THE FLORIDA STATE UNIVERSITY

COLLEGE OF ENGINEERING

A PHYSIOLOGICALLY BASED TOXICOKINETIC (PBTK) MODEL FOR INHALATION EXPOSURE TO BENZENE AND ITS ENGINEERING APPLICATIONS

By

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A Dissertation submitted to the
Department of Civil & Environmental Engineering
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Degree Awarded:
Spring Semester, 2004

UMI Number: 3137446

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For Aniket, who deserves this Ph.D. more than I do.

ACKNOWLEDGEMENTS

A number of factors and people have helped in making this dissertation a successful one. First and foremost, I would like to gratefully thank my advisor and mentor throughout my entire doctoral program, Dr. Andrew Dzurik, for his unflinching support of all my ideas, for his patience and kindness, for thoughtful comments and suggestions and for just being there with me throughout this journey, carrying my hand through the end.

My committee members deserve much appreciation and credit for all their help. Dr. Danuta Leszczynska's questions led me to new avenues of research; her words of encouragement always came just as I despaired. Dr. Amy Chan Hilton taught me structure, time management and the path to an organized analytical process in research. My minor area advisor Dr. Malak Kolta's constant encouragement and belief in me is the only reason I could dare to study the field of Toxicology with such ease and comfort and I am inspired to keep working in this field despite my limited background in it. Dr. Krishna Arora deserves a special mention and my gratitude in agreeing to serve as my outside committee member and helping me out in my last few days.

I am also thankful to Dr. Hall, Dr Wekezer, Dr Nnaji, Mrs. Ross, Mrs. Morris and Mrs. Gallon from the Civil and Environmental Engineering department, who in their own ways kept my morale up and helped out in any way possible.

Friends and family often play the biggest role in a person's academic success and I am indebted to all my friends for constantly encouraging me with their love and support. Shubha Kashinath, my dearest friend, Tanya Green, Claudia Wilson, Omar Beckford, Hafiz Ahmad and Dr. Saif Haroon in engineering and at DEP, Dawn Marcus,

Tamara Blyden and Shannan Gunoe, all stood by me and helped me in their own ways and I am really thankful for their advice, caring and friendship.

While hard work has its place, blessings, prayers and belief are sometimes stronger and that is exactly what my two sets of parents have always showered on me; the parents whom I was born to Maya and Vijendranath Shenoy, and the parents that I gained by marriage Pratibha and Pramod Kulkarni. My parents have sacrificed a lot to see me in this place. Raising three daughters to be strong, independent women is a tall order in a patriarchal society, but they have been relentless in their belief that we were meant to do great things. They set me on this path of academic curiosity, desire for aiming and achieving high and untiring hard work and I hope that with this success I have fulfilled at least a part of their dream for me. My parents in law have been an incredible source of support and encouragement and I am blessed to be a part of their family. They have been a part of my final semester ups and downs, taking over all my duties at home, as I tried to complete all my research, write my dissertation, defend it and race to the finish line.

I would like to thank my sisters Suchitra Shenoy Mukherjee and Madhuri Shenoy and brothers by marriage Amey Kulkarni and Sudipto Mukherjee for being my role models in many ways without even realizing it. My sisters will always be my big inspirations, my dearest friends, critics and counselors. I get my zeal for life and learning from them and they are the ones who have shown me that teaching is my calling in life.

Most of all I dedicate this piece to my husband Aniket; my best friend and soulmate, because without him, this truly would not have happened. He has walked by my side from the very beginning, supporting both financially and emotionally, my decision to enroll and get through this program. He has double checked my assignments, prepped me for quizzes and exams, patiently sat through my presentations practices, heard out my project ideas and helped make them better, critiqued my term papers, accompanied me on my innumerable trips to the library and advised and helped me shape this research. In this final semester, he has spent many a sleepless night with me, helping in any way possible and then some. Words fall short of describing his role in this research and I truly believe that he deserves much of the credit for this work if not more.

Overall I feel blessed to have had all these wonderful people around me, who have made this phase of my life seem effortless and forever memorable.

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ABSTRACT

Physiologically Based Pharmacokinetic/Toxicokinetic (PBPK/TK) Models are commonly used in the pharmaceutical and toxicological sciences to understand the fate and disposition of drugs in the human body. However, they are not being applied in environmental engineering studies involving risk, except in some health risk assessment studies. The risk posed by the adverse effects of a xenobiotic on the human body can be quantified using PBPK/TK models and such numbers can be used in engineering situations such as determining clean up levels at remediation sites, locating hazardous waste facilities and installing pollution control and monitoring devices. Thus the PBPK/TK modeling can serve as an appropriate tool in global initiatives such as Risk Based Corrective Action (RBCA).

This study presents two areas of new and contributory research. The first is a toxicological model for benzene and its major metabolites considering different doses and forms (continuous and intermittent) of inhalation exposure in male and female subjects of the human population. This model considers the bone marrow as a separate compartment and uses an extended version of the Michaelis- Menten kinetics for enzymes, both areas of study, not strongly addressed before. The results of this part of the study reveal that PBPK/TK models can successfully simulate intermittent exposures, which had not been done before. The benzene concentration levels are not significantly different in male and female exposures. The addition of the bone marrow as a separate compartment is recommended in all benzene models. The sensitivity analysis based on the Monte Carlo technique indicate that most of the rate constants tested, that are involved in the metabolic processes are stable; 2 are highly sensitive. The partition coefficient of benzene for the fat compartment is somewhat stable, but the model probably over-predicts the benzene concentrations in the fat compartment.

The second part of the study is to determine the possible use of PBPK/TK models in environmental engineering studies. Verbal communication from personnel of the Florida Department of Environmental Protection encouraged the theory that the model developed in this study can be potentially used in RBCA aspects of environmental engineering. So, an environmental engineering case study was presented to demonstrate its use as a screening tool at a petroleum contaminated site. Benzene concentration levels are estimated in male workers, at the site working on a remediation project for 3 months and getting exposed to 8 hours/day, 5days/week of continuous benzene exposure. An example on the potential use of the model in air pollution engineering problems is also presented. The results from this portion of the study as well as personal communication with FDEP personnel indicate that the PBPK/TK model developed in this study will benefit environmental engineering studies.

CHAPTER 1

1. INTRODUCTION

1.1 Risk Based Corrective Action (RBCA)

Environmental engineering studies, particularly those involving hydrocarbon contamination sites are looking at risk based corrective actions (RBCAs) to optimize engineering solutions needed to solve complex environmental pollution and contamination problems. The RBCA initiatives follow a three-tiered approach to protecting human health and welfare in conjunction with site specific conditions that influence the current and potential land uses, and institutional and environmental controls. Under RBCA new standards are being developed such as the ones for soil cleanup target levels (SCTLs) that are not as conservative as the ones currently in existence (Chapter 62-777, Florida Administrative Code (F.A.C.); Chapter 62-771, F.A.C). The idea is to implement only as many environmental engineering techniques in the clean up process, thereby cleaning up only as much as would optimally protect the human health, instead of remediating to the conservative near zero standard levels currently imposed by law.

As more and more states are realizing the benefits of RBCA, legislatures are passing laws encouraging states to develop rules and implement RBCA. In Florida, the Governor passed the "Global RBCA" bill into law on June 20, 2003. The Florida Department of Environmental Protection (FDEP) is in the process of rule making for RBCAs related to dry cleaning, petroleum and Brownfield activities among others (Ruddell, 2003).

The RBCA initiatives have shifted the focus of environmental engineering from highly conservative cleanup numbers to engineering for realistic risk reduction. Issues such as ineffective resource allocation, high cost and moderate gain, overly conservative cleanup goals, and questionable risk reduction – all factors in hindering the development of new remediation technologies due to the pre-existing risk structure are addressed by RBCA (Society of Risk Analysis, 2004). The results translate into savings of time, money and efforts for all parties involved in the contamination cleanup. Thus engineering and health risk assessments using processes such as RBCA go hand in hand.

1.2 Physiologically Based Pharmacokinetic/Toxicokinetic Models

With the growing advent of health risk assessments in engineering, tools commonly used to assess risk in non-engineering areas are becoming more mainstream in engineering. One such tool is a form of modeling called "Physiologically Based Pharmacokinetic or Toxicokinetic (PBPK/TK) modeling. The words "pharmacokinetic (PK) and toxicokinetic (TK) are used interchangeably throughout the text as explained in chapter 2. Models developed using the PK/TK principles are used in estimating the toxicity of chemicals. These models simulate the fate and disposition of a chemical, for example an environmental pollutant, through the human body, as it is absorbed, distributed, metabolized and excreted from the body. The results from such models are chemical concentrations that will eventually stay in the body post-exposure, which will help determine the risk of that individual to the specific chemical that was modeled.

Non-cancer toxicological risk analysis is currently based on reference doses and reference concentrations based on animal studies performed using specific routes of exposure. When extrapolation is needed between routes of exposure, rudimentary calculations are performed that do not account for the toxicokinetic properties of the chemical. A PBPK model accounts for the physiology of the body and the toxicokinetic properties of the chemical and thus provides realistic risk numbers.

For example, consider a situation where an underground petroleum storage tank has leaked and benzene vapors are being released into the environment through outlets (e.g.

cracks or fissures in the ground) present in the vicinity. This can happen because benzene volatilizes easily and tries to escape into the ambient environment in vapor form. The PBPK models estimate the concentrations of benzene as well as its major metabolites in the human body after exposure to the benzene vapors. These concentrations help in determining the potential nature and extent of adverse effects from the chemical exposure. Based on the concentration results from the model and the corresponding adverse health effects, an optimum cleanup level can be determined.

Thus, PBPK/TK models help determine the human body concentration levels from chemical exposures. These concentration values can then be applied in risk calculations, which in turn can be used in determining the appropriate engineering technique to be used for remediation. Cost- benefit analyses can be performed to reveal benefits such as lowered amount of excavation, transportation, instrumentation, monitoring or other kinds of installation costs that form a bulk of the environmental engineering expenses.

Such models are finding acceptance with many organizations including the United States Environmental Protection Agency (USEPA). In fact, USEPA's inhalation RfC (reference concentration) for vinyl chloride is based on a pharmacokinetic modeling study on vinyl chloride (Clewell et. al., 2001). As more and more uses and applications of these models are discovered, their significance will be greatly heightened.

1.2.1 PBPK/TK in Engineering

A thorough literature review, at the present time, revealed that PBPK/TK models are rarely being used in environmental engineering studies, especially in areas such as health risk assessment. A number of assessments are still being based on equations that were developed several years ago which included many assumptions. These assumptions might not necessarily be relevant in lieu of new research and information that have been published since. Moreover, it was surprising to see that while a number of new models are developed to study multiple pathway exposure through different environmental media, existing ones such as the PBPK/TK models have not been paid as much attention by environmental engineers. This can possibly be explained by the fact that the PBPK models require a detailed understanding of the physiology of the human body, the xenobiotic characteristics as well as an idea of how to apply such modeling in

engineering. Since, no studies are reported in literature where PBPK/TK models have been used in environmental engineering situations, this research attempts to fill in such a gap. It is hoped that the study presented here can draw the attention of environmental engineers towards the benefits and flexibility of PBPK/TK modeling, by showing readers details on the model development process as well as some possible engineering applications of such models.

In this study, a six-compartmental PBPK/TK model is developed for the chemical benzene. Benzene is a known human carcinogen and is a significant environmental pollutant (USEPA, 2002). Human beings are mostly exposed to benzene by the inhalation route of exposure from occupational sources or personal exposure sources such as automobile exhaust, fueling cars, cigarette smoking, and from some indoor sources such as paints, adhesives, etc (Wallace et. al., 1996). Benzene is reported to cause a number of blood disorders such as pancytopenia, aplastic anemia, acute myeloid leukemia, etc. The organ most targeted by benzene is the bone marrow (ATSDR, 2003).

The model developed in this study divides the body into six compartments, including the bone marrow and studies the effects of benzene as well as its major metabolites on the human body. Potential applications of this model in environmental engineering studies are then presented.

1.3 Problem Statement and Solution Methodology

1.3.1 Problem Statement

PBPK/TK modeling is used in the pharmaceutical industry to determine the exact dosing regimen of drugs for treatment of patients. Despite the widespread use of PBPK modeling in many disciplines, the United States Environmental Protection Agency (USEPA) and most national and international environmental organizations have spent only the last few years allocating funds or encouraging the use of PBPK modeling in various environmental studies. Even then, the funds are devoted to PBPK/TK modeling in environmental science areas than in environmental engineering research. However, as interest in this form of modeling increases, it is hoped that engineering can benefit from

the flexibility afforded by the PBPK/TK models. This research hopes to assist the decision making process of whether or not the model can be applied in environmental engineering studies.

In toxicology, as well there are a number of gaps in the research pertaining to toxicokinetic models. It is a research area that has emerged over the past 20 years or so, as computers became increasingly used for modeling and as more experiments helped discover new information on chemicals. One issue with previously developed PBPK>TK models is that they model for long continuous exposure duration. Environmental exposures that are non-occupational in nature are usually intermittent, with brief periods of exposure followed by longer periods of non-exposure. Thus PBPK/TK models should model both intermittent and continuous exposures. Both these forms of exposure are addressed in the model developed here.

Specifically for the chemical benzene there are some gaps in research. Over the past decade or so newer research has introduced the bone marrow as the main target organ for benzene toxicity and is also the organ where the carcinogenic effects of benzene are manifested. However, a number of previous PBPK/TK models for benzene do not study the bone marrow as a separate compartment. Only two previous models have considered the bone marrow as an individual compartment in their studies (Travis, 1990 and Bois and Paxman, 1992).

It has also been determined that benzene is not as toxic as some of its metabolites, making benzene by-products such as phenol, hydroquinone and benzoquinone the more toxic chemicals. To completely explain the metabolite kinetics, the simple Michaelis Menten expression for saturation kinetics of enzymes does not suffice, because this equation does not account for the effects of the metabolic interactions (Lovern et. al., 1999, Cole, 2001). While a number of previous PBPK/TK models did not even account for the benzene metabolites, the Bois and Paxman, (1992) model studied benzene metabolites using the standard Michaelis Menten expression, which is insufficient to provide accurate results. Since it is now proven that the toxic effects of benzene occur mainly as a result of its metabolism to toxic metabolites, this analysis is important. To date, the only model that has accounted for metabolic kinetic interactions for benzene is the Cole model (2001).This is done by extending the standard Michaelis Menten

expression so that other metabolite concentrations are considered in each metabolic process. However, this model is also lacking because the bone marrow is not studied as a separate compartment.

Hence a number of challenges are posed because of gaps in literature and research to date. A systematic process was adopted to try and answer some of these unknowns in research and more importantly to determine the suitability of such models in environmental engineering.

1.3.2 Solution Methodology

Given the lack of research supporting the benefits of PBPK modeling in environmental engineering situations, this research aims to develop a multi-compartmental PBPK model. The solution methodology for the problems posed in the previous section can be divided into several steps based on the issues in question. These are organized in the following manner:

1.3.2.1 Why benzene? While any environmental pollutant can be modeled using the PBPK approach and for any route of environmental exposure, this research models inhalation exposure to benzene.

Benzene is the chemical of choice based on several different reasons such as:

i) It is a known human carcinogen as defined by the USEPA (Group A) and the International Cancer Research Agency (IARC) (Class 1) (IARC, 1987; Hricko, 1994; USEPA, 1998).

ii) It is among the 20 most produced substances in the US, with the latest Toxic Release Inventory (TRI) quoting a value of 5,894,659 pounds as the amount of benzene present in the air over the year 2001 (TRI, 2001) (See Appendix A)

iii) Benzene is a significant environmental pollutant in all environmental media. In addition to the benzene concentrations released to the air as described above, accidental spills, leaks from underground petroleum storage tanks has led to widespread soil, water and groundwater contamination.

iv) In the human body, the metabolites of benzene are supposed to cause the more serious adverse effects, even more than benzene itself because of their complex interactions with the each other and with the human body. These processes make for an interesting and challenging model development process.

1.3.2.2 Bone marrow and enzyme kinetics – the new areas of research. A significant contribution made by this research will be the separation of the bone marrow as a separate compartment and the using an extended Michaelis Menten equation for saturation enzyme kinetics.

The inclusion of bone marrow as a separate compartment is considered pertinent based on the fact that research over the past decade or so has shown that in addition to the liver, the bone marrow is also capable of metabolizing benzene (cit). This is because of the presence of the cytochrome P450 enzymes, primarily responsible for the benzene metabolism. The bone marrow is also an organ where the most severe adverse effects of benzene exposure in humans are observed, namely pancytopenia, aplastic anemia, acute myeloid leukemia, etc (ATSDR, 2003). Most of the research and data available on bone marrow and benzene concentrations in bone marrow are from laboratory experimentation using animals as research subjects. The results from such studies have only been modeled in the Bois and Paxman study (1992).

The extended Michaelis Menten expression is a newer finding (Lovern et. al., 1999; Cole, 2001) which accounts for the effect of one metabolite's breakdown rate on that of another. This kind of study has been conducted only once before in modeling and only for the study of liver kinetics.

The bone marrow metabolic interactions have only been studied by Bois and Paxman (1992) using PBPK modeling. However, in the study the authors used the standard Michaelis Menten which can compromise the results of the model.

Thus, the results from this model with the inclusion of bone marrow and the extended Michaelis Menten kinetics used to explain metabolite interactions should add significantly to the pool of literature on benzene analysis.

1.3.2.3 Inhalation as a route of exposure. After selecting benzene as the choice xenobiotic, the choice of inhalation as a route of exposure is a simple one. This is because the highest potential for risk from benzene is by inhalation exposures (Wallace (1986), ATSDR (2003)). While ingestion and dermal contact do account for some adverse effects (IARC, 1987), the risk from inhalation exposure is much higher. This is especially true because of the volatile nature of benzene and its ability to easily diffuse into the air over vast distances affecting large populations. Even when the primary exposure route may be ingestion or dermal contact, inhalation cannot be ruled out as a sub-entry route, which increases the significance of modeling the inhalation exposure of benzene.

1.3.2.4 Intermittent and continuous exposures. In most PBPK/TK modeling studies, exposures are usually modeled as continuous. However, in environmental engineering situations exposures can be continuous or intermittent depending on the exposure conditions. Hence the model is executed to produce results based on both intermittent and continuous exposures.

1.3.2.5 Data Sources for human exposure to benzene. Experimentation was not conducted for this research. Therefore, most data used in the model are obtained from current literature and/or calculated based on the available resources. A number of research studies have been performed to provide data on benzene concentration levels in different parts of the human body following varied periods of exposure. For example *in vivo* studies using animal models and in vitro studies, epidemiological studies that have followed population groups for varying periods of time or laboratory analyses that have used the gas chromatography mass spectroscopy (GC MS) equipment to determine levels of benzene and/or its metabolites in blood and urine.

The different sources of data used for different parameters in the model are referenced as they are cited in the text of this document. The data sources for the model validation are cited and discussed in chapter 6.

1.3.2.6 Modeling for male and female human subjects. The model uses both healthy adult male and female physiological data to predict benzene concentrations in

both population groups. It is anticipated that future research in this area will allow the modeling of other population groups including pregnant women (significant because benzene does cross the placenta and adversely affect the fetus) and children. Other routes of exposure such as dermal or ingestion exposures may also be considered in future projects and is currently beyond the scope of this research.

1.3.2.7 Monte Carlo Simulations for Sensitivity Analysis. There are a number of uncertainties involved in developing and running PBPK/TK models and often times such uncertainties might have a significant impact on the model results. Hence the Monte Carlo simulation technique is applied in this model to perform a sensitivity analysis of some model parameters as described in chapter 5. The technique involves the use of a random number generator to select parameter values from a known or suspected distribution. After the selection, the model is run and the simulation results are saved. Iterations are performed until a statistically significant number are recorded. The results help determine the mean and standard deviation values, which explain the uncertainty of all parameter errors (Schnoor, 1996).

1.3.2.7 Use of Advanced Continuous Simulation Language. A commercially available software application Advanced Continuous Simulation Language (ACSL) is used for running the model and plotting the various graphs associated with the model results. The specific version of ACSL used in this research is ACSLxtreme. This is a highly sophisticated modeling tool, based on sound programming using the latest software technologies such as XML and Dot Net.

It would have been possible to develop a software application to perform the modeling. User friendly programming languages such as Visual Basic, provide the means to develop an easy-to-use model. However, it was decided to use ACSLxtreme in this research, for consistency with tools used by researchers in the field of PBPK/TK modeling. This will also help other researchers in the area validate this model against their data and models in the future.

1.4 Overall study objectives

The overall study objectives can be summarized as follows:

1. To build a multi-compartmental PBPK/TK model to simulate benzene inhalation exposure in healthy adult males and females for continuous and intermittent exposures.
2. To estimate the concentrations of benzene and all its major metabolites in all compartments of the model.
    1. To report the effects of separating the bone marrow as a separate compartment in the model.
    2. To validate the model against data from current literature (animal experiments, epidemiological studies and other models) as well as perform a sensitivity analysis using Monte Carlo simulation techniques to determine effects of variables on the model results.
    3. To demonstrate the possible use of PBPK/TK models in environmental engineering studies.
    4. To make the final determination on whether such models can lend their benefits to environmental engineering studies.

To summarize, the literature reviewed and presented in chapter 2 helps build the six –compartmental PBPK/TK model developed in chapter 3. The model results are presented in chapter 4. Chapter 5 presents the sensitivity analysis of the model while the discussion of all results is presented in chapter 6 along with a validation of the model. Chapter 7 presents possible applications of the model in environmental engineering areas. Chapter 8 states the conclusions of the study based on all the analyses and chapter 9 completes the research by pointing out the advantages and limitations of the study and providing a guide to future scope in this area of research.

CHAPTER 2

2. LITERATURE REVIEW

"The longer you look backward, the further you can look forward."

- *Winston Churchill*

2.1 Introduction

This chapter recounts the exhaustive literature reviewed for this study. It starts with a detailed history of toxicokinetic modeling, the different variations in such models, and describes their capabilities and limitations. Since this model uses inhalation as a route of exposure, a detailed explanation of the inhalation physiology is also provided. The chemical benzene is then placed under scrutiny and its sources, environmental fate and metabolism are explained in great detail especially in the context of benzene's potential to cause adverse health effects. Some of the PBPK/TK models that have studied benzene are then summarized and compared against the model presented in this research. Finally some background is provided on the data that will be used to perform the PBPK/TK modeling of benzene.

2.2. Pharmacokinetic/Toxicokinetic Models

Pharmacokinetics (PK) is defined as the study of the time course of drugs and their metabolites in the human body; as they move through the different bodily fluids and organ systems and get eliminated in urine and excreta. The definition also includes the mathematical relationships that form the building blocks required to develop models so as to be able to understand and interpret data obtained from such studies (Gibaldi and Perrier; 1975). In an environmental context, xenobiotics are studied in place of drugs and the field is termed as "toxicokinetics" (TK). In this document the terms PK and TK are used interchangeably throughout the text as are the terms PBPK and PBTK. PBPK modeling is widely used in the pharmaceutical sciences for determining the right amount of a drug dose to be prescribed to a patient suffering from malaise. Given the many benefits of such modeling, its flexibility in modeling any route of exposure and any environmental pollutant across and within species and population groups, potential use of the models in environmental engineering situations can be envisioned.

2.3 Forms of Pharmacokinetic/Toxicokinetic Models

There are several different PK/TK models, with advantages and limitations based on their capabilities. Some of these models are described in the following sections.

2.3.1 Data based models

One of the simplest forms of PK/TK modeling involves performing a mathematical analysis of the time course data for a population. A model is developed and the data are fitted so as to be able to predict the rate constants involved in the modeled processes such as the rates of metabolite formation. A diagrammatic representation is presented in figure 2.1 in which a plot of a chemical concentration versus time in an experiment is sketched. A model is then laid out and the data are fitted to estimate the rate constants k_1, k_2, k_{12} and k_{21} (Andersen, 2003).

2.3.2 Classical pharmacokinetic models

A second form of modeling is compartmental modeling, where the body is divided into different units called "compartments". The simplest of this form of modeling is the "single-compartment" model, where the entire body is considered to be one homogenous unit. This does not mean that the chemical concentration is the same in the entire body. It means that the change of chemical concentrations in the plasma will influence similar chemical changes in the organ systems of the body. The single compartment models also assume a linear or first-order rate of elimination of a chemical from the body. This elimination rate constant is based on the half life of the chemical in the body. Thus mathematically,

$$\frac{dC}{dt} = -k_e * C \qquad (2.1)$$

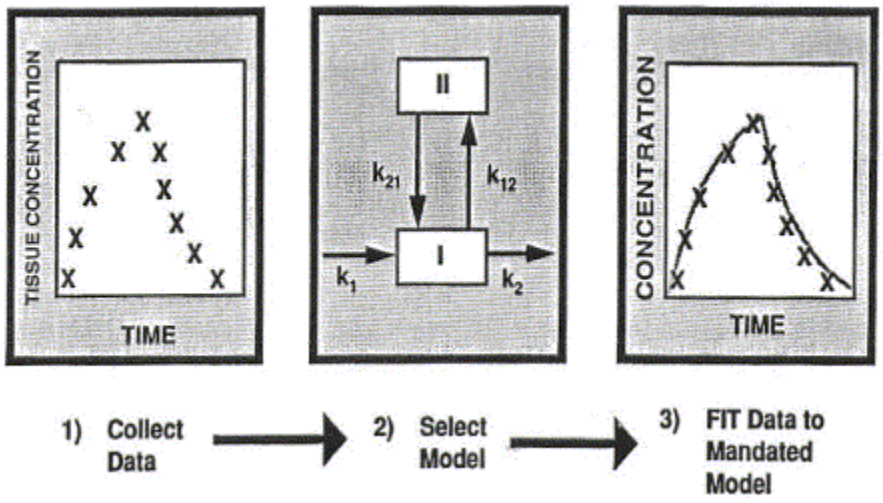

Figure 2.1 Data based PK modeling (Andersen, 2003).

$$C = C_o e^{-k_e * t} \qquad (2.2)$$

where, $\frac{dC}{dt}$ = the rate of change of chemical concentration in the compartment (mass/vol/time)

k_e = the first order elimination rate constant (/time)

C = concentration of chemical in the body at time t (mass/vol)

C_o = initial concentration in the body (mass/vol)

t = time (minutes)

Figure 2.2 depicts a typical single compartmental model.

A slightly modified version of this model is the "two-compartment" model, in which the body is composed of two compartments. The division into two compartments is based on the premise that some organs in the body are rapidly perfused tissues (RPT), i.e. they receive a higher supply of blood, for example the brain, kidney and bone marrow and are able to interact with the chemical earlier than the poorly perfused tissues (PPT), i.e. organ systems with a lower supply of blood such as the skin, muscle, etc. In such situations, the RPT are grouped together and form the "central" compartment and the PPT and other organ systems form the "peripheral compartment". Together the two systems make up the two-compartment model.

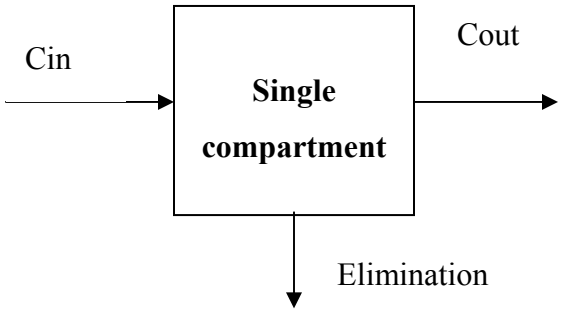

Figure 2.2 Single Compartmental Model

Three distinct formations of the two-compartment model are possible based on whether elimination occurs from the central, peripheral or both compartments. Mathematically, in case of two-compartment models, two mass balance equations are cited for the two compartments in question. These are

$$\frac{dC_1}{dt} = k_{21} * C_2 - (k_{12} + k_e) * C_1 \text{ and} \quad (2.3)$$

$$\frac{dC_2}{dt} = k_{12} * C_1 - k_{21} C_2 \quad (2.4)$$

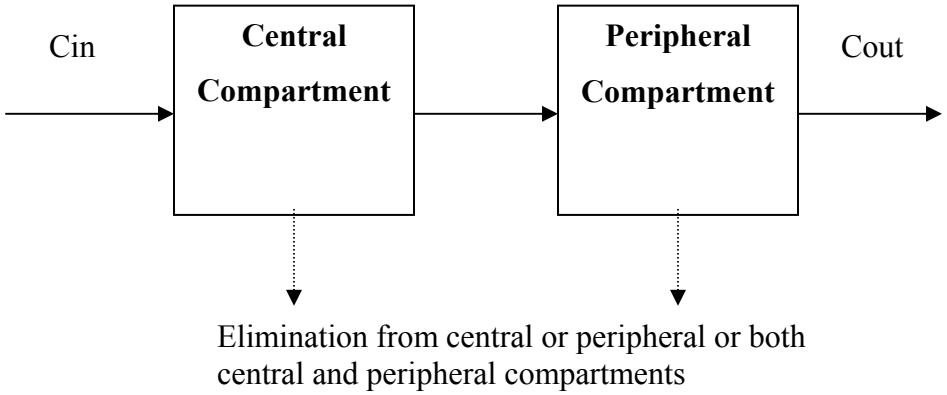

Figure 2.3 Two-Compartmental Model

where, C_1 and C_2 stand for the chemical concentrations in compartment 1 and compartment 2 respectively;

$\frac{dC_1}{dt}$ = rate of change of chemical concentration in the 1st compartment

$\frac{dC_2}{dt}$ = rate of change of chemical concentration in the 2nd compartment

and all other terms are as defined before.

When equations (2.3) and (2.4) are integrated simultaneously, chemical concentration values are obtained for the two compartments. For additional details on these forms of modeling, the reader is referred to the classic book on pharmacokinetics by Gibaldi and Perrier (1975). A typical two-compartment model is presented in figure 2.3.

Before the more advanced multi-compartmental and physiologically based compartmental models are discussed, it is important to point out the problems that existed

in the pre-digital era that led to a delay in the development of these mathematically superior and advanced models. Kohn (2000) points out some such problems which include:

i) Lack of computational skills to solve the complex mathematics involved in expressing the chemical interactions with the human body.

ii) Simplifications based on assumptions that strayed from reality.

iii) Scientists' limitations in the use of computers which urged them away from modeling and towards experimentation.

iv) Problems related to the extrapolation between doses, population groups of a species and between different species.

Such single and two compartment models along with multi-compartment models, where more peripheral compartments are added on to the single and two-compartment pharmacokinetic models are all called classical PK/TK models. These models consider only compartmental volumes and do not account for organ physiology. Although they contributed greatly to the overall field of PK/TK, and can be used for extrapolations between different exposures with a reasonable degree of accuracy, they still falter when it comes to the inter-species or inter-population modeling. The failure of classical PK/TK models to consider physiological or biochemical factors means that growth, sexual maturity, aging and such other phenomena cannot be satisfactorily explained using such models. Hence a newer form of the PK/TK models was developed which accounts for the physiology and biochemistry of the organ systems (O'Flaherty, 1987).

2.3.3 Physiologically based pharmacokinetic/toxicokinetic (PBPK/TK) models

These are models in which the body is divided into compartments based on their anatomic and/or physiological similarities. PBPK/PBTK models may also be called biologically based tissue dosimetry models (Liao et. al., 2002). These models are being developed over the past 70 years starting with modeling volatile anesthetics by Haggard in 1924, pharmaceutical agents by Teorell in the thirties, gases posing occupational exposure hazards by Fiserova-Bergerova in the seventies, followed by other classes of chemicals by other scientists (Aarons et. al., 1999). In these models, the chemical dose levels in the human tissues are calculated by integrating equations that use data on

chemical properties and metabolic pathways from literature to estimate some of the parameters used in the model.

The most important facet of designing such PBPK/TK models is determining the number of compartments that the model will have. In determining how to group organ systems into compartments, the anatomy as well as the physiology of the body are considered. However, this is not an easy determination to make because often times sufficient information on the chemical kinetics and interactions in the human body are unavailable. Thus, the preliminary choice is often made based on the pool of information available in literature through experiments.

Following the determination of compartments, mass balance equations are written for the various compartments accounting for the initial, free (and thus mobile) and bound portions of the chemical. These compartments are all flow-limited as is explained below.

2.3.3.1 Flow limited compartments. When the membrane permeability for a particular chemical is much greater than the blood flow rate into the compartment, then the compartment is said to be flow limited or perfusion limited. This means that the rate of chemical uptake into a compartment is influenced more strongly by the blood flow rate into that compartment (Q_i) rather than the rate at which the chemical partitions into the compartment, which depends on the cell membrane permeability [PA].

$$[PA] >> Q_i \tag{2.5}$$

where Q_i = blood flow rate into compartment i (vol/time)

[PA] = membrane permeability

In such compartments, the movement into and out of a compartment by a chemical is described as

$$V_i * \frac{dC}{dt} = Q_i * (C_{in} - C_{out}) \tag{2.6}$$

where

V_i = volume of the compartment (vol)

$\frac{dC}{dt}$ = rate of change of concentration in the compartment in time (mass/vol/time)

Q_i= blood flow rate into the compartment (vol/time)

C_{in} = chemical concentration entering the compartment (mass/vol)

C_{out}= chemical concentration leaving the compartment (mass/vol)

Since this is a flow limited compartment, the C_{out} is equal to the free product or the mobile portion of the chemical that had entered the compartment and did not bind to the tissue.

Thus,

$$C_{out} = C_f = \frac{C_i}{P_i} \tag{2.7}$$

where C_{out} is as defined above

C_f = the free portion of the chemical (mass/vol)

C_i = chemical concentration in the compartment (the bound portion) (mass/vol) and

P_i = the tissue:blood partition coefficient of the chemical for any compartment i and has no units.

Substituting equation (2.7) in (2.6), the final mass balance equation can be obtained for compartment i

$$V_i = \frac{dC}{dt} = Q_i * (C_{in} - \frac{C_i}{P_i}) \tag{2.8}$$

where all terms are as previously defined.

The flow limited compartments are based on the assumption that the concentrations of a chemical in all parts of the tissue are in equilibrium.

A second form of compartments is called diffusion limited in which the membrane permeability of the compartment is much lower than the blood flow rate into the compartment, such that the [PA] becomes the rate limiting factor for the chemical uptake into the compartment. Since none of the compartments considered in this study are assumed to be diffusion limited, further details on this subject are not provided.

2.3.3.2 Advantages of PBPK/TK models. There are a number of advantages in using the PBPK/TK models. Summarized from various sources, some advantages have been presented as follows (Clewell (1995); Kassen (1995); Andersen(2003)):

i) PBPK/TK models are highly flexible and allow for simple modifications to models to be able to perform a number of extrapolations such as:

1. From experimental animal data to the human population groups. Barring some qualitative differences (for example the absence of a gall bladder in certain species) most organ weights are in proportion with body weights for most mammals as is the blood flow diagram to and from different mammalian organ systems (Bischoff, 1989). This geometric similarity helps explain the applicability of PBPK models across species.
2. Between species and within population groups of a species.
3. Between dose levels
4. Between routes of exposure

ii) PBPK/TK models can help avoid the high cost experimentation that is otherwise required to understand toxicity.

iii) PBPK/TK models help save time because epidemiological studies for example, can take several years to provide reasonable amounts of data and reliable results in toxicity studies.

iv) PBPK/TK models also address the ethical issue of using animals in scientific studies by lowering, if not completely eliminating, the numbers of animals used in studies as well as the number of animal studies themselves.

v) PBPK/TK models are also effective tools in estimating the risk of chemical mixtures.

2.3.3.3 Limitations of PBPK/TK models. Some of the limitations in using PBPK/TK models include the following:

i) Models might be unreliable because of unknown, unavailable or unrealistic input parameters.

ii) Some chemical reactions within the body including biotransformation might be complicated processes, not easily represented in models.

iii) The use of average physiological parameters might be sufficient to model the sensitivity between the diverse population groups.

iv) Further experimental confirmation of the model results might be needed because the models are designed to simulate rather than fit real data.

v) The large amounts of data required to validate such models are sometimes unavailable and can create limitations for the use of the model.

vi) The high number of parameters can increase the possibility of errors.

2.3.4 Other forms of PBPK/TK models

There are some other forms of PBPK/TK models, for example ones that involve population-based modeling using Bayesian solution techniques. These are beyond the scope of discussion of this document.

2.4 Disposition of Gases and Vapors in the Human Body

2.4.1 Introduction

This discussion is required because of the fact that benzene is a highly volatile gas and exposure to benzene occurs chiefly by inhalation.

When a gas or vapor is inhaled, the lungs are the entry point for the gas or vapor into the human body. As the inhalation continues, the gas is carried into the arteries and is distributed to the rest of the body through the arterial blood. The tissues and organ systems in the body hold on to some amount of the gas and release the remaining into the veins from where it is carried back to the lung through the venous blood. Initially, the arterial concentration of the gas keeps on increasing as long as the exposure continues, or until steady-state is reached, while the venous blood concentration of the gas is relatively low. This is the saturation phenomenon.

When the exposure ceases, a reversal occurs. The veins carry the gas back to the lungs, where some of it is exhaled, making the arterial concentration of the gas much lower than the venous concentration. This occurs until the tissues are completely depleted of the gas or until equilibrium or steady-state conditions are reached. This is the desaturation phenomenon.

Such equilibria between the ambient environment and the body can be reached only in case of organ systems that do not metabolize or excrete. In case of organ systems that do metabolize or excrete, uptake continues in steady state. However, the amount of gas leaving the organs is lower than its concentration in the arterial blood (Fiserova-Bergerova, 1983).

2.4.2 Factors Affecting the Uptake, Distribution, Metabolism and Elimination of Inhaled Gases

Factors that most influence the amount of gas or vapor entering the human body are the alveolar concentration (Q_a), and the cardiac output (Q_c) of the individual as well as chemical specific parameters such as the chemical solubility and the blood:tissue partition coefficients for the chemical. For example, the blood flow rate into different organ systems of the human body will cause the gas concentrations in some compartments to rise sooner than in others. The total amount of chemical that passes through the body depends on the duration of exposure. Thus the main factors affecting the disposition of inhaled gases can be summarized as:

i) Rate of transfer of the environmental concentration of the gas to the tissues and vice-versa.

ii) The concentration of inhaled substance that the body is able to hold on to and

iii) Any metabolism and elimination that occurs in the organs.

The following sections describe in detail the important parameters involved in the inhaled gas kinetics; namely the alveolar ventilation, the gas exchange process in the lung, the cardiac output and the partition coefficients. The chemical specific parameters; in this case parameters that describe the kinetics of benzene are described in the section on benzene.

2.4.2.1 Alveolar ventilation. The alveolar ventilation is in reality a cyclic process with inspiration and expiration cycles. However, for the purpose of this modeling, the alveolar ventilation is considered as a continuous process. This is because of an experiment conducted by Eger and described by Fiserova-Bergerova (1983) in which he

studied the effect of respiratory rate and the rise of alveolar concentrations for an inert non-soluble gas. In this experiment he concluded that the rise in alveolar ventilation deviated very little for cyclic and continuous ventilation. This might be untrue in case of humans with some pathological conditions. However this assumption is still valid in this model because the model results are for healthy adult males and females.

2.4.2.2 Gas exchanges in the lungs. To understand how the gas behaves with the blood and the lung tissue it is necessary to first know the definitions of some respiratory volumes (Thibodeau and Patton, 2003).

i) Tidal Volume (TV): The volume of air that moves into and out of the respiratory tract during a normal respiratory cycle (500 ml).

ii) Inspiratory Reserve Volume (IRV): The maximum volume that can be moved into the respiratory tract after a normal inspiration (3000-3300 ml).

iii) Expiratory Reserve Volume (ERV): The maximum volume that can be moved out of the respiratory tract after a normal inspiration (1000-1200 ml).

iv) Residual volume (RV): The volume remaining in the respiratory tract after maximum expiration (1200 ml).

A diagrammatic representation of the volumes is shown in figure 2.4.

For this analysis, it is assumed that the alveoli are the only places where gas exchange occurs and the respiratory tubes carrying the inhalation and exhalation gas concentrations are inert tubes. This indicates that there is a certain amount of dead space in the respiratory airways. This dead space holds on to approximately 33% of the gas concentration that leaves the alveoli at the end of the previous expiration. As the figure explains, in normal adult males, approximately 150 ml of alveolar air in dead space re-enters the lung and mixes with approximately 350 ml of ambient air. During exhalation about 150 ml of ambient air retained in the dead space from the previous inspiration is exhaled first, followed by 350 ml of alveolar air; i.e. only about 2/3 of the effective tidal volume reaches the alveoli. The effective tidal volume is the tidal volume after subtracting the physiological dead space. The flow of ambient air that reaches the alveolar air in one minute is alveolar ventilation (Qa). Alveolar ventilation (= effective TV * respiratory rate) accounts for about 2/3rd minute ventilation (= total TV *

respiratory rate) under resting conditions. Hence the model uses alveolar ventilation instead of the minute ventilation to account for the physiological dead space.

2.4.2.3 Cardiac Output. Cardiac output is defined as the volume of blood pumped out with each contraction of the heart multiplied by the heart rate/minute. The heart rate can be defined as the number of times the heart beats per minute (American Medical Association, 2003).

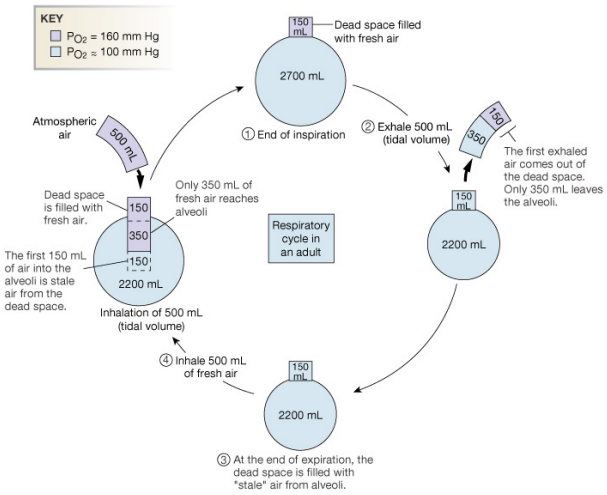

Figure 2.4 Pulmonary Volumes (www.med.howard.edu/physio.biophys/)

The cardiac output affects the blood flow rate to the different compartments in the model. The blood flow rate in turn affects the concentration of chemical reaching the different compartments of the body. Hence one of the common ways to divide the organs into compartments and group specific organs together is based on their perfusion, i.e. the amount of blood flow that they receive. For example, organs such as the brain, the kidney, bone marrow and liver receive a large supply of blood, which means that the blood flow rate to these organs is very high (richly perfused organs). Hence in a number of PK/TK studies these organs are grouped in a single compartment. Only when specific organs in such a group are target organs or need to be studied in greater detail, are they separated from the grouping and considered individual compartments. For example, in the model presented in this research, although the bone marrow is a richly perfused

tissue, it is an organ where benzene manifests its adverse effects, because the metabolism of benzene occurs to some extent in the bone marrow. Hence it is considered as a separate compartment.

2.4.2.4 Partition coefficients. The transfer of gases from the ambient environment to the lung (alveoli) through inhalation, the movement from the lung into the blood and specifically the arteries and finally the transfer of the gases from the arterial blood to the different compartments depends on the different partition coefficients for such transfers.

A partition coefficient is a ratio. For example, the concentration of a gas entering the fat compartment depends on the tissue:blood partition coefficient for the gas for that compartment. In case of benzene, the partition coefficient for benzene to partition from the fat compartment into blood is 54.5 in men and 51.8 in women.

2.5 Modeling Benzene using PBPK/TK Models

2.5.1 Introduction

In order to understand the PBTK modeling of benzene, especially in the context of human exposure to benzene by inhalation, it is important to get a perspective on the chemical benzene. Hence the following section discusses benzene, its sources, environmental fate and disposition, adverse effects caused by benzene, its kinetics along with a detailed analysis of all of its metabolites and modeling benzene using PK/TK studies.

2.5.2 Benzene sources

Benzene is a highly flammable, organic solvent, ubiquitous in the Environment (Lovern et. al., 2001; ATSDR 1997; USEPA, 2002). It is among the twenty highest produced chemicals in the United States (ATSDR, 1995). It originates from a number of natural sources such as crude oil seeps, forest fires and plant volatiles. However, the chief cause of concern is the benzene emission from man-made sources. The major ones include industrial operations, automobile refueling operations, environmental tobacco smoke, and automobile exhaust. Petroleum refining and petrochemical industries alone

account for approximately 98% of the benzene produced anthropogenically (Gist and Berg, 2003). Table 2.1 lists some of the common applications of benzene.

In an environmental engineering context benzene is a significant environmental pollutant given its presence in gasoline and petroleum products. These are products that can cause major environmental disasters when they leak from storage tanks and are spilled into the environment. Case studies are available of remediation engineering work being conducted across several different regions to clean up petroleum and related spills involving benzene (Federal Remediation Technologies Roundtable, 1995). While these incidents focus on the surface and groundwater and soil contamination by benzene, benzene's volatility makes it a significant air pollutant with an even higher inhalation hazard. In fact because benzene so readily partitions into air (99%), inhalation is the predominant pathway of human exposure accounting for more than 99% of the daily intake (ATSDR, 1997). The relatively high vapor pressure of benzene creates a significant hazard when adequate workplace safeguards are not in place (IARC, 1987).

There is a definite lack of research in this area pertaining to the inhalation hazard posed by the hydrocarbon vapors such as benzene fumes that may be present at such sites and their potential to cause adverse health effects in the exposed population. It is hoped that the modeling performed in this research can be applied at sites to safeguard the health of workers on site as well as the human population in the vicinity.

2.5.3 Benzene's environmental fate and disposition

Benzene is released in the environment in large quantities as indicated in Appendix A. In the atmosphere benzene degrades rapidly reacting principally with atmospheric radicals. It undergoes limited photolysis, which is not a major degradation process for benzene. This is because the upper atmosphere filters out wavelengths of light less than 290 nm and benzene does not absorb wavelengths of light greater than 260 nm (ATSDR, 2001). The physical and chemical properties of benzene as summarized in Table 2.2 are mainly responsible for the fate of benzene in the environment. Benzene easily advances from water surfaces to the atmosphere on account of its slight solubility

in water and its Henry's law constant. Benzene exists in the atmosphere mainly in the vapor phase, making inhalation the most prevalent form of human exposure.

Table 2.1 Uses and applications of benzene (past and present) (ATSDR, 2001)

Category	Applications
Industrial	Industrial paints, paint removers, adhesives, degreasing agents, denatured alcohol, rubber cements, and arts and crafts supplies
Solvent	Laboratory reagent
Housing	Building material and furnishings
Others	Environmental tobacco smoke, photocopier and laser printed paper, particle board furniture, floor adhesives, paints, wood paneling, caulking, and paint remover

2.5.4 Human Exposure to Benzene

A study by USEPA (Wallace, 1989 and Wallace et. al., 1996) called the Total Exposure Assessment Methodology (TEAM) study was conducted over a ten-year period and provides a large data source for human exposure to benzene, especially in a non-occupational setting. The TEAM study indicates that cars burning petroleum products produce nearly 85% of atmospheric benzene in outdoor air and the remaining 15% is produced by industry. For most people, the most common route of human exposure to benzene is through inhalation of air polluted with emissions from traffic and at gas stations (pumping gasoline and being exposed to benzene from gas vapors). Exposure from wood burning sources such as stoves also accounts for outdoor benzene exposure. Air around hazardous waste sites and industries also form a major source of exposure.

People directly involved in the use or manufacture of benzene containing products are the most at risk to benzene exposure. Average daily water intake of benzene is 0.2 mg/day while dermal contact or ingestion account for a rather insignificant amount of benzene exposure.

Table 2.2 Physical and chemical properties of benzene (ATSDR profile, 1997)

Property	**Value**
CAS Number	1076-43-3
Molecular Weight	78.11
Color	Clear, colorless liquid
Physical state	Rhomboid prisms
Melting point	5.5 degrees C
Boiling Point	80.1 degrees C
Density at 15 degrees C, g/cm^3	0.8787
Odor	Aromatic
Odor threshold in Water Air	 2.0 mg/l 4.9 mg/m^3
Taste threshold	0.5-4.5 mg/L
Solubility in Water at 25 degrees C Organic solvents	 W/w 0.188% Alcohol, acetone, galactic acid, acetic acid, chloroform, carbon disulfide, carbon tetrachloride, oils, ester
Partition coefficients Log K_{OW} Log K_{OC}	 2.13 1.8-1.9
Vapor pressure at 20 degrees C	75 mm of Hg

Table 2.2 (Continued)

Property	Value
Henry's law constant at 25 degrees C	5.5 * 10^{-3} atm m^3/mol
Autoignition temperature	498 degrees C
Flashpoint	-11 degrees C (closed cup)
National Fire Protection Association (NFPA) Hazard Classification	
Health	2.2
Flammability	3.3
Reactivity	0
Flammability limits in air	1.2 % lower; 7.8% upper
Explosive limits	1.4% lower; 8% upper

Thus, all outdoor sources including automobile exhaust and stationary sources account for only 20% of the total population exposure to benzene. As against this, 18% exposure is attributed to personal sources, which include smoking cigarettes, driving automobiles and using products that emit benzene such as adhesives, paints, rubber products, marking pens and tapes (ATSDR, 1996).

Some important findings from the TEAM study are:

i) Inhalation accounts for more than 99% of total human exposure to benzene. Exposure from sources such as food and water are minimal if not non-existent.

ii) The personal exposure levels (global average = 15 μg/m^3 (range 7-29 μg/m^3)) were much higher than indoor exposure levels of benzene (global average = 10 μg/m^3), which in turn exceeded the outdoor exposure levels (global average = 6 μg/m^3 (range 2-19 μg/m^3)).

Benzene exposure from cigarette smoke inhalation has been studied by EPA and other researchers over several decades and a broad range of data are available from such studies. Hence the benzene inhalation data from cigarette smoking studies are used as a starting point for this modeling project.

Table 2.3 states some of the occupation exposure limits to benzene set by the Occupational Safety and Health Administration (OSHA).

Table 2.3 Exposure limits for benzene
(http://www.osha.gov/dts/chemicalsampling/data/CH_220100.html**)**

Agency/Criterion	**Exposure Limit**
OSHA (Occupational Safety and Health Administration) Permissible Exposure Limit (PEL) for General Industry	1 ppm TWA; 5 ppm STEL; 0.5 ppm Action Level
American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Value (TLV)	0.5 ppm TWA; 2.5 ppm STEL; Skin; Appendix A1 (Confirmed Human Carcinogen)
National Institute for Occupational Safety and Health (NIOSH) Recommended Exposure Limit (REL)	0.1 ppm TWA, 1 ppm Ceiling (15 Minutes); Appendix A - Potential Carcinogen

2.5.4.1 Benzene at Petroleum Contaminated Sites. One of the biggest environmental contamination issues of recent times has been the accidental release of petroleum and other hydrocarbon products into the surface and subsurface environments from underground storage tanks (USTs). There are currently about 3 million USTs storing petroleum products in US, and as many as 500,000 may be leaking petroleum into the ground (Kao and Prosser, 2001). Vapors from such releases might pose a significant inhalation hazard during the initial periods of release. In case the vapors intrude into residential basements, not only do they constitute an important inhalation hazard, but they are also a significant indoor air pollution problem. For example, Sevigny et. al., (2003) studied the Volatile Petroleum Hydrocarbon (VPH) inhalation exposure by an adult receptor in a hypothetical, commercial building for occupational tenures of 16 to 70 years.

Integrated risk assessment approaches including the RBCA initiatives are proposed as possible ways to deal with the petroleum spill and related risk issues. In integrated risk assessment techniques, it is common to look at all possible exposure routes and determine the risk from each exposure route in determining the final risk. Inhalation is mostly overlooked in case of petroleum and other hydrocarbon spills

assuming that these are surface and groundwater as well as soil contamination problems and the only risks posed to people are through ingestion or dermal contact with petroleum contaminated waters and dermal contact with the contaminated soils.

Hence this study studies the impact of inhaling vapors from such releases and making a determination of how they contribute to the overall risk and hence cleanup of the site.

2.5.5 Benzene's Adverse Effects

While the biggest risks from benzene exposure include cancer, aplastic anemia and other blood disorders, it can cause a wide variety of adverse effects, not as serious but equally life threatening, if not attended to. These range from some of the commonly reported inhalation adverse effects such as mucous membrane irritation, dyspnea or nasal irritation and sore throat to some high dose fatalities resulting in hemorrhagic, edematous lungs or acute pulmonary edema observed in autopsy. Some human poisoning cases have pointed at ventricular fibrillation as the cause of death. Inhalation exposure and hematotoxicity have been linked by leukopenia, anemia, and thrombocytopenia.

Long term inhalation exposure of benzene has been linked to

i) Pancytopenia which results from a reduction in the ability of the red bone marrow to produce adequate number of these mature blood cells.

ii) Aplastic anemia which is a more severe effect of benzene and occurs when the bone marrow ceases to function and the stem cells never reach maturity. Aplastic anemia can progress to a type of leukemia known as acute myelogenous leukemia.
Some musculoskeletal effects such as myelofibrosis, hepatic effects such as enlarged livers, and renal effects such as acute kidney congestion have also been reported. Hemorrhagic respiratory tissues and second degree burns of the face, trunk and limbs are some rare dermatological effects while skin irritation is most common on benzene inhalation exposure.

Eye irritation, some changes in body weight, immunological effects such as leukocytopenia, some central nervous system effects such as drowsiness, dizziness, headache, vertigo, tremor, delirium and loss of consciousness and rarely death have also been associated with inhaling benzene over varying periods of time.

Under reproductive effects, women's fertility impairment, menstrual disorders and changes in ovaries have been observed while in men, changes in testes including weight increases, and severe gonadal lesions have been observed.

The results from fetotoxicity studies for developmental effects are inconclusive.

Besides the chromosomal aberrations, other genotoxic effects include benzene inhibition of DNA synthesis in certain cell types; although inhibition of DNA synthesis is not always indicative of genotoxic damage.

While these effects have received some attention and studies are ongoing to understand their link with benzene, cancer is by far the most adverse effect of benzene, since the chemical is a known human carcinogen. The exact metabolites or a combination thereof has not yet been definitely labeled as the ones to cause cancer in animals or humans. Studies have speculated on benzene oxide being the key carcinogenic agent, others have mostly attributed the blame on hydroquinone and its subsequent metabolism to 1,4 p-benzoquinone in the bone marrow. There have also been reports that phenol leads to an increase in the hydroquinone induced toxicity. However, Bois et. al., (1991) conducted a study that proved why benzene and not its major metabolite phenol is carcinogenic in rats, by developing a pharmacokinetic model. They concluded that the phenol-hydroquinone pathway by itself is not sufficient as a cancer inducer.

The most characteristic effect of benzene in both human and animal models is the depression of the bone marrow, leading ultimately to aplastic anemia. Abnormalities of humoral and cell-mediated immune responses following benzene exposure in mice are presumably caused by a defect in the lymphoid stem cell precursors of both T and B lymphocytes. Studies have also observed that bone marrow cellularity and the number of thymic T-cells increased, presumably as a compensatory response in these cell lines in response to benzene exposure. This compensatory proliferation may play a role in the carcinogenic response of mice to inhaled benzene.

A summary of some of the adverse effects of benzene and the concentrations at which they occur are described below.

2.5.6 Benzene's Toxicokinetics

Since only the inhalation exposure route is addressed in this research, only the inhalation toxicokinetics of benzene is discussed. It should be reiterated that this study looks only at the toxicokinetic aspects of benzene, which involves determining the chemical concentrations of benzene and its metabolites in different parts of the human body. The effects of those concentrations on the organs and the ability of the human to deal with them are subject matters studied in a separate allied discipline called "pharmacodynamics/toxicodynamics". The overall TK process involves only the absorption, distribution, metabolism and elimination of benzene to determine the amount of benzene that remains in specific compartments after inhalation exposure.

2.5.6.1 Absorption. In humans absorption by inhalation ranges from 70 to 80% in the first 5 minutes and then decreases to approximately 50% thereafter (IARC, 1987). The absorption of inhaled benzene in rodents as indicated in several studies is approximately 50% (ATSDR, 1997). This implies that the remaining 50% of inhaled benzene must be exhaled during exposure and after it ceases, such that only the absorbed 50% is "bioavailable". Thus, 50% of the inhaled benzene is absorbed and will be available for distribution, metabolism and elimination.

Benzene retention decreases at higher dose levels. Sabourin et. al., (1987); have noted that the percentage of inhaled benzene that was absorbed and retained during a 6-hour exposure period decreased from 33 to 15 % in rats and from 50 to 10% in mice when the exposure was increased from about 10 to 1000 ppm. Absorption of inhaled benzene proceeds rapidly at first and then decreases. It has been noted that at benzene concentrations greater than 200 ppm, zero-order kinetics were observed (i.e. uptake became non-linear, indicating saturation of the metabolic capacity) (ATSDR, 2001).

2.5.6.2 Distribution. Absorbed benzene is rapidly distributed throughout the body regardless of the route of exposure. The rapid distribution is usually followed by relative uptake by the tissues depending on the perfusion rate of the tissue by the blood and the total potential uptake dependent on fat content and metabolism.

2.5.6.3 Metabolism. As an aromatic hydrocarbon, benzene is the smallest and most stable. In order for benzene to manifest its toxic effects it needs to be metabolized (Snyder et. al., 1993; ATSDR, 2001; USEPA, 1998). This has been strongly evidenced in several studies. For example, when toluene is co-administered with benzene it competes for the CYP2E1 isoenzyme bringing about a marked decrease in benzene metabolism and toxicity (Tunek et. al., (1982). Another study demonstrated that even a partial hepatectomy in rats lowered benzene metabolism as well as toxicity implying that a metabolite(s) formed in the liver are required to manifest benzene toxicity (Sammett et. al., 1979).

Benzene metabolism has been studied in several different species of animals as well as in humans. Different doses and routes of exposure have been considered in order to best understand benzene metabolism. Benzene metabolism varies with the species, the route of exposure and is dose dependent. At low doses, more of the benzene is converted to putative toxic metabolites than at higher doses. At high doses, benzene inhibits phenol metabolism to hydroquinone, apparently through competition for a common site on the CYP2E1 isoenzyme to which hydroquinone and catechol also bond.

However, it still remains a complicated process that eludes complete clarity. The following section explains benzene metabolism in the context and scope of this research project.

2.5.6.3.1 Metabolic Pathways. Hepatic metabolism of benzene is mediated by the cytochrome P-450 dependent mixed function oxidases. The predominant form of the cytochrome P-450 isoenzymes is CYP2E1. Studies have demonstrated that the role of CYP2E1 is a pre-requisite for the development of cytotoxicity and genotoxicity. Humans vary in their expression of CYP2E1 activity and thus the isoenzyme plays an important role in human variability, genetic polymorphism and resultant differential risk from benzene exposure (E.P.A., 1998).

Benzene metabolism begins with the initial formation of the epoxide benzene oxide. Several alternative pathways might then continue the metabolism process. One process involves the formation of hepatic metabolites (phenol, catechol, hydroquinone) in the ring hydroxylation process. Another entails the ring opening, which leads to the formation of putative toxic metabolites (ATSDR, 2001).

Benzene oxide re-arranges itself non-enzymatically to form phenol, which is the major product of initial benzene metabolism. Phenol is further oxidized into hydroquinone, by CYP2E1 catalysis. Hydroquinone then undergoes catalysis by myeloperoxidase to oxidize into p-benzoquinone.

Another possibility emerges with benzene oxide reacting with glutathione to produce phenyl mercapturic acid. Two separate pathways are then likely to occur. If phenyl mercapturic acid undergoes enzymatic conversion by epoxide hydrolase then benzene dihydrodiol is formed which further produces catechol. On the other hand, if phenyl mercapturic acid undergoes an iron-catalyzed ring-opening reaction then *trans-trans*-muconaldehyde is produced which further metabolizes to form *trans-trans* muconic acid. The metabolic pathways of benzene are shown in figure 2.5 (Henderson et. al, 1989).

2.5.6.3.2 Sites of metabolism. Metabolism of benzene occurs primarily in the liver. However, the bone marrow has also been quoted a site of secondary benzene toxicity. The above pathways for benzene metabolism occur in the liver. The metabolites of benzene produced in bone marrow cells by the microsomal cytochrome *P*-450 are thought to be phenol, catechol, hydroquinone and *p*-benzoquinone (Rushmore et. al., 1984). The bone marrow has the ability to undergo a specific form of oxidation called "oxidative burst", in which numerous microsomal and peroxidative enzymes as well as oxidants such as hydrogen peroxide are released into the phagosomes and extracellular space.

The myeoloperoxidase (MPO) enzymes in combination with the hydrogen peroxide or alone have been attributed to activate some of benzene's phenolic metabolites and cause hematotoxicity (Smith et. al., 1989).

The paper also points to 1,4 p-benzoquinone as a metabolite of hydroquinone produced in the bone marrow that might be specifically responsible for the myelotoxicity as represented in figure 2.6. In figure 2.6, a simplified model is shown where benzene from the lung moves into the blood and from the blood into the liver. Here it metabolizes into phenol, which is further metabolized to hydroquinone. The metabolites phenol and hydroquinone enter the blood and are thus available to the bone marrow.

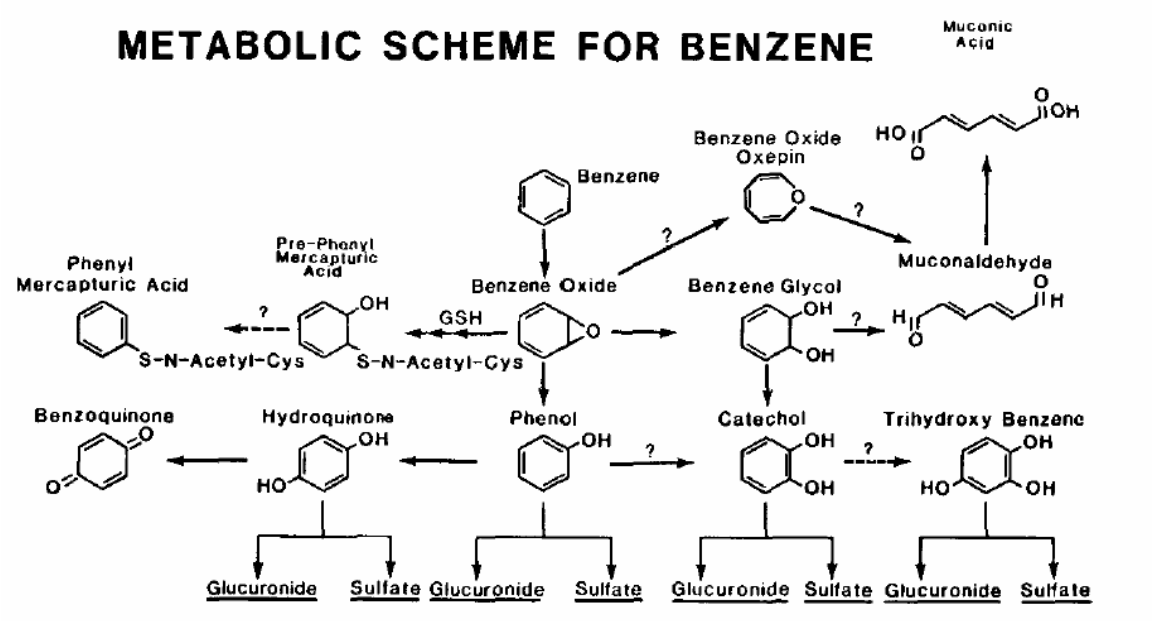

Figure 2.5 Metabolic Scheme for Benzene (Henderson et. al., 1989).

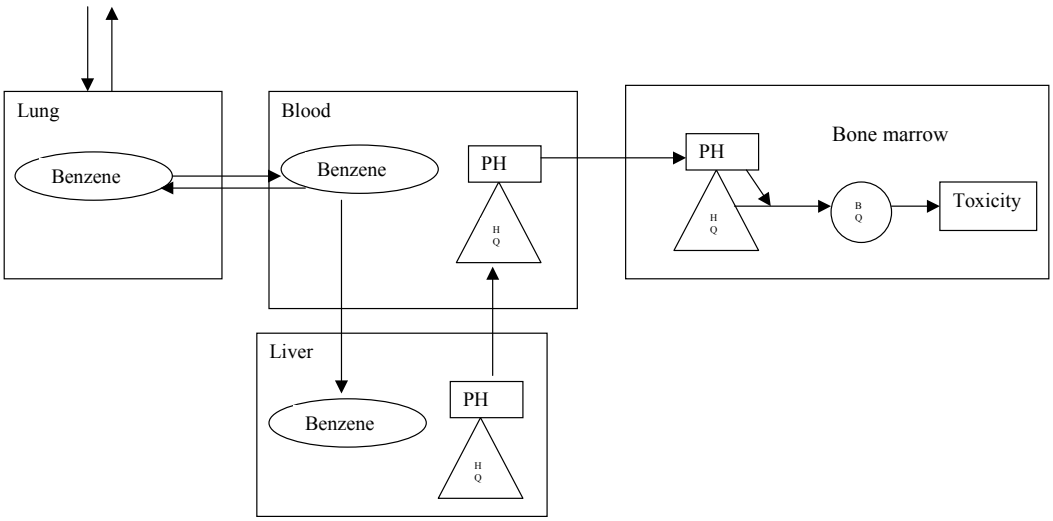

Figure 2.6 The toxicity of benzene's phenolic metabolites (Smith et. al., 1989).

The bone marrow further metabolizes the hydroquinone into the 1,4 p-benzoquinone with the help of the MPO to cause the myelotoxicity. Snyder et. al., (1989) also suggest the macrophages, a form of bone marrow stromal cells are a primary cellular target for benzene. The macrophages release a wide range of cytokines and growth factors which modulate the activation and proliferation of stem cells. These release levels are enhanced when the specific macrophages such as the phagocytes are activated on account of antigen stimulation. These activated phagocytes produce the highly reactive and potentially toxic oxygen intermediates including superoxide anion, hydrogen peroxide and hydroxyl radicals. Based on this hypothesis they present a model for the potential role of bone marrow phagocytes in benzene induced hematotoxicity as presented in figure 2.7.

Tunek et. al., (1982) studied the effects of bone marrow toxicity induced by benzene and its metabolites using mice. Only low level exposures could be studied because higher doses caused adverse effects such as tremors. They observed that the adverse effects produced by hydroquinone occurred at a different sequence than in benzene. Their conclusions were that hydroquinone is a hemotoxic metabolite of benzene in mice *in vivo*, but that other metabolites, or benzene itself, also probably contribute to the toxicity. Catechol gave no effects.

While a number of studies and experiments have established this metabolism of hydroquinone to 1,4 p-benzoquinone, it has never before been modeled as part of a PBPK/TK model. Also, the kinetics used in the metabolism of hydroquinone to 1,4 p-benzoquinone, are explained with the extended Michaelis Menten expression which accounts for the effects of benzene, phenol and hydroquinone concentrations on the rate of metabolism from hydroquinone to 1,4 p-benzoquinone, which has also never been reported before in all the literature that was reviewed for this study. This part is thus a new contribution to this field and to the body of knowledge on this subject. Knowing the concentration of 1,4 p-benzoquinone that forms in the bone marrow, researchers will be able to explain some of the biological effects of the metabolite such as its ability to find to DNA and protein, cause single stranded DNA breaks, interfere with the microtubule assembly, inhibit RNA and DNA synthesis, interfere with the growth of bone marrow stromal cells, etc (Henderson et. al., 1989).

2.5.6.3.3 Metabolism kinetics. Enzymes are proteins, which provide active sites for benzene and the metabolites to bind to; i.e. benzene and its metabolites serves as the substrates for the enzymes. The enzymes (E) form a temporary enzyme-substrate (S) complex (E-S) with its substrates by means of the hydrogen bonds at its active site. This complex is oriented just right so that the reaction occurs. As the reaction is completed, the reaction products are released as well as the free unchanged enzymes, which are now free to participate in another reaction. By providing several active sites to the substrates, the enzymes act as catalysts and enhance the rate of reactions. They also facilitate the breakdown of chemicals in the same manner.

Most enzyme kinetic data in literature are obtained from in vitro studies in which the functions and activities of enzymes are studied outside the animal or human bodies. The relationship between the rate of a reaction and the concentration of the substrate is graphically expressed as shown in figure 2.8.

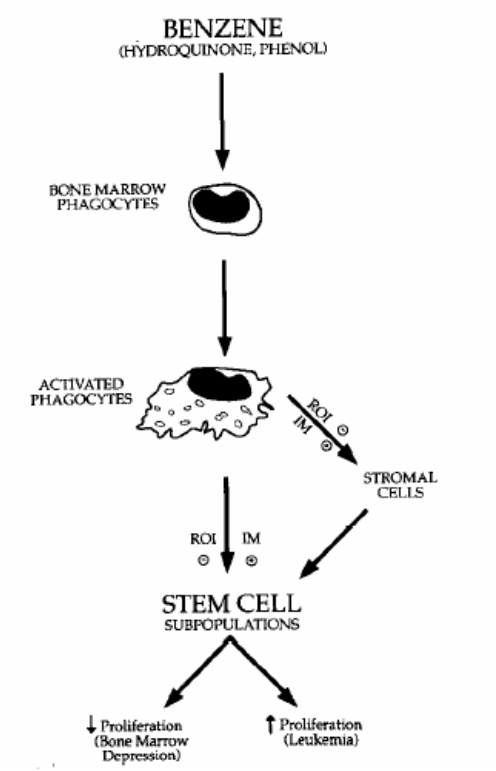

Figure 2.7 A model for the potential of bone marrow phagocytes in benzene induced hematotoxicity (Snyder et. al., 1989).

As observed from the graph, in the beginning, the reaction rate increases rapidly with increasing substrate concentrations, because its capacity to bind to the substrate is very high. Hence breakdown occurs and reaction products are formed. Eventually a maximum reaction rate (V_{max}) is reached, which means that any further increase in the substrate concentration will not have any significant impact on kinetics of the reaction, i.e. all active sites on the enzyme are used up and it is working at its maximum capacity (Rhoades and Pflanzer, 1996).

The graph also shows K_m, the Michaelis Menten constant, which can be defined as the substrate concentration that produces half the maximum reaction rate; i.e. K_m indicates the affinity of an enzyme for a particular substrate. A higher K_m value indicates a low affinity and vice versa. The standard form of the Michaelis Menten equation is presented in Appendix B.

In this study, an extended form of the Michaelis Menten equation is used. This extended version is also provided in Appendix B. The extended version is used because as reported by Medinsky et. al., (1996); Lovern et al.,(1999); and Cole (2001), the metabolism of benzene to benzene oxide, benzene oxide to phenol and phenol to hydroquinone and catechol are all mediated by the CYP2E1 isoenzymes. This means that their rates of metabolism are bound to be inter-dependent and will affect each other's rates of metabolism.

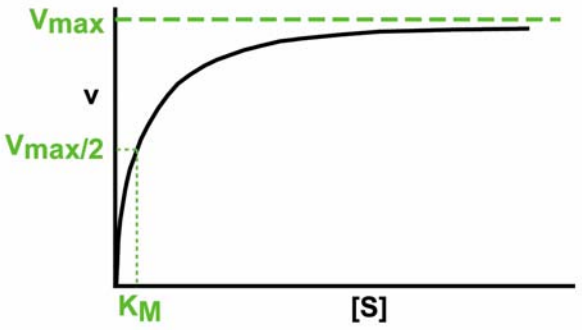

Figure 2.8 The Michaelis-Menten relationship
(http://www.le.ac.uk/by/teach/biochemweb/tutorials/michment1.html**)**

2.5.6.4 Elimination. Exhalation and urinary excretion serve as the major elimination routes for benzene and its metabolites. The half-life of exhaled benzene in humans varies depending on the benzene exposure concentration and duration. It has been experimentally determined that to 99 ppm for 1 hour resulted in an initial phase half-life of 42 minutes, and exposure to 6.4 ppm for 8 hours resulted in an initial phase half-life of 72 minutes, with a terminal phase half-life (from 10 to 100 hours after exposure) of 23 to 31 hours. Exposure to 99 ppm for 1 hour are reported to result in an initial phase half-life of 42 minutes, and exposure to 6.4 ppm for 8 hours resulted in an initial phase half-life of 72 minutes, with a terminal phase half-life (from 10 to 100 hours after exposure) of 23 to 31 hours (IARC, 1987).

As part of the detoxification process, the phenyl mercapturic acid is eliminated by biliary elimination. The water-soluble urinary metabolites of phenol and benzene dihydrodiol are excreted as sulfate or glucoronide conjugates and are also considered detoxification products (ATSDR, 2001). The breakdown of phenol into its sulfate conjugates are shown in figure 2.9.

Also as noted before, most of the benzene oxide is non-enzymatically converted to phenol. In a human study 16.4 to 41.6% of retained benzene was eliminated through the lungs within five to seven hours after a two- to three-hour exposure to 47 to 110 ppm and only 0.07 to 0.2% of the remaining benzene was excreted unchanged in the urine. After exposure to 63 to 405 mg/m^3 of benzene for 1 to 5 hours, 51 to 87% was excreted in the urine as phenol over a period of 23 to 50 hours (IARC, 1987).

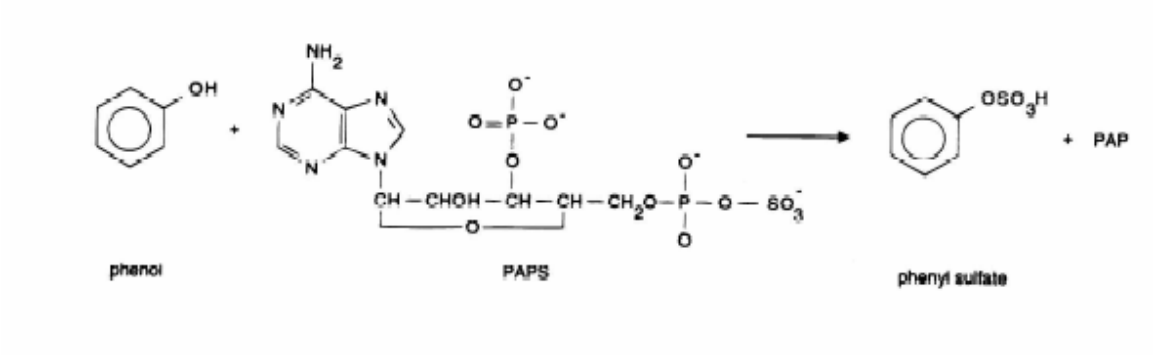

Figure 2.9 Phenol breakdown to phenyl sulfate
(http://aquaticpath.umd.edu/appliedtox/metabolism.pdf)

2.5.7 PBTK models for benzene

Pharmacokinetic models for benzene have been developed by a number of people such as Sato et. al. (1991), Snyder et. al., (1996), Beliles and Totman (1992) and Sabourin et. al., (1987) (ATSDR, 2003). Sato et. al., (1991), developed the first model for benzene. In an experiment, they exposed three men to 25 ppm and 100 ppm benzene vapor for 2 hours and then observed a triexponential decay of benzene from their blood. The investigators constructed a three-compartment model consisting of richly perfused tissues, poorly perfused tissues and fat, which acted as a major sink for benzene. Other researchers sampled benzene in blood and urine of different animal species by different routes of administration and for a range of doses and created the base for more advanced biologically based models to better study benzene (Snyder, 1993).

Some of the PBPK/TK models developed for benzene exposure were by Medinsky (1989); Travis (1990); Bois and Paxman (1992), each going one step ahead compared to the previous model in terms of the parameters modeled and the results obtained. A summary of these models and a comparison against the one presented in this research is presented below. Another research study was included in this comparison sheet.

Table 2.4. Comparison between the previous benzene models (Medinsky (1989); Travis (1990) and Bois and Paxman (1992)

Description	**Medinsky**	**Travis**	**Bois and Paxman**
Model subjects	Rats and mice	Rats, mice and humans	Rats
Number of compartments	4	5	5

Table 2.4 Continued

Description	Medinsky	Travis	Bois and Paxman
Names of compartments	1) liver 2) PPT 3) RPT including bone marrow, 4) fat	1) liver, 2) fat, 3) bone marrow, 4) muscle and 5) RPT	1) liver, 2) bone marrow, 3) fat, 4) PPT and 5) RPT
Metabolic compartment	Liver	Liver	Liver and bone marrow
Metabolic kinetics	Michaelis Menten	Michaelis Menten	Michaelis Menten except linear for BO
Limitations	Did not account for metabolism in the bone marrow and did not model the metabolites.	Did not model the benzene metabolites. Concentration in fat was also poorly predicted.	Modeled metabolites using standard Michaelis Menten

2.6 How PBPK/TK models fit in with the RBCA discussion

As presented in chapter 1, the main purpose of this study is to determine whether the PBPK/TK models such as the one developed here can be implemented in environmental engineering situations; i.e. whether the PK/TK flexibilities and capabilities will benefit the environmental engineering area or whether the modeling limitations will make such models useless in environmental engineering analyses. Hence this section discusses RBCA and the role PBPK/TK models can potentially play in this new aspect of risk based engineering.

RBCA as defined by the USEPA is "A streamlined approach in which exposure and risk assessment practices are integrated with traditional components of the corrective action process to ensure that appropriate and cost-effective remedies are selected, and that limited resources are properly allocated" (USEPA, 2002).

The term RBCA refers to the standard entitled Guide For Risk-Based Corrective Action Applied at Petroleum Release Sites [E-1739-95] that was published by the American Society for Testing and Materials (ASTM) Subcommittee on Storage Tanks.

The goals of a RBCA process are:

1. Protection of human health and environment
2. Practical and cost-effective application of risk-based decision-making
3. Consistent and technically-defensible administrative process

The ASTM standard specifies the three-tiered approach that RBCA follows http://www.zymaxusa.com/technotes/rbca-aug97.html#What%20is%20RBCA):

Tier 1:

1. A preliminary assessment of the site is performed
2. The site specific conditions are then compared to the look-up tables provided by state regulatory agencies
3. An initial response is framed and priorities are determined.

Tier 2:

1. The site specific SCTLs are developed. These may be developed by several different methods
2. Decide on the remediation method to implement

In case the first two tiers provide insufficient information to make a decision regarding the cleanup levels, the analysis continues with tier 3.

Tier 3:

1. More data is collected and analyzed from the site
2. Experimentation and modeling can be performed to help make the final determination.

Connor and McHugh (2002), report that nationwide study on the environmental cleanup performance using RBCA was positive. The study demonstrated faster case processing rates, reduced environmental cleanup costs, and more effective targeting of resources toward higher-risk sites. This study determines whether the PBPK/TK modeling would be applicable at environmental engineering sites using RBCA and how.

2.7 Summary of the literature Review

To summarize, the literature reviewed in this section presented background information on PBPK/TK models and modeling including the PBPK/TK models developed for benzene to date, the physiology of gas exchange in the lungs, toxicokinetics of benzene and its major metabolites and how PBPK/TK models fit in within the realm of environmental engineering.

CHAPTER 3

3. MODEL BUILDING

3.1 Introduction

As explained in chapter 2, model building is a complex process that requires an accurate understanding of the physical processes being modeled as well as reliable sources of data that can be used in the models. Physical processes are often explained by virtue of the rates at which they occur. For example, in calculus based mathematical modeling the inter-relationships between the processes are equations and the rates are derivatives. These derivatives are differential equations, which form the building blocks of the mathematical modeling of physical processes.

In constructing such mathematical models certain steps are followed. These include the following:

1. The independent and dependent variables are determined and letters are assigned to represent the variables.
2. The appropriate unit of measurement for each variable is selected.
3. The basic principle describing the process is written. The principle is then expressed mathematically in terms of the earlier defined variables. This may involve the introduction of physical constants or parameters and determination of appropriate values for them, or it may involve the use of auxiliary or intermediate variables that must then be related to the primary variables.

4. The solution of these functional interrelationships provides the model outcome.

3.2 The Model Building Steps for this Research

The steps involved in developing the model described in this research are presented in this section. They can be listed as:

1. Understand the kinetics of the chemicals involved; namely benzene and its major metabolites,
2. Decide how many compartments the model should have and why specific organs should be grouped in specific compartments,
3. Write equations to explain the toxicokinetics for each compartment, for each chemical,
4. Search the literature for data on parameter values such as compartment volumes, blood flow rates to the compartments and the partition coefficients and
5. Determine what mathematical program should be used to solve the ordinary differential equations that will result from the toxicokinetic analyses.

Based on these steps the model is developed. The flow diagram is shown in Appendix C.

3.2.1 Benzene kinetics

The first step in any PBPK/TK modeling exercise is obtaining a clear understanding of the chemical to be modeled. An in-depth review of benzene and its metabolites, their kinetics and interactions with the human body was performed as presented in chapter 2.

3.2.2 Compartments

Once the kinetics of the chemicals in question are analyzed, the next step is to decide the number of compartments the model should have, the compartments that should be grouped together into a compartment along with justification for such grouping, and

why and then define these compartments in terms of their specific parameter values for example their sizes (volume in liters) based on values available in literature.

Many researchers in the field of PBPK/PBTK modeling have explained that determining the number of compartments a model should have is not an easy task. In this model, six compartments were finalized based on reasons provided in the following paragraphs. The six compartments are:

1. Lung
2. Fat
3. Rapidly Perfused Tissues
4. Poorly Perfused Tissues
5. Liver
6. Bone Marrow

The following sections 3.2.2.1 to 3.2.2.6 explain the reasoning behind the selection of these organ systems and their grouping into these specific compartments.

3.2.2.1 Lung. Since inhalation was the route of entry considered in this study, it was imperative to include the lung as a compartment. The lung serves as the central compartment of the model, because it is the entry point for benzene. The benzene from the lungs is distributed via the arterial blood, throughout the other organ systems. The remaining benzene concentration from the different organ systems is then brought back into the lung through the venous blood (Klassen, 1996).

The lung is also an important compartment to design because a significant portion of the inhaled benzene is exhaled and so the lung serves as an organ of elimination. The presence of cytochrome P450 isoenzyme CYP2E1 enzyme in the lung is another reason why some researchers have speculated that the lung might also possibly be an organ of benzene metabolism (Cole, 2001). However, since there is much debate on this issue and because data could not be obtained for benzene metabolism in the lung, it was decided to assume that the lung did not function as a metabolic organ for the sake of this model. In the future, if more experiments are performed and it is proved that the lung is a significant contributor to benzene metabolism, the model will need to be modified and re-run to more accurately reflect the research of the time.

3.2.2.2 Fat. The fat tissue is widely considered as a separate compartment in most PBPK/TK models. For example all previous models for benzene cited in this document have included the fat tissue as a separate compartment. Some other non benzene models that have also included the fat tissue as a compartment include a Bayesian toluene PBPK model by Jonsson and Johanson (2001) and the trichloroethylene model in rats and mice by Keys et. al., (2003) to name a few. The adipose tissue or fat is usually considered as a separate compartment in such studies on account of its ability to hold on to chemicals. This is especially true of lipid soluble chemicals such as benzene. As benzene passes through the fat compartment, a significant portion of it binds to the macromolecules in the fat compartment, such that the benzene concentration in the venous blood leaving the fat compartment is significantly lower than the arterial concentration of benzene that entered it.

3.2.2.3 Rapidly perfused tissues (RPT). The rapidly perfused tissues are modeled as a separate compartment in a number of PBPK/TK models. These are tissues are sometimes also called richly perfused tissues. This is because almost all PBPK/TK models are flow-limited and hence organs that receive rich amounts of blood supply are typically grouped together. These organs include the kidney, intestines, brain, etc. The grouping of organs might differ from one model to another depending on the final use, purpose and objectives of the model. For example, as stated before, the bone marrow is a highly blood rich organ, hence it is grouped in the RPT compartment in most studies. An case in point is a PBPK model for tetrachloroethylene exposure in lactating mothers and their breast fed infants (Byczkowski, 1996) in which the rapidly perfused tissues compartment includes the bone marrow. However, in this model it forms a separate compartment as explained in the section 3.2.2.6 Bone Marrow.

3.2.2.4 Poorly Perfused Tissues (PPT). As is the case with the fat and RPT compartments, most tissues that do not receive an ample supply of blood are grouped separately into the compartment called poorly perfused tissues (PPT). These are also sometimes called the slowly perfused tissues. These include organs such as the skin, muscle, bones, etc. Typically these organs have a large volume, but receive limited blood

flow. Again, if any of the poorly perfused tissues are significant for a specific study or are target organs for a specific environmental pollutant, in that case, they would be separated out as an individual compartment. For example, studies considering the dermal exposure of xenobiotics would likely consider the skin a separate compartment. This is demonstrated in the PBPK model for percutaneous absorption to perchloroethylene in rats by Poet et. al., (2002). In some such studies, besides being the target organ, the skin is also considered as an organ of elimination. For example, gases can leave through the pores of the skin. In this model since the skin is not a target organ for benzene and because the concentrations, if any, of any gases that would be eliminated through the skin would be very small, it is not considered as a separate compartment.

3.2.2.5 Liver. The liver is considered as a separate compartment despite being a richly perfused organ system because of its capability to metabolize benzene and because it is a target organ for benzene, which causes hepatotoxicity. The liver contains the P450 cytochrome enzymes which are primarily involved in benzene metabolism, specifically the CYP2E1 isoenzyme. Details on how the metabolism occurs, and the steps involved in the formation of the metabolites have been already explained in chapter 2.

3.2.2.6 Bone marrow. As stated before, the bone marrow is a richly perfused tissue. However, in case of benzene, it is the site of secondary metabolism and more importantly it is a target organ and hence in this study it is considered as a separate compartment. Even benzene models developed previously have grouped the bone marrow in the RPT compartment, except for a couple of models that kept it separate (Travis (1990), Bois and Paxman (1992)). The grouping of the bone marrow into the RPT compartment can be considered to be one of the limitations of such benzene models. Even the Travis model that did separate the bone marrow as an individual compartment, did not model the kinetics of benzene metabolites. They only looked at the benzene kinetics. The Bois and Paxman model did consider the kinetics of benzene metabolites; however, they used the Michaelis Menten kinetics which does not consider the effects of the rate of metabolism of one benzene metabolite on another. Cole (2001) uses such a modified Michaelis Menten kinetic equation for the benzene metabolite analysis;

however she does not separate the bone marrow as a separate compartment and groups it along with the RPT. Hence the new contribution that this research attempts to accomplish is the separation of the bone marrow as a separate compartment and the study of the interactions of the benzene metabolites using the extended Michaelis Menten kinetics. The separation of the bone marrow and the use of the extended Michaelis Menten kinetics are expected to have a significant impact on the results of the model and as determined from the literature review no one has modeled such information in any study previously. The modified version of the Michaelis Menten kinetics equations are as presented in Appendix B with permission.

3.3 Mass balance equations

Once the compartments and their grouping is determined with the background knowledge of the chemical kinetics, mass balance equations can be written for each of the compartments for all the chemicals in question. The equations for this model are developed based on the model for styrene inhalation in rats previously developed by Ramsey and Andersen (1987) and the more recent PBPK model developed by Cole (2001). The format of the equations from these sources is adopted and they are then modified and extended based on the modeling needs of this study. These equations form the heart of the model and involve a number of assumptions. These assumptions are listed as follows:

1. The modeling is performed only for healthy adult male (body weight 70 kg) and non-pregnant female humans (body weight 60 kg).
2. Only the inhalation route of exposure is considered (justified in chapter 1).
3. Metabolism occurs primarily in the liver and to some extent in the bone marrow (from literature cited in chapter 2).
4. Each compartment is a homogenous well mixed unit (justified in chapter 2).
5. The inhalation concentration is considered half of the exposure concentration in the model, to account for the 50% exhalation of benzene.
6. The metabolites once produced are not exhaled from the lung.

Additional assumptions, for example the assumptions for the lung compartment are listed as they occur in the text.

The followings section is subdivided according to the model compartments and each subsection contains all equations for that specific compartment for all the chemicals that pass through it.

3.3.1 Lung

The lung is a special compartment because it serves as the central compartment in this study, which focuses on inhalation exposure to benzene. It is divided into two sub sections; namely the alveolar region of the lung and the blood region of the lung as is depicted in figure 3.1 below. Assumptions for developing the equations for this model are based on the ones listed in Klasses (1996) and can be listed as follows:

a) Ventilation is continuous and not cyclic.
b) Conducting airways act as inert tubes, carrying vapor to pulmonary or gas exchange regions.
c) Diffusion of vapors across lung cell and capillary wells is rapid compared with blood flow through the lung.
d) All chemicals from the inspired air appears in arterial blood (i.e. there is no significant storage of chemicals in the lung tissue and insignificant lung mass).
e) Vapor in alveolar air and arterial blood with lung are related by the blood air partition coefficient (PB). (e.g. $C_{alv} = C_{art}/PB$)

The most important variable in the kinetics of this compartment is PB. It is a thermodynamic parameter. It quantifies the partitioning of benzene into the blood as compared with the air.

The partitioning of benzene from the air into the blood will depend on how much benzene is inhaled which is further dependent on the alveolar ventilation rate (Q_a) and the inhaled concentration (C_{inh}). Similarly benzene exhalation from the lungs is a function of the alveolar ventilation rate and the benzene concentration in the alveoli or air sacs (C_{alv}). The venous blood finally carries the remaining benzene concentration from different

organ systems back to the lung and this returning concentration (C_v) is a function of the cardiac output (Q_c) and the arterial concentration of benzene (C_{art}). All of the above can be seen in the diagram depicted in figure 3.1 below.

Thus, we have two sets of mass balance equations, one for each of the alveolar and blood region of the lungs. Putting these four mass balance differential equations together, we get the rate of change in the amount of benzene in the lung compartment.

$$\frac{dL}{dt} = Q_a * (C_{inh} - C_{alv}) + Q_c * (C_v - C_{art}) \quad (3.1)$$

where dL/dt = rate of change of chemical mass in the lung and all other terms are as described before.

Previously stated assumptions indicate that, $V * \frac{dC}{dt} = 0$, the C_{alv} can be replaced by C_{art}/PB and the differential equation can be solved for the arterial blood concentration.

$$C_{art} = \frac{(Q_a * C_{inh} + Q_c * C_{ven})}{\frac{(Q_c + Q_a)}{PB}} \quad (3.2)$$

As the PB increases so does the maximum concentration of benzene in the blood as does the time required to reach steady-state concentration and the time required to clean the chemical.

The total cardiac output Q_c can be given as the sum of the individual blood flows to all the compartments; i.e.

$$Q_c = Q_f + Q_{rpt} + Q_{ppt} + Q_l + Q_b \quad (3.3)$$

where,

Q_f = blood flow rate into the fat compartment

Q_{rpt} = blood flow rate into the RPT compartment

Q_{ppt} = blood flow rate into the RPT compartment

Q_l= blood flow rate into the liver compartment

Q_b = blood flow rate into the bone marrow compartment

Thus, this section on the lung compartment basically provides us with an equation for the arterial concentration of benzene, which is the concentration that is carried to all the other 5 compartments in the model. Although, the same concentration of

benzene reaches all the organs through the arterial blood, the amount entering each compartment will be limited by the individual model characteristics as explained in the following sections. The amount of benzene that is removed from each of the organ systems and reaches back to the lung is the venous concentration of benzene (CV) term which can be written as

$$CV = \frac{(Q_f * CV_f) + (Q_{rpt} * CV_{rpt}) + (Q_{ppt} * CV_{ppt}) + (Q_l * CV_l) + (Q_b * CV_b)}{Q_f + Q_{rpt} + Q_{ppt} + Q_l + Q_{bm}} \quad (3.4)$$

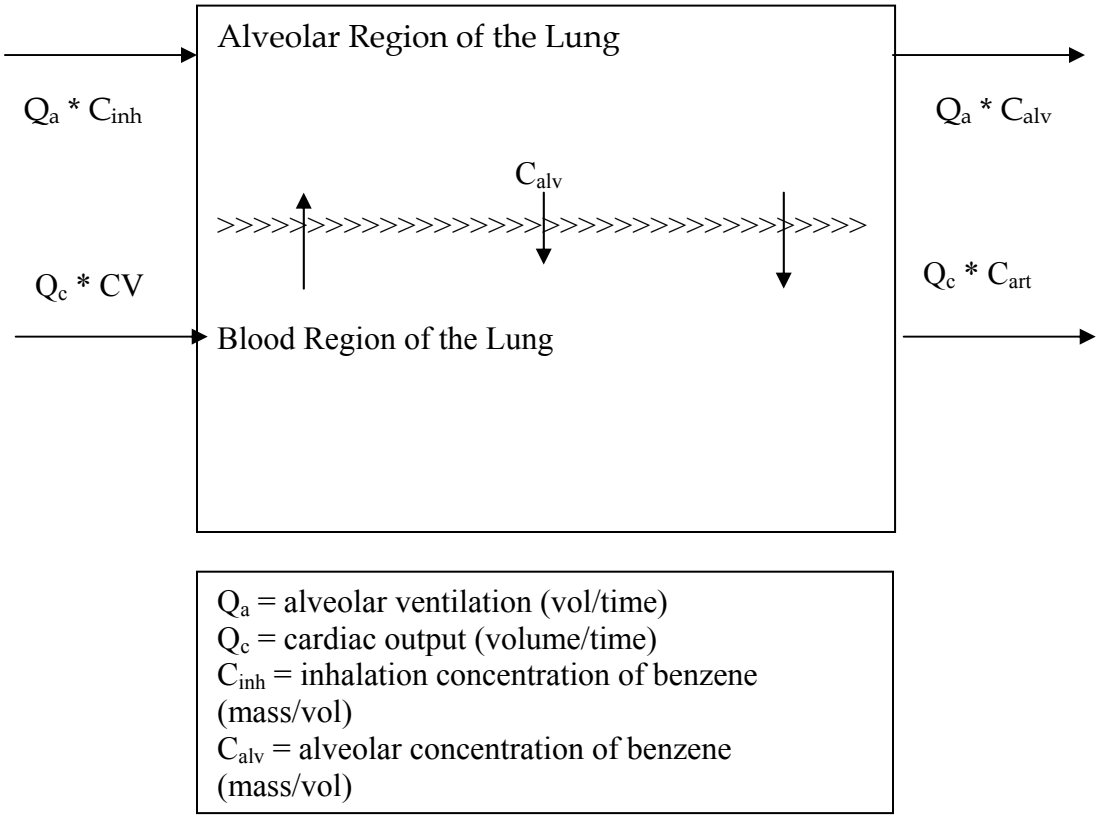

Figure 3.1 Flow between the alveolar and blood regions of the Lung (Modified from the original diagram in Medinsky & Klaassen, 1996).

The benzene oxide, phenol and hydroquinone; metabolites of benzene that are produced in the liver (C_{lbo}, C_{lph}, and C_{lhq} respectively) are circulated back to through the blood to all other organ systems. When they reach the lung, some of these metabolites are exhaled and the remaining is the metabolite concentration that is bioavailability for the

other organs to induce toxic effect. The concentration of this exhaled metabolite is expressed as C_{ebo} for benzene oxide, C_{eph} for phenol, and C_{ehq} for hydroquinone. These recirculation concentrations (i.e. the new C_{artbo}, C_{artph} and C_{arthq}, the corresponding CV_{bo}, CV_{ph} and CV_{hq} are presented for each of the metabolites. The metabolite amounts that are exhaled can be calculated by integrating the last equations of each set.

For benzene oxide:

$$C_{artbo} = C_{lbo} - C_{ebo} \tag{3.5}$$

$$CV_{bo} = \frac{((CV_{fbo} * Q_f) + (CV_{rptbo} * Q_{rpt}) + (CV_{pptbo} * Q_{ppt}) + (CV_{lbo} * Q_l) + (CV_{bmbo} * Q_{bm}))}{(Q_f + Q_{rpt} + Q_{ppt} + Q_l + Q_{bm})} \tag{3.6}$$

$$\frac{dC_{ebo}}{dt} = Q_c * (CV_{bo} - C_{lbo}) \tag{3.7}$$

where, all terms are as described in the preceding paragraph.

For phenol:

$$C_{artbo} = C_{lph} - C_{eph} \tag{3.8}$$

$$CV_{ph} = \frac{((CV_{fph} * Q_f) + (CV_{rptph} * Q_{rpt}) + (CV_{pptph} * Q_{ppt}) + (CV_{lph} * Q_l) + (CV_{bmph} * Q_{bm}))}{(Q_f + Q_{rpt} + Q_{ppt} + Q_l + Q_{bm})} \tag{3.9}$$

$$\frac{dC_{eph}}{dt} = Q_c * (CV_{ph} - C_{lph}) \tag{3.10}$$

For hydroquinone

$$C_{arthq} = C_{lhq} - C_{ehq} \tag{3.11}$$

$$CV_{hq} = \frac{(CV_{fhq} * Q_f) + (CV_{rpthq} * Q_{rpt}) + (CV_{ppthq} * Q_{ppt}) + (CV_{lhq} * Q_l) + (CV_{bmhq} * Q_{bm})}{(Q_f + Q_{rpt} + Q_{ppt} + Q_l + Q_{bm})} \tag{3.12}$$

$$\frac{dC_{ehq}}{dt} = Q_c * (CV_{hq} - C_{lhq}) \tag{3.13}$$

3.3.2 Fat

The fat compartment receives the benzene concentration from the arterial blood as an input (C_{art}). In the fat compartment some amount of the benzene binds to the macromolecules (C_f) and is the concentration that stays in the fat compartment. This is the concentration that we are interested in for further analyses. The concentration of benzene that is not involved in the binding process is the free or mobile part of the benzene that exits the fat compartment into the venous blood. This venous blood concentration of benzene leaving the fat compartment (CV_f) is recirculated back to the lung. The equations are presented below.

3.3.2.1 Equations for benzene.

$$V_f * \frac{dC_f}{dt} = Q_f * (C_{art} - CV_f) \tag{3.14}$$

This equation indicates that the partitioning of benzene from the arterial blood to the fat compartment depends on the size of the fat compartment expressed in terms of its volume in liters, the blood flow rate into the compartment as well as the partition coefficient of benzene for the fat compartment; i.e. the tissue: blood partition coefficient. Now, since our final objective is to find the concentration of benzene that remains in the fat C_f, let us start by considering the above equation again in terms of the rate of change of the **mass** of benzene in the fat compartment. It can be expressed as:

$$\frac{dA}{dt} = Q_f * (C_{art} - CV_f) \tag{3.15}$$

$$CV_f = \frac{C_f}{P_f} \tag{3.16}$$

i.e. the amount of benzene that leaves the fat compartment (CV_f) depends on how much benzene binds to the fat (C_f) and how much of it partitions into the venous blood (P_f). Now integrating equation (3.15):

$$Af = \int Q_f * (C_{atr} - CV_f)\, dt \tag{3.17}$$

This integration is performed by Advanced Continuous Simulation Language (ACSL), which was the software program used in performing the mathematical calculations of this model.

The final concentration C_f that we need can then be expressed as:

$$C_f = \frac{A_f}{V_f} \tag{3.18}$$

This completes the mass balance for benzene in the fat compartment. Similar mass balance equations can be written for the other chemicals which are routed through the fat compartment i.e. benzene oxide, phenol and hydroquinone. These equations are presented as follows. The explanation for the recirculation and actual concentrations of the metabolites is presented in the liver section, where they originate.

3.3.2.2 Benzene oxide equations.

$$\frac{dA_{bo}}{dt} = Q_f * (C_{artbo} - CV_{fbo}) \tag{3.19}$$

$$CV_{fbo} = \frac{C_{fbo}}{P_f} \tag{3.20}$$

$$Af = \int Q_f * (C_{atrbo} - CV_{fbo})\, dt \tag{3.21}$$

$$C_{fbo} = \frac{A_{fbo}}{V_f} \tag{3.22}$$

3.3.2.3 Phenol equations.

$$\frac{dA_{ph}}{dt} = Q_f * (C_{artph} - CV_{fph}) \tag{3.23}$$

$$CV_{fph} = \frac{C_{fph}}{P_f} \tag{3.24}$$

$$Af_{ph} = \int Q_f * (C_{atrph} - CV_{fph})\, dt \tag{3.25}$$

$$C_{fph} = \frac{A_{fph}}{V_f} \tag{3.26}$$

3.3.2.4 Hydroquinone equations:

$$\frac{dA_{hq}}{dt} = Q_f * (C_{arthq} - CV_{fhq}) \tag{3.27}$$

$$CV_{fhq} = \frac{C_{fhq}}{P_f} \tag{3.28}$$

$$Af_{hq} = \int Q_f * (C_{atrhq} - CV_{fhq})\, dt \tag{3.29}$$

$$C_{fhq} = \frac{A_{fhq}}{V_f} \tag{3.30}$$

These sets of equations complete the entire analysis for the fat compartment for benzene and all of its metabolites

3.3.3 Rapidly Perfused Tissues (RPT)

The RPT compartment receives the benzene concentration from the arterial blood as an input into it (C_{art}). In the RPT compartment some amount of the benzene binds to the tissues (C_{rpt}) and is the concentration that stays in the RPT compartment. This is the concentration that we are interested in for further analyses. The concentration of benzene that is not involved in the binding process is the free or mobile part of the benzene that exits the RPT compartment and joins the venous blood. This venous blood concentration of benzene leaving the RPT compartment (CV_{rpt}) is recirculated back to the lung. The equations are presented below.

3.3.3.1 Equations for benzene.

$$V_{rpt} * \frac{dC_{rpt}}{dt} = Q_{rpt} * (C_{art} - CV_{rpt}) \tag{3.31}$$

This equation indicates that the partitioning of benzene from the arterial blood to the RPT compartment depends on the size of the RPT compartment expressed in terms of its volume in liters, the blood flow rate into the compartment as well as the partition coefficient of benzene for the RPT compartment; i.e. the tissue: blood partition coefficient.

Now, since our final objective is to find the concentration of benzene that remains in the RPT (C_{rpt}), let us start by considering the above equation again in terms of the rate of change of the **mass** of benzene in the RPT compartment. It can be expressed as:

$$\frac{dA}{dt} = Q_{rpt} *(C_{art} - CV_{rpt}) \tag{3.32}$$

$$CV_{rpt} = \frac{C_{rpt}}{P_{rpt}} \tag{3.33}$$

i.e. the amount of benzene that leaves the RPT compartment (CV_{rpt}) depends on how much benzene binds to the RPT (C_{rpt}) and how much of it partitions into the venous blood (P_{rpt}).

Now integrating equation (3.32):

$$A_{rpt} = \int Q_{rpt} *(C_{art} - CV_{rpt})\, dt \tag{3.34}$$

The final concentration C_{rpt} that we need can be expressed by integrating equation 3.34 as:

$$C_{rpt} = \frac{A_{rpt}}{V_{rpt}} \tag{3.35}$$

This completes the mass balance for benzene in the RPT compartment. Similar mass balance equations can be written for the other chemicals, which are routed through the RPT, compartment i.e. benzene oxide, phenol and hydroquinone. These equations are presented as follows. The explanation for the recirculation and actual concentrations of the metabolites is presented in the liver section, where they originate from.

3.3.3.2 Benzene oxide equations.

$$\frac{dA_{rpt}}{dt} = Q_{rpt} *(C_{artbo} - CV_{rptbo}) \tag{3.36}$$

$$CV_{rptbo} = \frac{C_{rptbo}}{P_{rpt}} \tag{3.37}$$

$$A_{rptbo} = \int Q_{rpt} *(C_{artbo} - CV_{rptbo})\, dt \tag{3.38}$$

$$C_{rptbo} = \frac{A_{rptbo}}{V_{rpt}} \tag{3.39}$$

3.3.3.3 Phenol equations.

$$\frac{dA_{ph}}{dt} = Q_{rpt} * (C_{artph} - CV_{rptph}) \tag{3.40}$$

$$CV_{rptph} = \frac{C_{rptph}}{P_{rpt}} \tag{3.41}$$

$$A_{rptph} = \int Q_{rpt} * (C_{artph} - CV_{rptph})\, dt \tag{3.42}$$

$$C_{rptph} = \frac{A_{rptph}}{V_{rpt}} \tag{3.43}$$

3.3.3.4 Hydroquinone equations:

$$\frac{dA_{hq}}{dt} = Q_{rpt} * (C_{arthq} - CV_{rpthq}) \tag{3.44}$$

$$CV_{rpthq} = \frac{C_{rpthq}}{P_{rpt}} \tag{3.45}$$

$$A_{rpthq} = \int Q_{rpt} * (C_{arthq} - CV_{rpthq})\, dt \tag{3.46}$$

$$C_{rpthq} = \frac{A_{rpthq}}{V_{rpt}} \tag{3.47}$$

These sets of equations complete the entire analysis for the RPT compartment for benzene and all of its metabolites.

3.3.4 Poorly Perfused Tissues (PPT)

The PPT compartment receives the benzene concentration from the arterial blood as an input into it (C_{art}). In the PPT compartment some amount of the benzene binds to the tissues (C_{ppt}) which is the concentration that stays in the PPT compartment. This is the concentration that we are interested in for further analyses. The concentration of benzene that is not involved in the binding process is the free or mobile part of the benzene that exits the PPT compartment and joins the venous blood. This venous blood

concentration of benzene leaving the PPT compartment (CV_{ppt}) is recirculated back to the lung. The equations are presented below.

3.3.4.1 Equations for benzene.

$$V_{ppt} * \frac{dC_{ppt}}{dt} = Q_{ppt} * (C_{art} - CV_{ppt}) \tag{3.48}$$

This equation indicates that the partitioning of benzene from the arterial blood to the PPT compartment depends on the size of the PPT compartment expressed in terms of its volume in liters, the blood flow rate into the compartment as well as the partition coefficient of benzene for the PPT compartment; i.e. the tissue: blood partition coefficient.

Now, since our final objective is to find the concentration of benzene that remains in the PPT (C_{ppt}), let us start by considering the above equation again in terms of the rate of change of the **mass** of benzene in the PPT compartment. It can be expressed as:

$$\frac{dA}{dt} = Q_{ppt} * (C_{art} - CV_{ppt}) \tag{3.49}$$

$$CV_{ppt} = \frac{C_{ppt}}{P_{ppt}} \tag{3.50}$$

i.e. the amount of benzene that leaves the PPT compartment (CV_{ppt}) depends on how much benzene binds to the PPT (C_{ppt}) and how much of it partitions into the venous blood (P_{ppt}).

Now integrating equation (3.49):

$$A_{ppt} = \int Q_{ppt} * (C_{art} - CV_{ppt})\, dt \tag{3.51}$$

On integrating equation 3.51, the final concentration C_{ppt} that we need can then be expressed as:

$$C_{ppt} = \frac{A_{ppt}}{V_{ppt}} \tag{3.52}$$

This completes the mass balance for benzene in the PPT compartment. Similar mass balance equations can be written for the other chemicals which are routed through the PPT compartment i.e. benzene oxide, phenol and hydroquinone. These equations are

presented as follows. The explanation for the recirculation and actual concentrations of the metabolites is presented in the liver section, where they originate from.

3.3.4.2 Benzene oxide equations.

$$\frac{dA_{ppt}}{dt} = Q_{ppt} *(C_{artbo} - CV_{pptbo}) \tag{3.53}$$

$$CV_{pptbo} = \frac{C_{pptbo}}{P_{ppt}} \tag{3.54}$$

$$A_{pptbo} = \int Q_{ppt} *(C_{artbo} - CV_{pptbo})\, dt \tag{3.55}$$

$$C_{pptbo} = \frac{A_{pptbo}}{V_{ppt}} \tag{3.56}$$

3.3.4.3 Phenol equations.

$$\frac{dA_{ph}}{dt} = Q_{ppt} *(C_{artph} - CV_{pptph}) \tag{3.57}$$

$$CV_{pptph} = \frac{C_{pptph}}{P_{ppt}} \tag{3.58}$$

$$A_{pptph} = \int Q_{ppt} *(C_{artph} - CV_{pptph})\, dt \tag{3.59}$$

$$C_{prptph} = \frac{A_{pptph}}{V_{ppt}} \tag{3.60}$$

3.3.4.4 Hydroquinone equations:

$$\frac{dA_{hq}}{dt} = Q_{ppt} *(C_{arthq} - CV_{ppthq}) \tag{3.61}$$

$$CV_{ppthq} = \frac{C_{ppthq}}{P_{prpt}} \tag{3.62}$$

$$A_{ppthq} = \int Q_{ppt} *(C_{arthq} - CV_{ppthq})\, dt \tag{3.63}$$

$$C_{ppthq} = \frac{A_{ppthq}}{V_{ppt}} \tag{3.64}$$

These sets of equations complete the entire analysis for the PPT compartment for benzene and all of its metabolites.

3.3.5 Liver

While the basic framework of equations for the liver compartment is the same as that of the above three compartments, it does differ significantly as far as the metabolite analysis is concerned, since all of the metabolic activity initiates in this compartment.

The benzene equations for the liver are still somewhat similar to the ones described above for the previous three compartments. These can be though of as representative equations for the liver if it was a non-metabolizing compartment.

3.3.5.1 Equations for benzene.

$$V_l * \frac{dC_l}{dt} = Q_l * (C_{art} - CV_l) \tag{3.65}$$

This equation indicates that the partitioning of benzene from the arterial blood to the liver compartment depends on the size of the liver compartment expressed in terms of its volume in liters, the blood flow rate into the compartment as well as the partition coefficient of benzene for the liver compartment; i.e. the tissue: blood partition coefficient.

Now, since our final objective is to find the concentration of benzene that remains in the liver C_l, let us start by considering the above equation again in terms of the rate of change of the **mass** of benzene in the liver compartment. It can be expressed as:

$$\frac{dA}{dt} = Q_l * (C_{art} - CV_l) \tag{3.66}$$

$$CV_l = \frac{C_l}{P_l} \tag{3.67}$$

i.e. the amount of benzene that leaves the liver compartment (CV_l) depends on how much benzene binds to the liver (C_l) and how much of it partitions into the venous blood (P_l). Now integrating equation (3.66):

$$A_l = \int Q_l * (C_{art} - CV_l)\, dt \tag{3.68}$$

The final concentration C_l that we need can then be expressed as:

$$C_l = \frac{A_l}{V_l} \tag{3.69}$$

However, this is just the initial set of mass balance equations for the liver compartment. When the equations for the metabolites are written, they will in fact change the equations written for benzene.

As described in the literature review chapter, this research does not use the standard Michaelis Menten expression for saturation kinetics of enzymes because the rates of metabolism of the benzene metabolites affect each other since the metabolites all compete for the same CYP2E1 isoenzyme. Hence different rate equations are needed for each of the non linear metabolic processes.

Rate equations are present for (Lovern et. al., 1999):

1) The rate of change of benzene to benzene oxide can be stated as

$$RM_{bo}^{B} = k_1 \frac{V_{2E1} C_l}{D} C^{MP} T_l \tag{3.70}$$

where,

RM_{bo}^{B} = the rate of metabolism of benzene to benzene oxide

k_1 = efficiency of CYP2E1 for specific oxidations relative to V_{2E1} (L/nmol)

V_{2E1} = CYP2E1 specific activity as determined by the oxidation of p-nitrophenol to p-nitrocatechol (L/nmol)

C_l = the concentration of benzene in the liver

C^{MP} = Concentration of mixrosomal protein per gram of tissue in the liver (mg/g)

T_l = total mass of the liver

$D = 1 + A^B * C_l + A^{ph} * C_{lph} + A^{hq} * C_{lhq}$

A^B was obtained from Cole (2001) and is the inverse of the K_m and the other rate parameters were taken from Lovern et. al., (1999).

To calculate T_l, the density of the liver is assumed to be the same as that of water and the expression then becomes,

$$T_l = V_l * \frac{10^3 g}{1L} \tag{3.71}$$

The D term in the equation is the one that essentially helps to link the metabolic

rates of benzene oxide to the concentration of the other metabolites circulating through the body, namely phenol and hydroquinone.

Similar expressions can be written for the other metabolite rates

3.3.5.2 For benzene oxide metabolites

i) The rate of change of benzene oxide to phenol – linear

$$RM_{ph}^{bo} = k_2 C_{lbo} V_l \tag{3.72}$$

ii) The rate of change of benzene oxide to muconic acid

$$RM_{ma}^{bo} = k_3 C_{lbo} V_l \tag{3.73}$$

iii) The rate of change of benzene oxide to phenylmercapturic acid

$$RM_{pma}^{bo} = k_4 C_{lbo} V_l \tag{3.74}$$

where k_2, k_3, and k_4 are first order rates of metabolism (l/min)

3.3.5.3 For phenol metabolites

i) The rate of change of phenol to hydroquinone

$$RM_{hq}^{hp} = k_5 \frac{V_{2E1} C_l}{D} C^{MP} T_l \tag{3.75}$$

ii) The rate of change of phenol to catechol

$$RM_{cat}^{ph} = k_6 \frac{V_{2E1} C_l}{D} C^{MP} T_l \tag{3.76}$$

where all terms are as described before for the rate of metabolism from benzene to benzene oxide.

For the phenol conjugates – phenyl sulfate and glucoronide, it is assumed that since a larger concentration of phenyl sulfate is excreted than glucoronide, only the phenyl sulfate conjugate expression will be used. This expression uses two Michaelis Menten expressions in one and was developed to model the metabolism of phenol to phenyl sulfate.

$$RM_{conj}^{ph} = \left(\frac{V_{PH1} C_{lph}}{K_{m1ph} + C_{lph}} + \frac{V_{PH2} C_{lph}}{K_{m2ph} C_{lph}}\right) C^{CP} T_l \tag{3.77}$$

where, V_{PH1} and V_{PH2} = the maximum rates of metabolism of phenol by two sulfate transferases

K_{m1ph} and K_{m2ph} = the concentrations at half-saturation of phenol by the two sulfate transferases

C^{CP} = concentration of cytosolic protein per gram of liver

3.3.5.4 For hydroquinone metabolite

The rate of change of hydroquinone to trihydroxy benzene

$$RM_{thb}^{hq} = k_7 \frac{V_{2E1} C_l}{D} C^{MP} T_l \tag{3.78}$$

where all terms are the same as described above for the rate of metabolism from benzene to benzene oxide.

For hydroquinone, glucoronidation is assumed to be the conjugate and the expression for that metabolism is

$$RM_{conj}^{hq} = \frac{V_{hq} C_{lhq}}{K_{mhq} + C_{lhq}} C^{MP} T_l \tag{3.79}$$

where,

K_{mhq}= the concentration at half-saturation for hydroquinone (μmol/l). In the case of hydroquinone, it is assumed that the concentration of the sulfate conjugate produced is very small compared to the gloucoronide and the equation for the latter is assumed to be representative of both conjugates.

Now that the equations for all the metabolites of benzene in the liver have been presented, it should be noted that the metabolite kinetics change the equations originally written for the liver compartment at the top of this section. These are changed as follows:

$$V_l * \frac{dC_l}{dt} = \frac{Q_l}{P_l} *(C_{art} - CV_l) - RM_{bo}^{B} \tag{3.80}$$

The term for the metabolism of benzene to benzene oxide is added as a sink term in the mass balance for the benzene concentration in the liver compartment because once the metabolite benzene oxide is formed, the concentration of benzene is reduced, due to its loss during the break-down. This concentration of benzene oxide produced from the

benzene metabolism then enters the blood circulation and is distributed to all the model compartments. The C_{lbo} (i.e. the concentration of benzene oxide in the liver minus the concentration that is exhaled from the lungs, expressed in the equation below) is considered as the starting concentration for all the other compartments and the form of the mass balance equation still remains the same as that for benzene.

$$\frac{dA_{bo}}{dt} = Q_c * (CV_{bo} - C_{lbo}) \tag{3.81}$$

The amount of benzene oxide that is released back to the venous blood for recirculation is the CV_{ibo}, where i stands for the compartment. This amount can be calculated by integrating the following equation. The equation also accounts for the metabolism of benzene oxide to phenol, pheylmercapturic acid and muconic acid because the concentration of benzene oxide is reduced due to. The expression is

$$\frac{dAlbo}{dt} = Q_l * (C_{artbo} - \frac{C_{lbo}}{P_{lbo}}) + RM_{bo}^{B} - RM_{ph}^{bo} - RM_{ma}^{bo} - RM_{pma}^{bo} \tag{3.82}$$

The same occurs with phenol and hydroquinone as well.

The amount of phenol can also be calculated similar to the benzene oxide expression by integrating the equation

$$\frac{dAlph}{dt} = Q_l * (C_{artph} - \frac{C_{lph}}{P_{lph}}) + RM_{ph}^{bo} - RM_{hq}^{ph} - RM_{ct}^{ph} - RM_{conj}^{ph} \tag{3.83}$$

The amount of hydroquinone can be calculated by integrating

$$\frac{dAlhq}{dt} = Q_l * (C_{arthq} - \frac{C_{lhq}}{P_{lhq}}) + RM_{hq}^{ph} - RM_{thb}^{hq} - RM_{conj}^{hq} \tag{3.84}$$

3.3.6 Bone marrow

As stated before, the bone marrow is a rapidly perfused tissue. However, since it is also the main target organ for benzene carcinogenicity, in this model, its is considered as a separate compartment. It is also the organ of metabolism. The bone marrow equations are presented as follows.

3.3.6.1 Benzene equations. The basic mass balance equation for the bone marrow is;

$$V_{bm} * \frac{dC_{bm}}{dt} = Q_{bm} *(C_{art} - CV_{bm}) \quad (3.85)$$

This equation indicates that the partitioning of benzene from the arterial blood to the bone marrow compartment depends on the size of the bone marrow compartment expressed in terms of its volume in liters, the blood flow rate into the compartment as well as the partition coefficient of benzene for the bone marrow compartment; i.e. the tissue: blood partition coefficient.

Now, since our final objective is to find the concentration of benzene that remains in the bone marrow C_{bm}, let us start by considering the above equation again in terms of the rate of change of the **amount** of benzene in the bone marrow compartment. It can be expressed as:

$$\frac{dA}{dt} = Q_{bm} *(C_{art} - CV_{bm}) \quad (3.86)$$

$$CV_{bm} = \frac{C_{bm}}{P_{bm}} \quad (3.87)$$

i.e. the amount of benzene that leaves the bone marrow compartment (CV_{bm}) depends on how much benzene binds to the bone marrow (C_{bm}) and how much of it partitions into the venous blood (P_{bm}).

Now integrating equation (3.86):

$$A_{bm} = \int Q_{bm} *(C_{art} - CV_{bm})dt \quad (3.88)$$

Integrating equation 3.88, the final concentration C_{bm} that we need can then be expressed as:

$$C_{bm} = \frac{A_{bm}}{V_{bm}} \quad (3.89)$$

3.3.6.2 Benzene oxide equations.

$$\frac{dA_{bm}}{dt} = Q_{bm} *(C_{artbo} - CV_{bmbo}) \quad (3.90)$$

$$CV_{bmbo} = \frac{C_{bmbo}}{P_{bmt}} \quad (3.91)$$

$$A_{bmbo} = \int Q_{bm} *(C_{artbo} - CV_{bmbo})\, dt \quad (3.92)$$

$$C_{bmbo} = \frac{A_{bmbo}}{V_{bm}} \tag{3.93}$$

3.3.6.3 Phenol equations.

$$\frac{dA_{ph}}{dt} = Q_{bm} * (C_{artph} - CV_{bmph}) \tag{3.94}$$

$$CV_{bmph} = \frac{C_{bmph}}{P_{bmt}} \tag{3.95}$$

$$A_{bmph} = \int Q_{bm} * (C_{artph} - CV_{bmph})\, dt \tag{3.96}$$

$$C_{bmtph} = \frac{A_{bmph}}{V_{bm}} \tag{3.97}$$

3.3.6.4 Hydroquinone equations:

$$\frac{dA_{hq}}{dt} = Q_{bm} * (C_{arthq} - CV_{bmhq}) \tag{3.98}$$

$$CV_{bmhq} = \frac{C_{bmhq}}{P_{bm}} \tag{3.99}$$

$$A_{bmhq} = \int Q_{bm} * (C_{arthq} - CV_{bmhq}) dt \tag{3.100}$$

$$C_{bmhq} = \frac{A_{bmhq}}{V_{bm}} \tag{3.101}$$

The only additional equation for the bone marrow compartment is that for the rate of metabolism from hydroquinone to 1,4 benzoquinone. It is presented as follows:

$$RM_{bq}^{hq} = k_7 \frac{V_{2E1} C_{bm}}{D} C^{MP} T_{bm} \tag{3.102}$$

Note that due to lack of research in this area, the same form of extended Michaelis Menten expression is used for this rate of metabolism from hydroquinone to 1,4 benzoquinone. However, the concentration of hydroquinone in the bone marrow is considered and the expression for the D in the denominator is

$$D = 1 + A^{B} * C_{bm} + A^{ph} * C_{bmph} + A^{hq} * C_{bmhq} \tag{3.103}$$

This expression is still kept the same except for considering the metabolite concentrations from the bone marrow because of the influence that these other

metabolites have on this metabolic rate as explained in chapter 2. The V_{2E1} value is also kept as is because this is specific for the enzyme and not the metabolite. The bone marrow density is also presumed to be the same as that of the liver and the T_{bm} value is calculated.

Now that the equations for the additional metabolites of benzene in the bone marrow has been presented, it should be noted that the metabolite kinetics change the equations originally written for the bone marrow compartment at the top of this section. This change is as follows:

$$V_{bm} * \frac{dC_{bm}}{dt} = \frac{Q_{bm}}{P_{bm}} *(C_{art} - CV_{bm}) + RM_{hq}^{ph} - RM_{bq}^{hq} \quad (3.104)$$

The concentration of BQ in the bone marrow can be estimated by integrating the equation:

$$\frac{dAbmhq}{dt} = Q_{bm} *(C_{arthq} - \frac{C_{bmhq}}{P_{bmhq}}) + RM_{hq}^{ph} - RM_{bq}^{hq} \quad (3.105)$$

3.4 Data from literature

3.4.1 Cigarette smoke data

The data on cigarette smoke came from Jenkins et. al. (2000); who explained that the concentration of benzene in cigarettes is comprised of two parts. The first is the mainstream cigarette smoke and the second is the sidestream cigarette smoke. Mainstream smoke is that part of the cigarette smoke that is actually inhaled by the smoker. The sidestream smoke is the smoke that is released into the ambient environment, of which the smoker only inhales a specific amount. For the input concentration in the model, but these concentrations were considered. They are calculated as follows:

Benzene concentration in the mainstream smoke of a cigarette is about 45 µg (Jenkins et. al., (2000). Although it is known that the benzene concentration in the sidestream smoke is 10 times higher than the amount in the mainstream smoke, the actual concentrations are not known for benzene. However they are known for nicotine (500

μg), and hence the nicotine numbers are used for this calculation; i.e. the benzene concentration in the sidestream cigarette smoke is assumed to be 3.3 % of 500 μg.

Thus the total benzene concentration inhaled by smoking one cigarette is calculated as

45 μg + 3.3 % of 500 μg.

= 45 μg + 0.33 *500 μg

= 61.5 μg

Thus for a smoker who smokes 20 cigarettes every day, the exposure concentration is calculated as 61.5 * 20 = 1230 μg of benzene everyday.

The Jenkins study also states that it takes an individual about 10 minutes to smoke a cigarette based on values used by the Federal Trade Commission (Jenkins et. al., 2000). Hence, the concentration of benzene inhaled per cigarette smoked per minute is 61.5 μg/10 = 6.15 μg/min.

However, this is not the actual concentration of benzene inhaled by the smoker, because the inhalation depends upon the ventilation rate of the person (Q_a). This rate is 5 for healthy adult males (Travis, 1990). Hence the concentration of benzene inhaled becomes 6.15 μg/5 = 1.236 μg/min. Thus, this is the concentration used in the model as the input concentration.

The assumptions made in using these data are:

1. The cigarettes are smoked in the ambient environment.
2. The smoker smokes 20 cigarettes a day.
3. The smoker smokes 1 cigarette every fifty minutes, which means that the smoker is exposed to a concentration of 1.236 μg of benzene for 10 minutes, then no exposure for the next 40 minutes, then another 10 minutes of exposure, followed by 40 minutes of no exposure and so on.
4. Since 20 cigarettes are smoked in a day and the smoking pattern is as described above, all 20 cigarettes are smoked in a total of 960 minutes, or just about 16 hours. This is also assuming that the smoker is asleep in the remaining 8 hours of the day.

3.4.2 All other model data

As stated before, no experimentation was performed for this study. All data used in the study are from available current literature. This section explains where all the data used in the study were taken from and presents all these data in the tables 3.1 and 3.2. While several data sets for humans were available, it was necessary to use data from reliable sources for use in the model. The data sets presented by Travis et. al., (1990) were selected as the main data source for all human and benzene specific parameters, except for the partition coefficients, since the Travis model considered the bone marrow as a separate compartment. Hence data were available for the bone marrow and for consistency; it was decided to use other data from that study as well. The study as well as many other studies by the same authors have been published in several well known peer reviewed journals such as the Journal of Toxicology, Journal of Toxicology and Applied Pharmacology, Toxicology Letters, Risk Analysis, Environmental Health Perspectives, etc. and hence the reliability of the data is assumed excellent.

The metabolic parameter data, including the partition coefficients of metabolites, their rate constants and other metabolite related information was taken from the latest research performed by Cole (2001).

Only one source of data on parameters for women was available and that was taken from Brown et. al., (1998). The weight value for women was taken from this source and the other parameters were adjusted for this body weight from the parameters obtained from Travis et. al., (1990). The other set of data obtained from this source were the benzene tissue:blood partition coefficients. Since reliable data from bone marrow:blood partition coefficient were not available, the same values as the RPT were taken for this parameter.

The data used in the model spanned a number of different sources. To ensure that the model results are not affected by the data a sensitivity analysis was performed for some of the model parameters. The rate constants for the metabolic reactions of benzene and its metabolites are taken from a single source and hence it was imperative to check these numbers for their sensitivity to the model.

Table 3.1 Physiological and Biochemical Parameters Used for Adult Males and Females for benzene

Parameter	Male	Female	Units
Body weight	70	60	Kg
Organ weights			l
Fat (V_f)	13.3	11.4	
RPT (V_{rpt})	3.5	3	
PPT (V_{ppt})	40.6	34.8	
Liver (V_l)	1.82	1.56	
Bone marrow (V_{bm})	2.8	2.4	
Alveolar ventilation rate (Q_a)	5.0	4.0	l/min
Blood flow rates			l/min
Cardiac output (Q_c)	6.2	5.4	
Fat (Q_f)	0.31	0.27	
RPT (Q_{rpt})	2.728	2.376	
PPT (Q_{ppt})	1.364	1.188	
Liver (Q_l)	1.55	1.35	
Bone marrow (Q_{bm})	0.2418	0.2106	
Partition coefficients			
Blood: Air partition coefficient (PB)	7.8	8.2	
For benzene and benzene oxide			
Fat:blood (P_f)	54.5	51.8	
RPT:blood (P_{rpt})	1.92	1.8	
PPT:blood (P_{ppt})	2.05	2	
Liver:blood (P_l)	2.95	2.8	
Bone marrow:blood (P_{bm})			

Table 3.1 Continued

Parameter	Male	Female	Units
For phenol (Cole, 2001)	1.92	1.8	
Fat:blood (P_{fph})			
RPT:blood (P_{rptph})			
PPT:blood (P_{pptph})	27.63	27.63	
Liver:blood (P_{lph})	2.17	2.17	
Bone marrow:blood (P_{bmph})	1.22	1.22	
	2.17	2.17	
For hydroquinone (Cole, 2001)	2.17	2.17	
Fat:blood (P_{fhq})			
RPT:blood (P_{rpthq})			
PPT:blood (P_{ppthq})	4.06	4.06	
Liver:blood (P_{lhq})	1.04	1.04	
Bone marrow:blood (P_{bmhq})	0.94	0.94	
	1.04	1.04	
	1.04	1.04	

Table 3.2 Metabolic parameters

Parameter	Value – same values are used for males and females	Unit	Value used in the model (converting to correct units)	Model units
V_{2E1}	141	Nmol/mg/hr	2.35	Nmol/mg/min
V_{ph1}	0.0221	µmol/mg protein/hr	0.034660	µg/mg protein/min

Table 3.2 Continued

Parameter	Value – same values are used for males and females	Unit	Value used in the model (converting to correct units)	Model units
V_{ph2}	0.295	µmol/mg protein/hr	0.462658	µg/mg protein/min
V_{hq}	1.0456	µmol/mg protein/hr	1.918676	µg/mg protein/min
K_{m1ph}	1.4	µmol/l	131.74	µg/l
K_{m2ph}	220	µmol/l	20702	µg/l
K_{mhq}	746	l/µmol	82134.6	µg/l
A_{bz}	0.0397	l/µmol	0.0005089	µg/l
A_{ph}	0.013	l/µmol	0.00013815	µg/l
A_{hq}	0.0000001	l/µmol	0.000000009	µg/l
k1	0.000042	l/µmol	0.00042	µg/l
k2	32.16	1/hr	0.536	l/min
k3	2.045	1/hr	0.034083	l/min
k4	0.85	1/hr	0.014166	l/min
k5	0.00004	l/µmol	0.00004	l/µmol
k6	0.00000213	l/µmol	0.00000213	l/µmol
k7	0.000000203	l/µmol	0.000000203	l/µmol
k8	7.3	1/hr	0.121666	l/min
k9	29.58	1/hr	0.493	l/min
k10	421	1/hr	7.0166	l/min
C^{cp}	57.3	mg/g-tissue	57.3	mg/g-tissue
C^{mp}	387	mg/g-tissue	387	mg/g-tissue

This software is the one commonly used by professionals in the field of PBPK/TK modeling. Some examples are the previously referenced PBPK model for tetrachloroethylene exposure in lactating mothers and breast fed infants (Byczkowski, 1996), a PBPK model for canine inhalation of halogenated hydrocarbons (Vinegar, 2000), a PBPK model for acrylonitrile. exposure in humans developed by Sweeney et. al., (2003) among others. Hence for consistency, the selection of this application is ideal. Moreover, the choice of this application is also beneficial to future researchers who may validate the model against their data.

3.5 Summary of the model

To summarize, the six compartmental benzene inhalation PBPK/TK model comprises of the lung, fat, RPT, PPT, liver and bone marrow compartments. Mass balance analyses are performed for each chemical for each compartment and the equations are developed. They are converted to ordinary differential equations, which have to be solved simultaneously to obtain the benzene and metabolite concentrations in the compartments. The data used in the model are presented.

These model equations developed here extensions and improvements over existing models such as the Medinsky model (1989), Travis (1990), Bois and Paxman (1992) and Cole (2001) and the overall model contributes greatly to the field of PBPK/TK models for benzene.

CHAPTER 4

4. RESULTS AND VALIDATION

4.1 Introduction

Model results are presented in this chapter. Model runs are performed in accordance with the study objectives. The starting point for model development in this study was the use of cigarette smoke data to simulate intermittent exposure scenarios. Thus, this is the first run of the model. This run is followed by several situations that demonstrate the capabilities of the model. For example, runs for low and high benzene inhalation concentrations (50 ppm and 490 ppm), runs for six hour and eight hour benzene inhalation exposure at a concentration of 490 ppm to validate the model against published data and runs with and without the bone marrow to demonstrate the effect of including the bone marrow as a separate compartment in the model.

Each scenario is run for healthy, adult male and female exposures. Based on all the male and female runs, it was observed that there is no significant difference in the model prediction of benzene and its major metabolite concentrations in men and women. The only exception to this was the case of continuous 8 hour exposure in females to a concentration of 490 ppm.

Hence not all cases of female exposure are shown. However, results from all the female exposure runs not presented in this document are available with the author and can be made available for perusal.

Scenarios that the model is run for and presented in this chapter can be listed as follows:

Case 1: Modeling benzene concentrations in males and females for 1.236 μg/l

benzene (inhaled from cigarette smoke) intermittently for 10 minutes intervals over a total period of 16 hours.

Case 2: Modeling benzene concentrations in males and females for 490 ppm (1470 μg/l) of continuous 8 hour benzene exposure.

Case 3: Modeling benzene concentrations in males and females for a 490 ppm (1470 μg/l) of continuous 6 hour benzene exposure.

Case 4: Comparative modeling of benzene inhalation in males and females exposed to 10 ppm of benzene, continuously for 4 hours, intermittently at a regular interval of 1 hour for 10 minutes and intermittently at an irregular interval of 10 minutes of exposure, followed by 1 hours of no exposure, then 20 minutes of exposure followed by 30 minutes of no exposure followed by 1 hour exposure, then 15 minutes of exposure followed finally by 45 minutes of no exposure.

Case 5: Model runs considering the bone marrow as a separate compartment and considering that it is a part of the rapidly perfused tissues compartment in males and females.

While each of the above cases was run for both male and female exposure in this chapter results from only male exposure are presented. The results from female exposure are shown on for case 4A on continuous 8 hour inhalation exposure to benzene at a concentration level of 50 ppm or 150 μg/l.

Additional runs are performed to determine the sensitivity of model parameters on model results. These runs are based on the Monte Carlo simulation technique and are presented in chapter 5. All results are analyzed and discussed in chapter 7. All model runs presented in this chapter have the following graphs for each run.

1) Inhalation pattern (Figures 4.1, 4, 18, 4.22, 4.26, 1.55, 4.70).
2) Concentration of benzene in the arterial and venous blood (μg/l) (Figures 4.2, 4.19, 4.23, 4.27, 4.41, 4.56 and 4.71).
3) Concentration of benzene in the fat compartment (μg/l) (Figures 4.3, 4.20. 4.24, 4.28, 4.42, 4.57, and 4.72).
4) Concentration of benzene leaving the fat compartment (μg/l) (Figures 4.4, 4.28, 4.42, 4.57, and 4.72).

5) Concentration of benzene in and leaving the rapidly perfused tissues (μg/l) (Figures 4.5, 4.29, 4.43, 4.58, and 4.73).
6) Concentration of benzene in and leaving the poorly perfused tissues (μg/l) (Figures 4.6, 4.30, 4.44, 4.59, and 4.74).
7) Concentration of benzene in and leaving the liver (μg/l) (Figures 4.7, 4.31, 4.45, 4.60, and 4.75).
8) Concentration of benzene in and leaving the bone marrow (μg/l) (Figures 4.8, 4.21, 4.25, 4.32, 4.46, 4.61, and 4.76).
9) Concentrations of benzene oxide in the fat, PPT, RPT and bone marrow compartments (Figures 4.9, 4.33, 4.47, 4.62, and 4.77).
10) Concentrations of phenol in the fat, PPT, RPT and bone marrow compartments (μg/l) (Figures 4.10, 4.34, 4.48, 4.63, and 4.78).
11) Concentrations of hydroquinone in the fat, PPT & RPT compartments (μg/l) (Figures 4.11, 4.35, 4.49, 4.64, and 4.79).
12) Concentrations of hydroquinone in the bone marrow compartment. (This graph is plotted separately because its extremely small value caused it to fall outside the range of the scale for the other concentrations of hydroquinone) (μg/l) (Figures 4.12, 4.36, 4.50, 4.65, and 4.80).
13) Concentrations of metabolites in the liver (This graph is plotted separately to show the metabolite concentrations specifically in the metabolizing compartment) (μg/l) (Figures 4.13, 4.37, 4.51, 4.66, and 4.81).
14) Concentrations of phenol and hydroquinone in the liver (This graph is plotted separately for a clearer visual) (μg/l) (Figures 4.14)
15) The concentration of 1,4 p-benzoquinone in the bone marrow compartment (μg/l) (Figures 4.15, 4.38, 4.52, 4.67, and 4.82).
16) The concentration of the conjugates of benzene metabolites excreted in the urine (μg/l) (Figures 4.16, 4.39, 4.52, 4.48, and 4.83).
17) The concentration of muconic acid (MA) and phenylmercapturic acid (PMA) excreted in urine (μg/l) (Figures 4.17, 4.40, 4.54, 4.69, and 4.84).

4.2 Model runs for 1 day intermittent inhalation exposure to benzene concentrations of 1.236 μg/l benzene in adult male cigarette smokers.

The input concentration for this model run is 1.236 μg/l of benzene inhaled intermittently as depicted in figure 4.1 for male smokers. Assumptions related to this input concentration are as follows:

1. The smoker smokes a single pack of 20 cigarettes a day (16 hours).
2. The smoking pattern is uniform, with 10 minutes of smoking a cigarette followed by 40 minutes of no smoking and hence no exposure.

The results are a combination of several graphs as described above in section 4.1 and as shown below in figures 4.2 to 4.17 for male exposure. The graphs for female exposures to this scenario are not presented for brevity. These are available with the author.

4.3 Model runs for 8 hour and 6 hour continuous inhalation exposure to benzene concentrations of 490 ppm (1470 μg/l) exposure in adult males

The model was run for this exposure situation based on the data available to validate the model. The validation data were obtained from Spear and Bois (1992), which required model results using initial concentrations of 490 ppm (1470 mg/l) by running the model for continuous 8 hour and 6 hour exposure duration. These scenarios are represented as cases 2 and 3 respectively. Not all graphs are shown for these cases. Only graphs pertinent to validation are presented. Graphs for other exposure scenarios in both these cases as well as those for female exposure are available with the author.

4.4 Model runs for 8 hour continuous, regular intermittent and irregular intermittent inhalation exposure to benzene concentrations of 50 ppm (150 μg/l) exposure in adult males and females

The model was then run for a comparative analysis of three different situations. These were:

1. Continuous 8 hour inhalation exposure to 50 ppm (150 μg/l) benzene concentrations (represented as Case 4A).
2. Intermittent inhalation exposure to benzene concentrations at regular intervals of 30 minutes exposure then 30 minutes no exposure such that the total inhaled concentration over 8 hours is 50 ppm (150 μg/l) (represented as Case 4B).
3. Intermittent inhalation exposure to benzene concentrations at irregular intervals described as follows:
    i. Exposure for 5 minutes, then no exposure for 10 minutes.
    ii. Exposure for 7 minutes, then no exposure for 12 minutes
    iii. Exposure for 13 minutes, then no exposure for 62 minutes
    iv. Exposure for 25 minutes, then no exposure for 55 minutes
    v. Exposure for 50 minutes, then no exposure for 110 minutes
    vi. Exposure for 40 minutes

The overall exposure is such that the total inhaled concentration over 8 hours is 50 ppm (150 μg/l) (represented as Case 4C).

The graphs from these test runs are shown against a white background to differentiate this run from the previous 2 runs. For case 4A, graphs for female exposure are also shown.

4.5 Model Runs with and Without the Bone Marrow as a Separate Compartment for 8 hour continuous inhalation exposure to benzene concentrations of 490 ppm (1470 μg/l) exposure in males

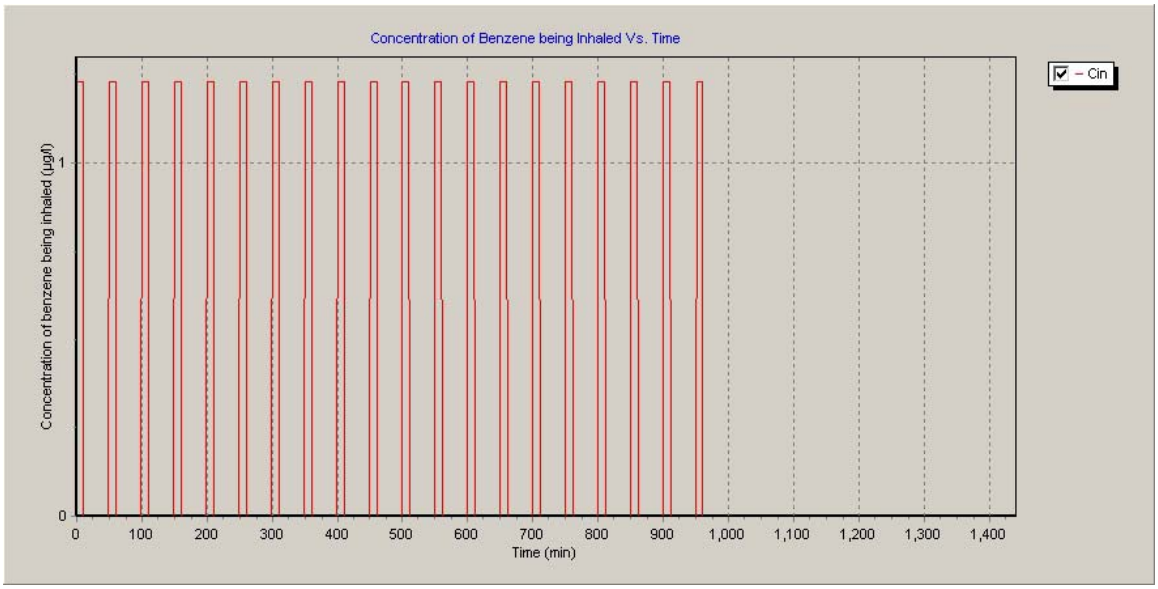

Figure 4.1 - Case 1 Benzene Inhalation Pattern.

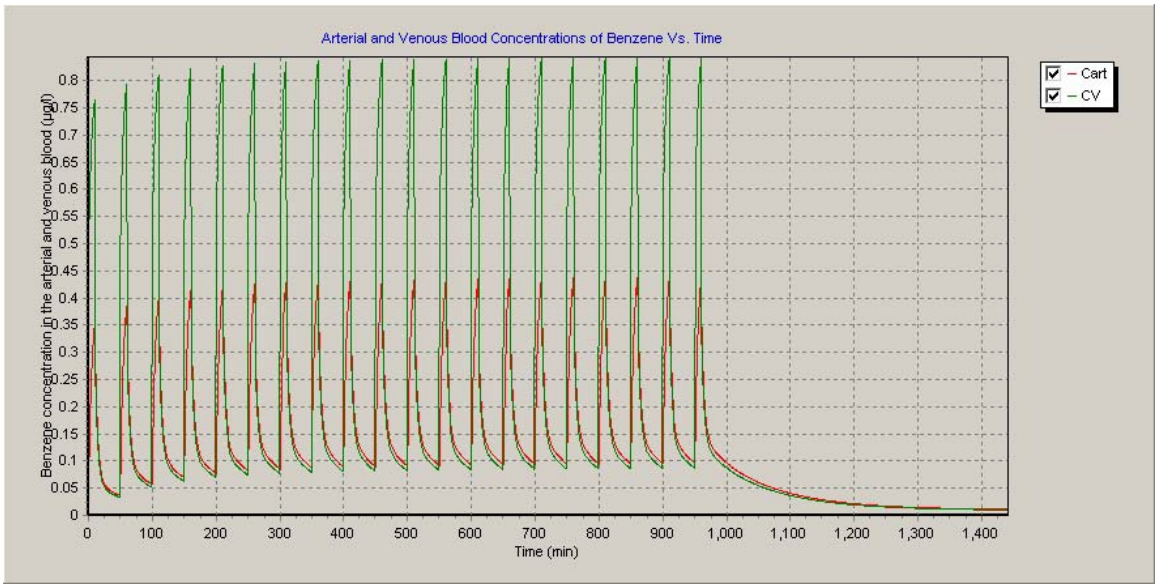

Figure 4.2 Case 1 - Arterial and Venous Blood Concentrations of Benzene.

Figure 4.3 Case 1 - Benzene Concentrations in the Fat Compartment

Figure 4.4 Case 1 – Benzene Concentrations Leaving the Fat Compartment

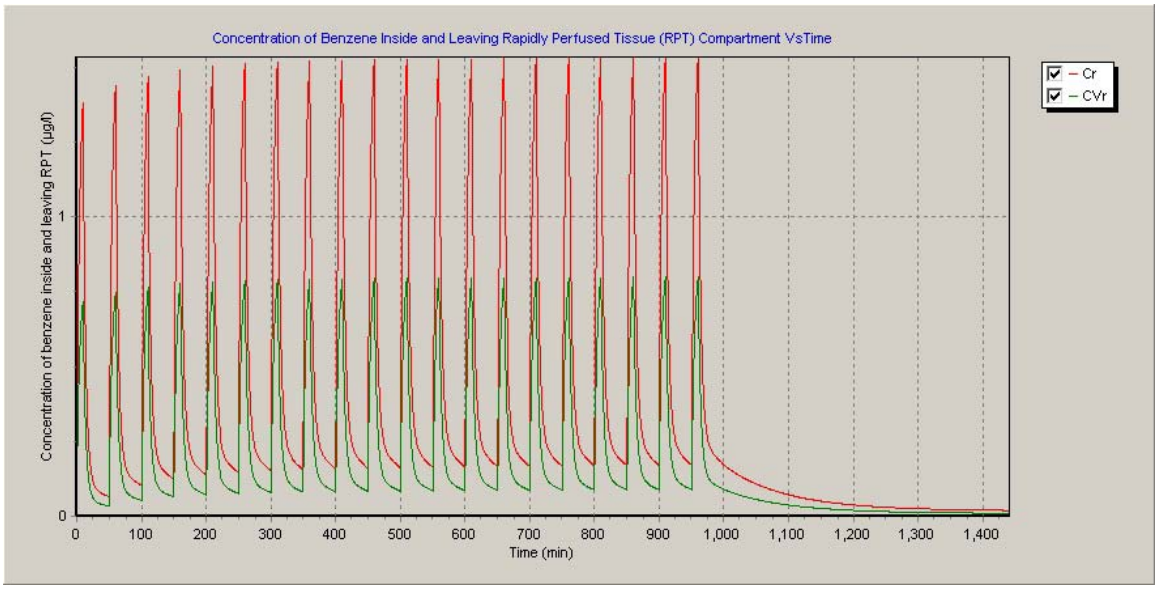

Figure 4.5 Case 1 – Benzene Concentrations in and Leaving the RPT Compartment.

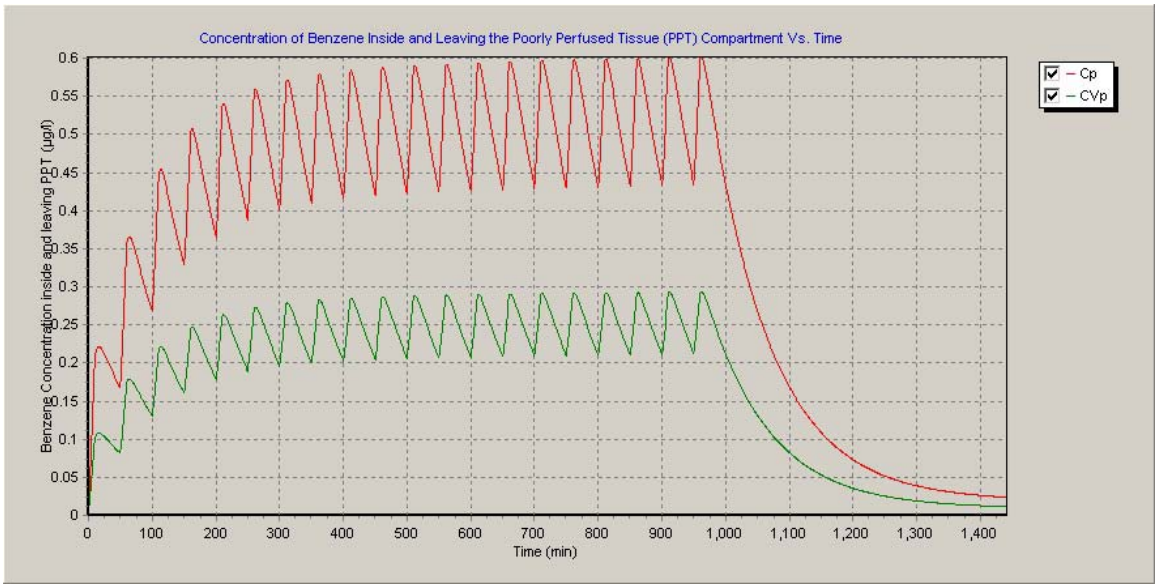

Figure4.6 Case 1 – Benzene Concentrations in and Leaving the PPT Compartment

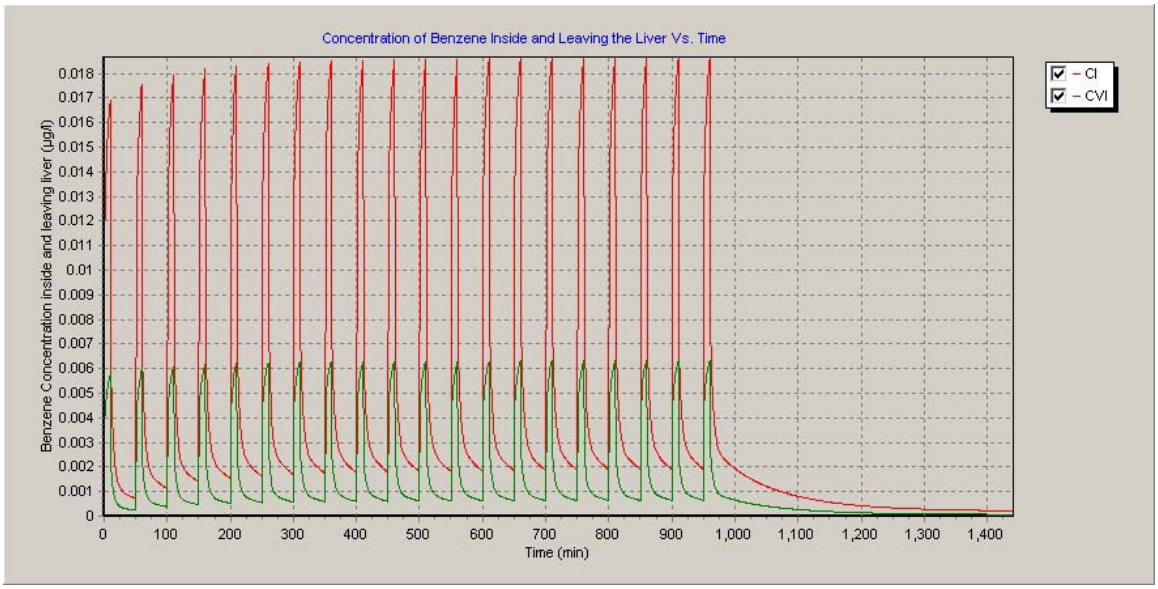

Figure 4.7 Case 1 – Benzene Concentrations in and Leaving the Liver Compartment

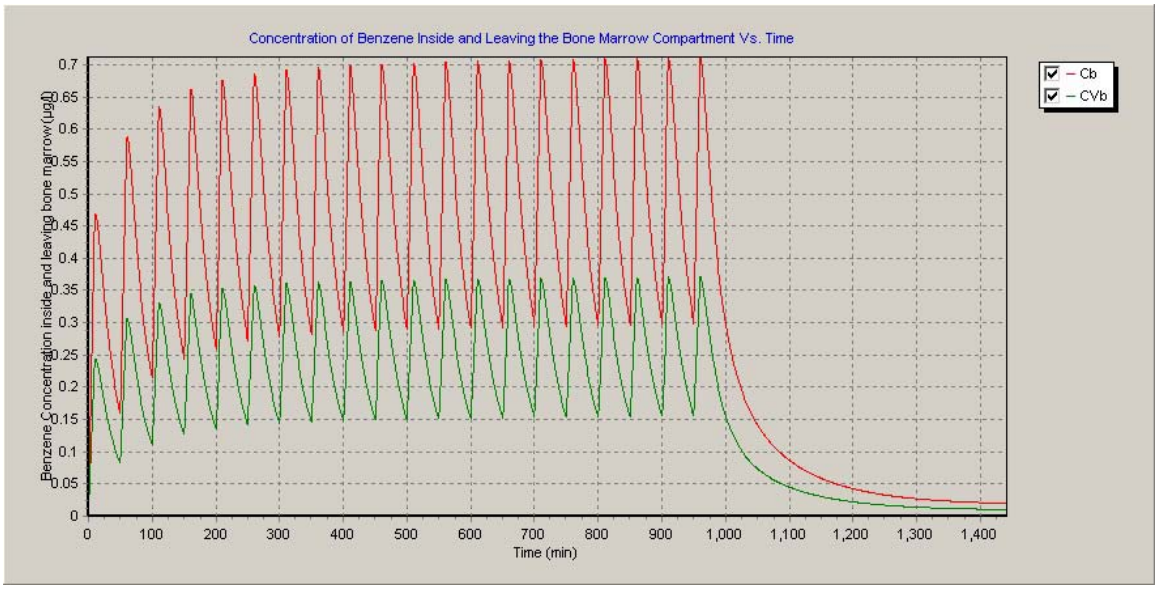

Figure 4.8 Case 1 – Benzene Concentrations in and Leaving the Bone Marrow Compartment.

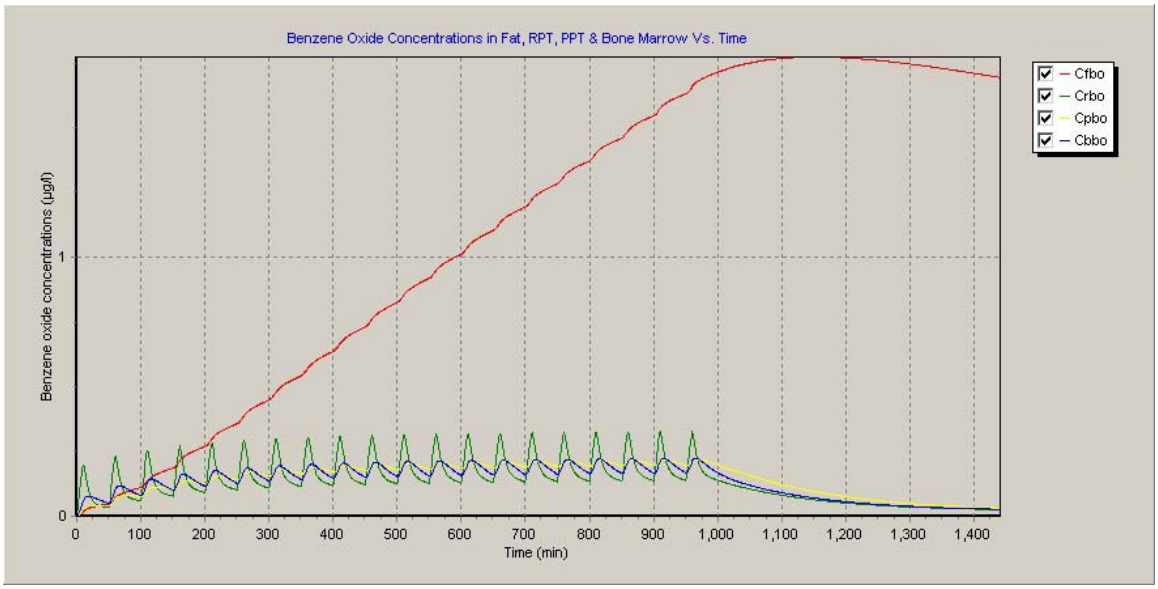

Figure 4.9 Case 1 – Benzene Oxide Concentrations in Fat, RPT, and Bone Marrow Compartments.

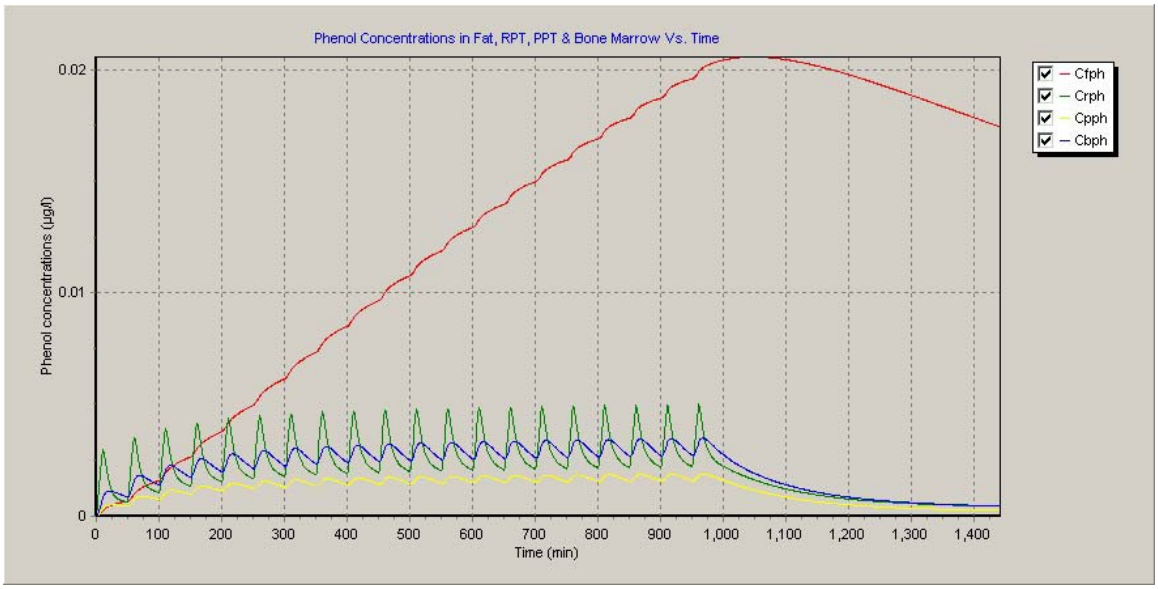

Figure 4.10 Case 1 – Phenol Concentrations in Fat, RPT, PPT and Bone Marrow Compartments.

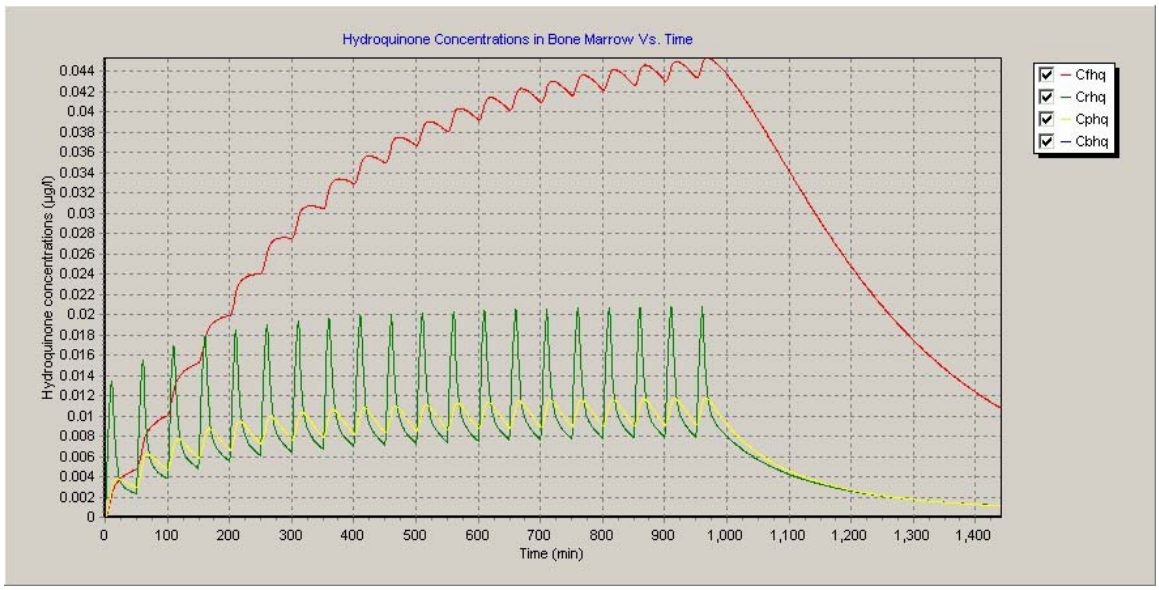

Figure 4.11 Case 1 – Hydroquinone Concentrations in Fat, RPT, and PPT Compartments.

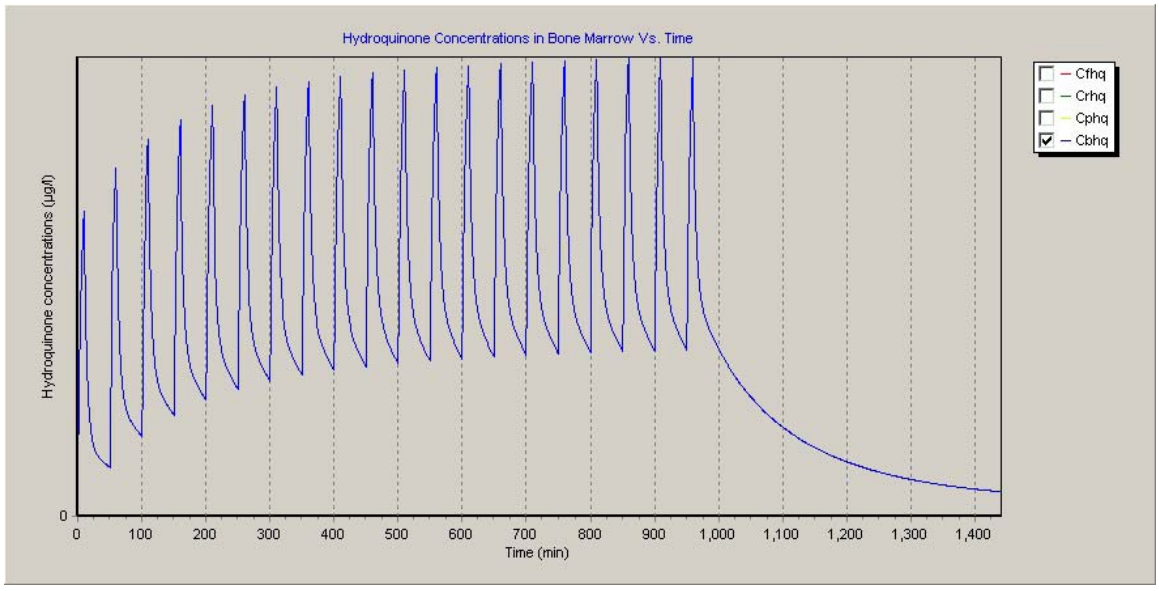

Figure 4.12 Case 1 - Hydroquinone Concentrations in Bone Marrow Compartment.

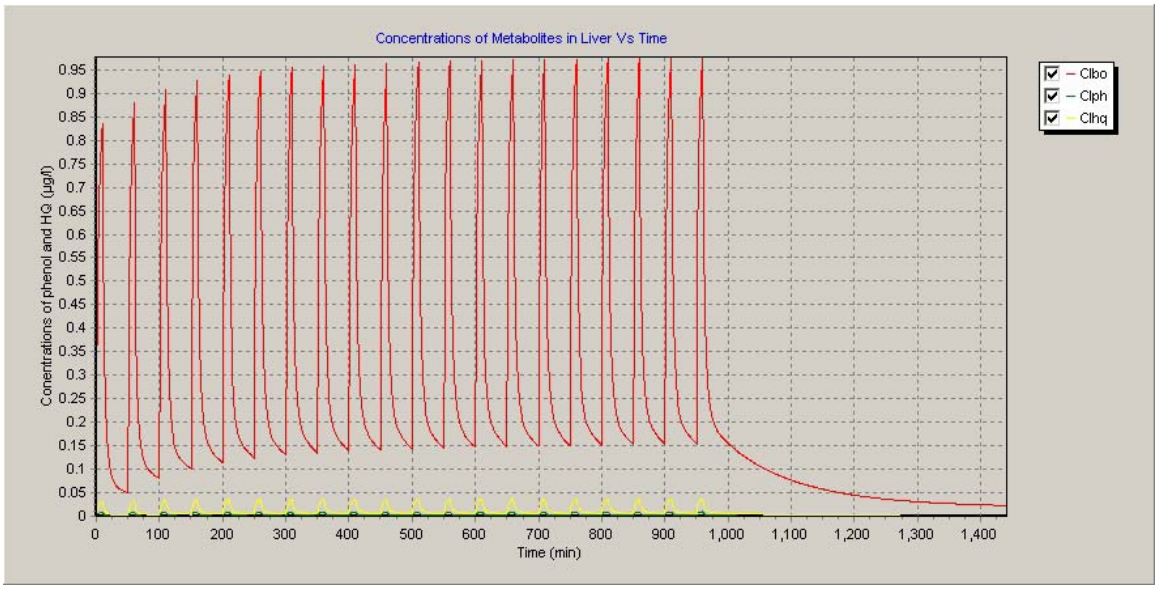

Figure 4.13 Case 1 – Metabolite Concentrations in Liver.

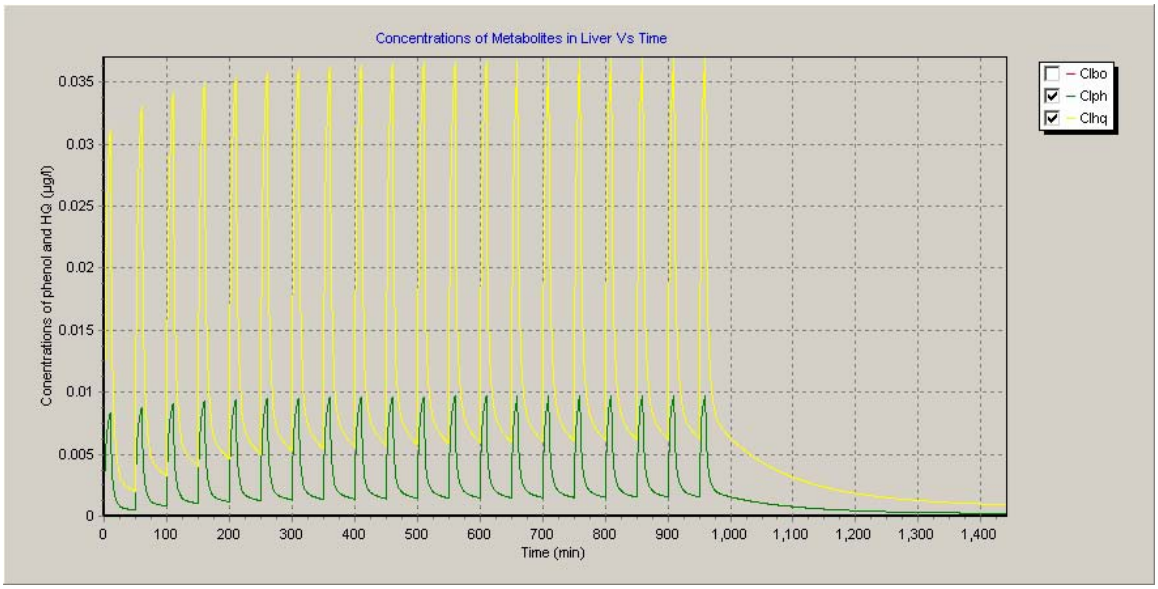

Figure 4.14 Case 1 – Phenol and Hydroquinone Concentrations in Liver.

Figure 4.15 Case 1 – Benzoquinone Concentrations in Bone Marrow Compartment.

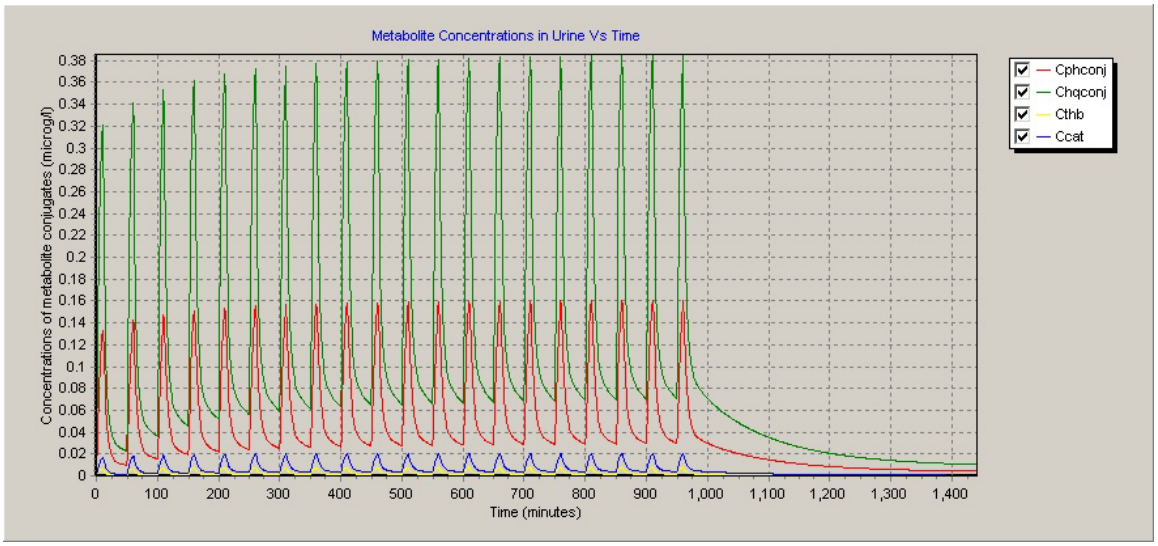

Figure 4.16 Case 1 – Concentrations of Metabolite Conjugates in Urine.

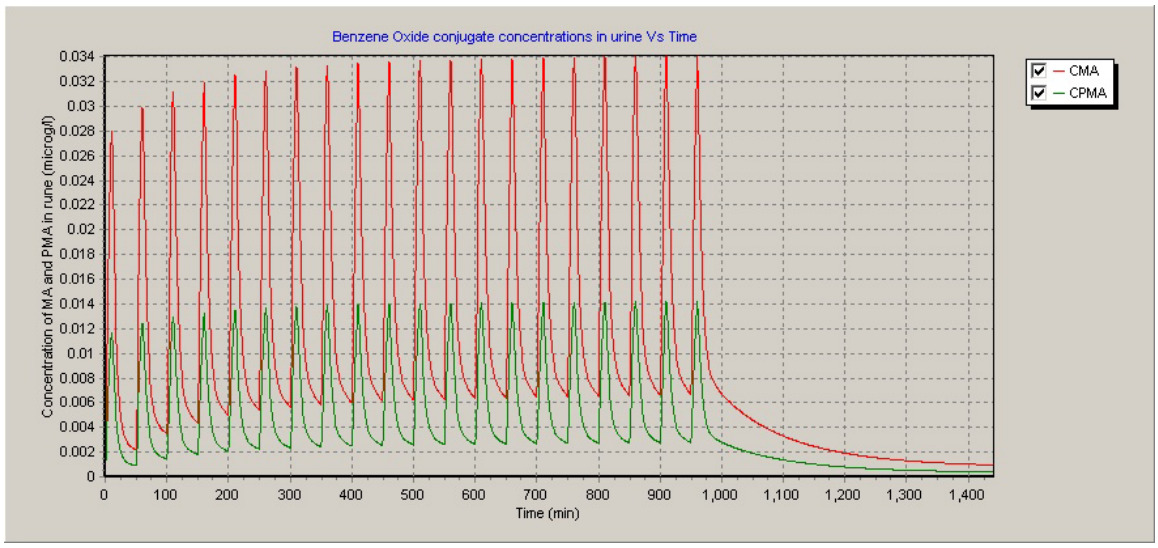

Figure 4.17 Case 1 – Concentrations of MA and PMA in Urine.

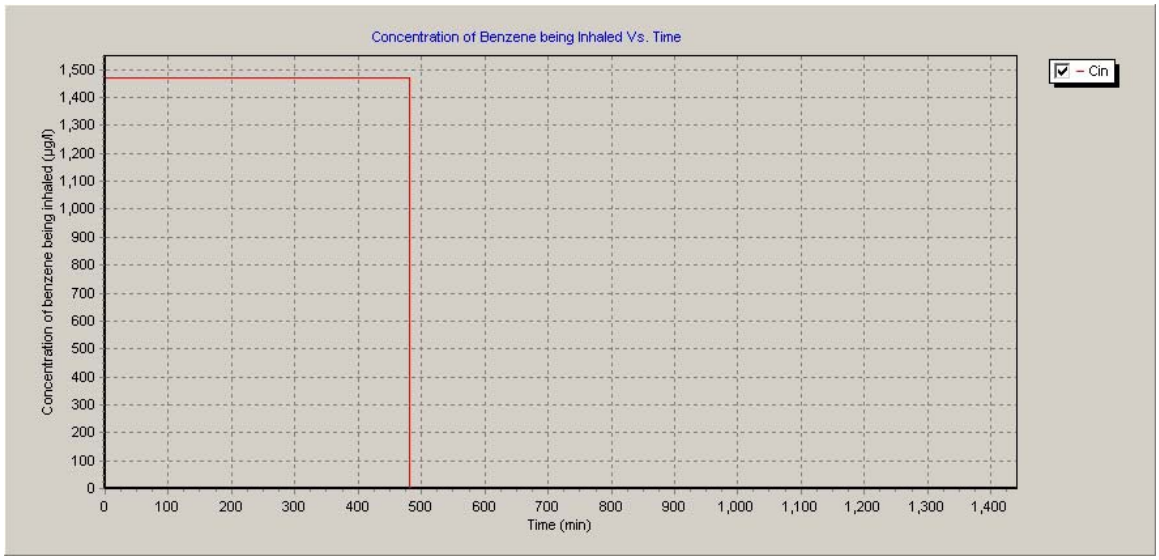

Figure 4.18 Case 2 – Benzene Inhalation pattern

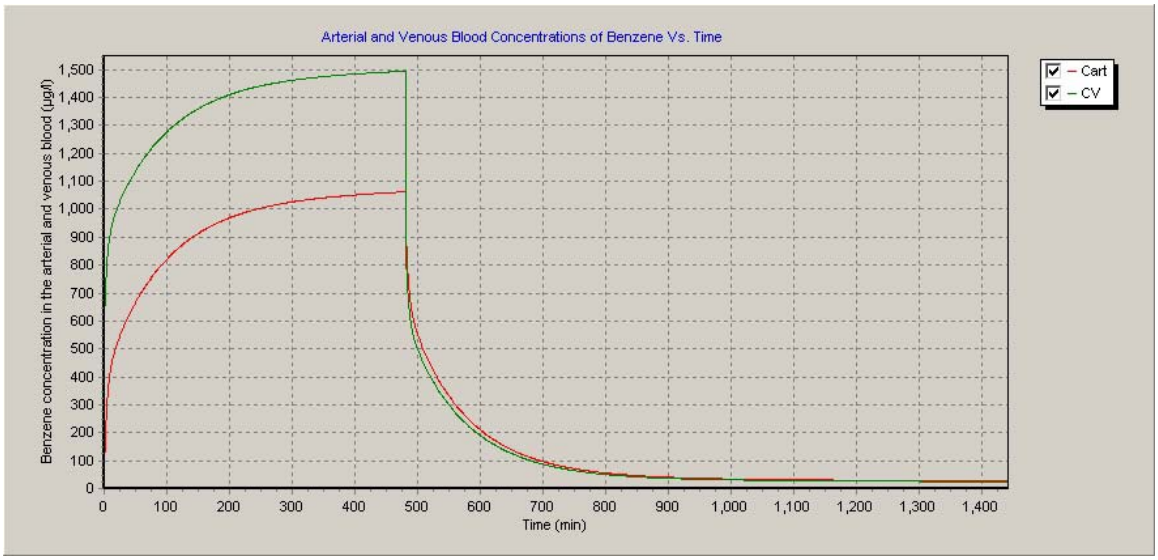

Figure 4.19 Case 2 – Arterial and Venous Blood Cocnentrations of Benzene.

Figure 4.20 Case 2 – Benzene Concentrations in Fat Compartment.

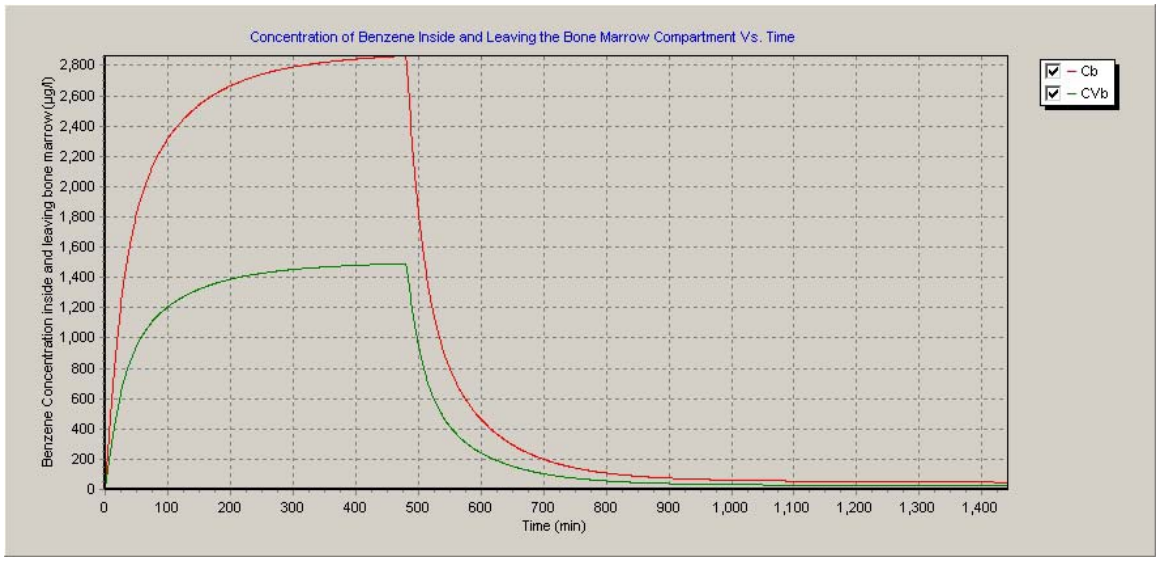

Figure 4.21 Case 2 – Benzene Concentrations in and Leaving Bone Marrow.

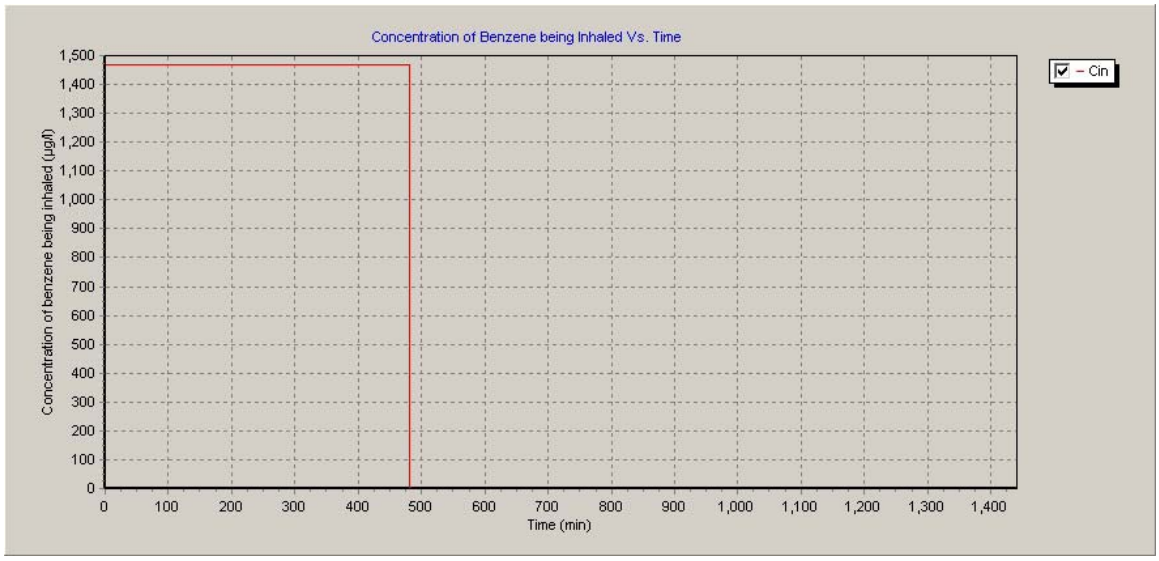

Figure 4.22 Case 3 – Benzene Inhalation Pattern.

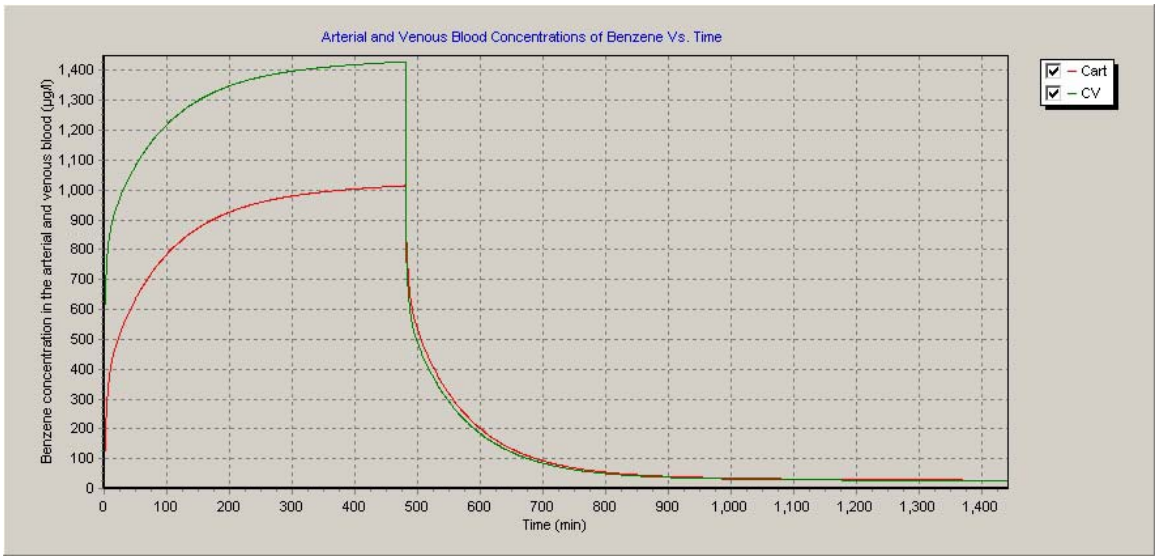

Figure 4.23 Case 3 – Arterial and Venous Blood Concentrations of Benzene.

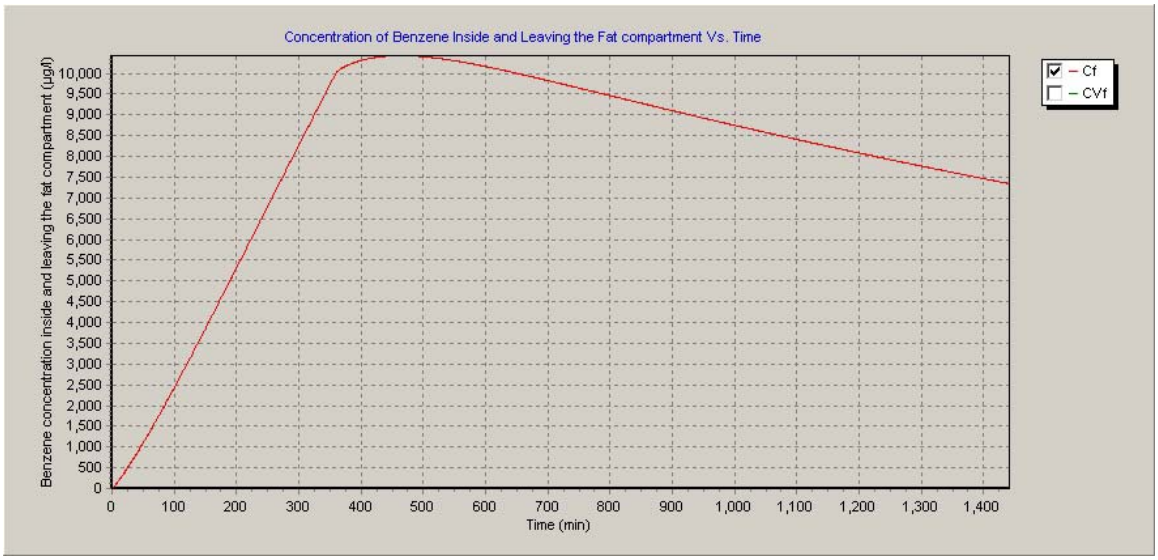

Figure 4.24 Case 3 – Benzene Concentration in Fat Compartment.

Figure 4.25 Benzene Concentrations in and Leaving Bone Marrow Compartment.

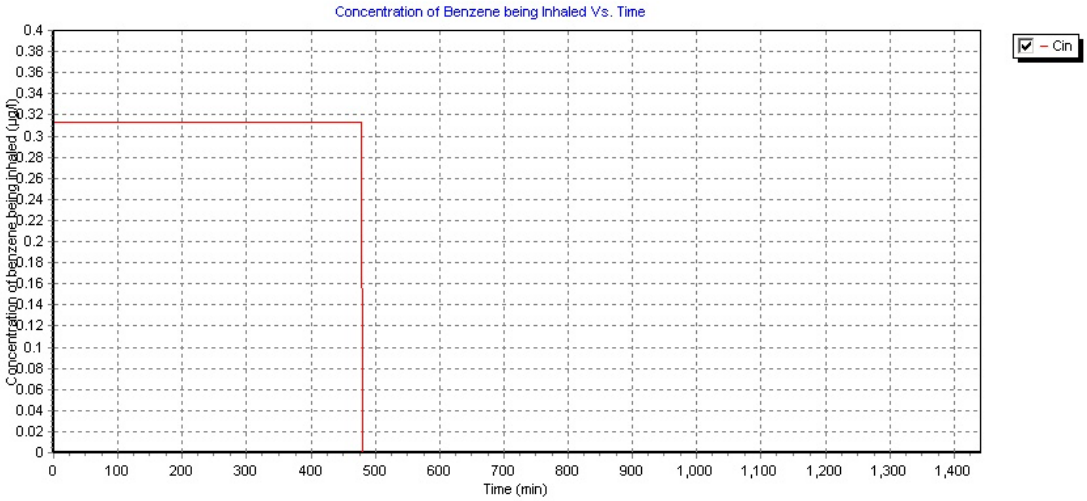

Figure 4.26 Case 4A Benzene Inhalation Pattern.

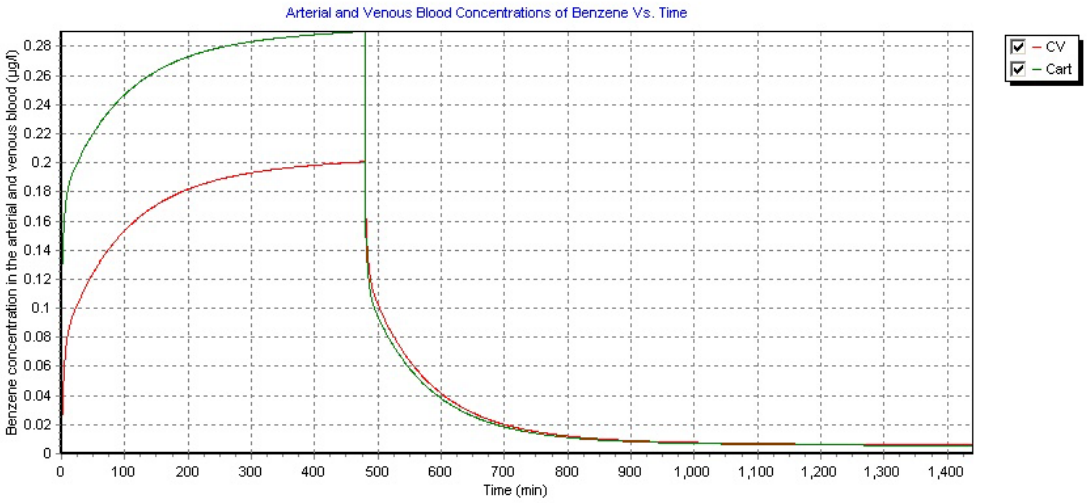

Figure 4.27 Case 4A – Arterial and Venous Blood Concentrations of Benzene.

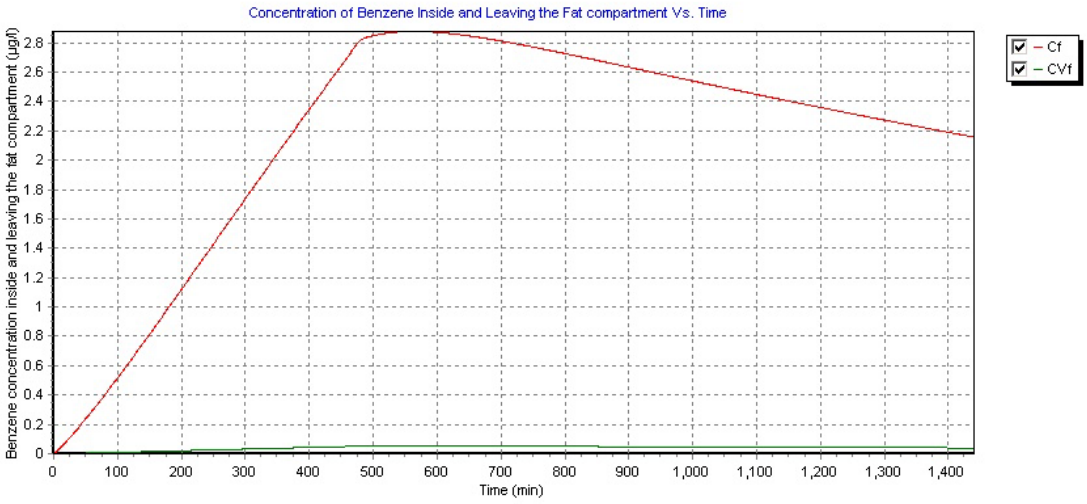

Figure 4.28 Case 4 A – Benzene Concentrations in and Leaving Fat Compartment.

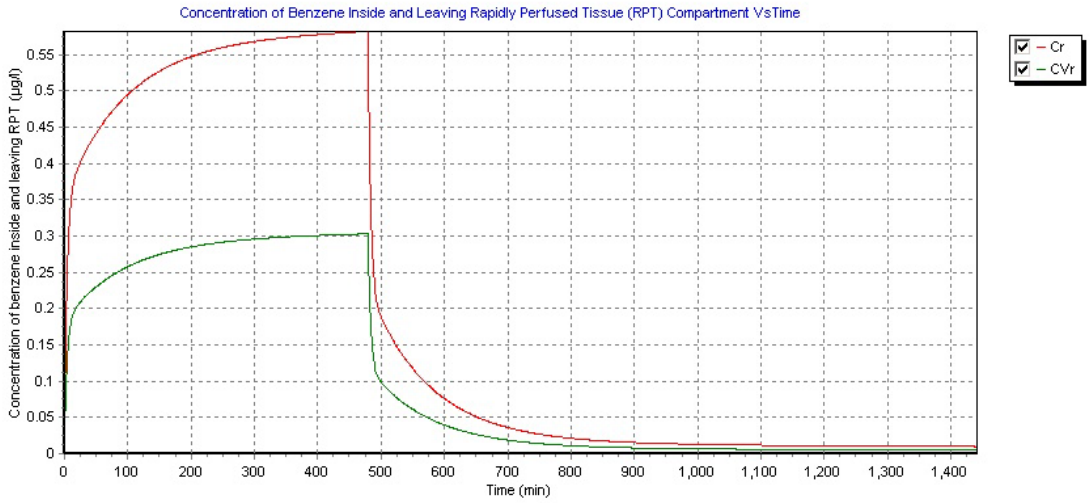

Figure 4.29 Case 4A – Benzene Concentrations in and Leaving RPT Compartment

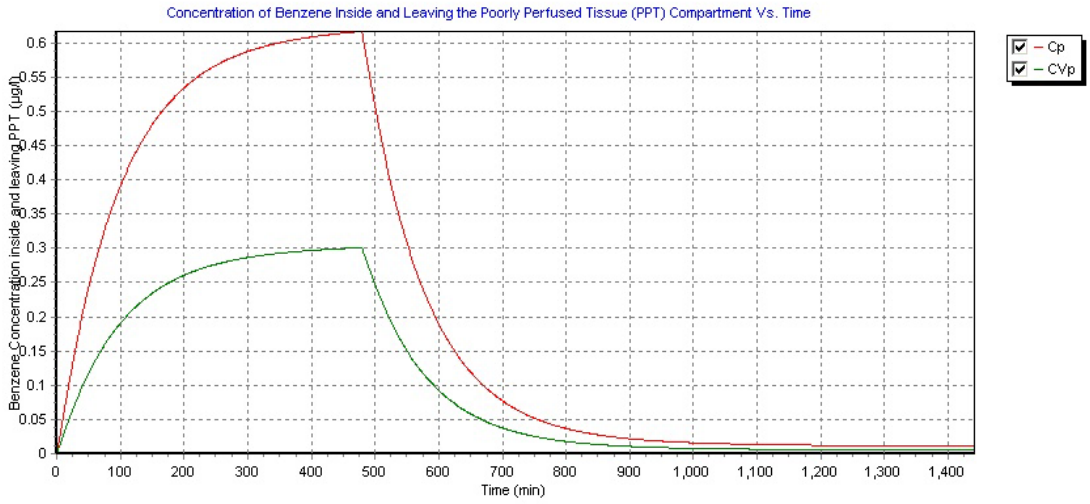

Figure 4.30 Case 4A – Benzene Concentrations in and Leaving PPT Compartment

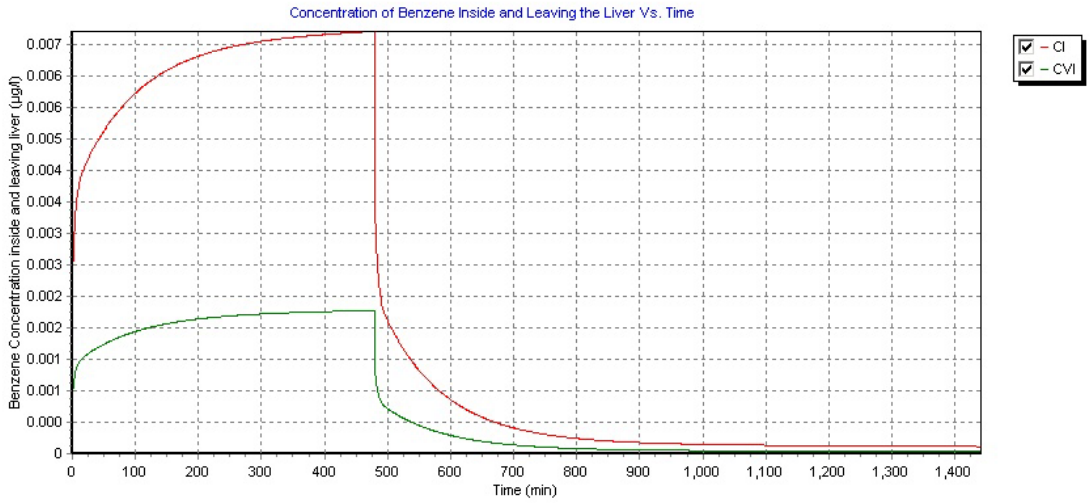

Figure 4.31 Case 4A – Benzene Concentrations in and Leaving Liver Compartment.

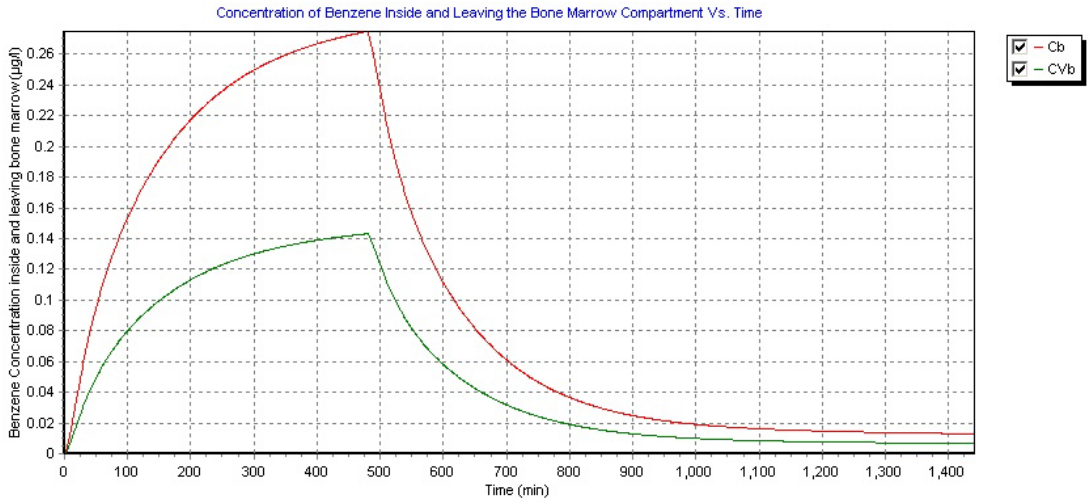

Figure 4.32 Case 4A – Benzene Concentrations in and Leaving Bone Marrow Compartment.

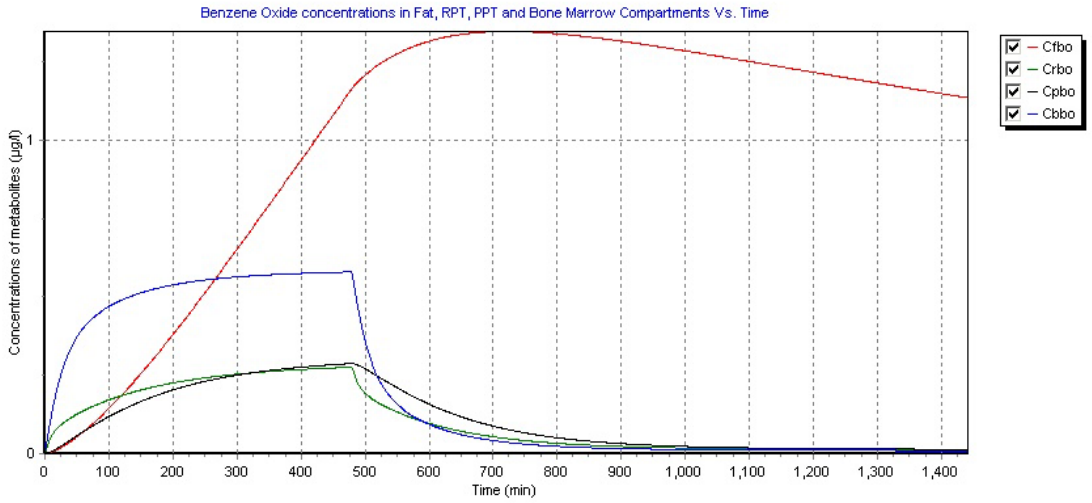

Figure 4.33 Case 4A – Benzene Oxide Concentrations in Fat, RPT, PPT and Bone Marrow Compartments.

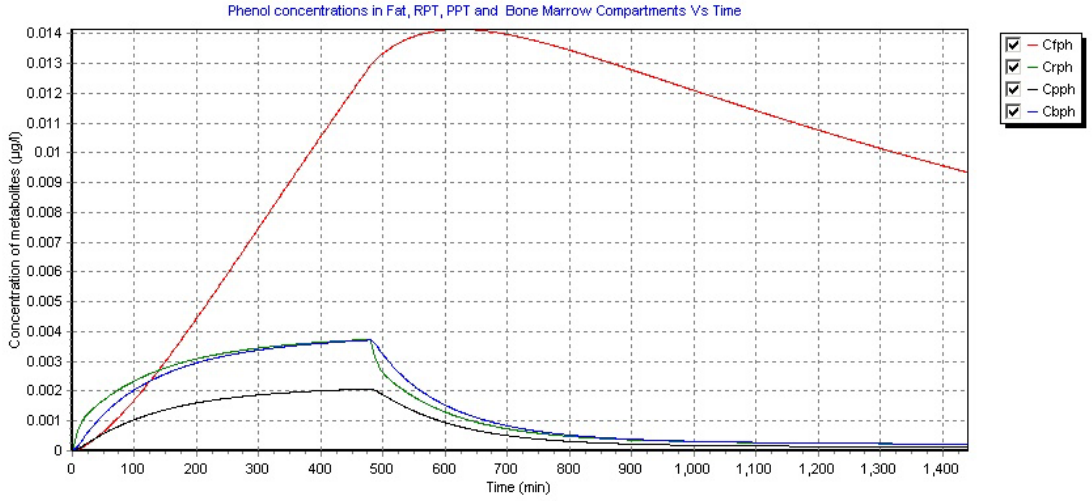

Figure 4.34 Case 4A – Phenol Concentrations in Fat, RPT, PPT and Bone Marrow Compartments.

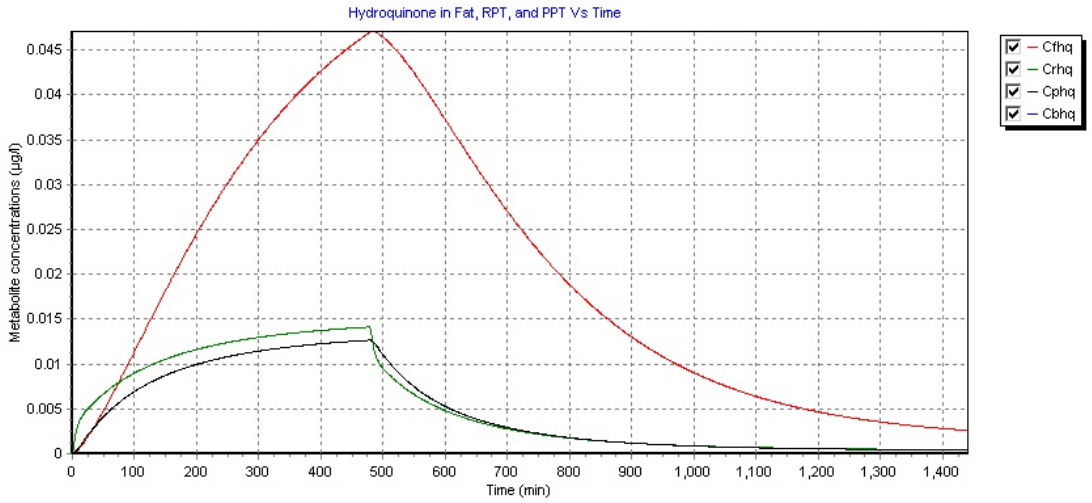

Figure 4.35 Case 4A – Hydroquinone Concentrations in Fat, RPT and PPT Compartments.

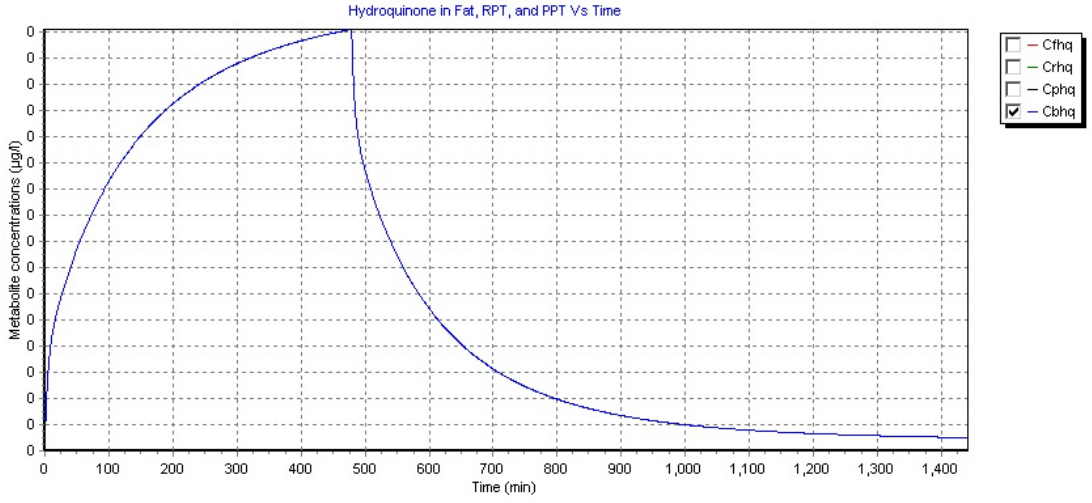

Figure 4.36 Case 4A – Hydroquinone Concentrations in Bone Marrow Compartment.

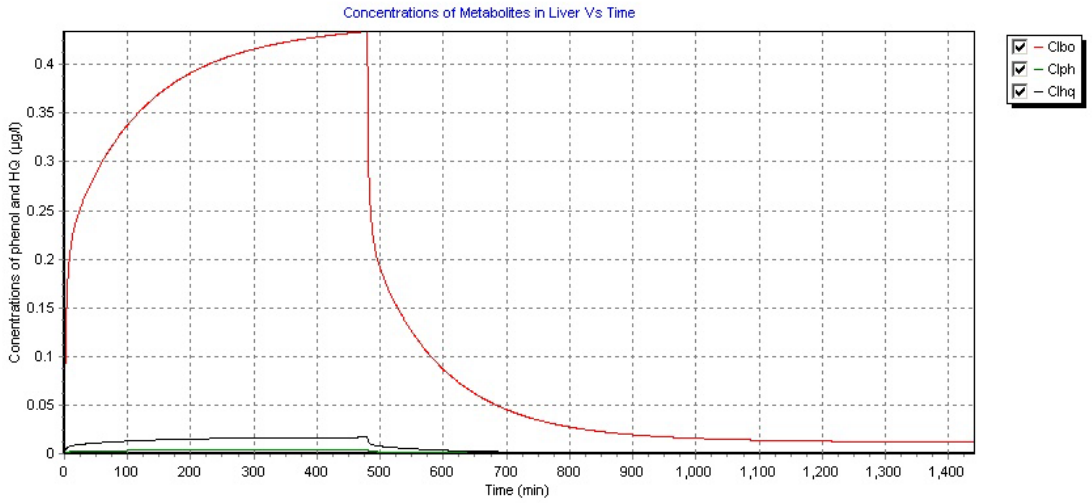

Figure 4.37 Case 4A – Benzene Metabolite Concentrations in Liver Compartment.

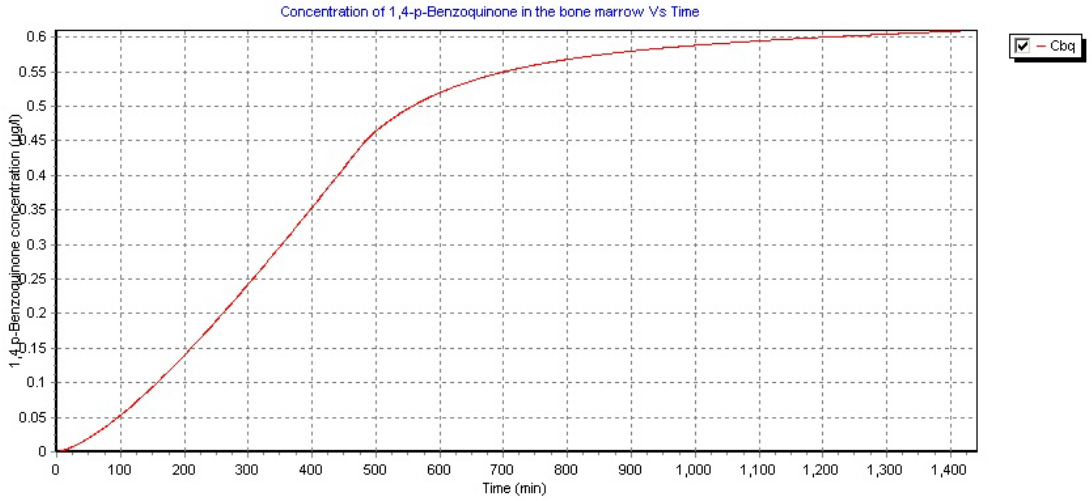

Figure 4.38 Case 4A – Benzoquinone Concentrations in Bone Marrow Compartment.

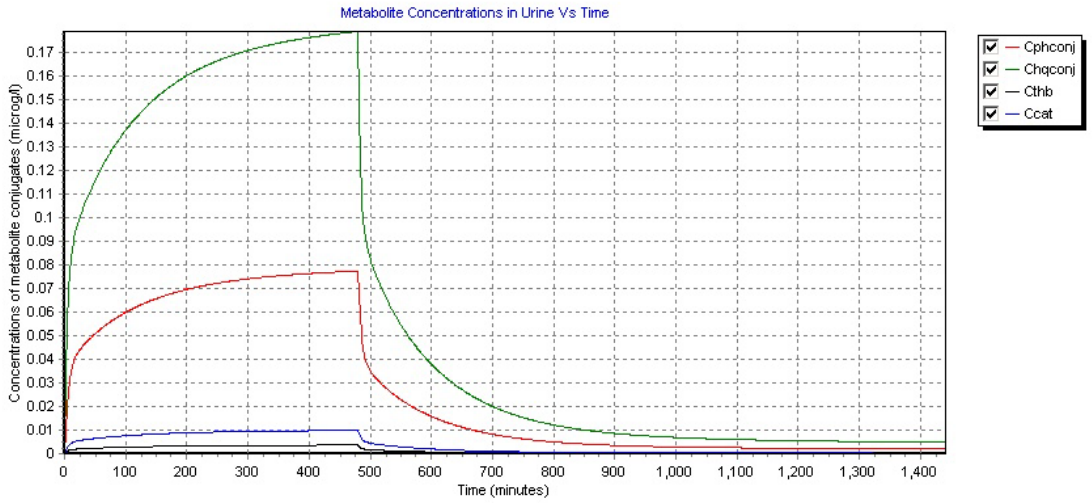

Figure 4.39 Case 4A Concentrations of Benzene Metabolite Conjugates in Urine.

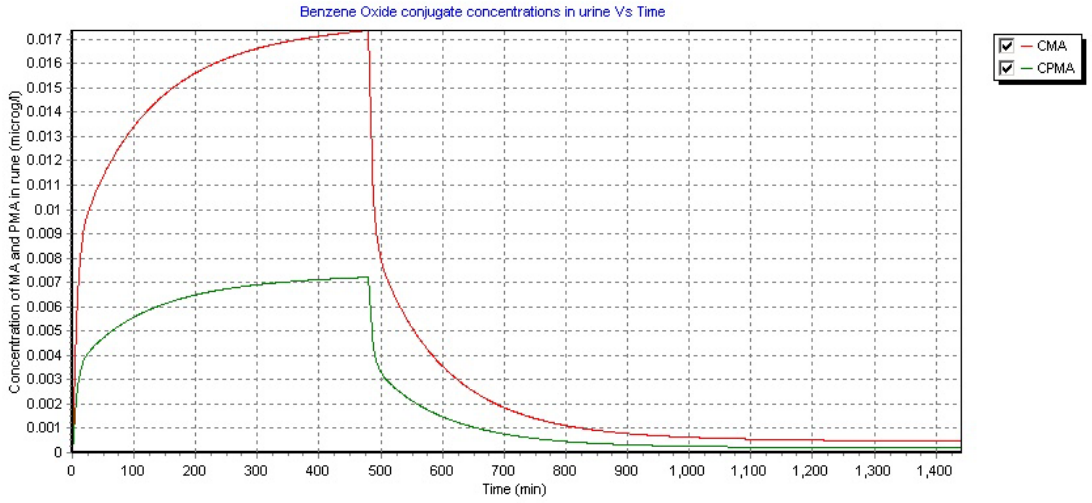

Figure 4.40 Case 4A Concentrations of MA and PMA in Urine.

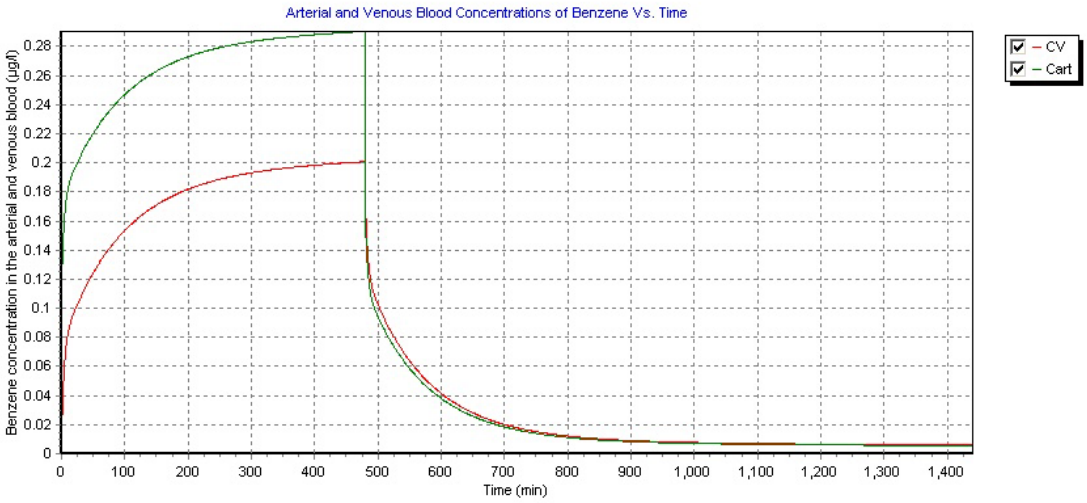

Figure 4.41 Case 4A (Female) - Arterial and Venous Blood Concentrations of Benzene.

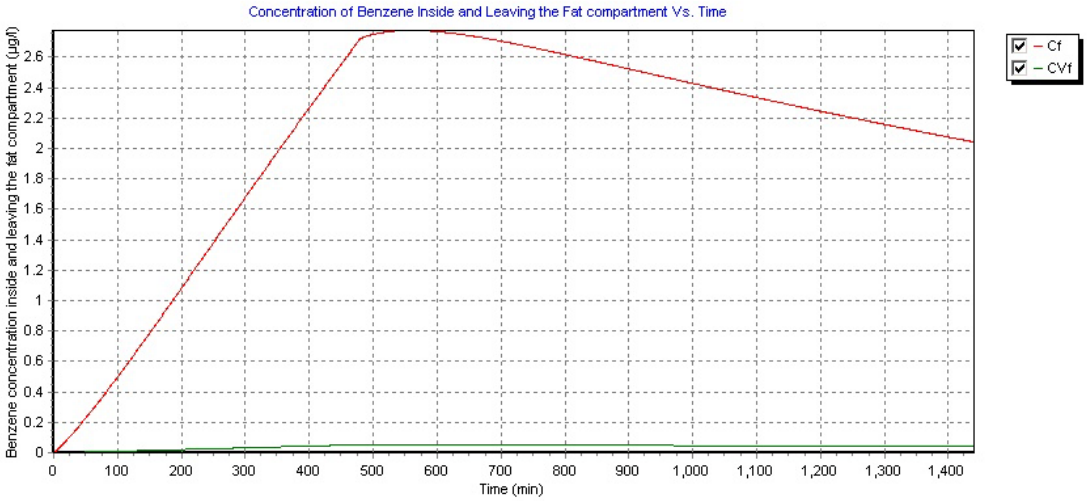

Figure 4.42 Case 4A (Female) – Benzene Concentrations in and Leaving Fat Compartment.

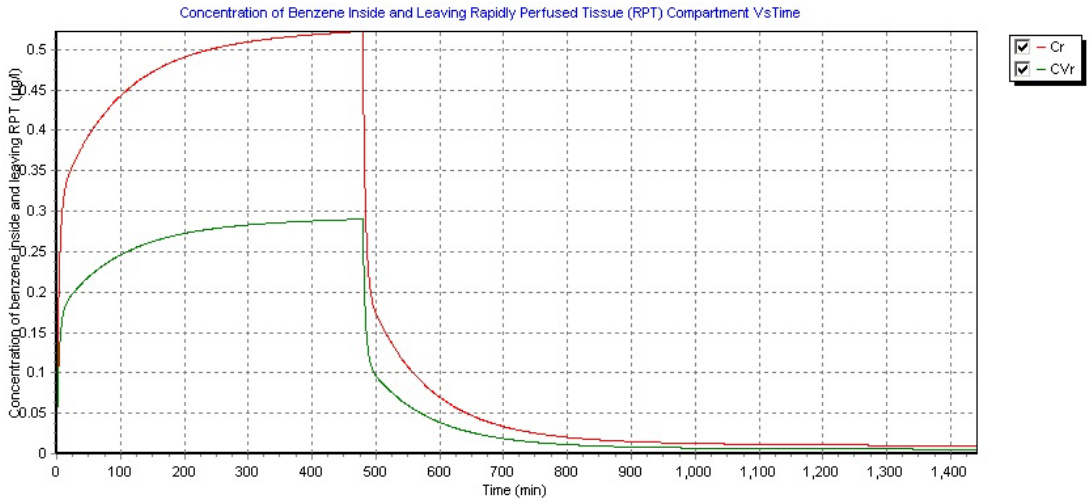

Figure 4.43 Case 4A (Female) – Benzene Concentrations in and Leaving RPT Compartment.

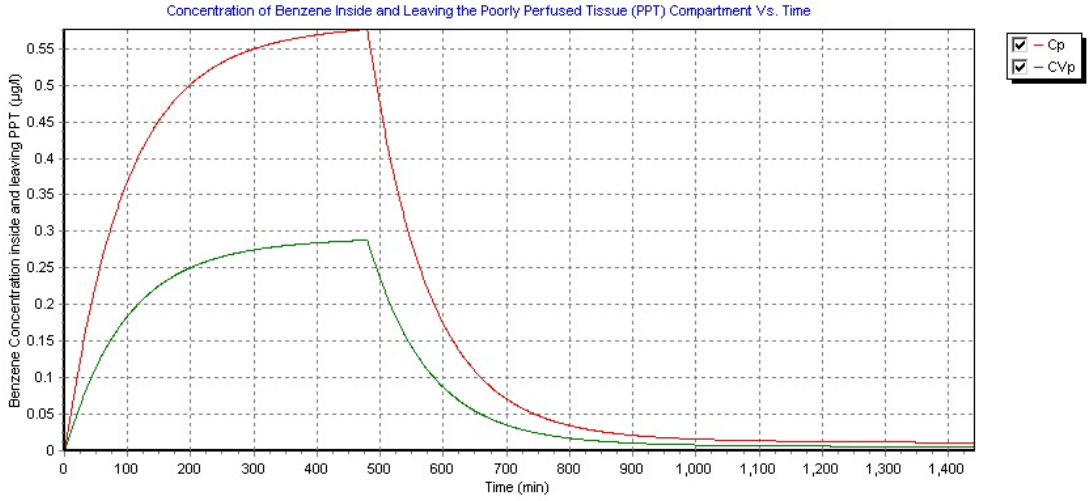

Figure 4.44 Case 4A (Female) – Benzene Concentrations in and Leaving PPT Compartment.

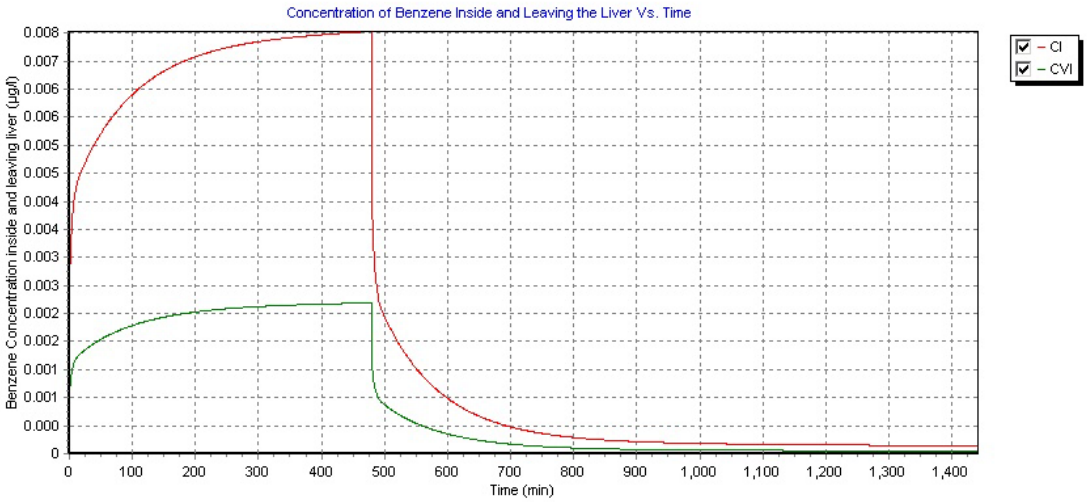

Figure 4.45 Case 4A (Female) – Benzene Concentrations in and Leaving Liver Compartment.

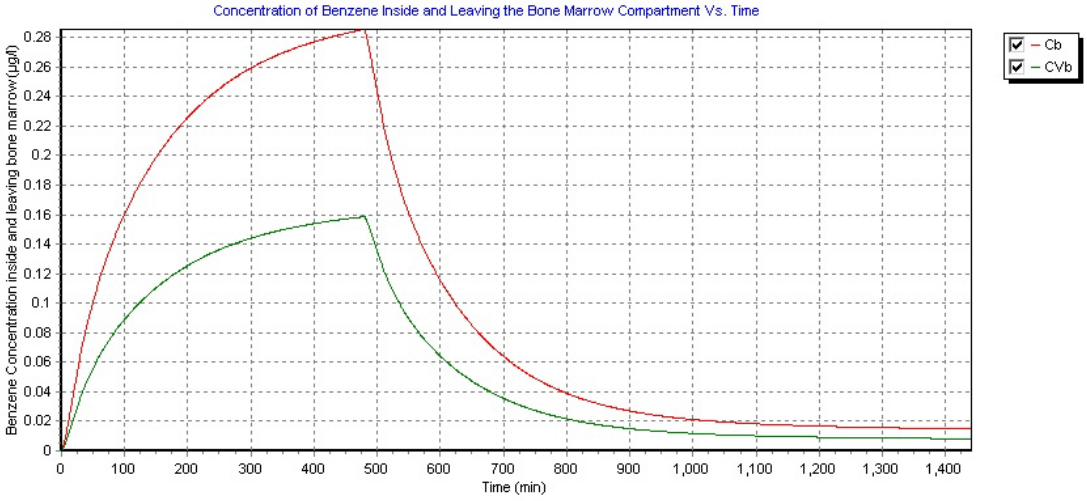

Figure 4.46 Case 4A (Female) – Benzene Concentrations in and Leaving Bone Marrow Compartment.

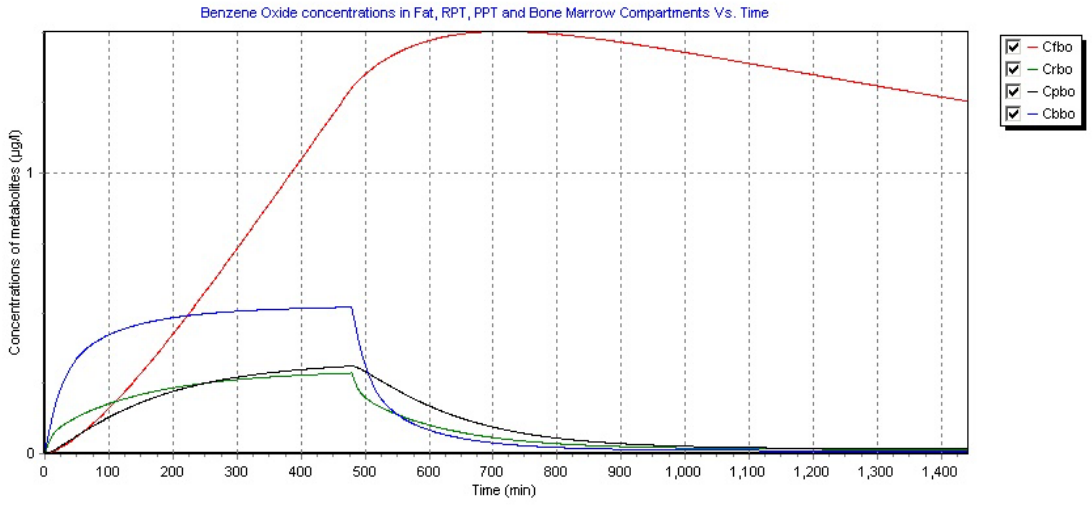

Figure 4.47 Case 4A (Female) – Benzene Oxide Concentrations in Fat, RPT, PPT and Bone Marrow Compartments.

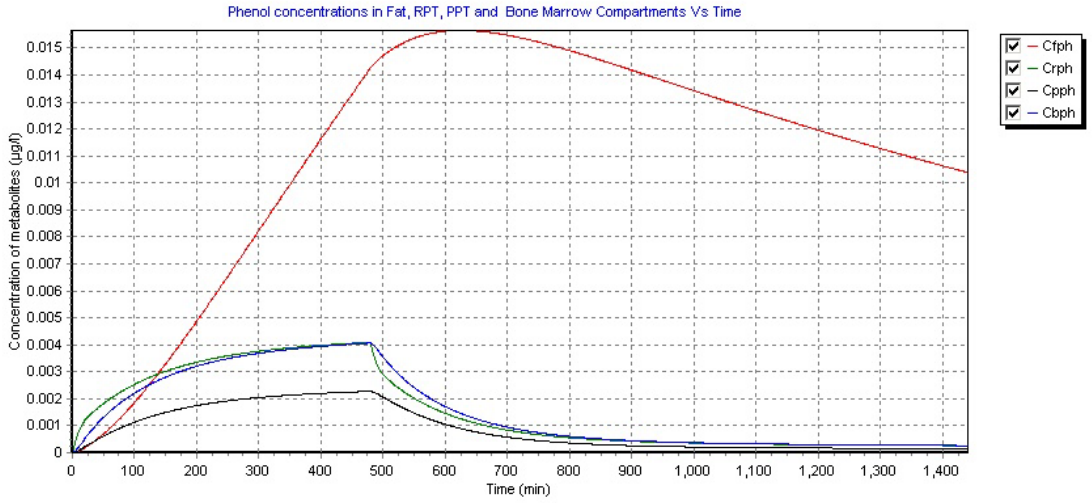

Figure 4.48 Case 4A (Female) – Phenol Concentrations in Fat, RPT, PPT and Bone Marrow Compartments.

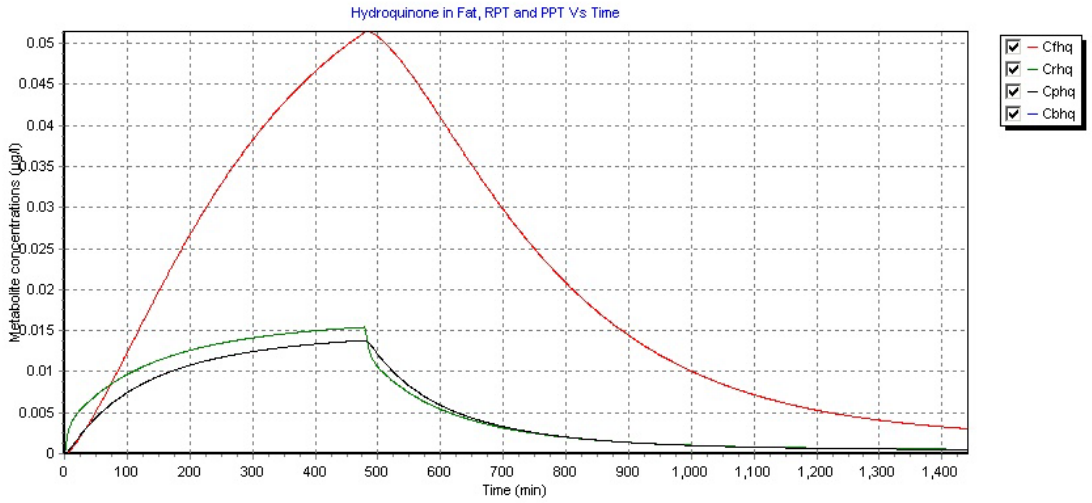

Figure 4.49 Case 4A (Female) – Hydroquinone Cocnentrations in Fat, RPT and PPT Compartments.

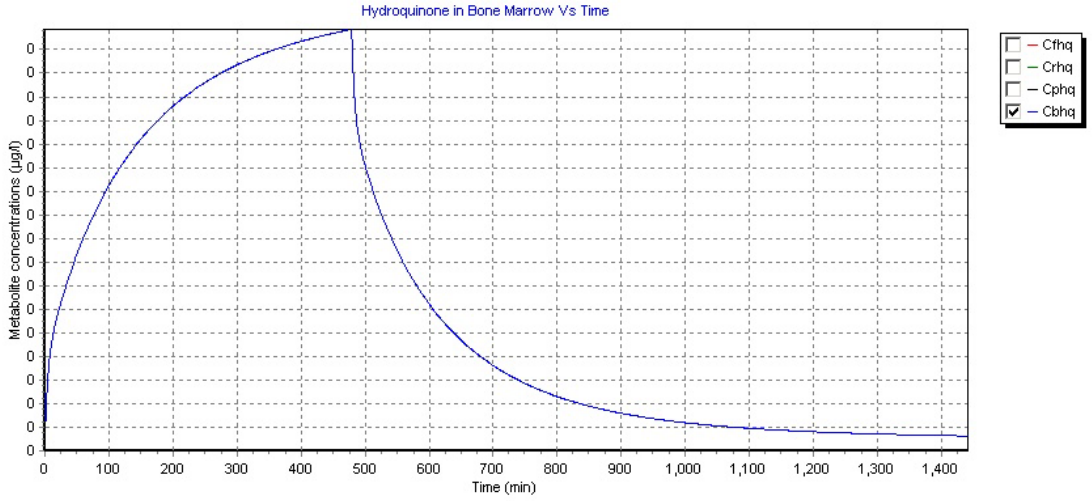

Figure 4.50 Case 4A (Female) – Hydroquinone Concentrations in Bone Marrow Compartment.

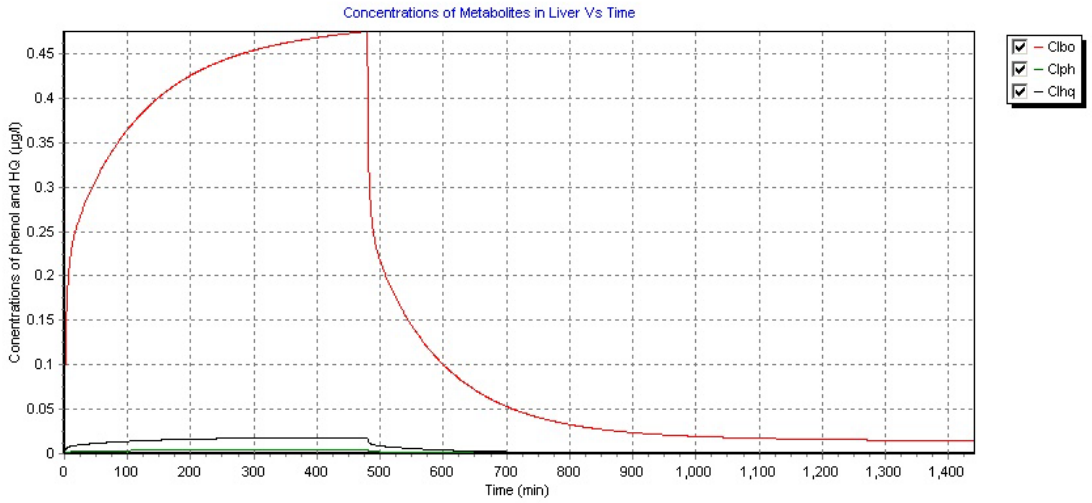

Figure 4.51 Case 4A (Female) – Benzene Metabolite Concentrations in Liver Compartment.

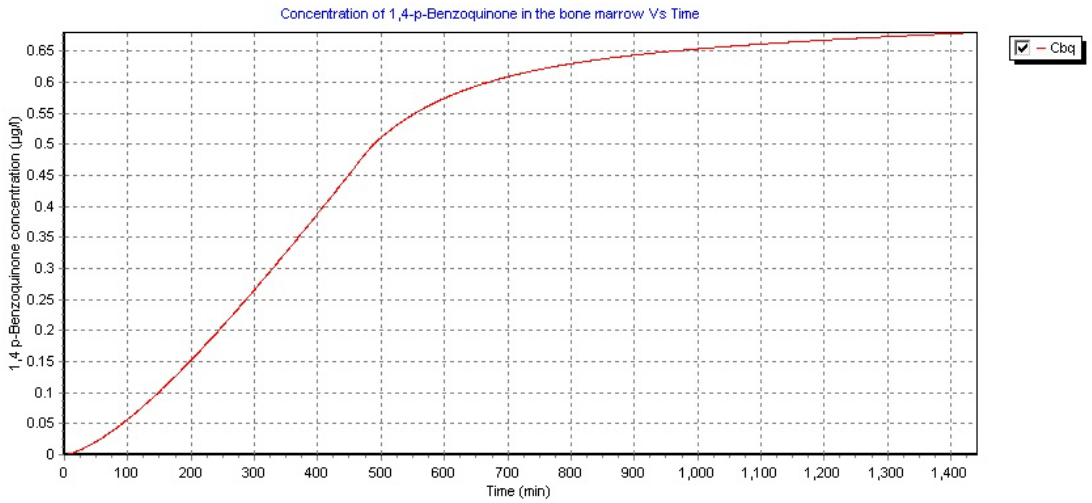

Figure 4.52 Case 4A (Female) – Benzoquinone Concentration in Bone Marrow Compartment.

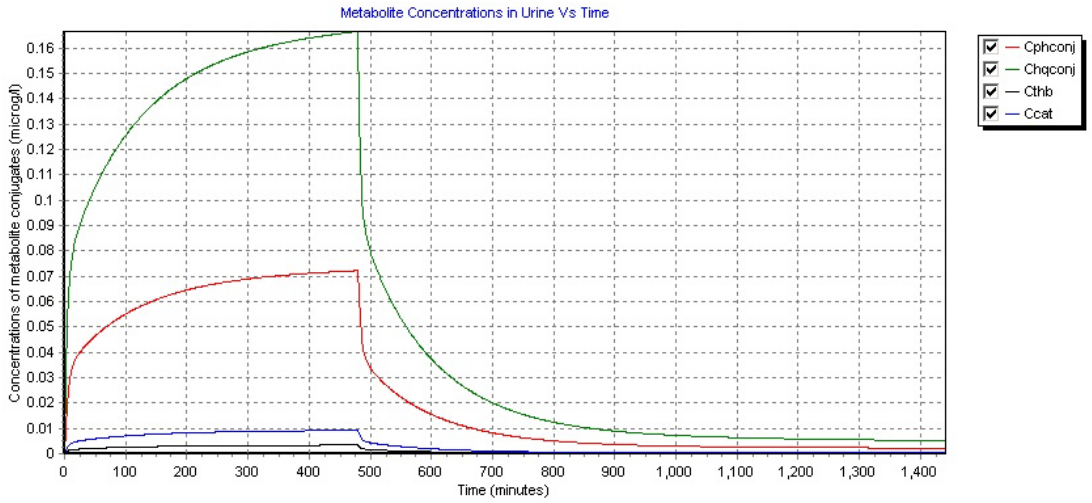

Figure 4.53 Case 4A (Female) Concentrations of Benzene Metabolite Conjugates in Urine.

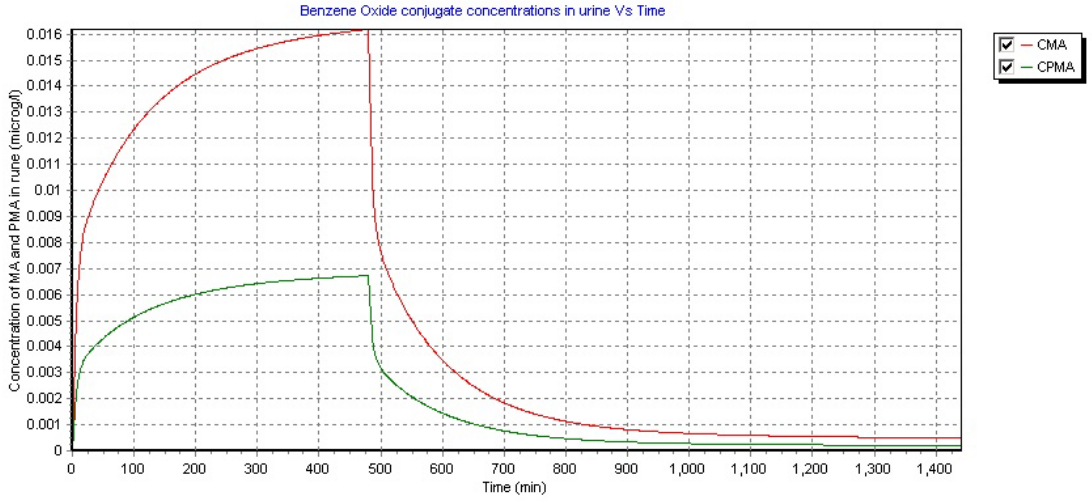

Figure 4.54 Case 4A (Female) Concentrations of MA and PMA in Urine.

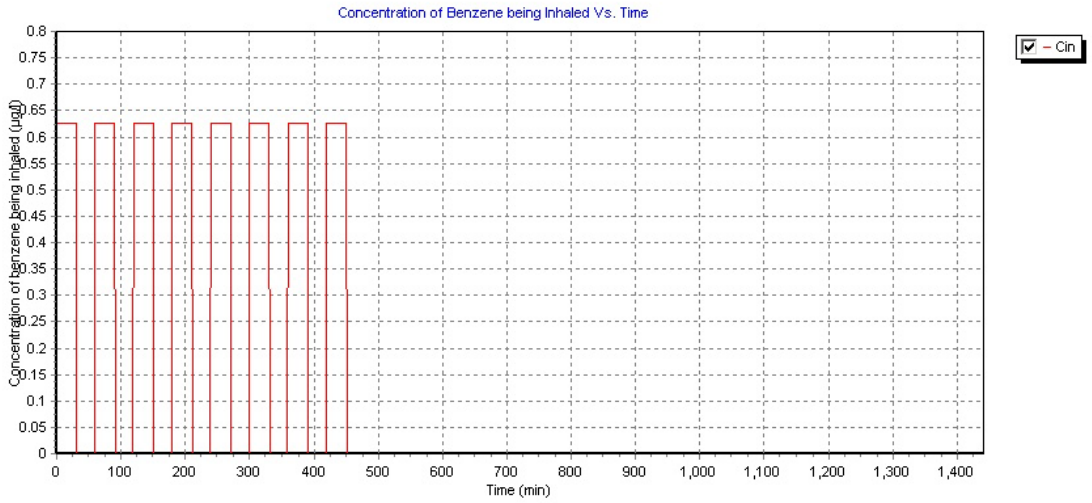

Figure 4.55 Case 4B – Benzene Inhalation Pattern.

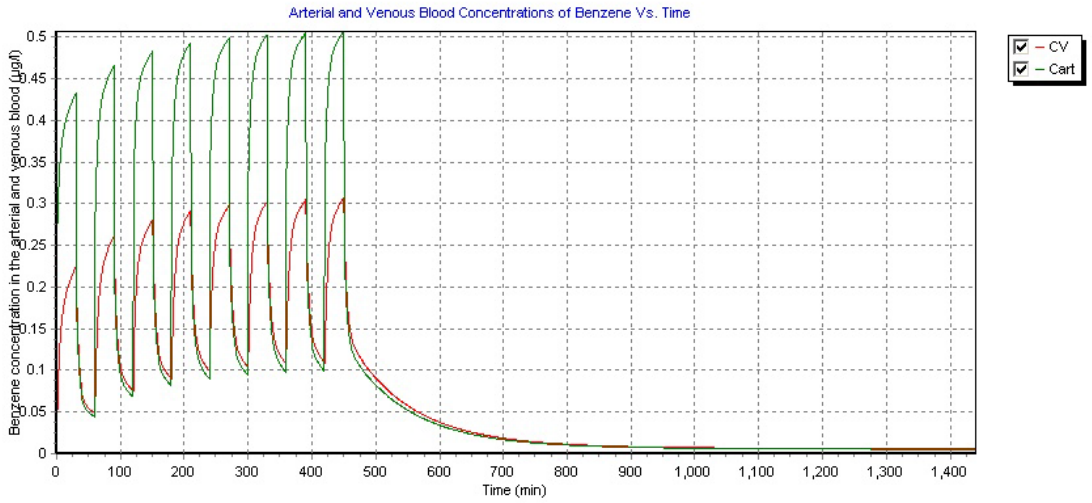

Figure 4.56 Case 4B – Arterial and Venous Blood Concentrations of Benzene.

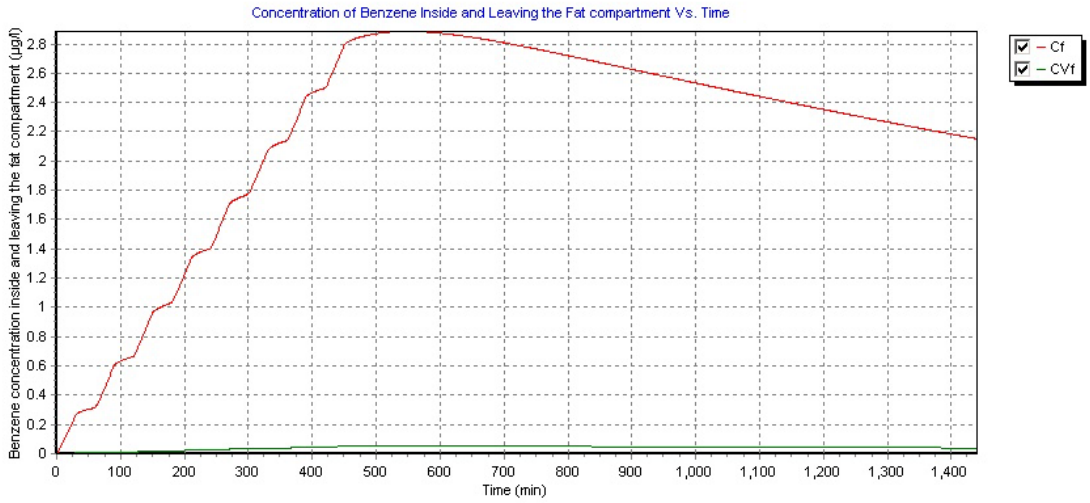

Figure 4.57 Case 4B – Benzene Concentrations in and Leaving Fat Compartment.

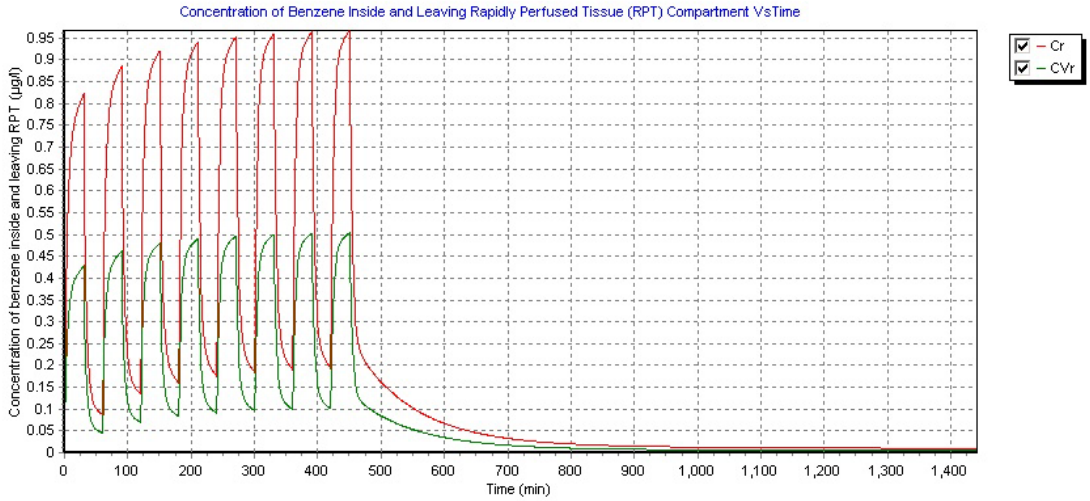

Figure 4.58 Case 4B – Benzene Concentrations in and Leaving RPT Compartment.

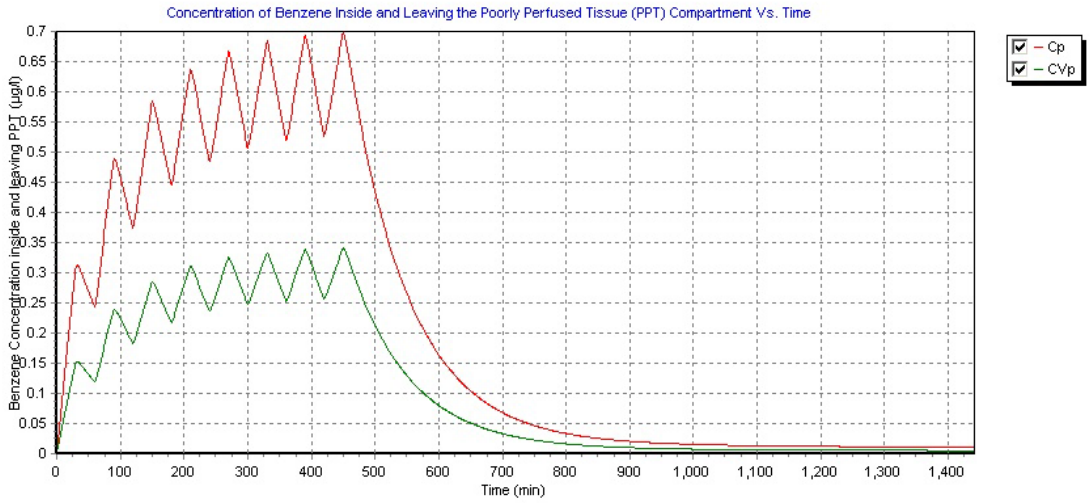

Figure 4.59 Case 4B – Benzene Concentrations in and Leaving PPT Compartment.

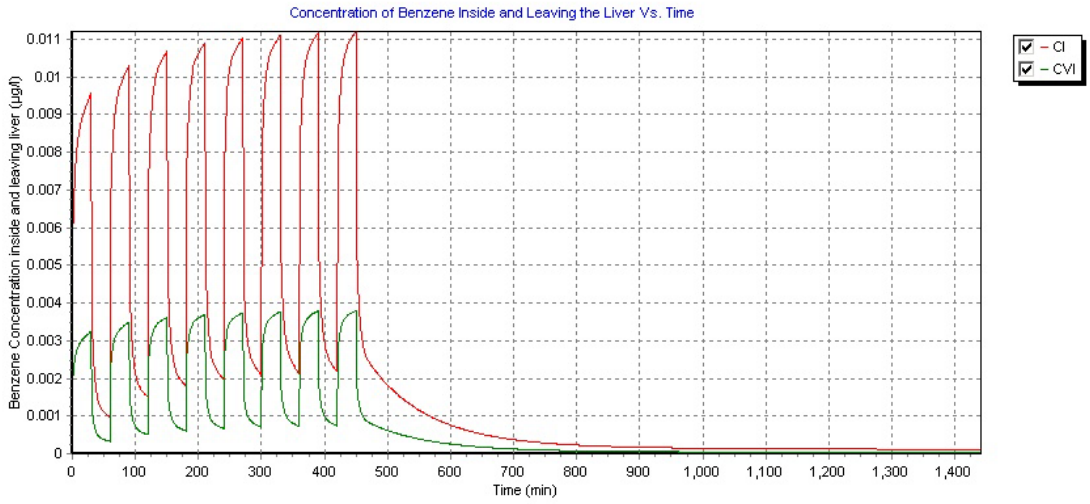

Figure 4.60 Case 4B – Benzene Concentrations in and Leaving Liver Compartment.

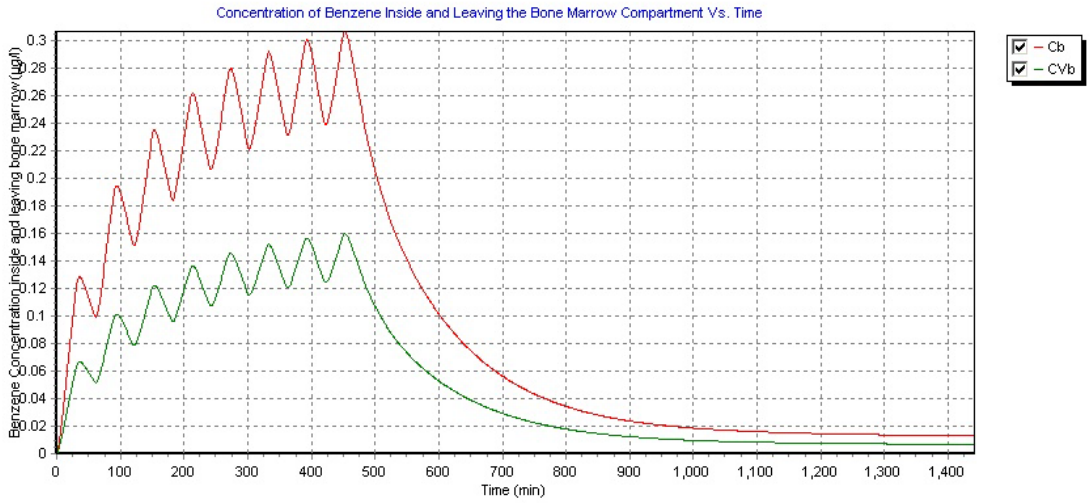

Figure 4.61 Case 4B – Benzene Concentrations in and Leaving Bone Marrow Compartment.

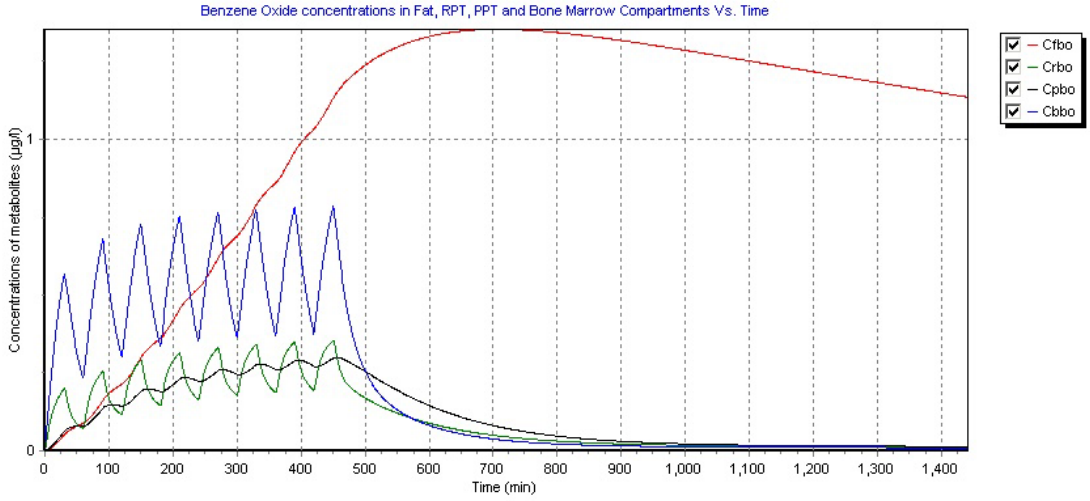

Figure 4.62 Case 4B – Benzene Oxide Concentrations in Fat, RPT, PPT and Bone Marrow Compartments.

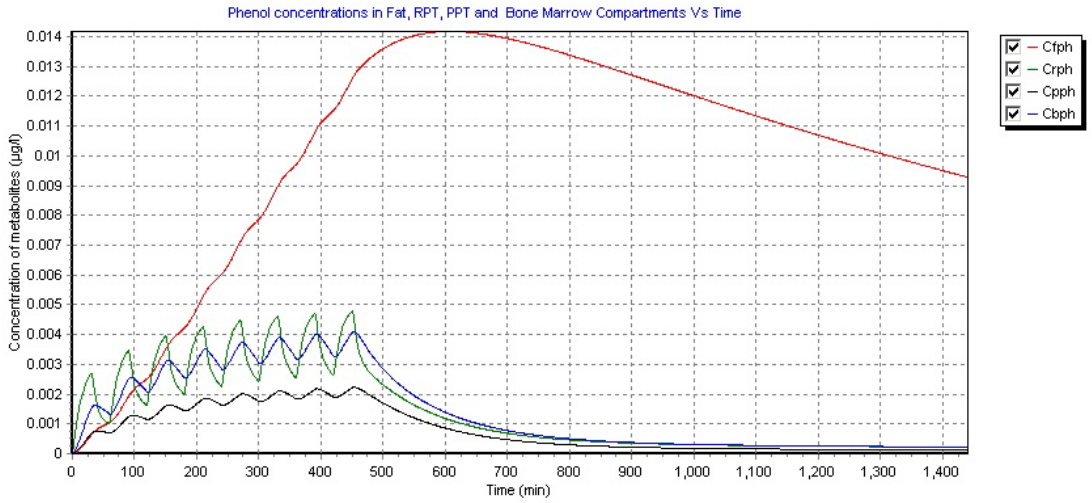

Figure 4.63 Case 4B – Phenol Concentrations in Fat, RPT, PPT and Bone Marrow Compartments.

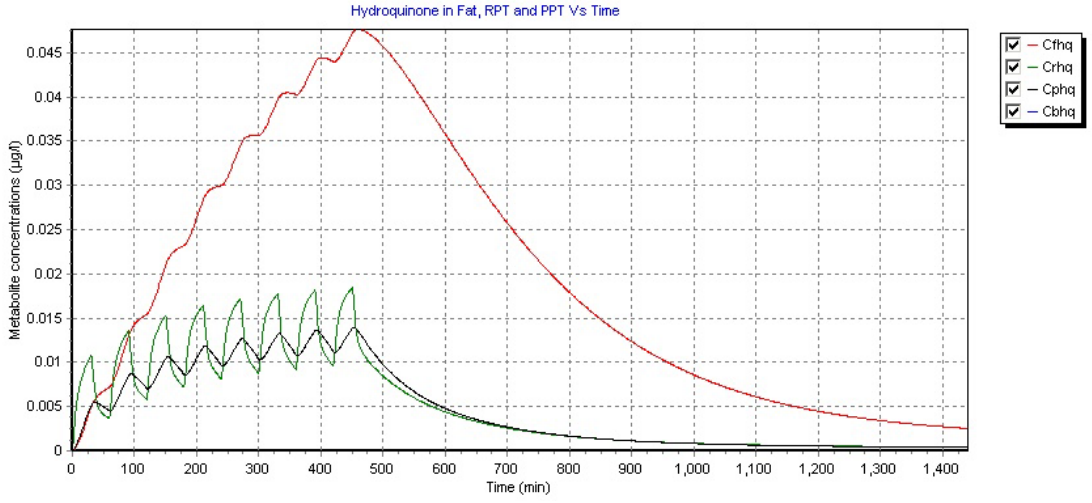

Figure 4.64 Case 4B – Hydroquinone Concentrations in Fat, RPT and PPT Compartments.

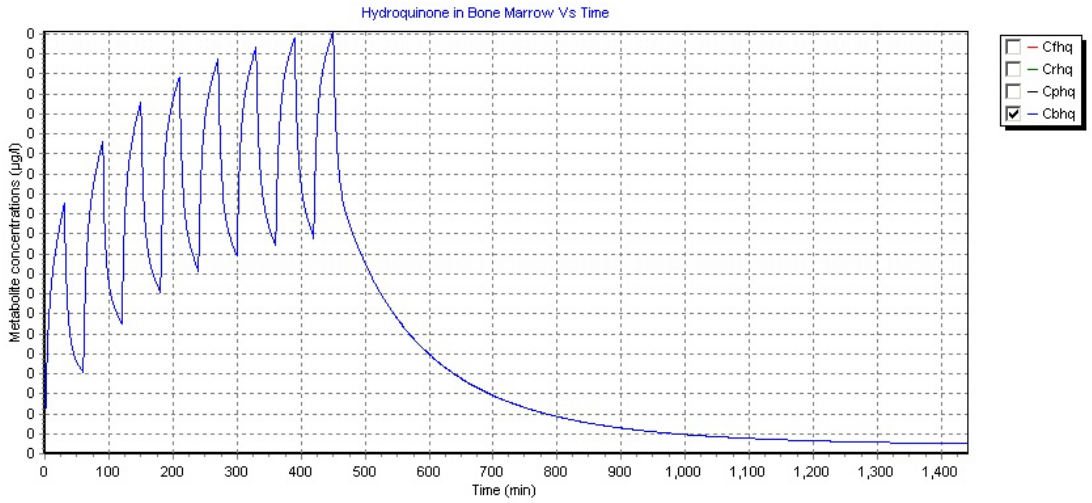

Figure 4.65 Case 4B – Hydroquinone Concentrations in Bone Marrow Compartment.

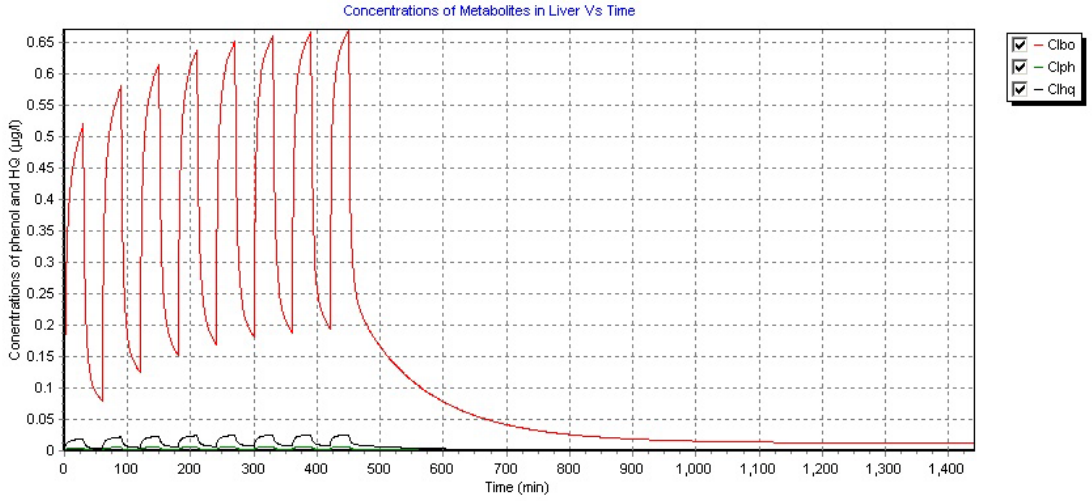

Figure 4.66 Case 4B – Benzene Metabolite Concentrations in Liver Compartment.

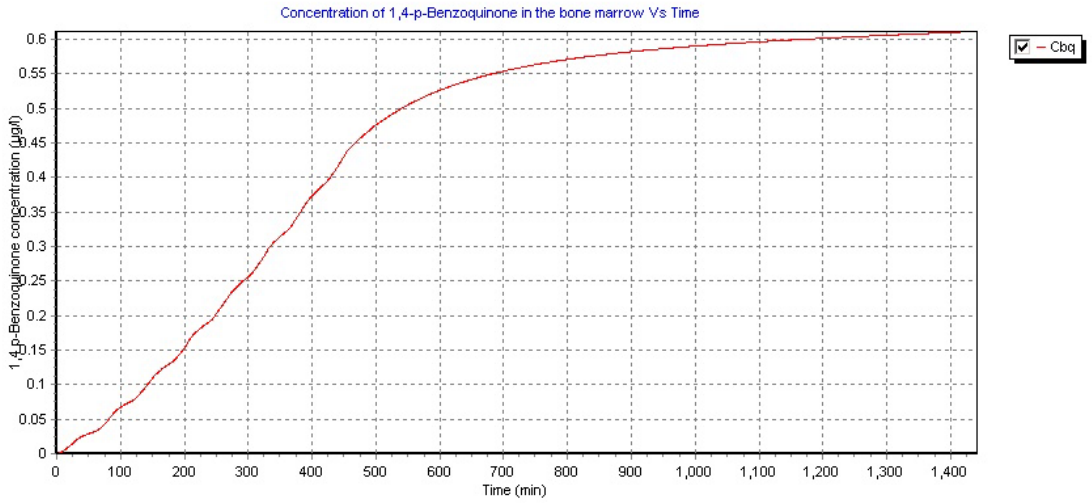

Figure 4.67 Case 4B – Benzoquinone Concentrations in Bone Marrow Compartment.

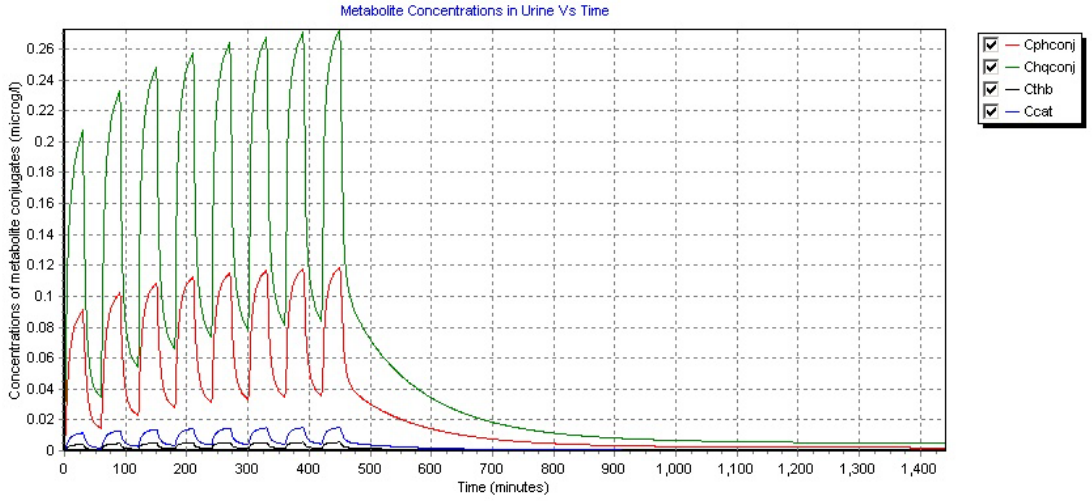

Figure 4.68 Case 4B – Concentrations of Benzene Metabolite Conjugate in Urine.

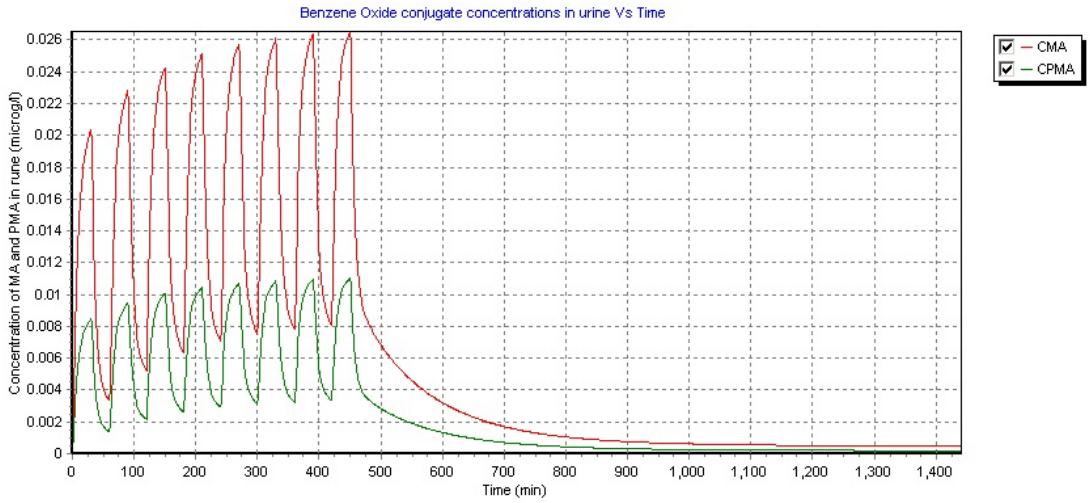

Figure 4.69 Case 4B – Concentrations of MA and PMA in Urine.

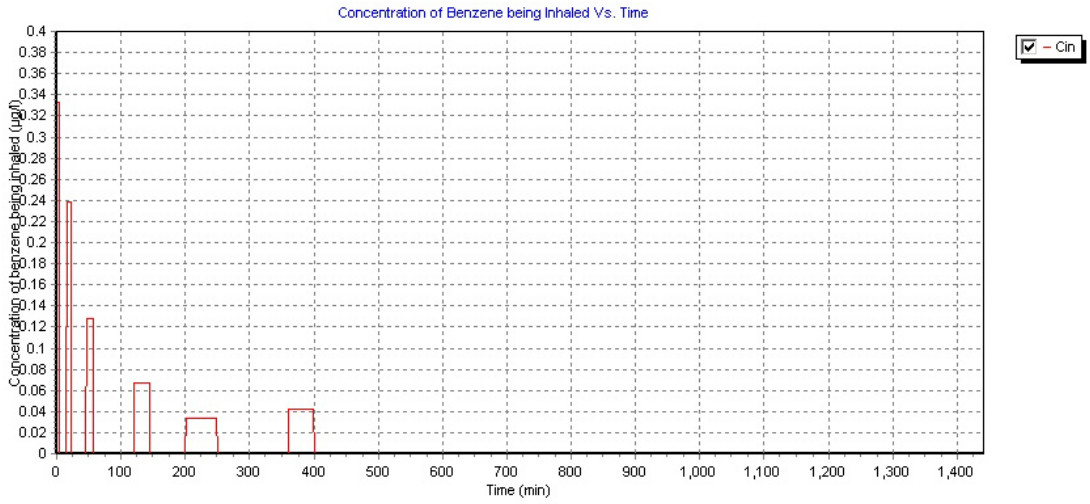

Figure 4.70 Case 4C – Benzene Inhalation Pattern.

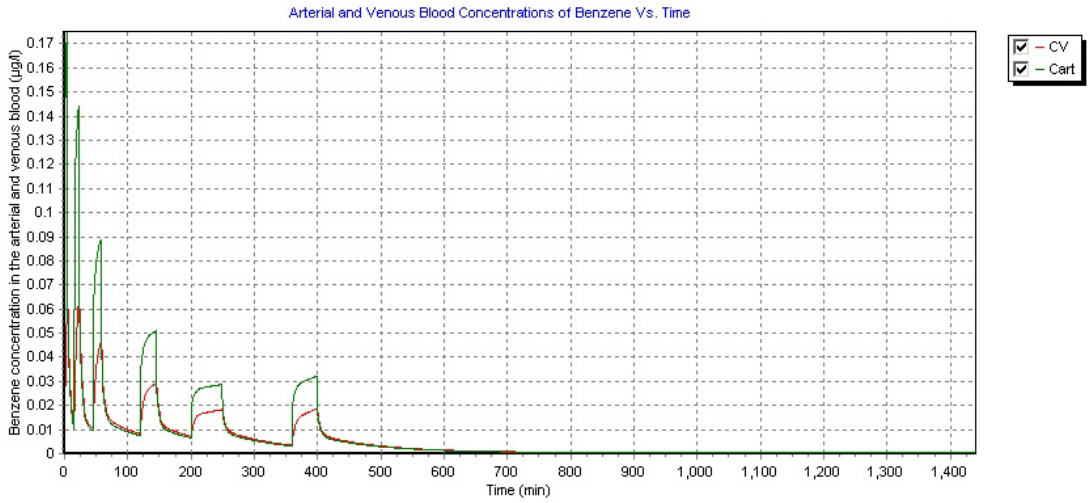

Figure 4.71 Case 4C – Arterial and Venous Blood Concentrations of Benzene.

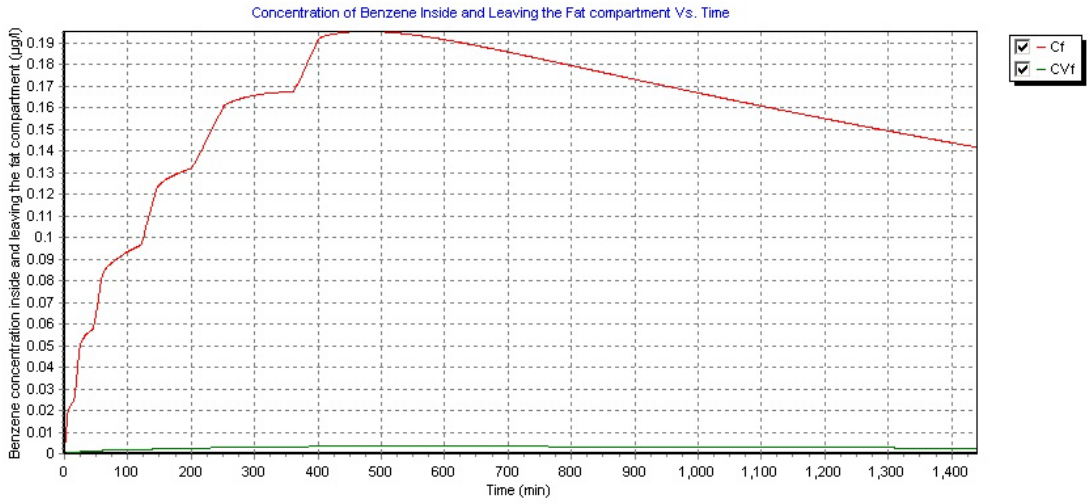

Figure 4.72 Case 4C – Benzene Concentrations in and Leaving Fat Compartment.

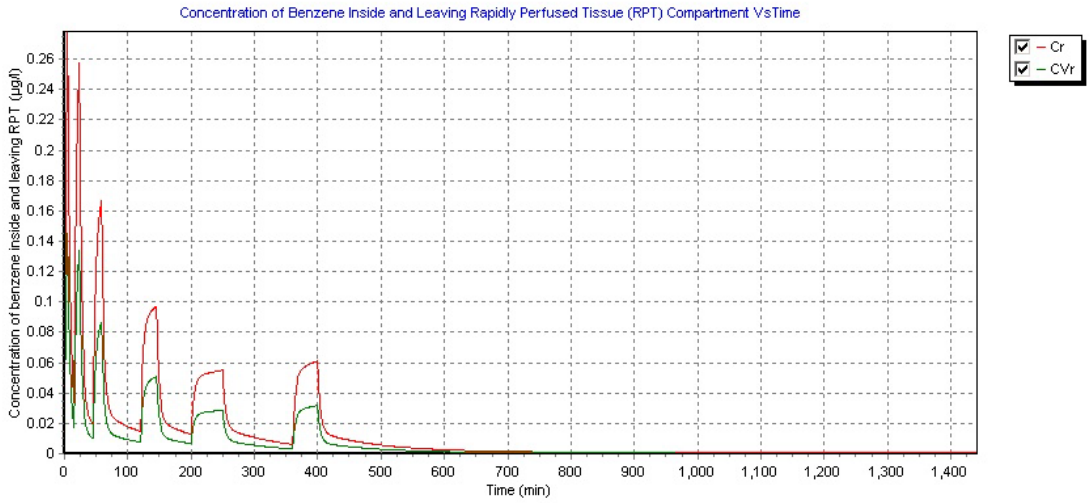

Figure 4.73 Case 4C – Benzene Concentrations in and Leaving RPT Compartment.

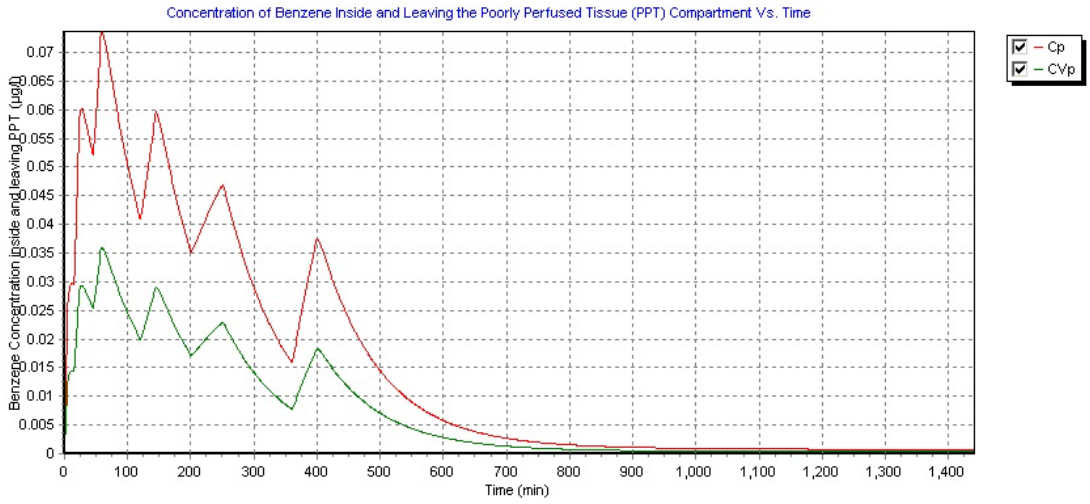

Figure 4.74 Case 4C – Benzene Concentrations in and Leaving PPT Compartment.

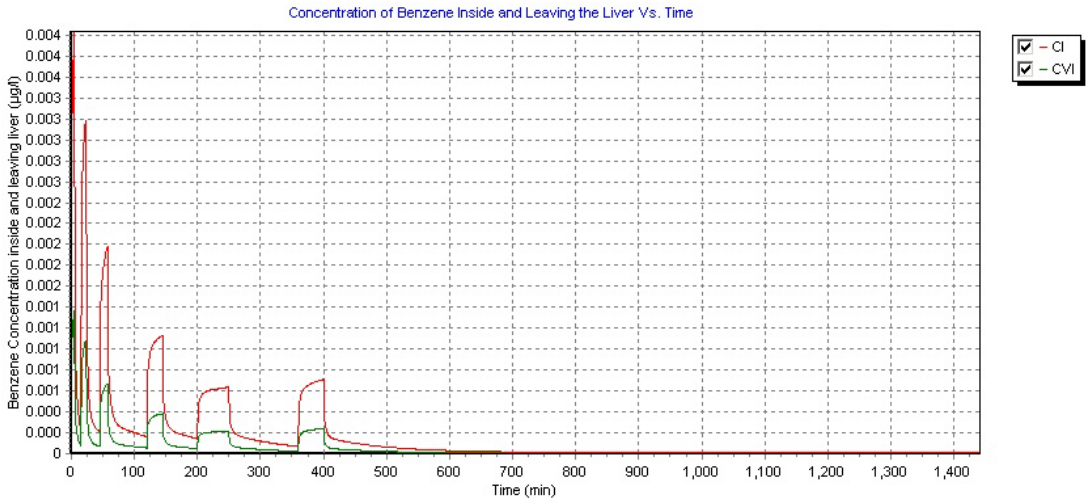

Figure 4.75 Case 4C – Benzene Concentrations in and Leaving Liver Compartment.

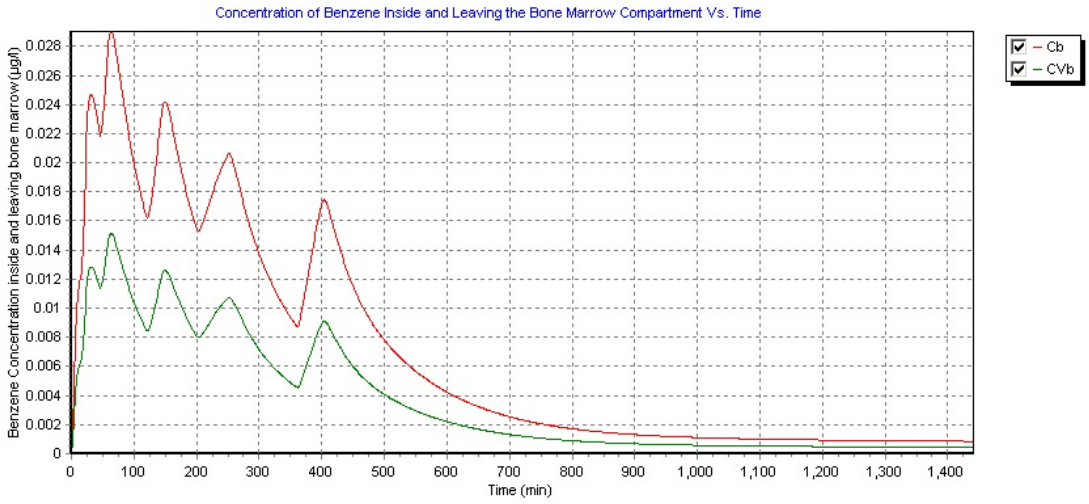

Figure 4.76 Case 4C – Benzene Concentrations in and Leaving Bone Marrow Compartments.

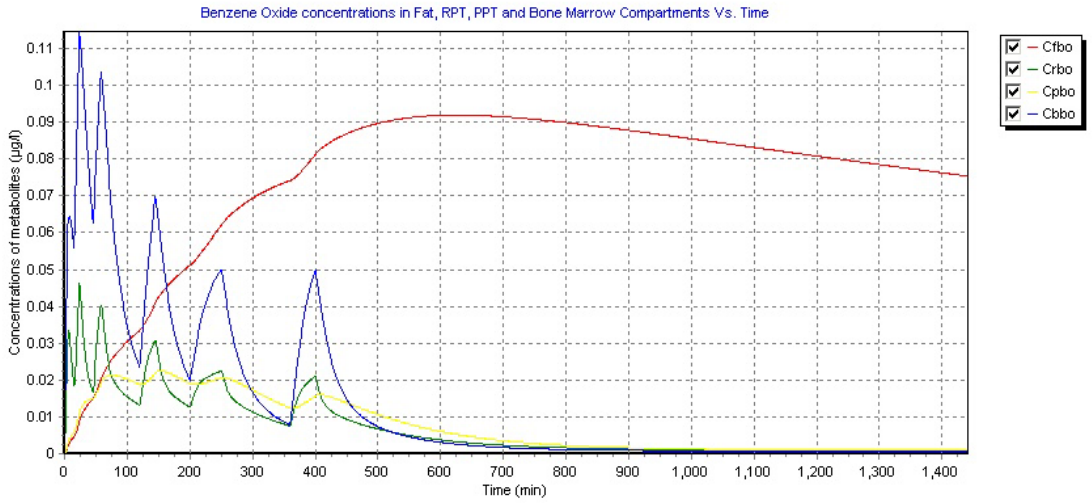

Figure 4.77 Case 4C – Benzene Oxide Concentrations in Fat, RPT, PPT and Bone Marrow Compartments.

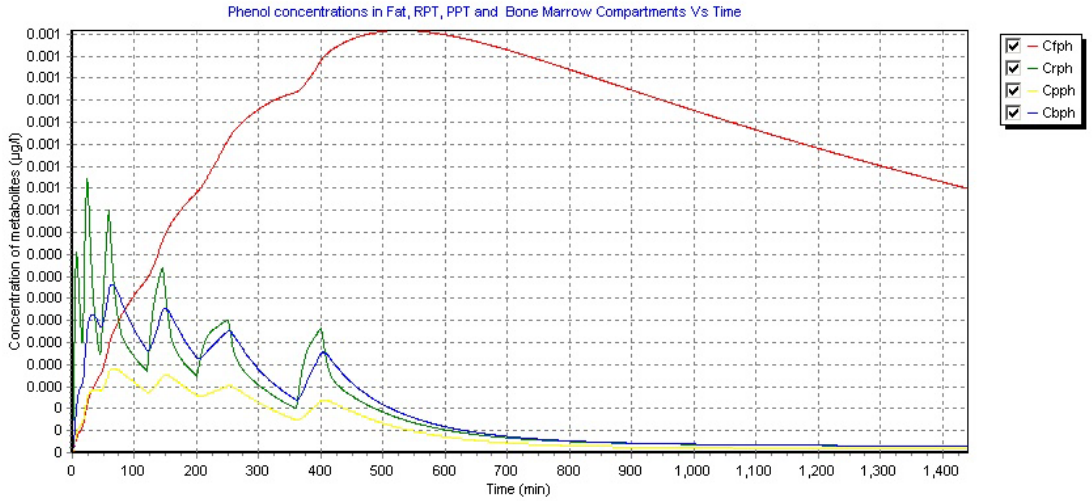

Figure 4.78 Case 4C – Phenol Concentrations in Fat, RPT, PPT and Bone Marrow Compartments.

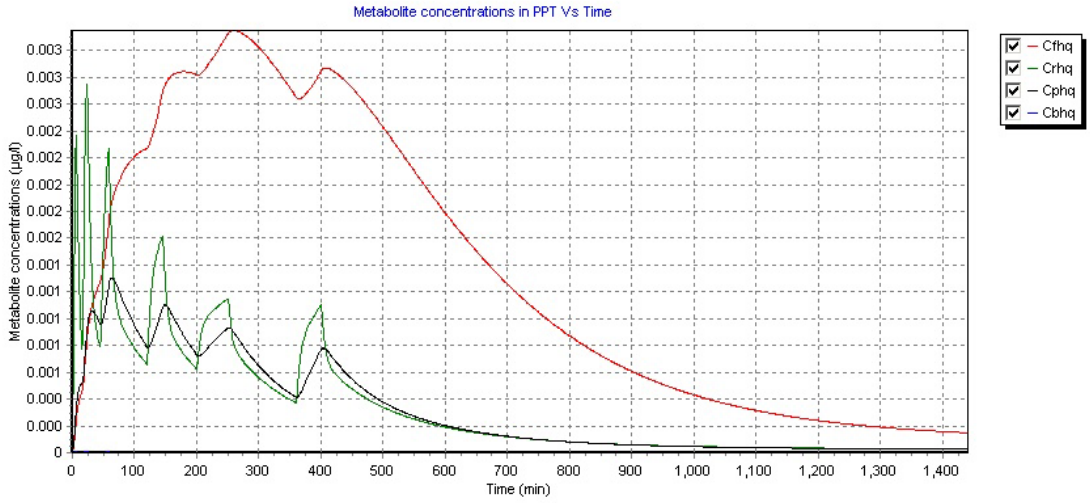

Figure 4.79 Case 4C – Hydroquinone Concentrations in Fat, RPT and PPT Compartments.

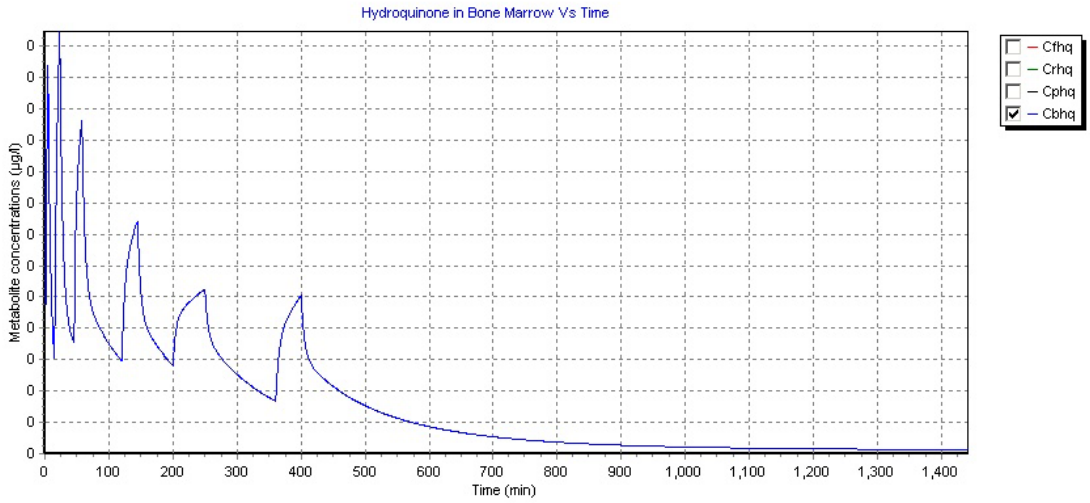

Figure 4.80 Case 4C – Hydroquinone Concentrations in Bone Marrow Compartment.

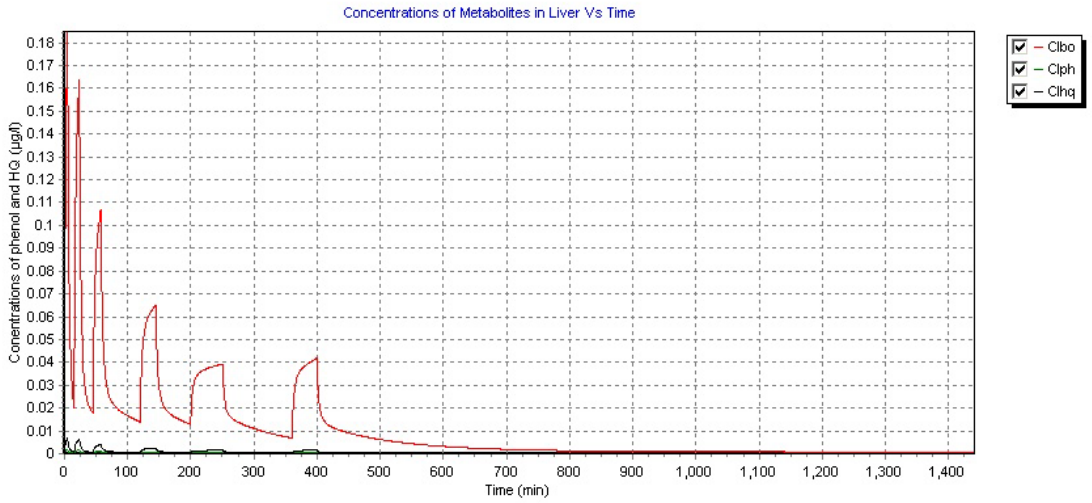

Figure 4.81 Case 4C – Benzene Metabolite Concentrations in Liver Compartment.

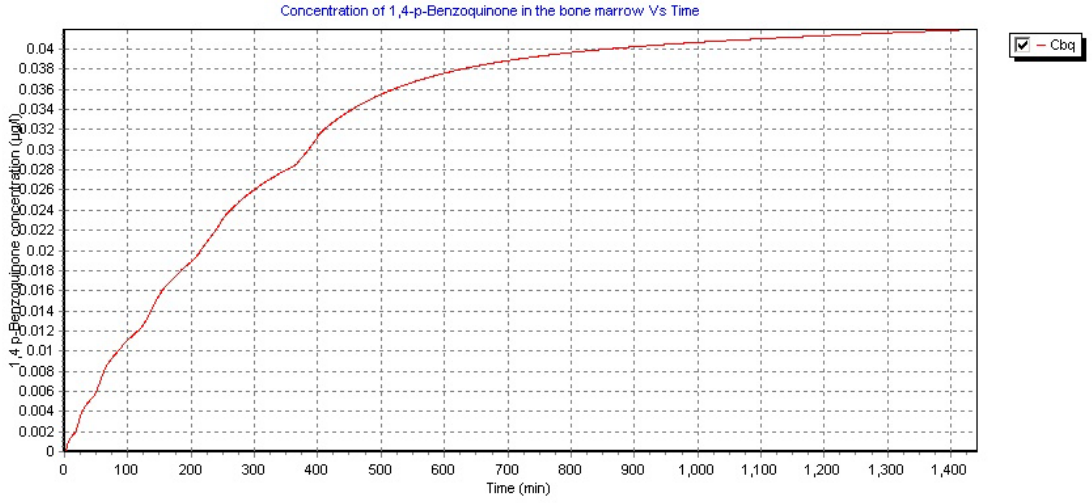

Figure 4.82 Case 4C – Benzoquinone Concentrations in Bone Marrow Compartment.

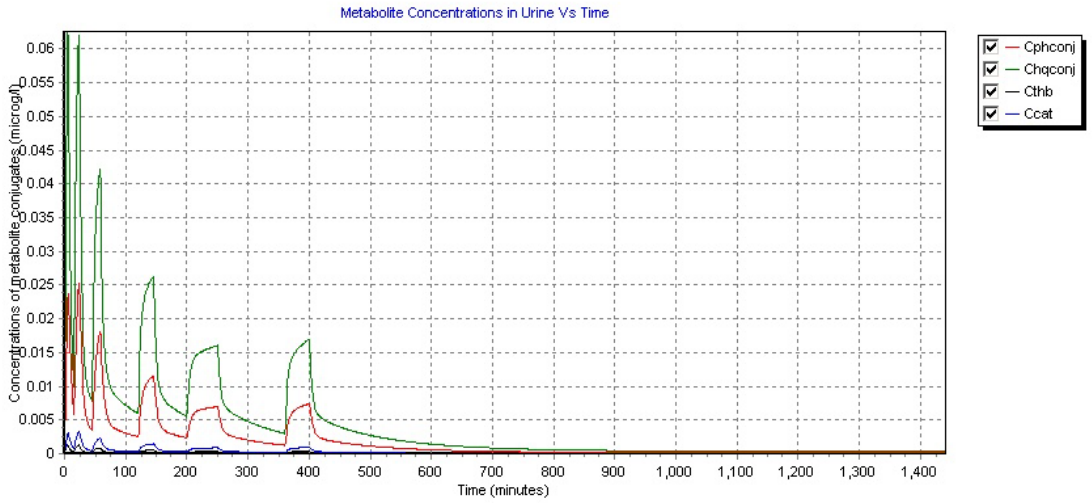

Figure 4.83 Case 4C – Concentrations of Benzene Metabolite Conjugates in Urine.

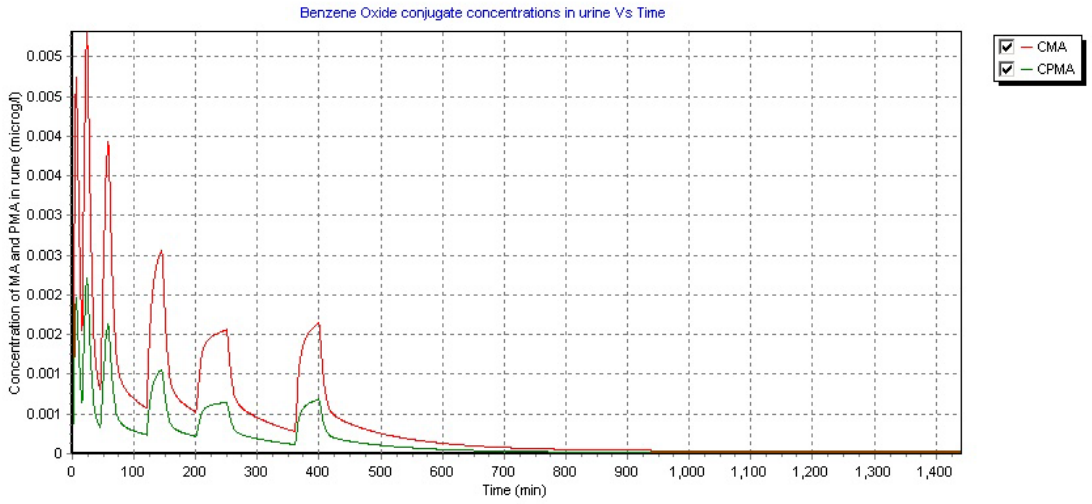

Figure 4.84 Case 4C – Concentrations of MA and PMA in Urine.

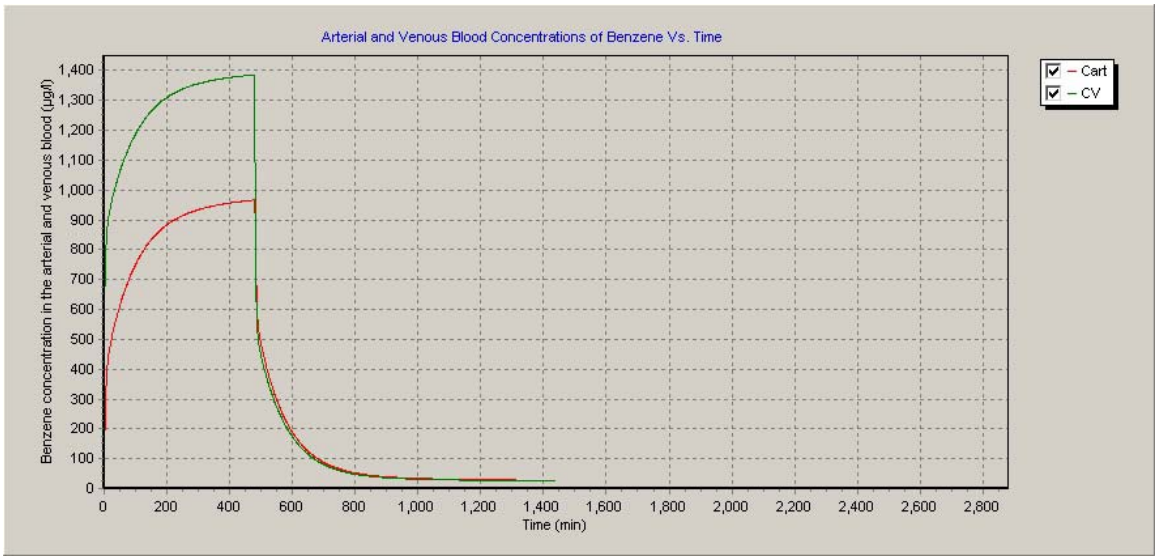

Figure 4.85 Case 5 – (No bone marrow) Arterial and Venous Blood Concentrations of Benzene.

Figure 4.86 Case 5 – (No bone marrow) Concentration of Benzene in Fat Compartment.

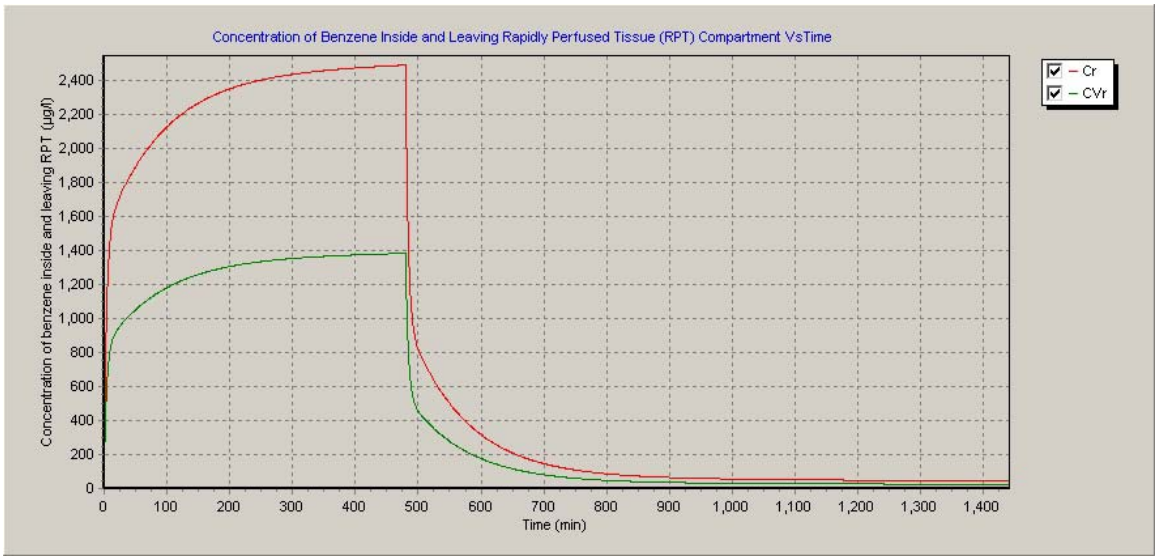

Figure 4.87 Case 5 – (No bone marrow) Concentration of Benzene in RPT Compartment.

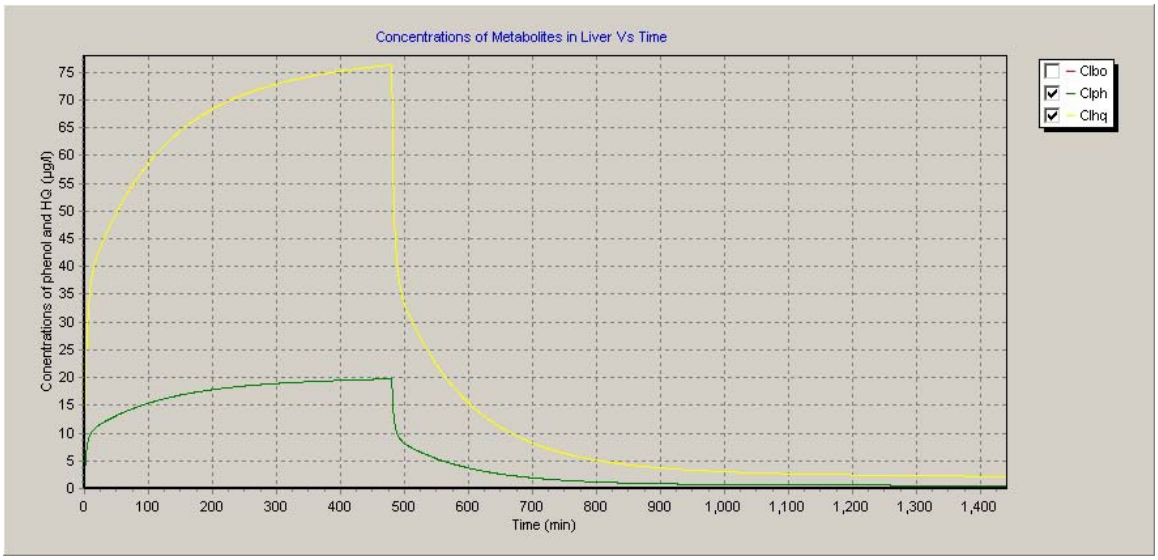

Figure 4.88 Case 5 – (No bone marrow) Concentrations of Phenol and Hydroquinone in Liver Compartment.

4.6 Model Validation

The model is validated against two sets of data. The first set of validation data is from USEPA's toxicological profile of benzene (USEPA, 2002). This profile summarizes all inhalation studies performed for benzene over the past 50 years including experimental and epidemiological studies and provide some general trends that these study results have indicated, regardless of the exposure concentration. Hence if the model presented in this research can predict concentrations in the range provided in this USEPA profile, it can be considered a good measure of the model's validity.

The profile refers to studies on respiratory absorption of benzene in humans performed by Nomiyama and Nomiyama (1974), Pekart et. al.; (1992), Srbova et. al.; (1950), and Yu and Weisel (1998) (USEPA, 2002). The trend indicated by all these studies, each of which use a different initial inhalation concentration and varied continuous exposure duration is as follows:

This validation is especially useful in ascertaining whether the model predicts concentrations of benzene's major benzene metabolites as correctly as possible. This is because, benzene absorption is usually verified on the basis of the amount benzene including its major metabolites that get excreted from the body through exhalation or in urine. Exhalation is already accounted for in the input concentration. So, the concentrations of major benzene metabolites in urine are measured. The leftover benzene and metabolites, post excretion can be considered as the amount of benzene that is effectively absorbed. If these excretion and absorption concentrations are accurately predicted it follows that the metabolic interactions must be predicted correctly by the model at least in terms of their concentrations. Also, this validation information can be used to validate any set of model results because the USEPA profile suggests that this is the general disposition observed for benzene from all the different studies. So, model runs are presented for varying initial concentration levels ranging from low to high and each set is validated against the same checks.

Another set of validation data is provided by Spear and Bois (1992), who performed a sensitivity analysis on several data sets obtained from different experimental and modeling studies. Based on their analyses, they set specific checks for model validation. Hence these checks and tests were used to validate the results of this model. The concentration to compare the validation data to was 490 ppm of continuous 6 hour and 8 hour exposure. Hence such runs were performed and numbers obtained for the required concentrations. These checks were specific for benzene concentrations in the fat compartment, the bone marrow compartment and venous blood. These data checks are provided in the table 2.

4.6.1 Model Validation Analyses:

The model validation results from both data sets mentioned before are presented below.

4.6.1.1 Validation for Benzene Absorption. The first validation was performed for the absorption of benzene. The benzene absorption results from a model run for 6 hours of continuous exposure to 10 ppm (30 μg/l) benzene inhalation in healthy adult male humans are compared with numbers from USEPA's summary of a number of respiratory absorption studies on benzene with different exposure concentrations and different continuous exposure periods (USEPA, 2002). This set of validation data is applicable regardless of initial concentration or duration of exposure.

The respiratory absorption was measured based on the concentrations and thus percentage of benzene metabolites excreted in the urine. These are summarized as follows in table 4.3. The absorption percentages were calculated based on these excreted concentrations from table 4.3 and are presented in table 4.4.

4.6.1.2 Validation for Specific Benzene Concentrations. Additional validation is also provided for benzene concentrations in the venous blood and in the fat and bone marrow compartments from data and tests for models provided in Spear and Bois (1992).

This analysis for the 6 hour exposure to 490 ppm of benzene by inhalation is presented in table 4.5 and that for the 8 hour exposure data validation is presented in table 4.6.

Table 4.1 Validation Data on Respiratory Absorption of Benzene (USEPA, 2002).

Time (minutes)	Benzene Absorption Range
After 5 minutes	70-80 %
After 1 hour	20-60 %
After 2 hours	20-50 %

Table 4.2 Validation Data from Spear and Bois (1992)

Variable	Observation Time (minutes)	Required Concentration Range (%)
During 8 – hour exposure		
Bone marrow concentration	0.2	15-55
Fat concentration	2	5-35
Venous blood concentration	1.5	30-70
During 6 – hour exposure		
Bone marrow concentration	6.5 9	20-60 0.02-5
Fat concentration	8 15	30-70 1-10
Venous blood concentration	6.5 10	30-70 1-10

Table 4.3 Concentrations of Benzene Metabolites Excreted in Urine.

Time (min)	C_{MA} (µg/l)	C_{PMA} (µg/l)	C_{PHconj} (µg/l)	C_{HQconj} (µg/l)
5	1.889	0.785	8.345	17.948
60	48.516	20.615	215.681	486.214
120	113.074	46.997	502.991	1805.728

Table 4.4 Validation of Benzene Absorption

Time (minutes)	Inhaled benzene concentration (µg/l)	Benzene concentration excreted (µg/l)	Excreted Percentage (%)	Absorbed percentage calculated by the model (%)	Absorbed percentage from USEPA's review(%)
After 5 minutes	30 * 5 = 150	28.2605	18.8	81.16 **CHECKS!**	70-80
After 60 minutes	30*60 = 1800	770.576	42.8	57.19	20-50
After 120 minutes	30*120 = 3600	1805.728	50.15	49.8 **CHECKS!**	20-50

Table 4.5 Benzene Concentrations after 6 hours exposure to 490 ppm Benzene

For Benzene Concentrations in the Venous Blood				
Time (minutes)	**Concentration at the time (µg/l)**	**Concentration at the end of exposure (µg/l)**	**The % benzene concentration in the venous blood from the model**	**The % concentration range from Spear and Bois (1992)**
6.5	420.132	1412.713	29.74 **CHECKS!**	30-70
10	66.291	1412.713	4.7 **CHECKS!**	1-10

Table 4.5 Continued

For Benzene Concentrations in the Bone Marrow Compartment				
6.5	1266.279	2534.609	49.5 **CHECKS!**	20-60
10	139.485	2534.609	5.5 **CHECKS !**	0.02-5
For Benzene Concentrations in the Fat Compartment				
8	10415.602	10049.923	103.6	30-70
15	9102.37	10049.923	90.57	1-10

Table 4.6 Benzene Concentrations after 8 hours exposure to 490 ppm Benzene

Time (minutes)	**Concentration at the time (μg/l)**	**Concentration at the end of exposure (μg/l)**	**The % benzene concentration in the venous blood from the model**	**The % concentration range from Spear and Bois (1992)**
For Benzene Concentrations in the Venous Blood				
1.5	1197.375	1429.614	83.75	30-70
For Benzene Concentrations in the Bone Marrow Compartment				
0.5	1279.487	2569.204	49.8 **CHECKS!**	5-55
For Benzene Concentrations in the Fat Compartment				
2	2994.477	13417.604	22.32 **CHECKS!**	5-35

4.7 Summary of Results

The model was run for different scenarios as listed in section 4.1. The results from each run for male exposures are presented throughout the chapter. Results from only 1 case study on females are presented. This is the 8 hour continuous inhalation exposure to benzene scenario. All other results from female exposure cases are available with the author and can be made available for perusal. The model is validated against two sets of data and is presented in section 4.6. Sensitivity analysis results are presented in chapter 5. Discussion on all results depicted in this chapter are discussed in chapter 6.

CHAPTER 5

5. SENSITIVITY ANALYSIS USING Monte Carlo SIMULATION TECHNIQUES

5.1 Introduction

PBPK/TK models rely heavily on experimental data to obtain results. Often, ethical issues and practical reasons stand in the way of experimenting using human subjects and animals are used in laboratories. The results from the animal models are then extrapolated and used to predict concentrations in human bodies. The extrapolation involves uncertainty or safety factors. This leads to uncertainties in the accuracy of results obtained from the models developed for human beings. Moreover, data from several different sources are often used in one model. These data may come from different experimental setups leading to further uncertainty because each laboratory might have different experimentation environments and techniques and results from one laboratory experiment might not directly co-relate to that of another. Hence it is very important to verify the effects of model parameter data on the results of the model. In this regard, one of the most popular sensitivity analysis tools available for use in the scientific community is Monte Carlo analysis. This simulation technique is also widely used in PK/TK work.

5.2 Uncertainty in PBPK/TK Models

There are many different kinds of uncertainties possible in PBPK/TK models (Edler and Heinzl, 2003). Some of them include:

i) Structural uncertainty – Occurs due to incomplete or insufficient knowledge or understanding of the biological and toxicological mechanisms

ii) Statistical uncertainty – Occurs when parameters are estimated based on variable data

iii) Technical uncertainty – Occurs due to inconsistent estimation procedures, or due to mathematical or analytical approximations used in the model or because of software limitations

iv) Model parameter uncertainty – occurs due to imperfect knowledge of the actual value of a parameter, leading to incorrect concentration calculations.

5.3 Monte Carlo Technique

The Monte Carlo simulation technique used in sensitivity analyses is simple. It involves the use of a random number generator to select parameter values from a known or suspected distribution. After the selection, the model is run and simulation results are saved. Iterations are performed until a statistically significant number are recorded. The results then help determine the mean and standard deviation values, which explain the uncertainty of all parameter errors (Schnoor, 1996). The computation steps involved in the process can be listed as follows:

1. Determine the uncertainty distributions for each parameter, input function and variable to be analyzed
2. Then use the random number generator to account for the probability of each value.
3. Use the set of parameter values selected as input for the first simulation. Run the model and save the output to the tape or file.

4. Run iterations of steps 2 and 3 until the statistical output remains unchanged with any further repetition.
5. Sort the stored output data and plot the output as the mean value plus or minus the probability range that is desired.

5.4 Monte Carlo in the Present Study

The Monte Carlo simulation technique has been used before for PBPK modeling and even specifically for benzene modeled using PBPK (Woodruff and Bois, 1993; Thomas et. al., 1996). In this study the same software model ACSLXtreme used to perform the model runs is also used for sensitivity analysis. The software in turn uses the Monte Carlo simulation technique to perform the analyses. Other commercial software are also available that can be used to perform sensitivity analyses. However, given the capabilities of ACSLXtreme and for consistency, the same software is used for model runs and runs for sensitivity analyses.

ACSLextreme uses the normal distribution to generate the maximum, mean and minimum concentration levels based on the new parameter values as varied over the 100 iterations. Normal distributions in a variate x, with mean μ and variance σ^2 has a probability function of

$$P(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-(x-\mu)^2/(2\sigma^2)} \tag{3.106}$$

The normal distribution is the most used statistical distribution. It is also called a Gaussian distribution or a bell curve. These distributions tend to have scores concentrated towards the middle than in the tail ends of the graphs (Glantz, 2002).

5.5 Sensitivity Analysis

Sensitivity analyses are performed for the partition coefficient of benzene for the fat compartment (P_f), the blood flow rate into the fat compartment (Q_f) and some of the rate constants (k values) for the metabolic reactions. The P_f parameter is selected because the fat compartment failed one of the three validation tests used to determine the accuracy of the model. Even though the fat is reported to absorb a great amount of benzene compared to the other compartments, it is believed that this study model is possibly over-predicting the values of benzene concentrations in the fat compartment. The over-prediction of concentration values in the fat compartment can also be time dependent. The time dependency is associated with the blood flow rate into the compartment, and so Q_f is also tested for its sensitivity on the model results for the fat compartment.

The rate parameters (k – values) for some of the important metabolic conversion equations are selected because these data are from a single study and it is prudent to test for their sensitivity.

In each case, the initial value for the sensitivity run is the parameter value used in the model; i.e. the value obtained from literature. This value is varied 10-15% in the random number generation process and 100 iterations are performed for each run. At the end of the run, the maximum and minimum concentrations of benzene/metabolite in the fat or liver compartments or excreted in urine are plotted. The results obtained from all analyses are presented below. The discussion is presented at the end of the graphs.

5.5.1 Results of the Analyses

The sequence of the graphs is as follows:

1. P_f - for benzene concentration in the fat compartment (Figure 5.1).
2. Q_f - for benzene concentration in the fat compartment (Figure 5.2).
3. k1 for benzene oxide concentration in the liver compartment (Figure 5.3).
4. k2 for phenol concentration in the liver compartment (Figure 5.4).
5. k3 for the muconic acid concentration excreted in the urine (Figure 5.5).
6. k4 for the phenylmercapturic acid concentration excreted in the urine (Figure 5.6).

7. k5 for the hydroquinone concentration in the liver compartment (Figure 5.7).
8. km1ph for the phenol conjugates concentration excreted in the urine (Figure 5.8).
9. kmhq for the hydroquinone conjugates concentration excreted in the urine (Figure 5.9).

Of these, the graph for the P_f has the maximum, minimum and mean concentration values for benzene in the fat compartment all in the same graph.

A separate graph for the maximum and minimum concentrations of phenol is provided because the parameter is highly sensitive as indicated by the huge difference in scale from maximum to minimum.

In case of phenylmercapturic acid, the minimum concentration excreted was predicted at 0. Hence a separate mean concentration graph is provided for clearer presentation.

Again, for brevity only sample graphs are provided so as to indicate the different analyses performed on the parameters, even though all parameters listed above were analyzed for their sensitivities and all results are described in this chapter. The additional plots not shown in the chapter are available for review from the author.

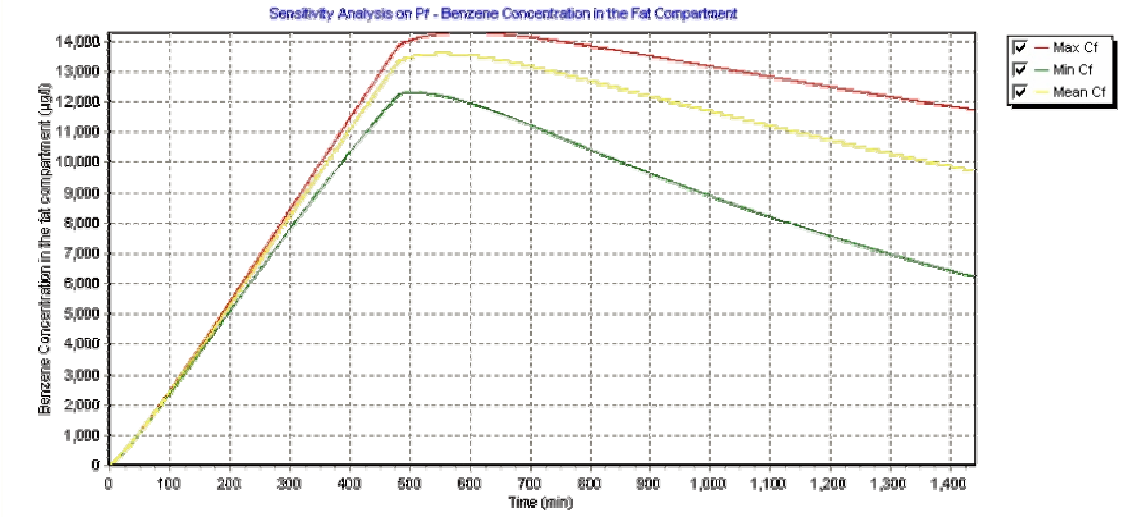

Figure 5.1 The Maximum, Mean and Minimum Concentrations of Benzene in the Fat Compartment (based on P_f values).

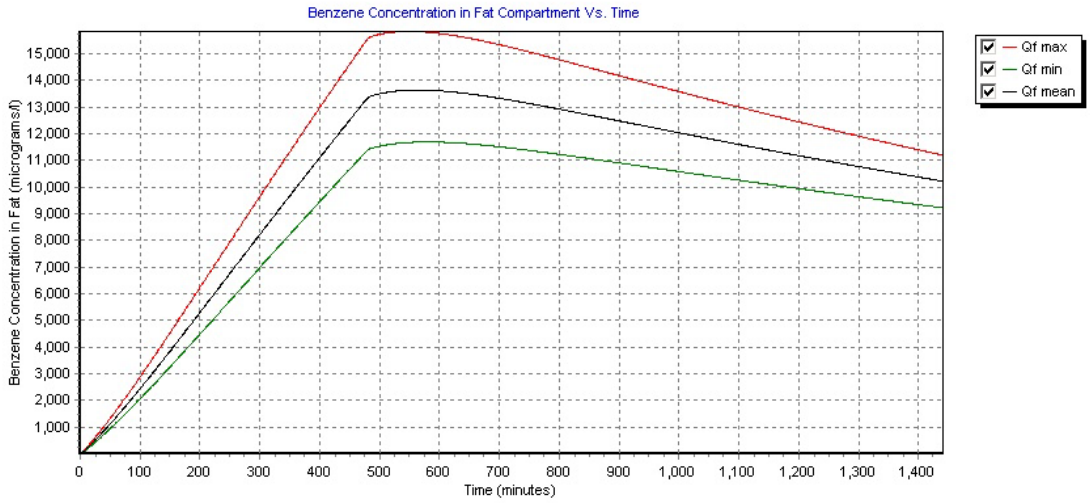

Figure 5.2 The Maximum, Mean and Minimum Concentrations of Benzene in the Fat Compartment (based on Q_f values).

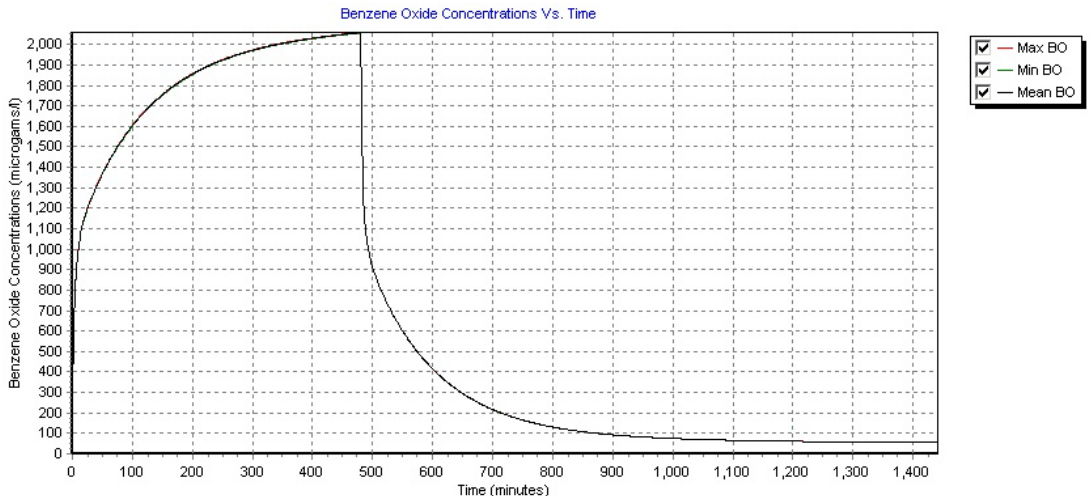

Figure 5.3 The Maximum, Mean and Minimum Concentrations of Benzene Oxide in the Liver.

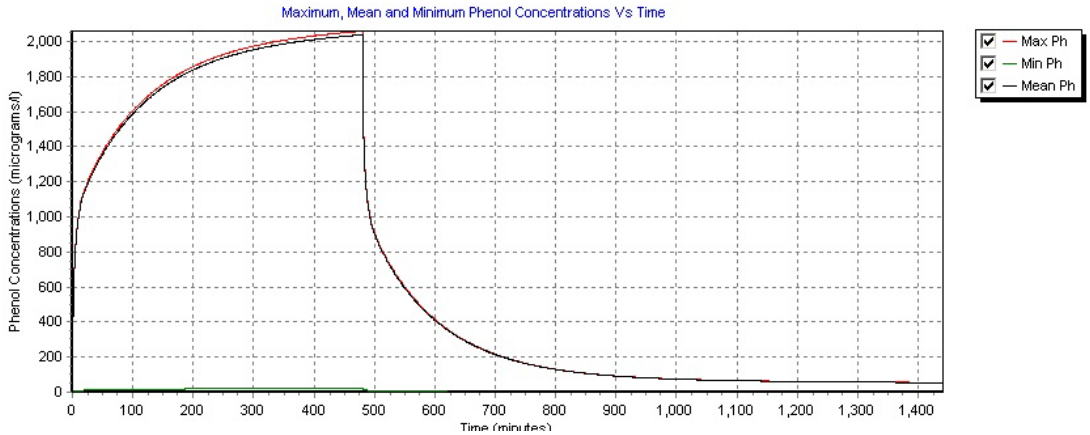

Figure 5.4 The Maximum, Mean and Minimum Concentration of Phenol in the Liver Compartment.

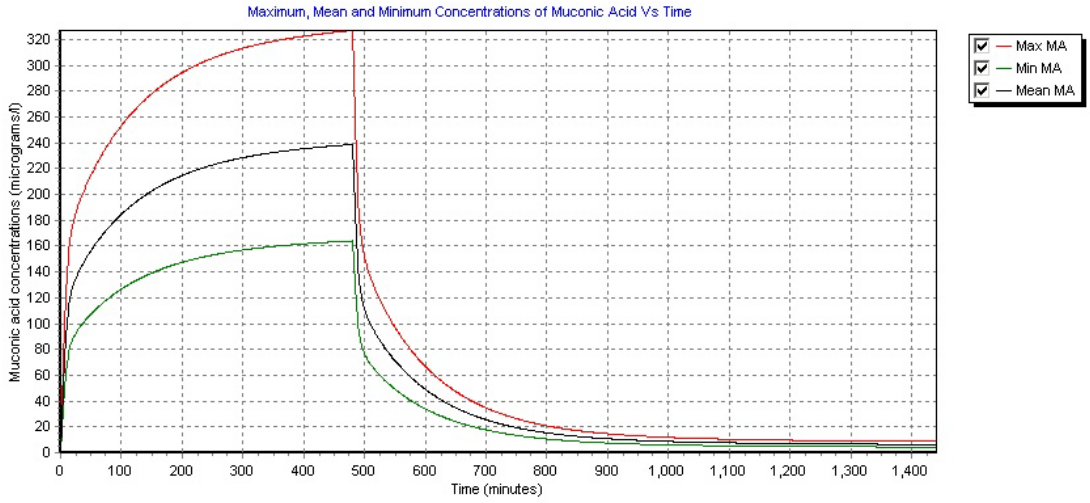

Figure 5.5 The Maximum, Mean and Minimum Concentrations of Muconic Excreted in the Urine.

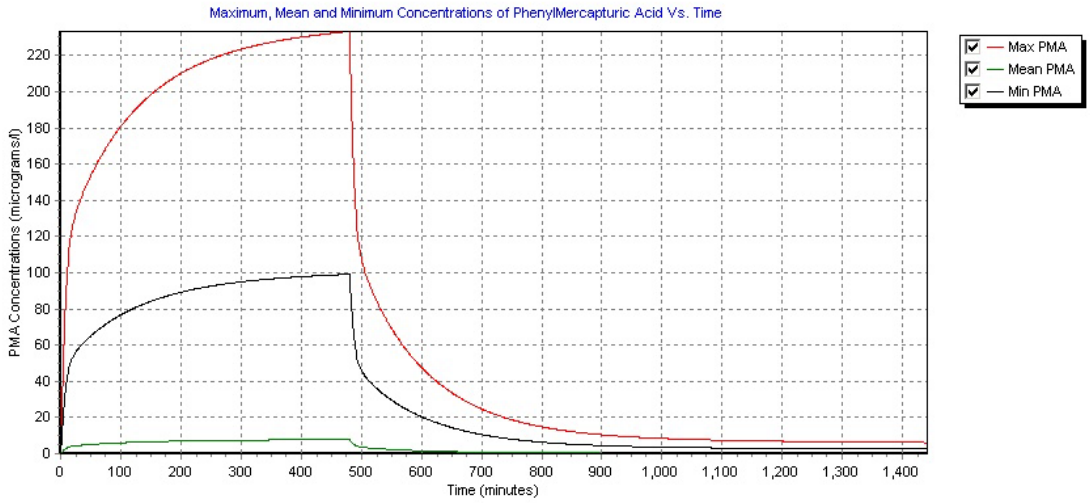

Figure 5.6 The Maximum, Mean and Minimum Concentrations of Phenylmercapturic Acid Excreted in the Urine.

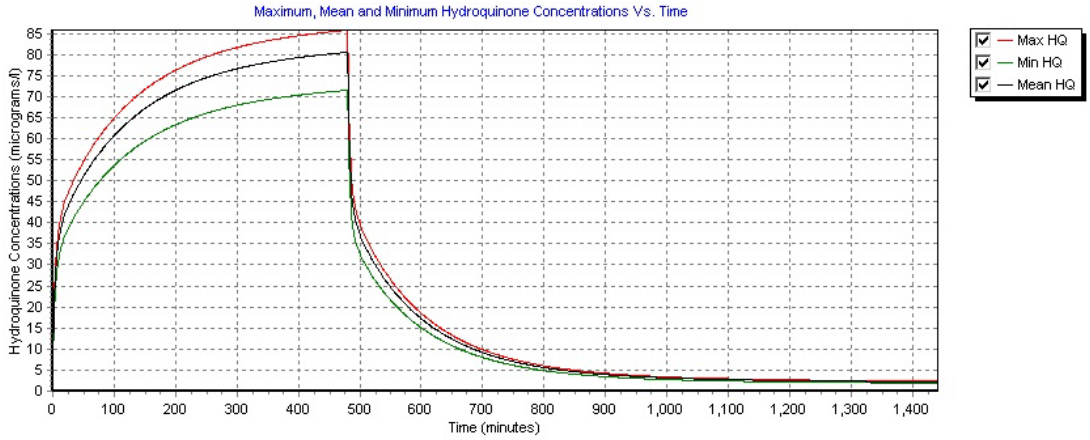

Figure 5.7 The Maximum, Mean and Minimum Concentrations of Hydroquinone in the Liver Compartment.

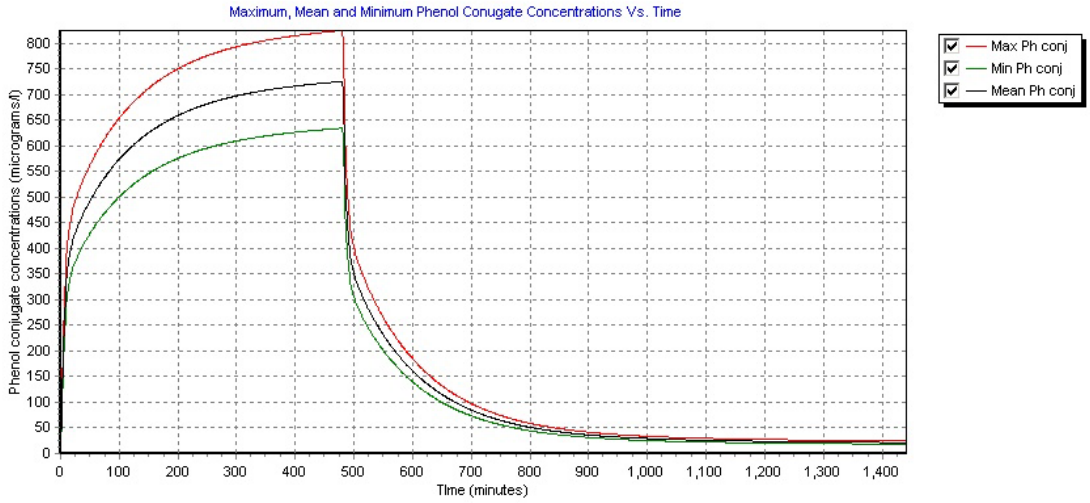

Figure 5.8 The Maximum and Minimum Concentrations of the Phenol Conjugates Excreted in the Urine.

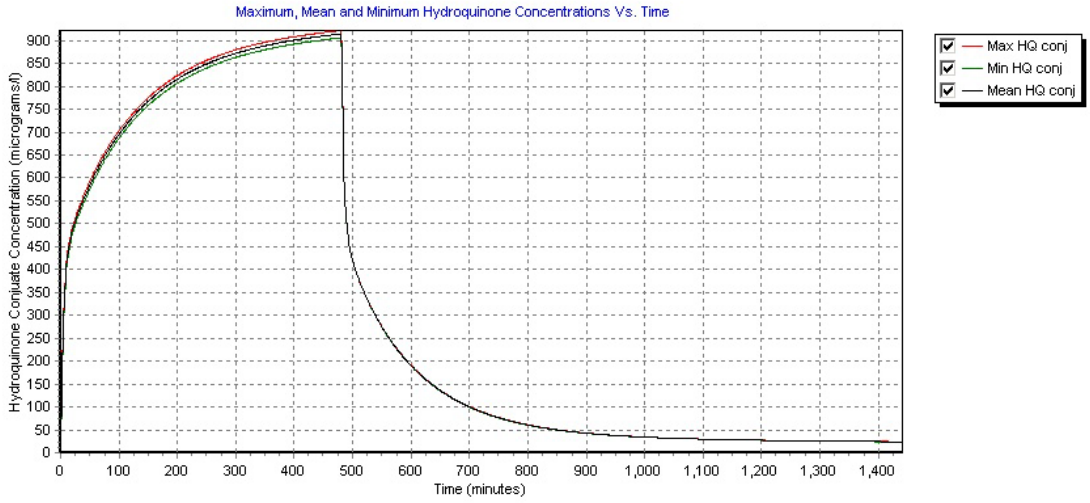

Figure 5.9 The Maximum, Mean and Minimum Concentrations of the Hydroquinone Conjugates Excreted in the Urine.

5.5.2 Discussion of the Sensitivity Results

The results obtained from the sensitivity analyses can be explained as follows:

5.4.2.1 For the P_f parameter. The P_f value run produced results which indicated that the maximum P_f value used in the sensitivity graph is 78 and the minimum value is 24. These values are used by the software to plot the final maximum and minimum concentration graphs for benzene in the fat compartment respectively. As indicated in the graph in figure 5.1, the maximum concentration value was accurately predicted in the analysis and is the same as the one estimated by the model. The minimum concentration is lower than the one predicted by the model, but the difference is not vast. Thus, it is believed that this parameter might be somewhat sensitive, especially in the lower range to change in the P_f values.

5.4.2.2 For the Q_f parameter. As seen in figure 5.2, the maximum and minimum concentration levels in the fat compartment based on varying the blood flow rate into the fat compartment (Q_f) by 10 to 30 % of the value used in the model, are not somewhat different indicating that this is a somewhat sensitive parameter. While the difference in concentrations is not vast, still the parameter cannot be said to be completely stable. However, it cannot be said with absolute certainty whether the Q_f parameter causes the differences in concentration observed in the validation runs for the 6 hour and 8 hour exposure duration.

The conclusion from the 2 runs for the fat compartment is that the reason for the over-prediction of the benzene concentration in the fat compartment is not very clear. It cannot specifically be attributed to the 2 parameters that are variable in nature for the fat compartment (P_f and Q_f). Each is found to be somewhat sensitive and might cause the over-prediction but this cannot be stated with complete certainty.

5.4.2.3 For the k1value. The results from this analysis indicates that the rate constants for the metabolism from benzene to benzene oxide, is accurate. Despite varying the value of the initial k1 of 0.000042 by 10-15 %, the graphs of maximum and minimum

benzene oxide concentrations nearly merge. They predict the same concentration as before, thus reflecting that the parameter is sound and is not sensitive to changes.

5.4.2.4 For the k2 value. The fact that 2 graphs were required to clearly represent the maximum and minimum phenol concentrations in the liver is in itself a confirmation that this parameter is highly sensitive to change. However, the minimum concentration tallied with the one modeled before, but not the maximum concentration. This indicates that lowering the k2 value by 10-15% will not have a significant effect on the concentration levels. However, even a slight increase in the value will cause large spikes in the concentration levels.

5.4.2.5 For the k-3 values. The maximum concentration of muconic acid predicted in the sensitivity analysis is close to the one originally estimated in the model. The lower value however, is off by about 50%, which means that in case of the constant k-3 lowering the k-3 value will have a large impact on concentration levels, but raising it by 10-15% will have only a small impact on the overall concentration levels.

5.4.2.6 For the k-4 values. The k-4 value seems to be highly sensitive to change since it over-predicts or under predicts the concentrations of the phenylmercapturic acid based on the change in the parameter value. This can potentially have a significant impact on model results if not used correctly.

5.4.2.7 For the k-5 values. This value is for the metabolism of phenol to hydroquinone. The hydroquinone concentrations provided in the sensitivity analysis closely match the numbers estimated by the model. Both the upper and lower concentration values are close to the original model prediction. Hence this is a stable parameter.

5.4.2.8 For the km1ph and kmhq value. These values indicate the metabolism of phenol and hydroquinone to their sulfate and glucoronide conjugates. Both these parameters are stable because of their accurate prediction of the concentrations of the

conjugates excreted in the urine. Both the minimum and maximum values of each parameter are very close to the ones predicted by the model, indicating their insensitivity to fluctuations in values by 10-15%.

CHAPTER 6

6. DICUSSION OF RESULTS

6.1 Introduction

The model runs performed in the study for the different scenarios are presented in chapter 4. The sensitivity analysis is presented in chapter 5. This chapter discusses the results obtained from the modeling exercise and the significance of those impacts on the study in question as well as the larger implications of the results beyond the present study.

6.2 The Model Results

The model was run for the different scenarios. This section analyses results from all those different scenarios.

6.2.1 Discussion on case 1 – Intermittent inhalation exposure to 1.236 μg/l benzene for 16 hours of a 24 hour day.

In this scenario, the exposure is assumed intermittent (10 minutes of exposure followed by 40 minutes of no exposure) for 16 hours of a 24 hour day. Such intermittent exposures have not been modeled in PBPK/TK models, since most previous models focused on continuous exposure concentrations. These runs not only demonstrate the capability of the model to perform simulations for intermittent exposures, but also provide a tool that can realistically be used in environmental engineering conditions,

where exposure conditions are variable. This theme is further enhanced in case 4c, where irregular intermittent exposures are modeled, which is the most practical form of exposure in non-occupational situations.

In this case, a very low concentration of benzene is considered, since the concentration of benzene in each cigarette is just 61.5 μg and the ventilation rate of humans, makes the actual inhalation concentration even lower at 1.236 μg/l or 0.4 ppm. The results indicate that the maximum benzene concentration in any organ is 4.6 μg/l or approximately 1.5 ppm, as seen in the fat compartment at the end of the 16 hour exposure in figure 4.3. At this concentration based on data in literature, no adverse effects are observed. Epidemiological studies performed by Tsai et. al., (1983) and Collins et. al; (1997) for continuous exposure concentrations of 0.53 and 0.54 ppm, for time periods of over 5 years in both cases did not indicate any adverse effects in the observed worker population any different form those in the controls. The total count of all red blood cells, white blood cells and platelets, were normal in both cases. This seems to imply that if people are intermittently exposed to such low concentrations of benzene, there is no possibility of developing any adverse effect. This finding is also in keeping with the USEPA's No Observed Adverse Effect Level (NOAEL) for benzene which is 7.8 ppm (USEPA, 2002).

Thus the results from this section of the study imply that when a person is exposed to benzene from cigarette smoke alone, there is no potential to develop any adverse effect. This does not however mean that cigarette smoke is harmless or does not cause adverse effects. A cigarette actually contains at least 63 known human carcinogens in addition to other chemicals that cause a myriad of adverse effects. However, just the benzene and that too from cigarette smoke alone does not cause adverse effects because of the extremely low exposure concentration. It should also be noted that if there are other sources of benzene exposure besides cigarette smoke, then the benzene levels from cigarette smoke might cause an additive effect on the overall benzene levels and contribute to possible adverse effects.

6.2.2 Discussion on cases 2 and 3 – Continuous inhalation exposure to 490 ppm (1470 μg/l) benzene for 8 hours and 6 hours respectively.

This section discusses compartment by compartment, the effect of the 8 hour and 6 hour continuous inhalation exposure to benzene on all benzene and its major metabolite concentration levels. The discussion applies to both the 8 hour and 6 hour model runs because of similar trends in all graphs. These cases were particularly chosen to validate the model results and are discussed in the validation context following the description of all compartment concentrations of benzene and its major metabolites.

6.2.2.1 The fat compartment. The runs from all scenarios consistently show that the fat compartment is the one that absorbs most of the benzene. This can be observed from the high C_f and low CV_f values in the graphs. The C_f values predicted by the model are much higher than the values indicated by the CV_f graph. This is observed in all continuous and intermittent exposures studied. These finding are corroborated by other research. The high partition coefficient of this compartment is the probable reason for the high concentrations. However, it should be stated that it not clearly validated whether the model is predicting accurate results for the fat compartment. This is based on the fact that the fat compartment failed one of the validation tests for the different exposure concentrations at the lower duration of exposure but is successfully validated in the longer 8 hour duration study.

An additional model run for a continuous 490 ppm exposure to benzene modeled over a seven day time period implied that it takes a little over seven days to get the benzene in the fat compartment down to near zero levels, after just a single exposure as described.

Another issue raised by this first set of runs on the cigarette smoke data for male and female exposures, is the difference in benzene disposition in males and females. This study found that there is not a significant difference in benzene behavior in men and women. It is true that the overall absorption of benzene is slightly higher in women than in men, however the values are close enough to disqualify any significant difference in the two. Thus the overall results from the male and female exposures indicate that the

concentration levels inside and leaving the different model compartments as well as the concentration levels of the metabolites and benzene conjugates that are excreted in urine are not significantly different in men and women. This finding is consistent in all the different scenarios tested in the model runs.

However, the disposition of benzene in males and females is a subject of some debate. While a few studies have indicated some differences in male and female absorption of benzene and have accounted this to the higher fat content in women, other studies have found no differences in the disposition of benzene in male and female subjects. Also, the studies in animals such as male and female rats and mice are strong in their bases, but the human studies are probably unreliable in this area because of factors such as the limited number of study subjects, or insufficient controls (USEPA, 2000).

6.2.2.2 The arterial and venous blood concentrations. The arterial and venous concentrations of benzene vary depending on the inhaled concentration, the alveolar ventilation rate, the cardiac output and the blood: air partition coefficient for benzene in humans. The benzene concentration in the arterial blood is always lower than its concentration in the venous blood because the venous blood concentration of benzene is a sum of all the venous blood concentrations of benzene coming from all the different compartments. Thus, the pattern of the graphs is similar in both cases, but the C_{art} is always lower than the CV.

6.2.2.3 The RPT and PPT Compartments. These compartments seem to absorb and release similar amounts of benzene, although the rates at which absorption and elimination occur are clearly different. In case of the RPT, a higher blood supply to these organs brings in larger concentrations of benzene, but not a lot of it is absorbed. In case of PPT, even though the blood supply received is rather small, the benzene is brought into a large volume and thus it is possibly supplied to the PPT in the same concentration as in the case of RPT, only at a slower rate. Benzene oxide concentrations are high in both compartments.

6.2.2.4 The Liver Compartment. This is the key compartment because the main benzene metabolism occurs here. High levels of benzene oxide are found in the liver. The results also show the main metabolites of benzene, phenol and hydroquinone and their concentrations. The percentage of phenol and hydroquinone produced in the liver at different concentrations are proportional in each modeling scenario. After metabolism the liver holds on to less than 3 % of the benzene. Most of it is released back into the bloodstream in the form of benzene or metabolites and some of the hydroquinone partitions in to the bone marrow.

At such high concentrations are used in this model run, hematotoxicity has been established in many animal experimental studies for continuous exposures over long periods of time.

6.2.2.5 The Bone Marrow Compartment. The bone marrow compartment is the place for secondary metabolism of benzene. Hydroquinone converts to 1,4 benzoquinone in the bone marrow. Since the bone marrow is a richly perfused tissue it is interesting to note that much of the benzene concentration levels are the same in the RPT and the bone marrow. As is the case with the other organ systems, benzene oxide is present in the bone marrow in high concentrations. The phenol concentrations are higher than hydroquinone indicating that the rate of metabolism of phenol to hydroquinone is a slow one. The benzoquinone concentrations in the bone marrow are the highest among all the benzene metabolites. This is mostly because the elimination of benzoquinone from the body has not been modeled because of lack of research in this area. No data are currently available regarding the breakdown of benzoquinone in the bone marrow or its half life time in the human body. This makes it difficult to determine hwo much benzoquinone leaves the body. Thus the benzoquinone concentration levels predicted by the model are those which are the highest possible for the given duration and concentration level of exposure considering that no elimination occurs. If elimination data become available and are added in the model equations, then more accurate results will be obtained on the exact concentration of benzoquinone that actually stays in the body and cause any adverse effect. Effects of considering the bone marrow as a separate compartment are discussed in section 6.1.4.

6.2.3 Model Validation Discussion Based on Case 2 and Case 3.

This set of runs included simulations of exposures to the 490 ppm (1470 μg/l) benzene concentration for 8 hours (case 2) and 6 hours (case 3). Since the validation data had numbers for continuous exposures, the model was run for the same. The data based on these results are noted in tables 4.1 - 4.6 towards the end of chapter 4 in section 4.6. The numbers in these tables indicate that the model predicts most concentrations accurately. Most observed concentration levels were in data ranges suggested in the sources of validation data. However, the one check that was no validated was that of the concentration of benzene in the fat compartment following a benzene inhalation exposure concentration for 6 hours.

High benzene concentration levels in the fat compartment were expected. The fat tissue does have a high affinity for lipophilic substances such as benzene and its metabolites. Moreover, animal experiments for benzene exposure and previous benzene PBPK/TK models have also made similar predictions. However, the concentration level predicted for the 6 hour exposure duration was much higher than any previous estimates. The other factor that led to further questions was that the model accurately predicted the fat compartment benzene concentration level for the 8 hour exposure period. Thus, while the initial speculation was that the over-prediction is a possible result of the high value of the blood:fat partition coefficient for benzene (P_f), the fact that the 8 hour exposure duration was validated led to the possibility that the blood flow rate into the fat compartment (Q_f) might be the possible reason for the higher concentration levels. Thus a sensitivity analysis was performed on both these parameters to determine whether they had any influence on the concentration levels in the fat compartment. The sensitivity analysis results using Monte Carlo simulation technique is presented in chapter 5 and those results are analyzed in the next section.

All other concentration ranges for the 6 hour exposure period were accurately predicted. In case of the 8 hour exposure period, the venous blood concentration of benzene was predicted slightly higher than the expected range, but concentration levels in

the fat and bone marrow compartments were correct. The benzene absorption validation also checked out in the expected ranges.

6.2.4 Discussion on the Sensitivity Analysis of Model Parameters

Details on sensitivity analysis using the Monte Carlo simulation technique are provided in chapter 5. As described in that chapter, ACSLXtreme was used to perform the analyses. The sensitivity analysis was performed for the blood:fat partition coefficient (P_f), blood flow rate into the fat compartment (Q_f) as well as the rate constants for all major benzene metabolic rates. Even though all rate constants were analyzed, graphs are plotted only for some of the constants. The remaining are available with the author and can be made available upon request. The results from the sensitivity analyses are in the form of graphs that plot the maximum, mean and minimum concentrations of benzene/metabolite in specific compartments, when parameter values are varied randomly over 100 iterations.

The fat compartment's concentration levels failed 2 of the validation tests for the 6-hour duration period. However, the fat compartment concentrations are validated for the 8-hour exposure period. Thus, it can be speculated that the model can make better predictions for the fat compartment at higher duration of exposure than at a lower duration. Additionally, the sensitivity analysis results show the higher values of the P_f predicted the same concentrations as estimated by the model indicating some sensitivity if the P_f values are lowered.

The rate constants had variable results. It is noted that the parameters k1, k3, k5, k6 and km1ph and kmhq are all stable parameters and changes in their values by 10-15% does not impact the concentration levels predicted using these constants. The k2 and k4 values, however are sensitive to change. The discussion for these parameters and their sensitivities are provided in chapter 5.

6.2.5 Discussion on case 4A, 4B and 4C - Model runs for 8 hour continuous, regular intermittent and irregular intermittent inhalation exposure to benzene concentrations of 50 ppm (150 μg/l) exposure in males and females

A comparative case study was conducted for observing the differences in benzene and its major metabolite concentration levels in all model compartments for the same overall benzene inhalation exposure concentration but different patterns of exposure. As described in section 4.4, the three cases are:

The model was then run for a comparative analysis of three different situations. These were:

4. Continuous 8 hour inhalation exposure to 50 ppm (150 μg/l) benzene concentrations (represented as Case 4A).
5. Intermittent inhalation exposure to benzene concentrations at regular intervals of 30 minutes exposure then 30 minutes no exposure such that the total inhaled concentration over 8 hours is 50 ppm (150 μg/l) (represented as Case 4B).
6. Intermittent inhalation exposure to benzene concentrations at irregular intervals described as follows:
    i. Exposure for 5 minutes, then no exposure for 10 minutes.
    ii. Exposure for 7 minutes, then no exposure for 12 minutes
    iii. Exposure for 13 minutes, then no exposure for 62 minutes
    iv. Exposure for 25 minutes, then no exposure for 55 minutes
    v. Exposure for 50 minutes, then no exposure for 110 minutes
    vi. Exposure for 40 minutes

The overall exposure is such that the total inhaled concentration over 8 hours is 50 ppm (150 μg/l) (represented as Case 4C).

The expectation from these runs was to have results that would indicate high value for the continuous exposure, followed by concentration levels in the regular intermittent exposure scenario and lowest concentration levels in case of the irregular intermittent case. This is based on the fact that when exposure to a pollutant ceases, the body gets a chance to recover from the toxic shock and the enzymes get time to break down the chemical into simpler by-products that can be eliminated before exposure

continues again. The results from these three runs do follow along these lines. However, while the irregular intermittent exposure produced the lowest concentrations of benzene and its major metabolites in the body, most concentration levels of benzene and its major metabolites were comparable in the continuous and regular intermittent exposure scenarios except for small differences in case of benzene concentration levels in the PPT and liver compartments and those of metabolite concentrations in the compartments and of their conjugates in urine. However these differences were not significant as can be seen in Table 6.1 presented below.

Also seen in this table is the difference in concentration levels of benzene and its major metabolites in case of male and female exposures. Again, the differences in concentration levels are not significant. The values are slightly higher in females than in males in most cases of any difference. This is usually accounted for because of the higher fat levels in females as compared to males and the tendency of females to hold on to higher concentrations of lipophilic substances such as benzene and its major metabolites. If concentrations are very high and there is a significant difference in concentration levels in males and females, then the higher concentrations in females also mean that they are at a higher risk as compared to males for same concentrations of inhalation exposure from benzene.

No significant differences were observed in the continuous exposure and regular intermittent exposure scenarios. In all likelihood, this can be explained on the basis that after exposing the body to benzene inhalation for 30 minutes, a 30 minute break before the next exposure begins is a rather small interval in which the body does not get sufficient time to recover from the first exposure and breakdown the concentration of benzene from this first exposure. In fact the somewhat higher concentrations observed during the regular intermittent exposure suggest that when the exposure is discontinuous but occurs at close intervals, the body holds on to more of the toxic chemical than in case of continuous exposure without any breaks.

The irregular intermittent exposure scenario considered a random exposure interval for benzene inhalation.

Table 6.1 Differences in Concentration Levels in Model Runs for Case 4A, Case 4B and Case 4C.

Parameter	Case 4A	Case 4A Female	Case 4B	Case 4C
C_{art}	0.29	0.29	0.3	0.06
CV	0.2	0.2	0.5	0.14
C_f	2.9	2.9	2.9	0.19
C_{rpt}	0.6	0.6	0.95	0.28
C_{ppt}	0.65	0.6	0.7	0.08
C_l	0.0075	0.008	0.011	0.004
C_{bm}	0.28	0.28	0.3	0.028
C_{bomax}	1.3	1.3	1.3	0.11
C_{phmax}	0.014	0.0155	0.014	0.001
C_{hqmax}	0.05	0.055	0.05	0.003
C_{bq}	0.6	0.65	0.6	0.045
$C_{caturine}$	0.1	0.1	0.018	0.002
$C_{thburine}$	0.005	0.005	0.002	0.001
$C_{phconjurine}$	0.07	0.07	0.12	0.025
$C_{hqconjurine}$	0.18	0.17	0.27	0.06
$C_{maurine}$	0.018	0.016	0.026	0.006
$C_{pmaurine}$	0.07	0.065	0.01	0.002

The results in this case suggest that when the intervals of exposure are erratic such that some are closely spaced (within minutes) while others are more apart (1 hour or more) the adverse effects will be less severe than continuous exposure or regular intermittent exposure to the same concentration level over the same overall duration of exposure.

Another point to be noted is that while the irregular intermittent pattern was randomly adopted, the exposure is more frequent earlier in the 8 hours and gets spaced

out more over the latter part of the 8 hour exposure period. This may also have given the body to recover after the initial busts of benzene exposure to reduce concentrations levels in time and cause less severe adverse effects than in the other cases.

6.2.6 Runs performed to understand the effect of including the bone marrow as a separate compartment.

Since one of the objectives of the study is to include a separate bone marrow compartment while developing the model for inhalation exposure to benzene, the model is tested for the effects of such an inclusion.

Model results from the runs so far consider the bone marrow as a separate compartment. However, the bone marrow as also considered as part of the RPT compartment and then the model was run for both male and female exposures. The results are shown in some of the representative graphs plotted in section 4.5 of chapter 4 with and without including the bone marrow compartment are presented in chapter 4.

As seen from the graphs presented in figures 4.85 - 4.88 and compared against results obtained from the previous runs considering the bone marrow as a separate compartment, the data presented in table 6.2 are obtained.

Table 6.2 Data Comparison between Models Runs with and Without the Bone Marrow

Parameter	Concentration considering the bone marrow (μg/l) as a separate compartment	Concentration considering the bone marrow (μg/l) as part of the RPT compartment
C_{art}	1500	1500
CV	1080	1080
C_f	34000	13000

Table 6.2 continued

Parameter	Concentration considering the bone marrow (μg/l) as a separate compartment	Concentration considering the bone marrow (μg/l) as part of the RPT compartment
C_{rpt}	7000	2500
$C_{phliver}$	22	20
$C_{hqliver}$	80	75

As seen from table 6.2, the concentration levels of benzene in the arterial and venous blood are not different whether the bone marrow is considered as a separate compartment or whether it is considered as a part of the RPT compartment. As opposed to that the concentrations of benzene in the all model compartments are higher when the bone marrow is included in the modeling exercise, while the metabolite concentrations are low in all model compartments. This means that when the bone marrow is not considered a separate compartment the model can under-predict concentration levels in the other organs giving a false sense of lower risk. For example the benzene concentration in the fat compartment is much higher considering the bone marrow as a separate compartment than when it is considered as a part of the RPT compartment. Even though these the concentration levels might not be significantly different overall in the model run provided here, the bone marrow should still be considered as a separate compartment. This is because it is a target organ for benzene toxicity and keeping the bone marrow as a separate compartment, helps determine the exact concentration levels of benzene and its major metabolites in the bone marrow. This in turns helps determine the exact nature of adverse effect and the corresponding prevention or treatment necessary to protect against the adverse effect.

6.3 Summary of the Model Results

Overall from the different runs of the model, several different pieces of information became available, all adding to gaps in research as well as contributing to a better understanding of the subject area. For example:

1. Thus the results from model run with the cigarette smoke data imply that when a person is exposed to benzene from cigarette smoke alone, there is no potential to develop any adverse effect attributed to benzene from the intermittent exposures typical in cigarette smoking. To reiterate cigarette smoking is harmful to health and can cause a number of adverse health effects including cancer from the complex mixture of chemicals present in every cigarette. However, if the exposure is only to benzene and only from the benzene in cigarettes then the above statement holds true.
2. Results from all runs indicated that the concentration levels of benzene and its metabolites are not significantly different in male and female exposures.
3. The intermittent form of exposure is modeled, which has not been done before in the PBPK/TK form of modeling. Regular and irregular intermittent exposures are modeled. The results indicate that there is not much difference when the exposure is continuous and when it is intermittent at a regular closely spaced interval. If the intervals are irregularly intermittent, then the risk of adverse health effects is lower.
4. The concentration levels of benzene are highest in the fat compartment.
5. The results of keeping the bone marrow as a separate compartment avoid any under-prediction of benzene concentrations in other organs as well as help study in depth the concentration levels of benzene in the bone marrow and corresponding adverse effects for those levels. This is especially useful since the bone marrow is a target organ for benzene toxicity.

CHAPTER 7

7. ENGINEERING APPLICATIONS OF THE DEVELOPED MODEL

7.1 Introduction

Environmental engineering studies use a number of models and tools to simplify calculations and assessments. However, in areas such as risk management, evaluations are often an art than a science and the best professional judgement of the engineer is often called upon in decision making processes. Whether the engineering project involves approval of a hazardous waste incinerator location, or remediating a leaking underground storage tank, decisions are made not only on the basis of sound engineering design, but also the capability of the engineering design to address issues such as protection of the potentially exposed population in the vicinity of the project. The USEPA has a myriad of resources, including guidance manuals, technical reports and other publications, databases, and mathematical models to bridge the gap between engineering and science. PBPK/TK modeling is finding acceptance in the environmental sciences over the past decade or so and the goal of this part of the study is to determine whether the models can be effectively used in environmental engineering projects.

The model presented here integrates research from areas such as chemistry, biology and toxicology, in order to simulate chemical behavior in the human body with potential applications in environmental engineering. Whether the application is in the area of air or water engineering or subsurface cleanup or hazardous waste facility

location, each engineering activity needs to be protective of human health as defined within the term "environmental engineering".

In an effort to validate the potential engineering significance of the model, three interviews were conducted with Florida Department of Environmental Protection employees including two engineers (Douglas Jones, Chief, Bureau of Waste Cleanup and Thomas W. Conrardy; PE Administrator; Petroleum Cleanup Section Three) and one toxicologist (Ligia Mora-Applegate; MSP, MPA, MPH – Risk Assessment) (Personal communication, 2004). The common consensus of all interviewees was that the model developed in this study can be used in environmental engineering studies. When asked specifically about potential engineering applications, all three professionals believed that the model can be used in combination with existing tools. It can be used not only as a screening tool for risk determination but in case of areas such as soil vapor intrusion, to establish the limits of indoor inhalation exposure because no such standard currently exists for benzene. Based on such confidence expressed by the FDEP officials, and additional information provided by the engineers, two case studies are presented to demonstrate the use of the PBPK/TK model in environmental engineering.

7.2 The Groundwater Remediation Case Study

In most subsurface environmental contamination problems, the issue of vapors and risk posed by the inhalation pathway is mostly ignored. Sometimes, numbers based on limited subjects in animal experimentation or epidemiological studies are used as limits of exposure to determine risk levels or cleanup targets. However, these may not always represent the true inhalation hazard and might be over or under protective of human health and the environment.

With the advent of risk based initiatives such as RBCA and their growing use in environmental engineering work, the focus is on multi-media pathways of exposure, where inhalation is also given its due. The use of RBCA has also prompted more remediation studies to adopt natural attenuation as a common remediation engineering practice and that has only increased the potential of inhalation exposure. Connor et. al.;

(1996) present a series of situations where vapors can originate from surface and subsurface contamination and be a potential exposure pathway with a serious degree of risk. The three-tiered RBCA process can guide users of the program into following the appropriate steps as presented in figure 7.1.

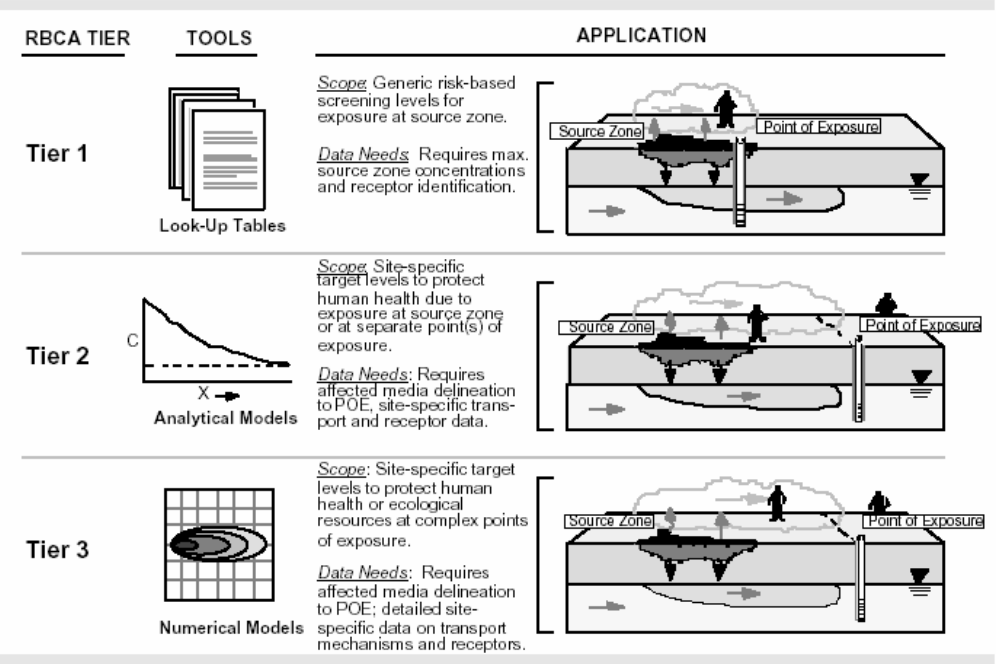

Figure 7.1 The Three Tiers of RBCA (Groundwater Services, Inc, 1996).

Depending on the severity of the contamination and the distance of the receptor from the source of contamination, different modeling exercises can be used in one of the three tiers mentioned above. For example, this PBPK/TK model can be used as a screening tool to determine the inhalation risk to receptors at a site.

Consider, for example the case presented in tier 1. This is an enlarged version of the same diagram from figure 7.1. In this case, as mentioned in figure 7.1, for any screening tool to function, information on the maximum concentration of the chemical of concern (COC) is required as well as the receptor information. These are the same kinds

of data needed as input values into the PBPK/TK model. So, say for example, there was a petroleum spill and benzene is the major COC.

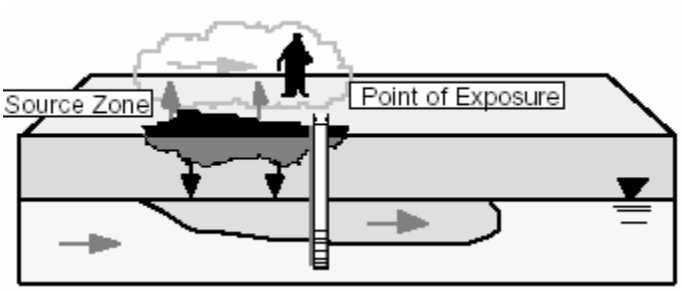

Figure 7.2 Tier 1 of RBCA (Groundwater Services Inc, 1996).

Let's assume that based on groundwater fate and disposition models and equations that calculate the soil to air volatilization factor of benzene, the concentration of benzene in air that a receptor will be exposed to is 10 ppm or (30 µg/l). Examples of such equations are taken from Connor et. al.; (1996) and presented as follows.

$$RBSL_{air}\left[\frac{\mu g}{m^3 - air}\right] = \frac{TRxBWxAT_c x365\frac{days}{y}x10^3\frac{\mu g}{mg}}{SF_i xIR_{air} xEFxED} \tag{7.1}$$

for direct exposure via inhalation and

$$RBSL_w\left[\frac{mg}{L - H_20}\right] = \frac{RBSL_{air}\left[\frac{\mu g}{m^3 - air}\right]}{VF_{wamb}}x10^{-3}\frac{mg}{\mu g} \tag{7.2}$$

for indirect exposure via inhalation

where,

$RSBL_i$ – Risk based screening level for the specific medium I (mg/kg soil, mg/l H_2O or µg/m^3 air).

TR – Target excess individual lifetime cancer risk (unitless)

BW – Body weight

AT_c – Averaging time for carcinogens (years)

SF_i – Inhalation cancer slope factor (mg/kg/day)

IR_{air} – Soil ingestion rate (mg/day)

EF – Exposure frequency (days/year)

ED – Exposure duration (years)

VF_{wamb} – Volatilization Factor:GW to ambient air [$(mg/m^3$ –air)/(mg/L- H_2O)]

In addition, the information that is needed on the COC itself, which in this case is benzene is already a part of the PBPK/TK model. Benzene specific information such as its blood flow rates, its partition coefficients, etc. are all known. This is the first part of the information needed.

In the second part, the receptor information is needed. Using the model developed, it is possible to estimate concentrations for male and female receptors. This is because the model already contains information on the body weight, organ volumes and other parameters of interest.

At this stage, most models apply empirical equations, using the above data and obtain values for the inhalation risk posed by benzene. However, with the PBPK model, it is possible to determine the exact concentrations in the different body parts of the individual exposed and determine whether there really is a risk or not. This is made especially significant by the PBPK/TK model, because not only does it account for the receptor and COC information but it can also model the exposures as they occur; i.e. the exact exposure pattern can be replicated and simulated. So consider the many situations possible leading to benzene exposure at such a site, for workers who are involved in the remediation efforts at the site.

1. If the workers take an hour long lunch break off-site in their regular work day, then the exposure would be 4 hours continuous, 0 for the next hour and then another 4 hours of continuous exposure. Concentrations based on such exposures can be assessed for the entire duration of the project/construction activities and the risk can be assessed.
2. If the workers do not take a lunch break, or if they take a break on site, then the exposure can be modeled as 8 hours continuous and the body concentrations can be determined for the entire duration of the project and the risk assessed

3. If the workers are people who are involved in sampling activities and are only going to be on site for a few hours everyday for a week, or once every month, etc., then again the exposure period can be set at that regular interval and the model can be run to estimate the risk from such exposures.

For this case study consider the worst case scenario, where the workers are on-site for the eight hours of the work shift and are thus constantly exposed to the 10 ppm benzene concentration. Also assume that the workers are all male for the purpose of this model run and that the project is a three month long remediation project, so that the model is run for a duration of 3 months or 129600 minutes. The results from the runs are presented as follows:

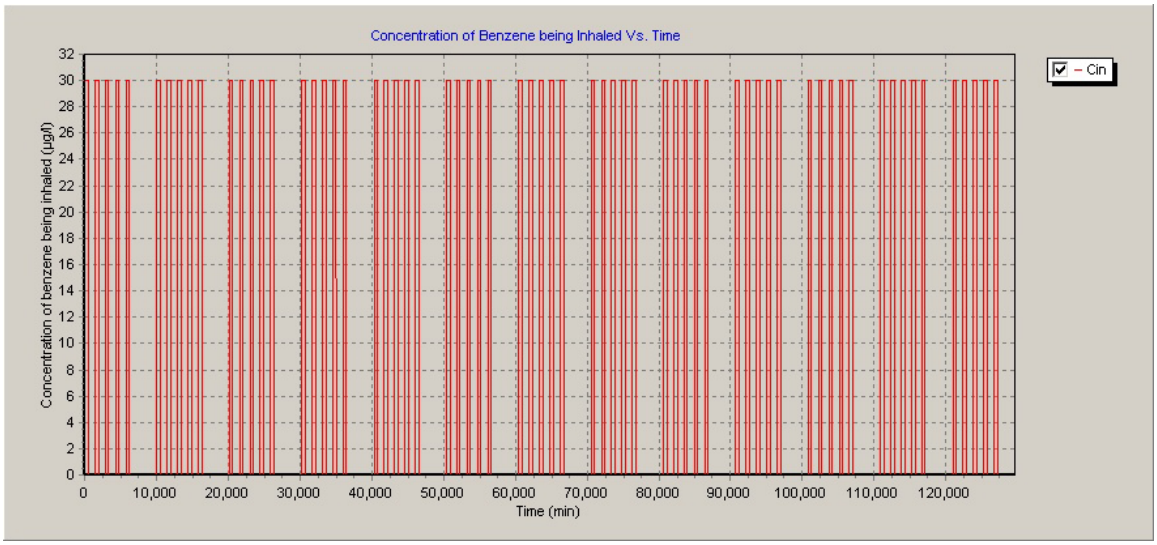

Figure 7.3 Input Concentration of 10 ppm for the Case Study

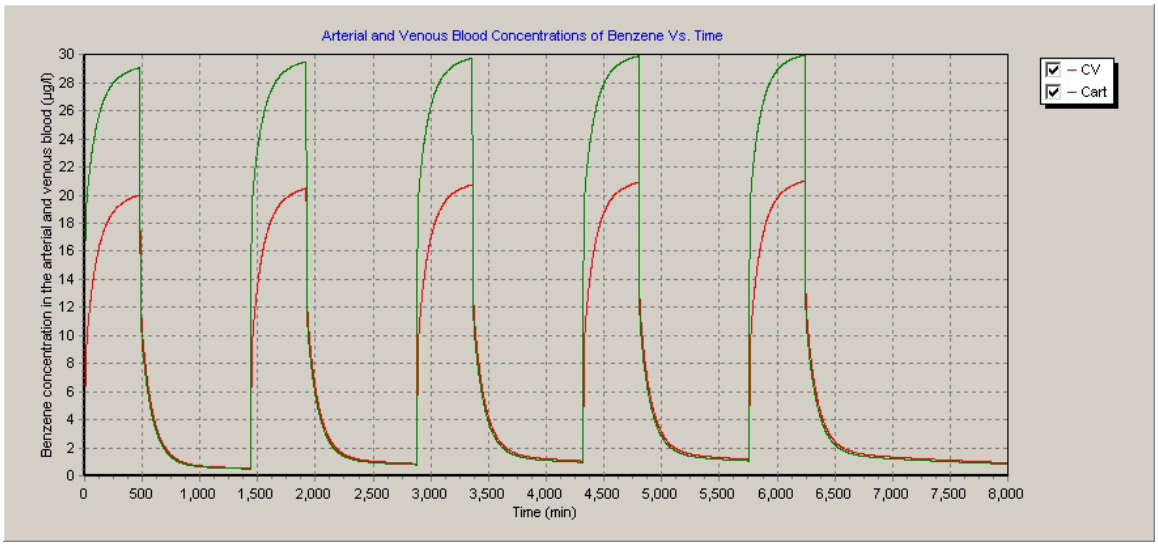

Figure 7.4 The Arterial and Venous Blood Concentration of Benzene

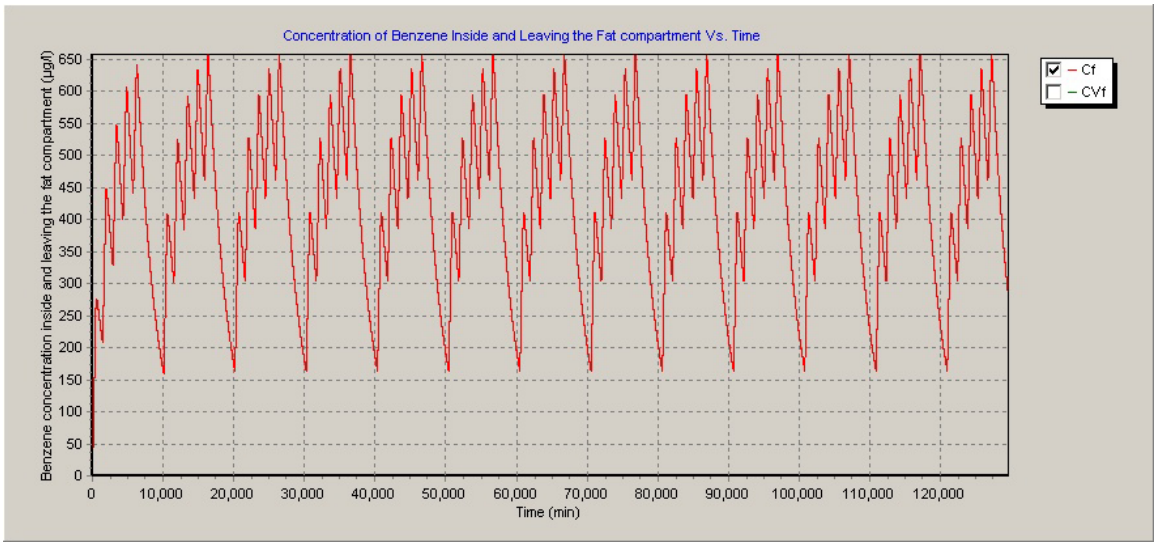

Figure 7.5 Concentration of Benzene in the Fat Compartment

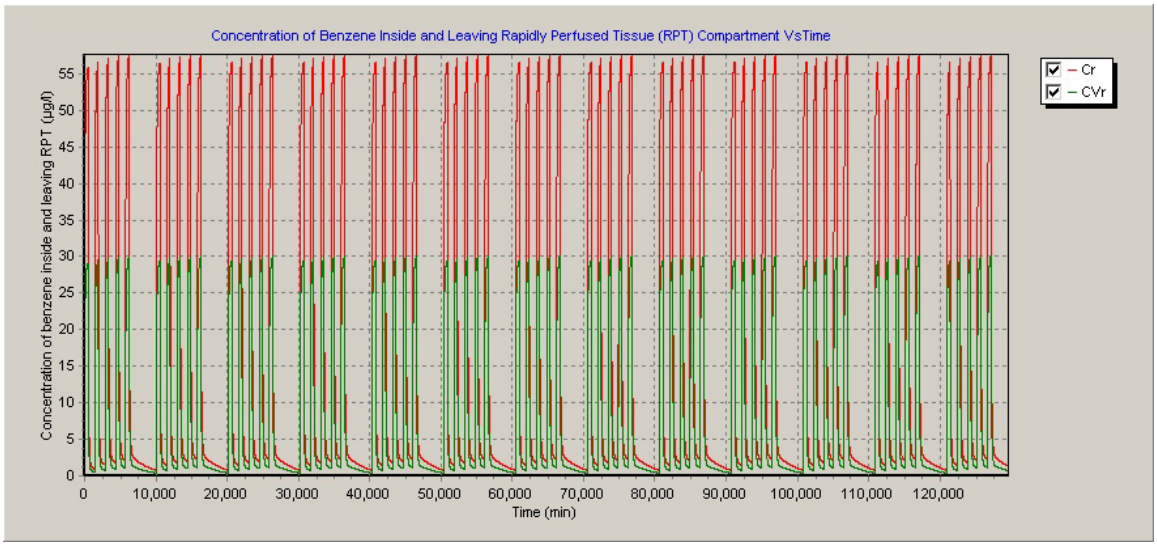

Figure 7.6 Concentration of Benzene in and Leaving the RPT Compartment

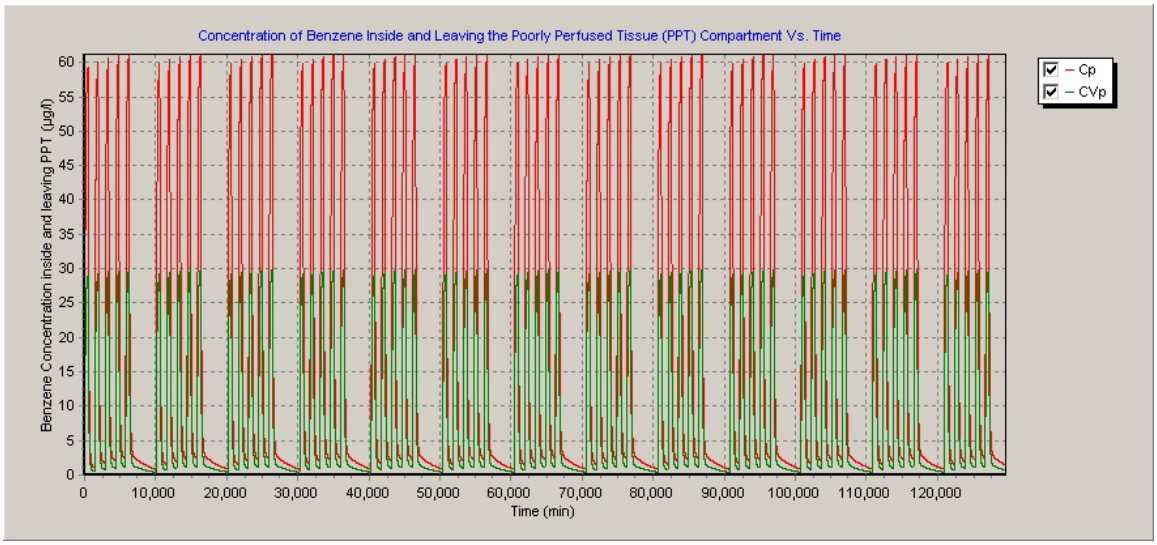

Figure 7.7 Concentration of Benzene in and Leaving the PPT Compartment

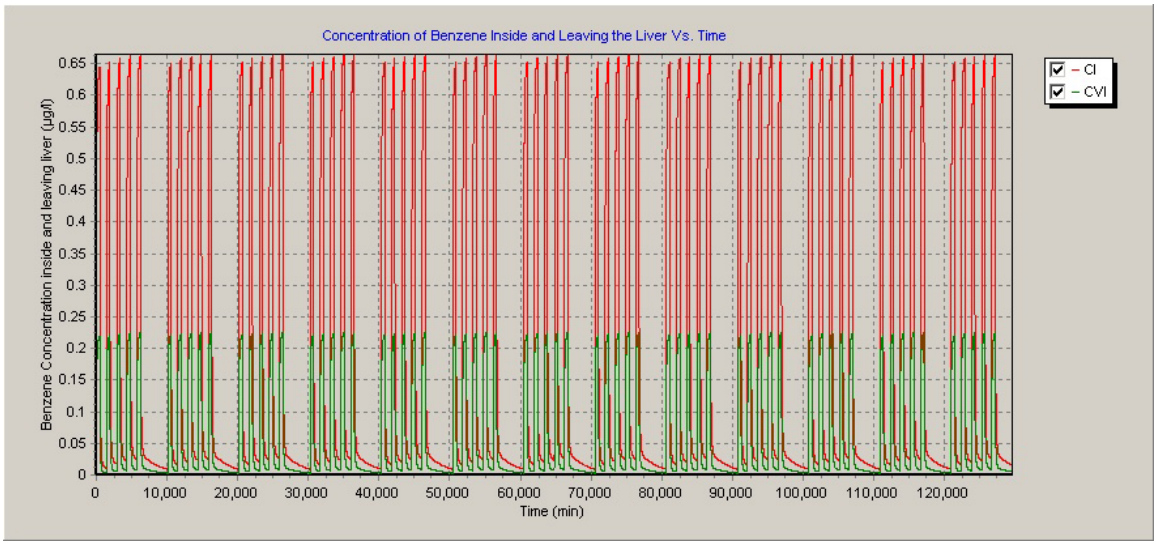

Figure 7.8 Concentration of Benzene in and Leaving the Liver Compartment

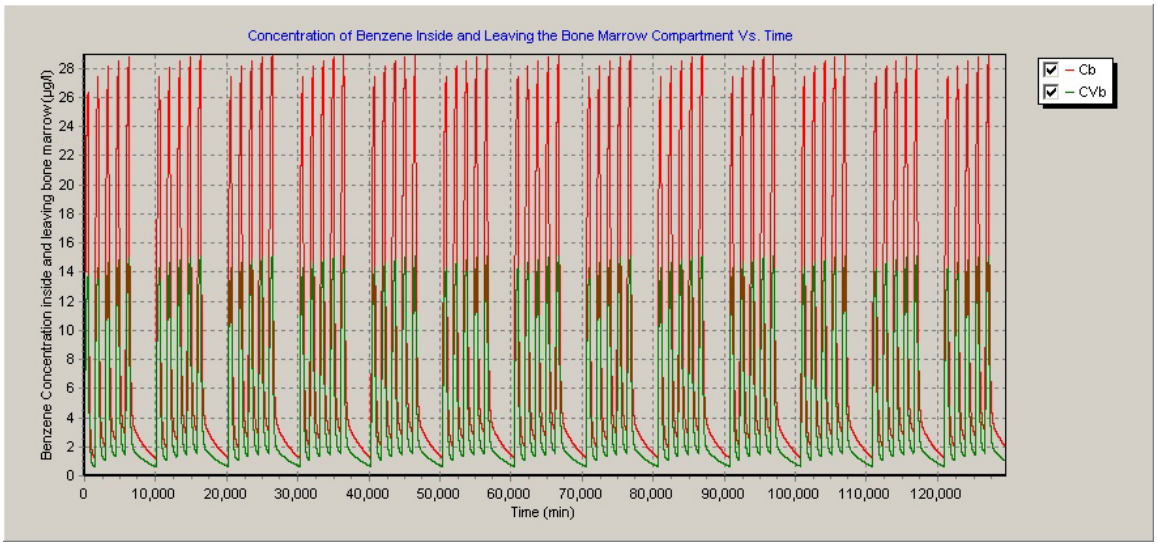

Figure 7.9 Concentration of Benzene in and Leaving the Bone Marrow Compartment

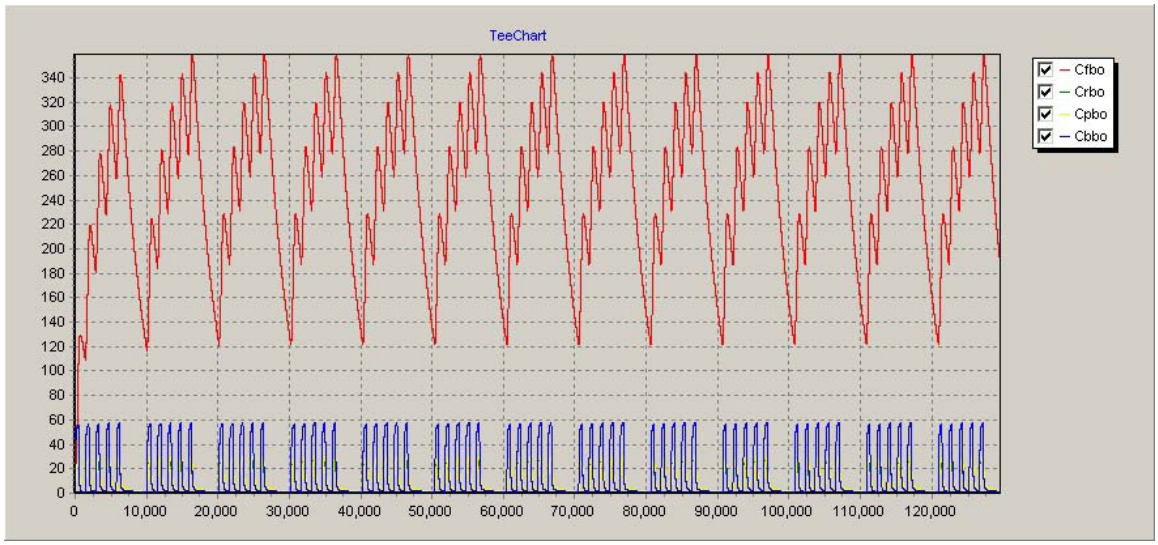

Figure 7.10 Concentration of Benzene Oxide in the Model Compartments

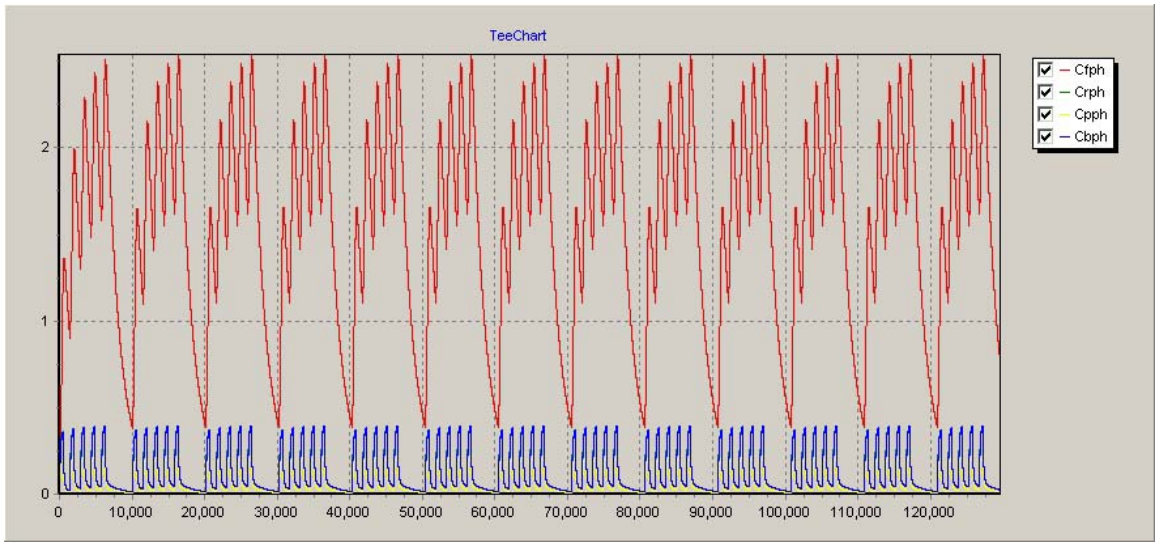

Figure 7.11 Concentration of Phenol in the Model Compartments

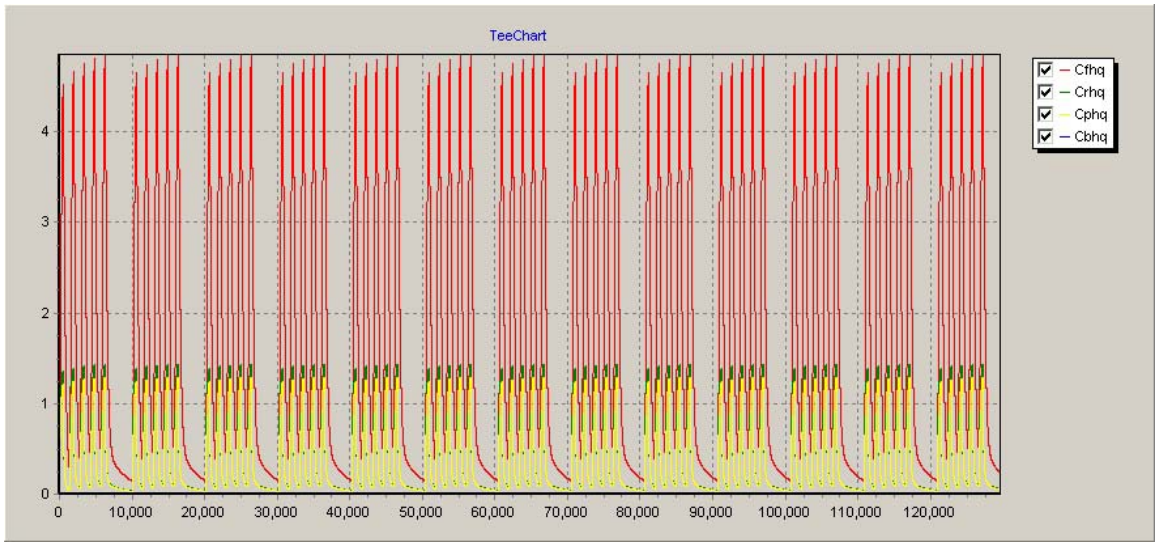

Figure 7.12 Concentration of Hydroquinone in the Model Compartments

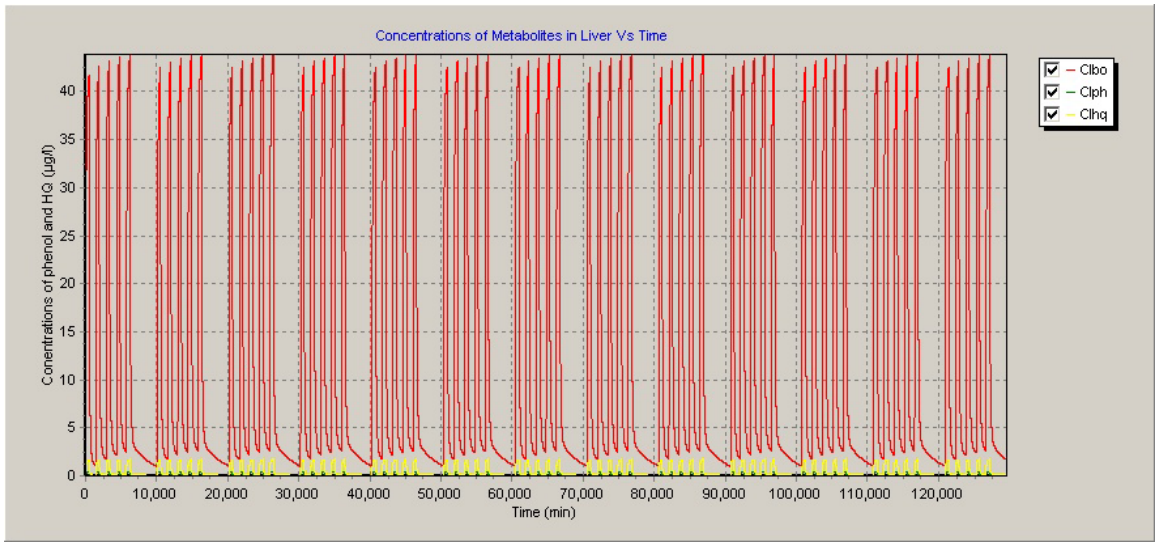

Figure 7.13 Concentration of Metabolites in the Bone Liver Compartment

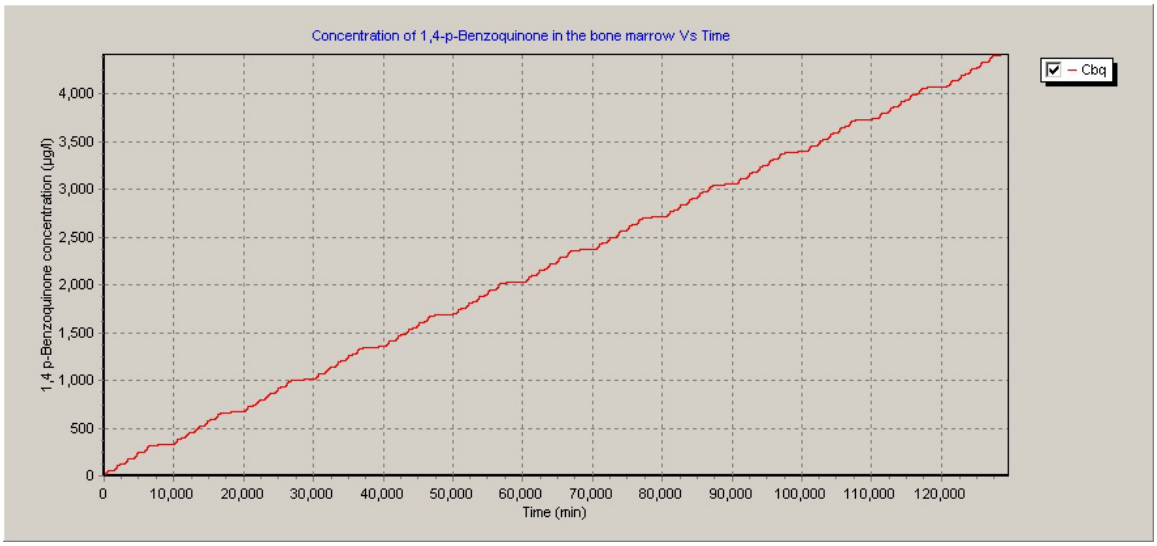

Figure 7.14 Concentration of 1, 4 – Benzoquinone in the Bone Marrow Compartment

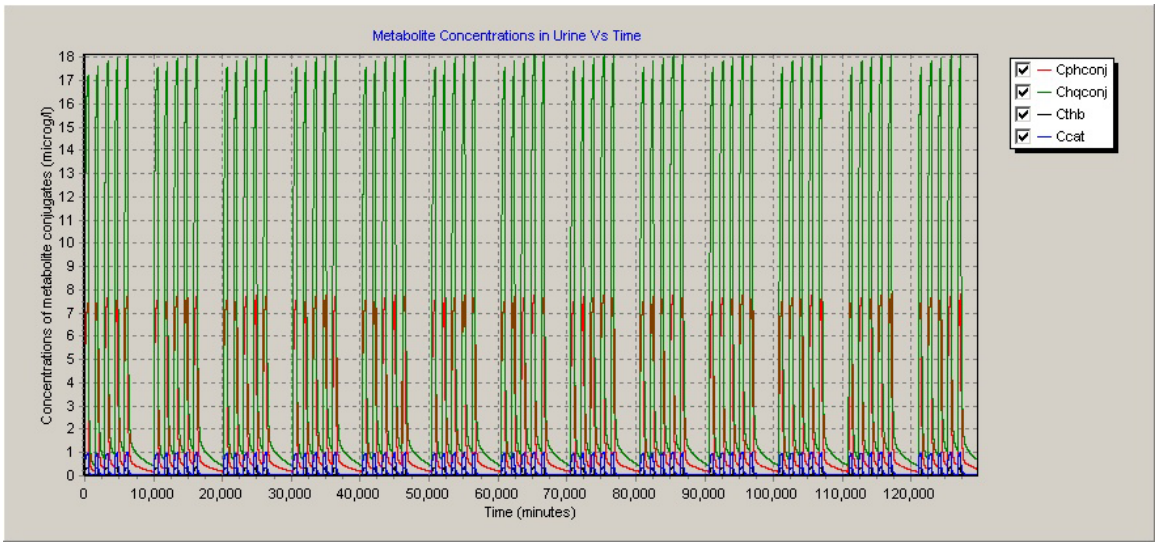

Figure 7.15 Concentration of Metabolites Excreted in the Urine

From the results obtained above, it can be seen that concentrations of benzene in the fat compartment are really high. At no point do they drop to near zero concentration levels like the other metabolites do. The same is true for benzene oxide. Thus at the end of the three months of occupational exposure to benzene the workers can expect to have a body burden of benzene that is higher than the usual background levels. As indicated in the previous model results, after a single exposure, of benzene, it took nearly a week for benzene concentration levels in the fat compartment to drop to zero. Even assuming that the model over-predicts the values in the fat compartment by a few percentage points, there would still be some benzene in the bone marrow in the form of 1,4 benzoquinone. The concentration of 1,4 benzoquinone is on the rise, because its elimination forms are not known and are not accounted for in the model as mentioned before. Overall, it means that some amount of benzene is present in the body of the exposed receptor for at least 1 week after the exposure has ceased. However, since these concentration levels do not persist much beyond the 3 months after exposure, no adverse effects should be observed. This is especially true of the carcinogenic effects. If at all they do occur, the possible adverse effects that the receptor might experience are related to the low level acute exposures such as headaches, dizziness, minor losses in the blood cell counts, etc.

Knowing that the risks posed aren't very significant, treatment processes such as natural attenuation can be implemented. Natural attenuation is specifically mentioned here, because it is a comparatively less expensive remediation process, given the reliance on the natural plume decay in the cleanup. It is also the one that poses a significant health hazard on account of its long lifespan in the surface/subsurface environment.

The components of gasoline besides benzene, such as toluene, ethylbenzene and xylene also pose significant hazards, alone or as part of the synergistic effects of being a component in gasoline. Similar models can be developed for each of these BTEX components or these other 3 chemicals can be incorporated within this model after the specific target organ systems for these chemicals are identified and placed in the model as separate compartments. For example, in case of ethylbenzene, Tang et. al.; (2000) reported that the greatest risk from ethylbenzene is from the inhalation route of exposure amounting to almost 99% of a total of 1.8 μg/kg/day. Stott et. al.; (2003) reported through their animal assays and from their summary of the National Toxciology Program (NTP)

study on ethylbenzene disposition on different animals that the target organs for ethylbenzene are the lungs, the liver and the kidneys. Thus the kidney would have to be added to this model as a separate compartment (it is currently a part of the RPT compartment) and the lung compartment would have to be dealt with in a different manner because it is a target organ for the manifestation of ethylbenzene's toxicity.

Thus, the use of PBPK modeling serves as a screening tool. For example, if the model had predicted very high benzene and metabolite values in the body, controls could have been adopted such as limiting the time spent by the worker on site, or adopting a different remediation technique. The use of PBPK also helps cut down on engineering expenses by potentially allowing the use of methods such as natural attenuation which but are often considered a health hazard because of the long time delay involved in the plume degradation when such a remediation technique is used.

7.3 The air pollution engineering case study

The PBPK/TK model developed in this study can be used effectively in the indoor air pollution problem of soil vapor intrusions. The problem occurs when vapors from environmental contaminants in the surface and sub-surface (water, groundwater, soils) enter residential areas and can pose an inhalation hazard to the residents. The common sources of such vapor intrusions are from leaking underground storage tanks, petroleum or other chemical spills in the water/groundwater or soils and buried waste.

The issue of vapor intrusion is a comparatively new one. In the past the only intrusive vapor that regulatory authorities had been concerned about was radon. However, there have been a number of cases in the recent past where vapors of hazardous, volatile chemicals in basements of residences have caused adverse health effects in residents and brought about public unrest regarding the situation including some class action law suits.

Thus understanding the risk from such contamination is very important. The whole emphasis of initiatives such as RBCA is to get away from set standards such as the drinking water Maximum Contaminant Levels (MCLs), and move towards the implementation of cleanup goals in keeping with the actual risk to human health and the

environment. In order to apply the RBCA concept, risk assessments are required to develop the appropriate cleanup levels. This means that any and all pathways that can possibly cause an adverse effect on people's health must be considered in an assessment to remedy the situation.

In this regard, there are several models that have been developed that can model the migration of the volatile gas from its surface/subsurface source to the indoor air in homes. For example, the American Society of Testing and Materials' (ASTM) first widely applied RBCA guide for an underground petroleum storage tank leak included several equations for calculating the indoor air concentrations of the migrating chemical. These estimates were based on the chemical concentration values in found in soils and groundwater based on the simplified form of the Johnson Ettinger model. The USEPA Superfund Guidance manual provides generic soil screening levels for most volatile organic compounds and provides site-specific equations for estimating the concentrations of chemicals in the indoor air from the contaminated groundwater (Folkes and Arell; 2003).

However, these models stop by predicting the indoor chemical concentration of the air. They do not calculate or model the actual risk posed by the chemical to the residents based on the toxicokinetic parameters as presented in this model; i.e. the existing models do not account for how much of that indoor air concentration will be inhaled and will actually be absorbed by the residents and what the adverse effects of such exposure would be on them.

Thus, the model developed here can be thought of as an extension to the vapor migration models already available and widely used. In combination with models such as the Johnson Ettinger model and other indoor air pollution measuring devices, each of which are not complete in themselves, the model developed in this study can be used to determine risk or set standards for cleanup rates.

A case study is presented of a school K-12 school in northern Florida, where leaking underground storage tanks (USTs) caused gasoline vapor migration into classrooms, imposing an inhalation hazard. The site details are presented in Table 7.1.

Table 7.1 Facility Details

Item	Description
Facility Location and type	Public school K-12
Facility Status	The site is currently active with two above ground storage tanks (ASTs) containing vehicular diesel and unleaded gasoline (1012 gallons each). A former school bus UST was closed in 1984 and an elementary classroom was built over the site
Land use	Well mixed commercial/residential in all directions, except to the north where the area is agricultural
Topography	Grand Ridge Physiographic Region; Facility terrain is relatively flat, gentle slopes to the west-southwest
Surface water bodies/hydrology	Surface flows towards the southwest. Groundwater flows to the west-southwest
Portable water supplies/groundwater usage	Two public water supply wells for the city are within a half mile radius of the facility
Fuels stored at site	Vehicular diesel and unleaded gasoline

The remedial action plan (RAP) for the facility obtained from FDEP indicates that benzene was found in several monitoring wells at levels higher than FDEP's ground and surface water cleanup target levels of 1 μg/l. The treatment was phased to progress in 2 stages. Multi-phase extraction (MPE) for groundwater and surface water vapors as well as vapor extraction (VE) using air sparging (AS) and down gradient in-situ oxygen diffuser technology were the chosen options. This is being accomplished by installing 2 MPE recovery wells, 9 VE wells, 6 AS wells, 2 Biosparge (BS) wells besides others incurring a total cost of $ 543800.

The way the model from this research can be implemented in a situation like this is as follows:

a. Place monitoring devices in classrooms to determine the vapor concentration of benzene in the air in classrooms.
b. Use the concentrations determined by the devices as the input concentration in the model.
c. Run the model for male and female exposures for the required duration of time.
d. The model results will include benzene concentration levels in the bodies of males and females exposed to the benzene vapors.
e. Compare the concentrations obtained versus the ones in databases such as the Integrated Risk Information System (IRIS) to determine the possible risk due to benzene exposure at the concentration levels predicted by the model.
f. Based on the nature of risk, determine the course of action such as evacuation or placing air ventilation devices in the affected classrooms.
g. Based on the nature of risk and mechanism adopted to protect human health, determine the remediation technique that would best fit to clean up the contamination while protecting human health under RBCA.

The model developed here thus serves as a useful tool, in conjunction with resources already available to determine and manage risk.

7.4 Summary of the Engineering Case Studies

The engineering case studies presented in this chapter serve as an example of how the model developed in this research can potentially be used in environmental engineering situations. In combination with other tools and resources available currently, the model can help in assessing risk by serving as a screening tool. It can also potentially be used as a resource in developing and setting exposure limits and inhalation exposure standards.

CHAPTER 8

8. CONCLUSIONS

8.1 Introduction

This research is comprised of two parts. The first involves the development of a PBPK/TK model to simulate inhalation exposure to different concentrations of benzene from continuous and intermittent sources in healthy adult male and female members of the human populations. All major metabolites of benzene were also studied for their toxicokinetic disposition in the human body. The bone marrow is presented as a separate compartment in the model and all metabolite interactions are explained with the help of an extended Michaelis Menten expression.

The second part of the study involved studying the possible application of such a model to environmental engineering problems. Two case studies are presented, one for an air pollution engineering problem related to soil vapor intrusion and the other based on a petroleum spill contamination. In each case, probable estimates of the hazard posed by such environmental contamination are provided based on model results and their co-relation with effects reported in literature.

The conclusions of this study are now presented.

8.2 Conclusions for the Toxicological Model Development Phase

The model development is a long and drawn out process that requires a good understanding of the physiology and anatomy of the human body, as well as the physical, chemical and biological interactions that occur between the chemical and the body. This

area of study is not common in engineering. However, the area of environmental risk assessments is closing in the gap between engineering and sciences such as toxicology. This phase of the research was taken up to encourage future work in such multi-disciplinary research areas.

The model tackled several different challenges and the conclusions from all of these are presented as follows:

1. The model looked at intermittent and continuous exposure situations. The intermittent form of exposure has not been modeled to date in PBPK/TK models and contributes significantly to this area of modeling. This is especially true of the irregular intermittent form of exposure modeled in case 4C as shown in chapter 4.The results indicate that continuous exposure and intermittent exposure at a regular and closely spaced interval provides same concentration levels of benzene and its major metabolites in the human body. When exposure is at irregular intervals that are not closely spaced, the body gets time to recover from constant exposure shocks and is able to break down the chemical pollutant and hence the concentration levels and corresponding risk due to benzene and its metabolites are lower.
2. The choice of using data on cigarette smoke, proved to be a good one, because it necessitated the development of an intermittent exposure model. The development started off by assuming a regular interval of cigarette smoking, which helped in developing the scenario on regular intermittent benzene exposure. However, given that most natural non-occupation environmental exposures are not regularly spaced but are irregular intermittent in nature, the earlier format was modified and this new scenario was modeled. This is an excellent contribution to the field of non-occupational modeling in environmental engineering. The cigarette smoke data run also showed that people who are just exposed to benzene from cigarette smoke are not at any risk to develop adverse health effects. If cigarette smoking is conducted in combination with occupational exposure to benzene these results will be different.
3. The model looked at male and female exposure to variable concentrations of continuous and intermittent exposure to benzene vapors. The study concludes that

benzene and its major metabolites do not differ significantly in their disposition in male and female members of the human population. While most concentration level differences are not significant in the different organs, for the initial exposure concentration levels used in this study, the trend suggests that higher concentration levels are possible in females on account of their higher body fat content. The fat tissue has a high affinity for lipophilic substances such as benzene and its metabolites. Hence the female members of the human population might be at a higher risk from benzene exposure that their male counterparts.

4. The model separated the bone marrow into an individual compartment and looked at the effect of the separation on the results of the overall concentrations. The study concludes that while the concentrations of benzene are somewhat higher when the bone marrow is considered as a separate compartment and concentrations of benzene metabolites are somewhat lower, a model that considers the bone marrow as part of the RPT compartment might under-predict the benzene concentrations in the body. This might lead to a false sense of lower risk. For such reason as well as the fact that bone marrow is a target organ for benzene and keeping it as a separate compartment helps its study in better detail, the bone marrow should be kept as a separate compartment, when modeling benzene exposures using PBPK/TK models.

From this modeling phase of the study the conclusion is that the model is a reasonably sound product that can be used in its existing form or can be modified depending on site specific conditions to simulate a wide range and type of benzene exposures.

8.3 Results from the Engineering Applications Phase

The use of the model developed in the first phase for benzene inhalation exposures is demonstrated in the second phase of the project. A model run was performed for an occupational exposure situation, and the results of the modeling exercise indicate that the model can serve as an effective screening tool for on-site exposures. Knowing the initial concentrations on site and the receptors that might get affected by the exposure, the

model can be run and benzene concentrations in the receptor bodies can be determined. These can then be used to compare against concentration levels known to cause adverse effects and the choice of controls can be made. Decisions can also be made regarding the use of less expensive remediation techniques, materializing in savings of time, efforts and labor.

Another case study of soil vapor intrusion in a school served to demonstrate that the model in conjunction with other tools and resources already available can be used to determine the risk of inhalation exposure. This knowledge can help determine the best remediation technique to clean up the contamination while being protective of human health and the environment.

8.4 Limitations of the study

Some of the limitations of the study include the following:

1. Probable high values are estimated for the benzene and benzene oxide concentrations in the fat compartment.
2. Benzoquinone behavior in the body is not completely modeled due to the current lack of information and studies on this chemical and its disposition in the human body. As more information on benzoquinone becomes available; for example its metabolic reactions and rates, the model can be modified.
3. Only male and female population groups are considered. Sensitive populations such as children or people with pre-disposed ailments cannot be studied using this model in its current form.
4. Only a single chemical (benzene) and its major metabolites are modeled. This model cannot determine risk due to a chemical other than benzene or if benzene exists in combination with other chemicals such as BTEX.
5. Only the inhalation route of exposure is analyzed. The dermal and ingestion routes of exposure are not modeled in this study. Thus this is not a multiple exposure pathway exposure model.

As new research is published addressing some of the core issues relating to the metabolism of benzene, it is expected that this model will be revised to always be current and useful. These limitations help in setting a future course of research direction for these kind of studies as explained in chapter 9.

CHAPTER 9

9. FUTURE SCOPE

9.1 Introduction

While this study has covered a lot of ground and addressed many issues in trying to accomplish the objectives set forward in chapter 1, there is still a long way to go in making this form of modeling commonplace in environmental engineering studies. Some of the exclusive features of the study presented here which help in contributing to the scientific and technical knowledge base include the following:

1. The focus on inhalation exposure, from gases or vapors, which is not commonly considered in environmental engineering work related to soils, water and waste; for example in remediation studies and hazardous waste or Superfund site analyses for human health risks.
2. The inclusion of the bone marrow as a separate compartment in the multi-compartmental benzene model since secondary metabolism of benzene does occur in the bone marrow and some of the metabolites produced there are responsible for some of the most adverse health effects such as aplastic anemia, and pre-carcinogenic activities.
3. The use of the modified form of the Michaelis Menten equation for enzyme activity and metabolism, in place of the standard Michaelis Menten expression, because benzene and most of its major metabolites compete for the CYP2E1 enzyme which is the primary enzyme for benzene metabolism.
4. The modeling of intermittent exposures that is more relevant in environmental engineering in addition to the continuous exposure situations typical in PBPK/TK.

However, the study was also limited by a number of factors which presents opportunities to conduct research in this area in the future.

9.2 Future Studies

The results from this study are applicable only to the healthy adult male and female members of the population. However, in most situations where this model will be applied a diverse population can exist. These population groups will also need to be accounted for and modeled in future molding studies. For example, some of the other population groups that can be modeled include women, pregnant women, children, people with pre-disposed respiratory and related ailments and people of different races and age levels amongst adults. Some such experimental and modeling studies have been formed with varying results (Corti and Snyder, 1998; Wong, 1995; ATSDR, 2001).

Age is also a factor because the effects of specific chemicals are quite different in the bodies of adults and children. Often, children are at a higher risk to environmental pollutant exposures on account of their higher absorption capacities compared to their body weights. For example, in the case study of soil vapor intrusion in the school as presented in chapter 7, the effects will be different in the student population of the school as compared to the staff and faculty of the school because of different physiologies and other age related factors.

Since this form of modeling relies heavily on data from experiments, a pre-cursor to modeling for the above-mentioned population groups, would be to conduct experiments on relevant laboratory animals and then extrapolating the results to the corresponding population groups. Once data are obtained from experiments, models can be developed and run and results can be analyzed. For example, McMahon et. al., (1994) determined the age related variations in disposition to benzene exposure in male rats, Multi-generation studies are performed with mice for example, where the disposition is traced in young mice as they grow into adults, and in their offsprings as they grow into adults and the offsprings of the first set of offsprings for a total study of 3 generations of mice to understand any sort of genetic effects of chemicals.

Additional routes of exposure can also be considered in the same study such as the dermal and ingestion routes of chemical exposure. For example, in case of benzene, it

is indicated in chapter 1 that dermal contact of liquid benzene can be attributed to specific adverse health effects. Hence a combination of the inhalation route of exposure from benzene vapors and dermal exposure to liquid benzene can be modeled together. Since benzene is a common solvent and present in a number of household products as well, ingestion of benzene can be an exposure route for the chemical.

Environmental pollutants are rarely present as single chemicals and hence it is anticipated that most future environmental models will look at mixtures of chemicals and their interactions with each other as well as with the human body. Even for benzene, it will be interesting to model and study the effects of the benzene in petroleum as a mixture of benzene, toluene, ethylbenzene and xylene (BTEX). Results from this model can be then compared to the BTEX model to understand the contribution of benzene specifically to the adverse effects caused by the BTEX mixture. A study of this sort was conducted by Haddad et. al., (2000). Their work showed that extrapolating the chemical kinetics from one mixture of chemicals to another set which might be different in complexity and composition, based on mechanistic considerations of interactions elucidated at the binary level was possible using the PBPK modeling framework. A recent study by Dennison et. al., (2003) also used PBPK modeling effectively to study the complex hydrocarbon mixtures in gasoline.

Further modifications to such modeling could be made by adding the ambient air conditions, such as weather conditions, wind direction and flow velocity and other parameters that would influence the exposure concentration of individuals. The existing ambient air models can be used in combination with the model developed here and a more comprehensive and complete form of model can be developed.

As can be seen from the preceding paragraphs, modeling work can never be complete. As new discoveries are made about the way our body works or the way physical processes occur, modelers will be challenged to take on the responsibility of simulating such behaviors for applications in several disciplines such as engineering. The hope is that with each surge in the research and development process, there will be even more opportunities to perform engineering activities while protecting human life and the environment in the best possible manner.

APPENDICES

APPENDIX A 2001 TOXIC RELEASE INVENTORY DATA

This is a screenshot from USEPA's Toxic Release Inventory (TRI) database that indicates the total and distributed emissions of benzene from all major sources in the year 2001.

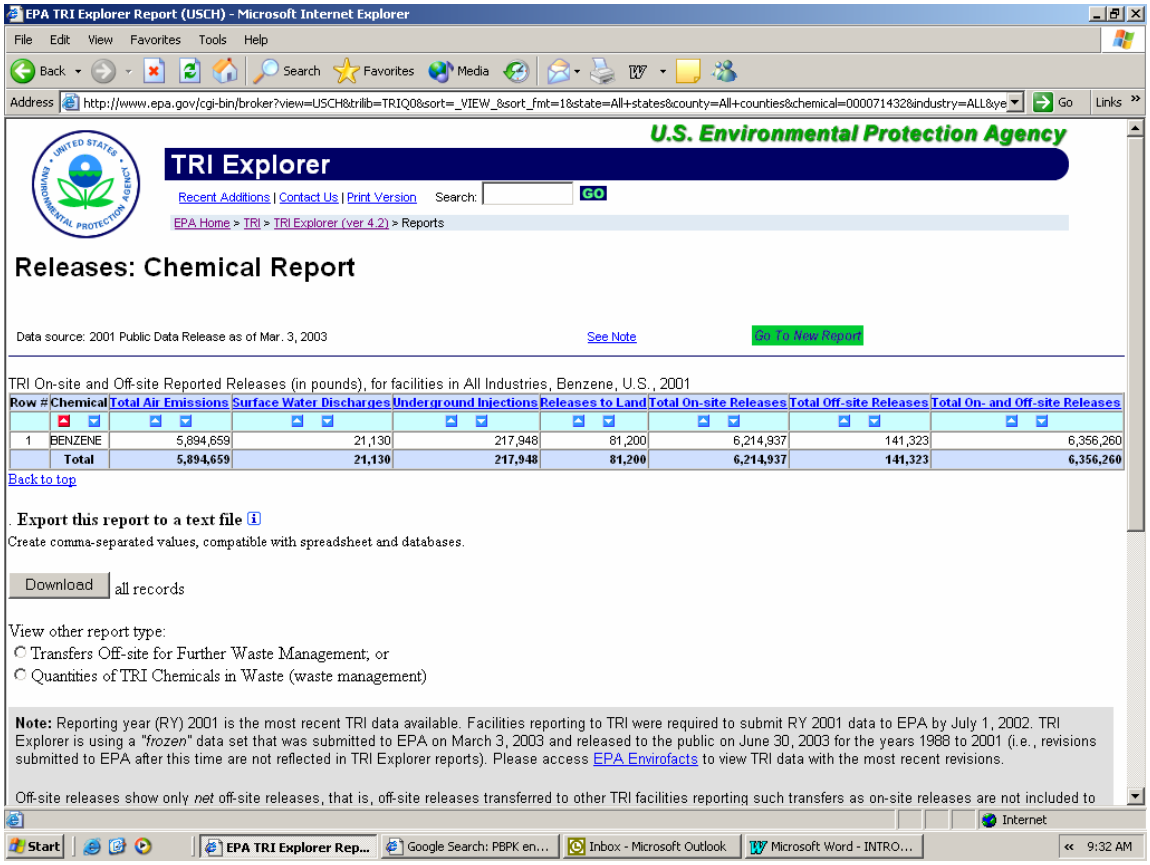

Figure A.1 Toxic Release Inventory Data on Benzene Levels (2003)

APPENDIX B- MODIFIED MICHAELIS MENTEN EXPRESSION

The equation for each rate of metabolism is determined by considering the process by which that particular metabolism occurs. For example, the metabolisms: benzene to benzene oxide, phenol to hydroquinone, phenol to catechol, and hydroquinone to trihydroxy benzene, which are mediated by the protein CYP_{2E1}, are governed by the following kinetics:

$$C^{BZ} + E_F \underset{k_2^{BZ}}{\overset{k_1^{BZ}}{\rightleftharpoons}} I^{BZ} \xrightarrow{k_3^{BZ}} C^{BO} + E_F$$

$$C^{PH} + E_F \underset{k_2^{PH}}{\overset{k_1^{PH}}{\rightleftharpoons}} I^{PH} \xrightarrow{k_3^{PH}} C^{HQ} + E_F$$

$$I^{PH} \xrightarrow{k_4^{PH}} C^{Cat} + E_F$$

$$C^{HQ} + E_F \underset{k_2^{HQ}}{\overset{k_1^{HQ}}{\rightleftharpoons}} I^{HQ} \xrightarrow{k_3^{HQ}} C^{THB} + E_F$$

where E_F represents the free enzymes and I is the intermediate substrate. The above chemical kinetics can be written as the system of the following differential equations:

$$\frac{dI^{BZ}}{dt} = k_1^{BZ} C^B E_F - (k_2^{BZ} + k_3^{BZ}) I^{BZ}$$

$$\frac{dI^{PH}}{dt} = k_1^{PH} C^{PH} E_F - (k_2^{PH} + k_3^{PH} + k_4^{PH}) I^{PH}$$

$$\frac{dI^{HQ}}{dt} = k_1^{HQ} C^{HQ} E_F - (k_2^{HQ} + k_3^{HQ}) I^{HQ}$$

$$E_F = E_T - I^B - I^{BO} - I^{PH} - I^{HQ},$$

where E_T are the total enzymes.

If we assume steady state conditions, $\frac{dI^B}{dt} = 0$, $\frac{dI^{PH}}{dt} = 0$ and $\frac{dI^{HQ}}{dt} = 0$, we obtain the following relations:

$$I^{BZ} = \frac{\frac{1}{K^{BZ}} E_T C^{BZ}}{1 + \frac{1}{K^{BZ}} C^{BZ} + \frac{1}{K^{PH}} C^{PH} + \frac{1}{K^{HQ}} C^{HQ}}$$

$$I^{PH} = \frac{\frac{1}{K^{PH}} E_T C^{PH}}{1 + \frac{1}{K^{BZ}} C^{BZ} + \frac{1}{K^{PH}} C^{PH} + \frac{1}{K^{HQ}} C^{HQ}}$$

$$I^{HQ} = \frac{\frac{1}{K^{HQ}} E_T C^{HQ}}{1 + \frac{1}{K^{BZ}} C^{BZ} + \frac{1}{K^{PH}} C^{PH} + \frac{1}{K^{HQ}} C^{HQ}}$$

where

$$K^{BZ} = \frac{k_2^{BZ} + k_3^B}{k_1^{BZ}}$$

$$K^{PH} = \frac{k_2^{PH} + k_3^{PH} + k_4^{PH}}{k_1^{PH}}$$

$$K^{HQ} = \frac{k_2^{HQ} + k_3^{HQ}}{k_1^{HQ}}.$$

We further denote 1/Ki by A^i to obtain the following expressions

$$I^{BZ} = \frac{A^{BZ} E_T C^{BZ}}{1 + A^{BZ} C^{BZ} + A^{PH} C^{PH} + A^{HQ} C^{HQ}}$$

$$I^{PH} = \frac{A^{PH} E_T C^{PH}}{1 + A^{BZ} C^{BZ} + A^{PH} C^{PH} + A^{HQ} C^{HQ}}$$

$$I^{HQ} = \frac{A^{HQ} E_T C^{HQ}}{1 + A^{BZ} C^{BZ} + A^{PH} C^{PH} + A^{HQ} C^{HQ}}$$

Thus, the rates for these metabolisms are given by

$$\begin{aligned} RM_{BO}^{BZ} &= k_3^B I^{BZ} \\ RM_{HQ}^{PH} &= k_3^{PH} I^{PH} \\ RM_{CA}^{PH} &= k_4^{PH} I^{PH} \\ RM_{THB}^{HQ} &= k_3^{HQ} I^{HQ} \end{aligned}$$

By introducing additional parameters such as C^{MP}, V_{2E1}, and T_L (see Appendix A for a description of these parameters as well as other parameters), we obtain the following rates of metabolism, which were also used by Seaton et al. [61]:

$$RM_{BO}^{BZ} = k_1 \frac{V_{2E1} C_L^{BZ}}{D} C^{MP} T_L$$

$$RM_{HQ}^{PH} = k_5 \frac{V_{2E1} C_L^{PH}}{D} C^{MP} T_L$$

$$RM_{Cat}^{PH} = k_6 \frac{V_{2E1} C_L^{PH}}{D} C^{MP} T_L$$

$$RM_{THB}^{HQ} = k_7 \frac{V_{2E1} C_L^{HQ}}{D} C^{MP} T_L$$

with,

$$D = 1 + A^{BZ} C_L^B + A^{PH} C_L^{PH} + A^{HQ} C_L^{HQ}$$

APPENDIX C MODEL DIAGRAM

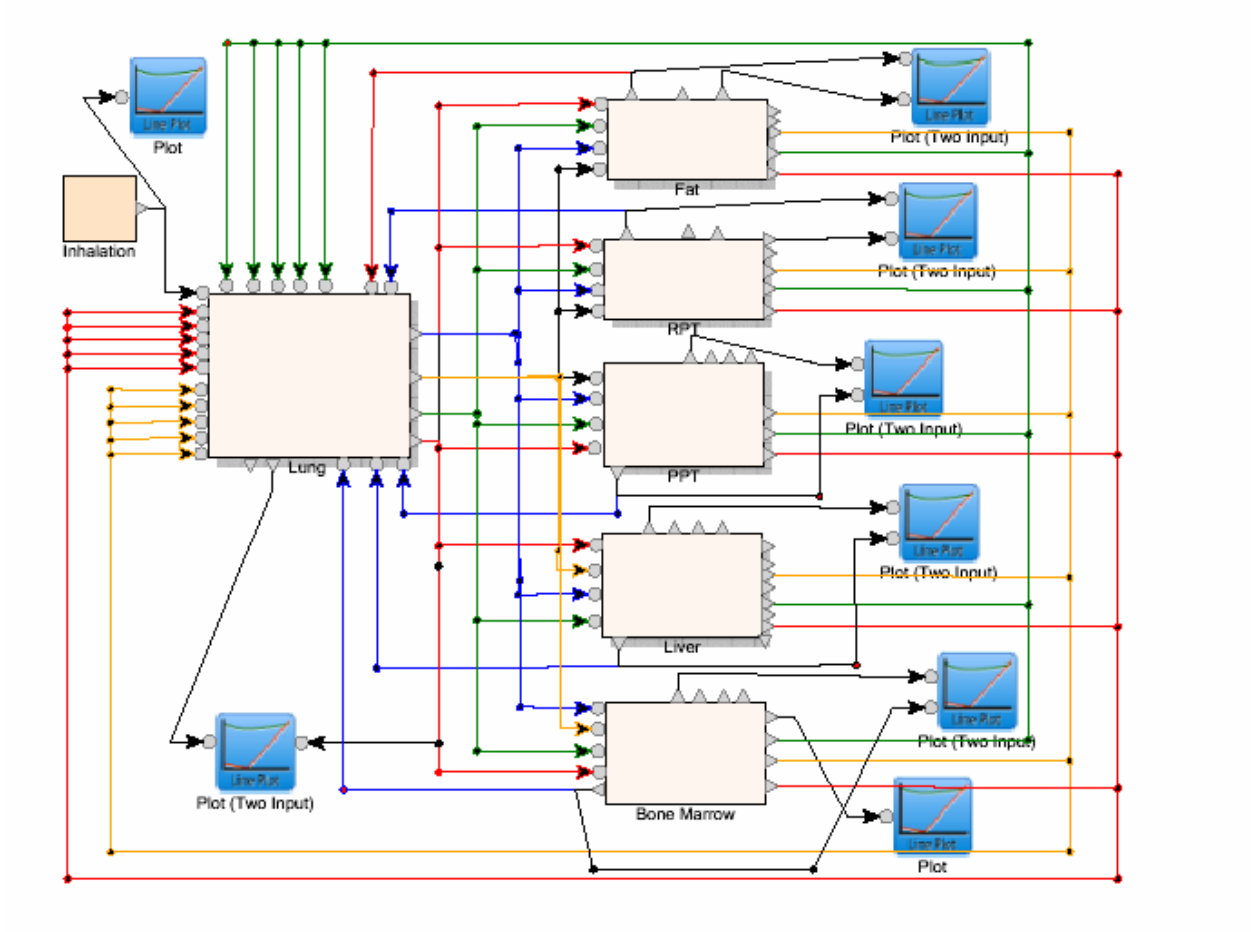

Figure C1. Model diagram

APPENDIX D. MODEL CODE

The following is the code developed for building the model using ACSLExtreme.

Inhalation:

```
Constant C = 1470      !Variable for concentration

!Set up the concentration
If (T.GT.360) then
        Cin = 0
Else
        Cin = C
End If


!i = T
!If (T.LT.960) Then
!myLabel: If (i.LT.10) Then
!                Cin = C
!                Go To Finish
!        Else IF ((i.GT.10).AND.(i.LT.50)) Then
!                Cin = 0
!                Go To Finish
!        End If
!        i = i - 50
!        Go To myLabel
!Finish: Continue
!Else
!        Cin = 0
!End If
```

Lung:

```
Constant PB = 7.8              !blood-air partition coefficient
```

```
Constant Qa = 5                 !alveolar ventilation rate
Constant Qc = 6.2               !cardiac output
Constant Qf = 0.31              !fat compartment blood flow rate
Constant Qr = 2.78              !rapidly perfused tissue comparment blood flow rate
Constant Qp = 1.364             !poorly perfused tissue comparment blood flow rate
Constant Ql = 1.55              !liver compartment bllod flow rate
Constant Qb = 0.2418            !bone marow compartment blood flow rate
Constant V = 7                  !volume of lung

! Benzene concetration is 50% of inhaled concentration
Cinh = Cin *0.5

!Venus concentration of benzene
CV = ((CVf * Qf) + (CVp * Qp) + (CVr * Qr) + (CVl * Ql) + (CVb * Qb)) / (Qf+ Qp +
Qr + Ql + Qb)

!arterial concentration of benzene
Cart = ((Qa * Cinh) + (Qc * CV))/(Qc + (Qa/PB))

!arterial concentration of benzene oxide
Cartbo = ((CVfbo * Qf) + (CVrbo * Qr) + (CVpbo * Qp) + (CVlbo * Ql) + (CVbbo *
Qb)) / (Qf + Qr + Qp + Ql + Qb)

!arterial concentration of phenol
Cartph = ((CVfph * Qf) + (CVrph * Qr) + (CVpph * Qp) + (CVlph * Ql) + (CVbph *
Qb)) / (Qf + Qr + Qp + Ql + Qb)

!arterial concentration of hydroquinone
Carthq = ((CVfhq * Qf) + (CVrhq * Qr) + (CVphq * Qp) + (CVlhq * Ql) + (CVbhq *
Qb)) / (Qf + Qr + Qp + Ql + Qb)
```

Fat:

```
CONSTANT Vf = 13.3              !volume of fat compartment
CONSTANT Pf = 54.5              !Blood:fat compartment partition coefficient for
benzene
Constant Pfph = 27.63           !Blood:fat compartment partition coefficient for
phenol
Constant Pfhq = 4.06            !Blood:fat compartment partition coefficient for
hydroquinone
CONSTANT Qf = 0.31              !fat compartment blood flow rate

!*********Benzene equations*********
```

```
!Concentration of benzene in the compartment
Cf = Af/Vf

!rate of change of benzene in the compartment
dA = Qf*(Cart - Cf/Pf)

!Amount of Benzene in the compartment
Af = integ(dA, 0.0)

!Concentration of benzene leaving the compartment
CVf = Cf/Pf

!*********Benzene oxide equations*********
!Concentration of benzene oxide in the compartment
Cfbo = Afbo/Vf

!rate of change of benzene oxide in the compartment
dAbo = Qf * (Cartbo - Cfbo/Pf)

!Amount of Benzene oxide in the compartment
Afbo = integ(dAbo, 0.0)

!Concentration of benzene oxide leaving the compartment
CVfbo = Cfbo/Pf


!phenol equations

Cfph = Afph/Vf

dAph = Qf * (Cartph - Cfph/Pfph)

Afph = integ(dAph, 0.0)

CVfph = Cfph/Pfph


!hydroquinone equations

Cfhq = Afhq/Vf

dAhq = Qf*(Carthq - Cfhq/Pfhq)

Afhq= integ(dAhq, 0.0)

CVfhq = Cfhq/Pfhq
```

RPT:

CONSTANT Vr = 3.5
CONSTANT Pr = 1.92
Constant Prph = 2.17
CONSTANT Qr = 2.78
Constant Prhq = 1.04

!-------Concentration in the compartment
Cr = Ar/Vr

!-------rate of change of benzene in the compartment
dA = Qr*(Cart - Cr/Pr)

!-------Amount of Benzene in the compartment
Constant a0 = 0.0
Ar = integ(dA, 0.0)

!-------Concentration leaving the compartment
CVr = Cr/Pr

!Benzene oxide equations

!-------Concentration in the compartment
Crbo = Arbo/Vr

dAbo = Qr*(Cartbo - Crbo/Pr)

!-------Amount of Benzene in the compartment
!Constant b0 = 0.0
Arbo = integ(dAbo, 0.0)

!-------Concentration leaving the compartment
CVrbo = Crbo/Pr

!Phenol equations

!-------Concentration in the compartment
Crph = Arph/Vr

dAph = Qr*(Cartph - Crph/Prph)

!-------Amount of Phenol in the compartment
Arph = integ(dAph, 0.0)

!-------Concentration leaving the compartment
CVrph = Crph/Prph

!hydroquinone equations

!-------Concentration in the compartment
Crhq = Arhq/Vr

dAhq = Qr*(Carthq - Crhq/Prhq)

!-------Amount of hydroquinone in the compartment
Arhq = integ(dAhq, 0.0)

!-------Concentration leaving the compartment
CVrhq = Crhq/Prhq

PPT:

CONSTANT Vp = 40.6
CONSTANT Pp = 2.05
Constant Ppph = 1.22
CONSTANT Qp = 1.364
Constant Pphq = 0.94

!-------Concentration in the compartment
Cp = Ap/Vp

!-------rate of change of benzene in the compartment
dA = Qp*(Cart - Cp/Pp)

!-------Amount of Benzene in the compartment
Constant a0 = 0.0
Ap = integ(dA, 0.0)

CVp = Cp/Pp

!Benzene oxide equations
Cpbo = Apbo/Vp

```
!-------rate of change of benzene in the compartment
dAbo = Qp*(Cartbo - Cpbo/Pp)

!-------Amount of Benzene in the compartment
!Constant b0 = 0.0
Apbo = integ(dAbo, 0.0)

CVpbo = Cpbo/Pp

!Phenol equations
Cpph = Apph/Vp

!-------rate of change of benzene in the compartment
dAph = Qp*(Cartph - Cpph/Ppph)

!-------Amount of Benzene in the compartment
!Constant b0 = 0.0
Apph = integ(dAph, 0.0)

CVpph = Cpph/Ppph

Carthq_plot = Carthq
!Hydroquinone equations
Cphq = Aphq/Vp

!-------rate of change of hydoquinone in the compartment
dAhq = Qp*(Carthq - Cphq/Pphq)

!-------Amount of hydoquinone in the compartment
Aphq = integ(dAhq, 0.0)

CVphq = Cphq/Pphq
```

Liver:

```
CONSTANT Vl = 1.82
CONSTANT Pl = 2.95
CONSTANT Ql = 1.55
Constant V2e1 = 2.35
Constant Cmp = 387
Constant Abz = 0.0005089
Constant Aph = 0.00013815
Constant Ahq = 0.0000000009082
Constant k1 = 0.000042
```

```
Constant k2 = 0.536
Constant k3 = 0.034083
Constant k4 = 0.014166
Constant k5 = 0.000040
Constant k6 = 0.00000213
Constant k7 = 0.000000203
Constant k8 = 0.121667
Constant k9 = 0.493
Constant k10 = 7.0166667
Constant Km1 = 131.74
Constant Km2 = 20702
COnstant Kmhq = 82134.6
Constant Ccp = 57.3
Constant Vph1 = 0.034660
Constant Vph2 = 0.046266
Constant Vhq = 1.918068
Constant Plhq = 1.04
Constant Plph = 2.17

!-------Concentration in the compartment
Cl = Al/Vl

!-------rate of change of benzene in the compartment
dA = Ql*(Cart - Cl/Pl) - RBOr

Constant a0 = 0.0
Al = integ(dA, 0.0)

CVl = Cl/Pl

!D = (1 + (Abz * Cl) + (Aph * Clph) + (Ahq * Clhq))
D = (1 + (Abz * Cl) + (Aph * Clph) + (Ahq * Clhq))

RBO = (k1 * V2e1 * Cmp * Vl * 1000 * Cl)/D
!RBO = (59.205 * Cl)/ (1 + 0.0005089 * Cl)

!IF (RBO.GT.Al) Then
!  RBOr = Al
!Else
  RBOr = RBO
!End If


!Benzene oxide equations
Clbo = Albo/Vl
```

```
!Mass balance equation for benzene oxide
dAbo = Ql*(Cartbo - Clbo/Pl) + RBOr - RPH - RMA - RPMA

!Amount of Benzene Oxide in the compartment
Albo = integ(dAbo, 0.0)

!Concentration of Benzene Oxide leaving the compartment
CVlbo = Clbo/Pl

RPH = (k2 * Vl * Clbo)
!0.8308 * Clbo          !Rate of change Benzene Oxide to Phenol

RMA = (k3 * Vl * Clbo)
!0.05 * Clbo    !Rate of change of Benzene Oxide to Muconic Acid

RPMA = (k4 * Vl * Clbo)
!0.03 * Clbo    !Rate of change of Benzene Oxide to Phenyl Mercapturic Acid

!IF (RPH.GT.Albo) Then
!  RPHr = Albo
!Else
  RPHr = RPH
!End If

!Muconic Acid equations
Alma = integ(RMA, 0.0)

Clma = Alma / Vl

!Phenylmercapturic Acid equations
Alpma = integ(RPMA, 0.0)

Clpma = Alpma / Vl

!Phenol Equations
Clph = Alph/Vl

dAph = Ql*(Cartph - Clph/Plph) + RPHr - RHQ - RCT - RCONJ

Alph = integ(dAph, 0.0)

CVlph = Clph/Plph

RHQ = (k5 * V2e1 * Cmp * Vl * 1000 * Clph)/D

RCT = (k6 * V2e1 * Cmp * Vl * 1000 * Clph)/D
```

```
RCONJ = ((Vph1 * Clph)/(Km1 + Clph) + (Vph2 * Clph)/(Km2 + Clph)) * Ccp * Vl *
1000

!IF (RHQ.GT.Alph) Then
!  RHQr = Alph
!Else
  RHQr = RHQ
!End If

!Catechol Equations
Alct = integ(RCT, 0.0)

Clct = Alct/Vl

!Phenol Conjugates
Alconj = integ(RCONJ, 0.0)

Clconj = Alconj/Vl

!Hydroquinone Euqations
Clhq = Alhq/Vl

dAhq = Ql*(Carthq - Clhq/Plhq) + RHQr - RTHB - RHQCONJ

Alhq = integ(dAhq, 0.0)

CVlhq = Clhq/Plhq

RTHB = (k7 * V2e1 * Cmp * Vl * 1000 * Clhq)/ D


RHQCONJ = (Vhq * Clhq * Cmp * Vl * 1000)/(Kmhq + Clhq)

!Trihydroxy Benzene Equations
Althb = integ(RTHB, 0.0)

Clthb = Althb/V1

!Hydroquinone Conjugates
Alhqconj = integ(RHQCONJ, 0.0)

Clhqconj = Alhqconj/Vl


------------------------
```

Bone Marrow:

```
CONSTANT Vb = 2.8
CONSTANT Pb = 1.92
Constant Pbph = 2.17
CONSTANT Qb = 0.2418
Constant Pbhq = 1.04
Constant k5 = 0.000040
Constant V2e1 = 2.35
Constant Cmp = 387
Constant Ahq = 0.0005089

!-------Concentration in the compartment
Cb = Ab/Vb

!-------rate of change of benzene in the compartment
dA = Qb*(Cart - Cb/Pb)

!-------Amount of Benzene in the compartment
Constant a0 = 0.0
Ab = integ(dA, 0.0)

CVb = Cb/Pb

!Benzene oxide equations

Cbbo = Abbo/Vb

dAbo = Qb * (Cartbo - Cbbo/Pb)

!Constant b0 = 0.0
Abbo = integ(dAbo, 0.0)

CVbbo = Cbbo/Pb


!phenol equations

Cbph = Abph/Vb

dAph = Qb * (Cartph - Cbph/Pbph)

Abph = integ(dAph, 0.0)

CVbph = Cbph/Pbph
```

```
!hydroquinone equations

Cbhq = Abhq/Vb

dAhq = Qb * (Carthq - Cbhq/Pbhq) - RBQ

Abhq = integ(dAhq, 0.0)

CVbhq = Cbhq/Pbhq


!Benzoquinone

RBQ = (k5 * V2e1 * Cmp * Vb * 1000 * Cbhq)/(1 + (Ahq * Cbhq))

Abbq = integ(RBQ, 0.0)

Cbbq = Abbq/Vb

-------------------------
```

ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

AS	Air sparging
BS	Biosparging
COC	Contaminant of Concern
FDEP	Florida Department of Environmental Protection
IRIS	Integrated Risk Information System
MCL	Maximum Contaminant Level
NOAEL	No Observed Adverse Effect Level
NTP	National Toxicology Program
PBPK	Physiologically Based Pharmacokinetic Models
PBTK	Physiologically Based Toxicokinetic Models
PK/TK	Pharmacokinetic/Toxicokinetic
PPT	Poorly Perfused Tissues
RBCA	Risk Based Corrective Action
RPT	Rapidly Perfused Tissues
SCTLs	Soil Cleanup Target Levels
USEPA	United States Environmental Protection Agency

SYMBOLS

Symbol	**Description**
Aij	The amount of chemical "j" in compartment "i"
a0	Modeling constant
C	Concentration at time t
C_1	Compartment 1
C_2	Compartment 2

C_{alv}	Alveolar concentration

Symbols Continued

Symbol	Description
C_{art}	Arterial concentration
C_{artij}	Concentration in the arterial blood of chemical "j" in compartment "i"
C^{CP}	concentration of cytosolic protein per gram of liver
C_{ij}	Concentration of chemical "j" in compartment "i"
C_{inh}	Inhalation concentration
C^{MP}	Concentration of microsomal protein per gram of tissue in the liver (mg/g)
C_o	Initial concentration
CV_{ij} C_{venij} or C_{vij}	Concentration of chemical "j" in the venous blood from compartment "i"
$\frac{dC}{dt}$	The rate of change of concentration with time
dL/dt	Rate of change of concentration in the lung
k_{12}	rate constant of flow from compartment 1 to 2
k_{21}	rate constant of flow from compartment 2 to 1
k_1, k_5, k_6, k_7	efficiency of CYP2E1 for specific oxidations relative to V_{2E1} (L/nmol)
k_2, k_3, k_4, k_8, k_9, k_{10}	Rate constants
k_e	First order elimination rate constant
K_{m1ph} and K_{m2ph}	Concentrations at half-saturation of phenol by the two sulfate transferases
[PA]	Compartment membrane permeability
PB	Air: Blood partition coefficient
P_{ij}	Blood: Compartment Partition coefficient
RM_k^j	the rate of metabolism of chemical "j" to chemical "k"
Q_a	Alveolar ventilation
Q_c	Cardiac output
Qi	Blood flow rate into a compartment
t	Time
T_l	Total mass of the liver
V_{2E1}	CYP2E1 specific activity as determined by the oxidation of p-nitrophenol to p-nitrocatechol (L/nmol)
V_i	Volume of the compartment
V_{PH1} and V_{PH2}	Maximum rates of metabolism of phenol by two sulfate transferases

REFERENCES

Aarons; L.; Clewell; H.; J.; Conolly; R.; B.; Delic; J.; I.; Houston; J.; B.; Jarabek; A.; M.; Loizou; G.; Mason; H.; J.; Nesterov; I.; Rowland; M.; Tran; C.; L. and G.; T.; Tucker; **1999**; Physiologically Based Toxicokinetic Modeling: A potential tool for use in risk assessment; Risk Assessment and Toxicology Steering Committee; **Institute of Environment and Health**; pp.; 7.

Agency for Toxic Substances and Disease Registry (ATSDR); **1997**; Potential for Human Exposure; Toxicological Profile for Benzene; **ATSDR**.

Agency for Toxic Substances and Disease Registry (ATSDR); **2001**; National Exposure Registry; **Benzene Subregistry: Baseline Through Followup 4**; Technical report; U.S. Department of Health and Human Services.

American Medical Association; **2003**; **Complete Medical Encyclopedia**; Random House; pp. 1137-1139, 638-639.

Andersen; M.; E.; **2003**; Toxicokinetic modeling and its applications in chemical risk assessment; **Toxicology Letters**; 138(1-2); pp. 9-27.

Bois; F.; Y.; Smith; M.; T. and R.; C.; Spear; **1991**; Mechanisms of benzene carcinogenesis: Application of a physiological model of benzene pharmacokinetics and metabolism; **Toxicology Letters**; 56(3); pp. 283-296.

Brown; E.; A.; Shelley; M.; L. and J.; W.; Fisher; **1998**; A Pharmacokinetic Study of Occupational and Environmental Benzene Exposure with Regard to Gender; **Risk Assessment**; 18(2); pp. 205-214.

Byczkowski; J.; **1996**; A Linked Pharmacokinetic Model and Cancer Risk Assessment for Breast-Fed Infants; **Drug Information Journal**; 30; pp. 401-412.

Clewell; H.; J.; III; Gentry; P.; R.; Gearhart; G.; M.; Covington; T.; R.; Banton; M.; I. and M.; E.; Andersen; **2001**; Development of a Physiologically Based Pharmacokinetic Model of Isopropanol and its Metabolite Acetone; **Toxicological Sciences**; 63; pp.162-170.

Cole; E.; C.; **2001**; Single Compartmental Liver; Benzene and its Effects on Erythropoiesis: Models, Optimal Controls and Analyses; PhD Dissertation; **North Carolina State University**; pp. 5-15, 28.

Connor; J.; A. and T.; E.; McHugh; **2002**; Impact of Risk-Based Corrective Action (RBCA) on State LUST Corrective Action Programs; **Human and Ecological Risk Assessment**; 8(3); pp. 573-589.

Corti; M. and C.; N.; Snyder; **1998**; Gender- and Age-Specific Cytotoxic Susceptibility to Benzene Metabolites *in Vitro*; **Toxicological Sciences**; 41(1); pp. 42-48.

Dennison; J.; E.; Andersen; M.; E.; Dobrev; I.; E.; Mumtaz; M.; M.; R.; S.; H.; Yang; **2004**; PBPK Modeling of Complex Hydrocarbon Mixtures: Gasoline; **Toxicology & Applied Pharmacology**; Article in Press; Corrected Proof.

Fiserova-Bergerova; V.; **1983**; Introduction to Mathematical Modeling; **Modeling of Inhalation Exposure to Vapors: Uptake , Distribution and Elimination; Volume 1**; pp. 52-53

Folkes; D.; J. and P.; S.; Arell; **2003**; Vapor Intrusion: EPA's New Regulatory and Initiative and Implications for Industry; **Enviro Group Limited**.

Gibaldi; M. and D.; Perrier; **1975; Pharmacokinetics**; Marcel Dekker Inc., pp. v

Gist; G.; L. and J.; R.; Burg; **2003**; Benzene-A Review of the Literature from a Health Effects Perspective; **Agency for Toxic Substances and Disease Registry (ATSDR)**.

Glantz; S.; A.; **2002**; The Normal Distribution; **Primer of Biostatistics; Fifth Edition**; McGraw Hill; pp. 14.

Haddad; S.; Charest-Tardif; G.; Tardif; R. and K.; Krishnan ;**2000**; Validation of a Physiological Modeling Framework for Simulating the Toxicokinetics of Chemicals in Mixtures; **Toxicology and Applied Pharamacology**; 167(3); pp. 199-209.

Henderson; R.; F.; Sabourin; P.J.; Bechtold; W.; E.; Griffith; W.; C.; Medinsky; M.; A.; Birnbaum; L.; S. and G.; W.; Lucier; **1989**; The Effect of Dose, Dose Rate, Route of Administration and Species on Tissue and Blood Levels of Benzene and Metabolites; **Environmental Health Perspectives**; 82; pp. 9-17.

Hricko; A.; **1994**; Rings of Controversy around Benzene; **Environmental Health Perspectives**; 102 (3); pp. 276-281.

http://web.ead.anl.gov/sra-chicago/vol1-1/rbca.html Risk Based Corrective Action (RBCA); **2004; Society of Risk Analysis**, Chicago Regional Chapter.

http://www.dep.state.fl.us/waste/quick_topics/publications/wc/GlobalRBCA_Implemnt.pdf; Ruddell, J.; **2003**; Implementation of Global RBCA; **Florida Department of Environmental Protection**.

http://aquaticpath.umd.edu/appliedtox/metabolism.pdf ; Kane; A.; S.; Metabolism; Applied **Toxicology**.

International Agency for Research in Cancer (IARC); **1987**; Benzene; **IARC** Summary and Evaluation; Supplement 7.

Jenkins; R.; A.; Guerin; M.; R. and B.; A.; Tomkins; **2000**; Mainstream and Sidestream Cigarette Smoke; **The Chemistry of Environmental Tobacco Smoke: Composition and Measurement; Second Edition**; Lewis Publishers; pp. 49.

Jonsson; F. and G.; Johanson; 2001; Bayesian estimation of variability in adipose tissue blood flow in man by physiologically based pharmacokinetic modeling of inhalation exposure to toluene; Toxicology; 157(3); pp. 177-93.

Kao; C.; M. and J.; Prosser; **2001**; Evaluation of natural attenuation rate at a gasoline spill site; **Journal of Hazardous Materials**; 82(3); pp. 275-289.

Keys; D.; A.; Bruckner; J.; V.; Muralidhara; S. and J.; W.; Fisher**; 2003;** Tissue Dosimetry Expansion and Cross-Validation of Rat and Mouse Physiologically Based Pharmacokinetic Models for Trichloroethylene; **Toxicological Sciences**; 76; pp. 35-50.

Kohn; M; **2000**; Current directions in physiological modeling for environmental health sciences: an overview; **Environmental Health Perspectives**; 108(5); pp. 857-860.

Liao; K.; H.; Dobrev; I.; D.; Dennison Jr.; L.; E.; Andersen; M.; E.; Reisfeld; B.; Reardon; K.; F.; Campain; J.; A.; Wei; W.; Klein; M.; T.; Quann; R.; J. and R.; S.; H.; Yang; **2002**; Application of Biologically Based Computer Modeling to Simple or Complex Mixtures; **Environmental Health Perspectives**; 102 (S6); pp. 957-963.

Lovern; M.; R.; Cole; C.; E. and P.; M.; Schlosser; **2001**; A Review of Quantitative Studies of Benzene Metabolism; **Critical Reviews in Toxicology**; 31(3); pp. 285-311.

Lovern; M.; R,; Marvis; M.; E. and P.; M.; Schlosser; **1999**; Use of a Mathematical Model In Vitro Benzene Metabolism to Predict In Vitro Metabolism Data; **Carcinogenesis**; 20(8); 1511-1520.

McMahon; T.; F.; Medinsky; M.; A. and L.; S.; Birnbaum; **1994**; Age-related changes in benzene disposition in male C57BL/6N mice described by a physiologically based pharmacokinetic model; **Toxicology Letters**; 74(3); pp. 241 – 253.

Member Agencies of the Federal Remediation Technologies Roundtable; **1995**; **Remediation Case Studies**; PB95-182903.

O'Flaherty; E.; **1987**; Modeling: An Introduction; **Pharmacokinetics in Risk Assessment: Drinking Water and Health**; National Academy Press; pp. 28-30, 34.

Poet; T.; S.; Weitz; K.; K.; Gies; R.; A.; Edwards; J.; A.; Thrall; K.; D.; Corley; R.; A.; Tanojo; H.; Hui; X.; Maibach; H.; I. and R.; C.; Wester; **2002**; PBPK Modeling of a Percutaneous Absorption of Perchloroethylene From a Soil Matrix in Rats and Humans; **Toxicological Sciences**; 67; pp. 17-31.

Rhoades; R. and R.; Pflanzer; **1996**; Chemical and Physical Principles; **Human Physiology**; Saunders College Publishing; pp. 67-69.

Rushmore; T.; Snyder; R. and G.; Kalf; **1984**; Covalent binding of benzene and its metabolites to DNA in rabbit bone marrow mitochondria in vitro; **Chemical-Biological Interactions**; 49 (1-2); pp. 135-154.

Smith; M.; T.; Yager; J.; W.; Steinmetz; K.; L. and D.; A.; Eastmond; **1989**; Peroxidase Dependent Metabolism of Benzene's Phenolic Metabolites and its Possible Role in Benzene Toxicity and Carcinogenicity; **Envionmental Health Perspectives**; 82; pp. 23-29.

Sweeney; L.; M.; Gargas; M.; L.; Strother; D.; E. and G.; L.; Kedderis; **2003**; Physiologically Based Pharmacokinetic Model Parameter Estimation and Sensitivity and Variability Analyses for Acrylonitrile Disposition in Humans; **Toxicological Sciences**; 71; 27-40; pp. 27-40.

Thibodeau; G.; A. and K.; T.; Patton; **2003**; Physiology of the Respiratory System; **Anatomy and Physiology: Fifth Edition**; Mosby (an affliliate of Elsevier Science); pp. 716.

Thomas; R.; S.; Lytle; W.; E.; Keefe; T.; J.; Constan; A.; A. and R.; H.: S.; Yang; **1996**; Incorporating Monte Carlo Simulation into Physiologically Based Pharmacokinetic Models Using Advanced Continuous Simulation Language (ACSL): A Computational Method; **Fundamental and Applied Toxicology**; 31(1); pp. 19-28.

Travis; C.; L.; Quillen; J.; L. and A.; D.; Arms; **1990**; Pharmacokinetics of Benzene; **Toxicology and Applied Pharmacology**; 102; pp. 400-420.

Tunek; A.; Högstedt; B. and T.; Olofsson; **1982**; Mechanism of benzene toxicity. Effects of benzene and benzene metabolites on bone marrow cellularity, number of granulopoietic stem cells and frequency of micronuclei in mice; **Chemical-Biological Interactions**; 39(2); pp. 129-138.

U.S. Environmental Protection Agency; **2003**; Releases Chemical Report; **TRI Explorer; Data source: 2001 Public Data Release** as of Mar. 3, 2003.

Wallace; L.; **1989**; Major Sources of Benzene Exposure; **Environmental Health Perspectives**; 82; pp. 165-169.

Woodruff; T.; J. and F.; Y.; Bois; **1993**; Optimization Issues in Physiological Toxicokinetic Modeling: A Case Study with Benzene; **Toxicology Letters**; 69(2); pp. 181-196.

Wong O., **1995**; "Risk of acute myeloid leukaemia and multiple myeloma in workers exposed to benzene", **Occupational and Environmental Medicine**; 52; pp. 380-384.

BIOGRAPHICAL SKETCH

My name is Tara Aniket Kulkarni. My maiden name is Taramati Shenoy. I was born in India, in 1977, the oldest of my parents' three daughters. I got my bachelor's degree in Civil Engineering from Pune's Government College of Engineering in India and came to the United States in 1998 to pursue my master's degree in Civil and Environmental Engineering at the University of Toledo (UT). I met my future husband Aniket Kulkarni at UT and am now married for just about 4 years. I started with my Ph.D. program in Civil and Environmental engineering in 2000 at the Florida State University (FSU), where I have intermittently been a teaching and research assistant.

I currently work for the Florida Department of Environmental Protection (FDEP) in Tallahassee, FL, where I also interned from 2000 – 2002. My research interests include anything related to the environment, but specifically issues that relate to human health.