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A Pharmacokinetic Study of the Effects of Stress On Chemical Exposure

Sierra H. Suhajda

A PHARMACOKINETIC STUDY OF THE EFFECTS OF STRESS ON CHEMICAL EXPOSURE

THESIS

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AFIT/GEE/ENV/00M-16

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ABSTRACT (*Maximum 200 Words*)

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The purpose of this thesis was to model and predict the changes that will occur when stress is combined with chemical exposure. Physiologically-based pharmacokinetic (PBPK) modeling is one tool that can be used to visualize, predict, and generate a hypothesis about chemical exposures. A PBPK model was developed that simulated human tissue compartments during chemical exposure and different levels of exercise. The PBPK model developed is a valid tool for helping explain and predict the fate and transport of a chemical on an individual under stress. The results suggest that the brain compartment is of high importance when addressing the uptake of chemicals during exercise. The maximal uptake of a chemical from the blood-brain barrier, as well as the decreased enzymatic levels in the brain compartment have been identified as key parameters for further study.

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Presented to the Faculty of the Graduate School of Engineering and Management

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In Partial Fulfillment of the

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Master of Science in Engineering and Environmental Management

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Sierra H. Suhajda

Table of Contents

Page

List of Figures

Page

List of Tables

Page

Abstract

Following the Gulf War there were many concerns raised about human exposure to chemicals and how these chemicals may affect the body. When a soldier deploys to a foreign country during times of conflict, there will be stress related changes in his or her body. Stress causes several changes in basic functions of a human body, from breathing and blood flow changes to hormonal and enzyme changes, and increased permeability of the blood-brain barrier. Each of these changes can strongly influence chemical uptake, distribution, and accumulation in the body.

The purpose of this thesis was to model and predict the changes that will occur when stress is combined with chemical exposure. Physiologically-based pharmacokinetic (PBPK) modeling is one tool that can be used to visualize, predict, and generate a hypothesis about chemical exposures. A PBPK model was developed that simulated human tissue compartments during chemical exposure and different levels of exercise.

As a result of a conceptualization, formulation, and testing stage it appears that this PBPK model is a valid tool for helping explain and predict the fate and transport of a chemical on an individual under stress. The results suggest that the brain compartment is of high importance when addressing the uptake of chemicals during exercise. The maximal uptake of a chemical from the blood-brain barrier, as well as the decreased enzymatic levels in the brain compartment 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 ON CHEMICAL EXPOSURE

I. Introduction

Background

The word "stress" is derived from the Latin verb *strictus*, meaning to draw tight. The modern definition of stress, in the context of this thesis, originates from its use in physics where stress describes an applied force or system of forces that tend to strain or deform a body (The American Heritage College Dictionary, 1993:1343).

The concept of stress was introduced into the biomedical field by Canadian physiologist Hans Selye in the 1930's and stress soon became a recognized field of study (Opstad, 1995:9). Selye referred to stress as the nonspecific response of the body to any pressure that is vital to survival (Selye, 1976:35). Selye used the term nonspecific to describe stress due to the fact that the body will adapt to a given pressure irrespective of the problem (Fox, 1996:291). Stress prepares the body for any stressful situation by releasing a specific chemical response, also known as the "fight or flight" response (Gherman, 1981:9). The release of epinephrine and norepinephrine into the bloodstream and the resulting excitatory or inhibitory effects on various organs, such as increased heart rate and ventilation, are the most common observations during a stressful situation.

Stress can result from a wide range of internal or external stressors. A stressor can be defined as any perturbation that disrupts homeostasis (Sapolsky, 1992:3). These stressors can vary in many ways and impact the body along several different pathways. To make the matter more complicated stressors can also act differently on each human body (Gherman, 1981:4). External stressors require some sort of adjustment to a new set of external conditions, while internal stressors result from the internal demands that individuals may put on themselves (Gherman, 1981:6). Humans are equipped to deal with these stressors, but when these stressors trigger the stress response repeatedly, the effects begin to multiply and compound over a lifetime. Increased duration and intensity of stressors can cause several stress-related diseases to emerge (Sapolsky, 1992:6).

The long-term implications of chronic stress and the addition of chemical exposure have seen greater attention recently due to the Gulf War Syndrome. In past years, there have been many concerns raised about human exposure to chemicals and how these chemicals affect the body. When a soldier deploys to a foreign country during times of conflict there will be changes in his or her body. Stress may cause several changes in basic functions of the human body, from breathing and blood flow changes to hormonal and enzyme alterations. When under stress, the body's response to chemical exposure may be different than the response not under stress. This addition of stress may lead to an exaggerated negative response to chemical exposure. Recent human and animal studies have suggested specific physiological changes under stress. A number of these changes are known to strongly influence chemical uptake, distribution, and accumulation in the human body.

At the close of the Persian Gulf War in 1991, many soldiers returned home with a wide range of health complaints. These symptoms ranged from headaches and fatigue to memory loss and depression, and were collectively called the Gulf War Syndrome. In the eight years since the war there have been reports of approximately 100,000 of the 697,000 U.S. veterans displaying these symptoms, and most of these veterans attribute symptoms to the war (Haley, 1997:695). Prompted by the seriousness of the implications, the U.S. Department of Defense (DOD) formed a Presidential Advisory Committee on Gulf War Veterans' Illnesses and conducted an $80 million evaluation of the long-term health problems observed in soldiers who served in the Persian Gulf War (Pennisi, 1996:479). The Committee failed to identify a specific disease that could explain the symptoms or a specific cause, but they did attribute the variety of ailments to wartime stress (Haley, 1997:695). The main argument for stress as the cause of the Gulf War Syndrome was based on several studies of post-traumatic stress disorder (PTSD) in Gulf War Veterans (Haley, 1997:695).

Many veterans have rejected the suggestion that stress plays an important role in the Gulf War Syndrome, while several other studies have pointed to other explanations. Diseases behind the syndrome have included conditions such as chronic fatigue syndrome and multiple chemical sensitivity. Possible exposure to agents such as depleted uranium, insecticides, anti-nerve agents, and smoke from oil-well fires have also been investigated (Wegman, 1997:704). It is unlikely that any one of these theories will provide a unified explanation for the syndrome. However, studies have discussed the possible combinations of wartime stress and chemical exposure. Several animal studies in particular have shown that stress-induced changes in the body can disrupt the blood-

brain barrier. Disruption in the blood-brain barrier may allow chemicals, such as pyridostigmine bromide, to penetrate the brain due to an increased permeability and produce potentially damaging long-term consequences. Pyridostigmine bromide was administered to soldiers during the Gulf War as a pretreatment against chemical warfare (Friedman, 1996:1382). Future studies that focus on the changes in the permeability of the blood-brain barrier could aid in understanding the mechanisms behind stress and how environmental or occupational stress may actually play a role in the Gulf War Syndrome.

The search for explanations of the Gulf War Syndrome has created a need to protect military personnel from toxic and hazardous chemicals and practice preventive medicine, including the identification of environmental and occupational stressors. This new field of study is called deployment toxicology. Military personnel will face a growing number of threats in the operational environment in future years. Conflict prevention, humanitarian relief, and peacekeeping activities will require that military personnel enter areas where there will be an increased threat to human health and performance will become more prevalent. DOD Directive 6490.2 now ensures that each branch of the military conducts medical surveillance during military deployments. This will include developing a system to more rapidly forecast and assess risks that may be encountered before, during, and after deployments.

Problem Statement

An understanding of the etiology behind the Gulf War Syndrome will help fill the major gaps that have hindered the studies and immediate identification of the disease. The lack of objective data on environmental exposures and the lack of understanding the

mechanisms behind the behavior of a chemical in the human body are two deficiencies that are in need of further research (Jollenbeck, 1998:71). A better understanding of the mechanisms of stress and chemical exposure in the human body could help prevent future Gulf War Syndromes and aid in the development of deployment toxicology practices.

Purpose Statement

The purpose of this thesis is to model and predict the changes that will occur when stress is combined with chemical exposure. Physiologically-based pharmacokinetic (PBPK) modeling is one tool that can be used to visualize, predict, and generate a hypothesis about chemical exposures. This thesis effort will be directed towards understanding the concepts behind stress and chemical exposure, and then developing an initial PBPK model. The model will study internal interactions of the complex human body, investigate the effects of external stressors on the system behavior, and explore different scenarios of stress and chemical exposure to help understand system behavior under varying system conditions.

Understanding the responses that stress will solicit in the human body during chemical exposure will be very important in trying to prevent adverse effects, as well as aid in the performance of an individual. The model developed in this thesis will help predict the distribution of any chemical in an individual who is under a high degree of stress. The model developed will be a tool that can be used and applied to future exploration. This study may be one of the first steps in understanding stress and chemical distributions in the body. This may allow us to predict the influence of stress during wartime on chemical effects to the body.

Research Objectives

In order to develop a general model for predicting stress and chemical distributions in the human body as they relate to stress, there are four research objectives that are accomplished:

1. Determine the mechanisms of stress and chemical uptake/distribution in the human body.
2. Use the principles of system dynamics modeling to better understand the influences of stress on chemical uptake and distribution.
3. Through testing of a PBPK model support the hypothesis that stress will affect chemical concentrations in the human body.
4. Establish a framework for ongoing investigation of chemical exposure as modified by deployment and stress.

II. Literature Review

The purpose of this literature review is to present relevant literature background on the subjects of stress and chemical exposure. In order to emphasize this need, the following chapter has been organized with the four research objectives in mind. The first section will discuss the mechanisms of stress in the human body. Second, the literature and theories behind the Gulf War Syndrome will be presented. Third, literature on chemical exposure and distribution will be presented. Finally, a review of the use and capabilities of system dynamics and PBPK modeling as previously used in the study of chemical exposure will be discussed.

Mechanisms of Stress in the Human Body

Stress, as defined in the introduction, is the nonspecific response of the body to any pressure. Hans Selye felt that this nonspecific response was used in the body to readjust itself following any perceived imbalance or threatened disruption of homeostasis (Fox, 1996:291). Selye found that a stereotyped syndrome can be induced in rats when exposed to heat, cold, infection, trauma, and many other stimuli or stressors. This syndrome was referred to as the general adaptation syndrome (GAS). There are three stages in the GAS: 1) Alarm reaction - the activation of the adrenal glands and increased secretion of corticoid and epinephrine occur; 2) Stage of resistance - the body adapts to the stressor and readjustment occurs; and 3) Stage of exhaustion - exhaustion will occur if the body is not able to completely readjust to the stressor. These stressors can range from

acute to chronic or mild to severe and will have different effects in each individual (Cooper, 1983:6).

Selye's concept of stress has evolved over the years to include the specific mechanisms of the endocrine and nervous systems. It was found during stress experiments on rats that nervous stimulation caused a stress response, but denervated rats also showed the same response when stressed; this lead to the discovery of hormones (Cooper, 1983:6). A hormone is a regulatory molecule that is secreted into the blood by several organs in the endocrine system (Fox, 1996:272). The release of hormones follows a set chain of events that cause the specific changes in the body – the changes seen in Selye's GAS. This chain of events is controlled through positive or negative feedback loops via the hypothalamus-pituitary axis (Cooper, 1983:8). Figure 1 is a simplified graphic representation of the stress response in the human body.

The following sequence is an overview of the release of hormones and the interactions of the endocrine and nervous system, as seen in Figure 1 (Gherman, 1981:148-150):

1. A stressful event will produce a physiological and emotional response in the body. These responses trigger the initial release of endorphins, releasing or inhibiting hormones, from the hypothalamus.
2. Endorphins trigger the body's pituitary gland causing a release of adrenocorticotrophic hormone (ACTH) into the bloodstream. ACTH then travels on to the adrenal glands.
3. The external portion of the adrenal gland (adrenal cortex) secretes steroid hormones called corticoids, mainly glucocorticoids, such as cortisol or corticosterone. These

hormones are responsible for preparing the body for the adaptive reactions that take place due to a stressor. Corticoids facilitate enzyme reactions and affect immune reactions.

4. The inner part of the adrenal gland (adrenal medulla) receives a nerve impulse from the hypothalamus and immediately secretes epinephrine, also known as adrenaline, into the body. This specific sequence involving the adrenal medulla is referred to as the "fight or flight" or sympathetic response. Increased metabolic or cardiovascular activities such as increased alertness, increased physical strength, increased heart rate, increased blood flow, and faster breathing are the common results of an increased release of epinephrine. When an individual is exposed to chronic or intense stress the secretion of epinephrine will prolong the sympathetic response and a continual stream of corticoids are released from the adrenal cortex.
5. This in turn will stimulate the liver to release more blood glucose to supply extra energy for recovery. This process of releasing blood glucose from the liver is called gluconeogenesis. Gluconeogenesis causes the mobilization of fats and proteins into the blood.

This fifth event is the point where the continuation of exposure to a chronic or intense stress will produce damaging effects in the body. Overactivation of the stress response or the inability to terminate the secretion of hormones can result from a prolonged stress. It was found during several studies that prolonged stress would result in muscular wasting and impairment of the immune system by depletion of protein stores in the body. These protein stores are needed for development of white blood cells and antibodies. Prolonged stress also results in a high level of fat in the bloodstream due to

the gluconeogenesis process; which may enhance the risk of arteriosclerosis. Furthermore, the release of fats and proteins into the bloodstream decreases the use of glucose in the system resulting in an excess amount of glucose, which can then lead to diabetes (Gherman, 1981:150).

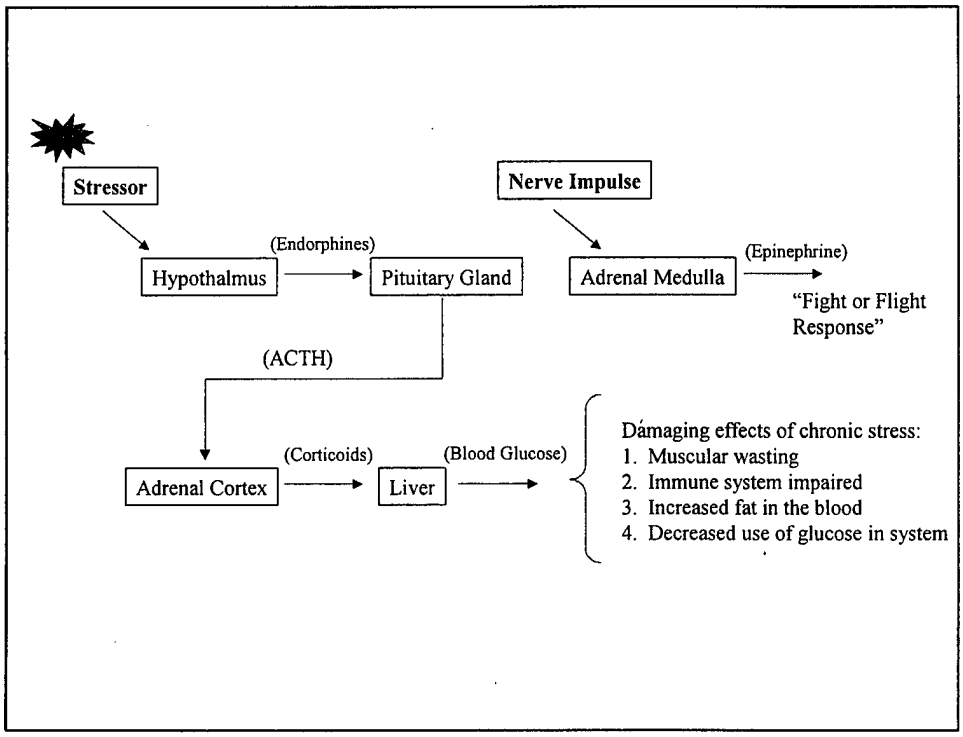


Figure 1. Stress Response in the Human Body

The results of stress can impact every part of the body. Stress is known to affect the cardiovascular, endocrine, immune, and central nervous system (Gherman, 1981:150). Stress and disease are interconnected in that every disease causes a certain type of stress, while stress plays a role in the development of disease. Stressors can disrupt the body's homeostasis by either going beyond the body's ability to adapt or by causing damage due to a particular weakness (Cooper, 1983:12). This is why every

individual is different in responding to stress. Due to heredity or external influences each individual will have a unique weakness. This has made the study of stress very difficult due to the fact that humans develop different diseases or symptoms under the influence of the same kind of stressor (Cooper, 1983:13).

A large amount of recent research has focused on the differences in stress response among various individuals. Studies suggest that people fall into different categories of responses when dealing with stress. In one particular study, men were subjected to five days of arithmetic tests. Cortisol concentrations were tested each day. Two different groupings of cortisol levels appeared with one group having a higher level than the other. The men categorized in the higher cortisol group also reported more health problems, and had larger variations in heart rate when under stress (Sgoutas-Emch, 1994:264).

Other studies have looked at the differences between men and women's response to stress. Women's blood pressure goes up less than men's blood pressure in response to stress, but women tend to react to a larger range of stressors. And finally, it has been found that childhood stress is on the rise. Research has found that childhood experiences with exposure to stress have a great influence on how adults deal with stress (Kalb, 1999:62).

Psychologist Sheldon Cohen completed the study that found an association between stress and reduced immune response in 1991. Individuals who ranked higher on a test of perceived stress were also more likely to develop colds. Cohen also found that chronic stress can increase the odds of getting sick by three to five times (Cohen,

1998:214). Everyday stresses accumulate and have a greater impact on the human body than just one isolated stressful event (Brosschot, 1994:216).

Another study performed by Zakowski and others found reduced antibody levels among individuals caring for spouses suffering from dementia. This study and several others have found that stress causes either an increase or decrease in the levels of natural killer (NK) cells, an increase in killer T-cells (CD8), and a decrease in helper T-cells (CD4) (Zakowski, 1992; Kiecolt-Glaser, 1992; Delahanty, 1996; and Shephard, 1998). These changes affect the human body's ability to regulate and enhance immune functions.

Shephard and others have performed numerous tests on military personnel during periods of physical stress or exposure to adverse environmental conditions. Results show that immune systems were more vulnerable, and a high incidence of "performance-impairing infections" developed (Shephard, 1998:545). Several other experiments have found that tumors will grow faster in animals subjected to stress, which is again related to corticoid levels (Fox, 1996:448). The strength of the immune system is also affected by stress in cancer progression studies done in humans (Delahanty, 1996:48).

Robert Sapolsky has researched several issues on the effects of stress. Sapolsky found that corticoids that are secreted in excess are damaging to the human brain. Corticoids, which are released from the adrenal cortex, have a large role in stimulating the hippocampus, an organ vital for memory and learning. Researchers have found that an excess secretion of corticoids, as is the case with depression or Cushing's syndrome patients, can actually damage the hippocampus. Memory and cognitive abilities are impaired, and significant reductions in the volume of the hippocampus appear in

magnetic resonance imaging (MRI) scans (Sapolsky, 1996:749). Studies of patients with posttraumatic stress disorder (PTSD) have also supported the evidence of the relation between corticoids and hippocampus size and function. Bremner and others found 12% atrophy in the hippocampus of adults with PTSD who were subject to childhood abuse. Bremner also found similar results in Vietnam veterans with PTSD. The veterans had 8% hippocampal atrophy. Longer periods of combat exposure in the veterans were associated with a smaller hippocampus (Sapolsky, 1996:749).

Sapolsky and Kaufer have also studied the relations between stress and a reduced level of acetylcholine (ACh) and cholinergic neuron expression (Kaufer, 1998:373). ACh is an excitatory or inhibitory neurotransmitter chemical that communicates from a neuron to another neuron, a muscle, or a gland. ACh is inactivated by acetylcholinesterase (AChE). AChE is vital in deactivating the nerve conduction performed by ACh (Fox, 1996:157). Kaufer and others found that a forced swim stress exposure on rats elicits two separate phases. The stress exposure was found to have the same affect as AChE inhibitors, which first results in an increase in excitatory ACh. After the stressor occurred, an unknown second phase was discovered where decreased neuron excitability (or increased AChE) and impaired cognition occur (Kaufer, 1998:373). Kaufer found that levels of AChE were still elevated eighty hours after the end of the swim stressor performed on the rats. This negative-feedback loop that results in impaired cognitive ability is a very important step in stress research, and also leads to questions of the possible synergistic effect of both stress and AChE inhibitors (Sapolsky, 1998:309).

Gulf War Syndrome

Between August of 1990 and July of 1991, approximately 697,000 U.S. military personnel served in the Persian Gulf during Operations Desert Shield and Desert Storm. For a significant portion of these individuals, the Persian Gulf experience was psychologically stressful – before, during, and after the deployments. The war-related environment has historically been associated with a wide range of stressors (Hyams, 1996:1). These stressors can range from the stress of combat or fear of attack to the stress of separation from family, long work hours, poor living conditions and different climate conditions. In many previous wars, physical and psychological stresses have been observed to lead to high rates of psychiatric illnesses. Disease, shell shock, combat fatigue, chronic fatigue syndrome (CFS) and posttraumatic disorder (PTSD) are just a few of the illnesses found to be more prevalent in combat veterans than in the general population (Hyams, 1996:1). PTSD and depression are found in a larger majority of Gulf War combat veterans (PAC, 1996:Ch. 3, 8).

Since the war an increasing number of veterans have complained of several neurocognitive symptoms, which have been collectively called the Gulf War Syndrome (Sapolsky, 1998:308). In 1996, the final report from the Presidential Advisory Committee (PAC) on Gulf War Veterans' Illnesses concluded that collecting exposure data and information on the risk factors in the Gulf War had been difficult, and most information available was based on recollections of Gulf War veterans (PAC, 1996:Ch.4, 1). Therefore, it has been difficult to link the syndrome to specific exposures or risk factors. The PAC focused on pesticides, chemical warfare agents, biological warfare

agents, vaccines, pyridostigmine bromide, infectious disease, depleted uranium, oil-well fire smoke, petroleum products, and psychological and physiological stress as the main risk factors (PAC, 1996:Ch. 4, 1). The PAC concluded that the collection of symptoms seen in Gulf War veterans can be attributed to the combinations of wartime stress and the possible exposure to variable risk factors, such as those listed above (Wickelgren, 1997:1404). It was also concluded by experts at the 1994 National Institutes of Health Technical Assessment Workshop on the Persian Gulf Experience and Health that "no single disease or syndrome is apparent, but rather multiple illnesses with overlapping symptoms and causes" (New Persian Gulf Possibilities, 1996:820).

Possible Chemical Exposures during the Gulf War

Due to the recommendations made in the PAC's final report numerous research projects have focused on chemical exposure and stress. Is the Gulf War Syndrome in some way tied to the psychological stressors that a soldier may be exposed to during times of deployment? Is the Gulf War Syndrome a result of chemical exposures? Or can the combination of stress and chemical exposure both be the culprit behind the syndrome? These questions and the growing concern from the public have led to several studies in pursuit of the cause behind the Gulf War Syndrome.

Four chemicals in particular have become the focus of research on the Gulf War Syndrome. Military personnel throughout the war used DEET, permethrin, pyridostigmine bromide, and organophosphate. These chemicals have also been the subjects of several studies addressing their potential toxicological impacts.

DEET

N,N-diethyl-m-toluamide, more commonly known as DEET, is an insect repellent that has been widely used both commercially and in the military. DEET is the active ingredient in commercial products such as Deep Woods Off and Cutter Insect Repellent. Several formulations and various concentrations of this insecticide exist including impregnated towlettes, solid sticks, liquids, and gels (Nelson, 1995:43). The most common formulations used by military personnel in the U.S. Army include 75% DEET liquid solution in ethanol, 33% DEET for extended duration formulation, and a 19% DEET stick (McCain, 1997:115). DEET is an EPA-approved insect repellent and has been linked with low levels of toxicity in animal studies (McCain and others, 1997:114). DEET crosses the blood-brain barrier, and neurotoxic damage has been reported (Verschoyle and others, 1992:80). Human health effects from exposure to DEET include: slowed respiration, slowed pulse rate, low blood pressure, depression, tremors, convulsions, skin rash, necrosis, and blistering (Nelson, 1995:39). The rat oral LD50 of DEET is 2-3 g/kg, the dermal LD50 is 5 g/kg, and the median lethal dose is 3.67 g/kg (Leach and others, 1988, and McCain and others, 1997).

Permethrin

Permethrin, a synthetic pyrethroid, is also an EPA-approved insecticide. The compound is used to impregnate battle dress uniforms. Most uses of this insecticide during the war were in the form of 0.5% aerosol spray, which provides protection for up to six washings or six weeks of wear (McCain and others, 1997:115). Wester and others found that insecticides impregnated in fabric could still be absorbed into the skin and then into the systems of the human body. The addition of water or other solvents to simulate

sweating or wet conditions caused an increase in skin absorption (Wester and others, 1996:734). Permethrin readily crosses the blood-brain barrier and acts upon transport mechanisms in the brain tissue. The median lethal dose in rats for permethrin is 1000 mg/kg (McCain and others, 1997:119).

Pyriodostigmine Bromide

Pyridostigmine Bromide (PB) is a quaternary ammonium carbamate that was used as a pretreatment against nerve agents during the Gulf War due to its ability to inhibit acetylcholinesterase (AChE) (McCain, 1997:115). PB used alone is not protective against nerve agent poisoning but must be used as a pre-treatment to counter the effects of atropine used by military forces. PB actually competes for binding sites of AChE. The key to PB's success is that instead of the irreversible bond that nerve agents have with AChE, PB has a reversible bond. PB's bond to AChE will detach more quickly, leading to reactivation of AChE and normal functioning (Cook and others, 1992:250). The quaternary structure prevents PB's permeation through the blood-brain barrier (Freidman, 1996:1382). The median lethal dose for PB lies in the range of 60 to 80 mg/kg (McCain and others, 1997:119). The LD50 of PB is 2.73 mg/kg (Blick and others, 1994:312). In 1995, the Food and Drug Administration (FDA) initially approved PB for treatment of myasthenia gravis (MG), a chronic neuromuscular disease (Fox, 1996:164). Based on the evidence that PB was safe when used as a drug with MG patients, the FDA concluded that PB was also safe for use by U.S. military personnel. MG patients typically take 1500 mg per day over several years, while military personnel take 90 mg per day over a maximum of seven days. The DOD estimated that approximately 250,000 military personnel took PB at some time during the war. Side effects of PB include

nausea, vomiting, dizziness, muscle weakness, and abdominal cramps. As with MG patients, the side effects of PB found in a DOD study ceased when PB use was discontinued. After several research efforts, the DOD has concluded that PB alone has no serious or long-term reactions (PAC, 1996:Ch. 4, 17).

Organophosphates

Many suspect exposures to organophosphates (OP) as a possible factor behind the symptoms found in the Gulf War Syndrome. The concern over OP exposure surfaced in the 1960's when farmers and industrial workers experienced memory and concentration problems after chronic occupational exposure of low levels of OP. Similar symptoms are now being found in Gulf War veterans. Several OP pesticides were used during the war to include diazinon and malathion (PAC, 1996:Ch. 4, 7). Buccafusco and colleagues have found in rat models that low levels of OP produce long term problems in the brain (Wickelgren, 1997:1404). Buccafusco found that OP reduced the number of receptors in the hippocampus region of the brain in rats, which hinders the response to nerve impulses. The rats also developed learning disorders that persisted for 3 weeks after the last OP injection. AChE is inhibited which leads to a build up of ACh in the nerve synapse; thus an overfiring of the nerves. High levels of OP can lead to death (LaPuma, 1999). Atropine, which blocks ACh receptors, counters the effects of OP. Atropine is currently used by the military in an auto-injector if personnel are exposed to nerve gas (LaPuma, 1999). Studies, such as the one performed by Buccafusco, now raises the concern over low level exposures of OP to military personnel.

Gulf War Related Research

Several areas of research conducted on the Gulf War Syndrome are relevant to the work presented in this thesis. Research on multiple chemical exposures, stress, and the blood brain barrier will be discussed.

Multiple Chemical Exposure

The need for more research into the toxicity of multiple chemical exposures has been emphasized after the Gulf War. Abou-Donia and others have found that exposure to two or more chemicals or drugs used by military personnel during the Gulf War have caused a synergistic type of effect in the nervous system of chickens (Abou-Donia and others, 1996:35). The researchers exposed chickens to DEET and permethrin as well as the anti-nerve agent called pyridostigmine bromide (PB) individually and in combination. Results showed that neurotoxicity was greater than what was expected with individual exposures. The compounds resulted in minimum toxicity when administered individually, but when combined adverse neurological effects occurred. Abou-Donia and others hypothesize that an exposure to multiple chemicals may have overwhelmed the animals' ability to break down chemicals through enzyme interactions. In particular, PB was found to bind to the enzyme butyrylcholinesterase. This enzyme neutralizes nitrogen-containing organic compounds, such as DEET, permethrin, and PB. PB can compete for butyrylcholinesterase, allowing the other chemicals to permeate the brain and nerves (Abou-Donia and others, 1996:52).

McCain and others also conducted experiments on the lethal interactions of DEET, permethrin, and PB in rats. A significant reduction effect occurred in the lethality when the chemicals were given at the same time. As in Abou-Donia's conclusions,

McCain found that competition among the three chemicals for enzymes actually reduced metabolism of the chemicals. Thus, there is an increased availability of chemicals to be taken up by the brain. The resulting lethal values and neurotoxicity exceeded the expected additive values (McCain and others, 1997:113).

Stress

Extensive literature on the topic of stress and its ability to compound neurological insults has supported the theory that stress may contribute to the Gulf War Syndrome (Sapolsky, 1998:309). Some researchers hypothesize that PB and stress may create central nervous system (CNS) effects. A study performed by Sharab and others looked at symptoms following intake of PB during the Gulf War. They found that most studies performed on the adverse effects of PB were done in nonstressful conditions. Evaluating soldiers during the war could lead to human data on PB and exposure to stressful conditions, such as the threat of chemical warfare attack. A correlation was found between PB intake and the symptoms reported by soldiers. Sharab and others hypothesized that during a stressful situation the human body becomes more sensitive to symptoms that may have been unnoticed in studies done during peacetime conditions (Sharab and others, 1991:656-658). Sharab and others concluded that PB administration during the Gulf War led to an increase in the severity of CNS symptoms seen in veterans (Sharab and others, 1991:657).

Previous animal studies have shown that stress tests resulted in a disruption of the blood brain barrier (BBB) (Sharma, 1991:211). Friedman reported that mice subjected to a forced stress swim test needed only $1/100^{th}$ of the usual dose of PB (1.50 to .01 mg/kg) for 50% of AChE activity to be inhibited. The stressful swim event that the mice were

exposed to allows PB penetration into the brain (Friedman, 1996:1382). The correlation between stress and PB-induced CNS effects was confirmed when nonstressed mice treated with PB had no affect on CNS functioning. They concluded that compounds that are limited in their ability to permeate the blood-brain barrier might become active in the CNS under stressful conditions (Friedman, 1996:1384). This study supports the theory that stress-induced changes in the blood-brain barrier increase permeability.

A concern with military personnel during wartime is decreased performance due to the intake of specific drugs. A study performed by Cook and others looked at the effects of PB in soldiers working in a desert environment. Small decreases in neurotransmission after muscle contraction were found. It was also noted in their study that PB had two effects on the body's ability to cool down. Sweating rates increased by 5% during PB administration, and the skin blood flow decreased. This impaired blood flow may be one of the reasons why the core temperature of individuals in this particular study increased (Cook and others, 1992:254). This also has the potential to increase the risk of heat stress in chemical warfare gear (Arad and others, 1992:213). It was concluded, even with the above findings, that administration of PB did not significantly reduce performance.

As mentioned earlier in this literature review, Kaufer and others have found that stress and AChE inhibitors, such as PB, can have a delayed long lasting effect in nervous system activity and cognitive ability (Kaufer, 1998:373). This delayed nervous system effect could possibly explain the delayed onset of PTSD and the delayed symptoms seen in Gulf War veterans. The combination of wartime stressors and exposure to PB could

compound the effects seen in Kaufer's study. Obviously, further studies are required to look at the effects of combined stressful situations and PB intake.

Blood Brain Barrier

Several studies reviewed in this literature review have discussed the possible repercussions from the increased permeability of the blood-brain barrier (BBB). The need for homeostasis is very important in maintaining functions in the brain. Paul Ehrlich, a bacteriologist, was the first to introduce the BBB. He found after injecting dye into the blood stream that most organs would stain, yet the brain always remained unstained. Ehrlich concluded that some sort of barrier to the brain prevented the flow of toxins and other substances that could affect the central nervous system (Hanin, 1996:1307). Unlike the porous capillary walls found throughout the body, the BBB consists of tight junctions between endothelial cells that make up the capillaries. This creates a very selective system, where only particular substances can cross through the endothelial cells. There are approximately 400 miles of these brain capillaries in a human (Introduction to the Blood-Brain Barrier, 1999).

The cell or plasma membranes of the endothelial cells are composed primarily of phospholipid and protein molecules. A double layer of phospholipids, which consist of polar and nonpolar heads, in the cell membrane result in the restriction of water and water-soluble molecules and ions through the membranes. Lipid-soluble molecules, on the other hand, pass through the membrane rather easily (Fox, 1996:48-49). This is also the case with the BBB where some chemicals can easily pass through, while others can not. It has been found that lipid-soluble molecules with a molecular mass under 400-600 Dalton are transported readily across the BBB (Pardridge, 1998:1782).

The high specificity of the BBB has caused several challenges in the medical field when patients with brain diseases, such as those with Parkinson's disease, are treated with drugs. Parkinson's disease can be treated with a chemical called dopamine, but it is not able to enter the brain. A drug called L-dopa is used instead. L-dopa is actively transported across the BBB and then converted to dopamine (Hanin, 1996:1307). Problems are also seen with specific antibiotics. Penicillin cannot cross the BBB, so when treating infections such as meningitis other antibiotics must be used (Fox, 1996: 151).

The tight junctions of the endothelial cells eliminate all transcellular bulk flow across the BBB (Introduction to the Blood-Brain Barrier, 1999). Under these conditions, a compound may gain access to the brain tissue via two pathways: lipid mediation (passive diffusion) or catalyzed transport (mediated/active transport) (Introduction to the Blood-Brain Barrier, 1999, and Fox, 1996). Astrocytes have also been found to interact with the BBB. The BBB is surrounded by the extensions of these neurological cells (Fox, 1996: 150-151). Thus, the brain is very protected against any chemical imbalance.

A more detailed understanding of the transport mechanisms across the BBB can be gained by understanding the structure of the endothelial cells that make up the BBB. Two membranes make up the endothelial cells. These membranes are separated by approximately 300 nm of endothelial cytoplasm, and are termed the lumenal and ablumenal membranes. The lumenal side faces the capillary or blood, while the ablumenal side faces out toward the brain tissue. Therefore, transport must exist across both lumenal and ablumenal membranes if a compound is to pass from the blood to the brain (Introduction to the Blood-Brain Barrier, 1999). The bi-directional flow of both

passive diffusion and mediated transport is also an important concept to be considered when studying the BBB (Smith and Stoll, 1998:189).

Figure 2 is a simplified pictorial representation of the BBB. As noted in Figure 2 another form of transport across the BBB is related to enzyme activity or metabolism where a chemical that initially may not be able to cross the BBB can be enzymatically altered to a form that is allowed to cross the BBB (Brightman, 1989:75). This metabolism of compounds acts as a detoxification system for the brain, but as discussed above in multiple-chemical and stress research, competition or saturation of enzymes may occur. Does this allow an increased amount of chemical into the brain tissue?

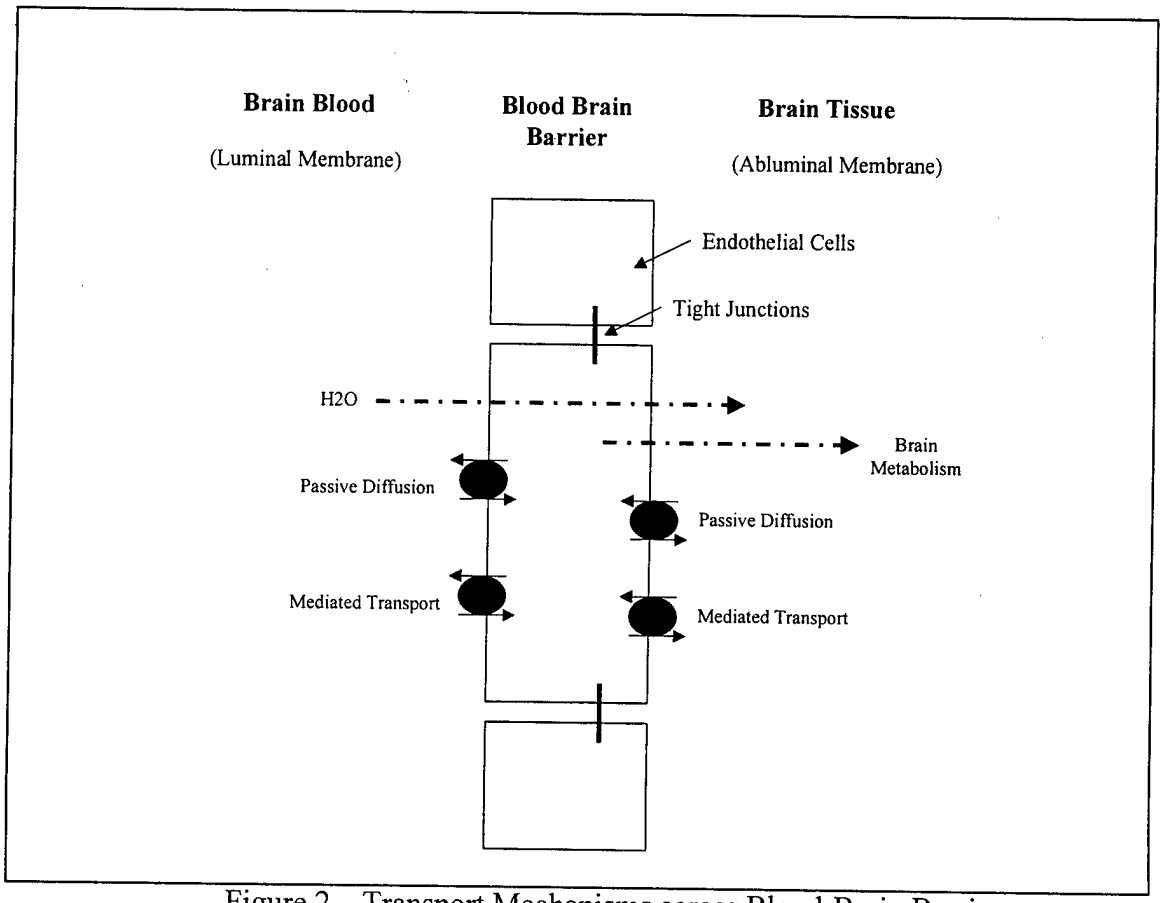


Figure 2. Transport Mechanisms across Blood-Brain Barrier

In a study performed by Belova and others the capacity of the BBB was investigated in the rat brain during normal conditions and stressful conditions. Acute immobilization (IMO) was used as the stress exposure. They found that the brain became more permeable after exposure to stress. With the use of dye they also found an increased level of dye penetration in the brain following the stress. Dye was found in specific brain regions involved in autonomic regulation. The autonomic nervous system controls the involuntary responses in the body, such as increased heart rate or increased blood glucose (Fox, 1996:216). The penetration of dye into the brain could explain the adverse effects or symptoms seen during stressful conditions (Belova and others, 1982:116).

In 1991 Sharma and others supported the conclusions reached in the above study. During a rat exposure to forced swimming, researchers found an increased BBB permeability in specific regions of the brain. Decreased metabolism, altered blood flow, and interactions with serotonin, a neurochemical compound, were cited as possible explanations behind the BBB permeability (Sharma and others, 1991:212,218). A decrease in BBB metabolism explains why an increase in dye was transported to the brain. The increased permeability in the BBB was only found during 30 minutes to 120 minutes after the forced swim exposure, and increased permeability was limited in younger rats; thus the BBB permeability appears to be time and age dependent and is thought to be reversible (Sharma and others, 1991:218).

Chemical Exposure and Distribution

The purpose of this portion of the literature review is to gain an understanding of the processes of chemical exposure and distribution in the human body. The study of environmental toxicology includes the hazardous effects that a foreign substance can have on human health. Physiologically-based pharmacokinetic (PBPK) modeling is one method that can be used in the study of toxicology or toxicokinetics. Toxicokinetics is concerned with the exposure, absorption, distribution, storage, biotransformation, and elimination of toxicants in an organism (Hughes, 1996:21). An understanding of these time-dependent processes is important when looking at the end effect a toxicant has on the organism's system. The organism's specific response is also important. Each species or individual will be affected differently. These effects can range from lethal to systemic, acute to chronic, or immediate to delayed.

Absorption is the process by which a toxic substance crosses an epithelial cell barrier in an organism. The three primary routes of absorption include absorption through the skin, respiratory system, and digestive system. Knowledge of each of these routes is vital in understanding the possible effects from a particular exposure.

Distribution through an organism's system occurs through the lymph or bloodstream. Duration of exposure, dose, and chemical characteristics must all be considered when determining the distribution of a toxicant to particular tissues in the body. Other factors that are also important would include the concentration gradient (concentration of the toxicant in the blood and tissues), volume of blood flow, volume of target tissue, cardiac output ratios to each tissue, and a toxicant's affinity for a particular tissue (Hughes, 1996:49-64).

Once a toxin is distributed through the body, other processes, such as storage, biotransformation, and elimination, occur. Storage occurs when a toxic substance accumulates in a specific tissue. Storage occurs most commonly in the bone, kidneys, liver, and fat (Hughes, 1996:67). Biotransformation is the process by which a toxic substance is changed to alternative forms making elimination from the body easier. And finally, elimination from the body occurs through several routes to include the following: urine, feces, sweat, saliva, exhaled air, and milk.

Modeling

There is a need to establish a basic pharmacokinetic study on stressful situations and the intake of chemicals. Estimates of health hazards during times of stress will be very important in future incidents of wartime deployment, as well as in developing the field of deployment toxicology. Pharmacokinetic modeling looks at the processes of absorption, distribution, metabolism, and elimination by the body. Physiologically-based pharmacokinetic (PBPK) modeling is a more recent technique that can be used to predict chemical concentrations in specific tissue compartments. PBPK modeling is a more accurate tool for exposure assessments since 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).

Adequate epidemiological studies are hard to find, so human exposure studies are usually based on animal studies. Animal studies require a tremendous amount of time and resources, and also pose difficulties when extrapolating to human exposures. PBPK modeling is one way to reduce the reliance on animal studies and provide more accurate

predictions. The combinations of time-dependent uptake, distribution, metabolism, and elimination of a chemical are used in PBPK modeling (Clewell, 1988:A125). Hazard assessments become less complicated, and the extrapolation from animal to human exposure becomes more accurate (Clewell, 1988:A125).

PBPK models compartmentalize various tissue groups into a system on the basis of physical and biochemical parameters. Mass-balance differential equations are used to represent the behavior of a substance within the system (Brown, 1994:Ch.2, 11). The first widely recognized PBPK model was completed by Ramsey and Andersen in 1983. They simulated the behavior of inhaled styrene in rats and humans (Ramsey and Andersen, 1984:159). Their model organized tissue into the following four groups: 1) highly perfused tissues, 2) moderately perfused tissues, 3) slowly perfused tissues, and 4) tissues that metabolize a large amount of styrene (liver). The model was first simulated to represent rats and then scaled up to represent human exposures. The rat model was found to be very accurate when compared to actual rat exposure data used in experiments. This provided a rational basis for the use of PBPK modeling in the extrapolation of animal data to chemical hazards in humans (Ramsey and Andersen, 1984:172).

The Ramsey and Andersen model has become the foundation of PBPK research (Brown, 1994:Ch.2, 13). Figure 3 is an example of a generic PBPK model. The alveolar space and lung blood compartments represent the initial intake of a lipophilic chemical through inhalation. The fat tissue compartment is very important due to a chemical's affinity for fat. The muscle tissue compartment falls under the slowly perfused, and represents lean tissues. The richly perfused tissue group would include those organs

where blood flow is rapid, such as the heart, kidney, and brain. And finally, the liver compartment represents significant metabolic activity. Each of these compartments is linked through arterial and venous blood flow (Q variables) and specific chemical concentrations (Cv and Cart variables) to each compartment.

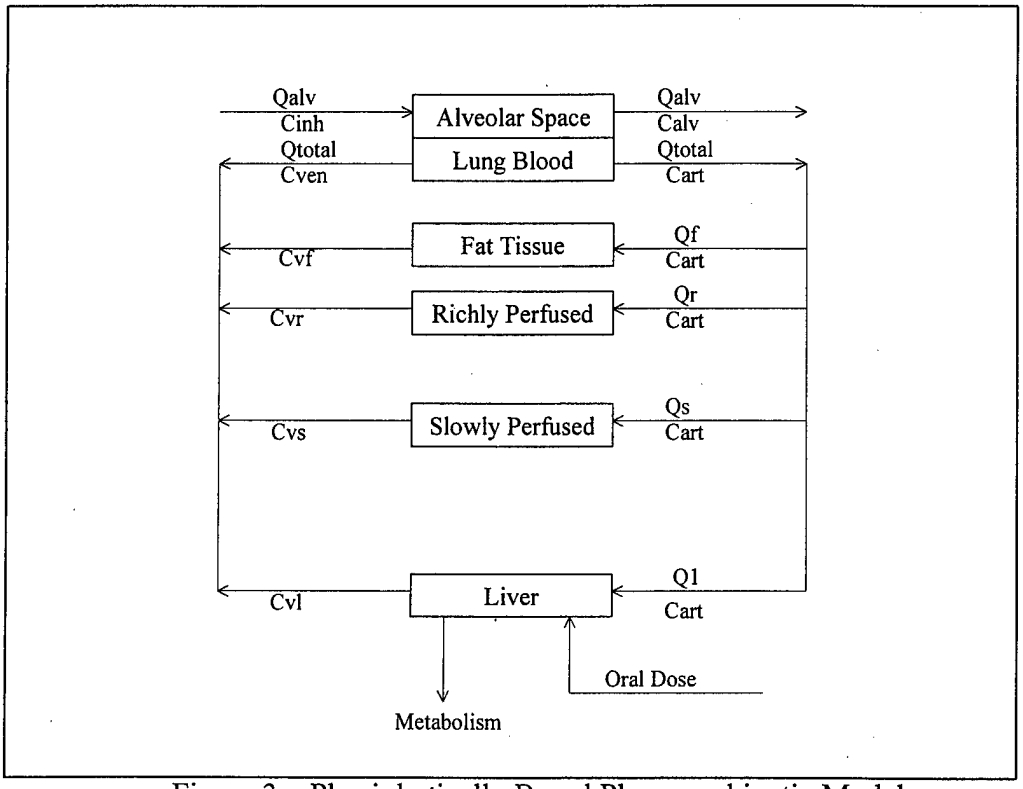


Figure 3. Physiologically Based Pharmacokinetic Model

Using numerical integration of a system of differential mass balance equations, the rate of input, output, and metabolic activity through each compartment in the model can be simulated (Brown, 1994:Ch.2, 11). This basic model can be changed to include more tissue compartments to address a specific chemical exposure, as well as account for the interactions of stress on the human body.

From the research that has been performed on the effects of stress on chemical exposure it has been found that the brain and the BBB may play a very important part in the chemical kinetics. Adding a single brain compartment to the model developed by Ramsey and Andersen will not accurately reflect the mechanisms of transport across the BBB from the blood into the brain tissue. Figure 4 contains the modified PBPK model that will be used in this study. The brain was divided into three sub-compartments (1-brain blood, 2-blood-brain barrier, and 3-brain tissue) to show a more accurate representation of the transport and metabolism that occurs in the human brain.

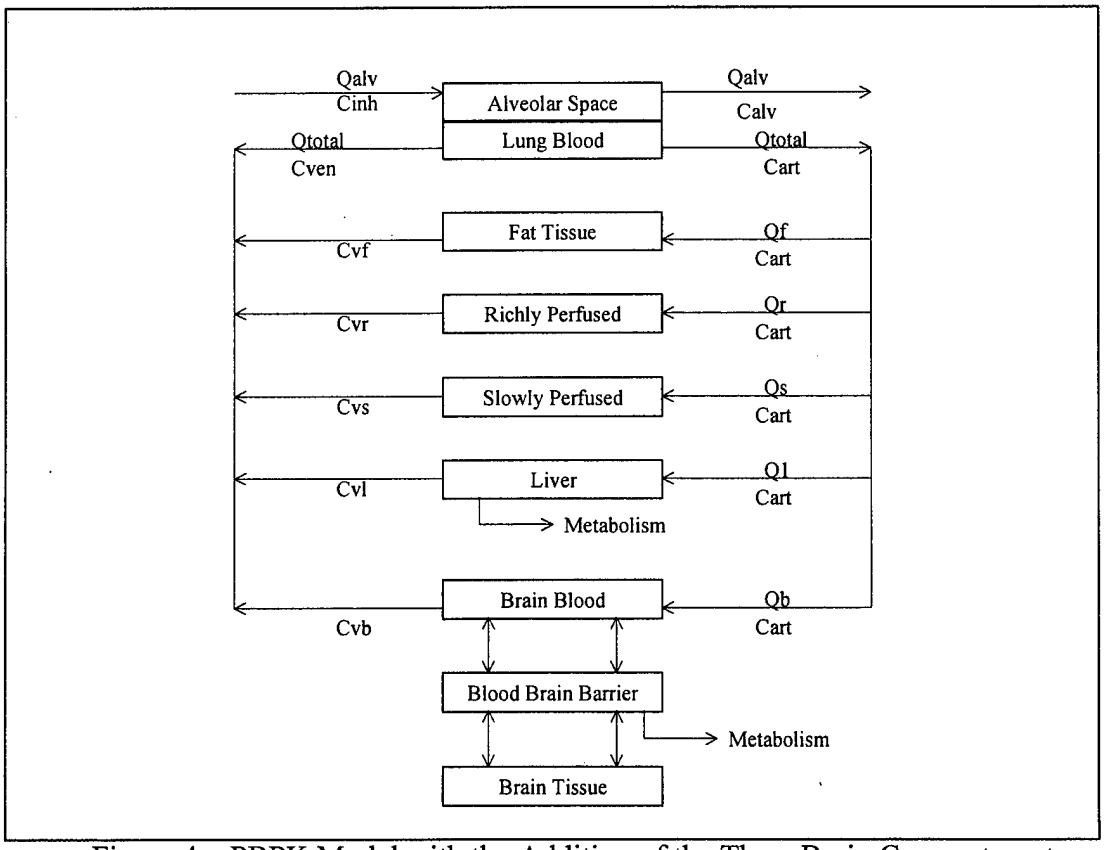


Figure 4. PBPK Model with the Addition of the Three Brain Compartments

A PBPK model like the one in Figure 4, which also addresses the interactions of stress and chemical exposure, can now explore the system behavior and help predict the causes behind the Gulf War Syndrome. Through the use of a developed model we can learn what variables are important and investigate changes in these variables and the effect they have on human health.

Model Parameters

The parameters necessary for the development of a PBPK model include physiological values, partition coefficients for the specific compound in question and metabolic values. Physiological values can be used with several different chemical compounds. Values for several chemical compounds have been determined experimentally for use in PBPK models. The compound-specific parameters in this particular model have initially been defined to match the chemical characteristics of trichloroethylene (TCE). Several PBPK models have been developed for TCE and human exposure. TCE was chosen due to the abundance of information and research performed on this chemical in PBPK models.

Several difficulties arise when considering the concept of stress. Little effort has been devoted to developing a specific classification of stress or stressors. There is also a lack of consistency with the stress research protocols that have been used in past experiments on rats and humans (McCarty, 1989:5). These issues should be considered when defining the parameters for this PBPK model. Table 1 summarizes one classification system that has been used to identify various stressors.

An individual under a deployment situation may undergo several types of stressors that fall within each of the categories in Table 1. The model developed in this

thesis will be a starting point for exploring the behavior and physiological changes that occur under emotional and physical stress. Physical activity or exercise is one type of stressor that is well understood in its effects on the human body. The physiological changes during exercise and the impact these changes have on chemical kinetics in the body will be addressed in this model.

Table 1. Classification of Stressors (Elliott 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

The study of exercise has been very important in the exploration of stress and how stress effects the human body. Animal studies have used the forced swim stress protocol and shaker stress test, while human studies have used the basic treadmill test. Physical activity is just one of the many types of stress that initiates the stress response and triggers changes in the cardiovascular system. Physical activity results in an increase in ventilation, cardiac output, and distribution of cardiac output. The flow to skeletal muscle or the slowly perfused tissue compartment will increase due to redistribution away from the fat and liver compartments, while the richly perfused tissue compartments will maintain the same amount of blood flow. This redistribution of blood flow is a very important mechanism that enables the body to maintain homeostasis for vital organs such as the brain and kidneys. Table 2 lists the physiological and biochemical parameters found in the literature for both rest and physical activity. These values will be used to

develop the initial PBPK model for stress and chemical exposure. It should be noted that the biochemical parameters could be redefined for a different chemical at any point in the modeling process.

Table 2. Physiological and Biochemical Parameters (TCE) 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[b]			
Blood/Air	PBloodAir	9.2	9.2
Fat/Blood	PF	73.3	73.3
Slowly Perfused/Blood	PS	2.3	2.3
Richly Perfused/Blood	PR	6.8	6.8
Liver/Blood	PL	6.8	6.8
Metabolic Parameters[b]			
Michaelis-Menten Constant (mg/l)	LKm	1.5	1.5
Max. Velocity of Metabolism (mg/hr -kg)	LVmax	291.58	291.58

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

With the addition of the three brain compartments to the PBPK model it is necessary to research the physiological and biochemical parameters for the brain as well. Unfortunately, the available research literature has not focused on the transport or metabolic mechanisms for the brain. The dashed boxes in Table 3 below are the values that are not known and have not been found in the literature. These unknown parameters will either be defined numerically or graphically.

Table 3. Brain Physiological and Biochemical Parameters (TCE)

	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	VBB	-	-
Blood Brain Barrier	VBBB	-	-
Brain Tissue	VBT	1.4	1.4
Partition Coefficients			
BrainBlood/Blood	PBB	1	1
Brain Tissue/Blood	PB	7	7
Metabolic Parameters			
Michaelis-Menten Constant (mg/l)	BKm	-	-
Max. Velocity of Metabolism (mg/hr -kg)	BVMax	-	-
Transport Parameters			
Passive Diffusions			
Transfer Rate	TR	-	-
Mediated Transports			
Maximum Transport	Max Transport	-	-
Maximum Transport2	Max Transport2	-	-
Transport Constant	Transport Constant	-	-
Transport Constant2	Transport Constant2	-	-

- Physiological Parameter Values for PBPK Models (1994)

The maximum transport values, and the brain Vmax will be defined based on a graphic relationship with exercise. The literature supports the theory that as exercise intensity

increases the permeability of the brain increases, and this relationship will be explored with these three parameters in the model.

Conclusions can be drawn about these parameters and any role they may play in chemical exposure through simulation and testing of the PBPK model developed in this study. The development and testing strategy of the model will be discussed in the following chapter. The testing will help gain confidence in a range of values for the brain compartments listed above in Table 3.

Scaling

The development of allometric relationships in a PBPK model can also be an important step in the testing of the model. The use of mathematical equations allows the modeler to scale the model parameters to a specific bodyweight. The recommended reference human body weight of 70 kg for males and 58 kg for females can be used initially in the model, but it is also nice to have simulated the model over a range of bodyweights. This helps the modeler gain confidence in the behavior of chemical concentrations in various tissue groups. The scaling factors for tissue group volumes, ventilation, cardiac output, and liver metabolic parameters that will be used in this study are listed below in Table 4 (Allen and Fisher, 1993:72). The brain tissue will be the only scaled parameter in the brain compartment, since scaling factors for the brain blood and blood-brain barrier were not available. The impact of exercise on the alveolar ventilation and cardiac output must be accounted for in this study since the major component of interest is in fact the effects of stress. A more thorough discussion on the scaling of the ventilation and cardiac output parameters is below in the "exercise" sub-heading.

Table 4. Scaling Parameters

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

a. Allen and Fisher (1993)

Exercise

The varying intensities of exercise are important to consider when modeling the effect of exercise on chemical exposure. Exercise may vary from moderate work to extremely strenuous work. In a report compiled by the International Life Sciences Institute the values for ventilation and cardiac output based on their linear relationship with exercise were discussed (Physiological Parameter Values for PBPK Models, 1994:44,79). These exercise values, based on a 100% scale, can be used to define the scaling parameters needed for cardiac output and ventilation. The scaling equation that was listed in Table 4 for both ventilation and cardiac output was used to calculate varying scaling factors (QCc and QPc) based on the standard male bodyweight of 70 kg (Fisher and Allen, 1993:75). For example, the cardiac output and ventilation rate of an individual weighing 90 kg and exercising at a level of 55% can now be calculated using

the scaling factors listed below in Table 5. This value and the determined bodyweight of the individual can then be input into the scaling equations listed above in Table 4.

Table 5. 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

Military personnel were exposed to varied risk factors. The studies performed on chemicals and chemical interactions and the years of stress research support the serious nature of the Gulf War Syndrome.

A large majority of the research hypothesizes that stress and increased permeability of the blood-brain barrier have a large contributing role in the Gulf War Syndrome. This may increase the effects of exposures to DEET, permethrin, pyridostigmine bromide, and organophosphates. Several theories have been discussed in this chapter, but the following three main issues will be the focus of the modeler as the PBPK model is developed:

1) Exposure to multiple chemicals as well as stress has an inhibiting effect on the enzymatic system in the brain. Altered metabolism may allow more chemicals to cross the blood-brain barrier (McCain and others, 1997, and Abou-Donia and others, 1996).
2) Stress increases permeability in the blood-brain barrier (Friedman and others, 1996, Belova and Jonsson, 1982, and Sharma and others, 1991). The increased permeability may be due to changes in the two types of transport across the blood-brain barrier (passive diffusion or mediated transport).
3) Stress also causes changes in cardiac output, alveolar ventilation, and fractional distributions to the tissue groups.

Determining the long-term behavior and understanding the mechanisms of stress and chemical exposure will help fill the major gaps that exist. These impacts can be assessed with the use of a PBPK model. A detailed methodology is provided in the next section.

III. Methodology

This section will define the concepts and elements of the system dynamics approach that will be used to develop a PBPK model. The proper modeling approach will first be explored. The four stages of a system dynamic model development will then be addressed. These stages are conceptualization, formulation, testing, and implementation (Randers, 1980:285). It must be remembered that the system dynamics process is iterative in nature (Shelley, 1999).

Model Approach

There are several different modeling approaches used to explore various system behaviors. Each of these modeling approaches is based on different paradigms on how to define, develop and interpret a problem (Meadows, 1980:24). The selection of a modeling approach will depend on the particular requirements of a model.

In this thesis a kinetic model will be developed to address the feasibility of generating a hypothesis of physiological changes during chemical exposure and stressful conditions. Since this modeling effort is one of the first attempts at modeling the combinations of chemical exposure and stress, it will be important to keep the focus fairly simple. The questions that need to be addressed would include the following: Does the addition of stress increase the chemical exposure to tissue groups in the body? Does stress play a role in the effects experienced after chemical exposures?

The dynamic nature of modeling chemical exposure in the human body with the addition of a stressor will be a mechanistic process. Through the literature review, it has

been shown that a chain of events occur during a stressful condition. This includes the specific mechanisms of the endocrine and nervous systems. An aggregate level of the endocrine and nervous system interactions will be portrayed to find the representative behavior during exposure to chemicals and stress.

Keeping the above requirements of this model in mind, the system dynamics approach will help provide the most insight into this thesis effort. This is based on several reasons. First, this model will provide a general understanding of a real-world system based on the fact that the model addresses a relatively new question in regard to human exposures. Every element and relationship in the model will be based on an identifiable real-world counterpart, thus leading to a better understanding of the system (Meadows, 1980:34). Second, the development of this PBPK model will be very process oriented, instead of product oriented (Meadows, 1980:28). Insights into the system will be gained through an iterative and modular development of the model. And third, the dynamic behavior of the model will arise from a causal structure within the system (Meadows, 1980:31). The internal structure of this PBPK model will be of value in understanding the overall behavior of the system.

Conceptualization

Conceptualization is the first stage in the system dynamics modeling process. This entails becoming familiar with the problem, defining the questions to be addressed, developing a reference mode, and describing the basic mechanisms in a causal diagram (Shelley, 1999). The questions that will be addressed have already been presented in the

research objectives in Chapter I. The remaining parts of the conceptualization stage will be discussed below.

Reference Mode

One of the initial steps in the modeling process is to conceptualize a mental view of the system forces that drive chemical exposure in the body. This mental view aids in the development of a reference mode. Through the literature review it was decided that there would be five main tissue groups in this system: fat, slowly perfused, richly perfused, liver, and brain. These key compartments or accumulation points in the model are associated with the following parameters: air concentration, ventilation, cardiac output, arterial concentrations, venous concentrations, partitioning coefficients, metabolism, and finally the fractional distributions to each tissue group.

A plot, or reference mode, representing the time development of the behavior of tissue concentrations is important in providing a focus for the development of the flow diagram. Chemical concentration in tissue (with no stress input) over time will rapidly increase and then level off as the concentrations reach a steady state. As displayed in Figure 5, it is hypothesized that stress will cause a more drastic increase in chemical concentrations in the different tissue groups.

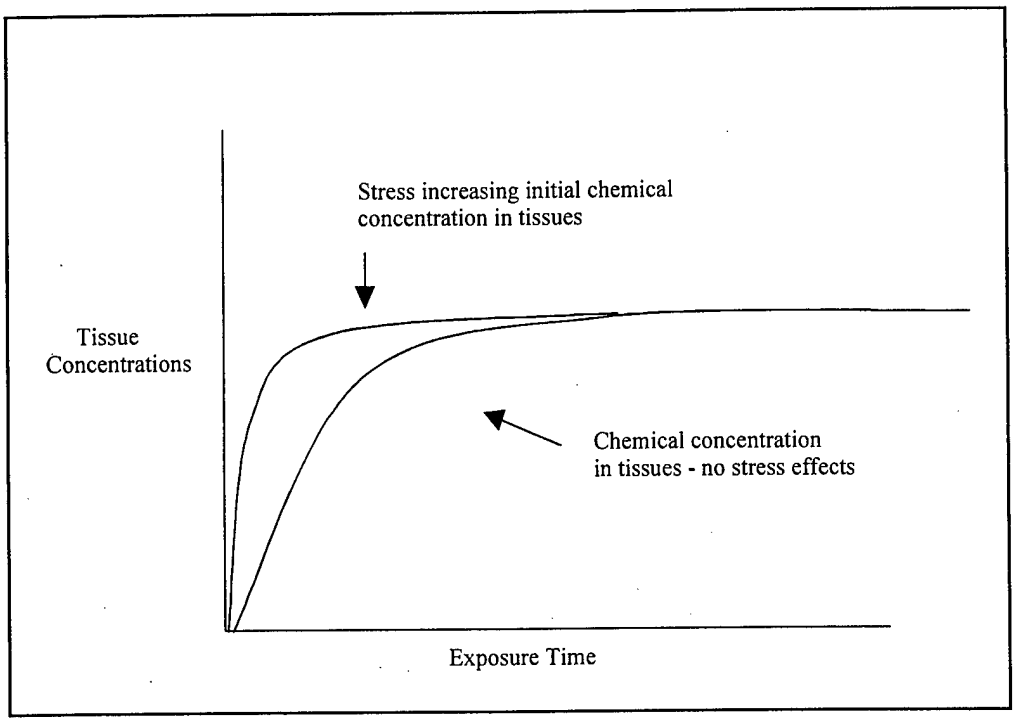


Figure 5. Reference Mode

Influence Diagram

The structure of any system dynamics problem is the interwoven chain of cause and effect loops necessary to explain the system behavior. It is very important to ensure that the loops within this influence diagram give rise to the behavior in the reference mode. The reference mode should always guide the problem or behavior development in the system dynamics process. Additional terms in the influence diagram will only be added to express the logic of the reference mode.

Figure 6 is a simple influence diagram representing the cause-and-effect relationships between arterial and venous concentrations and the chemical concentration in a tissue.

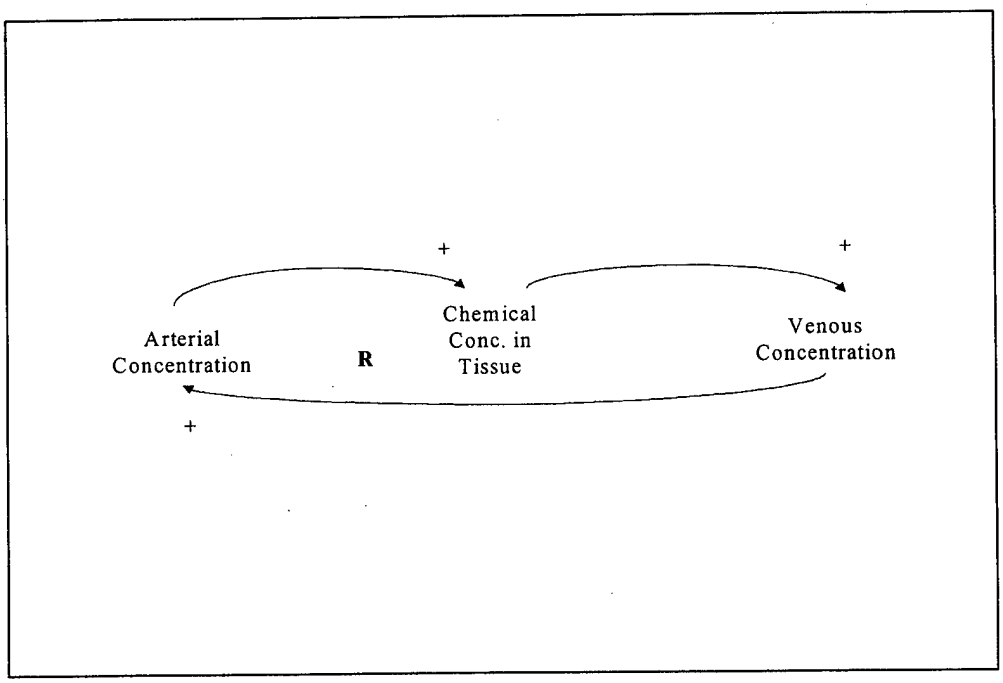


Figure 6. Simple Influence Diagram

Each arrow represents the cause and effect relationships between each affected parameter. The plus and minus signs symbolize either a positive or negative influence. These positive or negative influences define whether the given feedback loops are either reinforcing or compensating. A reinforcing loop (R), as seen in Figure 6, gives rise to an expanding or unstable behavior. So, the chemical concentration in the tissue in Figure 6 will keep increasing. A compensating loop (C) gives rise to a dampened or stable or goal-seeking behavior. The combinations of these loops can help explain the behavior seen in Figure 5. A compensating loop must be present to account for the leveling off of the tissue concentration to a steady state. It is also important to note that the closed loops in an influence diagram are the key to establishing the system boundaries.

Formulation

Formulation is the process of transforming the conceptual model to a real operating system representation (Shelley, 1999). The influence diagram will be mathematically coded into a modeling program, which uses numerical integration. The system dynamics modeling software used for this research is STELLA Research 5.0, by High Performance Systems. The flow diagram below in Figure 7 contains all relationships represented in the influence diagram, Figure 6. This diagram adds to the cause and effect loops by explicitly identifying accumulating stocks, flow rates, concentrations, and other parameters identified in the initial steps of the modeling process. The flow diagram should be consistent with the level of detail represented in the influence diagram.

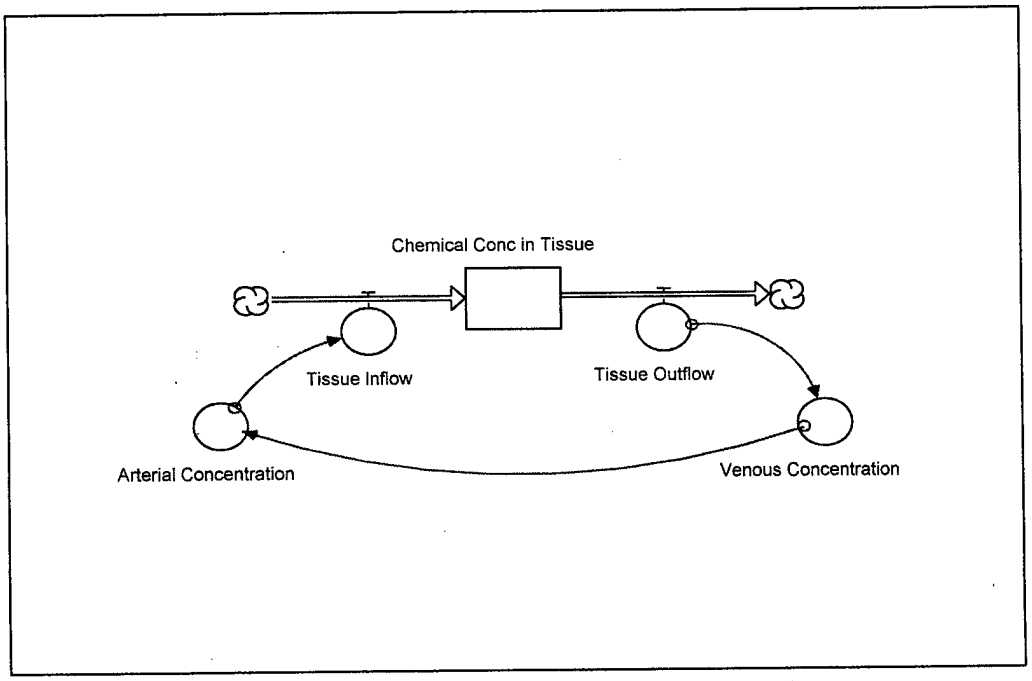


Figure 7. Simple Flow Diagram

At this stage in the system dynamics process it may also be helpful to list the steps that need to be taken from the initial flow diagram to the proposed final flow diagram. The following sequence is an overview of the modular approach that will be conducted towards completing this PBPK model.

1. Based on Ramsey and Andersen's PBPK model, a set of tissue groups will be developed in the model. For this scenario it will be important to include a brain compartment as discussed in Chapter 2. The model below in Figure 8 is the PBPK model that will be used in this study. To realistically represent the transport and metabolic mechanisms that occur, as shown in Figure 8, the brain tissue must be divided into the three sub-compartments.

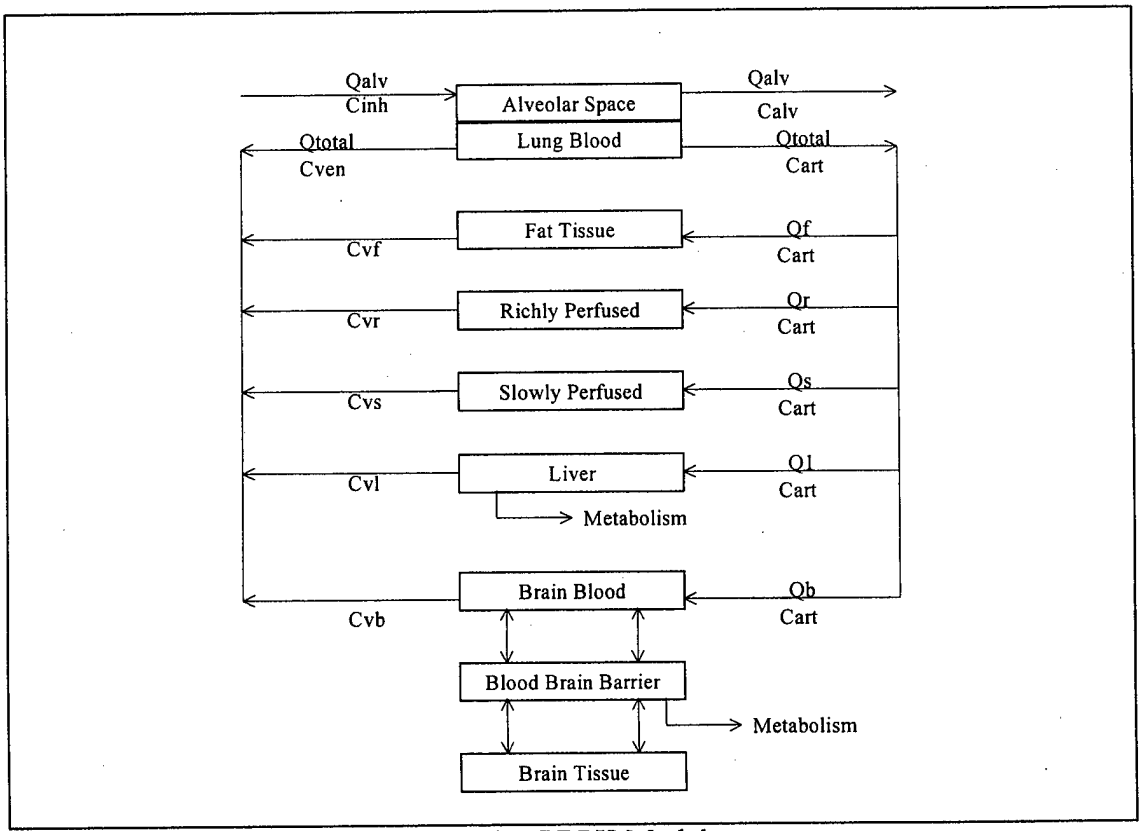


Figure 8. PBPK Model

2. Due to the large amount of PBPK modeling that has been conducted on TCE exposure, the model will be initially developed for solvent exposure under normal (non-stressful) conditions. This exposure scenario is important because past modeling literature is available that can be used for validation.
3. Clearly define flow parameters from the literature (i.e. – air concentration, ventilation, cardiac output, arterial concentrations, venous concentrations, partitioning coefficients, metabolism, and fractional distributions)
4. Comparing the behavior to actual exposure data will validate the model under normal conditions.
5. Once the model has been validated different parameters will be used to describe the exercise scenario. Using different cases of a stressed individual will aid in determining if stress does have a potential impact on the distribution of chemicals in the body.
6. Portions of the stress response will be sequentially added to the model and simulated. An initial example of this might be to address physical activity and how physical activity affects the behavior of the system under chemical exposure. The data presented in Chapter 2 will be used to simulate behavior.
7. The end result of this thesis effort will be a developed model that will be a theoretical prediction of behavior after stress exposure. Due to the fact that the previous models used to build up to this point have been validated there will be a higher level of confidence in the resulting behavior.
8. The simulation of these model scenarios and the manipulation of the PBPK model will aid in drawing conclusions between the effects of stress on chemical exposures,

specifically how chemical concentrations change in the brain tissues. Conclusions would also like to be drawn on the brain biochemical parameters.

Testing

The concept of model testing refers to the comparison of a system dynamics model to empirical reality for the purpose of either corroborating or refuting the model (Forrester and Senge, 1980:210). Model testing is conducted to determine if the model is an accurate representation of the system under study. Validation is the process of establishing confidence in the soundness and usefulness of the model.

Once the flow diagram is completed and the parameters are defined, the simulations can be run. Use of second opinion to avoid personal bias and a step by step check for expected behavior at each stage should be conducted during the simulation to insure verification. The testing should include further exploration of the behavior under a stress influence to watch the general trends in concentration levels in the tissues. Once an understanding of the general behavior is explored the process of building confidence in the model must be conducted. There are several tests that can be used to build confidence in a system dynamics model. The following validation tests will be used on this PBPK model: structure-verification test, behavior-reproduction test, parameter-verification test, extreme-conditions test, and behavior-anomaly. The first three validation tests check the model structure, while the latter two test the model behavior (Forrester and Senge, 1980:209).

Structure-Verification Test

Structure-verification compares the model structure directly with the structure of the real system. The structure of the model can be verified with the use of available literature, and it is important during this time to ensure that there are no contradictions between the model built and the real system. The system is a simple representation of the tissues in the human body, so this must be considered when comparing the structure to a real world, but the overall structure of the model should represent what would be expected in the real human body. Review of the model structure by experts and advisors may also be appropriate.

Behavior-Reproduction Test

The behavior-reproduction test will be applied throughout the simulation process in making sure that the behavior generated is arising as a consequence of the model structure. This test is used to examine how well the generated behavior matches the observed behavior of the real system (Forrester and Senge, 1980:218). For this model, it will be important to compare the generated behaviors to the reference mode developed during the conceptualization stage.

Parameter-Verification Test

Parameters in the model will be compared to the real-world system. The parameters must correspond either conceptually or numerically to the parameters in the real system. Conceptual verification means that the parameters match the elements of the system structure, while numerical verification matches model parameters to the appropriate real system parameters appropriately (Forrester and Senge, 1980:213). Due to the limited amount of research that has been performed on stress and chemical

exposure, it may be suitable to consult the available literature or experts for the plausibility of the parameter value ranges.

Extreme-Conditions Test

Extreme-conditions testing procedures examine the maximum and minimum conditions. The use of maximum and minimum conditions will be simulated for several parameters while the output behavior is monitored. If model behavior is unreasonable under extreme conditions, it indicates possible errors in the model logic and structure. For the PBPK model developed in this study, the steady state values of chemical concentrations in the tissues will be an important indicator for the extreme-conditions test. These steady state values can be determined by multiplying the air concentration, blood-air partition coefficient, and tissue-blood partition coefficients. Each simulation should match up with these steady state values for each tissue concentration even when the model is analyzed under conditions that may not be normally experienced. Testing with extreme values of exercise, bodyweight, and other physiological or biochemical parameters and confirmation of the steady state values will aid in building confidence during the extreme-conditions test.

Behavior-Anomaly Test

Behavior anomaly can be used through the initial development of the simulations in tracing anomalous behavior back to structural causes. These causes often reveal flaws in the model structure or model parameters, or in the case of this initial model an anomalous behavior may suggest a real system behavior that has not been observed yet.

Implementation

Implementation is the process of translating the information that was gained through this system dynamics process to a concept that is easily understood and useable by others (Randers, 1980:121). This basic PBPK may be an on-going tool to study the behavior of chemical exposure and stress during deployment situations.

In summary, the methods discussed in this chapter provide an organized process of building and testing a PBPK model that simulates stress and chemical exposure. It must be remembered that the model developed is a simple, but accurate representation of the human body and how the human body changes during stressful situations, such as in exercise. Insights will be gained, which will help future model revisions to more accurately depict the system behavior.

IV. Results and Analysis

Following the methods presented in Chapter 3 the PBPK model was developed and tested. This chapter presents the results and insights gained through the conceptualization, formulation, and testing stages of the system dynamics approach. The system dynamics modeling approach allowed the modeler to gain insights throughout the three stages, not just the testing stage, and these insights into stress and chemical exposure will be discussed in the sections below.

Conceptualization

As described in Chapter 3, the conceptualization stage allows the modeler to become familiar with the problem, define questions to be asked, and develop a reference mode and influence diagram. In the literature review for this thesis, information was found on the mechanisms of stress and possible theories behind the Gulf War Syndrome. The following reference mode and influence diagrams of the mechanisms and feedback loops together form the dynamic hypothesis of this study.

Reference Mode

The information found was used to initially conceptualize how chemical concentrations in the human body would behave during exposure to stress through the use of a reference mode. It has been known and studied for quite some time how a chemical concentration will behave in any particular tissue. Typically, the concentration will rapidly increase and then level off as the concentration reaches steady state over time. As already discussed and presented in Chapter 3 it is hypothesized that stress will

cause the chemical concentrations in tissue to drastically increase and then eventually level off at the same steady state value. It is important to remember at this point that the addition of parameters that remove chemical concentrations from the body, such as metabolism, will slightly alter the initial reference mode behavior. With the addition of metabolism, chemical concentrations in a tissue during stress will not level off at the same steady state value as the chemical concentration at rest, but the same basic behavior will occur. Stress will cause a more drastic increase in chemical concentrations before reaching a steady state. This steady state value can be calculated as the product of the air concentration, the blood-air partition coefficient, and the tissue-blood partition coefficient. Figure 9, the same reference mode as presented in Chapter 3 is the proposed reference mode for the PBPK model developed in this thesis effort. This reference mode represents the time development of the behavior of tissue concentrations and is important in providing a focus for the development of the influence diagram and flow diagram.

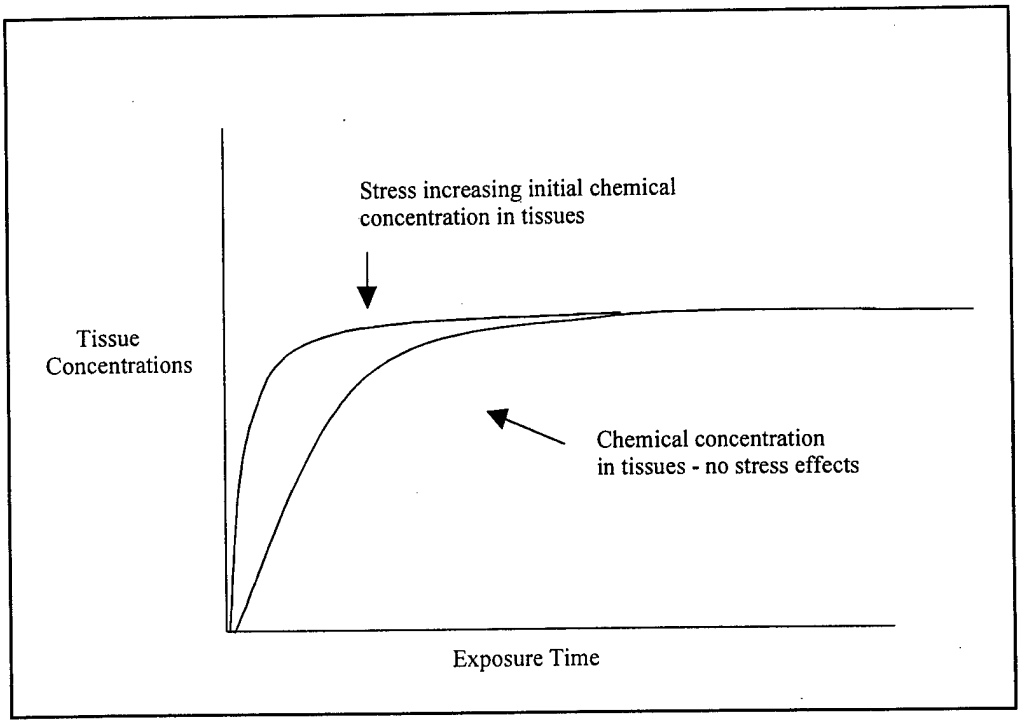


Figure 9. Reference Mode

Influence Diagram

The behavior of the chemical concentration over time in the above reference mode should be explained by an interwoven chain of cause and effect loops, or an influence diagram, as explained in Chapter 3. The addition of stress to the influence diagram should affect the initial uptake of chemical in the tissue groups. This structure is used to explore the system behavior and refine the structure as appropriate.

The basic causal mechanisms and feedback loops of the five tissue compartments with the addition of stress would make a very complex influence diagram. A portion of the dynamic system, to include the fat and slowly perfused tissue compartments, are displayed in the influence diagram below in Figure 10.

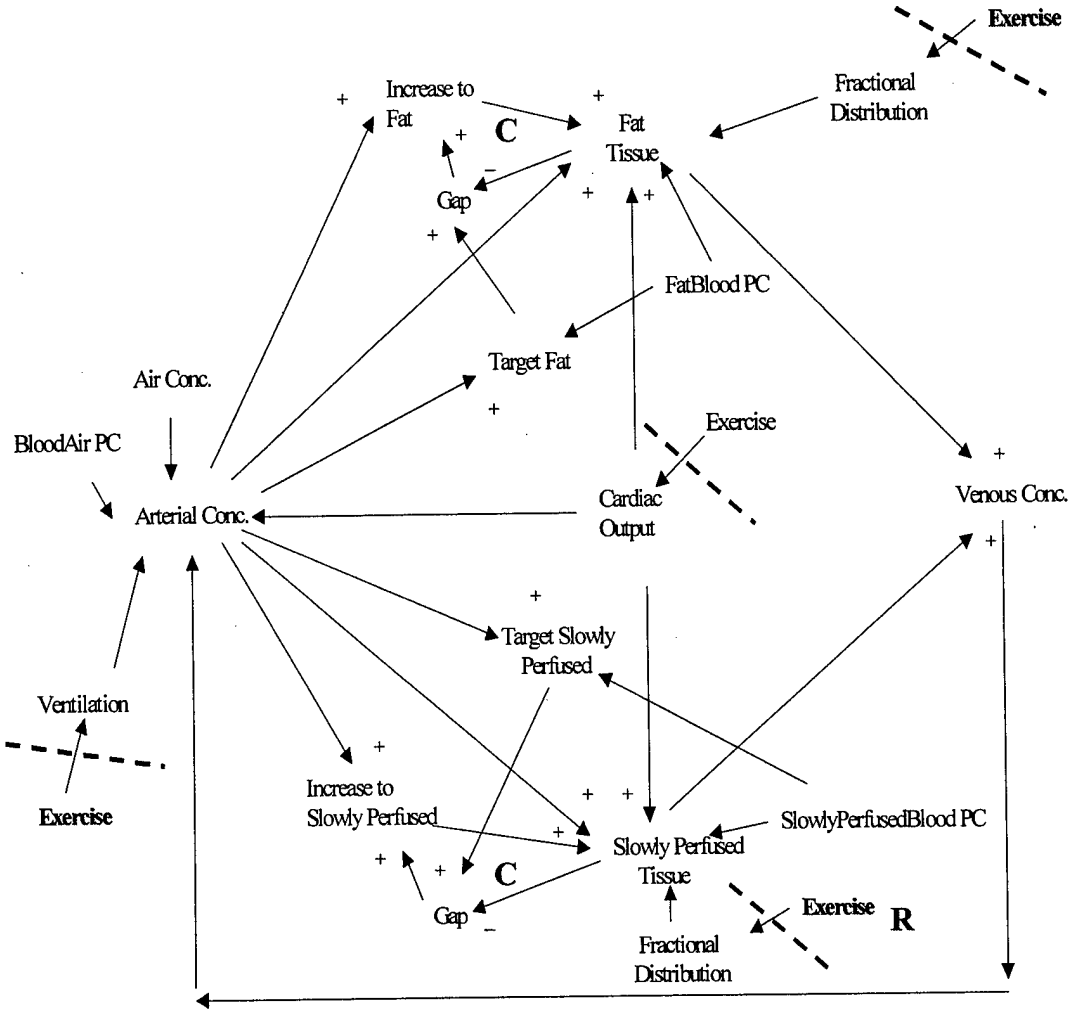


Figure 10. Influence Diagram of Fat and Slowly Perfused Tissues

The example influence diagram shown in Figure 6 of Chapter 3 is a reinforcing loop, where the chemical concentration will continue to increase in the tissue group. To properly represent the behavior seen in the reference mode a few concepts were added to the influence diagram in Figure 10. A goal-seeking behavior was added that acts upon the reinforcing loop. This results in a compensating loop that keeps the concentrations in the tissues under control. A good way to explain this is that a gap initially exists between

the tissue chemical concentration and the steady state value. As the concentration approaches the steady state, the gap value becomes smaller until steady state is attained. At this point, if chemical exposure continues, the arterial inflow of the chemical to the tissues will equal the venous outflow of the chemical. Exercise is also added to this scenario, which will change the cardiac output, ventilation rate and fractional distributions. These changes caused by exercise result in an increase in blood flow to the tissue groups, which means an increase in chemical being carried to the tissues.

An influence diagram of the brain compartment can also be found below in Figure 11. The brain compartment was split into three different sub-compartments: the brain blood, the blood-brain barrier, and the brain tissue. The same goal-seeking behavior that was described above also acts upon the brain tissue to control the unstable loop formed by the flow between the arterial concentration, brain blood compartment, and venous concentration.

Based upon the literature review, three more exercise influences have been added to the brain compartment. Exercise will decrease the brain metabolism at the blood-brain barrier by increasing the production of binding compounds. This increase in binding compounds results in a decrease in binding sites, which then results in a decreased brain Vmax value and brain metabolism. The decrease in metabolism will result in an increase of chemical past the blood-brain barrier. This behavior will continue, which results in a reinforcing loop. Exercise will also influence the mediated transport processes on either side of the blood-brain barrier. Exercise will decrease the maximum transport values. This will result in a decrease in mediated transport, which will then allow more chemical to pass through the blood-brain barrier. This is also an unstable or reinforcing behavior.

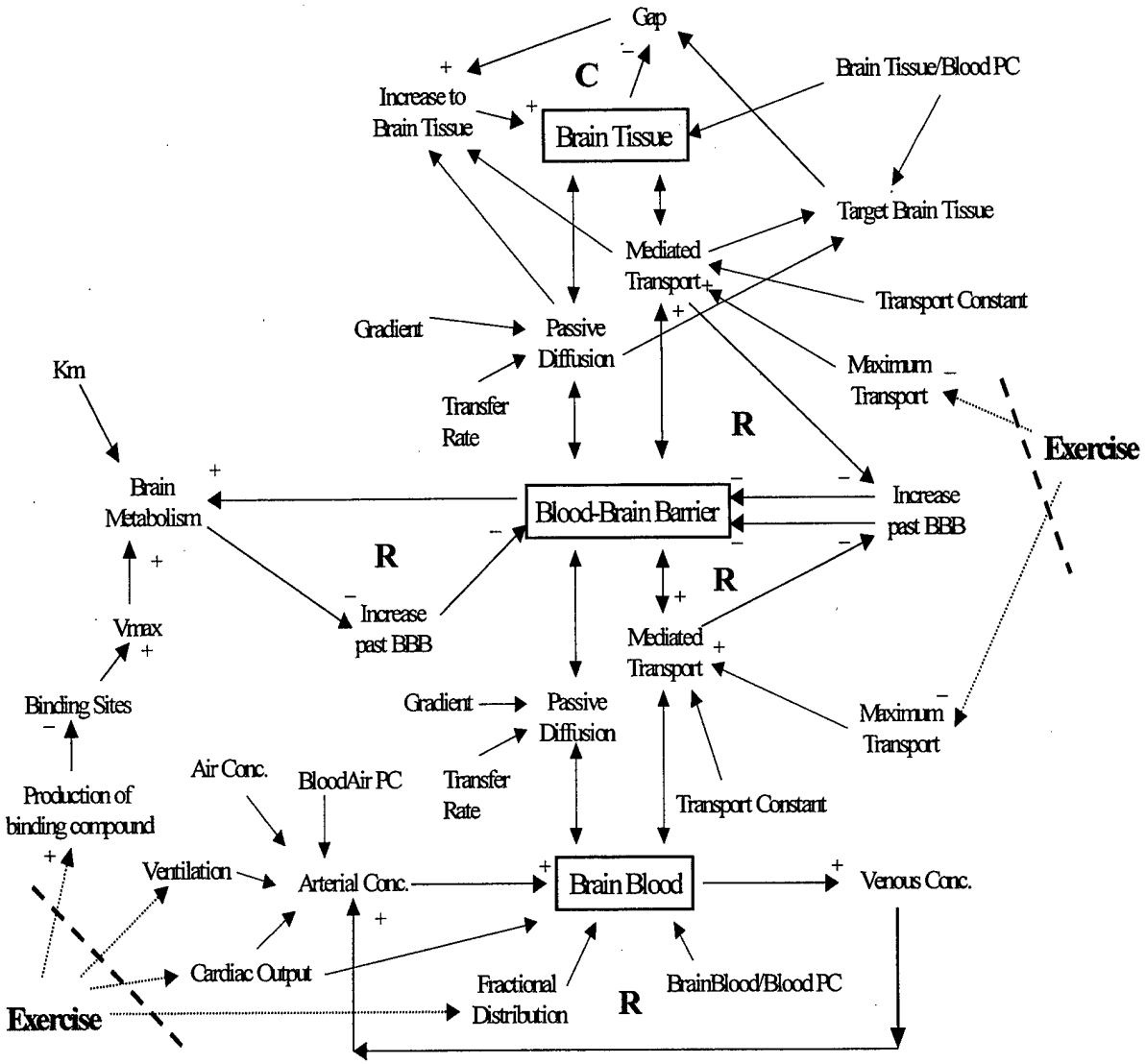


Figure 11. Influence Diagram of the Brain Compartment

Formulation

The influence diagrams were used to input the behavior and mechanisms of the system into the modeling program, STELLA. The resulting flow diagram should contain

all relationships discussed in the influence diagrams. The flow diagram will also add to the cause and effect relationships by explicitly identifying accumulating stocks, flow rates, concentrations, and other physiological and biochemical parameters identified in the initial steps of this modeling process. The flow diagram should be consistent with the level of detail shown in the influence diagrams.

A modular approach was used in the formulation of the PBPK model. The steps, as outlined in the methodology, built upon each other until the final product in STELLA matched the PBPK model that was modified in the literature review to include the three sub-compartments in the brain. The same figure is repeated here in Figure 12 for convenience to the reader.

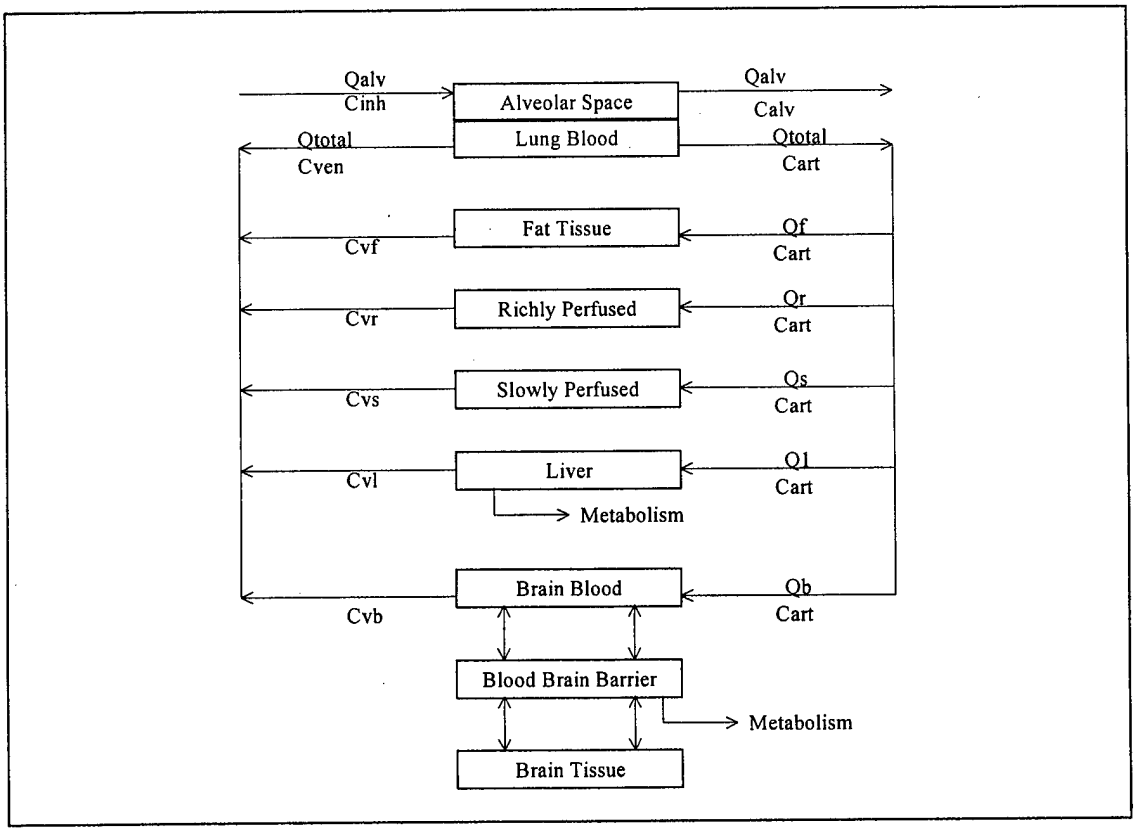


Figure 12. PBPK Model

The initial step taken in the formulation stage was to duplicate past work on PBPK modeling and exposure to trichloroethylene (TCE). Allen and Fisher developed a model for the solvent exposure under non-stressful conditions in 1993. The model contained the four tissue groups, as developed by Ramsey and Andersen, and explored the tissue concentrations of TCE and its metabolite, trichloroacetic acid (TCA) (Allen and Fisher, 1993:72). The duplication was performed in the STELLA modeling software and confidence was gained in the basic structure of the model developed. The model was simulated with the same conditions that Allen and Fisher used in their model. An air concentration of 140 ppm of TCE for an exposure time of 4 hours was used. Figure 13 contains the resulting behavior for the venous blood concentration. Confidence was gained when the behavior in Figure 13 was compared to the venous blood concentrations presented by Allen and Fisher's model (1193:78). Data from the Allen and Fisher's model is overlaid on the simulation performed and marked as "+" in Figure 13.

The four tissue concentrations were never presented in the work done by Allen and Fisher, but an important objective with this particular thesis effort was to produce the behaviors of the various tissue concentrations under exercise conditions. So, it was important to establish the four tissue concentrations in this initial model before adding the additional brain compartment and exercise. The confidence gained from duplicating the venous blood concentrations allowed the modeler to feel comfortable with simulating the four tissue groups under the same conditions that were used to produce the output behavior in Figure 13. Figure 14 contains a graph of the behavior of chemical concentrations for the fat, liver, richly perfused, and slowly perfused tissue groups.

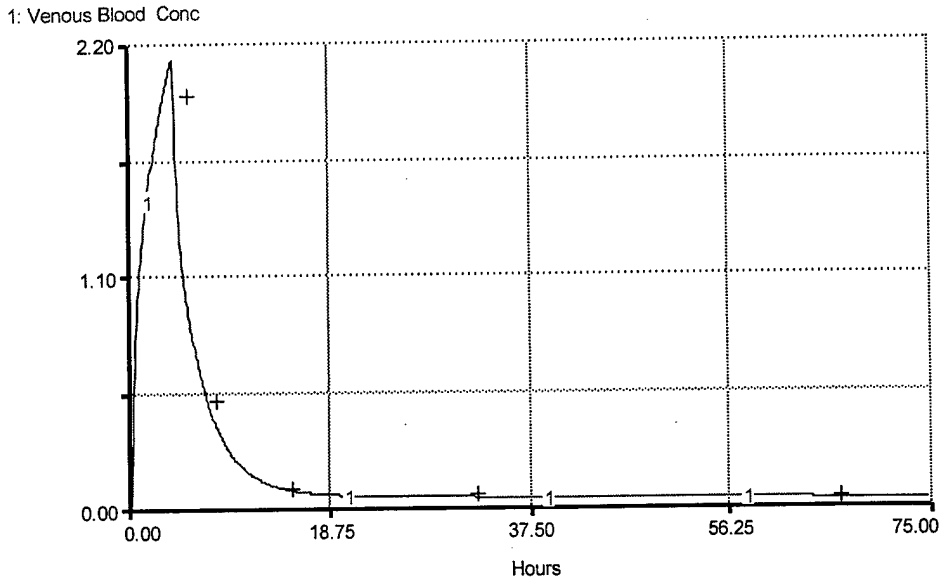


Figure 13. Venous Concentration after a 4 hr. exposure to 140 ppm of TCE

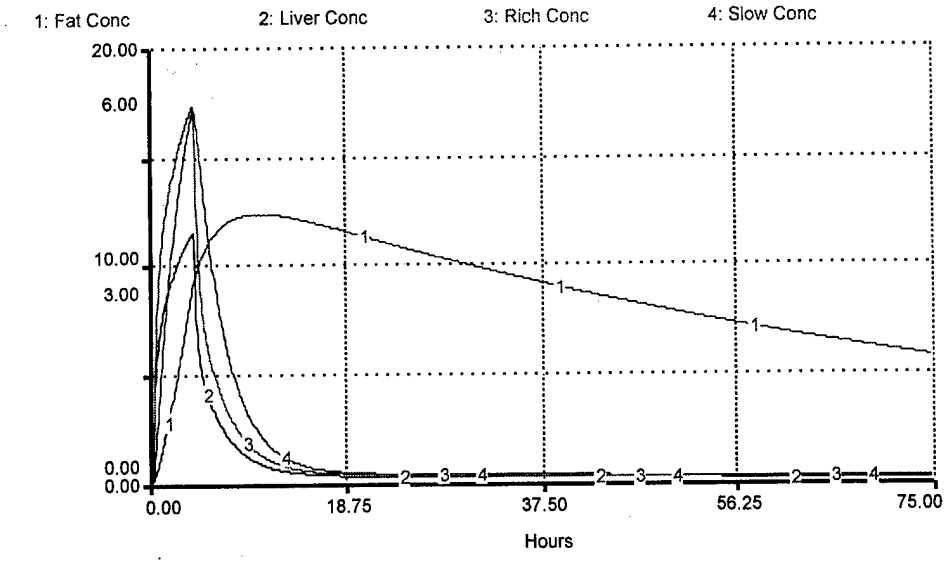


Figure 14. Tissue Group Concentrations after a 4 hr. exposure to 140 ppm of TCE

The second step taken was to modify the model with the addition of exercise. The exercise impacted the alveolar ventilation rates, cardiac output, and fractional distributions to the four tissue groups. The model was then run to insure a reasonable response in the expected behavior when stress was added to a chemical exposure scenario. The resulting output of chemical concentrations under resting conditions and exercise conditions was similar to the behavior predicted in the reference mode, Figure 9, lending credence to the model algorithm as a reasonable representation of the physiological system. Below, Figure 15 is the result of the fat concentration simulated at rest (line #1) and then simulated under exercise conditions (line #2).

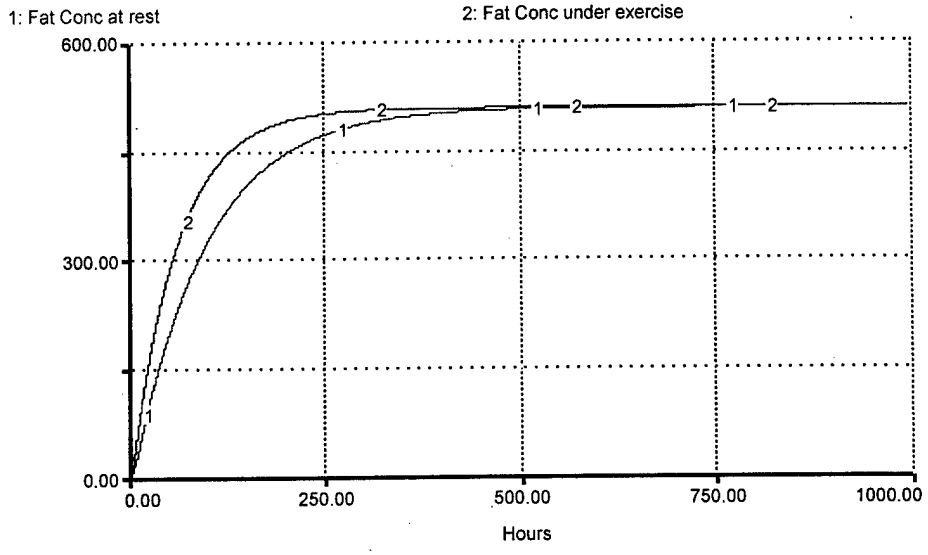


Figure 15. Fat Concentration in Initial PBPK model (1 – Rest, 2 – Exercise)

The next step taken was to add the brain tissue compartment and finally represent the model structure developed in Chapter 3. To accurately represent the transport

mechanisms across the lumen and ablumen membranes of the blood-brain barrier and the metabolic activity that takes place, the brain compartment was divided into three accumulating compartments. Difficulties with the software program arose at this point. The volume of the brain blood sub-compartment had such a small value that numerical integration problems developed. This resulted in nothing being transported to the brain tissue compartment. It was important to get around this software limitation and see the overall behavior of the chemical in the brain compartments. The volume of the brain blood sub-compartment was increased to a large enough value that behaviors produced could be studied. This unrealistically large volume for the brain blood sub-compartment is still relatively small compared to other tissues, and the inaccurate accumulation of chemical in the blood is not considered significant in characterizing system behavior.

Several assumptions were also made with the addition of the brain sub-compartments. As discussed in the literature review, minimal information and data was found on the biochemical and physiological parameters of the brain. These parameters were assigned realistic, but arbitrary values to enable simulation. It is hoped that conclusions can be drawn that will help narrow the range of the values assigned. Another large assumption pertained to the brain blood sub-compartment. It was assumed that this was a homogeneous and well-mixed compartment that represented the average of the arterial and venous concentrations over time. The blood-brain barrier was also assumed to be in the aqueous phase as intracellular fluid. This allowed the partition coefficient between the brain blood and blood-brain barrier sub-compartments to be defined as 1, an exchange between two similar phases.

The final steps in the formulation stage included the addition of exercise influences on the metabolism and transport processes in the brain and the addition of the scaling parameters so that the PBPK model could be simulated for any bodyweight. Based on the information in the literature review a linear relationship was established between exercise and the brain metabolism, passive diffusions, and mediated transports. As exercise increased each of these values would decrease in value. These relationships are supported by the several studies discussed in Chapter 2, which point to an increase in permeability or a decrease in metabolism during exercise (McCain, 1997; Abou-Donia, 1996; Friedman, 1996; Belova and Jonsson, 1982; and Sharma, 1991). The information found during the literature review was also used to scale the alveolar ventilation and cardiac outputs to a specific and exercise level. The tissue volumes and liver metabolic activity was also scaled to bodyweight. 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.

The modular approach used in this formulation stage allowed the modeler to gain confidence in the modeling process. The focus of this formulation was to represent the behavior presented in the reference mode and described through the influence diagrams. Portions of the stress or exercise response were sequentially added to the model, which also helped the modeler gain confidence as well as understand the specific mechanisms behind the influence of exercise on chemical concentrations in the five tissue groups. It was also important to keep the research goals, which were identified in the introduction of this thesis, in mind as the formulation stage was taking place.

Testing

After the development of the PBPK model, numerous simulations were conducted in order to gain an understanding of the behavior of chemical concentrations during exercise conditions. Several issues were kept in mind during the testing stage. First, the levels of exercise and the impact this had on the behavior of chemical concentration in the body were very important. Second, testing different bodyweights was important to ensure the model was performing as intended for any particular individual. And finally, the metabolic activity in the blood-brain barrier and the transport parameters were of high interest due to the possible impacts of increased exposure to the central nervous system, as well as the fact that this area of research is just beginning. Focusing on these issues and the five validation tests outlined in Chapter 3 will help increase the confidence in the resulting behaviors of the PBPK model.

The initial verification of the model compared the chemical concentrations in the seven compartments for resting conditions and heavy exercise conditions (25%). The exposure scenario for this test was an exposure of 140 ppm of TCE for 4 hours. This exposure scenario will be used for the remainder of the testing phase of this thesis. However, instead of the concentrations leveling off at a steady state they should drop back down after the 4 hours of exposure. Figure 16 represents the fat tissue compartment. The 1st line is the fat concentration of TCE at rest and the 2nd line is the fat concentration at heavy exercise.

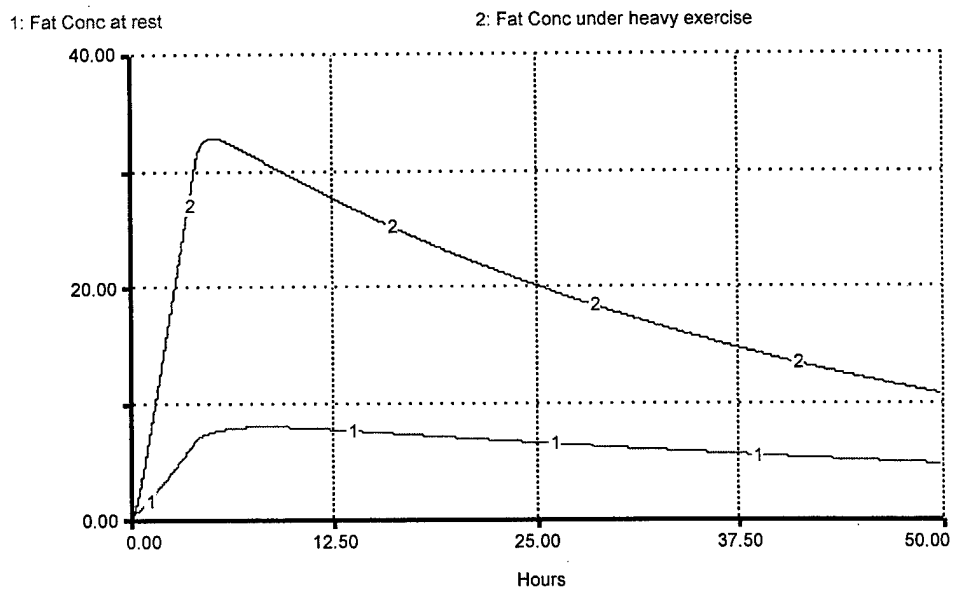


Figure 16. Fat Concentration (1-Rest, 2-Heavy Exercise)

Figure 16 does reflect the reference mode that was predicted in Figure 9. The time was extended to 50 hours to see the general behavior over time. The concentrations will eventually reach a steady state level of tissue concentrations, or as is the case above go back down to zero concentration. A definite difference between rest and heavy exercise conditions are noted during this simulation. Appendix C contains the remaining six compartments (Figures 35-40) for the same scenario as described above with the fat tissue. The remaining six compartments include: liver tissue, richly perfused tissue, slowly perfused tissue, blood-brain barrier, brain blood, and the brain tissue.

These simulation runs were the first steps taken in the initial verification process to determine if the model is performing as expected. Once the simulations have been run and an understanding of the general behavior is explored, the process of building confidence in the model must be conducted. Validation is the actual process of

determining whether the conceptual model is an accurate representation of the system under study (Shelley, 1999). This is a very important step in establishing the usefulness of the PBPK model. The results of the five validation tests will be analyzed in the following order: structure-verification test, behavior-reproduction test, parameter-verification test, extreme-conditions test, and behavior-anomaly test.

Structure-Verification Test

The structure of the PBPK model must accurately reflect the actual structure of the human body, and contain enough detail to resemble the processes that play a part during a chemical exposure in a real world scenario. Structure-verification can be performed a number of ways: comparison with the reference mode and influence diagrams, verification through the literature, and consultation with advisors. The model structure seen in Appendix A was built based upon each of these methods.

The reference mode and influence diagrams were developed based on literature in the study of chemical concentrations, as well as in the study of exercise and stress. The model was then modularly built upon this literature and the structure of the real system. For the brain compartment, there was not a lot of information available that could be used to characterize several of the parameters in this system dynamics model. The most logical hypotheses were drawn from this information to develop the brain compartment. The model was reviewed and confirmed by advisors at several points during the development of the model. Because the initial output of the model matches the reference mode, the model structure is assumed to be valid for representation of general system behavior at the chosen level of detail.

Behavior-Reproduction Test

This test examines how well the model matches the real system behavior over time. The first round of testing focused on the changes in chemical concentrations in each of the tissues during changing levels of exercise. Each of the seven compartments was simulated for each exercise value assigned in Chapter 2. The compartments were first simulated during resting conditions, and then each additional exercise level was added to the same graph so that observations and comparisons regarding the resulting behaviors would be easier to make. The simulations were first run for 30 hrs at the 140 ppm exposure of TCE for 4 hours. The simulation duration was then decreased to 15 hours so that the behavior of the tissue concentrations would be easier to observe. Figure 17 shows the results of the fat tissue concentrations for this simulation. Again, line #1 corresponds to 0% exercise, line #2 to 15% exercise, line #3 to 25% exercise, line #4 to 55% exercise and finally line #5 corresponds to 100% exercise. The concentrations in Figure 17 follow the behavior that was predicted in the reference mode. As the exercise intensity increases the concentrations in the tissues will initially increase at a faster rate, and the concentrations will peak at higher levels. The results for the remaining six compartments for this same simulation can be found in Appendix C (Figure 41-46). Each compartment had the same basic behavior as above in the fat tissue.

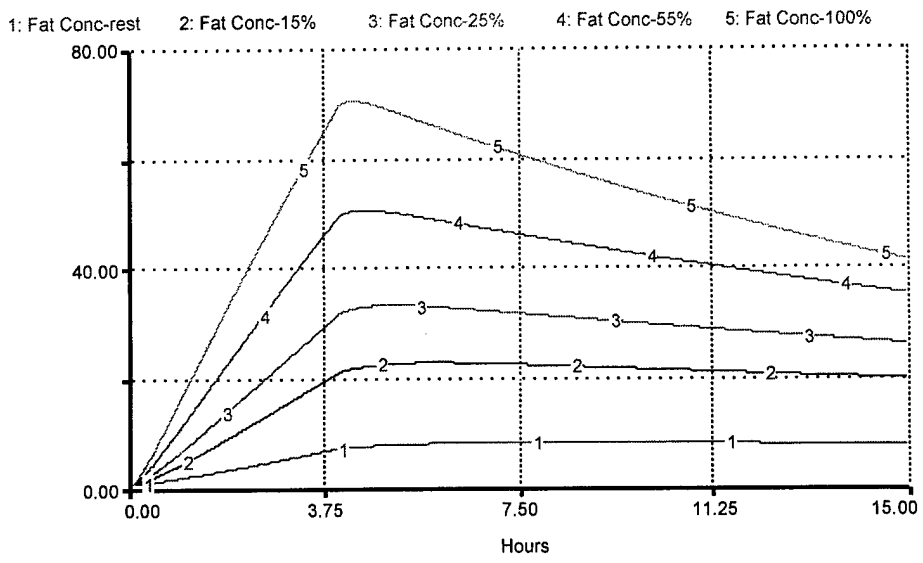


Figure 17. Comparative Graph of the Fat Concentrations for Five Exercise Levels

The second round of behavior-reproduction testing focused on the changes in chemical concentrations in each of the tissues during changing levels of bodyweights. During this portion of the testing an anomalous behavior was found, which will be discussed in the "behavior-anomaly test" section below.

Bodyweight was an important parameter to test to ensure that the tissue concentrations of a 60 kg individual during exercise were not only behaving similar to the reference mode and at slightly greater concentrations when compared to a 110 kg individual. A sensitivity analysis was simulated for each of the tissue compartments with the bodyweight starting at 60 kg and ending at a maximum of 110 kg. The results of the fat tissue concentrations are below in Figure 18 and, as expected, the tissue concentrations will decrease as the bodyweight increases. This relationship remains valid

in the remaining compartments as well, which can be found in Appendix C (Figures 47-52).

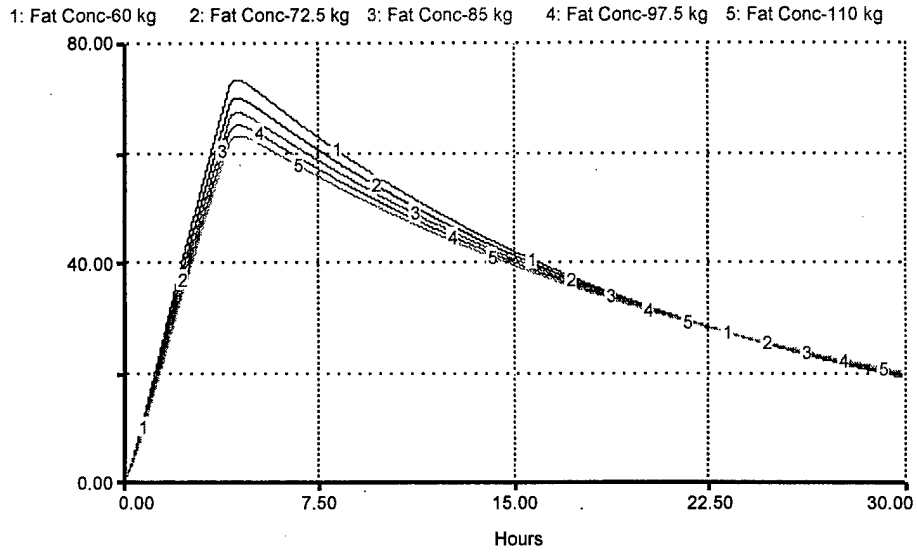


Figure 18. Fat Tissue Concentrations for Increasing Levels of Bodyweight.

At this point in the testing, confidence has been gained in the behaviors of the fat, liver, richly perfused, and slowly perfused tissue groups. The resulting behavior of each of these tissue groups with the addition of exercise and the model structure for these tissue groups is assumed to be correct. The remaining tests will focus on the three brain sub-compartments. There are still a lot of questions that need to be answered as to how the transport processes and the metabolic activity in the brain affect the brain tissue concentrations during exercise.

Parameter-Verification Test

Comparing the model parameters to knowledge of the real system is another test that aids in describing the real world system of interest. The parameters should

correspond conceptually and numerically to the real system (Forrester and Senge, 1980:212). This test was very important in the validation of this PBPK model. Minimum research has focused on stress and chemical exposure, specifically pertaining to the brain tissue. As discussed in Chapter 2, several of the brain parameters were not found in the literature, so determining a plausible range of values for the physiological and biochemical brain parameters will aid in gaining confidence in the model. This parameter-verification testing phase was also used as a behavior-sensitivity test. As each of the parameters was tested, the sensitivity of the model behavior to the changes in parameter values was also observed.

Before the following testing is discussed, it is appropriate at this point to review the roles and relationships of a few of the brain parameters and transport processes. There are two sets of transport processes in the brain compartment. There is both a mediated transport and a passive diffusion between the brain blood and blood-brain barrier, as well as another mediated transport and passive diffusion between the blood-brain barrier and brain tissue. Refer to Appendix A to see these transport processes in the brain compartment. These processes are duplicates of each other. And, since one of the objectives of this thesis effort is to understand the behaviors and interactions of the brain parameters, it is reasonable to focus on one set of transport processes (mediated transport and passive diffusion between the brain blood and blood-brain barrier sub-compartments) during the parameter testing.

The transfer rate defines the rate at which the flow of chemical is transferred between the brain blood and blood-brain barrier by passive diffusion. The transport constant and maximum transport values define the process of mediated transport between

the two compartments. These values are not metabolic parameters, but parameters that act upon the permeability across the blood-brain barrier. The mediated transport can change the permeability in either direction. This process can be described by an analogy of a gate that either closes or opens wider to allow more chemical in. Finally, the brain Km and Vmax parameters define the saturable metabolism process from the blood-brain barrier. Km is defined as the metabolic half-saturation rate or substrate affinity for a chemical within the blood-brain barrier endothelial cells (Allen and Fisher, 1993:73). Vmax defines the maximum rate of enzymatic metabolism that can occur from the blood-brain barrier.

The first round of parameters that were tested included the following: brain/blood partition coefficient, transfer rate, transport constant, and the brain Km parameter. Each of these parameters affected the overall behavior of the brain concentrations as expected. As each of these four parameters were increased, an increase in the blood-brain barrier and brain tissue concentrations occurred. There was no change or a very minimal change in brain blood concentration. This makes sense because the brain blood compartment in this model is functioning as a continuous transfer phase between the blood and blood-brain barrier. An example of the results observed can be found below in Figures 19-21 for the transport constant (TC). The parameter was simulated through a sensitivity analysis where the value was increased from .5 to 3 for the three brain sub-compartments.

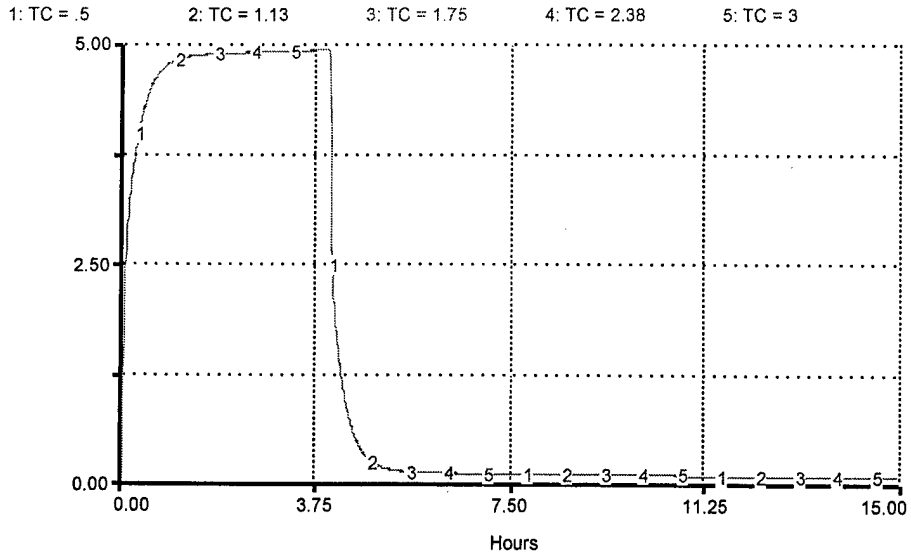


Figure 19. Sensitivity of the Transport Constant's Effects on Brain Blood

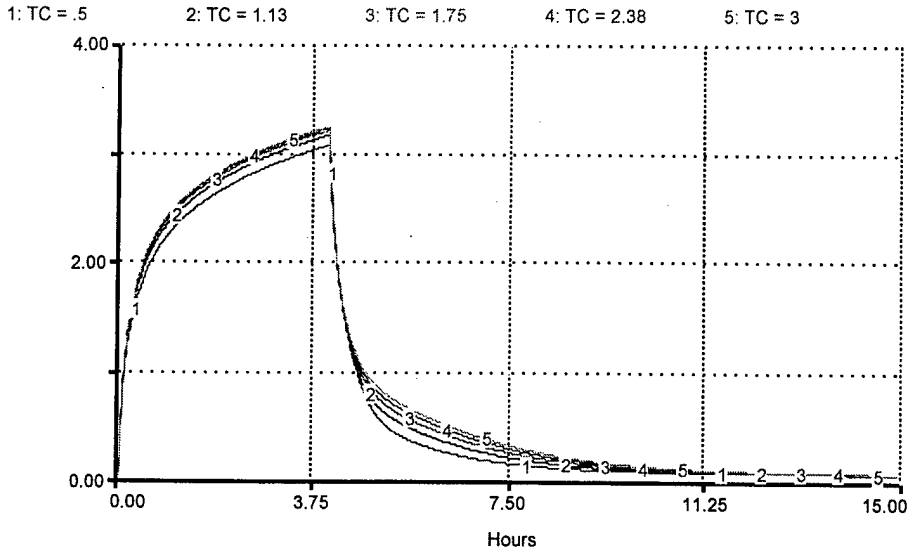


Figure 20. Sensitivity of the Transport Constant's Effects on BBB

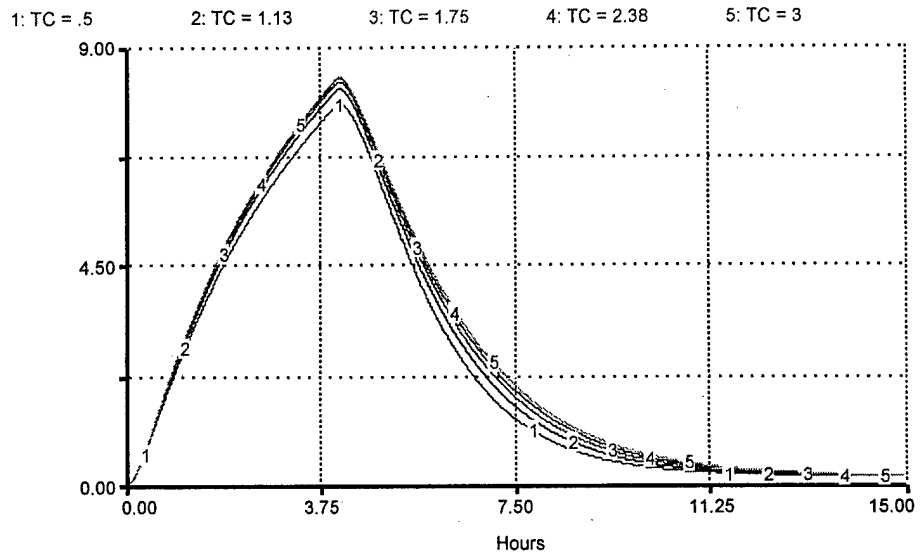


Figure 21. Sensitivity of the Transport Constant's Effects on Brain Tissue

The transport constant also plays a role in the behavior of the mediated transport process between the brain blood and blood-brain barrier sub-compartments. The effects of varying transport constant on the mediated transport between the brain blood and blood-brain barrier compartments can be seen in Figure 22. The behavior of the mediated transport in Figure 22 was expected. As the transport constant was increased the mediated transport would become more positive or less negative. This is due to the bi-directional flow of mediated transport between the brain blood and blood-brain barrier sub-compartments. A less negative mediated transport process correlates to a smaller amount of chemical being transported back to the brain blood from the blood-brain barrier; which results in a an increase in blood-barrier and brain tissue concentrations (Refer back to Figures 20 and 21).

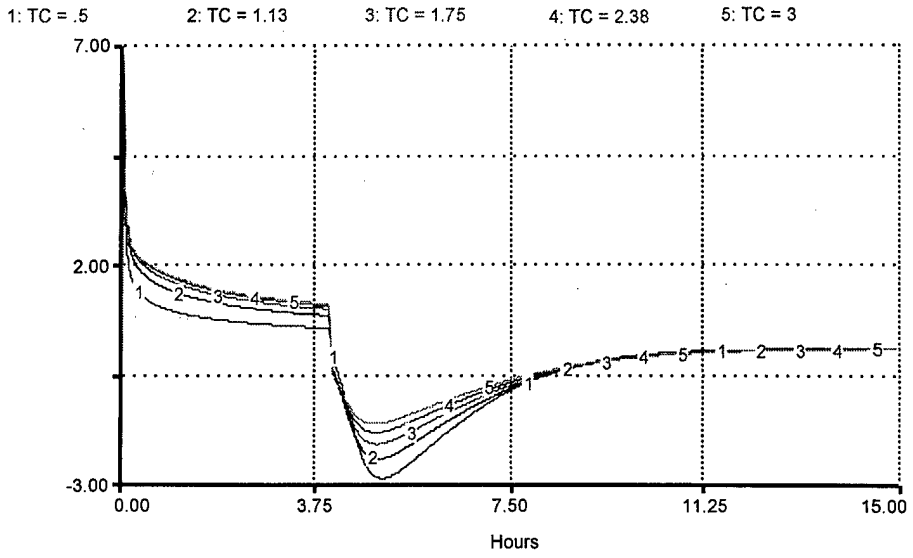


Figure 22. Transport Constant's Effects on Mediated Transport

The results from the remaining simulations, to include the brain/blood partition coefficient, the transfer rate, and the brain Km parameter can be found in Appendix C (Figures 53-61) respectively. These simulations include the three brain sub-compartment concentrations under varying brain/blood partition coefficients (2-20), varying transfer rates (2-25), and varying brain Km values (5-3).

One interesting observation was made during the simulation of the above parameters in the blood-brain barrier. As the parameters increased, an increase in the blood-brain barrier concentration occurred. But, as the concentrations increased, it was noted that there was also a more rapid decrease in concentration after the 4-hour exposure. In Figure 23, this behavior can be seen in the blood-brain barrier concentration during increasing transfer rate values. The fifth line corresponds to a transfer rate of 25, and it does in fact decrease at a greater rate than the three previous transfer rates (values – 7.75, 13.5, 19.3).

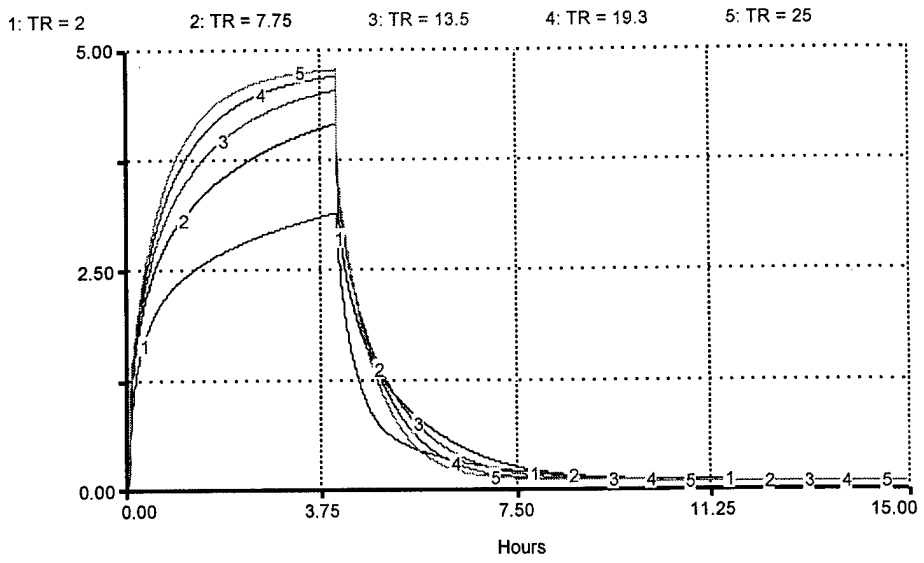


Figure 23. Rapid Decrease in BBB Concentration as the Transfer Rate Decreases

A sensitivity analysis of varying the transfer rate and its affects on passive diffusion was also simulated. The resulting behavior can be seen in Figure 62 in Appendix C. As the transfer rate increases the passive diffusion between the brain blood and blood-brain barrier increases in both directions.

The effects of increasing intensities of exercise on passive diffusion were also explored. As expected the passive diffusion from the brain blood increased and the amount of chemical transported through passive diffusion from the blood-brain barrier back into the blood increased as well. Figure 24 contains this behavior from the simulation. The behavior points to the theory that exercise causes the blood-brain barrier to become more permeable; thus allowing more chemicals through to the brain tissue. Note that during the first couple of hours of the 4-hour exposure the passive diffusion increases, so more is transported to the blood-brain barrier. Around the 3.5 – 4 hour point the passive diffusion process increases back towards the brain blood sub-

compartment. Since, exercise does not directly affect passive diffusion, this behavior could be explained by that fact that there is an increase in the blood flow rate that will affect the tissue concentrations, therefore affecting the rate of passive diffusion. Figure 63 in Appendix C contains the same simulation but for a bodyweight of 110 kg.

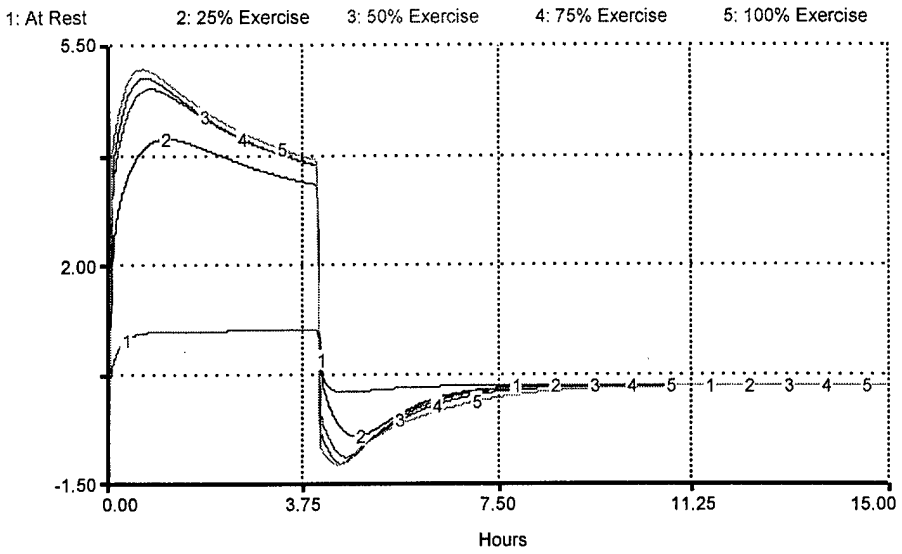


Figure 24. Passive Diffusion Effects to Increasing Exercise Levels

The final round of parameter testing focused on the parameters that were defined by a linear graphical relationship with exercise. The first parameter that was tested was the brain Vmax value, which directly affects the metabolic rate within the blood-brain barrier. This value defines the maximum rate of the saturable metabolic or enzymatic activity. As discussed in the literature review the brain Vmax may have some influence on the amount of chemical that is allowed into the brain during a stressful situation. A graphic relationship was developed between the brain Vmax and exercise, where Vmax

decreases in value as the intensity of exercise increases. The decrease in Vmax will then decrease the overall metabolic rate. Figure 25, shows the sensitivity analysis that was run for metabolism for a 70 kg individual. Exercise was increased from 0% through 100%. The initial range that was defined for the brain Vmax was 19.5 down to 11.9.

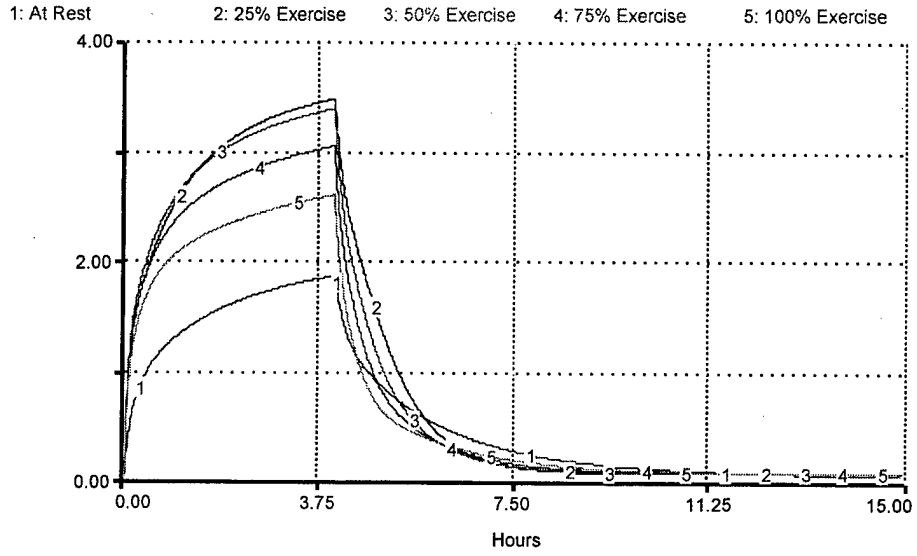


Figure 25. Metabolism Response to Increasing Exercise Levels

An interesting behavior appears above in Figure 25. The metabolism initially increases between rest and 25% exercise, which was not anticipated. There are several factors that could play into this behavior. It must be remembered that other transport processes are occurring in the brain compartment, and one of these processes could be more dominant during the initial exercise periods. The expected behavior is observed when exercise increases to 50% or greater – the metabolism of the chemical decreases which should result in an increase in the blood-brain barrier and brain tissue

concentrations. Figures 26 and 27 show these two brain concentrations. The concentrations increase as exercise increases and metabolism decreases, but the blood-brain barrier increases in very small increments as exercise exceeds 50%.

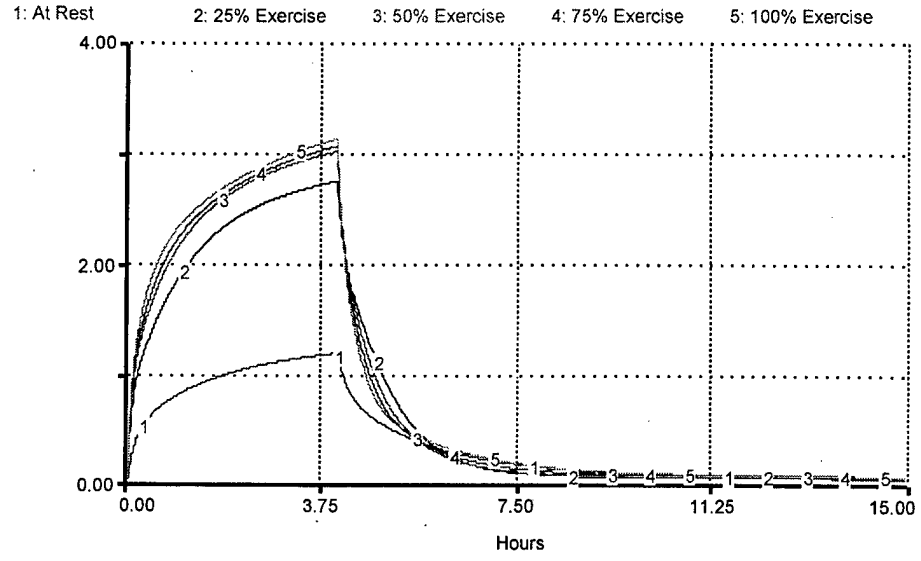


Figure 26. BBB Concentration as Exercise Increases

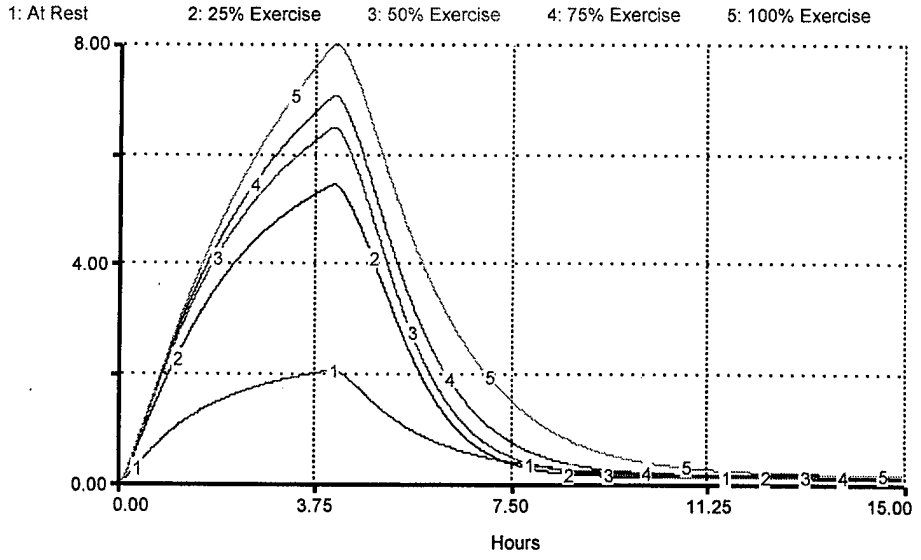


Figure 27. Brain Tissue Concentration as Exercise Increases

Another interesting observation was found when the same simulation was run for metabolism and increasing exercise, for a 110 kg individual. There is no difference between the metabolism for the two bodyweights tested. This finding helps build confidence in the model since there should not be a difference due to the way the model was built. As discussed in Chapter 2, the blood-brain barrier was not scaled to varying bodyweights. If the blood-brain barrier was scaled to bodyweight, then the behavior of the metabolic activity would vary based on different sizes of individuals. Scaling parameters were not found for the blood-brain barrier during the literature review, and this is just one of the few areas that can be pursued in further research to make the brain compartment more of a realistic representation of what actually occurs during chemical exposure and stress. The results of the simulation for 110 kg can be found in Appendix C as Figure 64.

After re-evaluating all the relationships that occur in the brain compartment during exercise, it became clear that since metabolism is a saturable process the concentrations in the blood-brain barrier will start to level off as the saturation point is met. The brain, on the other hand, will still increase in concentration due to the increased permeability of the blood-brain barrier. This behavior supports the predictions of several articles reviewed during the literature review.

The next graphical relationship that was explored was the maximum transport parameter, which affects the behavior of the mediated transport between the brain blood and blood-brain barrier. The same simulation was conducted where the exercise intensity was increased and as defined by the graphical relationship the maximum transport parameter would decrease, which in turn had an impact on the mediated transport. As

expected, the simulations initially resulted in an increased transport from the brain blood to the blood-brain barrier. After the four-hour exposure was complete an increased transportation from the blood-brain barrier to the brain blood occurred. This finding again supports the findings of several studies that focused on stress effects in the brain. This behavior of this mediated transport for a 70 kg individual can be seen below in Figure 28. Figure 65 in Appendix C contains the same simulation but for a 110 kg individual. Again the behavior for the heavier bodyweight is the same as the lighter.

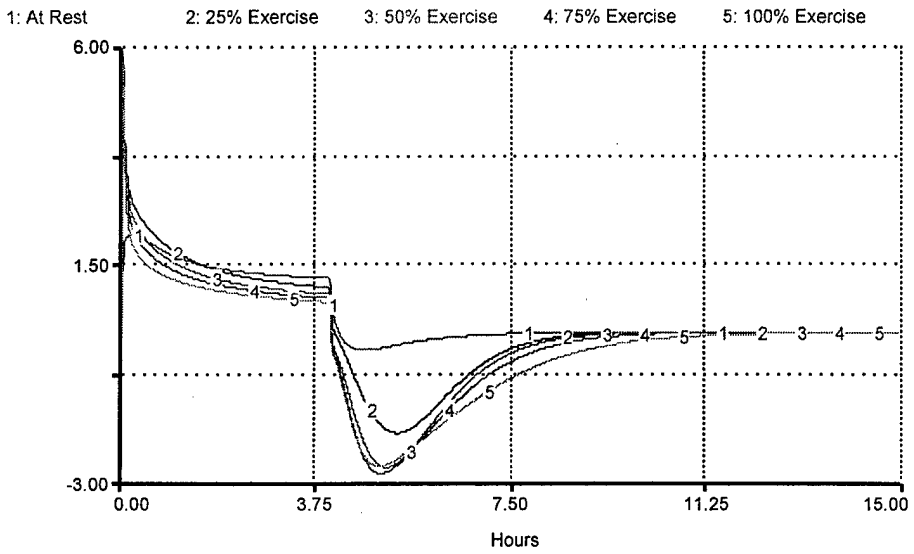


Figure 28. Mediated Transport Response to Increasing Exercise Levels

Extreme-Conditions Test

This test was fairly straightforward for this model due to the numerical integration difficulties found in the software program. If any values were either too small or too large, the resulting behavior during the simulations was very anomalous. As already discussed above an increase in exercise, which ranged from 0% to 100%, resulted in the

normal behavior expected. The model was tested with increasing levels of bodyweight, which resulted in a flat line behavior due to the small brain blood volume. The model was also tested under decreasing bodyweight conditions and increased air concentrations of TCE. The output behavior was normal in terms of what was expected for each of these simulations. Figures 66 and 67 in Appendix C contain the simulation output for a bodyweight of 40 kg and an air concentration of 300 ppm respectively.

Behavior-Anomaly Test

This test was used throughout the testing stage of the system dynamics process. When a behavior from the model deviated from what was expected of the behavior in the real system the anomaly was traced back to structural causes. This occurred at several points during the modular development of the model, but was crucial at two points during the process.

The first anomaly occurred when the brain was divided into the three sub-compartments to accurately reflect the metabolic and transport activities. When this step was taken the blood-brain barrier and brain tissue concentrations immediately flat lined at a zero concentration. The model was analyzed until it was concluded that there was not a problem with the model structure but with the limits of numerical integration within the modeling software. The volume of the brain blood sub-compartment was so small that blood flow through the capillaries cleared all mass from the sub-compartment before any opportunity to cross the blood-brain barrier. This problem was solved by increasing the brain blood volume until the chemical concentrations in the other brain sub-compartments were behaving as expected.

A similar behavior occurred again in the model when bodyweight was increased. An anomalous behavior was identified in the blood-brain barrier and brain tissue concentrations during a sensitivity analysis of the five exercise values. Figure 29 and 30 are examples of the anomalous behavior seen in these two sub-compartments. In Figure 29 the blood-brain barrier concentration increases as the exercise value is increased from 0% (line #1) to 15% (line #2), 25% (line #3), and 55% (line #4). This behavior is expected but when the exercise is increased to 100% (line #5) the blood-brain barrier decreases back below the output from 55% exercise. A similar behavior is seen in Figure 30 where the brain tissue concentrations increases as the exercise value increases, but at 100% the concentration increases at a slower rate then at 55%. This behavior can be seen where line #5 crosses over line #4 just before the maximum concentration is reached.

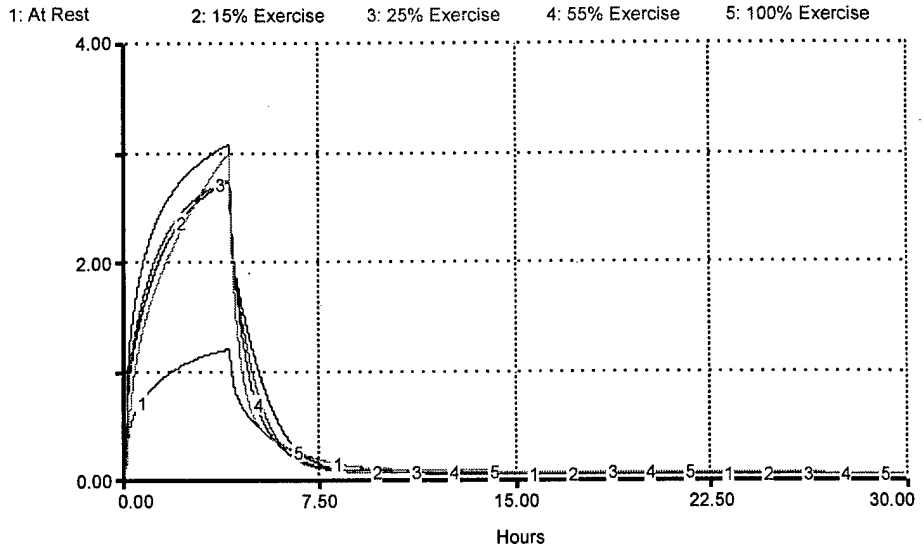


Figure 29. Anomalous Behavior in the Blood-Brain Barrier

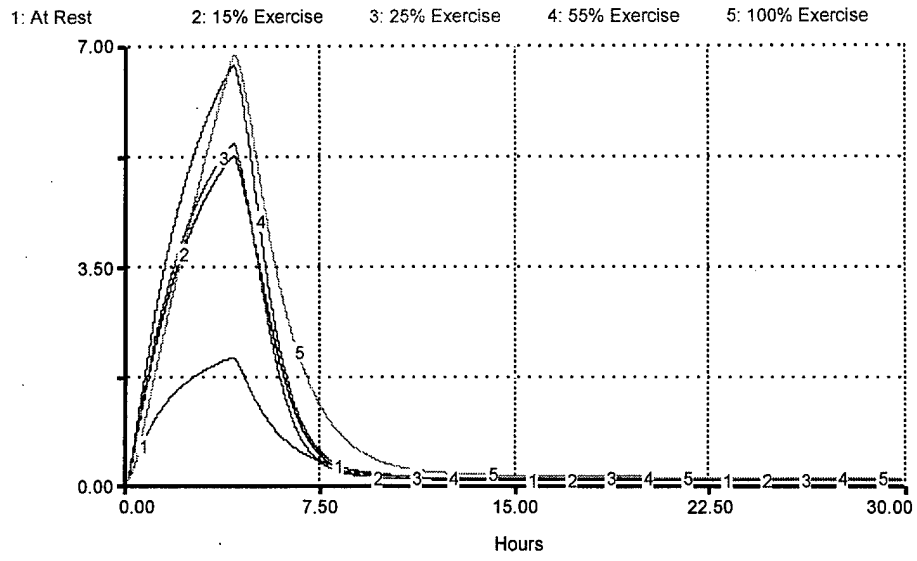


Figure 30. Anomalous Behavior in the Brain Tissue

The behavior for both these tissue concentrations was again tracked to the brain blood volume, which had already been increased to 2.17 liters. The problem was traced back to the numerical integration limitation with the STELLA software program. The mass of contaminant in the brain blood compartment was too small for the time step between calculations (DT), and this lead to an inaccurate simulation. The brain blood volume was increased again until the expected behavior was seen. The final value for the brain blood volume was 3.5 liters. This may be unrealistic in the real world, but the overall behavior was important in this thesis effort and increasing the volume will not impact the behavior. Figure 31 and Figure 32 show the blood-brain barrier and brain tissue concentrations simulated with this new brain blood volume. Notice how the anomalous behavior disappeared and the tissue concentrations increase, as they should with each increase in the exercise level.

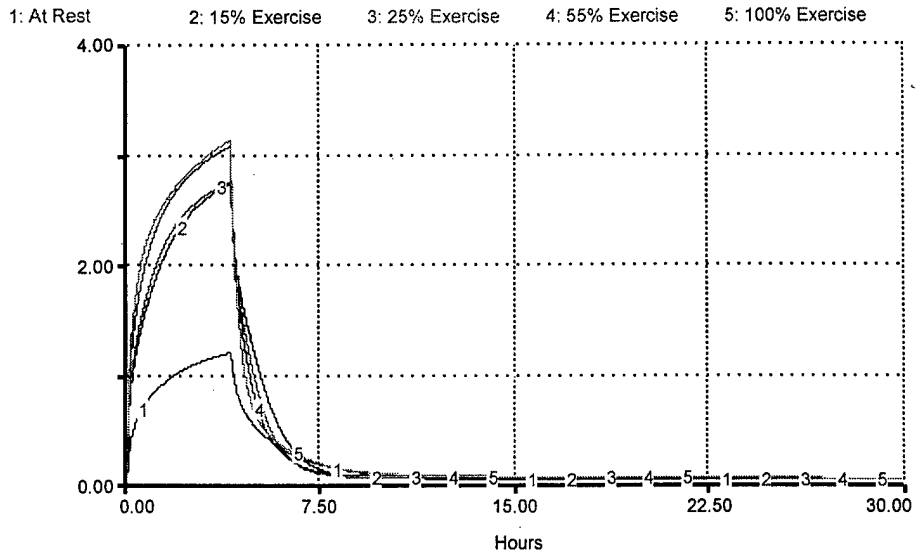


Figure 31. Expected Behavior in the Blood-Brain Barrier

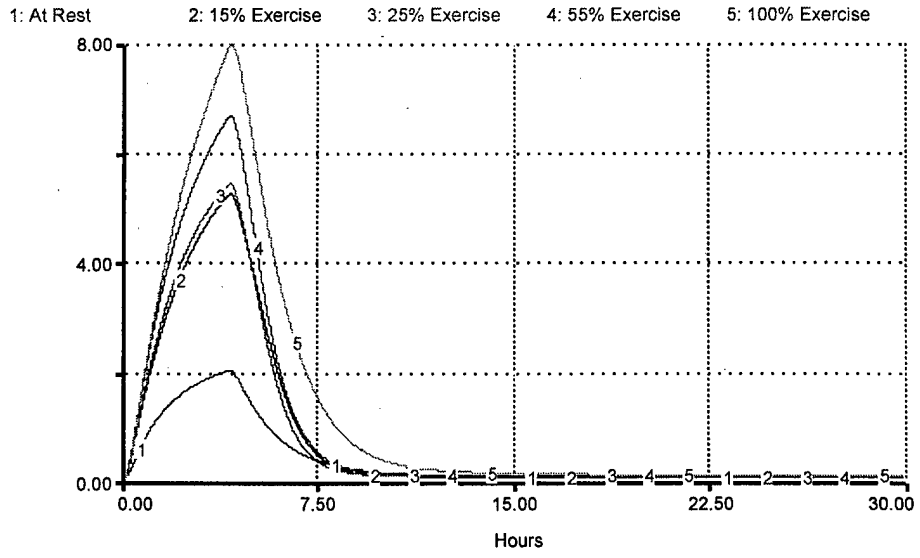


Figure 32. Expected Behavior in the Brain Tissue

The same simulation above was conducted again, but this time the brain blood volume was increased by one order of magnitude to insure that the same behavior was occurring in the blood-brain barrier and brain tissue compartments. The following figures, Figure 33 and 34, are the results of this simulation. The expected behavior occurred, which proves that the brain blood volume is not a sensitive parameter to the output in this model. The compartment acts as a significant accumulator, but the same behavior will occur with a wide range of values.

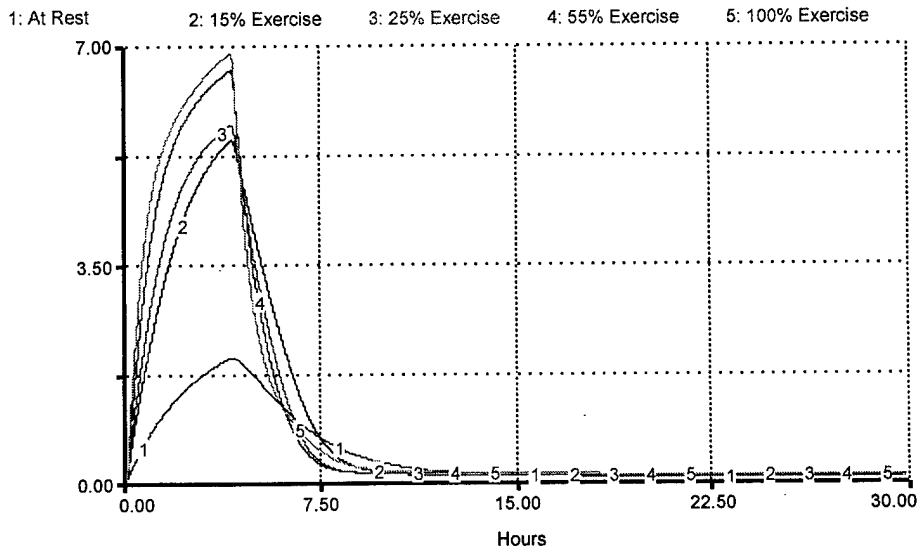


Figure 33. BBB output when Brain Blood Volume is Increased

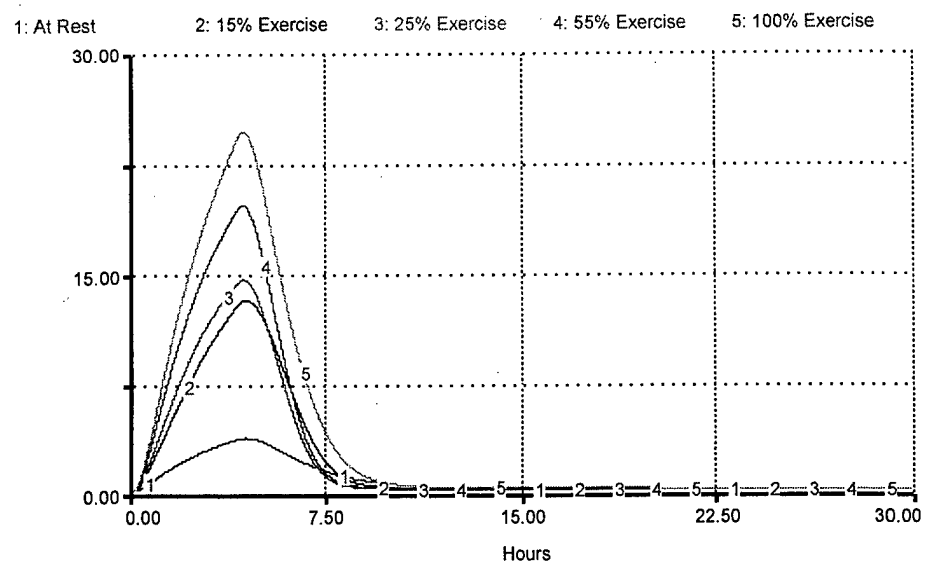


Figure 34. Brain Tissue output when Brain Blood Volume is Increased

The second anomaly occurred with the addition of the exercise influence in the model structure. In the first stages of the model development, the exercise parameter's affect on the maximum transport and brain Vmax parameters was defined incorrectly. Exercise was also defined in a complicated manner, which only made the model formulation more confusing. Initially, changes in exercise were defined by changes in either alveolar ventilation or cardiac output and then this exercise level was directly added on to the maximum transport gates and the Vmax in the brain. This definition was reverse of what occurs in reality where changes in the alveolar ventilation and cardiac output are a direct result of changes in physical activity. The simulation of the model also resulted in a behavior that was not expected. The tissue concentrations would increase and decrease in no particular order as the exercise level was increased. This anomalous behavior was traced back to the how exercise affected the maximum transport and Brain Vmax. A change to the structural development of exercise was done at this

point, which made it easier to change the way exercise impacted the brain parameters. Alveolar ventilation, cardiac output, the fractional distributions of blood flow, and the brain metabolism and transportation processes were all changed to have a direct relationship with exercise. If the exercise value changed in the model then these other values would be effected. Changing the exercise parameter in the model made the model structure more of an accurate representation of what happens in the real human body and the resulting behavior was once again what was expected.

V. Conclusions and Recommendations

The purpose of this research effort has been to build a model that predicts the changes that will occur when stress is combined with chemical exposure. A physiologically-based pharmacokinetic (PBPK) model is an excellent tool that can aid in understanding the concepts behind stress and chemical exposure, as well as further the research in understanding the etiology behind the Gulf War Syndrome.

A PBPK model has been constructed that provides the groundwork for studying the internal interactions of the human body and how stress can change these interactions to possibly increase the harmful effects of a chemical exposure. In this initial work the model has investigated the effects of a stressor, in this case exercise, on the system behavior. Tremendous insights have been gained concerning this system through the system dynamics modeling stages: conceptualization, formulation, and testing. This chapter will complete the final stage of the system dynamic process, implementation, by translating the insights and information gained with respect to the research objectives. Strengths and weaknesses of the model and areas for further study are also addressed.

Research Objectives

Throughout the literature review and the modular building of the model, knowledge was gained on the mechanisms of stress and chemical uptake and distribution in the human body. Several studies have theorized that a large player in the human body's response to stress is the brain. Homeostasis is crucial in the brain, and any changes in the uptake or metabolism of chemicals could potentially disrupt this

homeostasis and have many other harmful effects to the human body. A large amount of literature was reviewed to learn about the brain and the blood-brain barrier.

This information was used to build a PBPK model that addresses a topic that few if any PBPK models have done in the past. A quantitative description of the research was input into STELLA using the system dynamics process. This process included the conceptualization stage where a reference mode and influence diagrams were formed. The testing phase was then conducted to simulate the model over a range of reasonable parameter values. These simulations helped the modeler gain confidence in the model and the resulting behaviors.

A hypothesis can now be formed as to the interactions of stress and chemical exposure. It has been demonstrated through this thesis effort that exercise does have a large influence on the chemical concentrations in the body; and, the brain is an important component in these interactions. The blood-brain barrier is the gate to the brain tissue and exercise can open this gate. It has been demonstrated that mediated transport, passive diffusion, and metabolism in the brain are all contributing causes to an increase in concentrations in the brain.

The question now is how can we prevent this possible harm to the brain? And, how can we predict the consequences of stress on the human body? Hopefully, with some future work on this model we may be able to use it as a tool to help predict and explore these questions.

Model Strengths and Limitations

Model Strengths

1. Provides insights and the groundwork for studying the effects of chemical exposure and stress in combination.
2. Provides a simplified, but important basis for simulating the behavior of xenobiotic chemicals in the brain tissue in the context of circulation throughout the physiological system.
3. Suggests areas of future research that will be important in the implementation phase of this model.

Model Limitations

1. Rests upon many generalities and assumptions of parameters in the brain compartment portion of the model.
2. The stress response only addressed the physical aspect, not the emotional aspect.
3. The modeling software program had numerical integration limits, so the brain blood volume was defined as an unrealistic value during the testing of the model.

Areas of Further Research

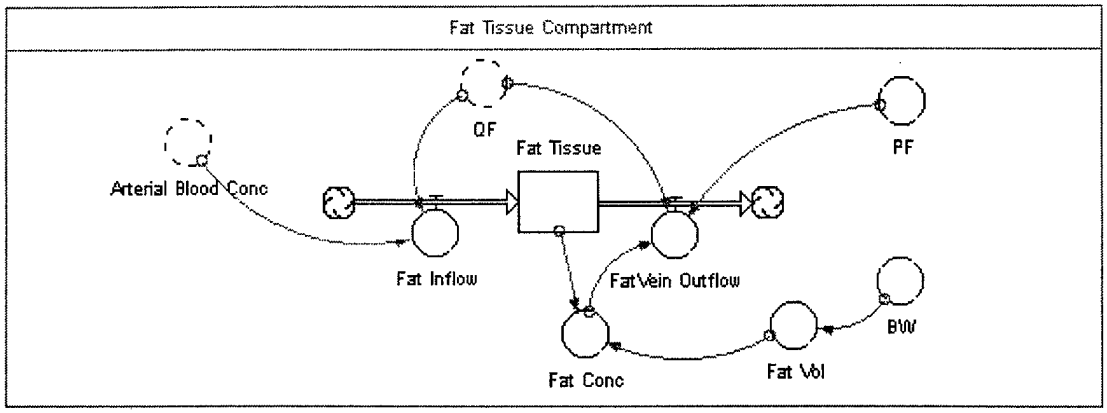
1. Expand upon the brain compartment portion of this model. The development of the three sub-compartments is a realistic, but very simple description of the brain, blood, and blood-brain barrier interactions. The transport systems may also be too simplistic in this model. Bi-directional transport for both passive diffusion and mediated transport was assumed. Uni-directional flows may be more appropriate in some cases.

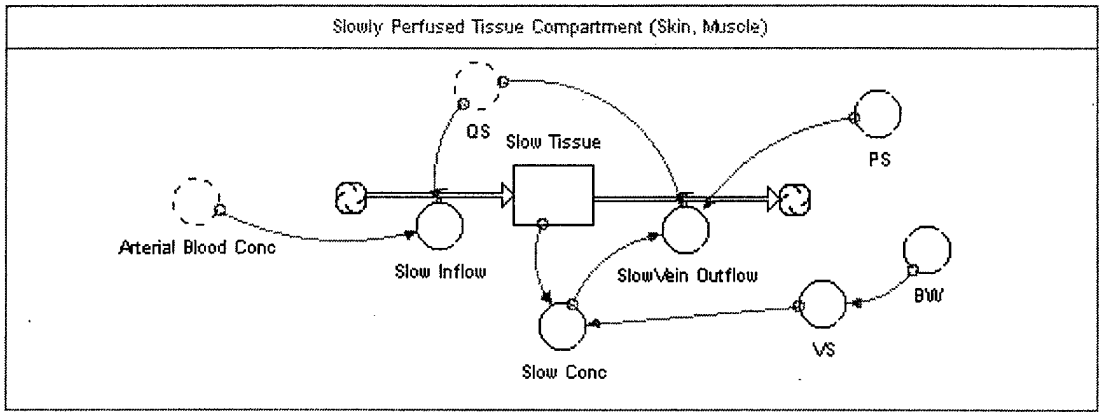
2. Explore behavior of other chemicals that may be more relevant to deployment toxicology concerns. Eventually PB, DEET and all the other chemicals in question could be used in the model to predict their effect in human tissue.
3. Using a more powerful version of STELLA or using a different software program for the testing of this model would be helpful. The problems with the numerical integration limits that were encountered during the testing of the model could be avoided with a more powerful software program; and, more realistic values for the parameters in the brain compartment could be used.
4. Add the mental or emotional aspects of stress to the model.

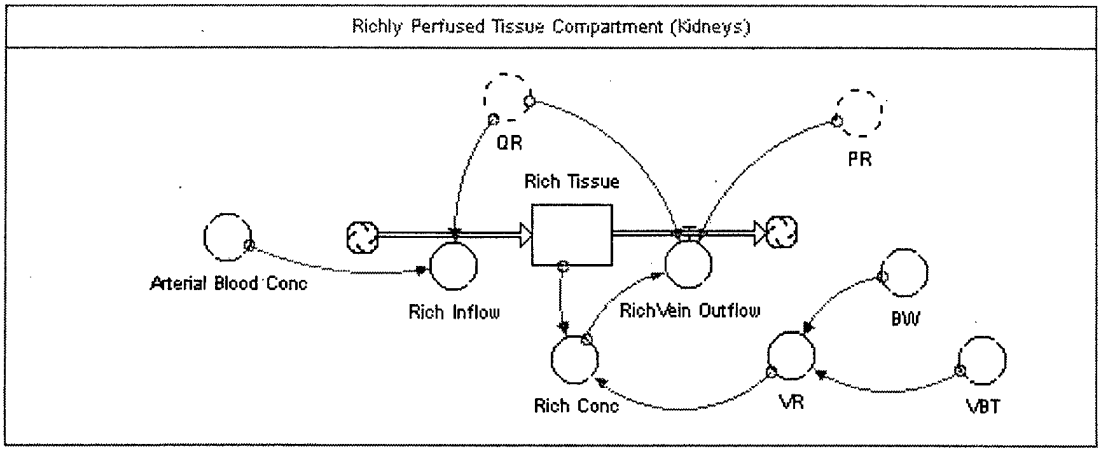
Conclusions

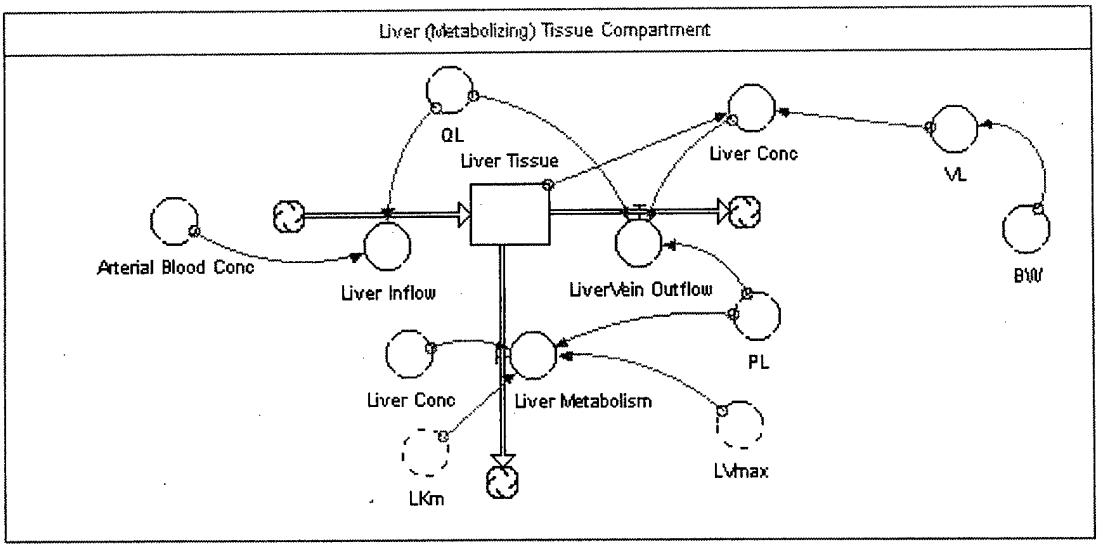
This model and the testing that has been performed have laid the groundwork for further research, and will aid in the pursuit to understand and be able to predict and control the effects of stress and chemical exposure during deployment scenarios, or in any situation that may apply. The model developed appears to be a valuable tool for gaining insights into the mechanisms behind stress and chemical exposure. It can also be used to help define plausible parameters in the brain tissue. This information could assist the military in understanding the etiology behind the Gulf War Syndrome, or at least help fill major gaps that have hindered immediate identification of the disease.

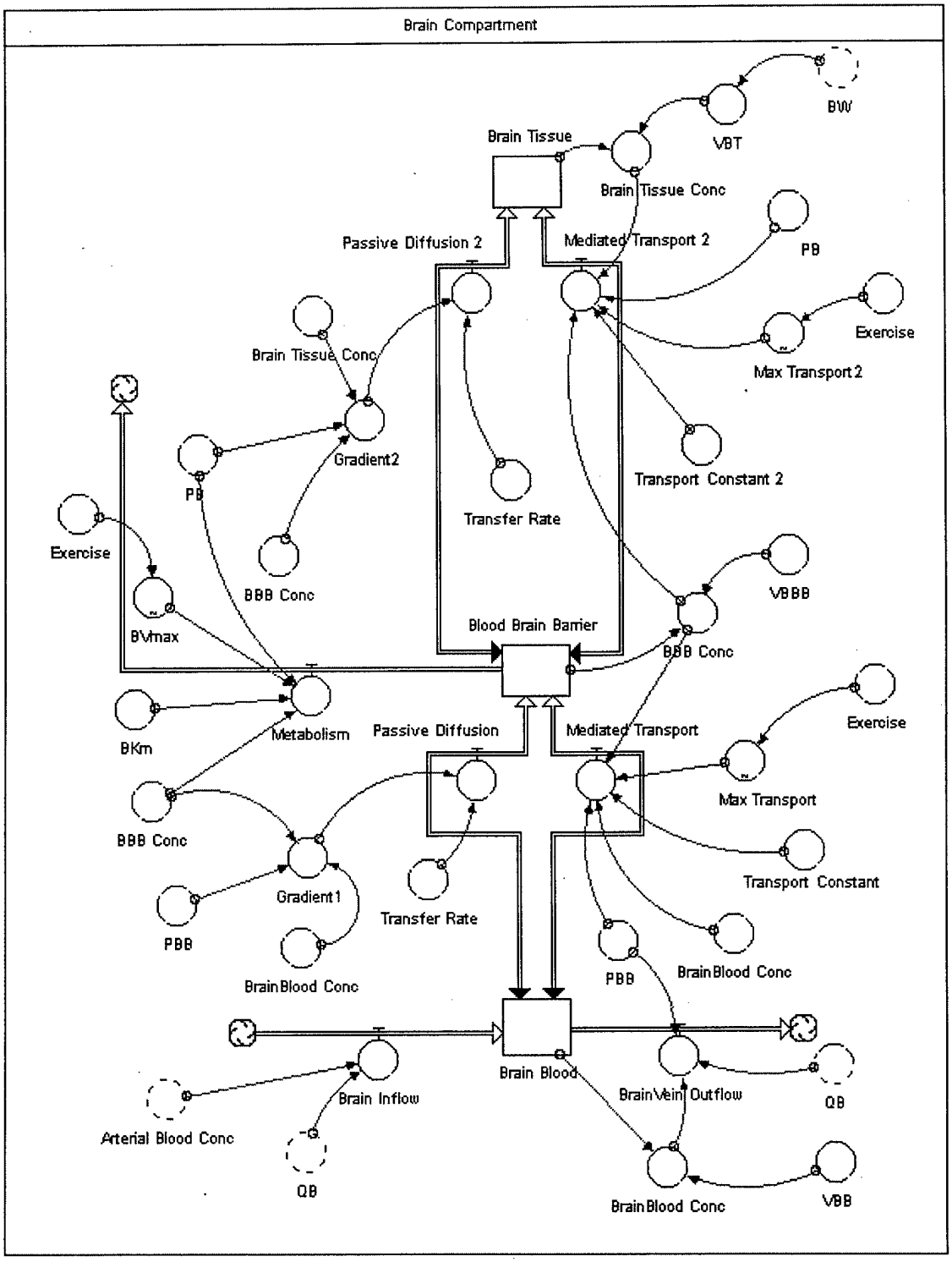
Appendix A – Model Flow Diagram

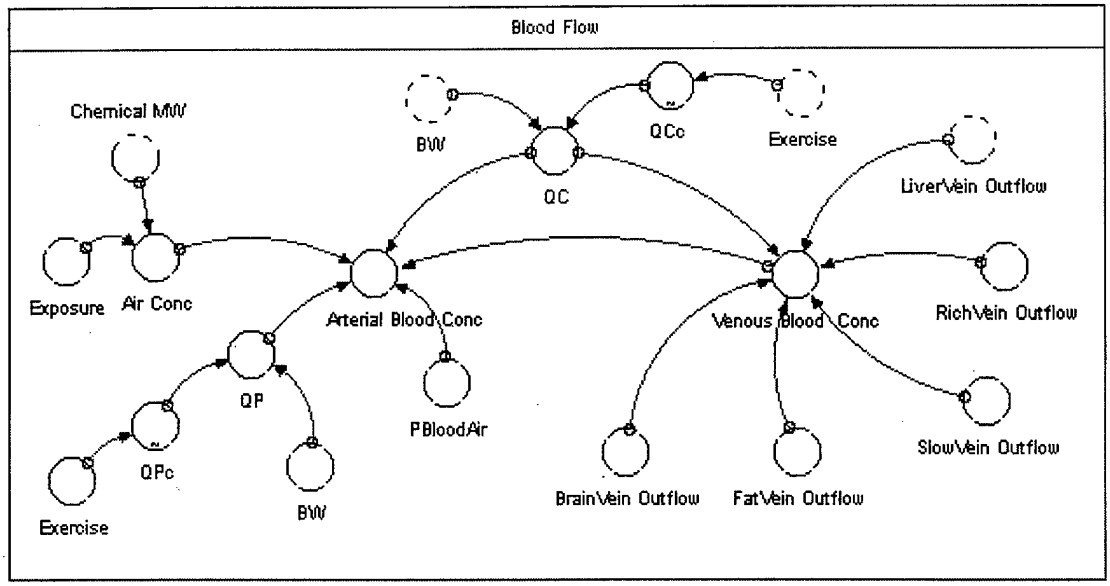


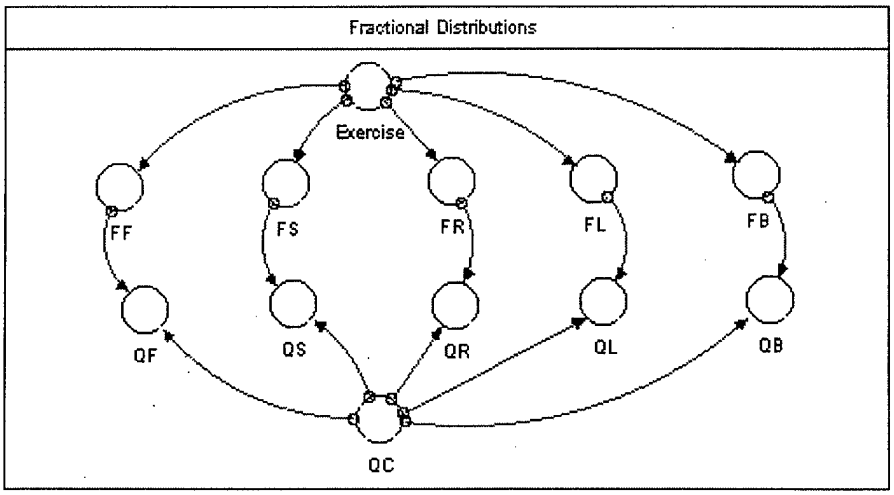


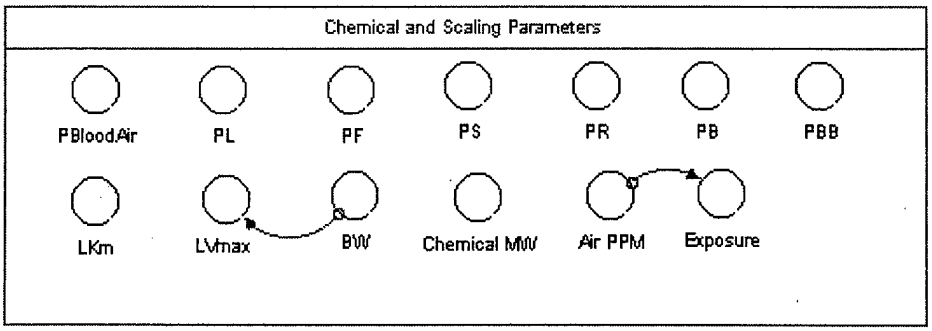



Liver (Metabolizing) Tissue Compartment
QL
Liver Tissue
Liver Conc
VL
BW
Arterial Blood Conc
Liver Inflow
LiverVein Outflow
PL
Liver Conc
Liver Metabolism
LVmax
LKm


Brain Compartment
BW
VBT
Brain Tissue
Brain Tissue Conc
PB
Passive Diffusion 2
Mediated Transport 2
Exercise
Max Transport 2
Brain Tissue Conc
Gradient2
PB
Transfer Rate
Transport Constant 2
Exercise
BBB Conc
VBBB
B\Vmax
Blood Brain Barrier
BBB Conc
BKm
Metabolism
Passive Diffusion
Mediated Transport
Exercise
Max Transport
BBB Conc
Gradient1
Transfer Rate
Transport Constant
PBB
BrainBlood Conc
PBB
BrainBlood Conc
Brain Blood
Brain Inflow
BrainVein Outflow
QB
Arterial Blood Conc
QB
BrainBlood Conc
VBB


Blood Flow
Chemical MW
BW
QCc
Exercise
QC
Liver\Vein Outflow
Exposure
Air Conc
Arterial Blood Conc
Venous Blood Conc
Rich\Vein Outflow
QP
PBlood/Air
QPc
Slow\Vein Outflow
Exercise
BW
Brain\Vein Outflow
Fat\Vein Outflow


Fractional Distributions
Exercise
FF
FS
FR
FL
FB
QF
QS
QR
QL
QB
QC


Chemical and Scaling Parameters
PBlood/Air
PL
PF
PS
PR
PB
PBB
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%
Strenuous 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
Blood_Brain_Barrier(t) = Blood_Brain_Barrier(t - dt) + (Passive_Diffusion + Mediated_Transport - Passive_Diffusion_2 - Mediated_Transport_2 - Metabolism) * dt
INIT Blood_Brain_Barrier = 0
DOCUMENT: This reservoir makes up the endothelial cells in the brain that make up the barrier. This consists of the lumen side (facing the brain blood) and the ablumen side (facing the actual brain tissue). This reservoir also represents the accumulation point in the blood brain barrier where there is a biflow of material (passive diffusion and mediated transport) passing between the blood brain barrier and brainblood.

Inflows
Passive_Diffusion = Gradient1*Transfer_Rate

Mediated_Transport = ((Max Transport*BrainBlood_Conc/PBB)/(Transport Constant+BrainBlood_Conc/PBB))-((Max Transport*BBB_Conc/PBB)/(Transport Constant+BBB_Conc/PBB))

Outflows
Passive_Diffusion_2 = Gradient2*Transfer_Rate

Mediated_Transport_2 = ((Max Transport2*BBB_Conc/PB)/(Transport Constant2+BBB_Conc/PB))-((Max Transport2*Brain_Tissue_Conc/PB)/(Transport Constant2+Brain_Tissue_Conc/PB))

Metabolism = (BVmax*BBB_Conc/PB)/(BKm+BBB_Conc/PB)
DOCUMENT: Metabolism was assumed to occur in the blood brain barrier for this particular model. The saturable metabolic transformation of any particular chemical in the blood brain barrier was defined using the Michaelis-Menten equation with the bio-chemical constants Vmax2 and Km2.

Stock
Brain_Blood(t) = Brain_Blood(t - dt) + (Brain_Inflow - BrainVein_Outflow - Passive_Diffusion - 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 that enters the brainblood portion of the brain tissue compartment.

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

Passive_Diffusion = Gradient1*Transfer_Rate

Mediated_Transport = ((Max Transport*BrainBlood_Conc/PBB)/(Transport Constant+BrainBlood_Conc/PBB))-((Max Transport*BBB_Conc/PBB)/(Transport Constant+BBB_Conc/PBB))

Stock
Brain_Tissue(t) = Brain_Tissue(t - dt) + (Passive_Diffusion_2 + Mediated_Transport_2) * dt
INIT Brain_Tissue = 0
DOCUMENT: This reservoir represents the accumulation point in the brain tissue where there is a biflow of material (passive diffusion and mediated transport) passing between the brain tissue and the blood brain barrier.

Inflows
Passive_Diffusion_2 = Gradient2*Transfer_Rate

Mediated_Transport_2 = ((Max Transport2*BBB_Conc/PB)/(Transport Constant2+BBB_Conc/PB))-((Max Transport2*Brain_Tissue_Conc/PB)/(Transport Constant2+Brain_Tissue_Conc/PB))

Parameters
BBB_Conc = Blood_Brain_Barrier/VBBB
DOCUMENT: This connector represents the concentration of chemical in the blood brain barrier.

BKm = 1.6
DOCUMENT: Km2 - Michaelis constant (mg/L). This parameter value is an assumption at this time due to lack of data in the literature.

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-(BBB_Conc/PBB))

Gradient2 = (BBB_Conc-(Brain_Tissue_Conc/PB))
Transport Constant = .8: This parameter value is an assumption at this time due to lack of data in the literature.

Transport Constant2 = 1.75: This parameter value is an assumption at this time due to lack of data in the literature.

Transfer_Rate = 2: This parameter value is an assumption at this time due to lack of data in the literature.
VBB = 3.5: This value is larger than the real-world volume of the brain blood compartment due to integration limitations with the software.

VBBB = .5: This parameter value is an assumption at this time due to lack of data in the literature.

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).

Graphs
BVmax = GRAPH(Exercise)
(0.00, 19.5), (10.0, 18.8), (20.0, 18.1), (30.0, 17.3), (40.0, 16.4), (50.0, 16.0), (60.0, 15.2), (70.0, 14.5), (80.0, 13.9), (90.0, 13.2), (100, 11.9)
DOCUMENT: Vmax2 (mg/hr) - maximum rate of enzymatic (saturable) metabolism

Max Transport = GRAPH(Exercise)
(0.00, 15.0), (10.0, 14.4), (20.0, 13.8), (30.0, 13.3), (40.0, 12.8), (50.0, 12.3), (60.0, 11.7), (70.0, 11.5), (80.0, 11.0), (90.0, 10.5), (100, 9.97)

Max Transport2 = GRAPH(Exercise)
(0.00, 29.8), (10.0, 28.5), (20.0, 27.0), (30.0, 25.3), (40.0, 23.6), (50.0, 21.8), (60.0, 20.3), (70.0, 18.4), (80.0, 16.8), (90.0, 14.9), (100, 12.3)

Chemical and Scaling Parameters

Parameters
Air_PPM = 300
DOCUMENT: This value is the concentration of a particular chemical exposure in parts per million (PPM).

BW = 40
DOCUMENT: The bodyweight of the individual used in the simulation of this model.
Chemical_MW = 131.40
DOCUMENT: Molecular Weight of Trichloroethylene

Exposure = Air_PPM-STEP(Air_PPM,4)
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 exposed to the Air PPM for four hours.

LKm = 1.5
DOCUMENT: Liver Km - Michaelis constant (mg/L). This was based on 'Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans' by Allen and Fisher (1993). The Vmax value for the liver can now be computed for any bodyweight.

LVmax = 14.9*(BW^7)
DOCUMENT: The scaling parameter of 14.9 was used to scale the Liver Vmax (mg/hr) - maximum rate of enzymatic (saturable) metabolism. This was based on 'Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans' by Allen and Fisher (1993). The Vmax value for the liver can now be computed for any bodyweight.

PB = 7
DOCUMENT: Brain-Blood Partition Coefficient
This value is a realistic assumption at this time. A richly perfused-blood partition coefficient of 6.8 has been used in previous PBPK models (Allen and Fisher-1993). The brain compartment was included in the richly perfused compartment in these previous models, so when the brain is separated into it's own compartment the partition coefficient should be fairly similar.

PBB = 1
DOCUMENT: BrainBlood-Blood Partition Coefficient
Assumption - Blood-Brain Barrier Compartment is an aqueous concentration (intracellular fluid) so this value will equal 1.

PBloodAir = 9.2
DOCUMENT: Blood-Air Partition Coefficient
Found in - Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans' by Allen and Fisher (1993)

PF = 73.3
DOCUMENT: Fat-Blood Partition Coefficient
Found in - Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans' by Allen and Fisher (1993)

PL = 6.8
DOCUMENT: Liver-Blood Partition Coefficient
Found in - Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans' by Allen and Fisher (1993)

PR = 6.8
DOCUMENT: Richly Perfused-Blood Partition Coefficient
Found in - Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans' by Allen and Fisher (1993)

PS = 2.3
DOCUMENT: Slowly Perfused-Blood Partition Coefficient
Found in - Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans' by Allen and Fisher (1993)

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.

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

Outflow
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

Parameters

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 - 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.

Inflow
Liver_Inflow = Arterial_Blood_Conc*QL

DOCUMENT: The fraction of the arterial blood flow that enters the liver tissue compartment.

Outflow

LiverVein_Outflow = QL*Liver_Conc/PL

DOCUMENT: This represents the fraction of venous blood flow that exits the liver tissue.

Parameters

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.

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.

Inflow

Rich_Inflow = Arterial_Blood_Conc*QR

DOCUMENT: The fraction of the arterial blood flow that enters the richly perfused tissue compartment.

Outflow

RichVein_Outflow = QR*Rich_Conc/PR

DOCUMENT: This represents the fraction of venous blood flow that exits the richly perfused tissue.

Parameters

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 perfused 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)

Slowly Perfused Tissue Compartment (Skin, Muscle)

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.

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

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

Parameters
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 – Model Testing

Initial Testing:

Comparative graphs of the tissue concentrations at rest (#1) and at heavy exercise (#2)

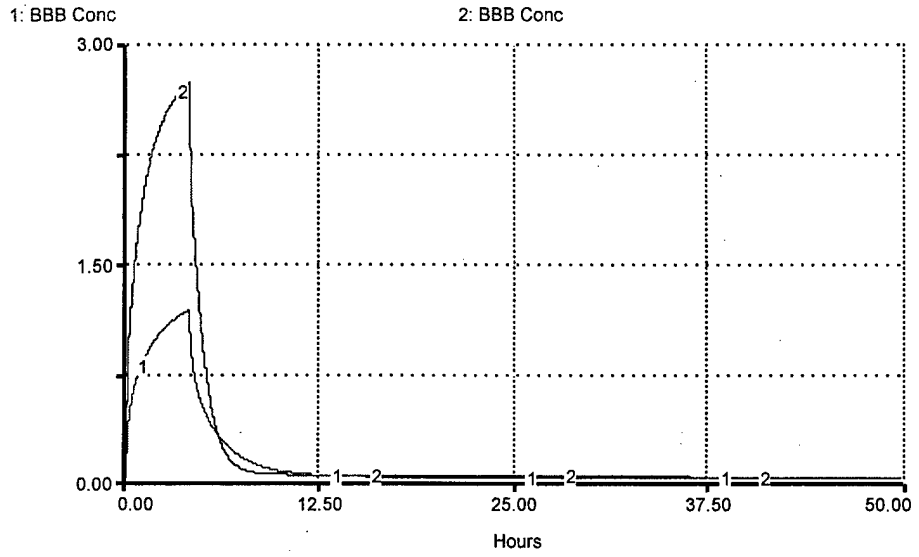


Figure 35. BBB Concentration (1-Rest, 2-Heavy Exercise)

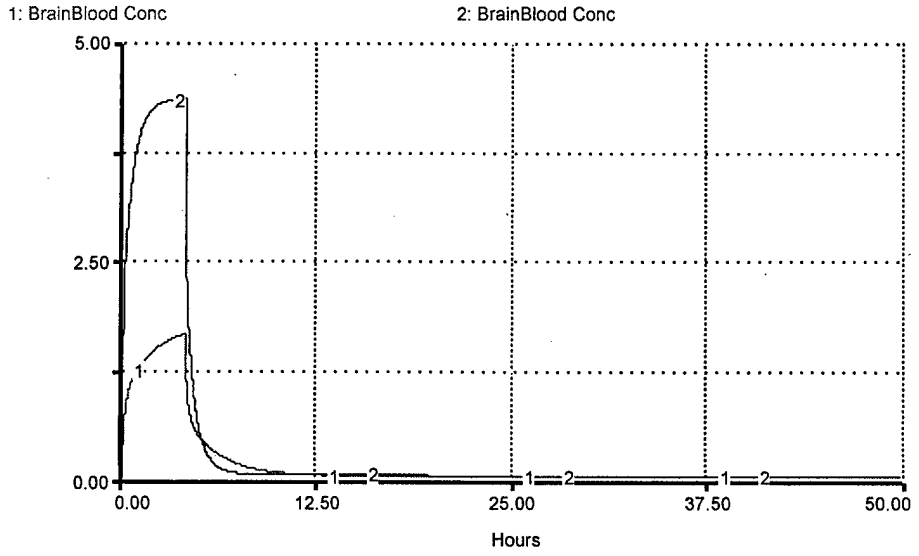


Figure 36. Brain Blood Concentration (1-Rest, 2-Heavy Exercise)

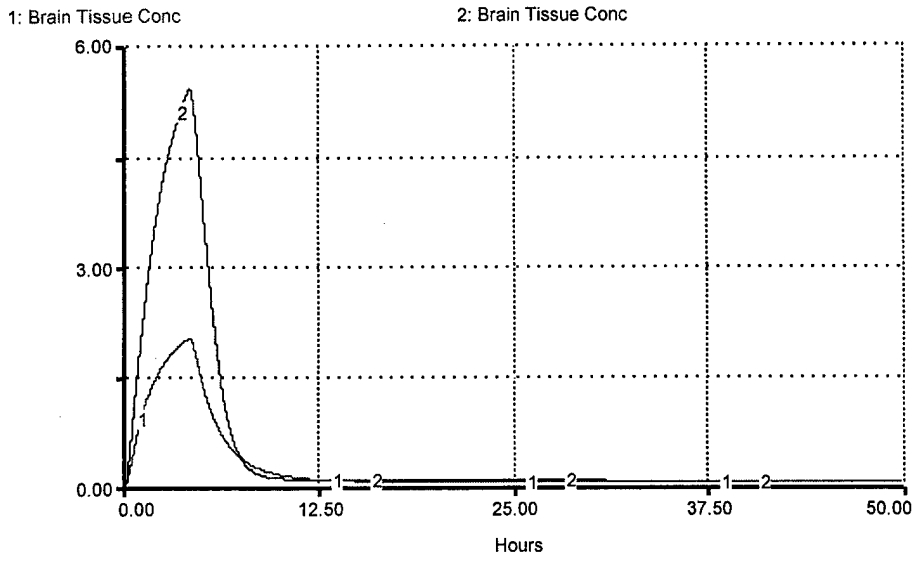


Figure 37. Brain Tissue Concentration (1-Rest, 2-Heavy Exercise)

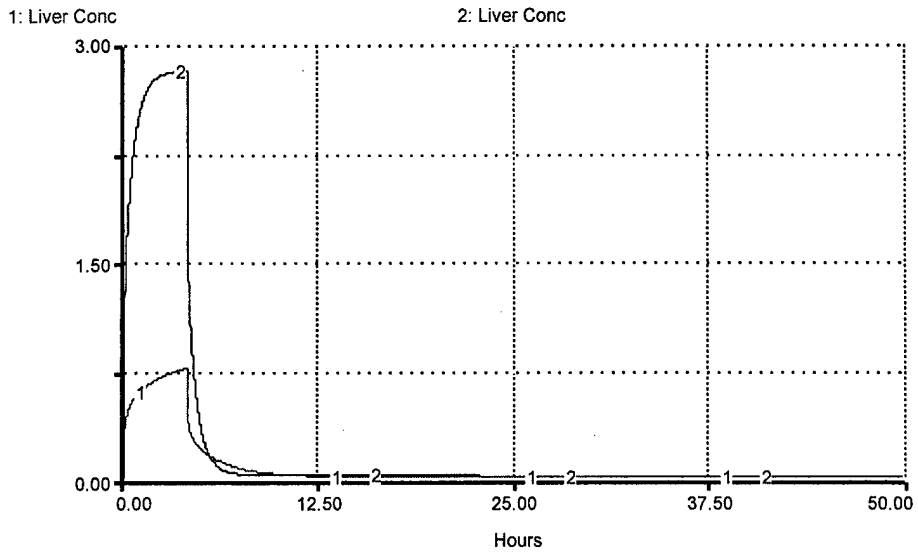


Figure 38. Liver Concentration (1-Rest, 2-Heavy Exercise)

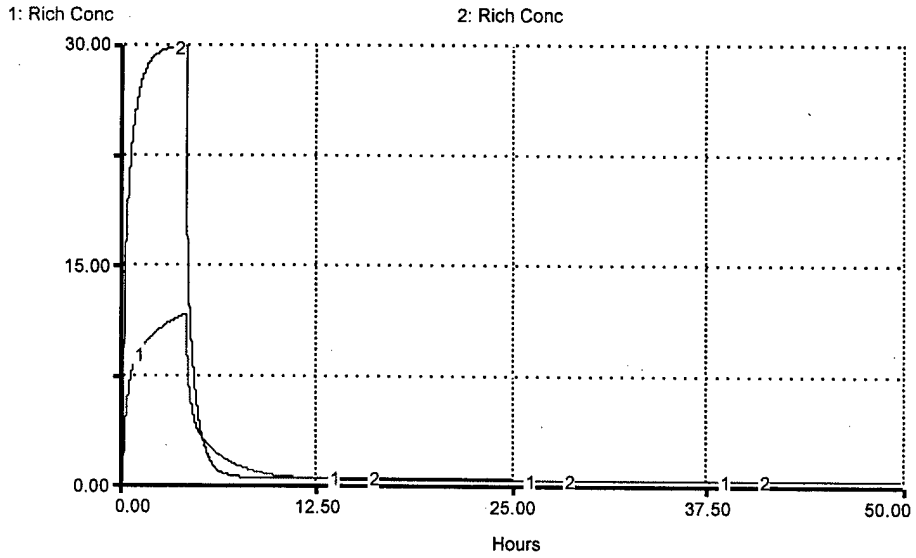


Figure 39. Richly Perfused Concentration (1-Rest, 2-Heavy Exercise)

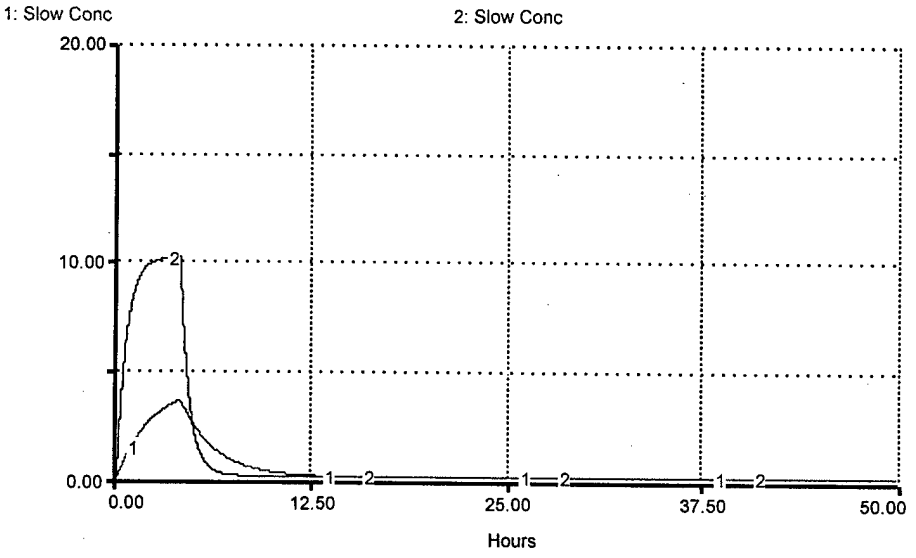


Figure 40. Slowly Perfused Concentration (1-Rest, 2-Heavy Exercise)

Exercise Testing:

Comparative Graphs for the varying tissue concentrations at the 5 Levels of Exercise:

1 Rest
1- Moderate Exercise (15%)
2- Heavy Exercise (25%)
3- Strenuous Exercise (55%)
4- Maximum Output (100%)

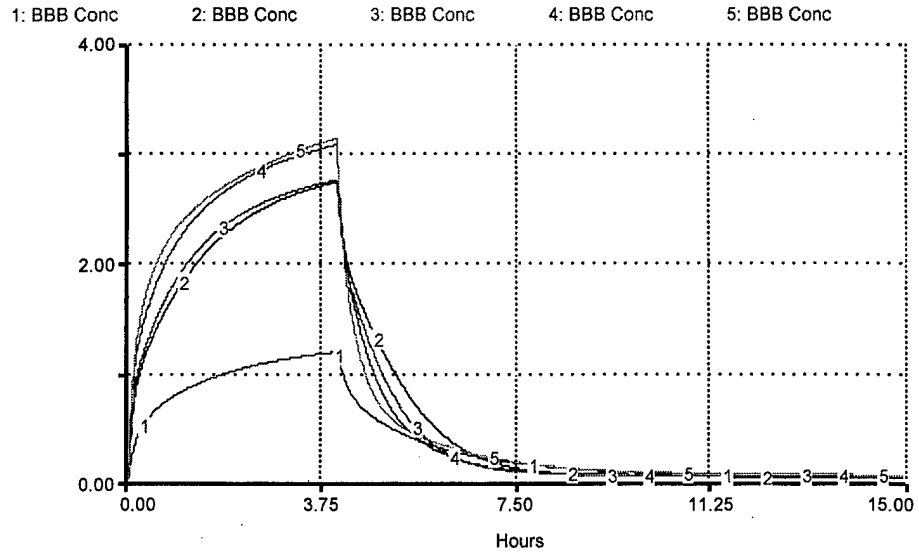


Figure 41. BBB Concentrations at 5 Levels of Exercise

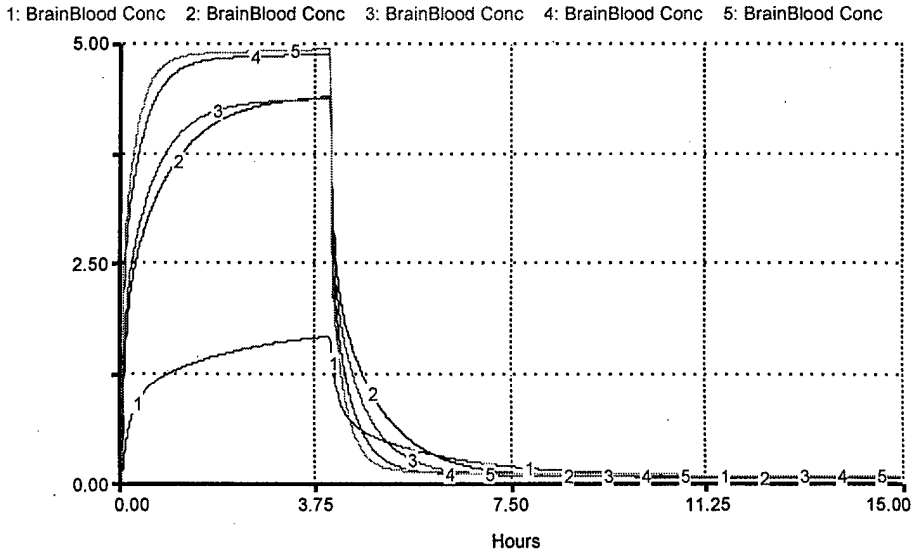


Figure 42. Brain Blood Concentrations at 5 Levels of Exercise

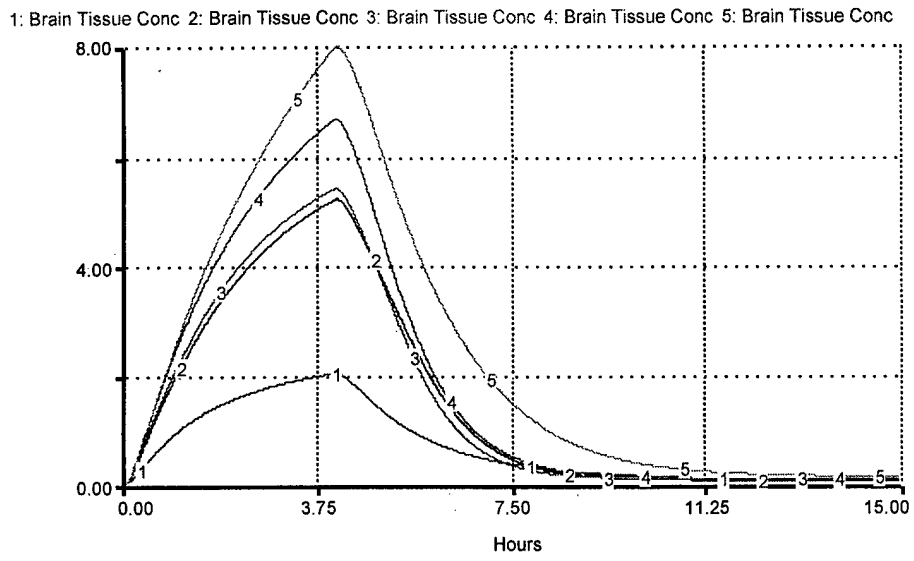


Figure 43. Brain Tissue Concentrations at 5 Levels of Exercise

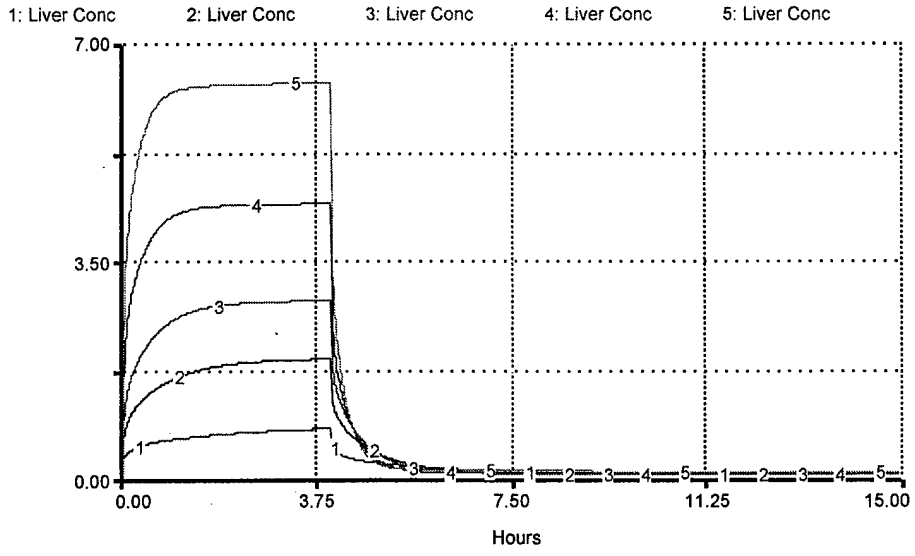


Figure 44. Liver Concentrations at 5 Levels of Exercise

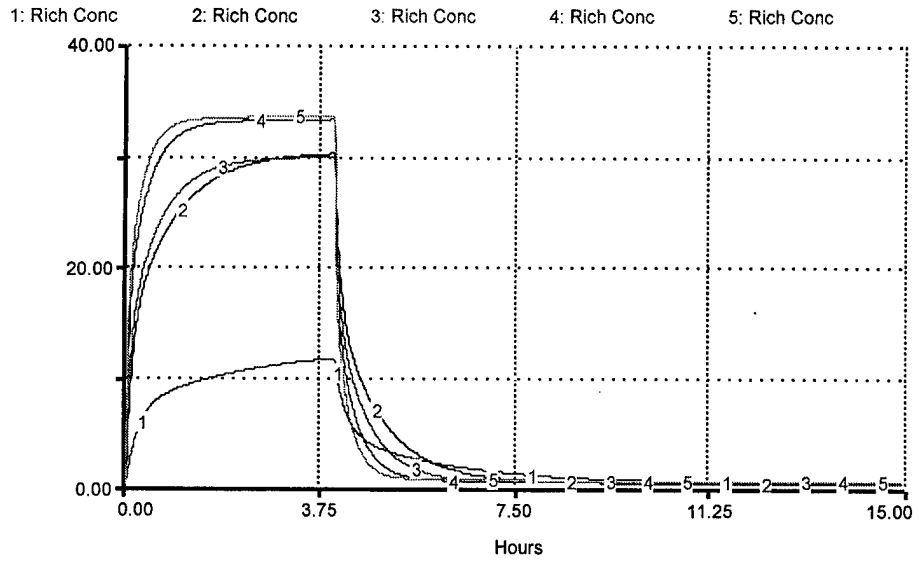


Figure 45. Richly Perfused Concentrations at 5 Levels of Exercise

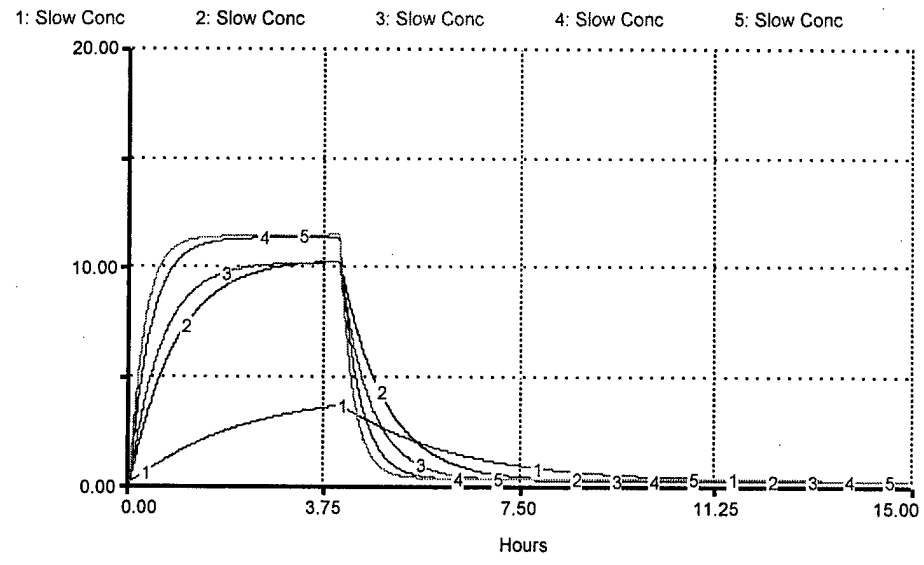


Figure 46. Slowly Perfused Concentrations at 5 Levels of Exercise

Bodyweight Testing:

Sensitivity Analysis of Bodyweight and Effect on Tissue Concentrations (Range = 60-110 kg)

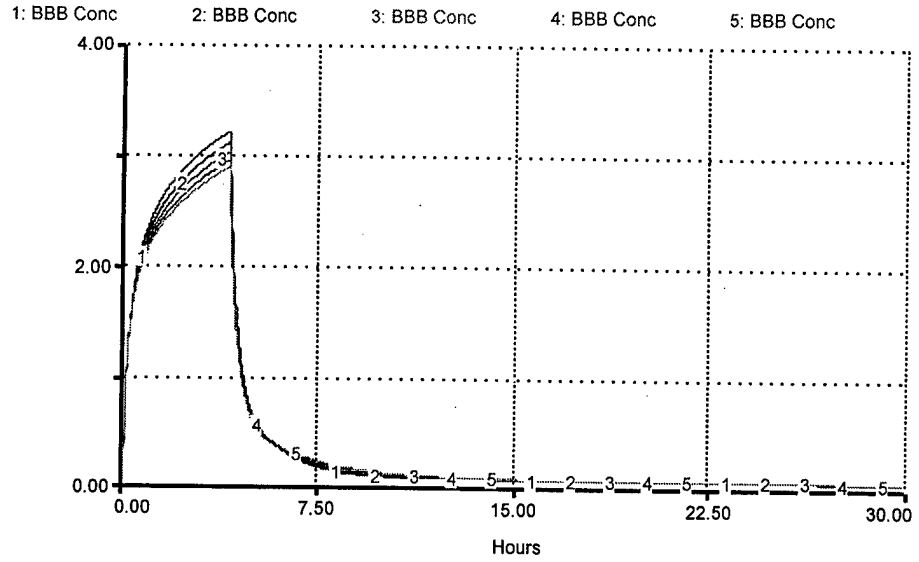


Figure 47. BBB Concentrations for Increasing Levels of Bodyweight.

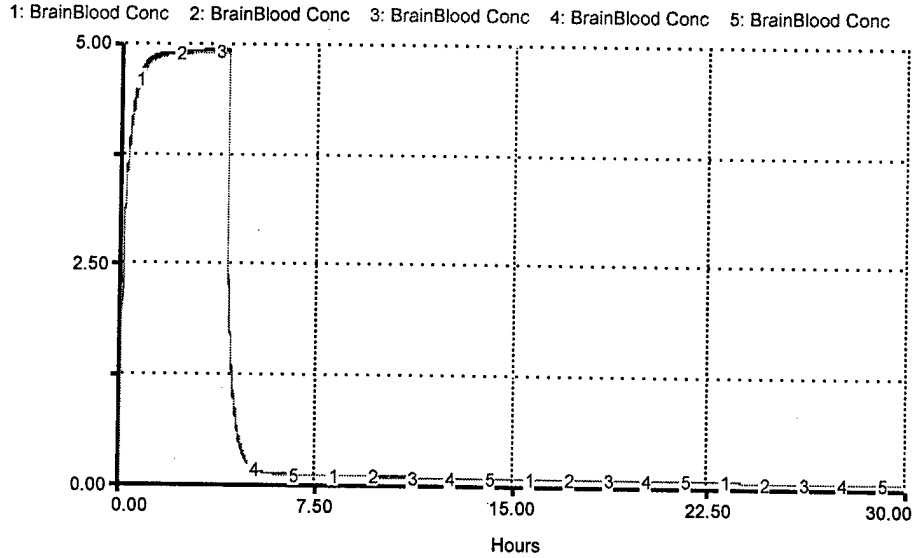


Figure 48. Brain Blood Concentrations for Increasing Levels of Bodyweight.

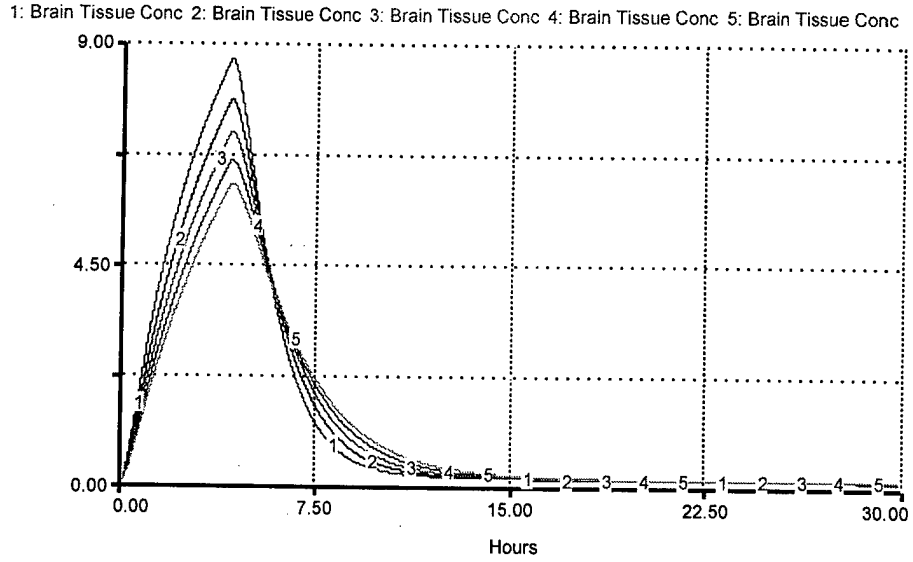


Figure 49. Brain Tissue Concentrations for Increasing Levels of Bodyweight.

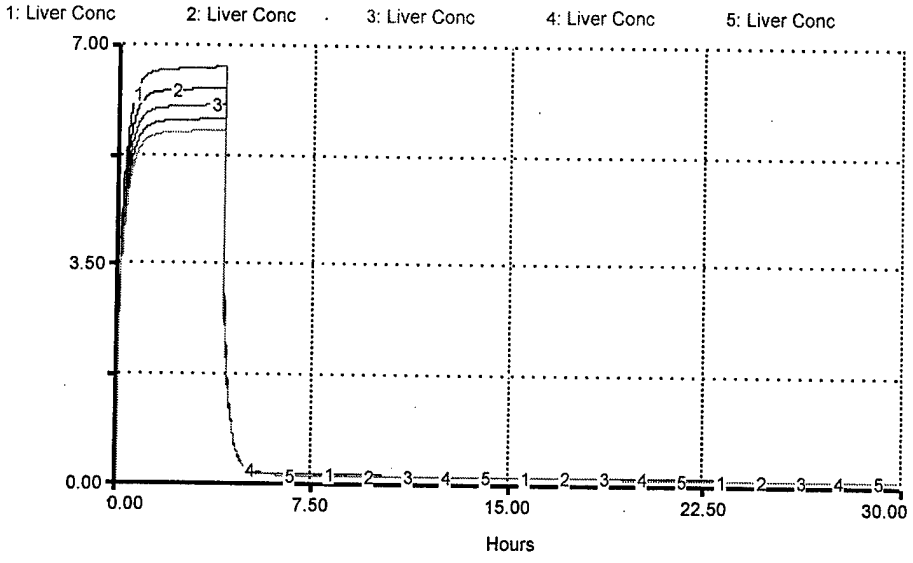


Figure 50. Liver Concentrations for Increasing Levels of Bodyweight.

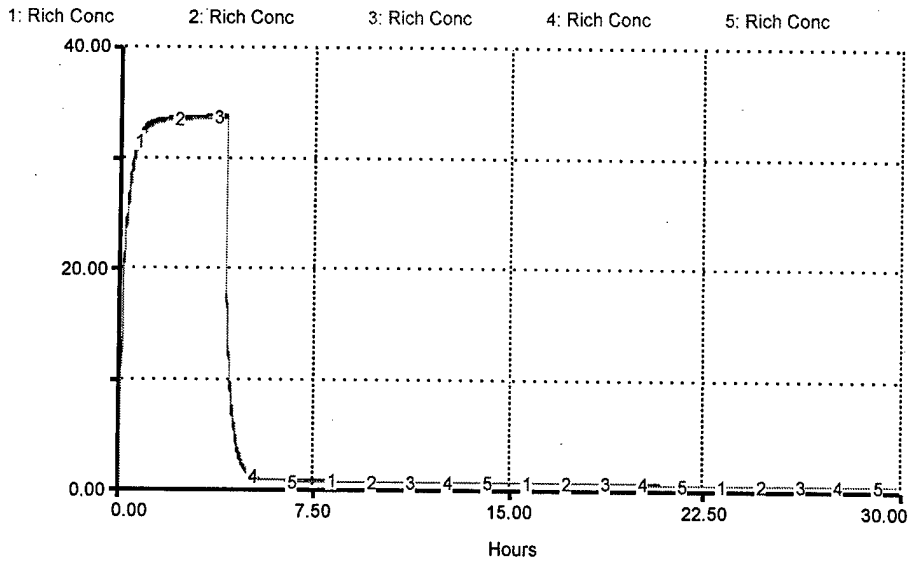


Figure 51. Richly Perfused Concentrations for Increasing Levels of Bodyweight.

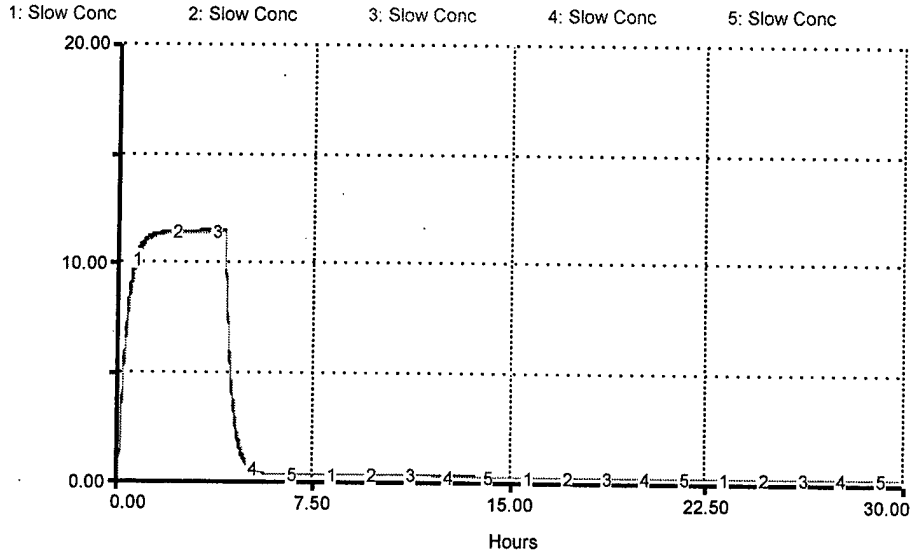


Figure 52. Slowly Perfused Concentrations for Increasing Levels of Bodyweight.

Parameter Testing:

Sensitivity Analysis of the Brain Tissue/Blood Partition Coefficient (Values = 2, 6.5, 11, 15, and 20)

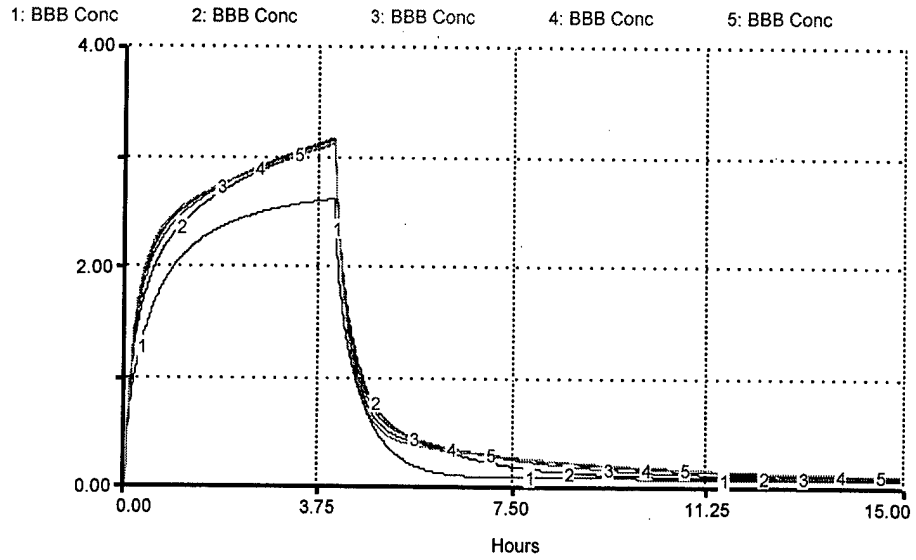


Figure 53. BBB Concentrations for Increasing Values of the Brain/Blood PC

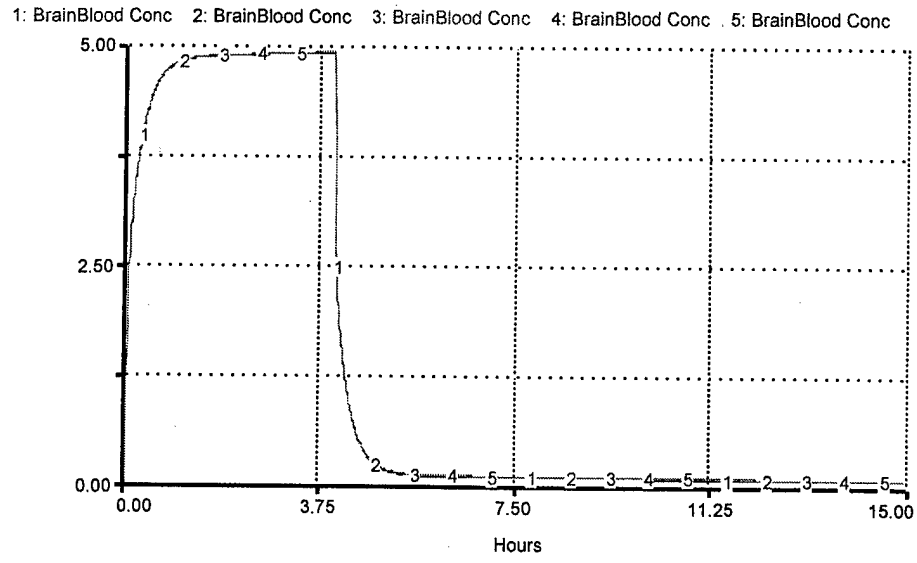


Figure 54. Brain Blood Concentrations for Increasing Values of the Brain/Blood PC

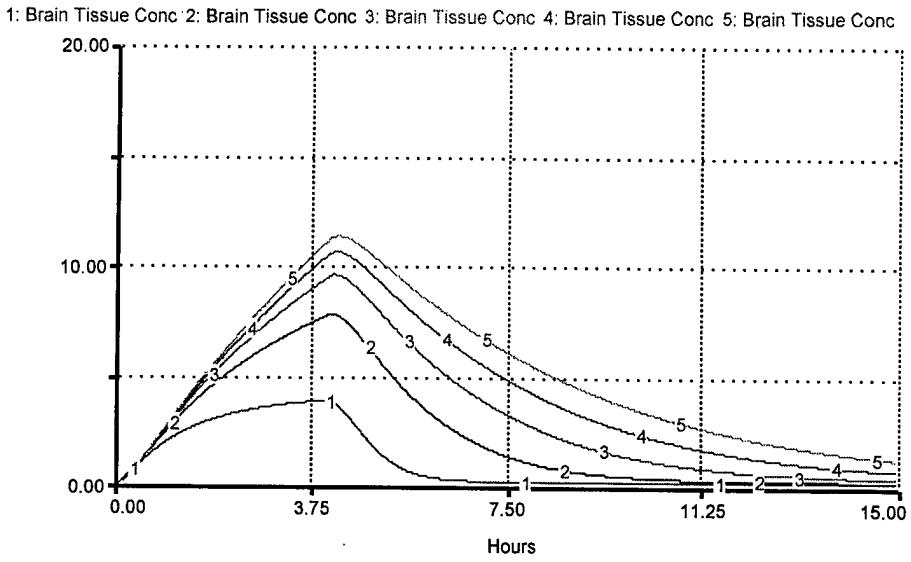


Figure 55. Brain Tissue Concentrations for Increasing Values of the Brain/Blood PC

Sensitivity Analysis of the Transfer Rate (Range = 2, 7.75, 13.5, 19.3, and 25)

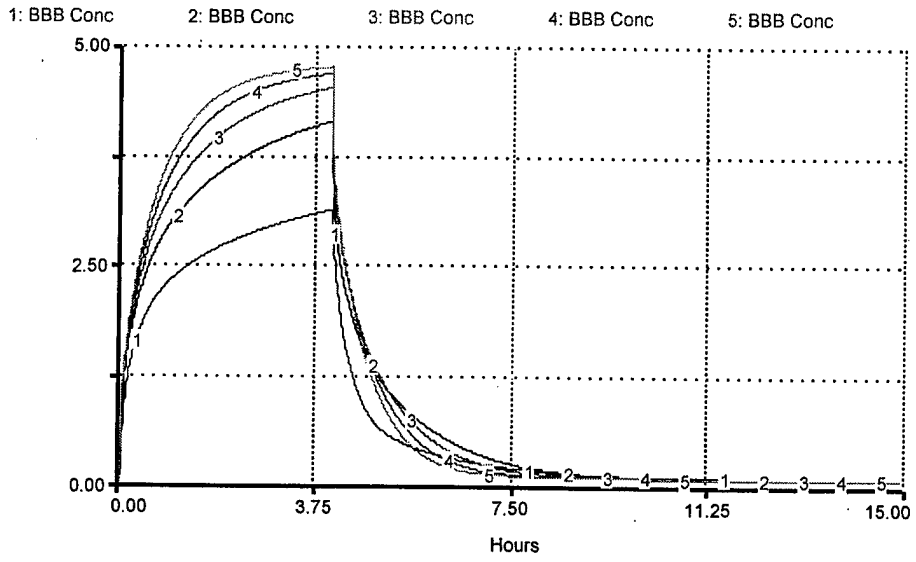


Figure 56. BBB Concentrations for Increasing Values of the Transfer Rate

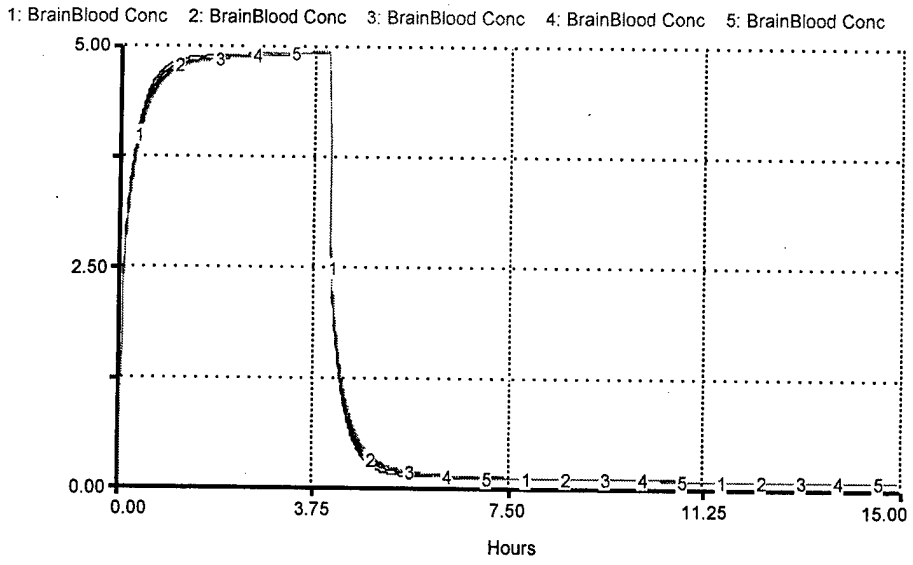


Figure 57. Brain Blood Concentrations for Increasing Values of the Transfer Rate

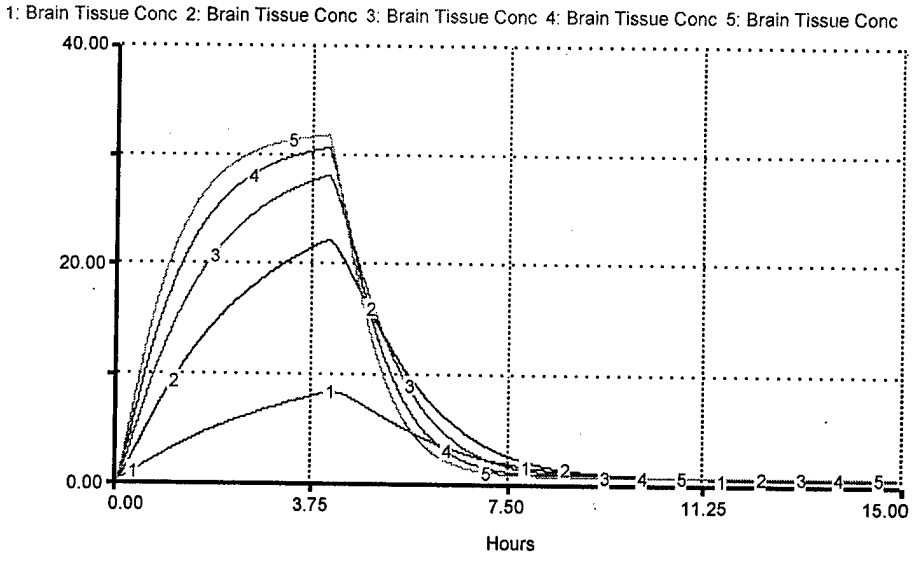


Figure 58. Brain Tissue Concentrations for Increasing Values of the Transfer Rate

Sensitivity Analysis of the Brain Km (Range = .5, 1.13, 1.75, 2.38, 3)

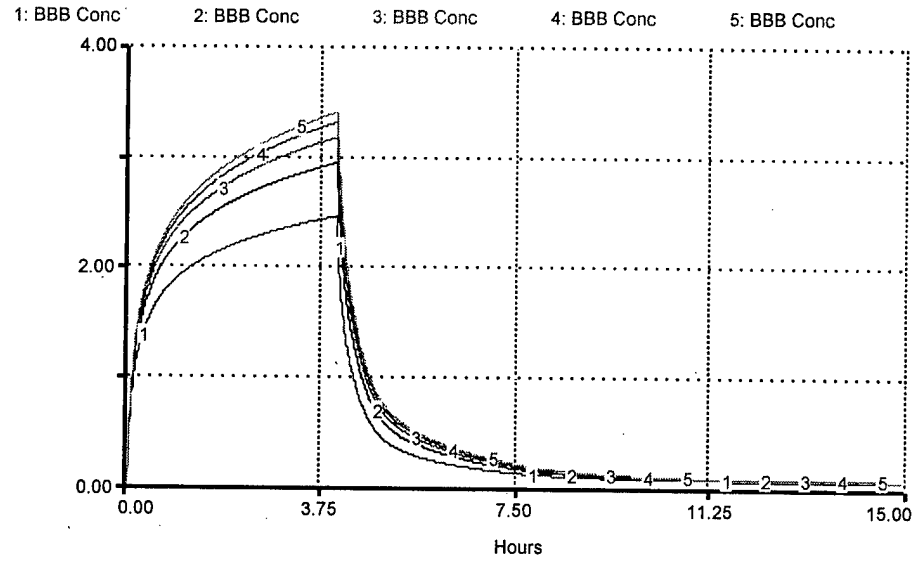


Figure 59. BBB Concentrations for Increasing Values of the Brain Km

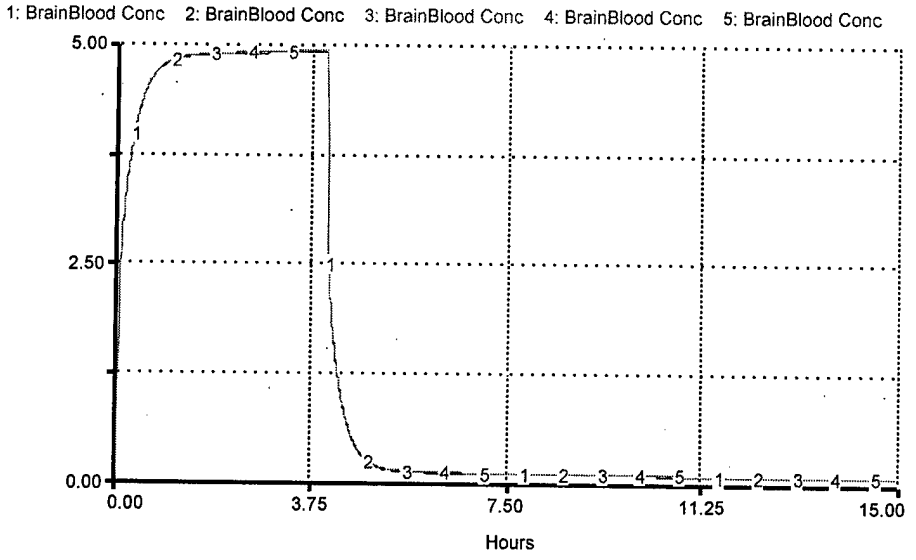


Figure 60. Brain Blood Concentrations for Increasing Values of the Brain Km

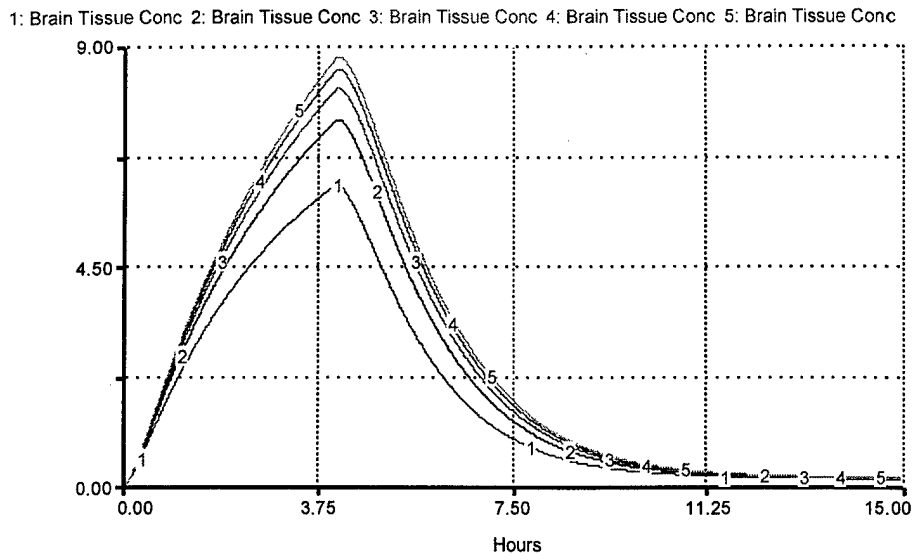


Figure 61. Brain Tissue Concentrations for Increasing Values of the Brain Km

Sensitivity Analysis of the effects of Transfer Rate (Range = 2-25) on Passive Diffusion

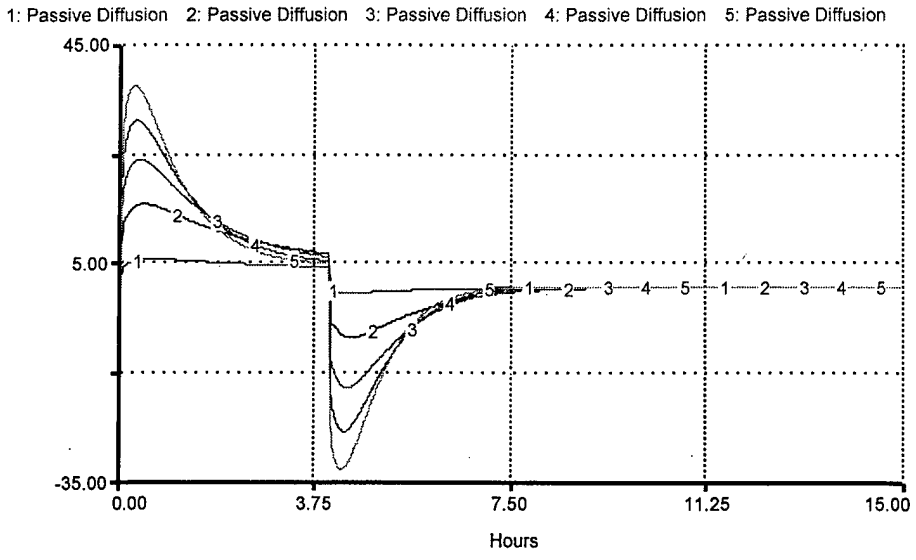


Figure 62. The Effects of Increasing Transfer Rates on Passive Diffusion

Sensitivity Analysis of the effects of increasing exercise on Passive Diffusion (110 kg)

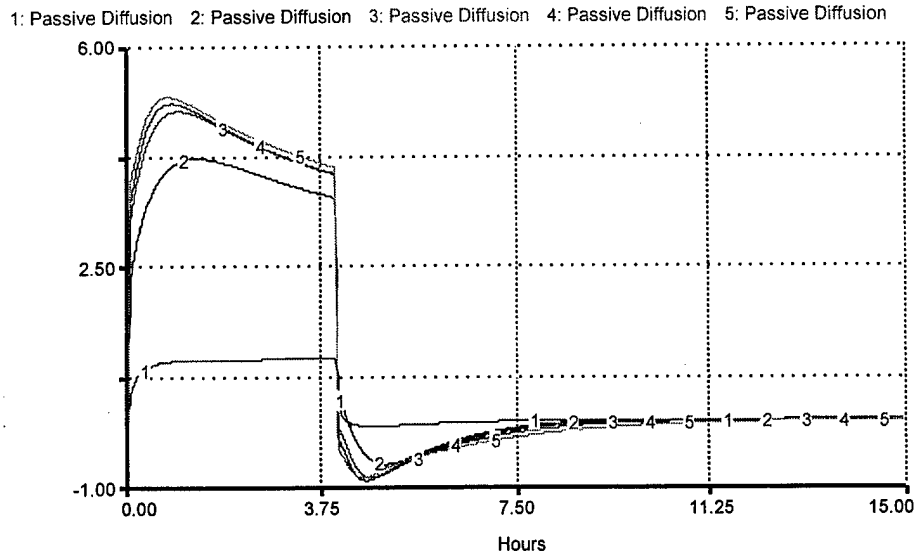


Figure 63. The Effects of Increasing Exercise Levels on Passive Diffusion

Sensitivity Analysis of the effects of the 5 levels of exercise on Brain Vmax, which then affects the overall behavior of Metabolism from the blood-brain barrier for 110 kg.

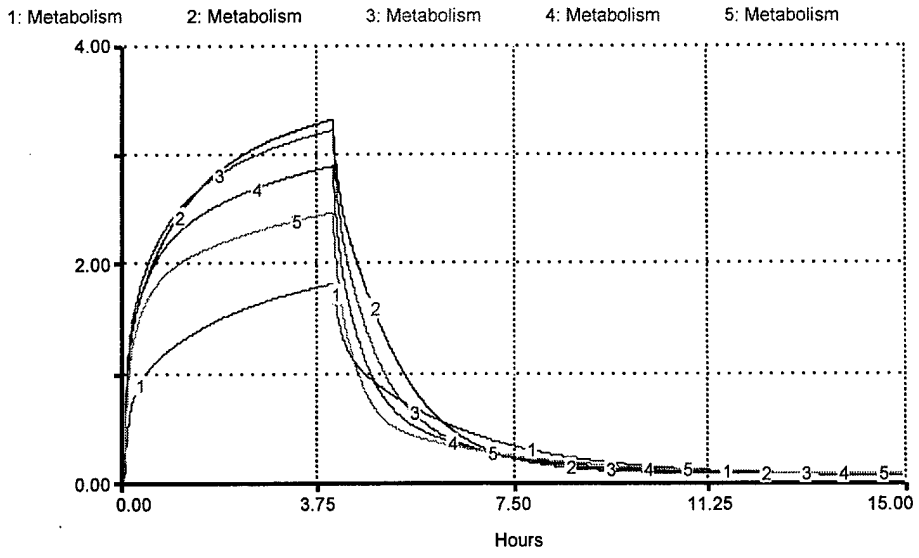


Figure 64. The Effects of Increasing Exercise Levels on Metabolism

Sensitivity Analysis of the effects of increasing exercise on Maximum Transport, which then affects the overall behavior of Mediated Transport between the brain blood compartment and the blood-brain barrier for 110 kg.

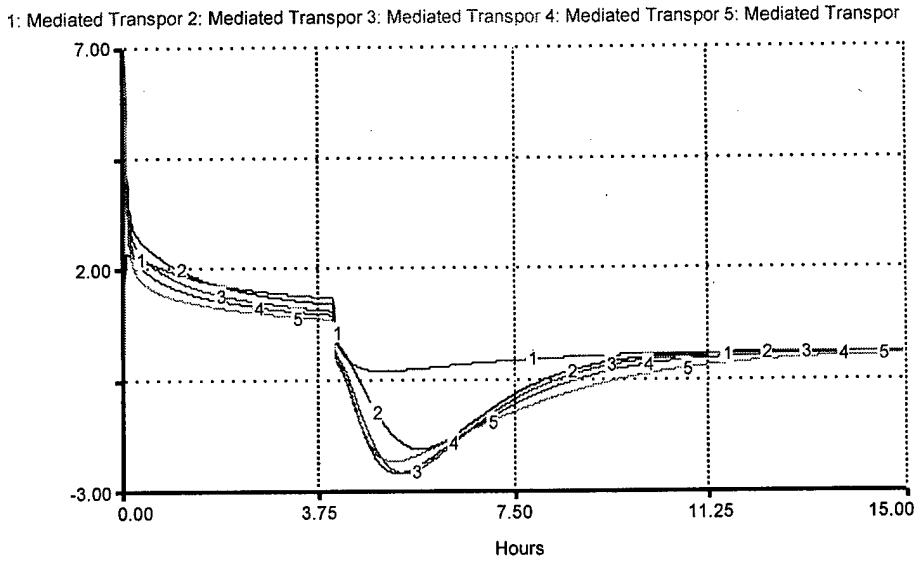


Figure 65. The Effects of Increasing Exercise Levels on Mediated Transport

Extreme Conditions:

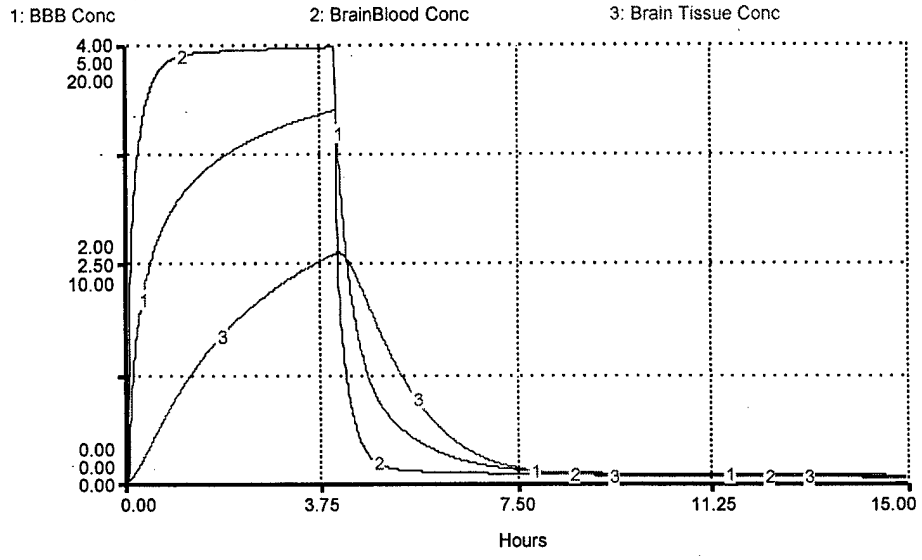


Figure 66. Brain Concentrations at a Minimum Bodyweight of 40 kg

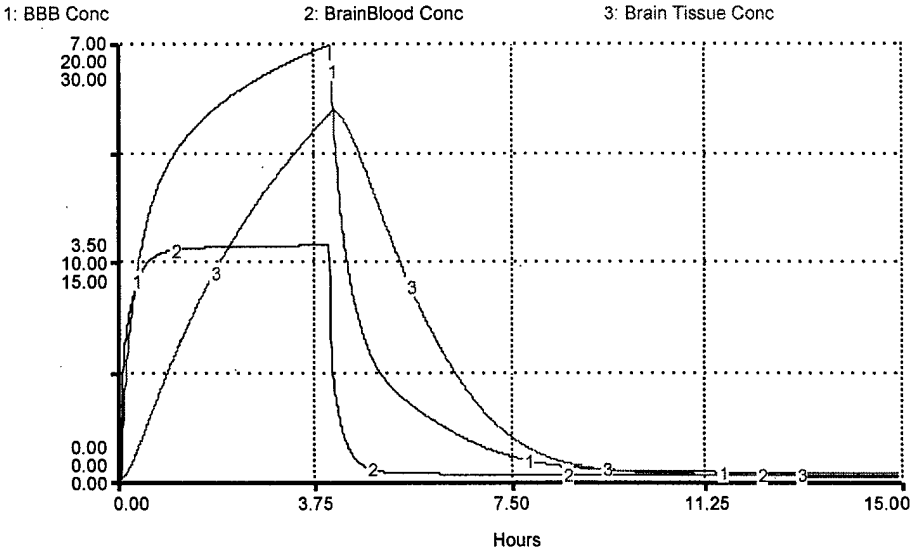


Figure 67. Brain Concentrations at a Maximum Exposure of 300 ppm

Bibliography

Abou-Donia, Mohamed B., Kenneth R. Wilmarth, Karl F. Jensen, Frederick W. Oehme, and Thomas L. Kurt. "Neurotoxicity Resulting From Coexposure to Pyridostigmine Bromide, DEET, and Permethrin: Implications of Gulf War Chemical Exposures," Journal of Toxicology and Environmental Health, 48: 35-56 (1996).

Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Trichloroethylene. DHHS Publication No. ATSDR/TP-88/24, 1989.

Allen, Bruce C. and Jeffrey W. Fisher. "Pharmacokinetic Modeling of Trichloroethylene and Trichloroacetic Acid in Humans," Risk Analysis, 13: 71-86 (1993).

Almog, Shlomo, Eyal Winkler, Yona Amitai, Sanda Dani, Menachem Shefi, Moshe Tirosh, and Joshua Shemer. "Acute Pyridostigmine Overdose: A Report of Nine Cases," Israel Journal of Medical Science, 27: 659-663 (November-December 1991).

The American Heritage College Dictionary (Third Edition). Boston: Houghton Mifflin Company, 1993.

Anger, W. Kent, Daniel Storzbach, Laurence M. Binder, Keith A. Campbell, Diane S. Rohlman, Linda McCauley, Craig A. Kovera, Kelly L. Davis, and the Portland Environmental Hazards Research Center. "Neurobehavioral Deficits in Persian Gulf Veterans: Evidence from a Population-based Study," Journal of the International Neuropsychological Society, 5: 203-213 (1999).

Aquilonius, S.M., S.A. Eckernas, P. Hartvig, B. Lindstrom, and P.O. Osterman. "Pharmacokinetics and Oral Bioavailability of Pyridostigmine in Man," European Journal of Clinical Pharmacology, 18: 423-428 (1980).

Arad, Michael, David Varssano, Daniel Moran, Ronen Arnon, Amnon Vazina, and Yoram Epstein. "Effects of Heat-Exercise Stress, NBC Clothing, and Pyridostigmine Treatment on Psychometer and Subjective Measures of Performance," Military Medicine, 157: 210-213 (1992).

Bell, Iris R. "Self-Reported Chemical Sensitivity and Wartime Chemical Exposures in Gulf War Veterans with and without Decreased Global Health Ratings," Military Medicine, 163: 725-732 (November 1998).

Belova, T.I. and B. Jonsson. "Blood-Brain Barrier Permeability and Immobilization Stress," Acta Physiologica Scandinavica, 116: 21-26 (1982).

Belova, T.I. and K.V. Sudakov. "Blood-Brain Barrier and Emotional Stress," in Stress: Neurochemical and Humoral Mechanisms. Ed. G.R. Van Loon, R. Kvetnansky, R. McCarty and J. Axelrod. New York: Gordon and Breach Science Publishers, 1989.

Blick, Dennis W., Michael R. Murphy, G. Carroll Brown, Michael G. Yochmowitz, John W. Fanton, and Stanley L. Hartgraves. "Acute Behavioral Toxicity of Pyridostigmine or Soman in Primates," Toxicology and Applied Pharmacology, 126: 311-318 (1994).

Bradbury, Michael W.B. Physiology and Pharmacology of the Blood-Brain Barrier. New York: Springer-Verlag, 1992.

Brennan, Mark A. and Michael L. Shelley. "A model of the uptake, translocation, and accumulation of lead (Pb) by maize for he purpose of phytoextraction," Ecological Engineering, 12: 271-297 (1998).

Brightman, Milton W. "The Anatomic Basis of the Blood-Brain Barrier," in Implications of the Blood-Brain Barrier and Its Manipulation. Ed. Edward A. Neuwelt. New York: Plenum Medical Book Company, 1989.

Brosschot, J.F., R.J. Benchop, G.L.R. Bodaert, M. Olff, M. DeSmet, C.J. Heijnen, and R.E.Ballieux. "Influence of Stress on Immunological Reactivity to Mild Psychological Stress," Psychosomatic Medicine, 56: 216-224 (1994).

Brown, Elizabeth A., Michael L. Shelley, and Jeffrey W. Fisher. "A Pharmacokinetic Study of Occupational and Environmental Benzene Exposure with Regard to Gender," Risk Analysis, 18: 205-213 (1998).

Clewell, Harvey J. III, Melvin E. Andersen, Michael G. MacNaughton and Bruce O. Stuart. "Toxicokinetics: An Analytical Tool for Assessing Chemical Hazards to Man," Aviation, Space, and Environmental Medicine, A125-A131, November 1988.

Cohen, Sheldon and Stephen B. Manuck. "Stress, Reactivity, and Disease," Psychosomatic Medicine, 57: 423-426 (1995).

Cohen, Sheldon, Ellen Frank, William J. Doyle, David P. Skonger, and Bruce S. Rabin. "Types of Stressors that Increase Susceptibility to the Common Cold in Healthy Adults," Health Psychology, 17: 214-223 (1998).

Cook, James E., Margaret A. Kolka, and C. Bruce Wenger. "Chronic Pyridostigmine Bromide Administration: Side Effects Among Soldiers Working in a Desert Environment," Military Medicine, 157: 250-254 (1992).

Cooper, Cary L. Stress Research: Issues for the Eighties. New York: John Wiley and Sons, 1983.

Cooper, Mary H. "Chemical and Biological Weapons," The CQ Researcher, 7: 75-95 (31 January 1997).

Delahanty, Douglas L., Angela Liegey Dougall, Leanne Hawken, John H. Trakowski, John B. Schmitz, Frank J. Jenkins, and Andrew Baum. "Time Course of Natural Killer Cell Activity and Lymphocyte Proliferation in Response to Two Acute Stressors in Healthy Men," Health Psychology, 15: 48-55 (January 1996).

Dobson, Clifford B. Stress: the Hidden Adversary. Ridgewood NJ: George A. Bogden and Son, Inc. Publishers, 1983.

Doull, John. "Specificity and Dosimetry of Toxicologic Responses," Regulatory Toxicology and Pharmacology, 24: S55-S57 (August 1996).

Elliott, Glen R. and Carl Eisdorfer. Stress and Human Health. New York: Springer Publishing Company, 1982.

Ember, Lois. "Gulf War Syndrome: Dueling studies focus on stress versus environmental exposures as cause of ills," Chemical and Engineering News, 75: 4-5 (13 January 1997).

Fiedler, Nancy, Howard Kipen, Benjamin Natelson, and John Ottenweller. "Chemical Sensitivities and the Gulf War: Department of Veterans Affairs Research Center in Basic and Clinical Science Studies of Environmental Hazards," Regulatory Toxicology and Pharmacology, 24: S129-S138 (August 1996).

Fisher, Jeffrey W., Michael L. Gargas, Bruce C. Allen and Melvin E. Andersen. "Physiologically Based Pharmacokinetic Modeling of Trichloroethylene and Its Metabolite, Trichloroacetic Acid, in the Rat and Mouse," Toxicology and Applied Pharmacology, 109: 183-195 (1991).

Forrester, Jay W. and Peter M. Senge. "Tests for Building Confidence in System Dynamics Models," TIMS Studies in the Management Sciences, 14: 209-228 (1980).

Fox, Stuart Ira. Human Physiology (Fifth Edition). Boston: Wm. C. Brown Publishers, 1996.

Friedman Alon, Daniela Kaufer, Joshua Shemer, Israel Hendler, Hermona Soreq, and Ilan Tur-Kaspa. "Pyridostigmine brain penetration under stress enhances neuronal excitability and induces early immediate transcriptional response," Nature Medicine, 2: 1382-1385 (December 1996).

Fukuda, Keiji, Rosan Nisenbaum, Geraldine Stewart, William W. Thompson, Laura Robin, Rita M. Washko, Donald L. Noah, Drue H. Barrett, Bonnie Randall, Barbara L. Herwaldt, Alison C. Mawle, and William C. Reeves. "Chronic Multisympton Illness Affecting Air Force Veterans of the Gulf War," JAMA, 280: 981-988 (16 September 1998).

Gherman, E.M. Stress and the Bottom Line. NewYork: AMACOM, 1981.

Haley, Robert W. "Is Gulf War Syndrome Due to Stress? The Evidence Reexamined," American Journal of Epidemiology, 146:695-703 (November 1997).

Hanin, Israel. "The Gulf War, Stress and a Leaky Blood-Brain Barrier," Nature Medicine, 2: 1307-1308 (December 1996).

Ho, C.W., J.L. Beard, P.A. Farrel, C.T. Minson, and W.L. Kenney. "Age, Fitness, and Regional Blood Flow During Exercise in the Heat," Journal of Applied Physiology, 82: 1126-1135 (1997).

Hughes, W. William. Essentials of Environmental Toxicology. Washington DC: Taylor and Francis, 1996.

Hyams, Kenneth C. and Robert H. Roswell. "Resolving the Gulf War Syndrome Question," American Journal of Epidemiology, 148: 339-342 (August 1998).

International Life Sciences Institute Risk Sciences Institute. Physiological Parameter Values for PBPK Models. Under Cooperative Agreement with the U.S. Environmental Protection Agency Office of Health and Environmental Assessment, December 1994.

"Introduction to the Blood-Brain Barrier." Excerpt from unpublished article, n.pag. http://www.med.ucla.edu/division/endo/TextOnly/Introduction.html. 11 November 1999.

Joellenbeck, Lois M., Philip J. Landrigan and Elaine L. Larson. "Gulf War Veterans' Illnesses: A Case Study in Causal Inference," Environmental Research, 79: 71-81 (November 1998).

Kalb, Claudia and Adam Rogers. "Stress," Newsweek: 56-63 (14 June 1999).

Kasl, Stan V. and Cary L. Cooper. Stress and Health: Issues in Research Methodology. New York: John Wiley and Sons, 1987.

Kaufer, Daniela, Alon Friedman, Shlomo Seidman, and Hermona Soreq. "Acute Stress Facilitates Long-lasting Changes in Cholinergic Gene Expression," Nature, 393: 373-376 (28 May 1998).

Keller, Jill R., Charles G. Hurst, and Michael A. Dunn. "Pyridostigmine Used as a Nerve Agent Pretreatment Under Wartime Conditions," JAMA, 266: 693-695 (07 August 1991).

Kiecolt-Glaser, J.K., J.T. Cacioppo, W.B. Malarkey, and R. Glaser. "Acute psychological stressors and short-term immune changes: What, why, for whom, and to what extent?," Psychosomatic Medicine, 54: 680-685 (1992).

LaPuma, Peter. Class Lecture for ENVR 621, Physiological Systems. School of Engineering, Air Force Institute of Technology (AU), Wright Patterson AFB OH, Spring 1999.

Lashof, Joyce C. and Joseph S. Cassells. "Illness among Gulf War Veterans: Risk Factors, Realities, and Future Research," JAMA, 280: 1010-1011 (16 September 1998).

Leach, Glenn J., R. David Russel, and John T. Houpt. "Some Cardiovascular Effects of the Insect Repellent N,N-Diethyl-m-Toluamide (DEET)," Journal of Toxicology and Environmental Health, 25: 217-225 (1988).

Maes, Michael, Dirk R. Van Bockstaele, Ann Val Gastel, Cai Song, Chris Schotte, Hugo Neels, Ingrid DeMeester, Simon Scharpe, and Aleksander Janca. "The Effects of Psychological Stress on Leukocyte Subset Distribution in Humans: Evidence of Immune Activation," Neuropsychobiology, 39: 1-9 (1999).

Meadows, Donella H. "The Unavoidable A Priori," in Elements of the System Dynamics Method. Ed. Randers, J. Cambridge, MA: Productivity Press, 1980.

McCain, Wilfred C., Robyn Lee, Mark S. Johnson, Janet E. Whaley, Jennifer W. Ferguson, Patricia Beall, and Glenn Leach. "Acute oral Toxicity Study of Pyridostigmine Bromide, Permethrin, and DEET in the Laboratory Rat," Journal of Toxicology and Environmental Health, 50: 113-114 (07 February 1997).

McCarty, R. "Stress Research: Principles, Problems, and Prospects," in Stress: Neurochemical and Humoral Mechanisms. Ed. G.R. Van Loon, R. Kvetnansky, R. McCarty and J. Axelrod. New York: Gordon and Breach Science Publishers, 1989.

Murphy, Frances M. "Gulf War Syndrome," British Medical Journal, 318: 274-275 (30 January 1999).

Nelson, R.A. Determining Types of Health Effects to Persian Gulf Veterans Due to Exposure to Occupational Hazards. Thesis No. AFIT/GEE/ENV/95D-13, Air University, USAF Institute of Technology, Wright-Patterson Air Force Base, OH, 1995

"New Persian Gulf Possibilities," Environmental Health Perspectives, 104: 819-820 (August 1996).

Opstad, Per-Kristian. Medical Consequences in Young Men of Prolonged Physical Stress with Sleep and Energy Deficiency. Norwegian Defence Research Establishment (NDRE). NDRE/Publication-95/05586, December 1995.

Pardridge, William M. "CNS Drug Design Based on Principles of Blood-Brain Barrier Transport," Journal of Neurochemistry, 70: 1781-1792 (1998).

Pennisi, Elizabeth. "Chemicals Behind the Gulf War Syndrome?," Science, 272: 479-480 (26 April 1996).

Peterson, S. and B. Rishmond. Stella II Technical Documentation. Hanover, NH: High Performance Systems, 1994.

"Physiological Parameter Values for PBPK Models," A Report Prepared by The International Life Sciences Institute Risk Science Institute, December 1994.

Pollet, Claudia, Benjamin H. Natelson, Gudrun Lange, Lana Tiersky, John Deluca, Therese Policastro, Parul Desai, John E. Ottenweller, Leo Korn, Nancy Fiedler, and Howard Kipen. "Medical Evaluation of Persian Gulf Veterans with Fatigue and/or Chemical Sensitivity," Journal of Medicine, 29: 101-112 (1998).

Presidential Advisory Committee (PAC) on Gulf War Veterans' Illnesses: final report. Washington, DC: US GPO, 1996.

Ramsey, John C. and Melvin E. Andersen. "A Physiologically Based Description of the Inhalation Pharmacokinetics of Styrene in Rats and Humans," Toxicology and Applied Pharmacology, 73: 159-175 (1984).

Randers, J. "Guidelines for Model Conceptualization," in Elements of the System Dynamics Method. Ed. Randers, J. Cambridge, MA: Productivity Press, 1980.

Rapoport, Stanley I. Blood-Brain Barrier in Physiology and Medicine. New York: Raven Press, 1976.

Robinson, Peter J. and Stanley I. Rapoport. "Blood-Brain Barrier," Encyclopedia of Human Biology, 1: 715-727 (1991).

Rowell, Loring B. Human Cardiovascular Control. New York: Oxford University Press, 1993.

----. Human Circulation Regulation During Physical Stress. New York: Oxford University Press, 1986.

Saltin, B., G. Radegran, M.D. Koskolou, and R.C. Roach. "Skeletal Muscle Blood Flow in Humans and its Regulation During Exercise," Acta Physiologica Scandinavica, 162: 421-436 (1998).

Sapolsky, Robert M. "The Stress of Gulf War Syndrome," Nature, 393: 308-309 (28 May 1998).

----. "Why Stress is Bad for your Brain," Science, 273: 749-750 (09 August 1996).

----. Stress, the Aging Brain, and the Mechanisms of Neuron Death. Cambridge, Massachusetts: The MIT Press, 1992.

Selye, Hans. The Stress of Life (Revised Edition). New York: McGraw-Hill Book Co, 1976.

Sharab, Yehonatan, Yehuda L. Danon, Haim Berkenstadt, Shlomo Almog, Aviva Mimouni-Bloch, Amnon Zisman, Sanda Dani, and Jacob Atsmon. "Survey of Symptoms Following Intake of Pyridostigmine During the Persian Gulf War," Israel Journal of Medical Science, 27: 656-658 (November-December 1991).

Sharma, Hari Shanker, Jorge Cervos-Navarro, and Prasanta Kumar Dey. "Increased Blood-Brain Barrier Permeability Following Acute Short-term Swimming Exercise in Conscious Normotensive Young Rats," Neuroscience Research, 10: 211-221 (1991).

Shelley, Michael L. Class Notes for ENVR 642, System Dynamics. School of Engineering, Air Force Institute of Technology (AU), Wright Patterson AFB OH. Spring 1999.

Shen, Z.-X. "Pyridostigmine Bromide and Gulf War Syndrome," Medical Hypotheses, 51: 235-237 (September 1998).

Shephard, Roy J. "Immune Changes Induced by Exercise in an Adverse Environment," Canadian Journal of Physiology and Pharmacology, 76: 539-546 (May 1998).

Shephard, Roy J., John W. Castellani, and Pang N. Shek. "Immune Deficits Induced by Strenuous Exertion under Adverse Environmental Conditions: Manifestations and Countermeasures," Critical Review in Immunology, 18: 545-568 (1998).

Sgoutas-Emch, S.A., J.T. Cacioppo, B.N. Uchino, W. Malarkey, D. Pearl, J.K. Kiecolt-Glaser, and R. Glaser. "The Effects of an Acute Psychological Stressor on Cardiovascular, Endocrine, and Cellular Immune Response: A Prospective Study of Individuals High and Low in Heart Rate Activity, " Psychophysiology, 31: 264-271 (1994).

Smith, James J. and John P. Kampine. Circulatory Physiology – the Essentials (Third Edition). Baltimore: Williams and Wilkins, 1990.

Smith, Quentin R. and James Stoll. "Blood-Brain Barrier Amino Acid Transport," in Introduction to the Blood-Brain Barrier. Ed. William M. Pardridge. New York: Cambridge University Press, 1998.

Suckling, A.J., M.G. Rumsby, and M.W.B. Bradbury. The Blood-Brain Barrier in Health and Disease. Chichester, England, 1986.

Ursin, Holger and others. Psychobiology of Stress: A Study of Coping Men. New York: Academic Press, Inc., 1978.

Van Haaren, Frans, Reinoud De Jongh, James B. Hoy, Janet L. Karlix, Charles J. Schmidt, Ian R. Tebbett, and Donna Wielbo. "The Effects of Acute and Repeated Pyridostigmine Bromide Administration on Response Acquisition with Immediate and Delayed Reinforcement," Pharmacology, Biochemistry and Behavior, 62: 389-394 (February 1999).

Verschoyle, R.D., A.W. Brown, C. Nolan, D.E. Ray, and T. Lester. "A Comparison of the acute toxicity of neuropathology, and electrophysiology of N,N-diethyl-m-tolumide and N,N-dimethyl-2,2-diphenylacetamide in rats," Fundamental Applied Toxicology, 18: 79-88 (1992).

Wegman, David H., Nancy F. Woods, and John C. Bailar. "How Would We Know a Gulf War Syndrome If We Saw One?," American Journal of Epidemiology, 146: 704-712 (November 1997).

Wester, R.C., D. Quan and H.I. Maibach. "In Vitro Percutaneous Absorption of Model Compounds Glyphosate and Malathion from Cotton Fabric into and through Human Skin," Food and Chemical Toxicology, 34: 731-735 (August 1996).

Wickelgren, Ingrid. "Rat Model for Gulf War Syndrome?," Science, 278: 1404 (21 November 1997).

Zakowski, S., M.H. Hall, and A. Baum. "Stress, stress management, and the immune system," Applied Preventive Psychology, 1: 1-13 (1992)