney Elimination Fractions

1: Fract=0 2: Fract=0.15 3: Fract=0.35 4: Fract=0.5 5: Fract=0.75



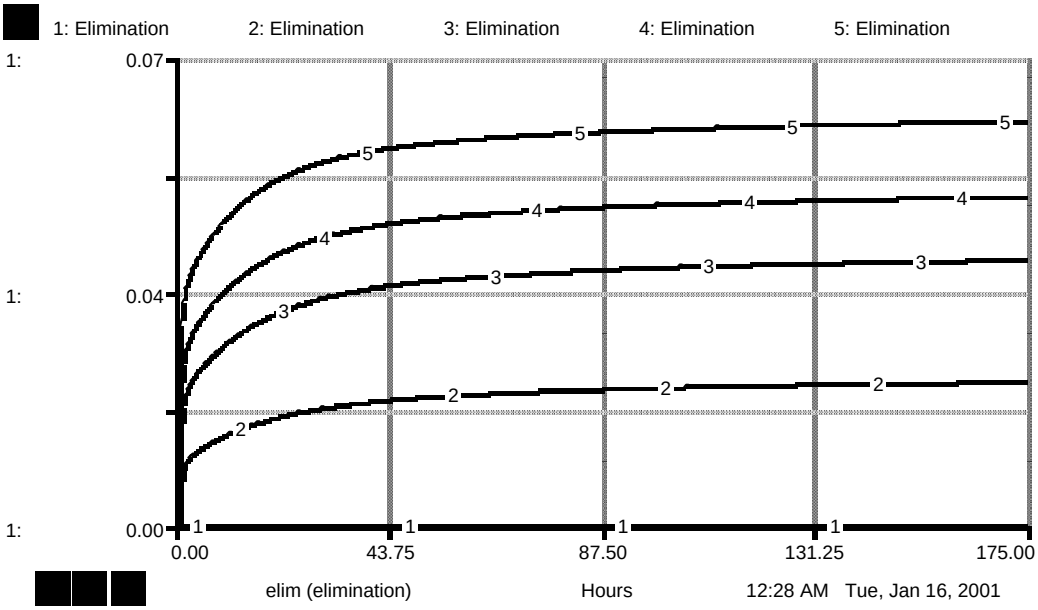
Slowly Perfused Tissue Level (mg) for Increasing Kidney Elimination Fractions

1: Fract=0 2: Fract=0.15 3: Fract=0.35 4: Fract=0.5 5: Fract=0.75



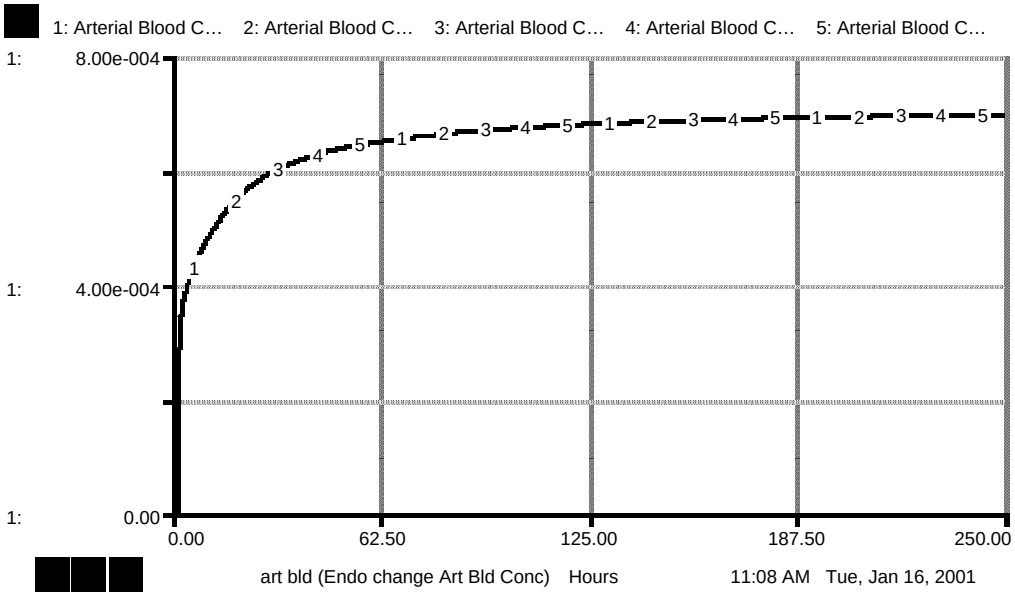
Richly Perfused Tissue Level (mg) for Increasing Kidney Elimination Fractions

1: Fract=0 2: Fract=0.15 3: Fract=0.35 4: Fract=0.5 5: Fract=0.75



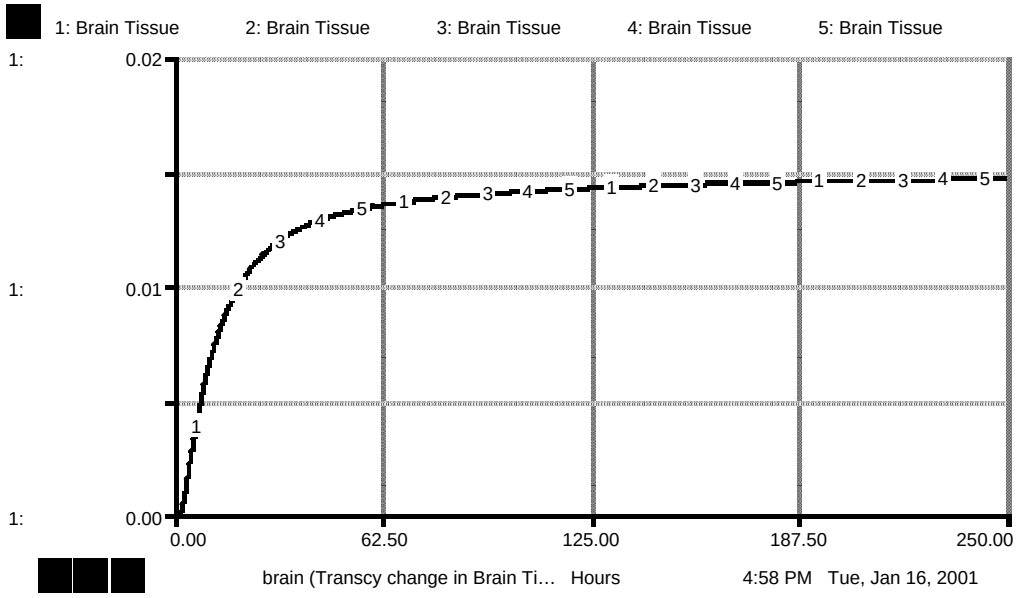
Elimination Flow (mg/hr) for Increasing Kidney Elimination Fractions

1: Fract=0 2: Fract=1E-12 3: Fract=1E-10 4: Fract=1E-8 5: Fract=1E-7



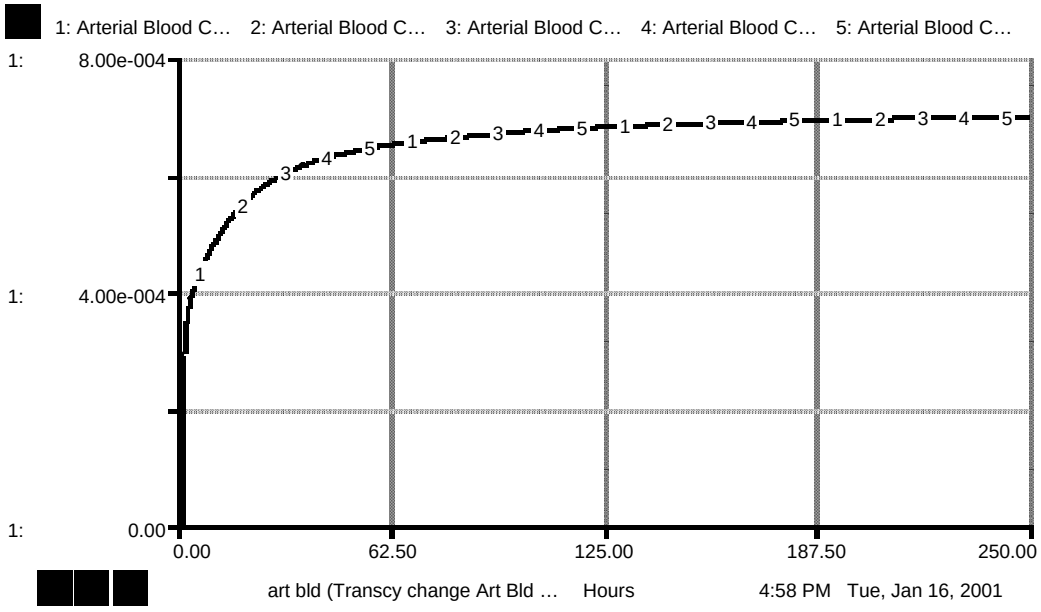
Arterial Blood Concentration (mg/L) for Increasing Endothelial Baseline Flow Fractions

1: Fract=0 2: Fract=1E-13 3: Fract=1E-11 4: Fract=1E-9 5: Fract=1E-8



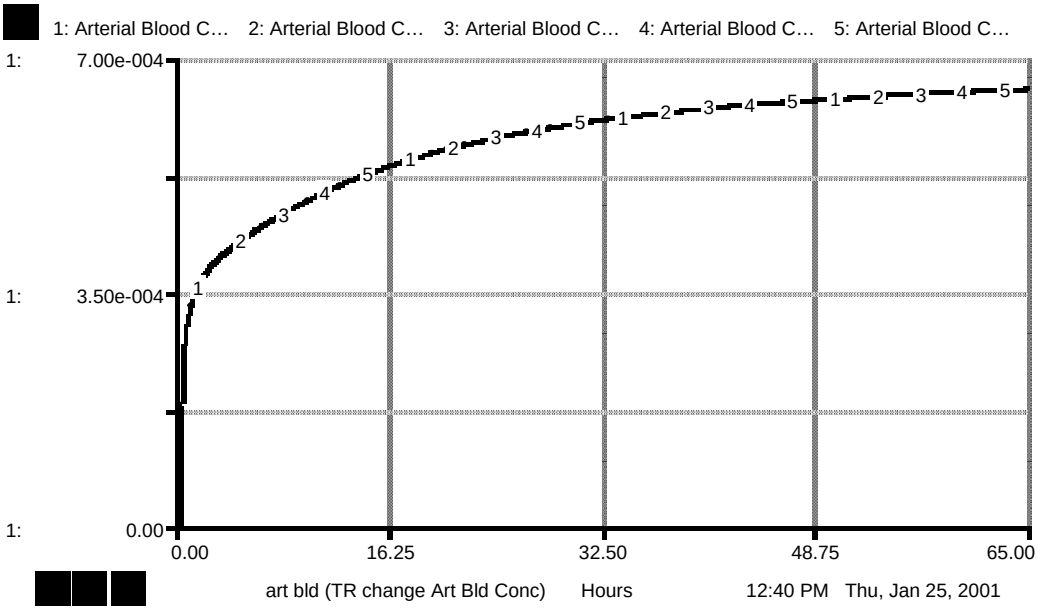
Brain Tissue Level (mg) for Increasing Transcytosis Baseline Flow Fractions

1: Fract=0 2: Fract=1E-13 3: Fract=1E-11 4: Fract=1E-9 5: Fract=1E-8



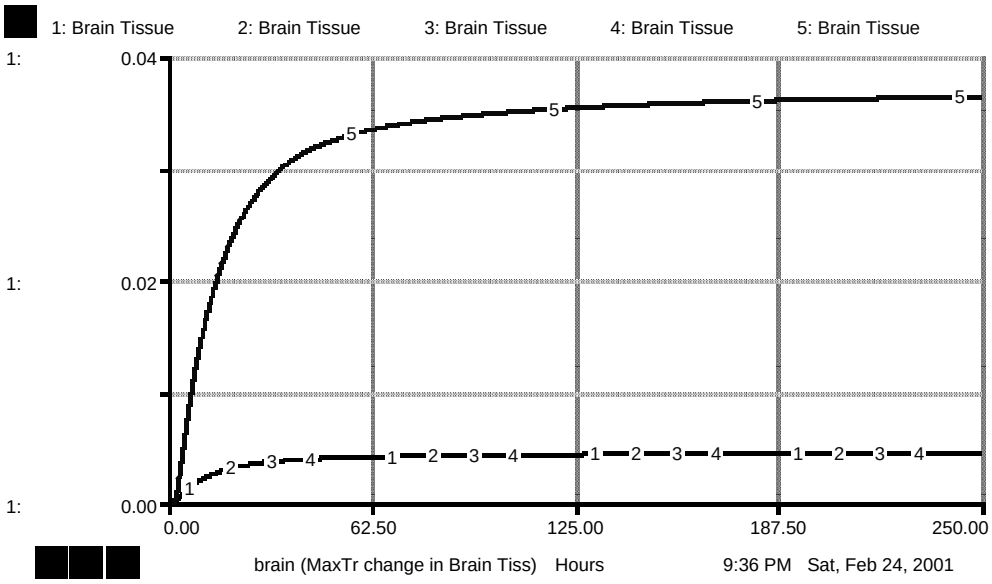
Arterial Blood Concentration (mg/L) for Increasing Transcytosis Baseline Flow Fractions

1: TR=0 2: TR=0.5 3: TR=1 4: TR=3 5: TR=5



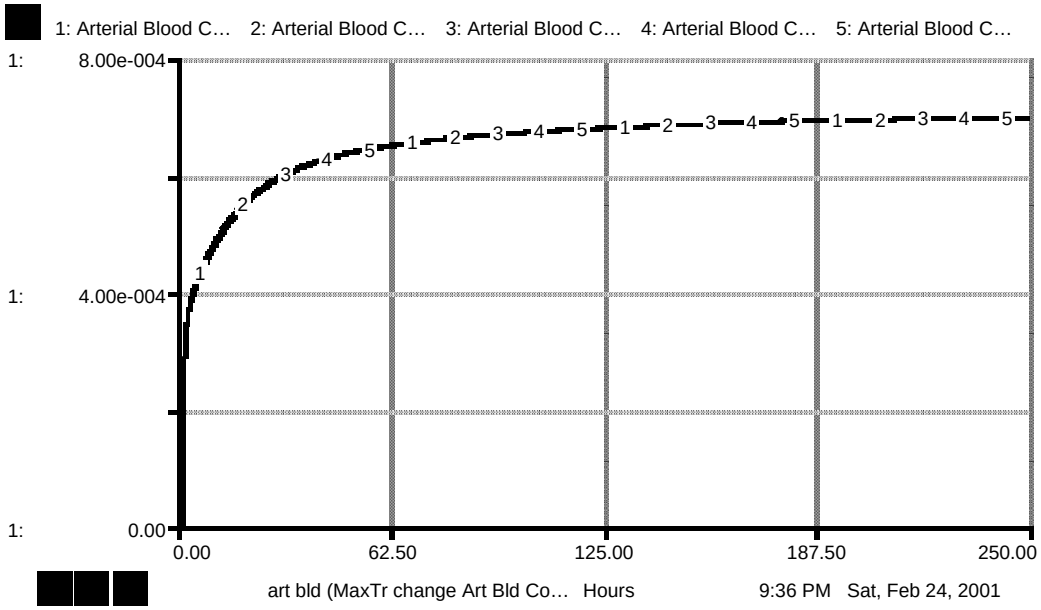
Arterial Blood Concentration (mg/L) for Increasing Passive Diffusion Transfer Rates

1: MT=0.5 2: MT=5 3: MT=10 4: MT=20 5: MT=30



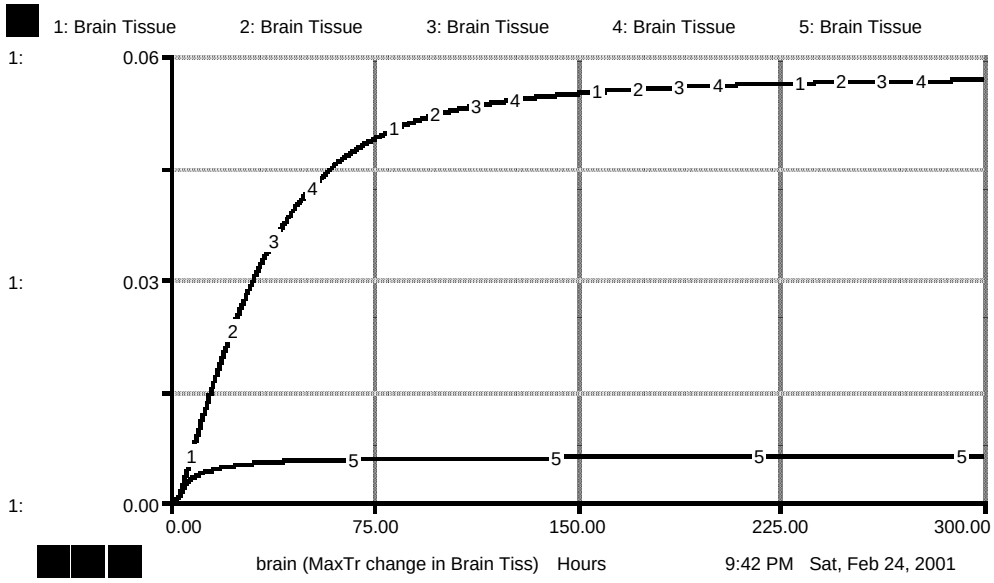
Brain Tissue Level (mg) for Increasing Mediated Max Transport Values

1: MT=0.5 2: MT=5 3: MT=10 4: MT=20 5: MT=30



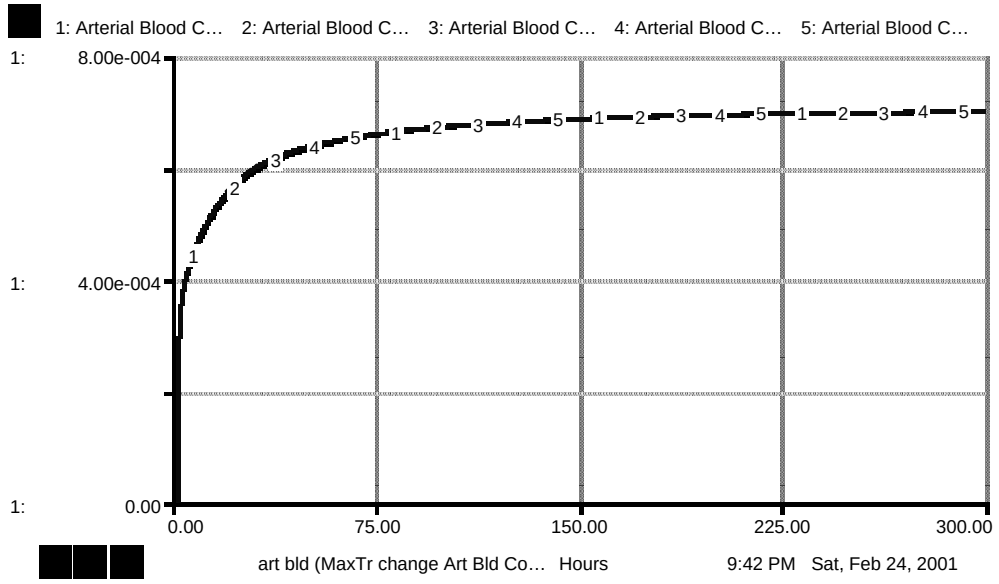
Arterial Blood Concentration (mg/L) for Increasing Mediated Max Transport Values

1: MT2=0 2: MT2=5 3: MT2=10 4: MT2=20 5: MT2=30



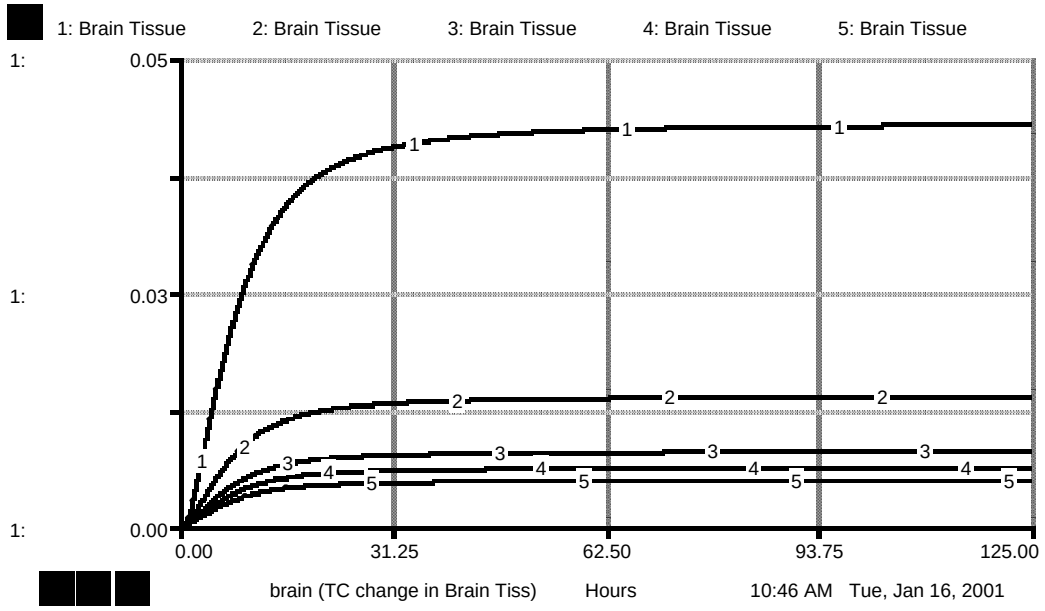
Brain Tissue Level (mg) for Increasing Mediated Max Transport2 Values

1: MT2=0 2: MT2=5 3: MT2=10 4: MT2=20 5: MT2=30



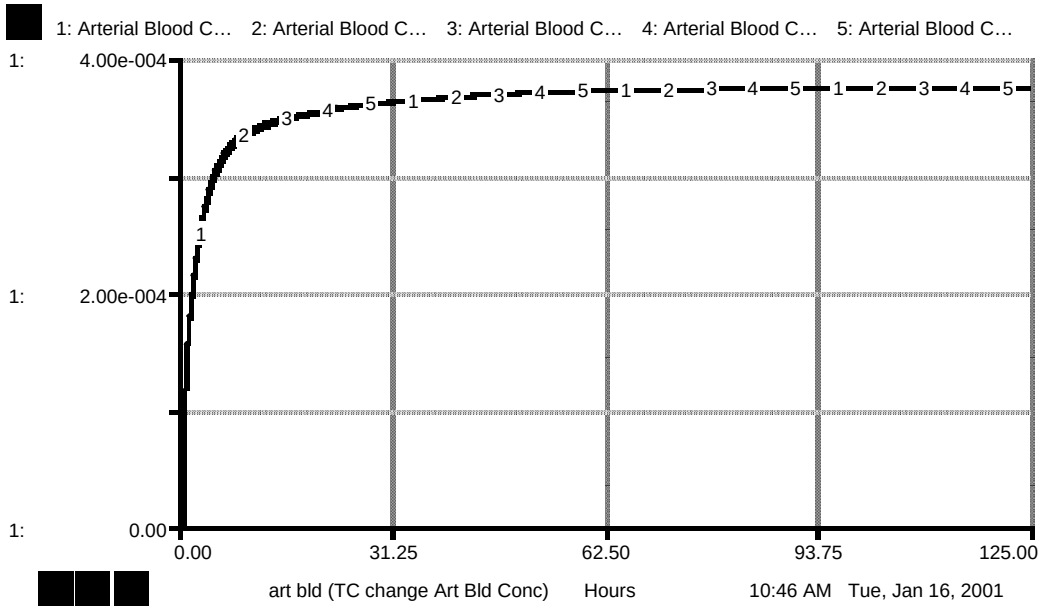
Arterial Blood Concentration (mg/L) for Increasing Mediated Max Transport2 Values

1: TC=0.5 2: TC=1.75 3: TC=3.5 4: TC=5 5: TC=7.5



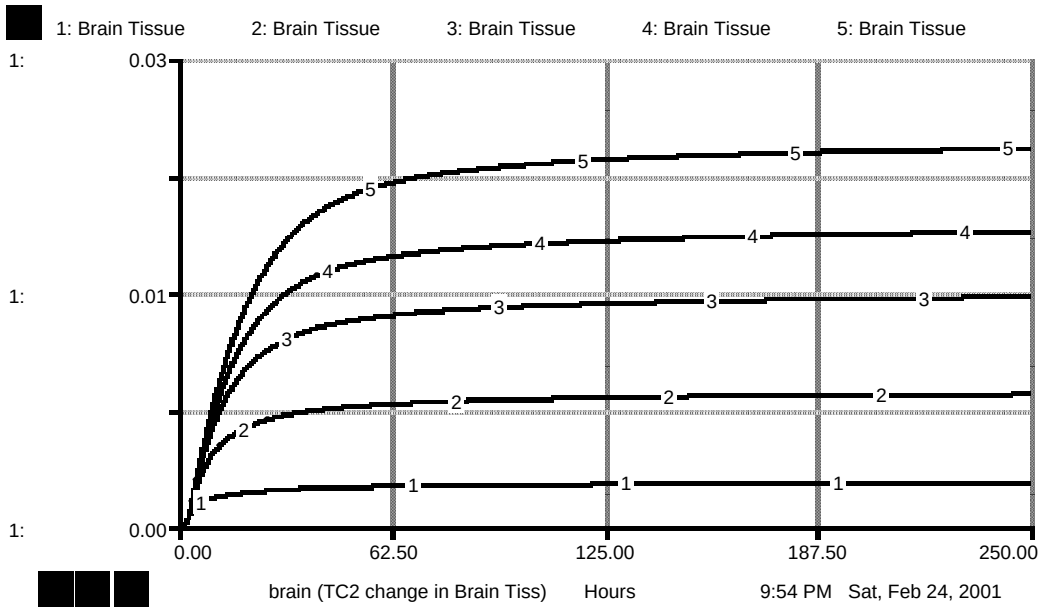
Brain Tissue Level (mg) for Increasing Mediated Transport Constant Values

1: TC=0.5 2: TC=1.75 3: TC=3.5 4: TC=5 5: TC=7.5



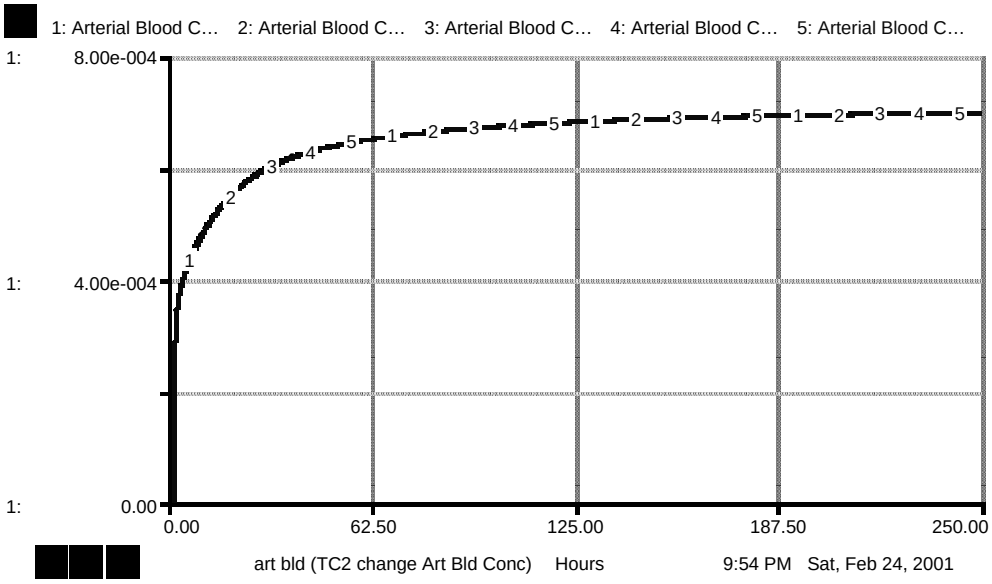
Arterial Blood Concentration (mg/L) for Increasing Mediated Transport Constant Values

1: TC2=0.5 2: TC2=1.75 3: TC2=3.5 4: TC2=5 5: TC2=7.5



Brain Tissue Level (mg) for Increasing Mediated Transport Constant2 Values

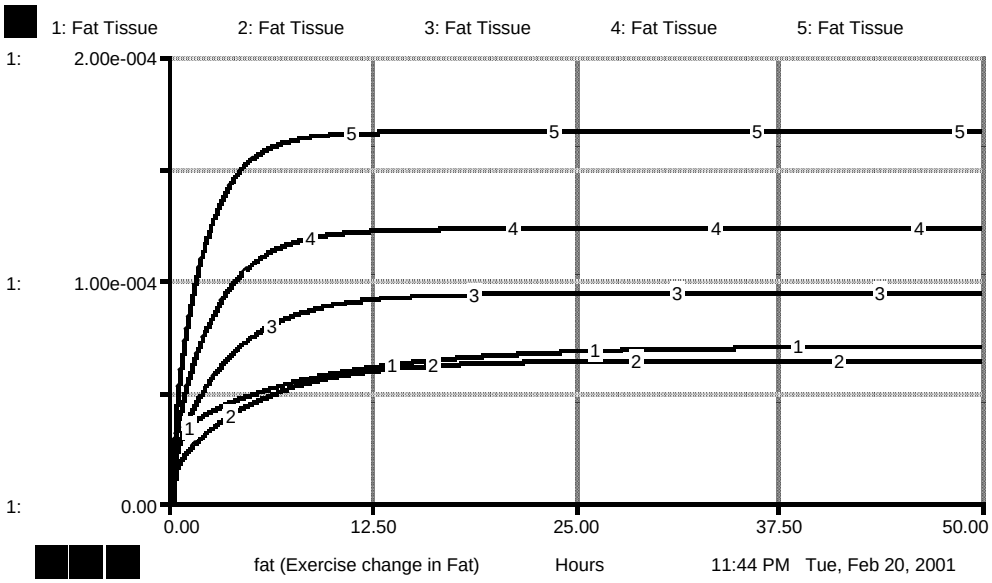
1: TC2=0.5 2: TC2=1.75 3: TC2=3.5 4: TC2=5 5: TC2=7.5



Arterial Blood Conc. (mg/L) for Increasing Mediated Transport Constant2 Values

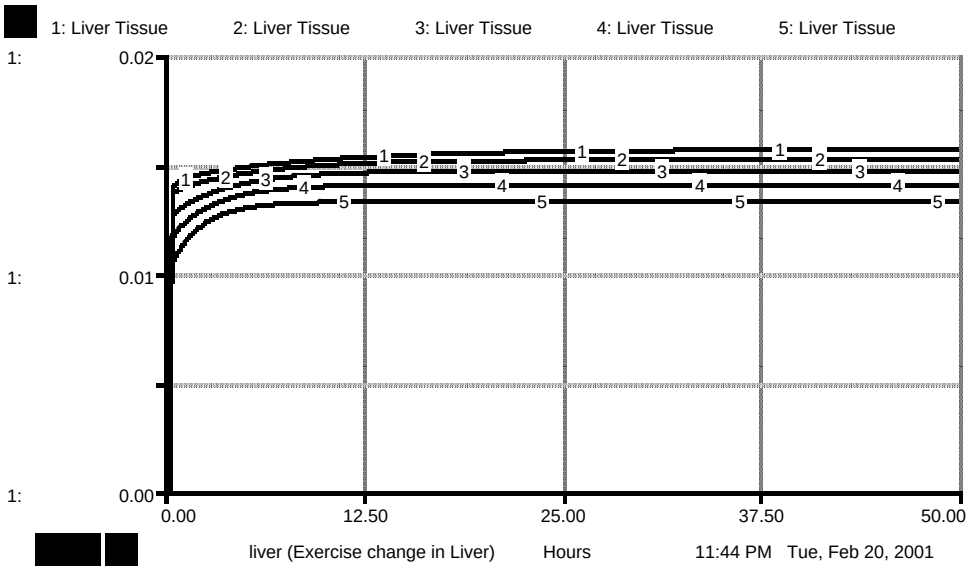
Appendix D – Scenario 2

1: At Rest 2: 15% Exercise 3: 25% Exercise 4: 55% Exercise 5: 100% Exercise



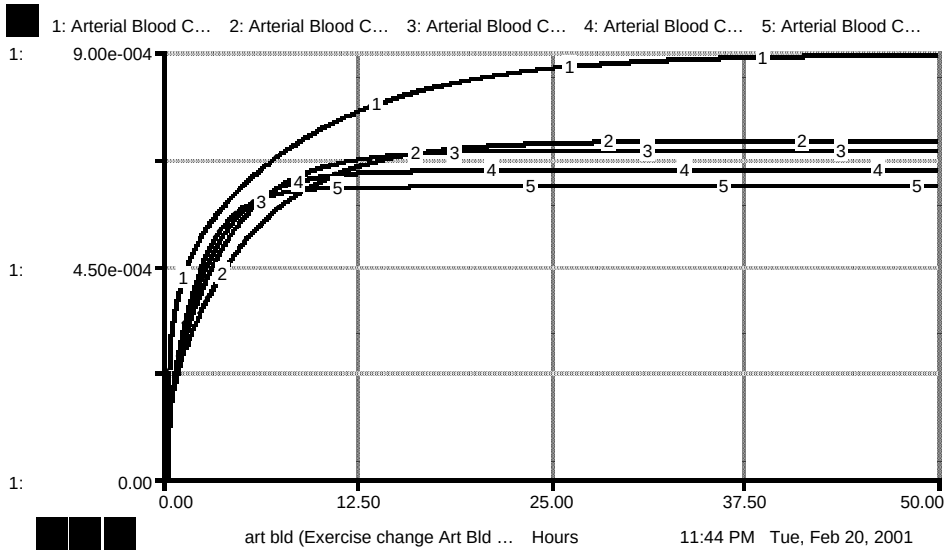
Fat Tissue Level (mg) with Increasing Exercise

1: At Rest 2: 15% Exercise 3: 25% Exercise 4: 55% Exercise 5: 100% Exercise



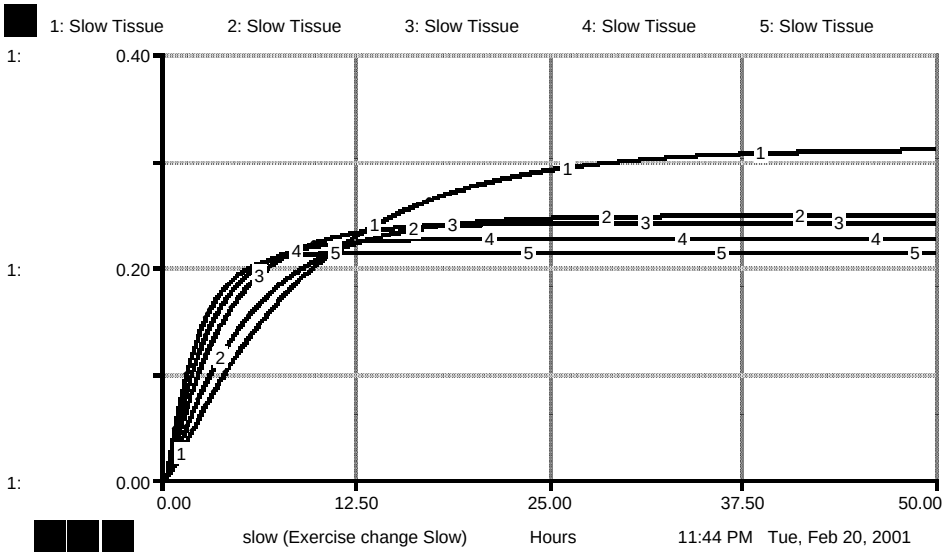
Liver Tissue Level (mg) with Increasing Exercise

1: At Rest 2: 15% Exercise 3: 25% Exercise 4: 55% Exercise 5: 100% Exercise



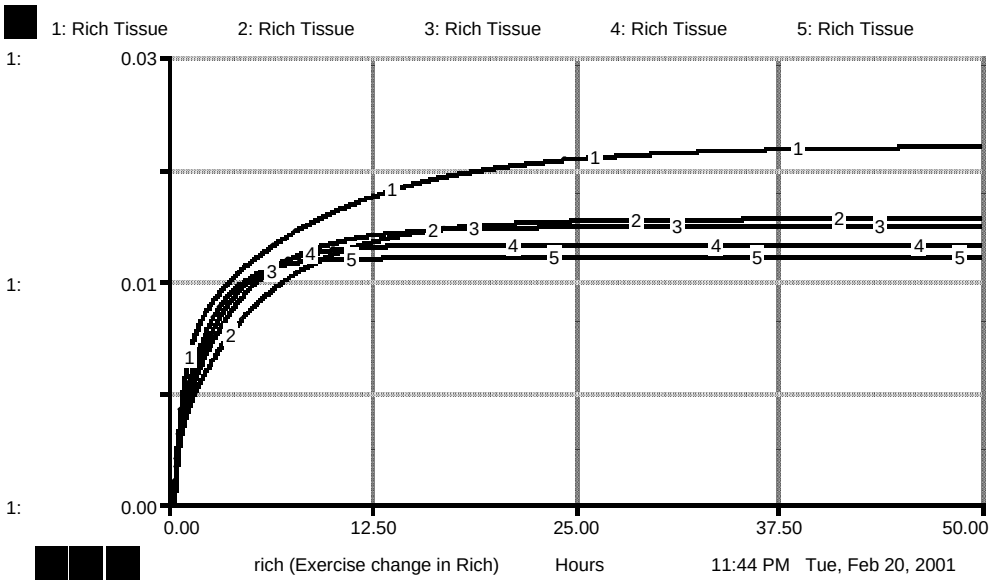
Arterial Blood Concentration (mg/L) with Increasing Exercise

1: At Rest 2: 15% Exercise 3: 25% Exercise 4: 55% Exercise 5: 100% Exercise



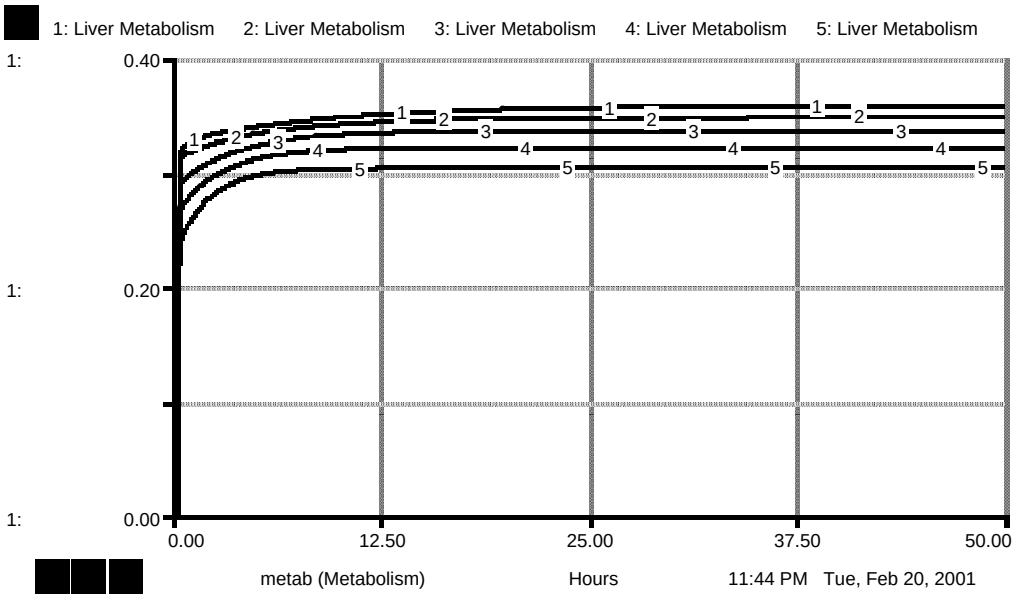
Slowly Perfused Tissue Level (mg) with Increasing Exercise

1: At Rest 2: 15% Exercise 3: 25% Exercise 4: 55% Exercise 5: 100% Exercise



Richly Perfused Tissue Level (mg) with Increasing Exercise

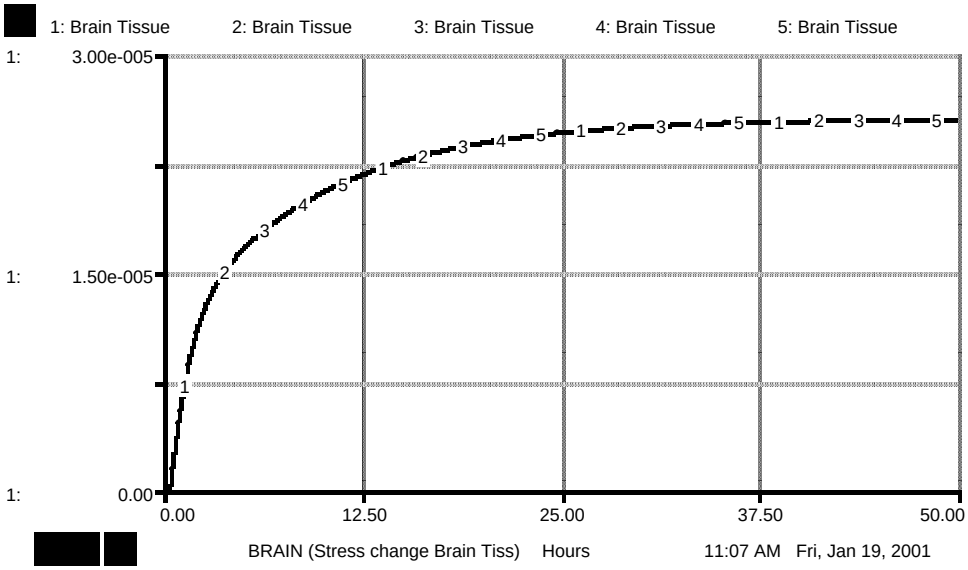
1: At Rest 2: 15% Exercise 3: 25% Exercise 4: 55% Exercise 5: 100% Exercise



Liver Metabolism (mg/hr) with Increasing Exercise

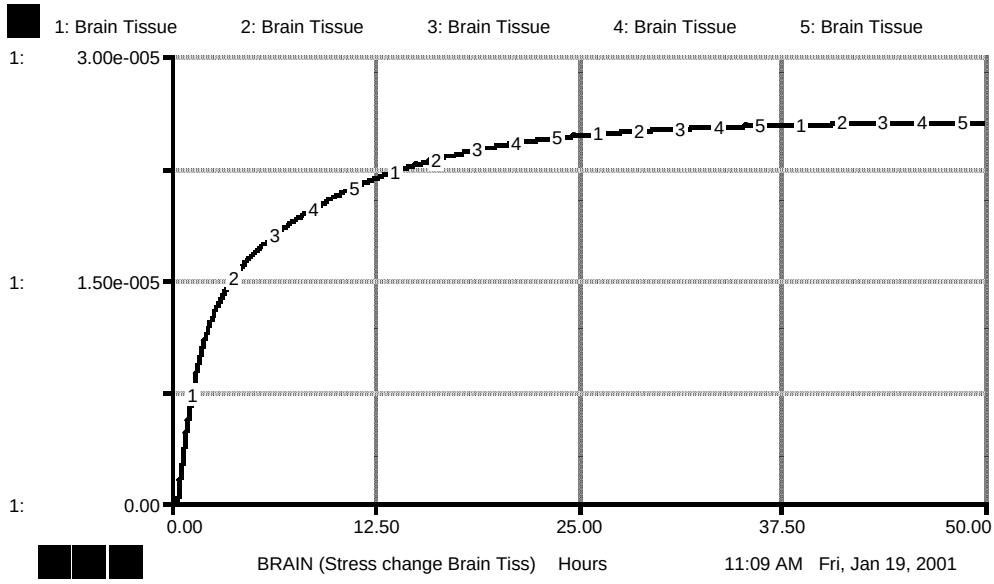
Appendix E – Scenario 3

1: Stress-Free 2: 15% Stress 3: 25% Stress 4: 55% Stress 5: 100% Stress



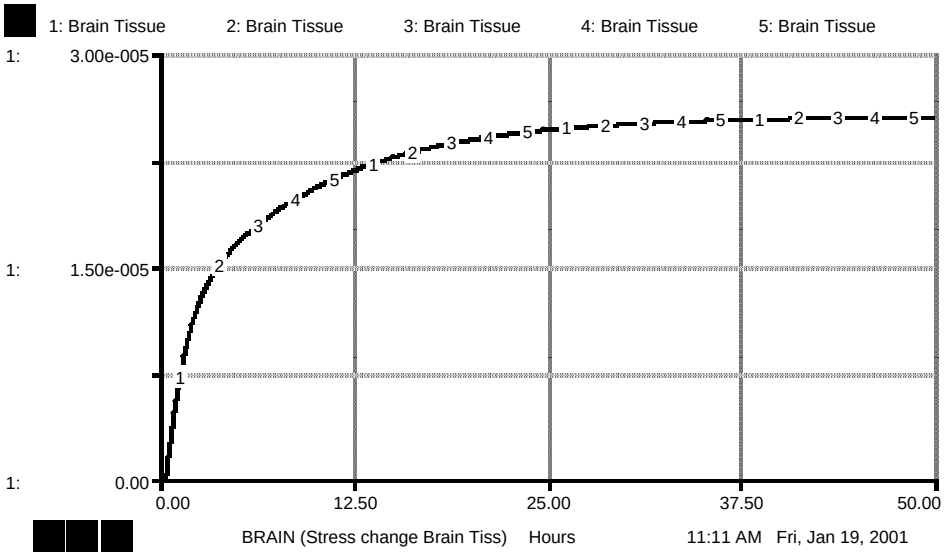
Brain Tissue Level (mg) with for Linearly Increasing Endothelial Flow Fractions (0 to 1E-8) with Increasing Stress

1: Stress-Free 2: 15% Stress 3: 25% Stress 4: 55% Stress 5: 100% Stress



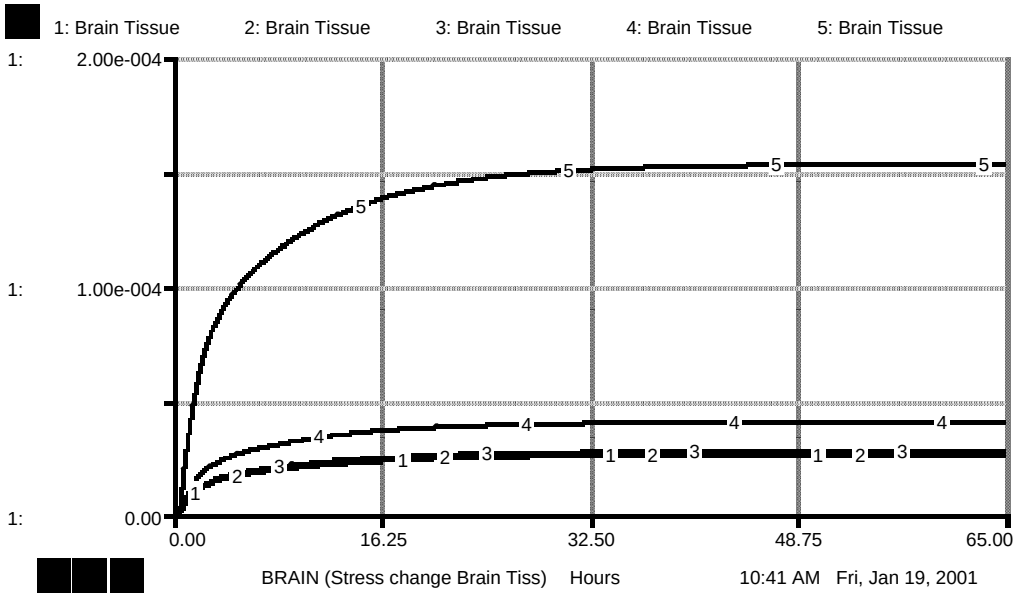
Brain Tissue Level (mg) with for Exponentially Increasing Endothelial Flow Fractions (0 to 1E-8) with Increasing Stress

1: Stress-Free 2: 15% Stress 3: 25% Stress 4: 55% Stress 5: 100% Stress



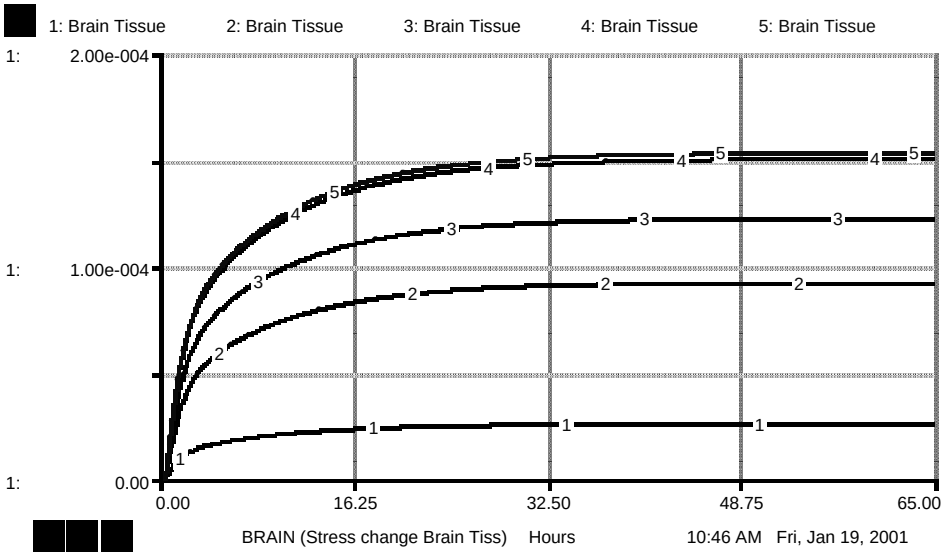
Brain Tissue Level (mg) with for Concave Up Endothelial Flow Fractions (0 to 1E-8) with Increasing Stress

1: Stress-Free 2: 15% Stress 3: 25% Stress 4: 55% Stress 5: 100% Stress



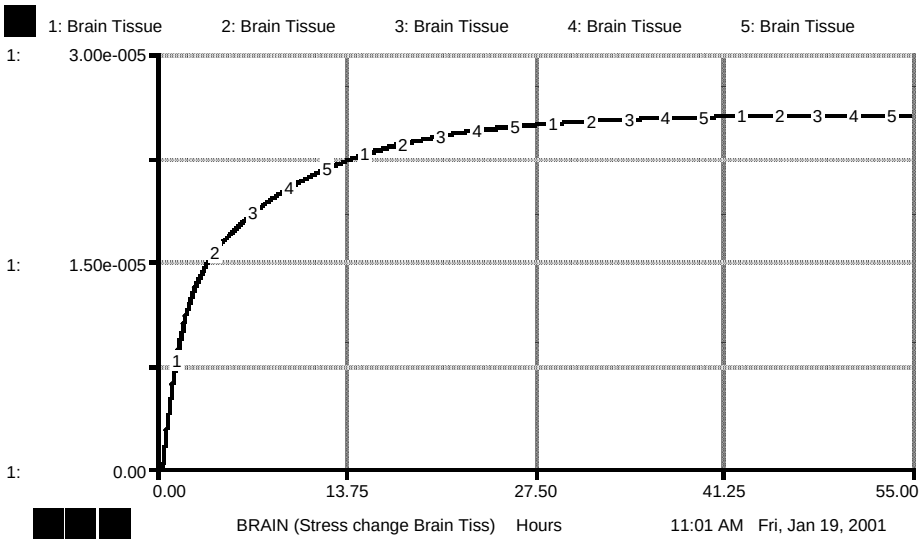
Brain Tissue Level (mg) with for Exponentially Increasing Mediated Maximum Transport (5 to 30) with Increasing Stress

1: Stress-Free 2: 15% Stress 3: 25% Stress 4: 55% Stress 5: 100% Stress



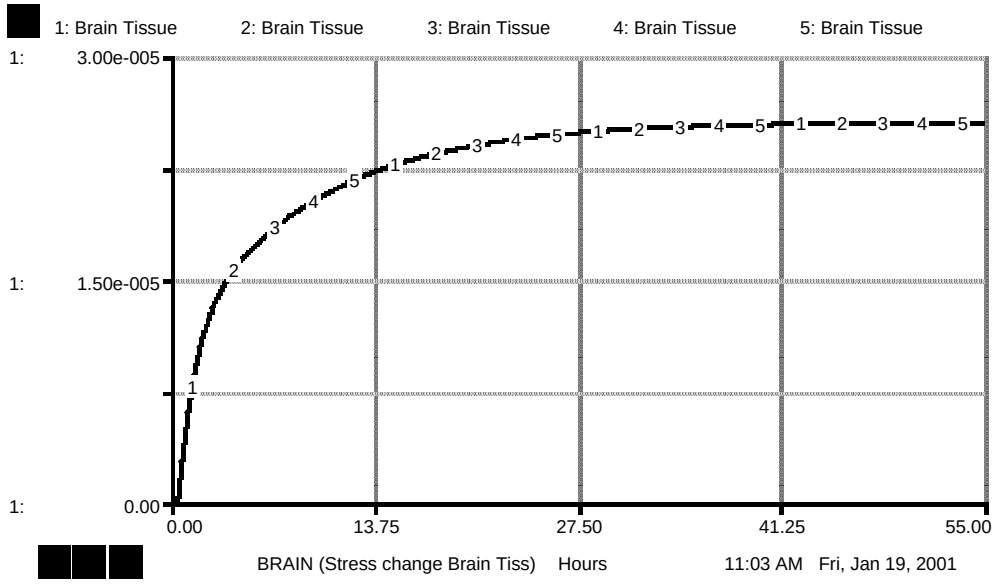
Brain Tissue Level (mg) with for Concave Up Mediated Maximum Transport (5 to 30) with Increasing Stress

1: Stress-Free 2: 15% Stress 3: 25% Stress 4: 55% Stress 5: 100% Stress



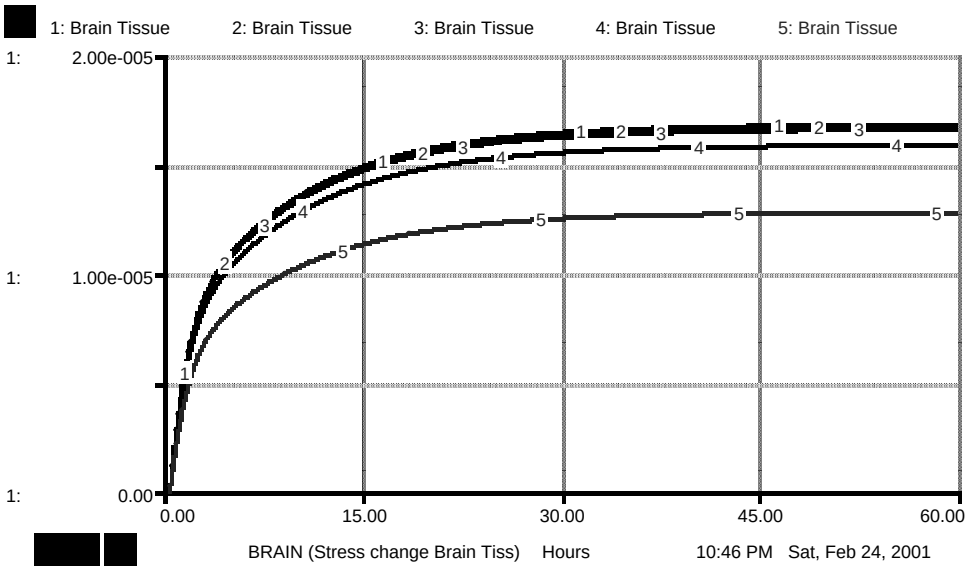
Brain Tissue Level (mg) with for Exponentially Increasing Transcytosis Flow Fractions (0 to 1E-9) with Increasing Stress

1: Stress-Free 2: 15% Stress 3: 25% Stress 4: 55% Stress 5: 100% Stress



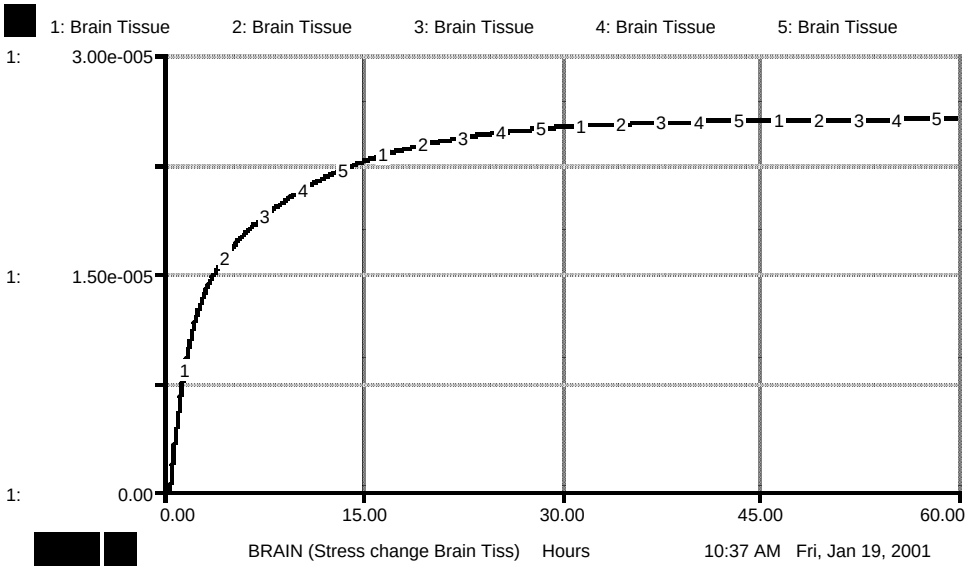
Brain Tissue Level (mg) with for Concave Up Transcytosis Flow Fractions (0 to 1E-9) with Increasing Stress

1: Stress-Free 2: 15% Stress 3: 25% Stress 4: 55% Stress 5: 100% Stress



Brain Tissue Level (mg) for Exponentially Increasing Passive Diffusion Transfer Rate (0.5 to 1) with Increasing Stress

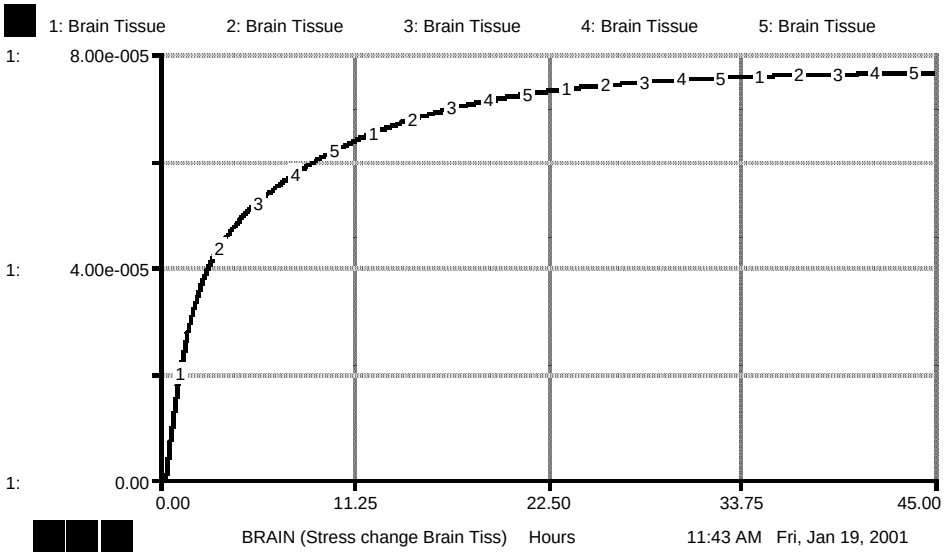
1: Stress-Free 2: 15% Stress 3: 25% Stress 4: 55% Stress 5: 100% Stress



Brain Tissue Level (mg) for Concave Up Passive Diffusion
Transfer Rate (0.5 to 1) with Increasing Stress

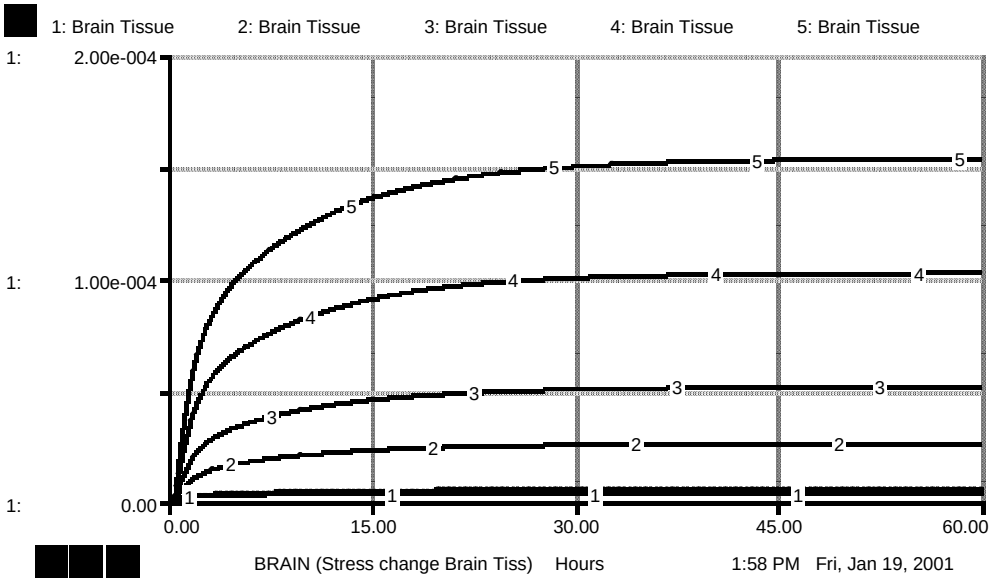
Appendix F – Scenario 4

1: Fract=0 2: Fract=1E-11 3: Fract=1E-9 4: Fract=1E-7 5: Fract=1E-5



Brain Tissue Level (mg) with Increasing Transcytosis Flow Fractions at Maximum Stress

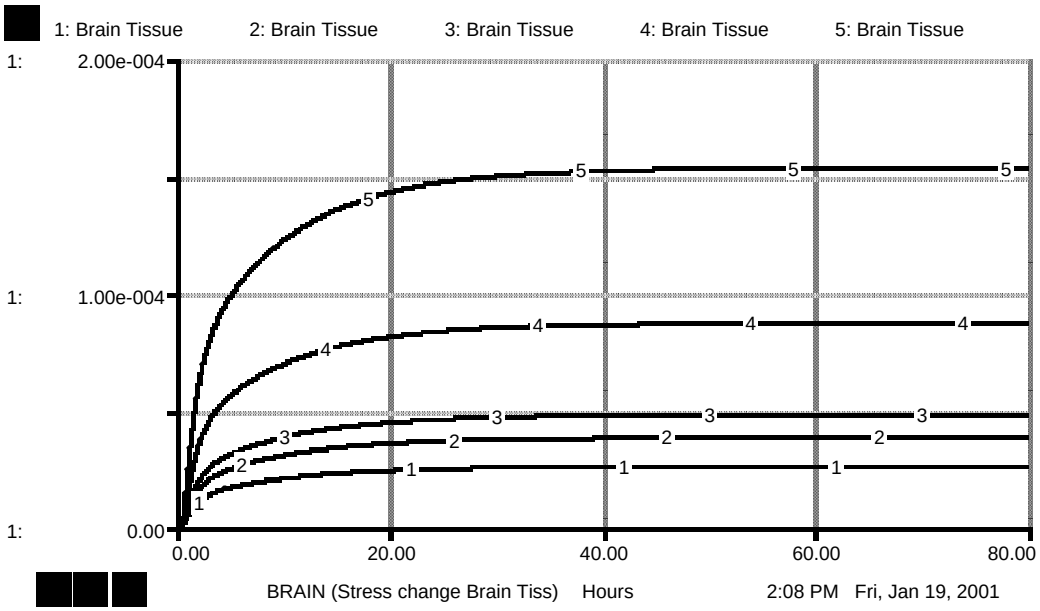
1: MT=0.5 2: MT=5 3: MT=10 4: MT=20 5: MT=30



Brain Tissue Level (mg) with Increasing Mediated Maximum Transport at Maximum Stress

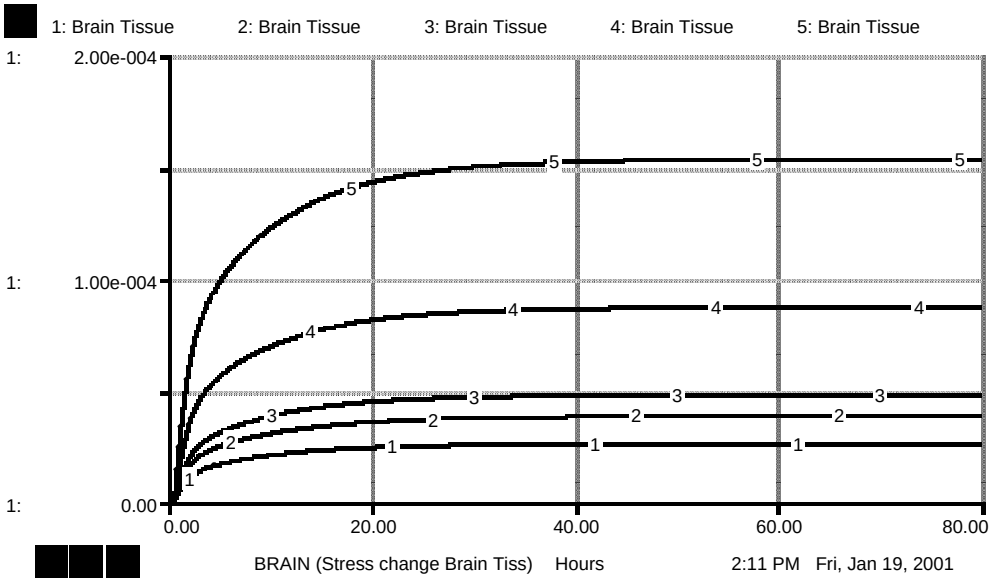
Appendix G – Scenario 5

1: Stress-Free 2: 15% Stress 3: 25% Stress 4: 55% Stress 5: 100% Stress



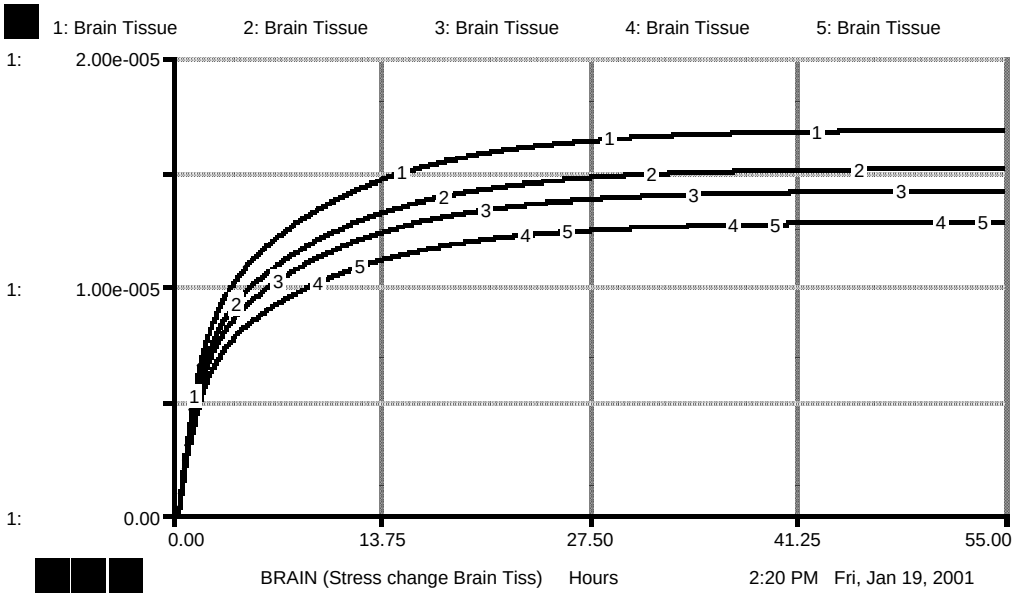
Brain Tissue Level (mg) for Linearly Increasing Mediated Transport/ Passive Diffusion Combination for Increasing Stress

1: Stress-Free 2: 15% Stress 3: 25% Stress 4: 55% Stress 5: 100% Stress



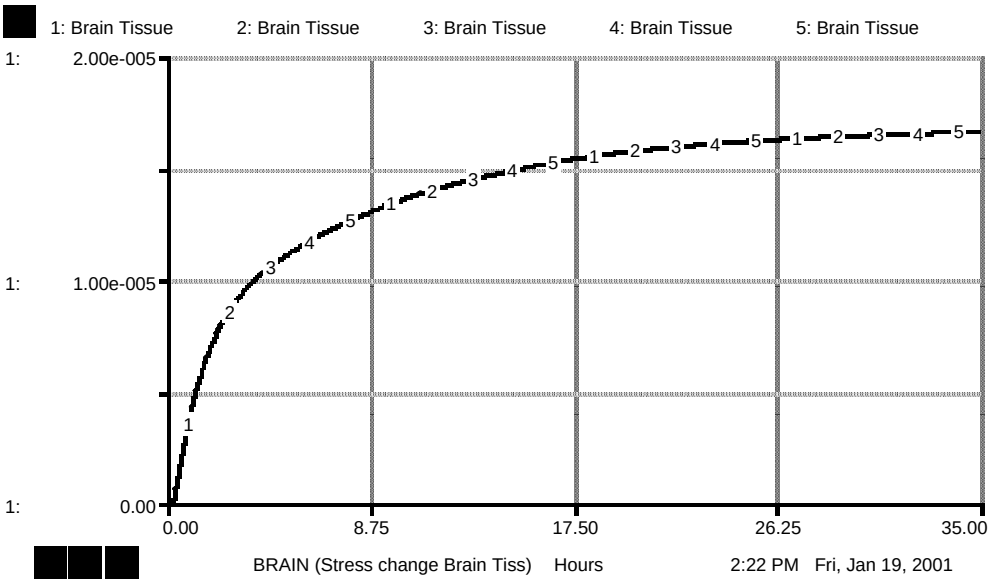
Brain Tissue Level (mg) for Linearly Increasing Mediated Transport/ Transcytosis Combination for Increasing Stress

1: Stress-Free 2: 15% Stress 3: 25% Stress 4: 55% Stress 5: 100% Stress



Brain Tissue Level (mg) for Linearly Increasing Passive Diffusion Transfer Rate/ Endothelial Transport Combination for Increasing Stress

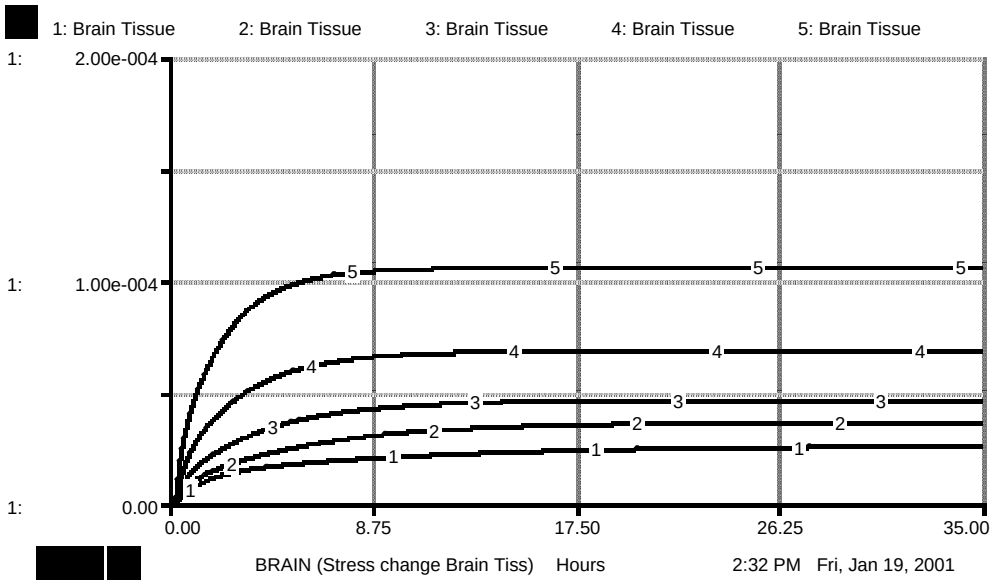
1: Stress-Free 2: 15% Stress 3: 25% Stress 4: 55% Stress 5: 100% Stress



Brain Tissue Level (mg) for Linearly Increasing Transcytosis/ Endothelial Transport Combination for Increasing Stress

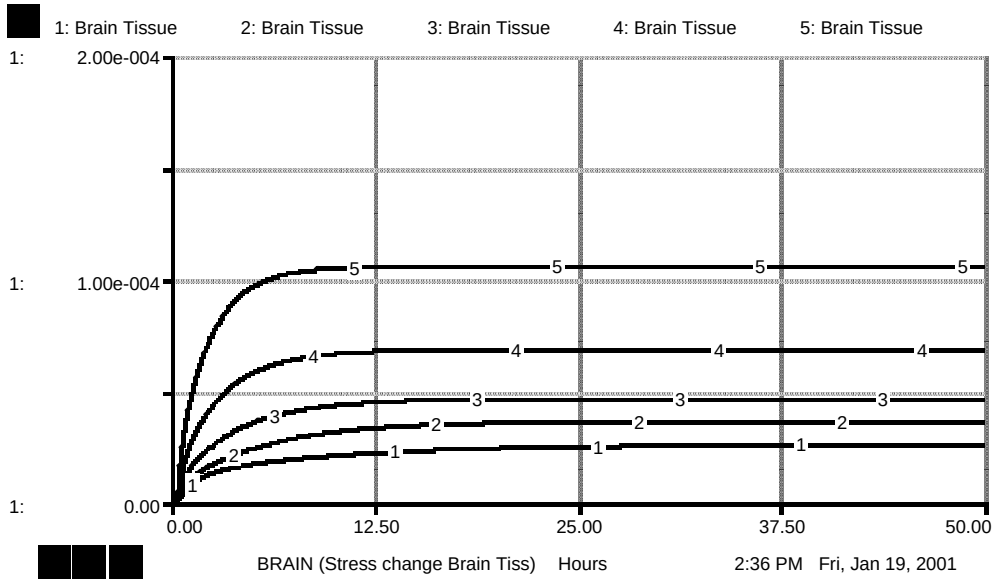
Appendix H – Scenario 6

1:Stress-Free/Rest 2:15% Str/Ex 3:25% Str/Ex 4:55% Str/Ex 5:100% Str/Ex



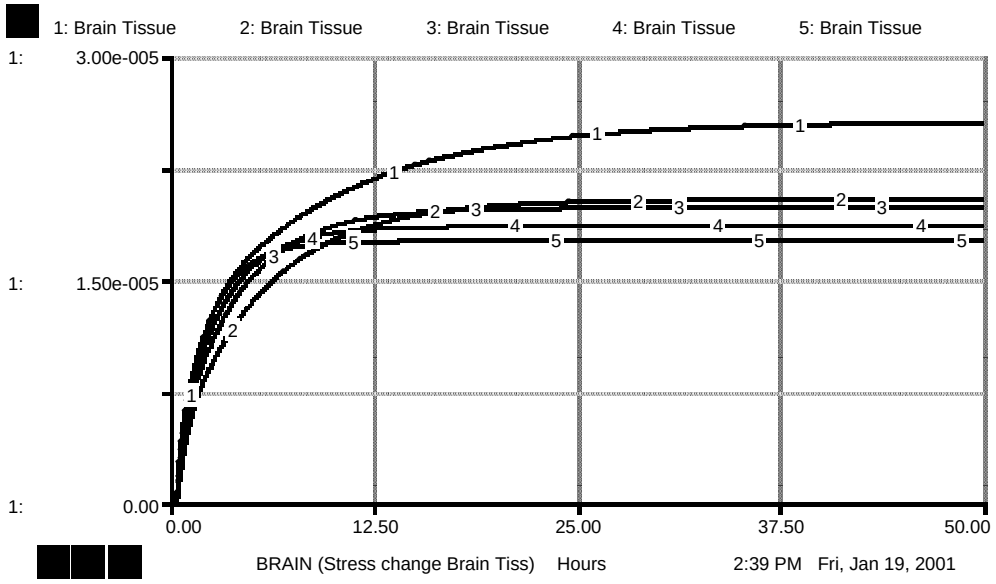
Brain Tissue Level (mg) for Linearly Increasing Mediated Maximum Transport/ Passive Diffusion Transfer Rate Combination for Increasing Stress and Exercise

1:Stress-Free/Rest 2:15% Str/Ex 3:25% Str/Ex 4:55% Str/Ex 5:100% Str/Ex



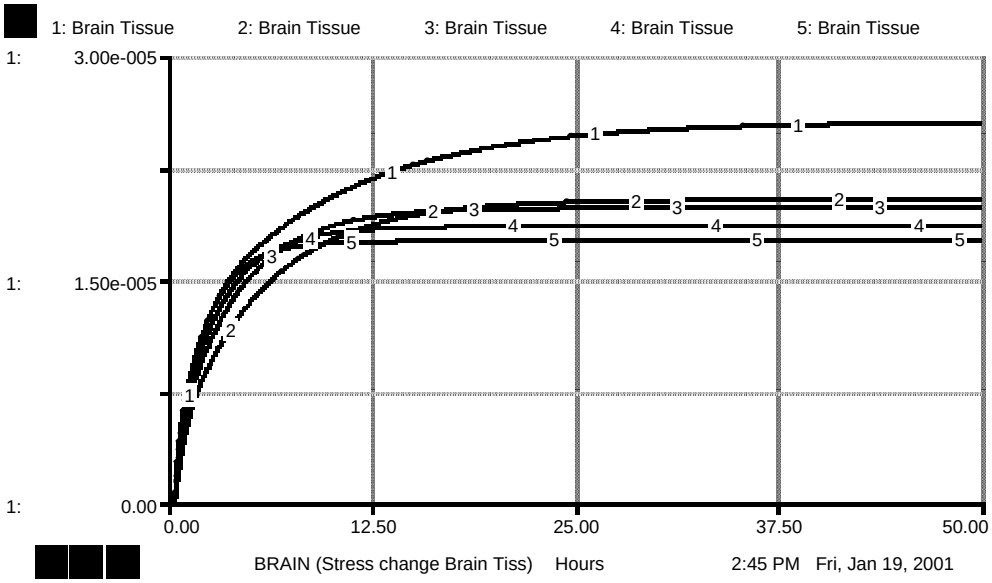
Brain Tissue Level (mg) for Linearly Increasing Mediated Maximum Transport/ Transcytosis Combination for Increasing Stress and Exercise

1:Stress-Free/Rest 2:15% Str/Ex 3:25% Str/Ex 4:55% Str/Ex 5:100% Str/Ex



Brain Tissue Level (mg) for Linearly Increasing Passive Diffusion Transfer Rate/ Endothelial Transport Combination for Increasing Stress and Exercise

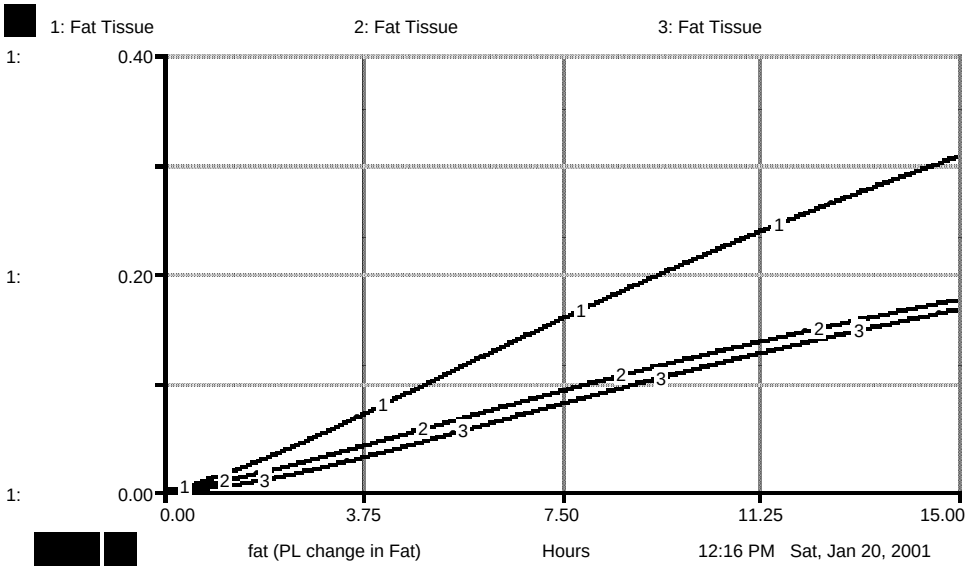
1:Stress-Free/Rest 2:15% Str/Ex 3:25% Str/Ex 4:55% Str/Ex 5:100% Str/Ex



Brain Tissue Level (mg) for Increasing Endothelial Transport/ Transcytosis Combination for Increasing Stress and Exercise

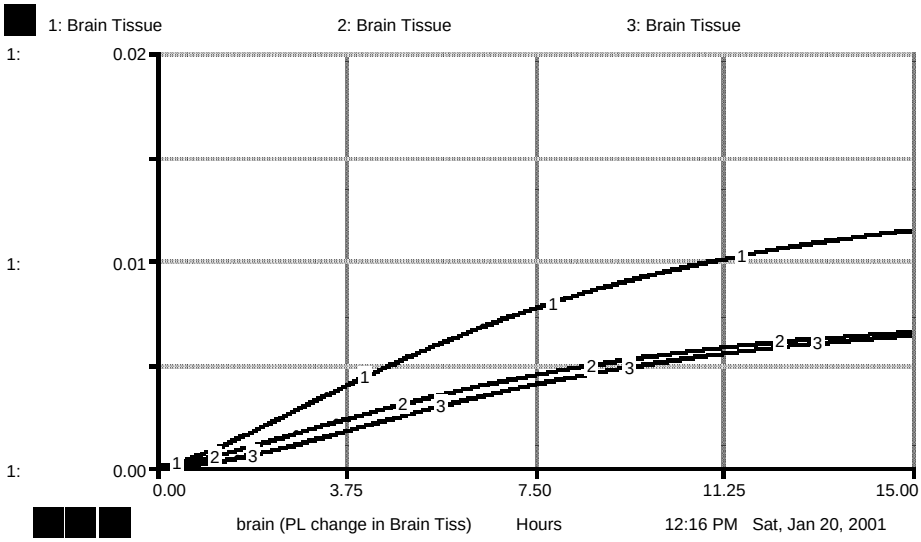
Appendix I – Scenario 7

1: PL =0.2 2: PL=15 3: PL=250



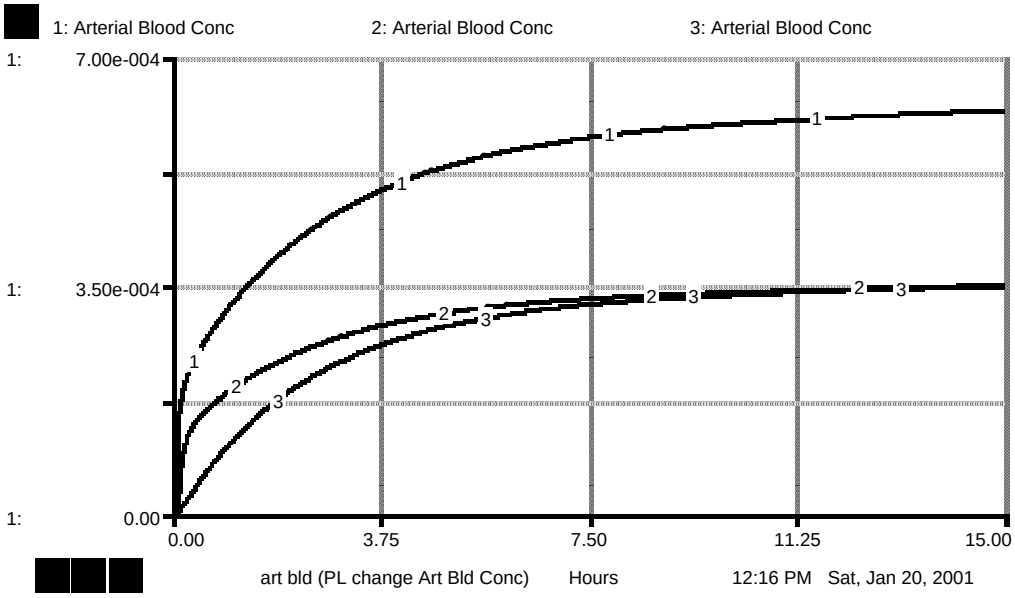
Fat Tissue Level (mg) for Increasing Liver/Blood PC Values
at Maximum Stress and Exercise

1: PL =0.2 2: PL=15 3: PL=250



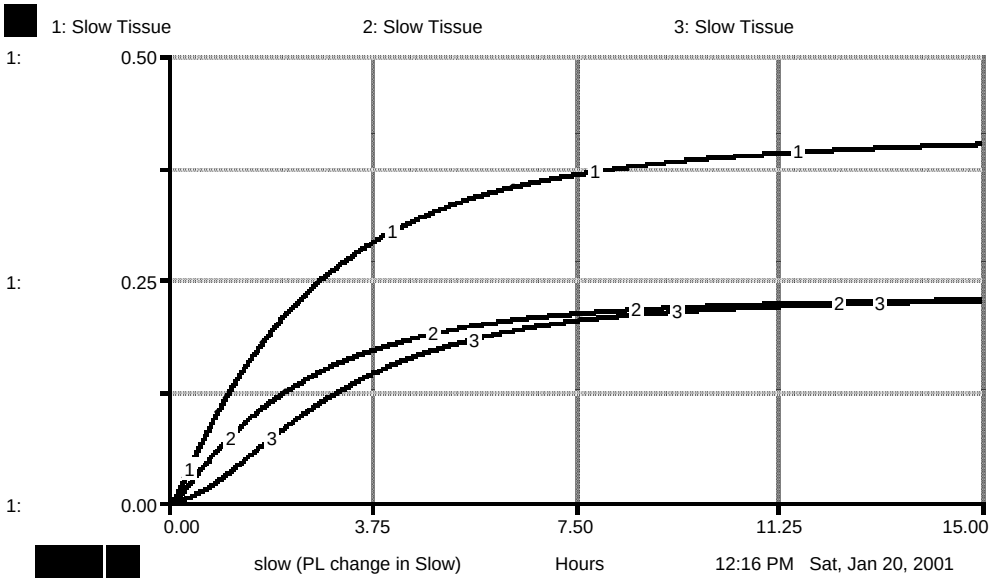
Brain Tissue Level (mg) for Increasing Liver/Blood PC Values
at Maximum Stress and Exercise

1: PL =0.2 2: PL=15 3: PL=250



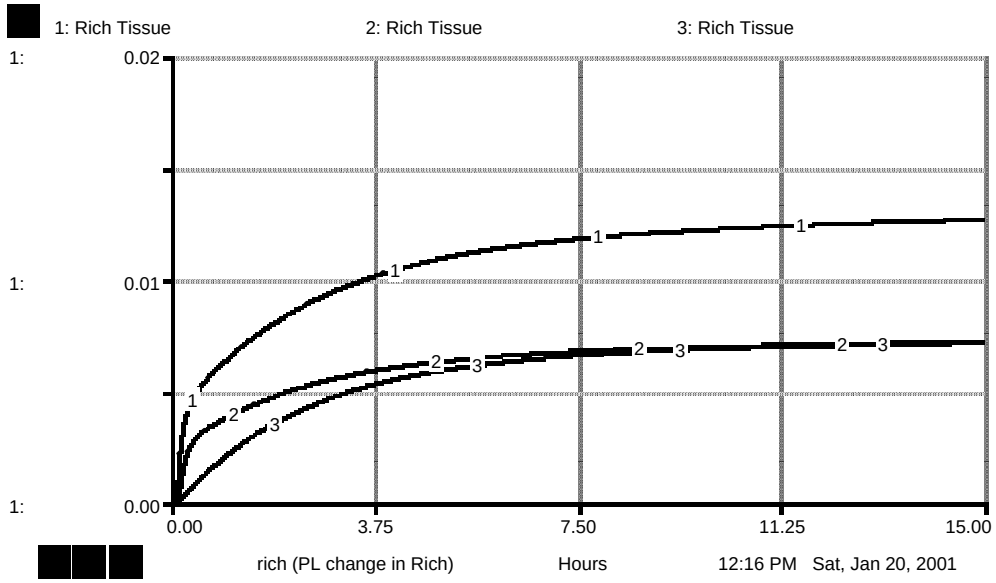
Arterial Blood Concentration (mg/L) for Increasing Liver/Blood PC Values
at Maximum Stress and Exercise

1: PL =0.2 2: PL=15 3: PL=250



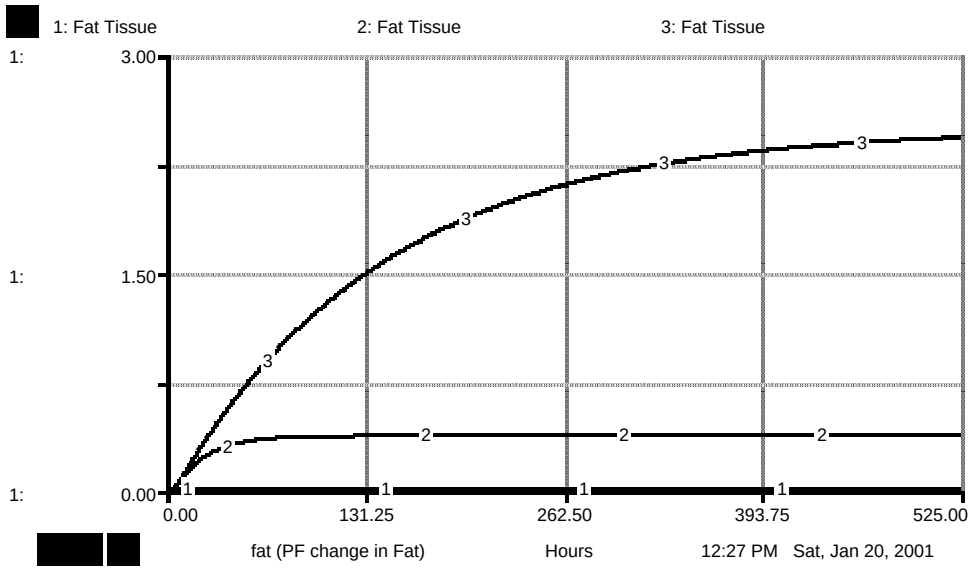
Slowly Perfused Tissue Level (mg) for Increasing Liver/Blood PC Values
at Maximum Stress and Exercise

1: PL =0.2 2: PL=15 3: PL=250



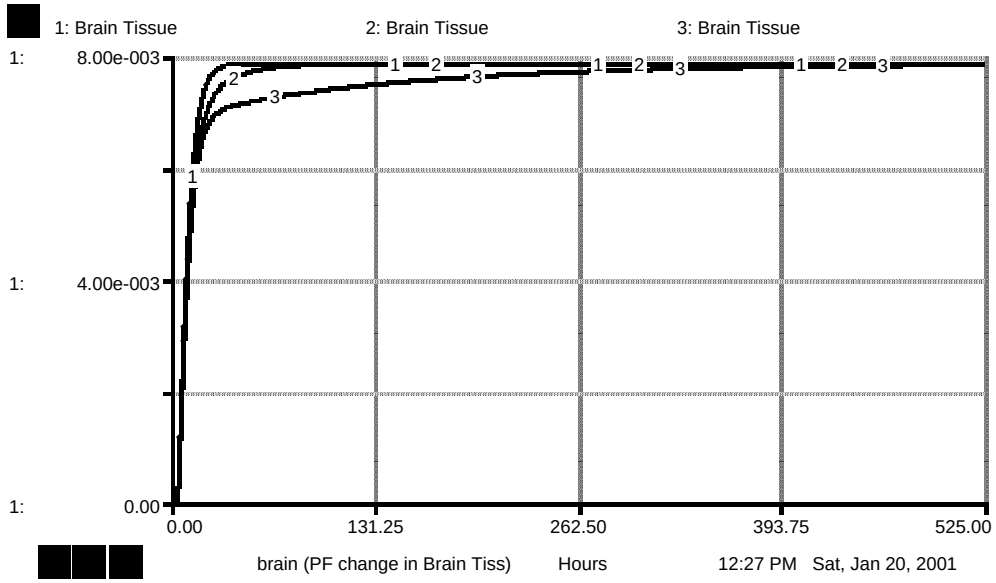
Richly Perfused Tissue Level (mg) for Increasing Liver/Blood PC Values
at Maximum Stress and Exercise

1: PF =0.002 2: PF=75 3: PF=500



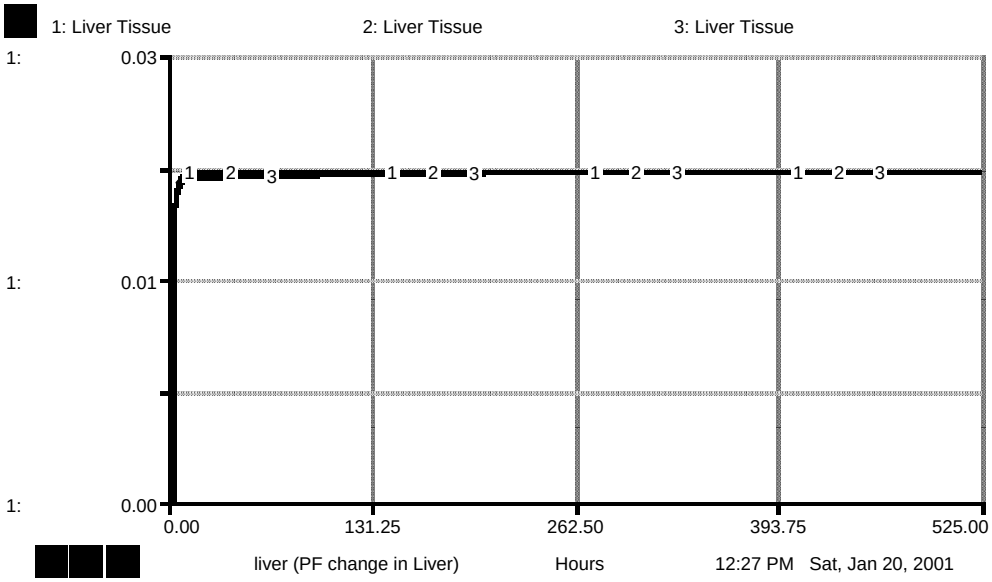
Fat Tissue Level (mg) for Increasing Fat/Blood PC Values
at Maximum Stress and Exercise

1: PF =0.002 2: PF=75 3: PF=500



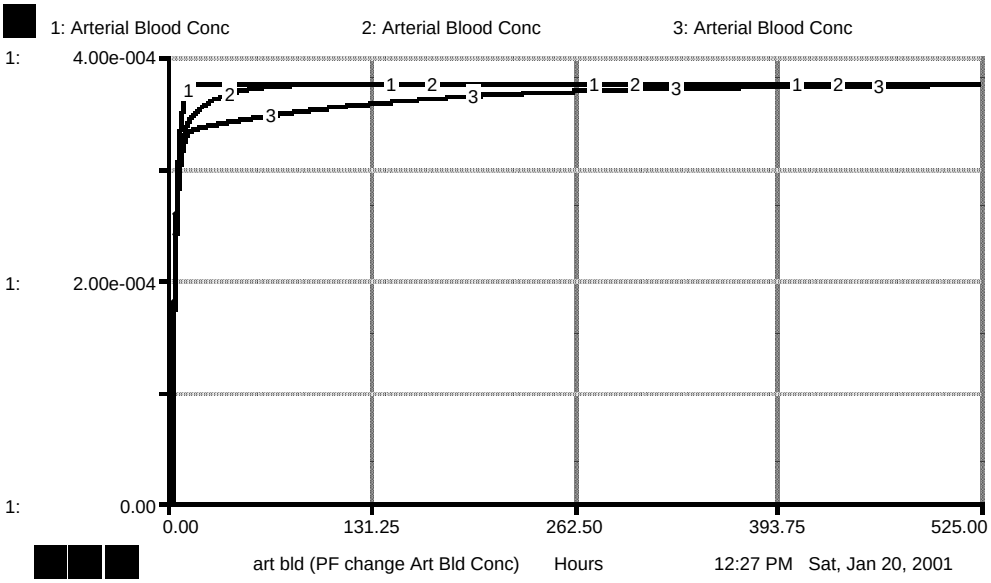
Brain Tissue Level (mg) for Increasing Fat/Blood PC Values
at Maximum Stress and Exercise

1: PF =0.002 2: PF=75 3: PF=500



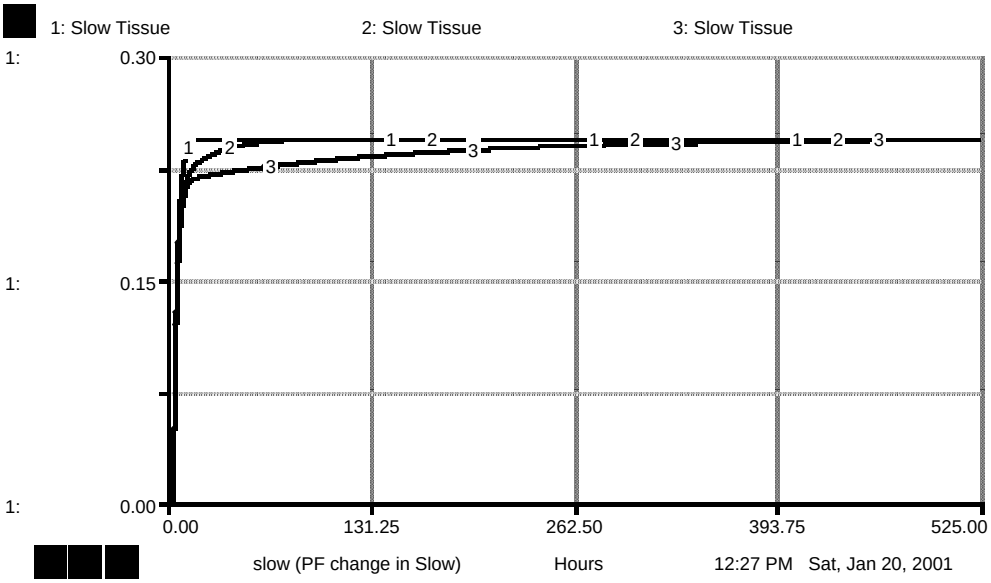
Liver Tissue Level (mg) for Increasing Fat/Blood PC Values
at Maximum Stress and Exercise

1: PF =0.002 2: PF=75 3: PF=500



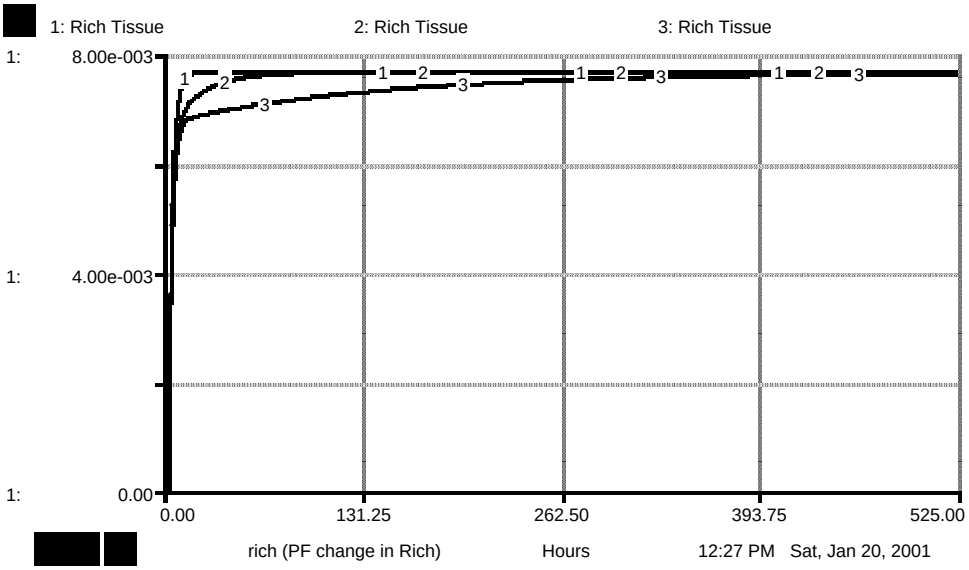
Arterial Blood Concentration (mg/L) for Increasing Fat/Blood PC Values
at Maximum Stress and Exercise

1: PF =0.002 2: PF=75 3: PF=500



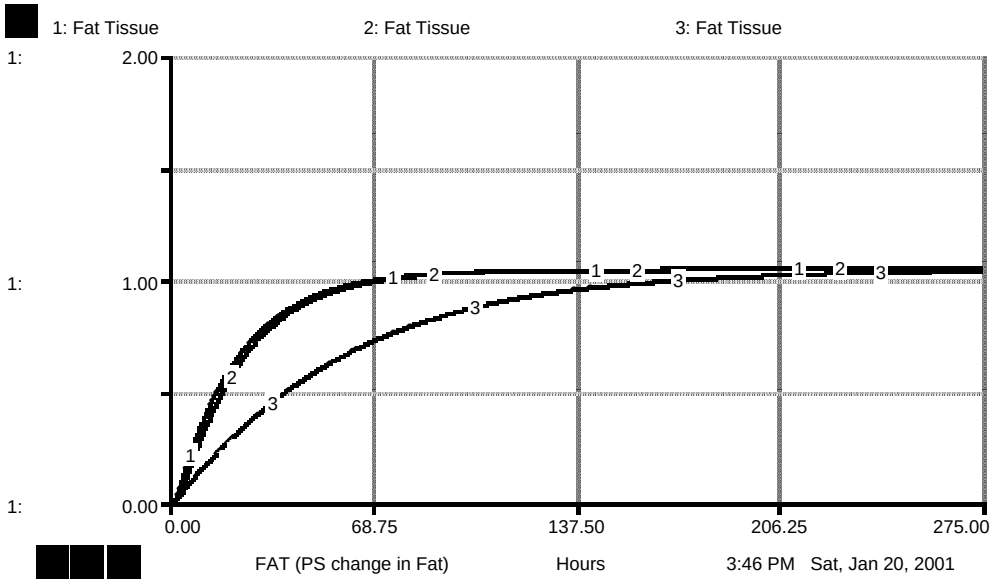
Slowly Perfused Tissue Level (mg) for Increasing Fat/Blood PC Values
at Maximum Stress and Exercise

1: PF =0.002 2: PF=75 3: PF=500



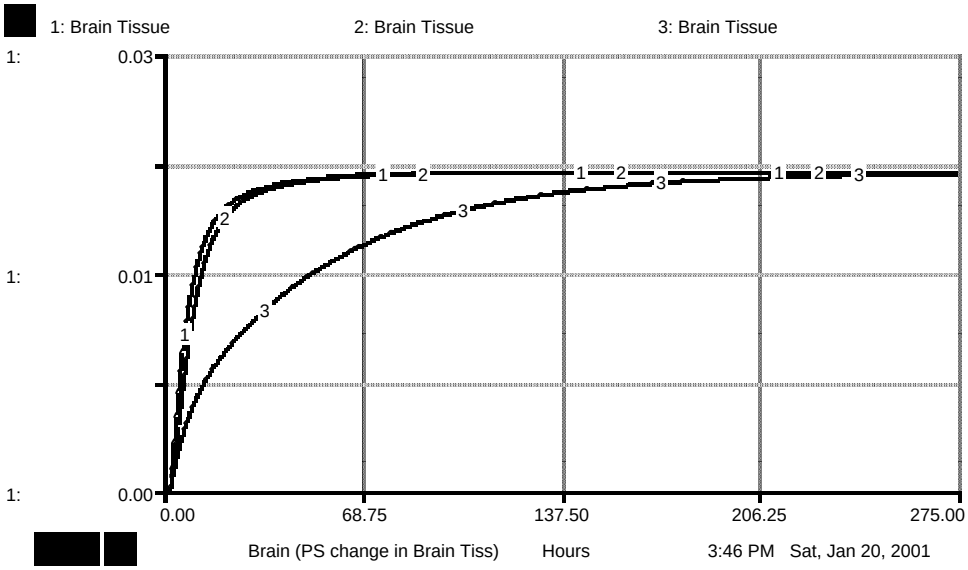
Richly Perfused Tissue Level (mg) for Increasing Fat/Blood PC Values
at Maximum Stress and Exercise

1: PS =0.02 2: PS=15 3: PS=250



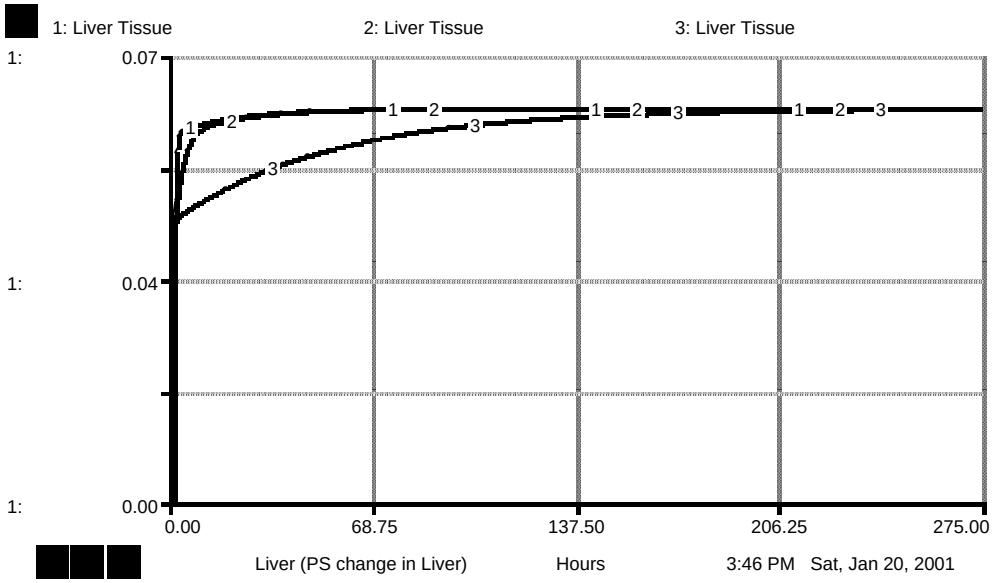
Fat Tissue Level (mg) for Increasing Slowly Perfused/Blood PC Values
at Maximum Stress and Exercise

1: PS =0.02 2: PS=15 3: PS=250



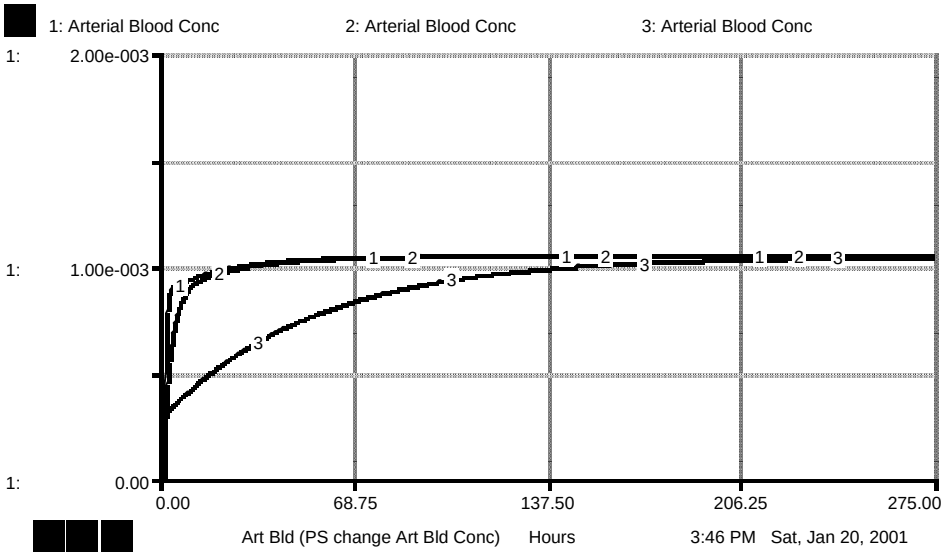
Brain Tissue Level (mg) for Increasing Slowly Perfused/Blood PC Values
at Maximum Stress and Exercise

1: PS =0.02 2: PS=15 3: PS=250



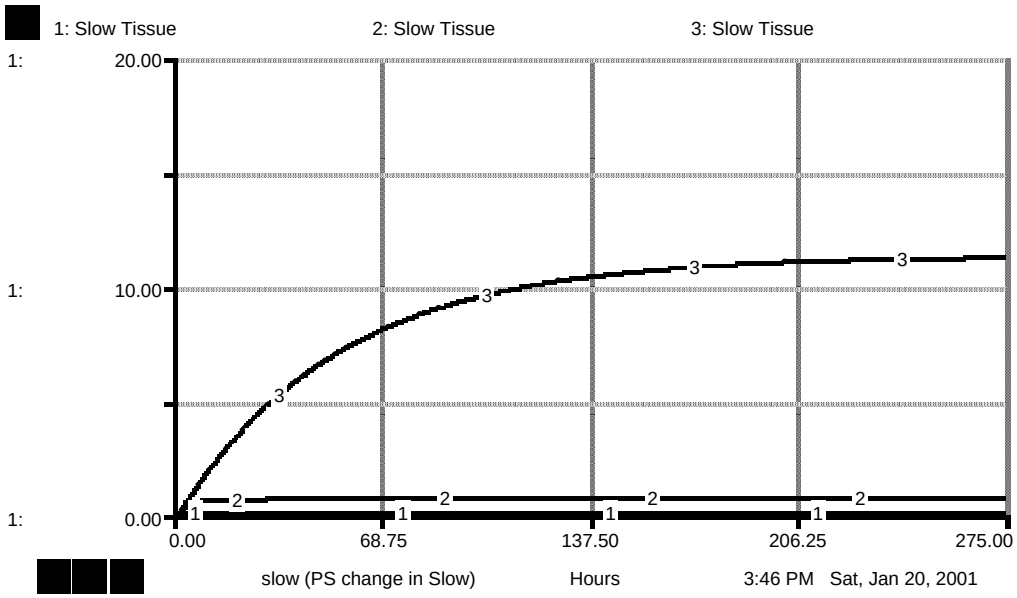
Liver Tissue Level (mg) for Increasing Slowly Perfused/Blood PC Values
at Maximum Stress and Exercise

1: PS =0.02 2: PS=15 3: PS=250



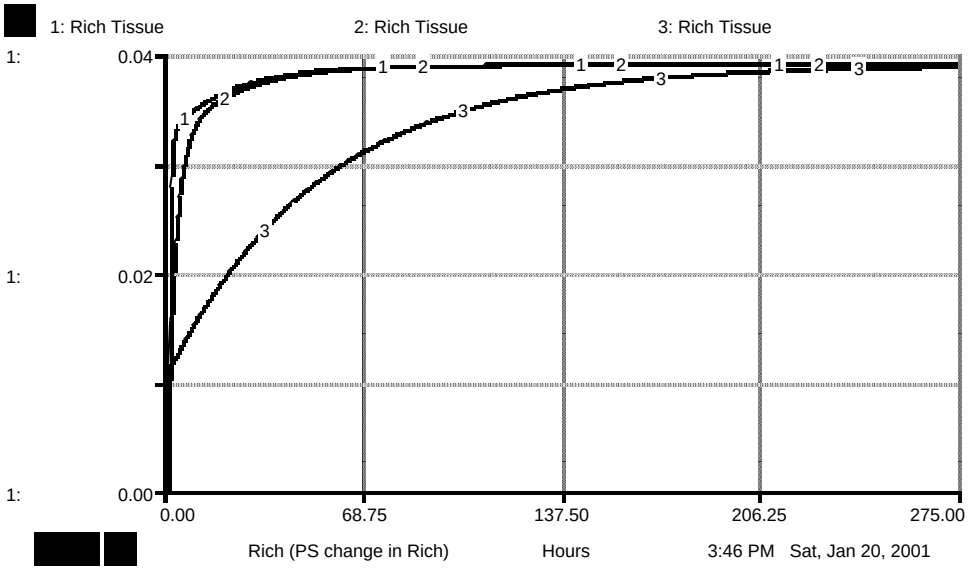
Arterial Blood Concentration (mg/L) for Increasing Slowly Perfused/Blood PC Values at Maximum Stress and Exercise

1: PS =0.02 2: PS=15 3: PS=250



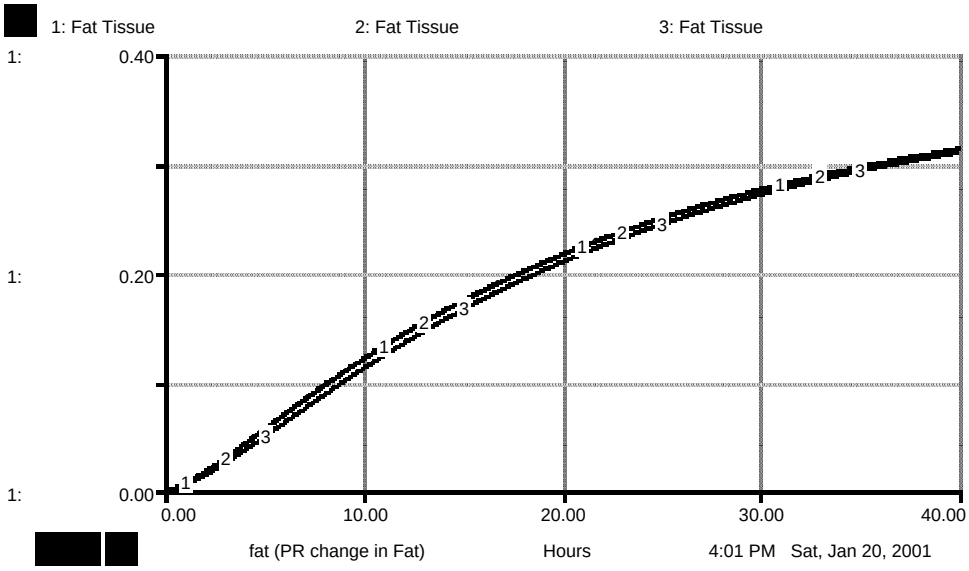
Slowly Perfused Tissue Level (mg) for Increasing Slowly Perfused/Blood PC Values at Maximum Stress and Exercise

1: PS =0.02 2: PS=15 3: PS=250



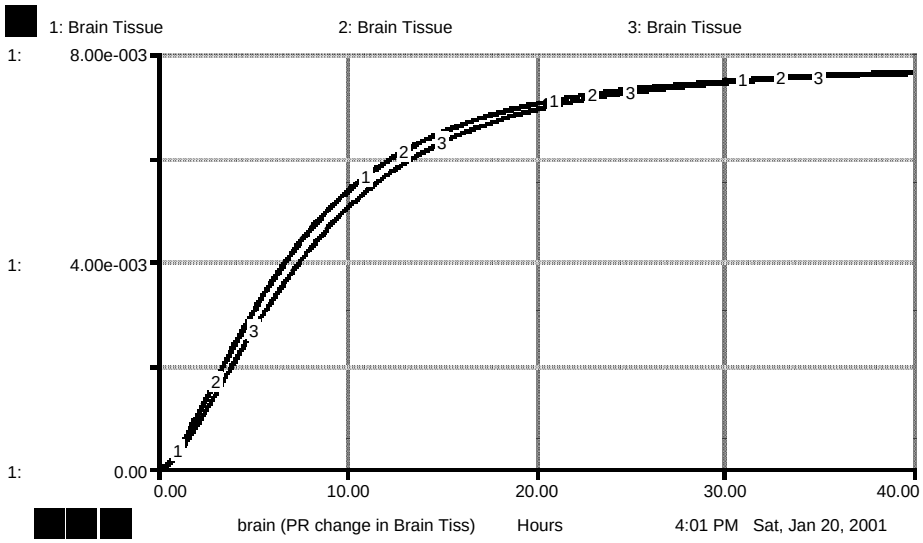
Richly Perfused Tissue Level (mg) for Increasing Slowly Perfused/Blood PC Values at Maximum Stress and Exercise

1: PR =0.2 2: PR=15 3: PR=250



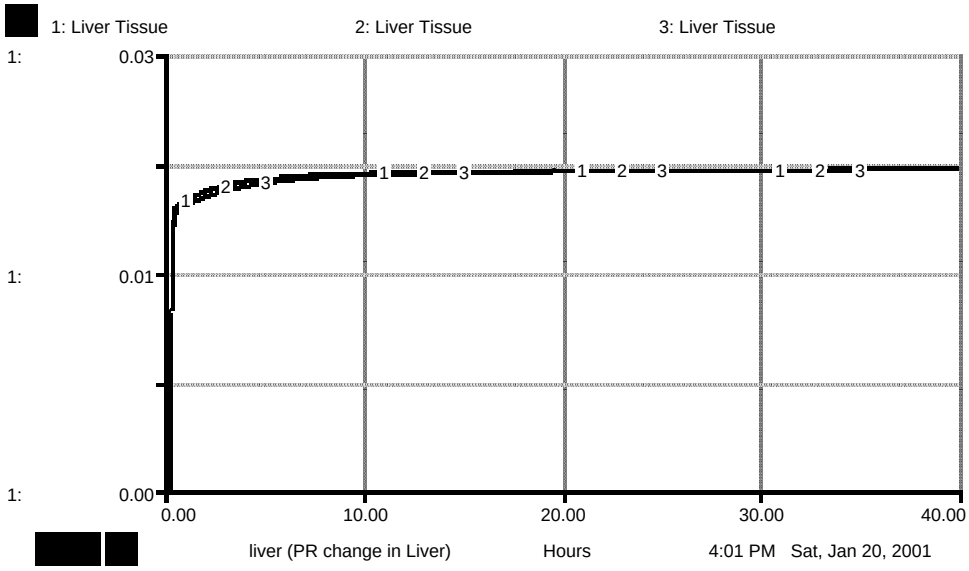
Fat Tissue Level (mg) for Increasing Richly Perfused/Blood PC Values at Maximum Stress and Exercise

1: PR =0.2 2: PR=15 3: PR=250



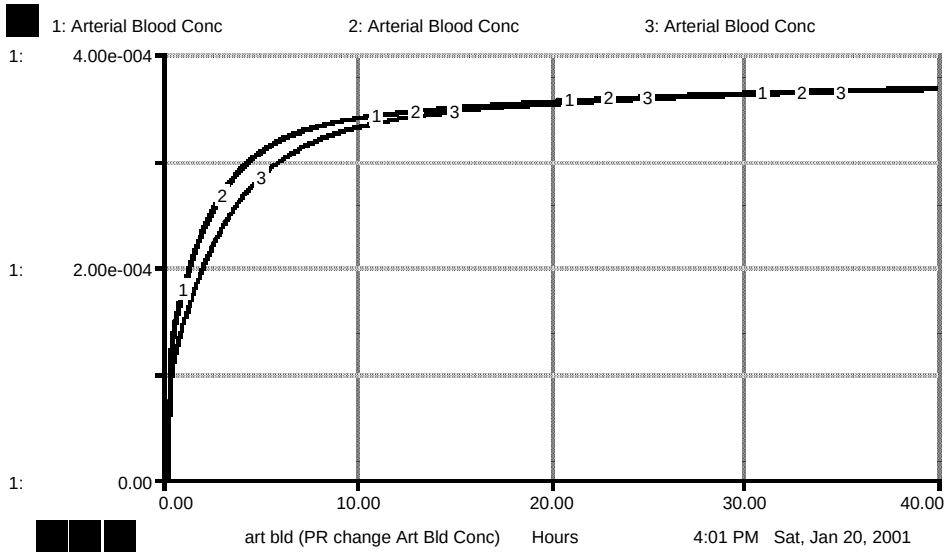
Fat Tissue Level (mg) for Increasing Richly Perfused/Blood PC Values
at Maximum Stress and Exercise

1: PR =0.2 2: PR=15 3: PR=250



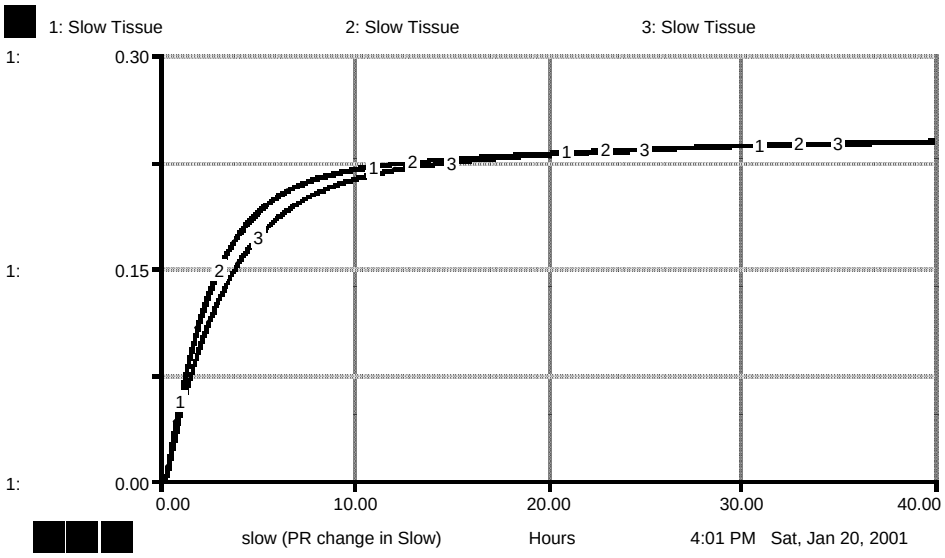
Liver Tissue Level (mg) for Increasing Richly Perfused/Blood PC Values
at Maximum Stress and Exercise

1: PR =0.2 2: PR=15 3: PR=250



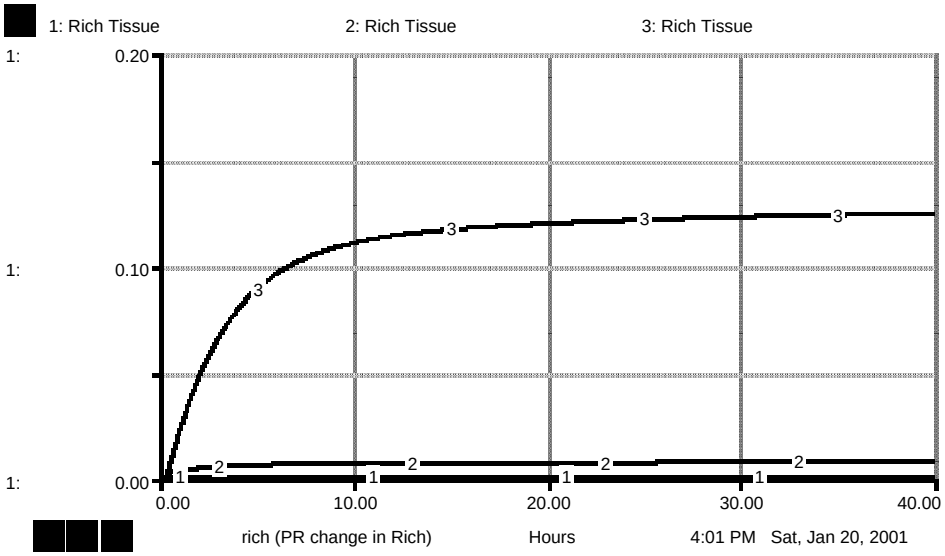
Arterial Blood Concentration (mg/L) for Increasing Richly Perfused/Blood PC Values at Maximum Stress and Exercise

1: PR =0.2 2: PR=15 3: PR=250



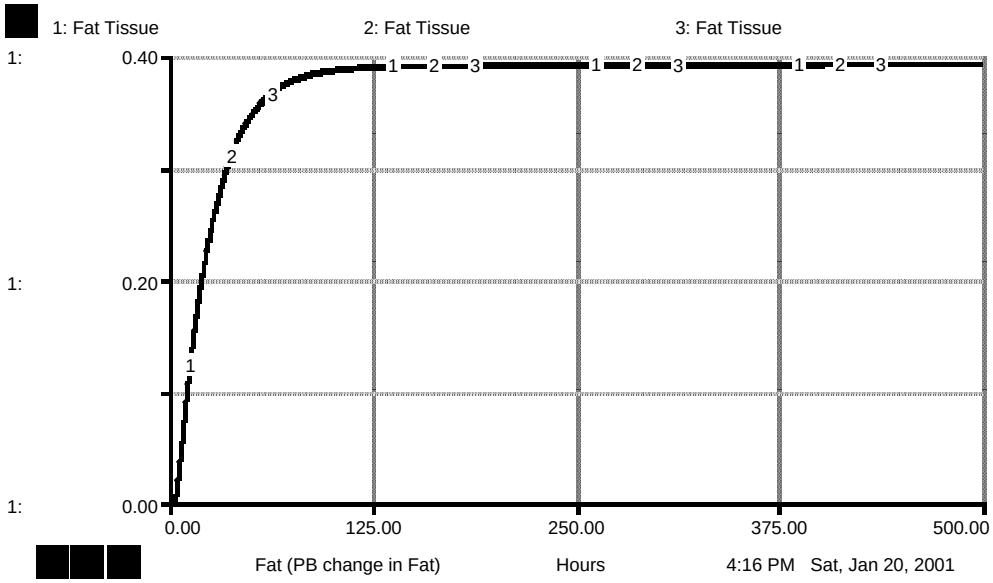
Slowly Perfused Tissue Level (mg) for Increasing Richly Perfused/Blood PC Values at Maximum Stress and Exercise

1: PR =0.2 2: PR=15 3: PR=250



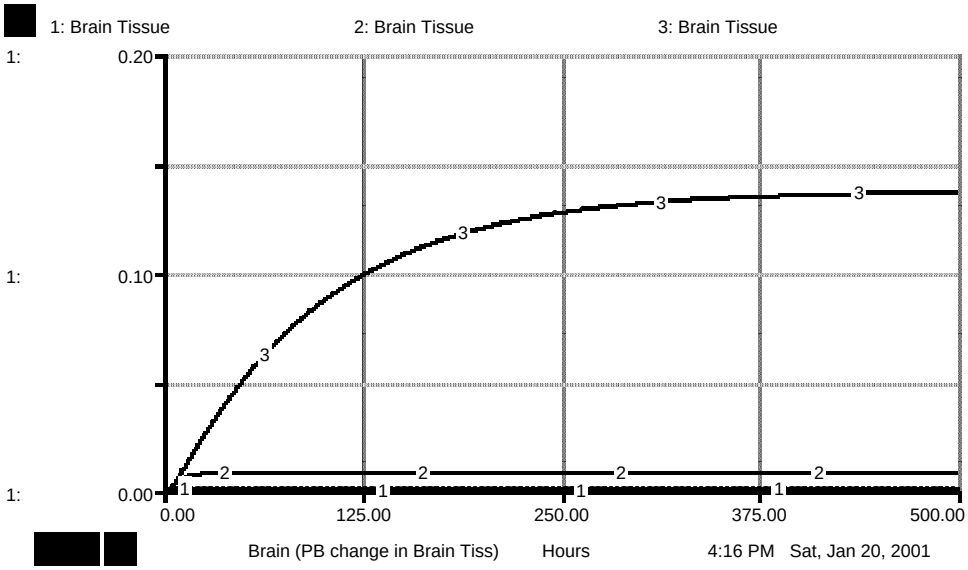
Richly Perfused Tissue Level (mg) for Increasing Richly Perfused/Blood PC Values at Maximum Stress and Exercise

1: PB =0.002 2: PB=15 3: PB=250



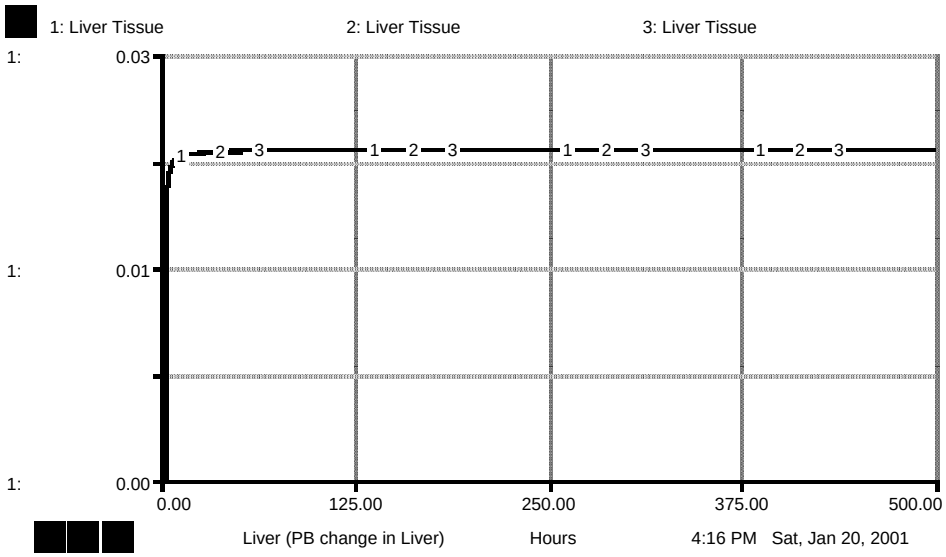
Fat Tissue Level (mg) for Increasing Brain/Blood PC Values at Maximum Stress and Exercise

1: PB =0.002 2: PB=15 3: PB=250



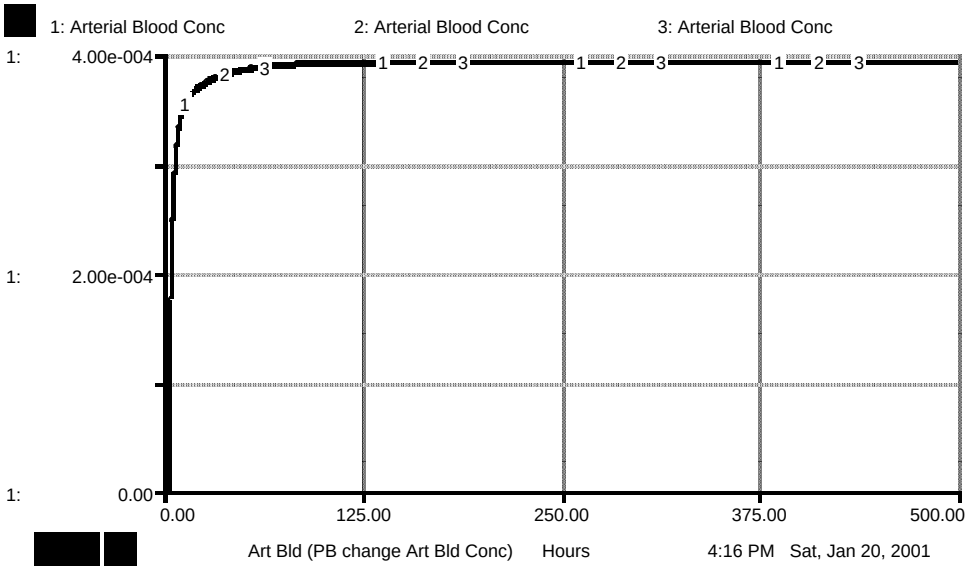
Brain Tissue Level (mg) for Increasing Brain/Blood PC Values
at Maximum Stress and Exercise

1: PB =0.002 2: PB=15 3: PB=250



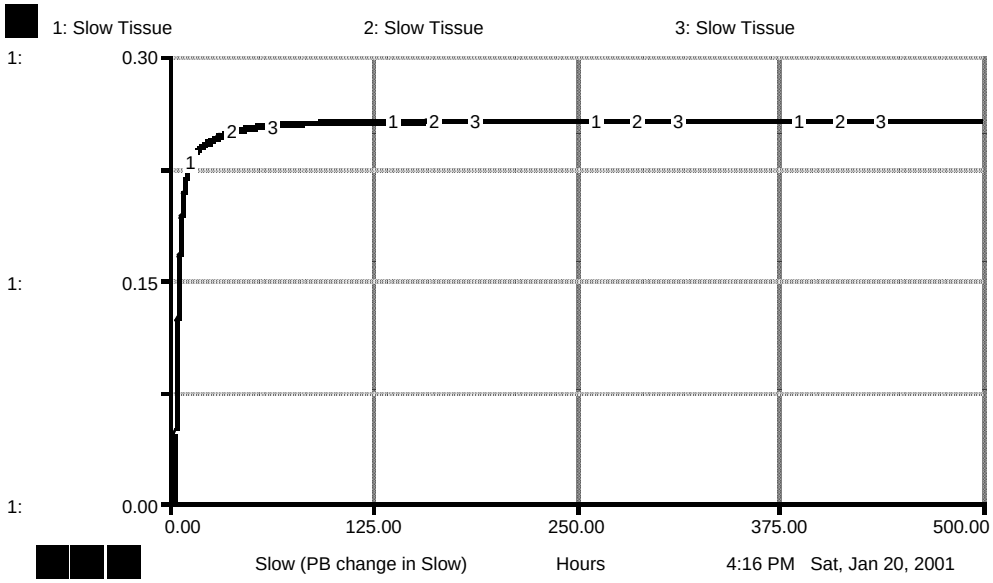
Brain Tissue Level (mg) for Increasing Brain/Blood PC Values
at Maximum Stress and Exercise

1: PB =0.002 2: PB=15 3: PB=250



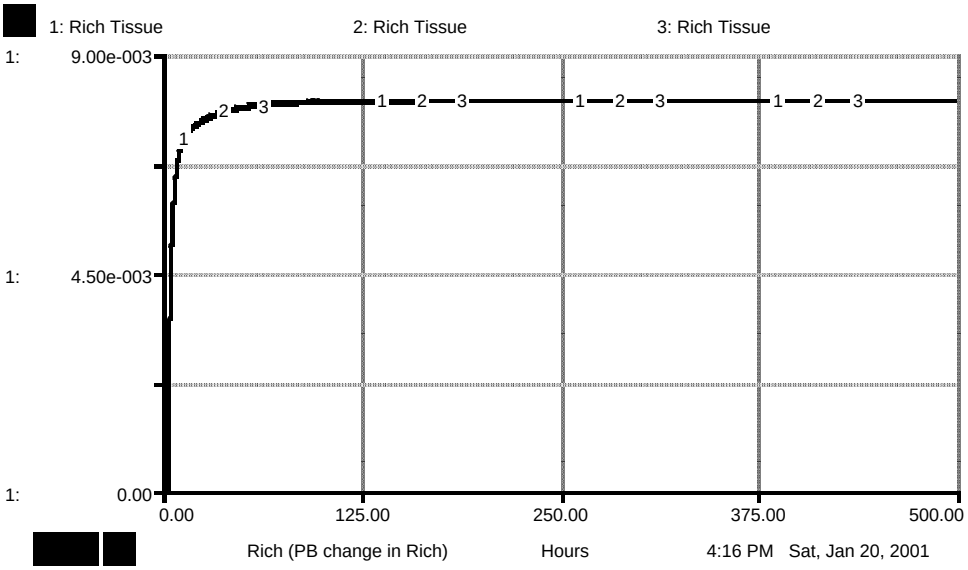
Arterial Blood Concentration (mg/L) for Increasing Brain/Blood PC Values at Maximum Stress and Exercise

1: PB =0.002 2: PB=15 3: PB=250



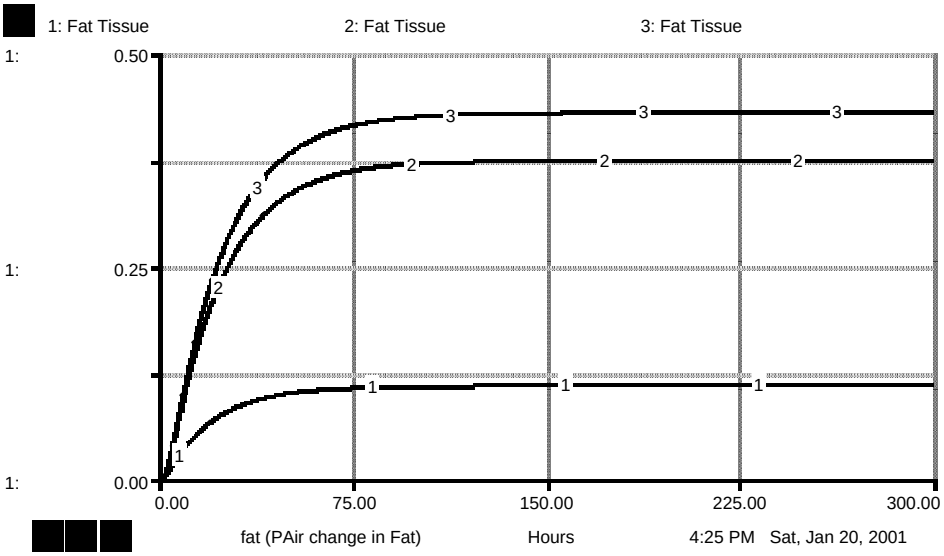
Slowly Perfused Tissue Level (mg) for Increasing Brain/Blood PC Values at Maximum Stress and Exercise

1: PB =0.002 2: PB=15 3: PB=250



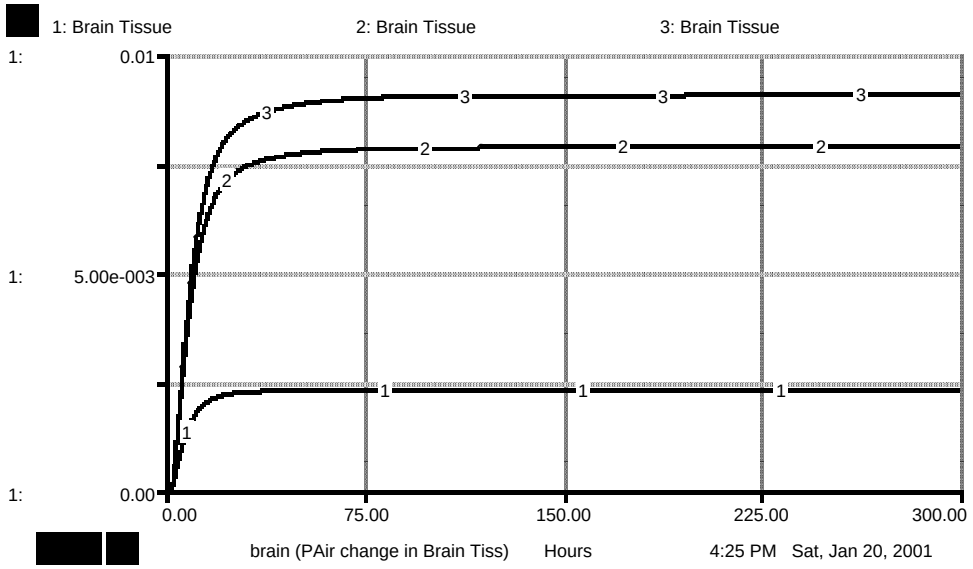
Richly Perfused Tissue Level (mg) for Increasing Brain/Blood PC Values
at Maximum Stress and Exercise

1: PB/A=5 2: PB/A=75 3: PB/A=300



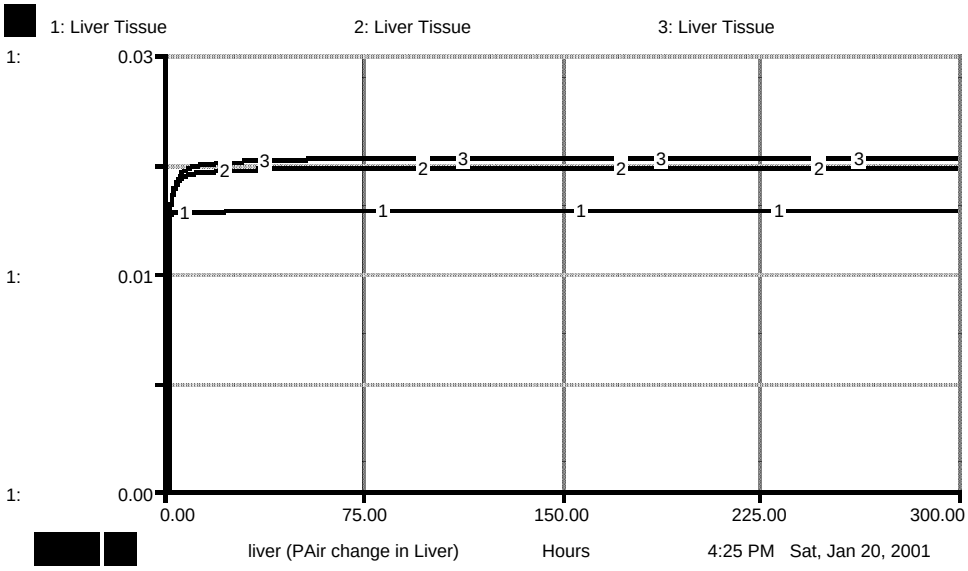
Fat Tissue Level (mg) for Increasing Blood/Air PC Values
at Maximum Stress and Exercise

1: PB/A=5 2: PB/A=75 3: PB/A=300



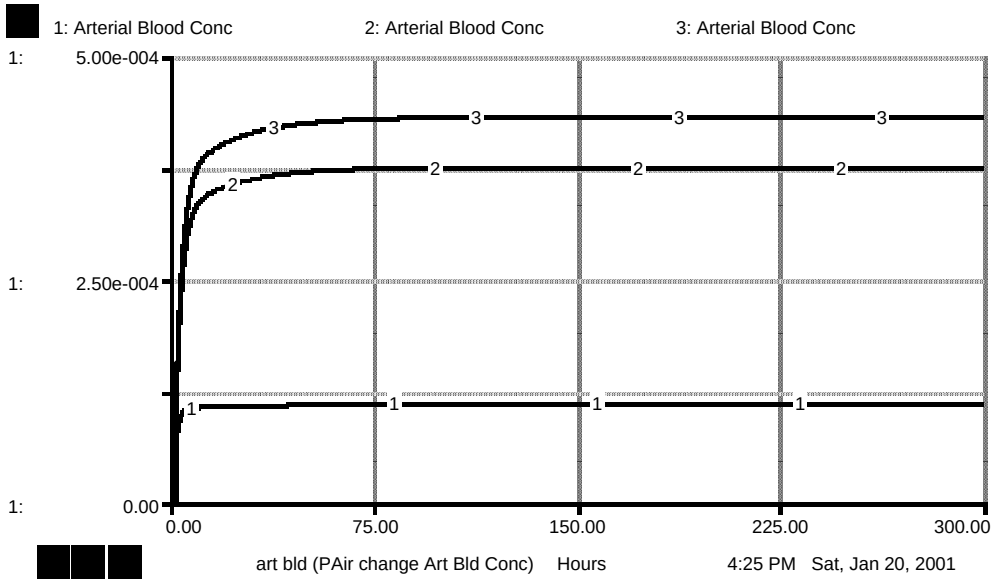
Brain Tissue Level (mg) for Increasing Blood/Air PC Values
at Maximum Stress and Exercise

1: PB/A=5 2: PB/A=75 3: PB/A=300



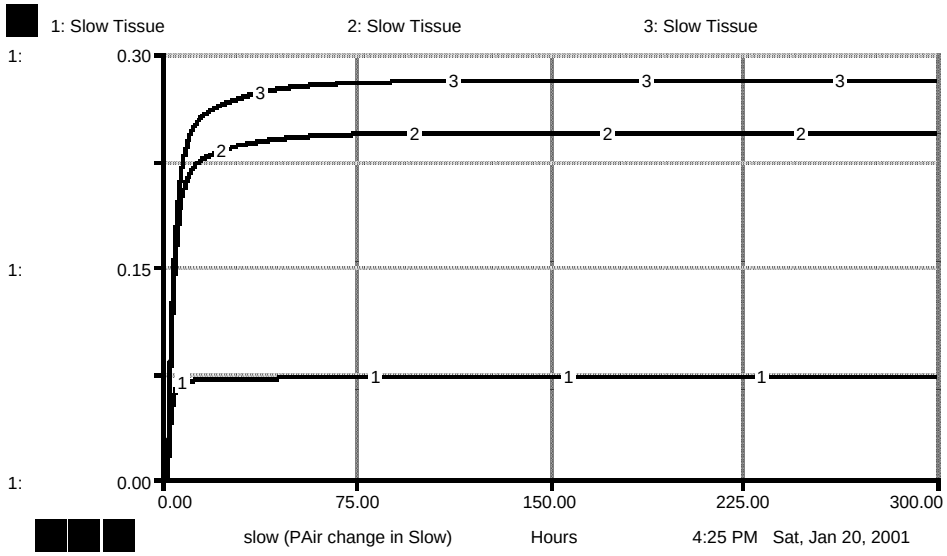
Liver Tissue Level (mg) for Increasing Blood/Air PC Values
at Maximum Stress and Exercise

1: PB/A=5 2: PB/A=75 3: PB/A=300



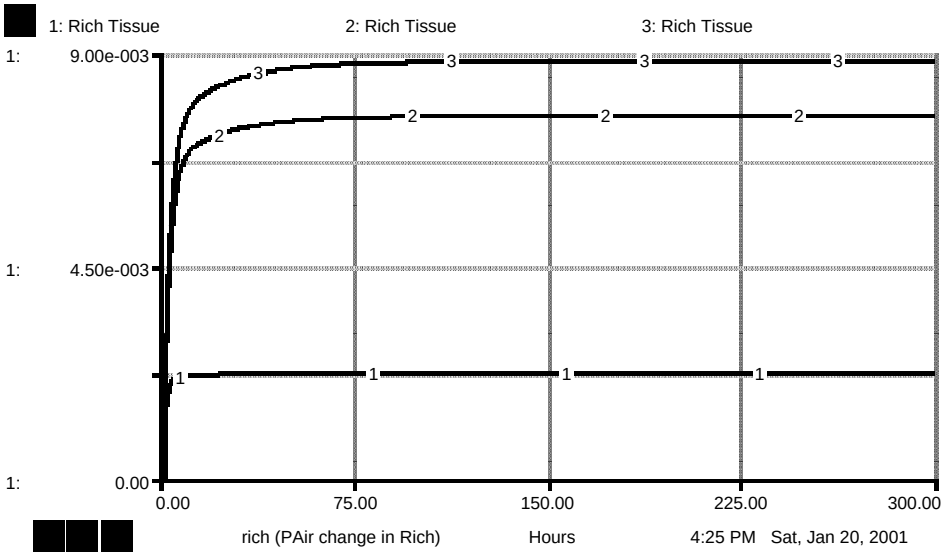
Arterial Blood Concentration (mg/L) for Increasing Blood/Air PC Values
at Maximum Stress and Exercise

1: PB/A=5 2: PB/A=75 3: PB/A=300



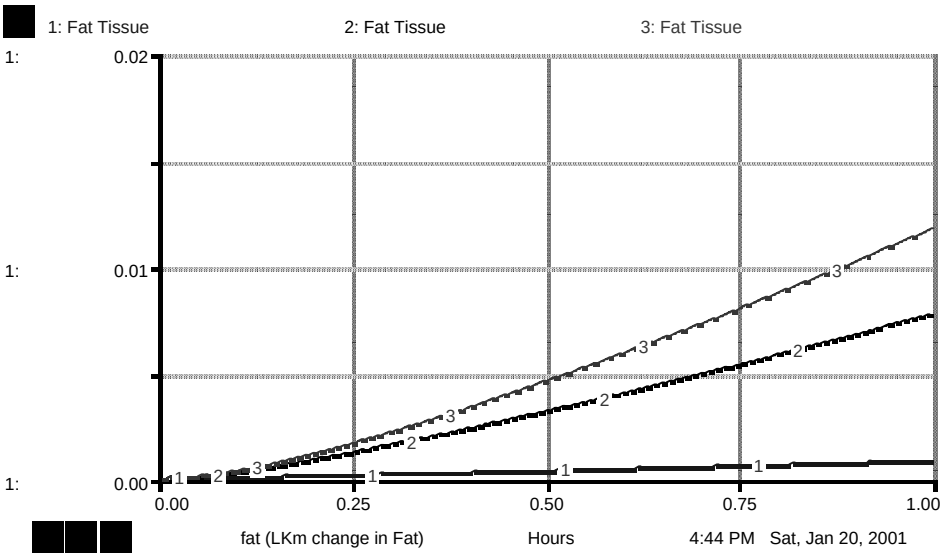
Slowly Perfused Tissue Level (mg) for Increasing Blood/Air PC Values
at Maximum Stress and Exercise

1: PB/A=5 2: PB/A=75 3: PB/A=300



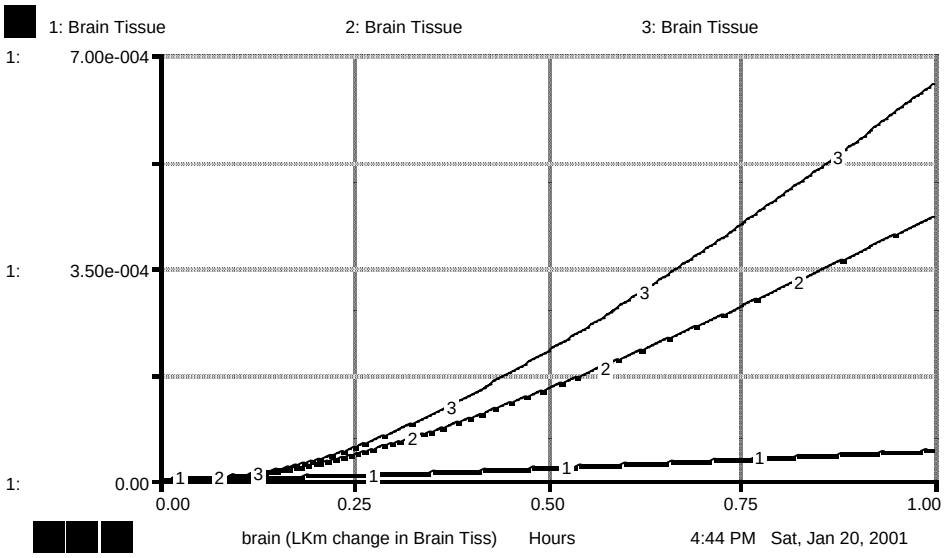
Richly Perfused Tissue Level (mg) for Increasing Blood/Air PC Values
at Maximum Stress and Exercise

1: LKm=0.1 2: LKm=1.5 3: LKm=3.5



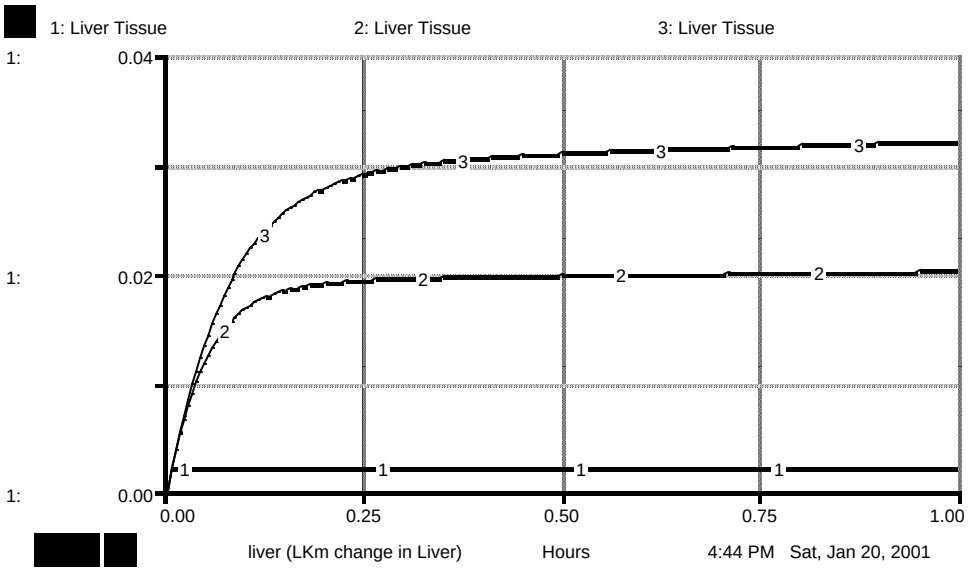
Fat Tissue Level (mg) for Increasing Liver Km Values
at Maximum Stress and Exercise

1: LKm=0.1 2: LKm=1.5 3: LKm=3.5



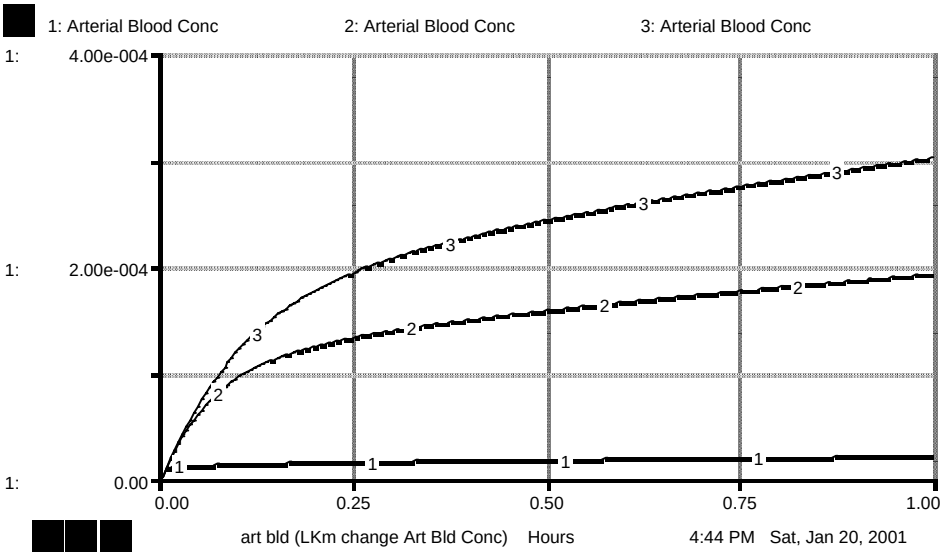
Brain Tissue Level (mg) for Increasing Liver Km Values
at Maximum Stress and Exercise

1: LKm=0.1 2: LKm=1.5 3: LKm=3.5



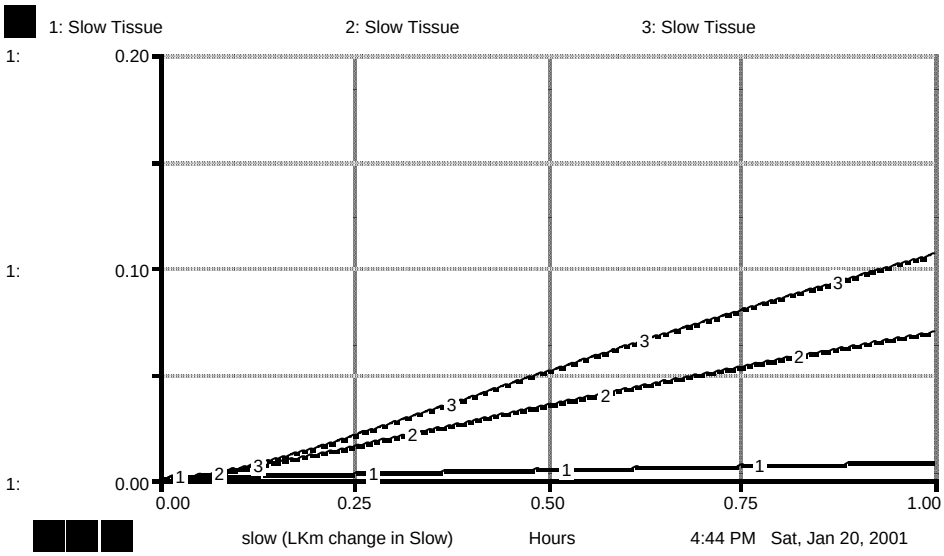
Liver Tissue Level (mg) for Increasing Liver Km Values
at Maximum Stress and Exercise

1: LKm=0.1 2: LKm=1.5 3: LKm=3.5



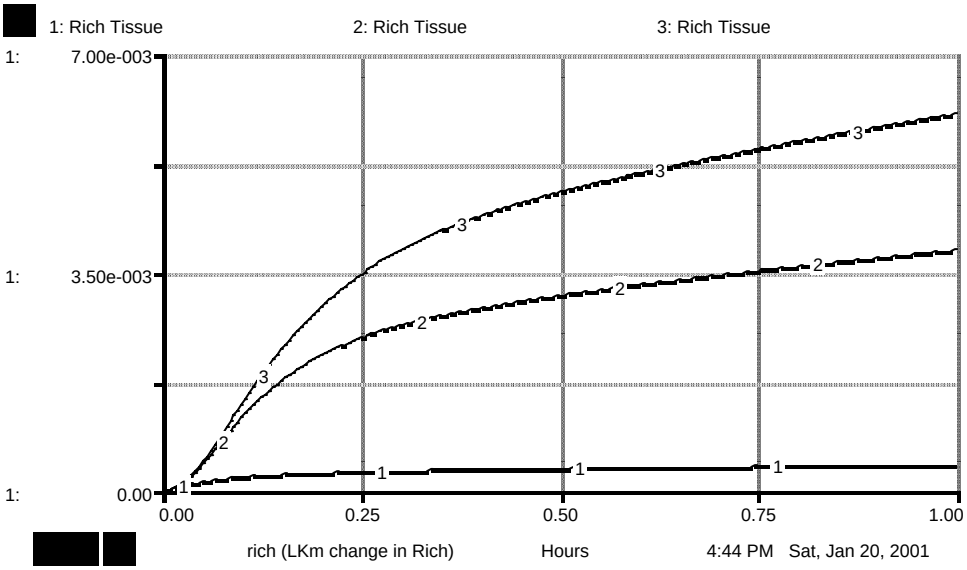
Arterial Blood Concentration (mg/L) for Increasing Liver Km Values
at Maximum Stress and Exercise

1: LKm=0.1 2: LKm=1.5 3: LKm=3.5



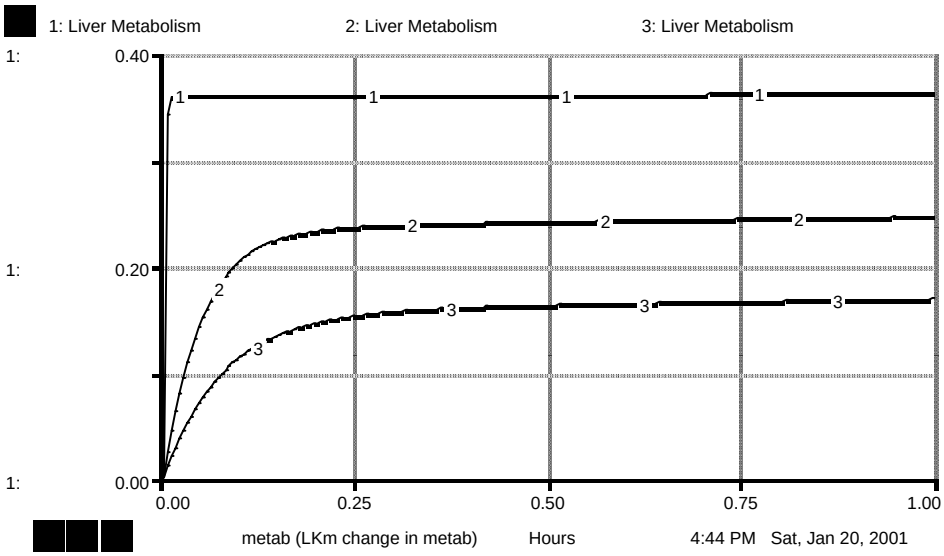
Slowly Perfused Tissue Level (mg) for Increasing Liver Km Values
at Maximum Stress and Exercise

1: LKm=0.1 2: LKm=1.5 3: LKm=3.5



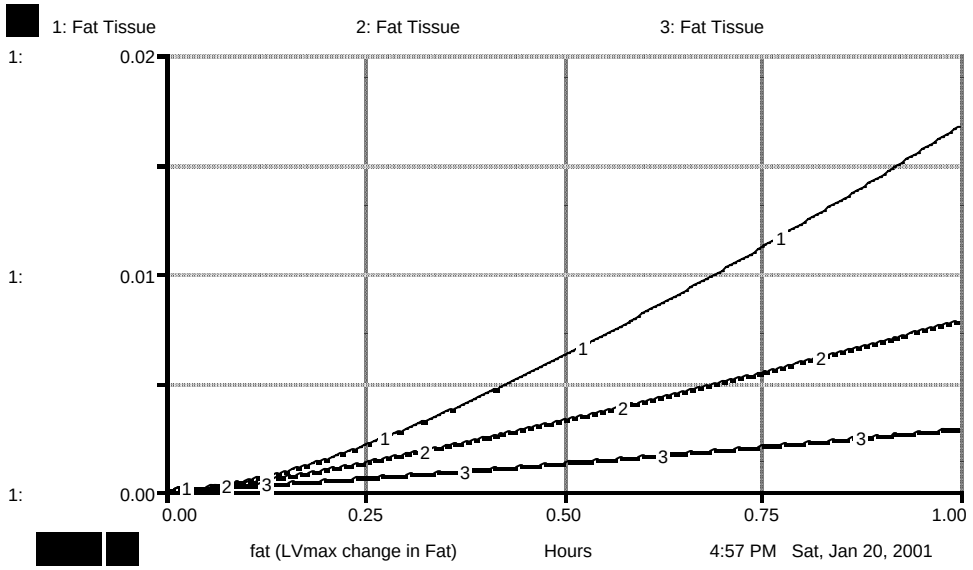
Richly Perfused Tissue Level (mg) for Increasing Liver Km Values
at Maximum Stress and Exercise

1: LKm=0.1 2: LKm=1.5 3: LKm=3.5



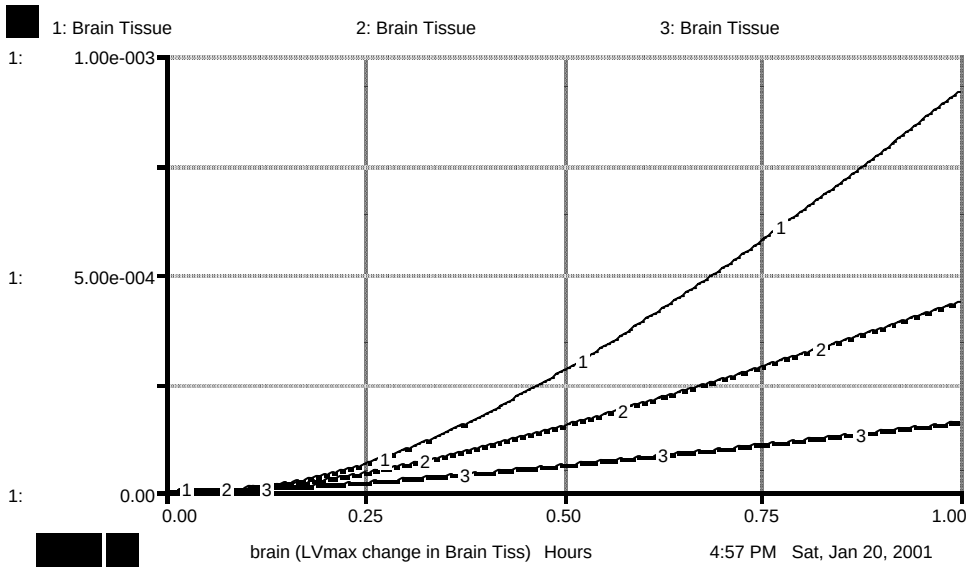
Liver Metabolism (mg/hr) for Increasing Liver Km Values
at Maximum Stress and Exercise

1: Lvmax=50 2: Lvmax=500 3: Lvmax=2000



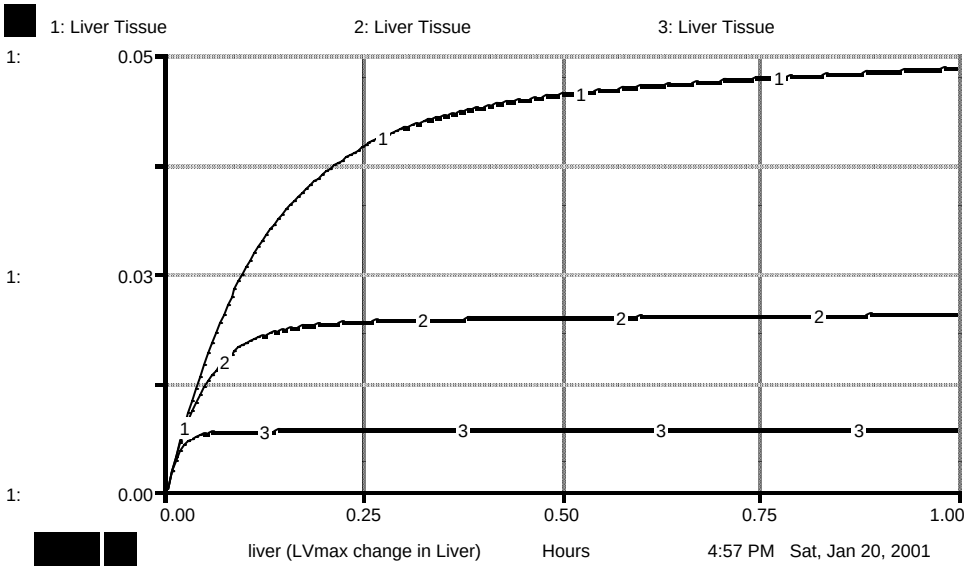
Fat Tissue Level (mg) for Increasing Liver Vmax Values
at Maximum Stress and Exercise

1: Lvmax=50 2: Lvmax=500 3: Lvmax=2000



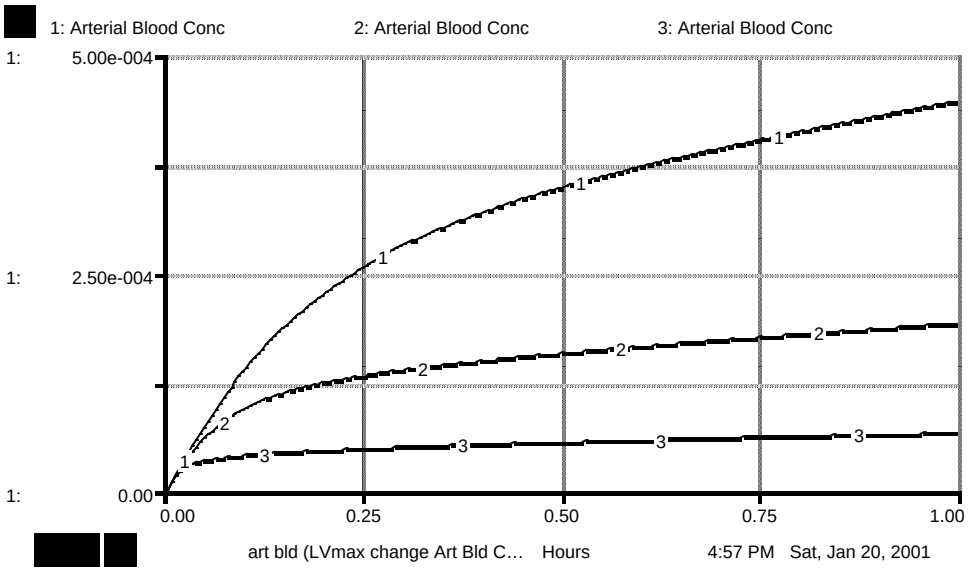
Brain Tissue Level (mg) for Increasing Liver Vmax Values
at Maximum Stress and Exercise

1: Lvmax=50 2: Lvmax=500 3: Lvmax=2000



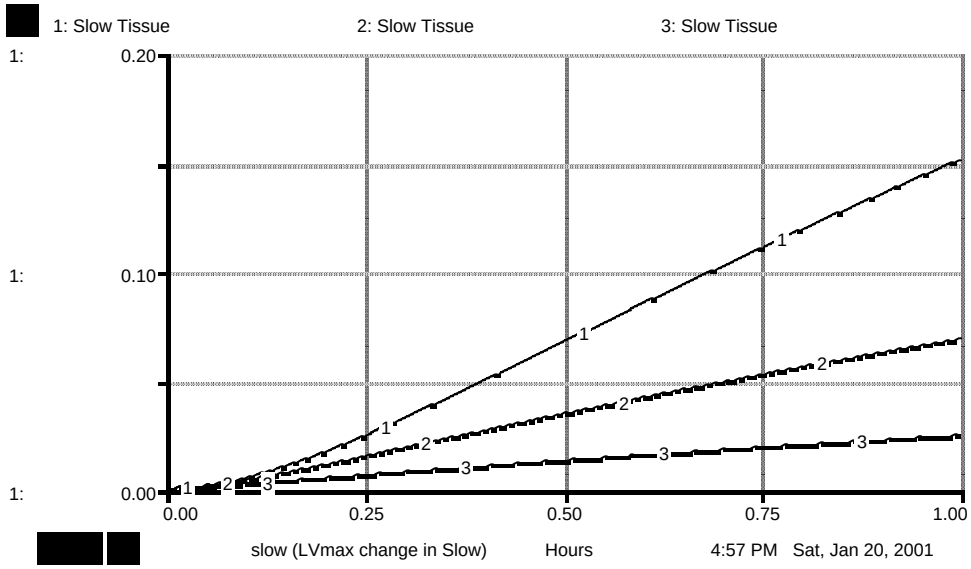
Liver Tissue Level (mg) for Increasing Liver Vmax Values
at Maximum Stress and Exercise

1: Lvmax=50 2: Lvmax=500 3: Lvmax=2000



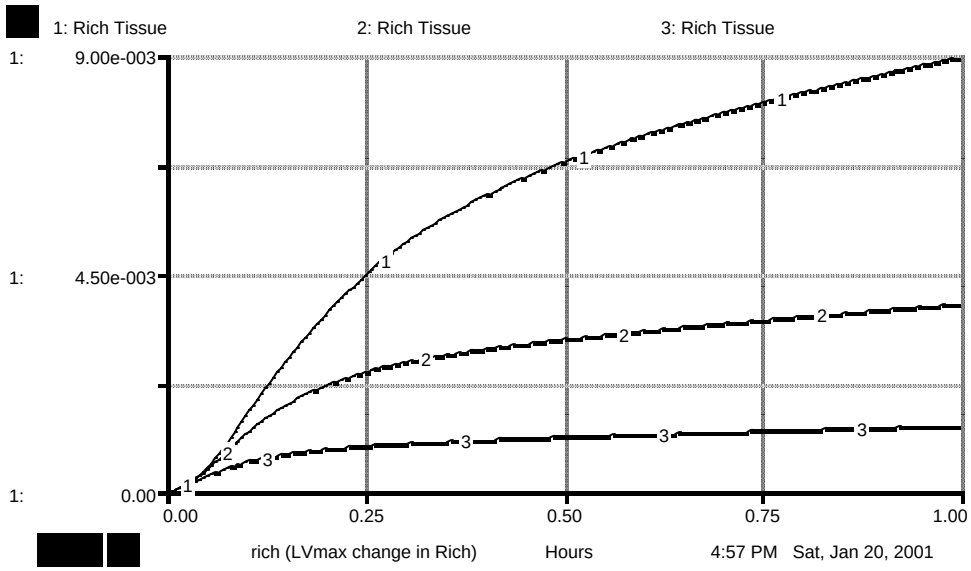
Arterial Blood Concentration (mg/L) for Increasing Liver Vmax Values
at Maximum Stress and Exercise

1: Lvmax=50 2: Lvmax=500 3: Lvmax=2000



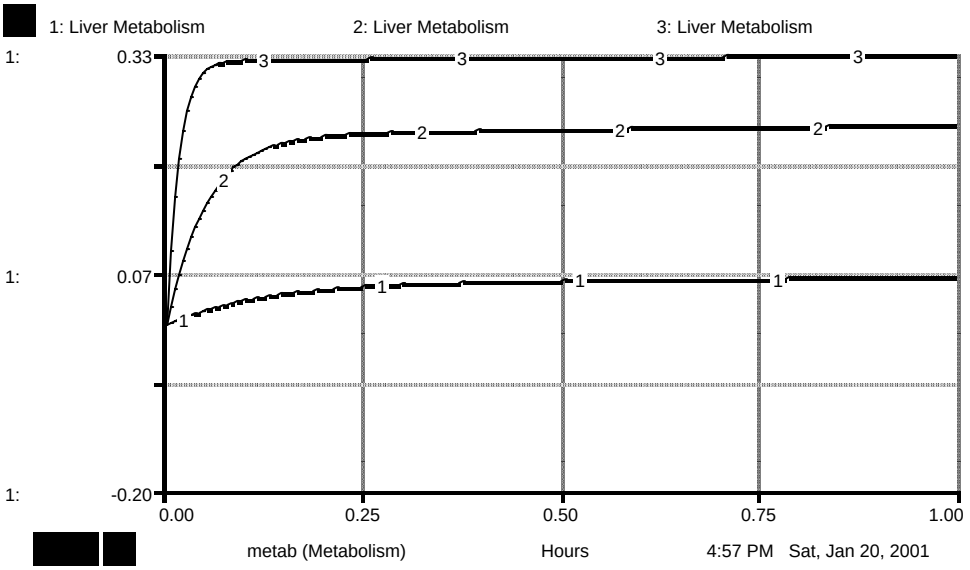
Slowly Perfused Tissue Level (mg) for Increasing Liver Vmax Values
at Maximum Stress and Exercise

1: Lvmax=50 2: Lvmax=500 3: Lvmax=2000



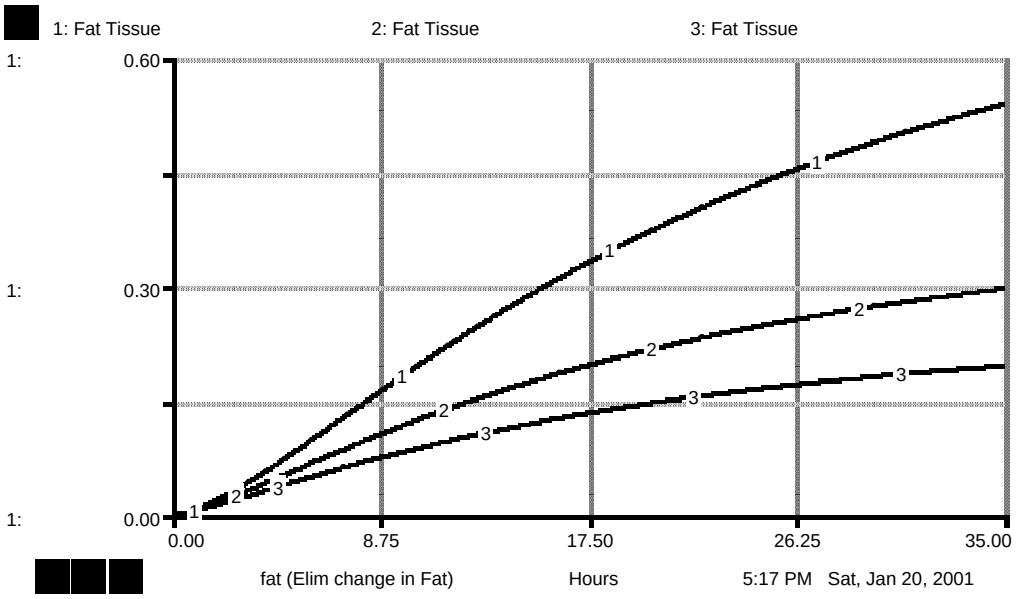
Richly Perfused Tissue Level (mg) for Increasing Liver Vmax Values
at Maximum Stress and Exercise

1: Lvmax=50 2: Lvmax=500 3: Lvmax=2000



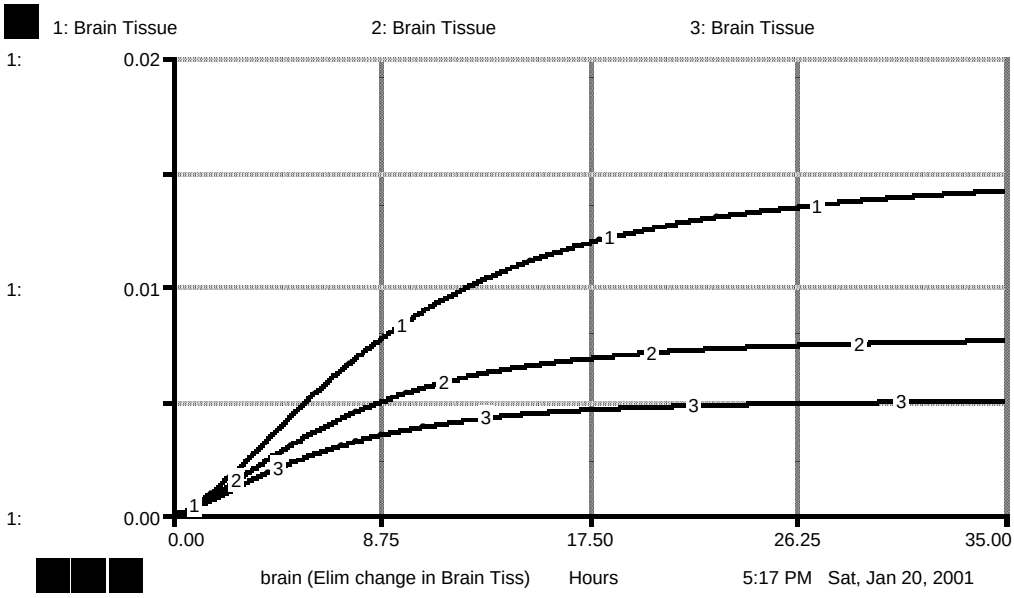
Liver Metabolism (mg/hr) for Increasing Liver Vmax Values
at Maximum Stress and Exercise

1: Elim Fract=0 2: Elim Fract=0.35 3: Elim Fract=0.75



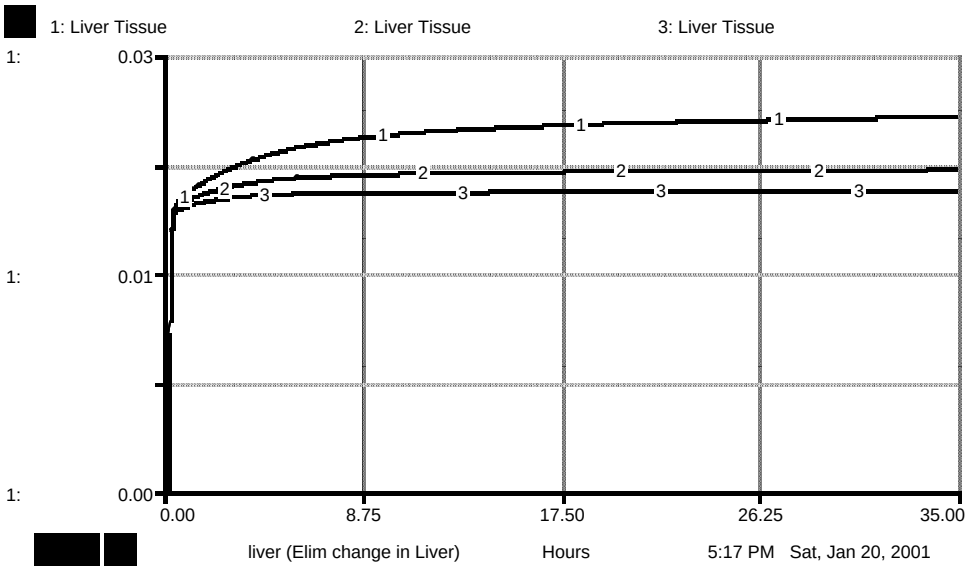
Fat Tissue Level (mg) for Increasing Kidney Elimination Fractions
at Maximum Stress and Exercise

1: Elim Fract=0 2: Elim Fract=0.35 3: Elim Fract=0.75



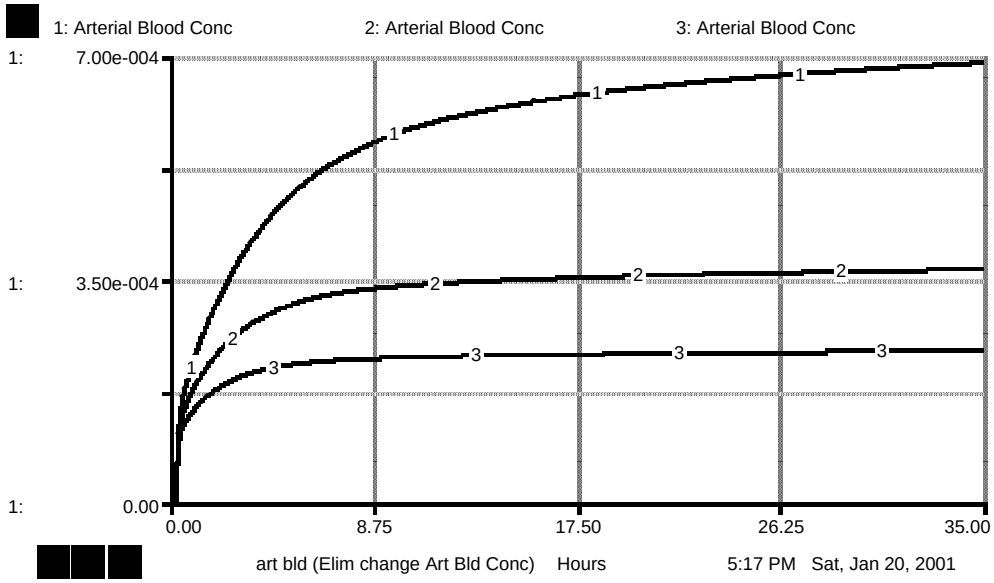
Brain Tissue Level (mg) for Increasing Kidney Elimination Fractions
at Maximum Stress and Exercise

1: Elim Fract=0 2: Elim Fract=0.35 3: Elim Fract=0.75



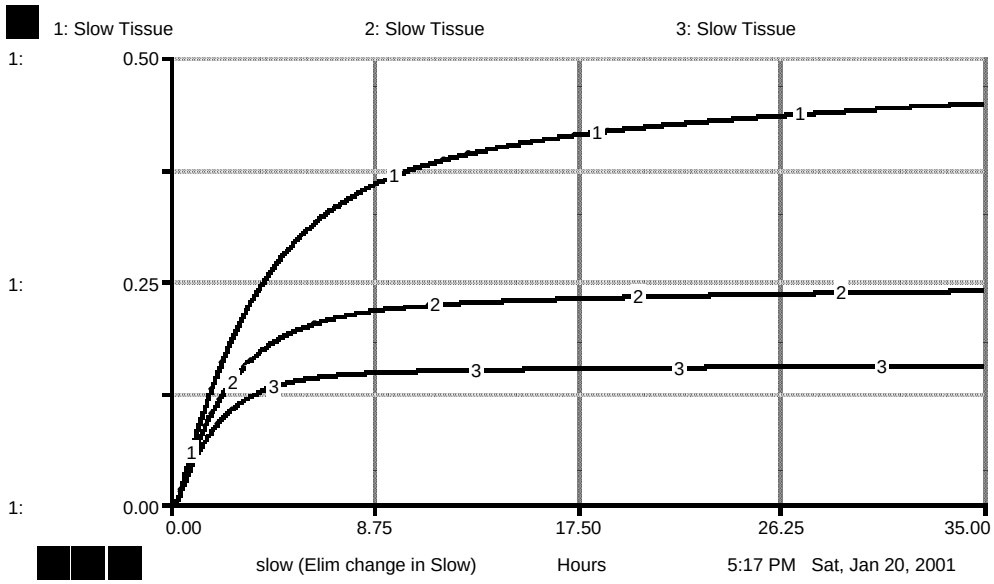
Brain Tissue Level (mg) for Increasing Kidney Elimination Fractions
at Maximum Stress and Exercise

1: Elim Fract=0 2: Elim Fract=0.35 3: Elim Fract=0.75



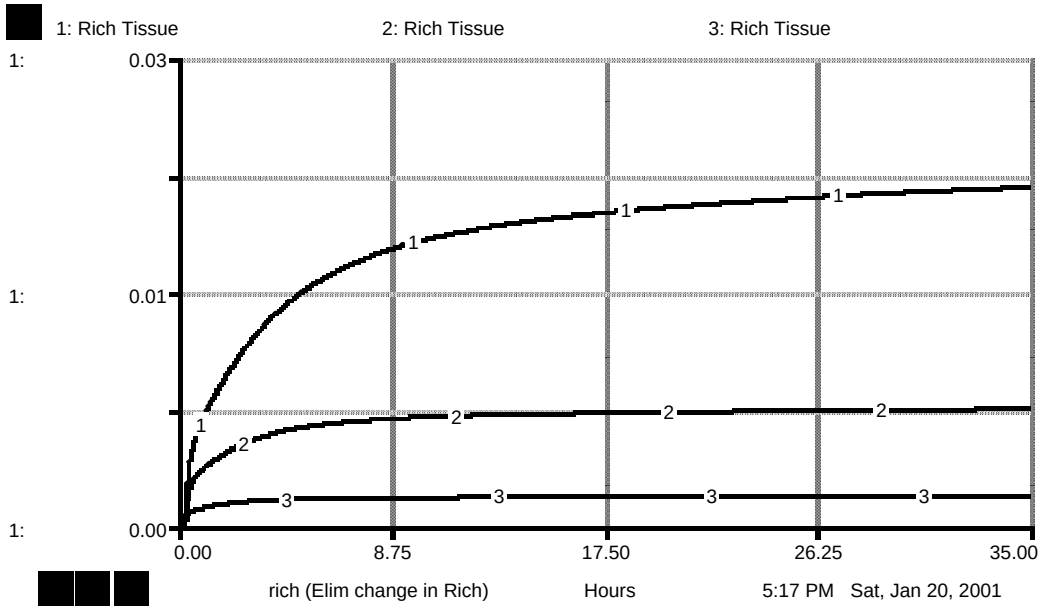
Arterial Blood Concentration (mg/L) for Increasing Kidney Elimination Fractions at Maximum Stress and Exercise

1: Elim Fract=0 2: Elim Fract=0.35 3: Elim Fract=0.75



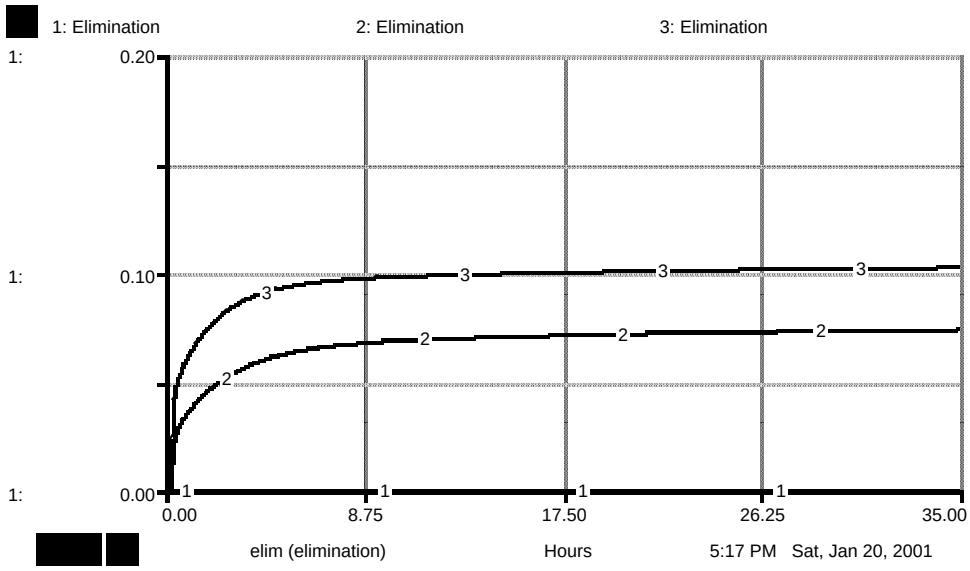
Slowly Perfused Tissue Level (mg) for Increasing Kidney Elimination Fractions at Maximum Stress and Exercise

1: Elim Fract=0 2: Elim Fract=0.35 3: Elim Fract=0.75



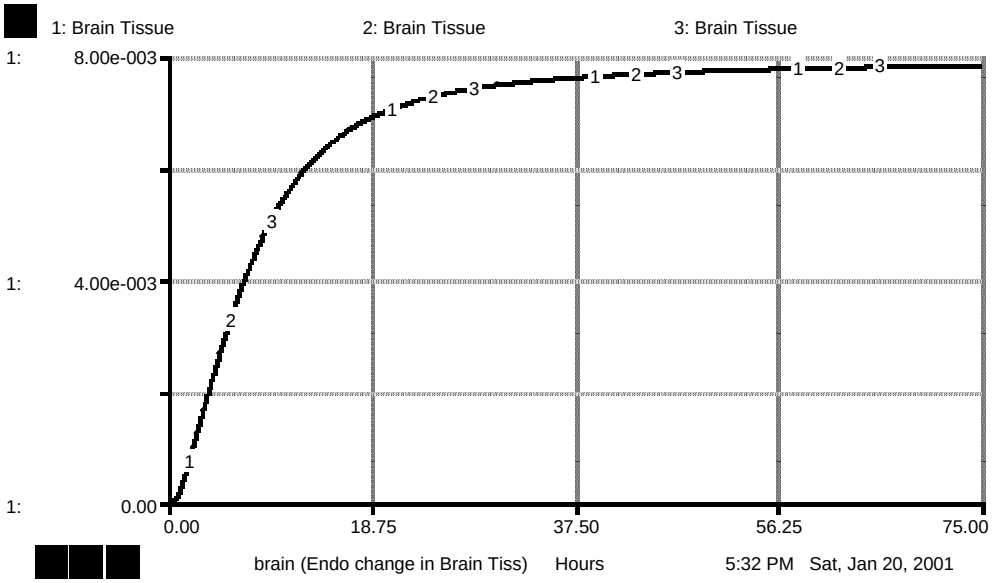
Richly Perfused Tissue Level (mg) for Increasing Kidney Elimination Fractions at Maximum Stress and Exercise

1: Elim Fract=0 2: Elim Fract=0.35 3: Elim Fract=0.75



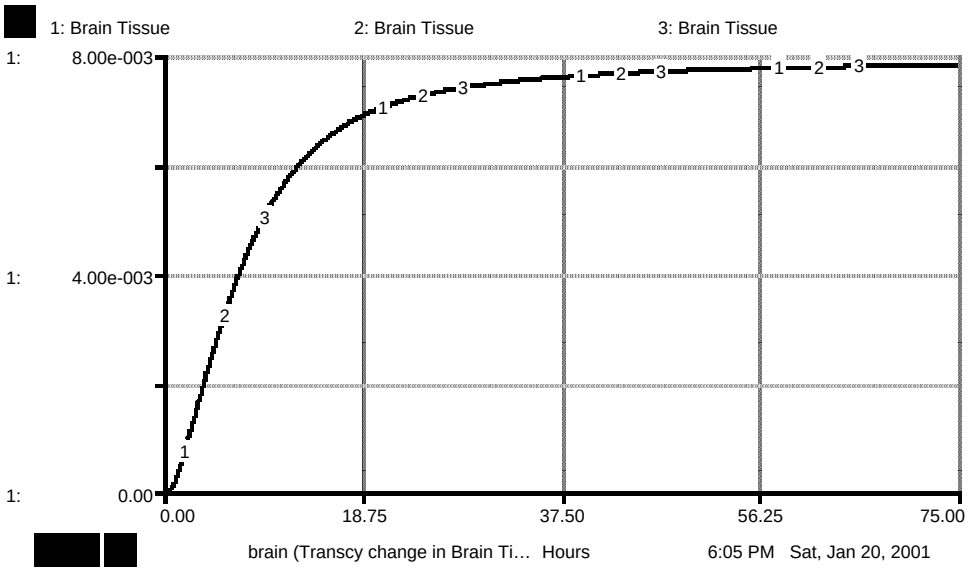
Elimination Flow (mg/hr) for Increasing Kidney Elimination Fractions at Maximum Stress and Exercise

1: Flow Fract=0 2: Flow Fract=1E-10 3: Flow Fract=1E-7



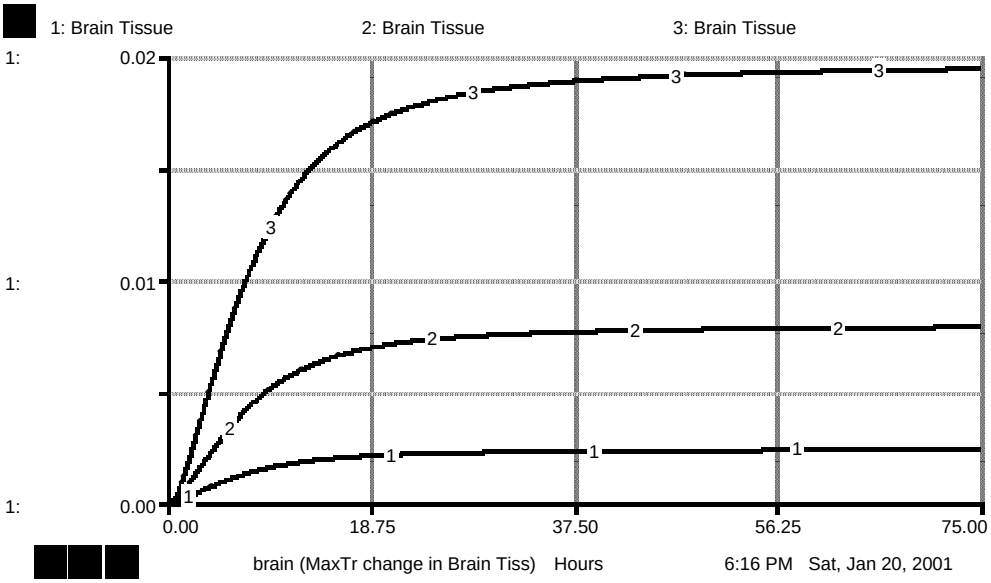
Brain Tissue Level (mg) for Increasing Endothelial Flow Fractions
at Maximum Stress and Exercise

1: Flow Fract=0 2: Flow Fract=1E-11 3: Flow Fract=1E-8



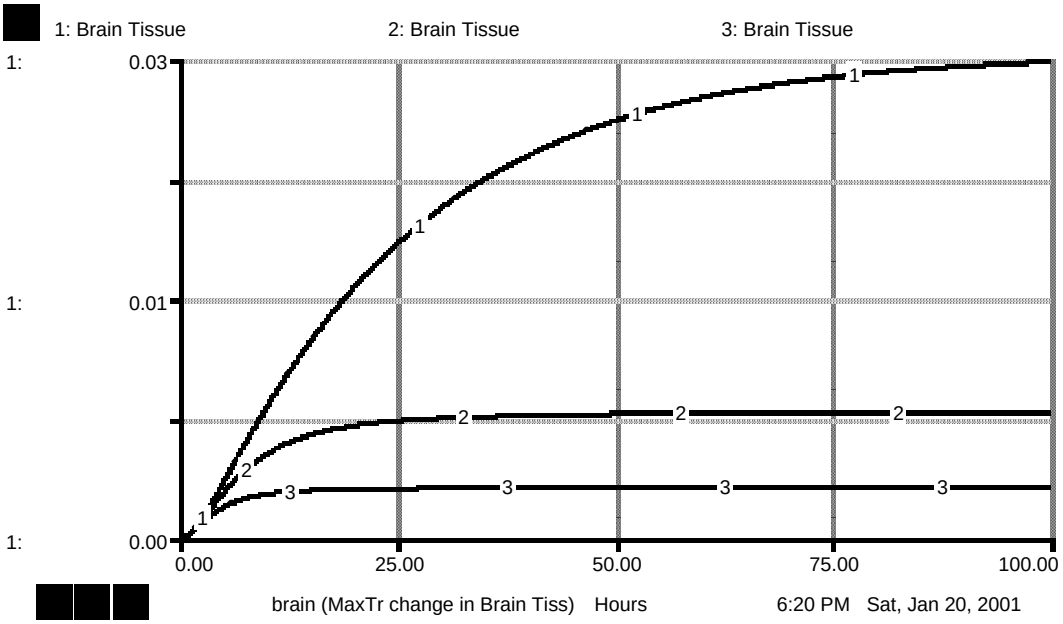
Brain Tissue Level (mg) for Increasing Transcytosis Flow Fractions
at Maximum Stress and Exercise

1: MT=0.5 2: MT=10 3: MT=30



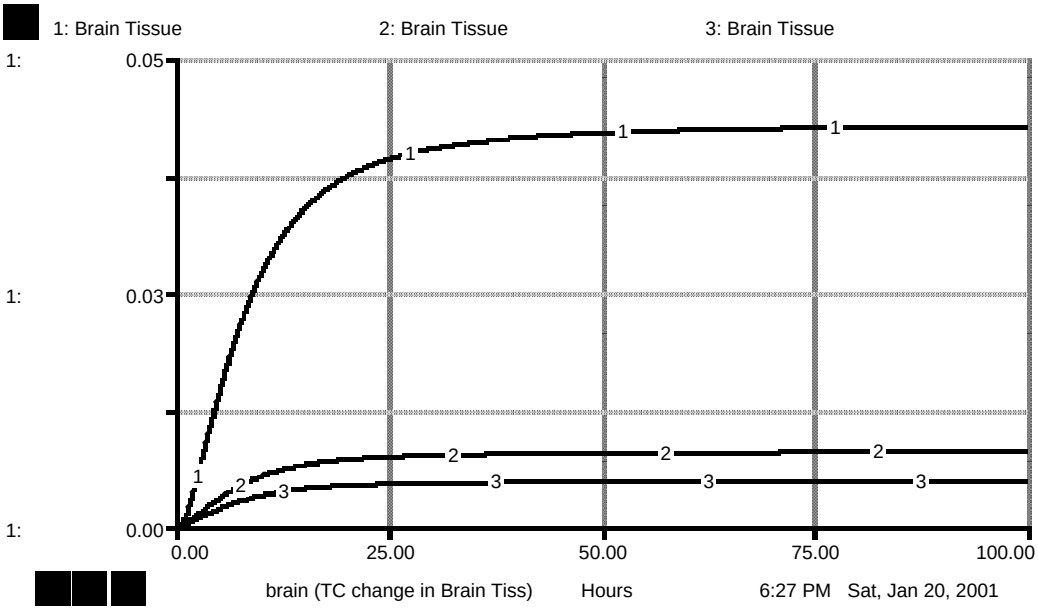
Brain Tissue Level (mg) for Increasing Mediated Maximum Transport
at Maximum Stress and Exercise

1: MT2=0 2: MT2=10 3: MT2=30



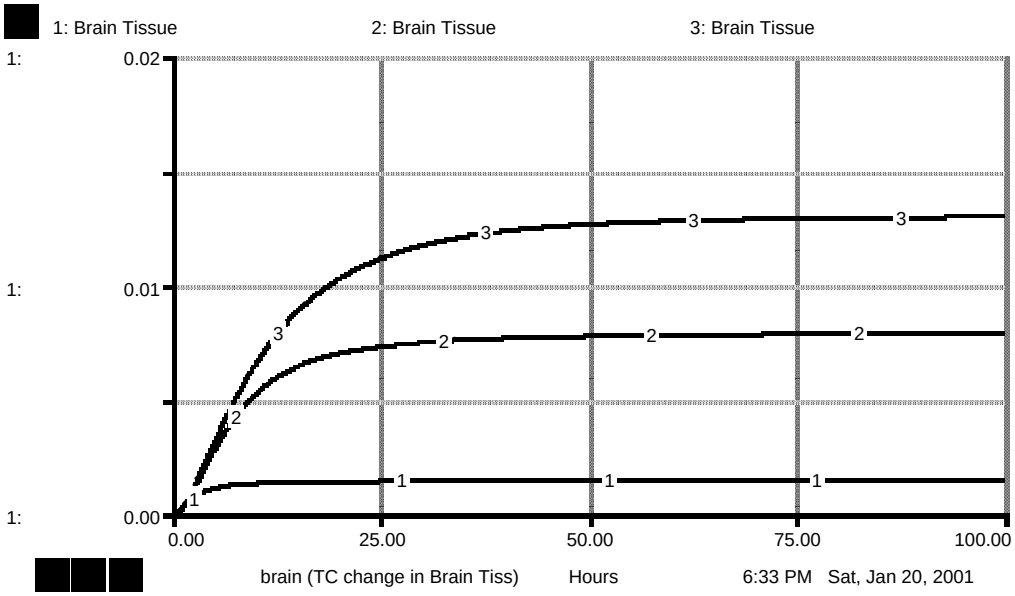
Brain Tissue Level (mg) for Increasing Mediated Maximum Transport2
at Maximum Stress and Exercise

1: TC=0.5 2: TC=3.5 3: TC=7.5



Brain Tissue Level (mg) for Increasing Mediated Transport Constant
at Maximum Stress and Exercise

1: TC2=0.5 2: TC2=3.5 3: TC2=7.5



Brain Tissue Level (mg) for Increasing Mediated Transport Constant2
at Maximum Stress and Exercise

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REPORT DOCUMENTATION PAGE

Form Approved
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1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From – To)*
20-03-2001	Master's Thesis	Aug 2000 – Mar 2001

4. TITLE AND SUBTITLE

A PHARMACOKINETIC STUDY OF THE EFFECTS OF STRESS AND EXERCISE ON CHEMICAL EXPOSURE

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Watson, Karen M., Captain, USAF

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES(S) AND ADDRESS(S)

Air Force Institute of Technology
Graduate School of Engineering and Management (AFIT/EN)
2950 P Street, Building 640
WPAFB OH 45433-7765

8. PERFORMING ORGANIZATION REPORT NUMBER

AFIT/GEE/ENV/01M-23

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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2856 G. Street
WPAFB OH 45433-7765
(937) 255-5150 x3143

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Several concerns about the effects of the combinations of human chemical exposures with the stressful conditions of the Gulf War have been raised. Stress causes changes in the human body, including blood flow, hormonal, and ventilation changes, and may increase the permeability of the blood-brain barrier. Each change influences the chemical uptake, distribution, and accumulation in the body.

The purpose of this thesis was to model and predict changes that occur when stress and exercise are combined with chemical exposure. A Physiologically-based pharmacokinetic (PBPK) model was used as a tool to visualize, predict, and generate a hypothesis about chemical exposures. The PBPK model developed simulated human tissue compartments during chemical exposure under varying stress and exercise conditions. The results suggest that the chemical concentrations in the brain are highly dependent on the transport mechanisms involved. The transport mechanisms and their respective strengths have been identified as key parameters for further study.

15. SUBJECT TERMS

Physiologically-based Pharmacokinetic (PBPK) Modeling, Stress, Exercise, System Dynamics, Chemical Exposure, Blood-brain barrier (BBB)

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Dr. Michael L. Shelley, ENV
U	**U**	**U**	**UL**	217	**19b. TELEPHONE NUMBER** *(Include area code)* (937) 255-3636, ext 4594

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Form Approved
OMB No. 074-0188