AFIT/GEE/ENV/93S-3

A REFINEMENT OF RISK ANALYSIS PROCEDURES FOR TRICHLOROETHYLENE THROUGH THE USE OF THE MONTE CARLO METHOD IN CONJUNCTION WITH PHYSIOLGICALLY BASED PHARMACOKINETIC MODELING

THESIS

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THESIS

Presented to the Faculty of the School of Engineering

of the Air Force Institute of Technology

In Partial Fulfillment of the

Requirements for the Degree of

Master of Science in Engineering and Environmental Management

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September 1993

The views expressed in this thesis are those of the authors and do not reflect the official policy or position of the Department of Defense or the U.S. Government.

Preface

The purpose of this study was to quantify and investigate the uncertainty associated with an excess lifetime cancer risk of one in one million for human exposure to trichloroethylene (TCE) to help improve risk analysis procedures. The uncertainty is quantified in two categories. First, a sensitivity analysis was conducted for the TCE Physiologically-Based Pharmacokinetic Model used to generate dose-metrics of interest. Monte Carlo generated parameters were then used in conjunction with the PBPK model to generate a range of mouse dose-metric values which, in turn, were used in a linearized multistage risk model to determine the range of human TCE exposure levels corresponding to a $1x10^{-6}$.

Without the instruction, guidance, and advice from others, this research wouldn't have been possible. We would like to acknowledge Lieutenant Colonel Michael Shelley for providing precursory instruction in the field of risk analysis, introducing us to the professionals at the Armstrong Laboratory Toxicology Division, and for occasionally bumping us back on course. Mr. Carlyle Fleming (ManTech Inc.) was a tremendous help. He spent many hours assisting us with the "nuts and bolts" of simulation languages and PBPK modeling concepts. His help probably reduced our computer work-load from thousands, to hundreds of hours. We would also like to thank Mr. Jeff Gearheart (ManTech Inc.) for his assistance. Most importantly, we acknowledge Dr. Jeffrey Fisher, Senior Scientist of the Armstrong Laboratory's Occupational and Environmental Health Directorate, Toxicology Division. Dr. Fisher's willingness to take time out of an extremely busy schedule; to provide computer software, hardware, and research literature; and allow personnel in his organization to support us is greatly appreciated.

It goes without saying that our families deserve tremendous thanks. Their patience and understanding seemed unending and didn't go unrecognized.

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Abstract

The purpose of this study is to quantify the uncertainty associated with a risk assessment for trichloroethylene (TCE) using physiologically based pharmacokinetic (PBPK) modeling. The authors use the PBPK model and physiological data presented in a 1993 study by Fisher and Allen. To quantify uncertainty the authors use the Monte Carlo method, in conjunction with the PBPK model, to generate a range of TCE exposure concentrations which result in a human cancer risk of $1x10^{-6}$. The general routes of exposure used in the study are inhalation and ingestion. To further quantify uncertainty, a sensitivity analysis of the model is also conducted.

Information about the means and distributions of the model parameters is used to generate two hundred mice (male and female) and humans using the Monte Carlo method. Parameter means were derived from the Fisher-Allen study while information on the type of distribution and variance of the parameters was obtained from the Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory. Next, a range of model output for mice is generated by simulating exposure of the mice to the TCE exposure scenarios used by the Environmental Protection Agency (EPA) in their health assessment of TCE. The model results are then correlated to cancer incidence rates determined in the studies and risk curves are generated for the upper, lower, and mean values of the 95% confidence interval of the results of the mouse study simulations. Human TCE exposures are found by iteratively running the model at different exposure concentrations until a risk of $1x10^{-6}$ is determined for the upper, lower, and mean value of the 95% confidence interval. This process results in a range of exposures which are used to quantify parameter uncertainty. The lower bound of the 95% confidence interval is used to compare to current EPA risk estimates.

For the model output value for total amount of TCE metabolized, used by the EPA in their assessment, the lower bound on the 95% confidence interval was greater than the EPA risk by a factor of 23 for continuos inhalation and by a factor of 1.6 for ingestion.

A sensitivity analysis of the model is conducted by first running the model at the mean value of the parameters. Each parameter is then increased by 1% and the model is run again for each parameter increase. The results of the increase of each parameter is then compared to the results from the mean and a percentage change is determined.

The results of the sensitivity analysis show the parameters which have a significant impact on the model output. These parameters should receive emphasis in determination of their types of distributions and variances as they have the greatest impact on model predictions. For inhalation, the most sensitive parameters are the elimination rate constant (KUPC), the alveolar ventilation rate (QPC), the rate of metabolism (VMC), and the cardiac output (QCC). Sensitive parameters for ingestion are KUPC and VMC.

The results of the study demonstrate that PBPK modeling can be used to improve the risk analysis process by reducing the need for qualitative discussions of uncertainty. This method for determining parameter uncertainty provides significantly more information than do traditional approaches. Although the method does not quantify all uncertainty associated with modeling, its adoption as a risk assessment standard could be used to promulgate, and better present, more realistic standards.

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1. *Introduction*

General Background

Predicting the quantitative nature of risk associated with chemical exposure is a difficult problem. Most current estimates of risk are based on extrapolation of laboratory animal bioassay data to humans. This extrapolation does not effectively take into consideration the manner in which humans are exposed to contaminants in the environment and the mechanisms of action in the human body. Because of the lack of knowledge about how chemicals act, safety factors and conservative estimates are used in calculating human risk. These safety measures can result in an unrealistic risk estimate posed by a chemical. This estimate is then translated by regulatory enforcement agencies (e.g. the EPA) into cleanup requirements at contaminated sites and human exposure standards. A more realistic approach to setting standards is available although it has not seen widespread regulatory use. This method is the use of Physiologically Based Pharmacokinetic (PBPK) modeling.

PBPK modeling has been used for a number of years as a method to predict dynamic toxic effects of various chemical substances in animals and humans.

With the increased power and availability of computer systems and numerical analysis techniques, PBPK modeling is enabling researchers to simulate the kinetic effects of chemicals on biological systems.

Only recently has the Environmental Protection Agency (EPA) integrated PBPK techniques into the process of promulgating regulations and standards. Although limited acceptance has been realized, clear guidelines for the use of such techniques do not exist. Preliminary exploratory research indicates that in some instances PBPK modeling predicts significantly lower chemical concentrations in humans than would otherwise be indicated by traditional bioassay studies.

Due to the uncertainty which is inherent in modeling, PBPK results have been viewed with skepticism and play a limited role in risk assessment. If PBPK data could be generated, formatted, and presented in such a manner as to quantify uncertainty, more realistic exposure standards might result. Realistic standards, in turn, could potentially minimize adverse economic effects and excess regulatory burden while, at the same time, provide a safe environment.

Pharmacokinetics. Before the advent of PBPK models, computers, and numerical algorithms capable of implementing these models, researchers relied heavily on simplified pharmacokinetic models. Pharmacokinetic models are still employed today and are quite useful in studying certain nonlinear dose-response relationships. These less complex models, however, correlate chemical exposure directly with response and are not useful for providing insight about specific target organs. A target organ is one for which a contaminant exerts adverse effects.

PBPK. Unlike most classical pharmacokinetics, PBPK modeling utilizes a multi-compartment approach. The biological specimen is represented by a number of lumped compartments including critical organs such as the heart, lungs, and liver. Other lumped compartments include fatty tissue, slowly perfused tissue, and richly perfused tissue.

Following a mass-balance approach, each compartment's input, output, and metabolic activity is calculated using a set of linear ordinary differential equations. Relationships within and between the various compartments establish a system of equations which can be solved simultaneously using numerical methods.

The equations described do not lend themselves to a closed form solution; therefore, the solution must be determined by computer systems and numerical algorithms. Fortunately, microcomputers have advanced to the point that most simulation algorithms can be realized on affordable systems.

Problem Background

Uncertainty can be defined as ***not having complete knowledge***. It is precisely this lack of knowledge that forms the core of this thesis. Uncertainty is inherent in all sciences and risk assessment is no exception. There are numerous sources of uncertainty in the process of risk assessment. The following examples are by no means exhaustive but should provide the reader with an appreciation of uncertainty as it relates to PBPK modeling and its role in risk assessment.

Experimental Data. Empirical data derived from experimentation is subject to procedural error, measurement error, background noise, and researcher bias to name a few. Experimental data derived from animal bioassays provides the information necessary to validate the PBPK model.

Carcinogenity. The physical properties of certain chemicals make them inherently able to cause damage to human cells. Some of these chemicals are either known or suspected to cause cancer in animals. These results have been used to extrapolate cancer risk to human beings. The extrapolation process presents significant uncertainty for the quantification of risk in humans.

Mathematical Models. The use of mathematical models necessarily includes certain assumptions concerning the behavior of the biological system under consideration.

The assumption of linearity, while certainly valid under certain conditions, can lead to a high level of uncertainty. In the case of PBPK models, dose-metric variability may differ from one concentration scenario to the next.

Metabolic systems can become saturated at high levels of target organ concentration; therefore, extrapolation from known behavior at high concentration to low concentration may not yield realistic results. The model's structure has to account for this behavior.

Numerical Integration. Computer algorithms used to solve differential equations can generate solutions with a high degree of accuracy. Conversely, integration routines can also generate solutions which are unlikely to reflect physiological reality. Choosing an inappropriate algorithm or an incorrect discrete time step size can result in answers with a high degree of uncertainty.

Parameter Estimation. The physiological and kinetic parameters used in a system of differential equations are often represented by their statistical means. The researcher must choose values for these parameters based on other research efforts and the target population. These parameters, and their statistical characteristics, are not known with certainty. Research methodologies vary greatly; this variation requires the researchers to distinguish between viable and questionable parameter values. Species parameters may vary widely from one group to another, thereby confounding their estimation. Parameter estimation is a significant source of uncertainty in PBPK modeling.

Specific Problem Background

Risk management decisions for maximum exposure concentrations are often based on limited data and associated studies accompanied by lengthy qualitative uncertainty analysis. In the case of Trichloroethylene (TCE), the current maximum exposure level for water ingestion is 5 parts per billion (Steinberg, 1992: 4). This level is based on limited bioassay studies which expose mice to very high concentrations of TCE (EPA, 1985).

A document prepared by the MITRE corporation for the Air Force Center for Environmental Excellence outlines a number of issues which have led to concerns about the conservative nature of TCE standards (Steinburg, 1992).

Recent studies show that trichloroacetic acid (TCA) and dichloroacetic acid (DCA), two major metabolites of TCE, are correlated with liver tumors in B6C3F1 mice (Bull et al, 1990; Herren-Freund et al, 1987). The inability of classical pharmacokinetic models to track the formation, concentration, and elimination of these metabolites has prompted development and use of PBPK models for TCE (Fisher-Allen, 1993).

PBPK modeling efforts often assume single values of kinetic and physiological parameters. This practice results in a single dose-metric, exposure concentration, or risk level. Single values of any modeling effort necessarily do not provide insight concerning uncertainty. The general absence of uncertainty quantification in PBPK modeling and in traditional risk assessment methods forms the basis for this research.

Specific Problem

Traditional risk assessment methods generally result in qualitative discussions of uncertainty. Although PBPK models, coupled with statistical methods such as Monte Carlo analysis, can be used to quantify physiological and kinetic parameter uncertainty, most methodologies do not allow for parameter variability. The resulting dose-metric values and associated risks are characterized by single-values and do not account for natural variability within the sample population. This thesis will attempt to incorporate parameter variability in a PBPK risk assessment for TCE to determine a range of TCE exposures which result in a human excess lifetime cancer risk of one-in-one million. The research will also attempt to determine an appropriate format for presenting the results of such an assessment.

Scope of Research

Limitations. As previously mentioned, there are numerous sources of uncertainty associated with PBPK modeling. A complete analysis of uncertainty is beyond the scope of this thesis; therefore, the focus of this research will be limited to model sensitivity and parameter variability. Chemical analysis shall be limited to TCE.

Assumptions. Many areas of this research are subject to controversy. The views of various researchers reflect differences associated with carcinogenic properties of certain chemicals as well as the PBPK techniques used to model these characteristics.

The concept of a risk threshold is controversial. Zero risk threshold implies that, no matter how small the exposure to a chemical, there always exists a finite risk that the agent of concern will cause some adverse effect. A zero threshold of risk is assumed with carcinogens. Chemicals are classified as potential or probable human carcinogens based on animal bioassay data. Often, the appearance of tumors within an animal population subjected to chemical exposure is interpreted as sufficient evidence to warrant the chemical being classified as a probable human carcinogen. This research will focus on a chemical which falls into this category, TCE. TCE is assumed to be a human carcinogen.

This research will not attempt to conduct a comprehensive survey of potential parameter means, intervals and distributions, but will use values suggested by researchers at the Harry G. Armstrong Aerospace Medical Research Laboratory, Wright Patterson Air Force Base, Ohio. These values are based on considerable research by professional toxicologists and as such are assumed to be accurate.

Objectives

In order to 1) determine model sensitivity, 2) address parameter uncertainty and its associated affect on risk prediction, and 3) quantify the uncertainty for a PBPK based risk assessment for TCE, this research will attempt to meet the following objectives:

1. Determine current EPA policies governing human exposure to TCE.

2. Identify appropriate dose-metrics for use in determining cancer risk.
3. Given validated PBPK model for inhalation and ingestion of TCE:
    a. Identify and quantify the physiological and kinetic model parameters to which the model is sensitive.
    b. Determine the range of dose-metrics and associated TCE exposure concentrations which lead to a 1×10^{-6} excess lifetime cancer risk in humans.
5. Present the resulting dose-metrics and exposure concentration ranges in an appropriate quantitative manner.
6. Suggest how a quantitative presentation can improve the understanding of uncertainty by reducing lengthy qualitative discussions currently included in risk assessment documents.

Possible Benefits

The selection of parameter values contributes to the uncertainty of a PBPK model output. Quantifying the nature of this uncertainty helps both the regulator and the researcher. PBPK modeling's ability to quantify uncertainty could reduce regulatory reliance on conservative safety factors and allow regulators to place more realistic limits on chemical exposures. From the researchers perspective, quantification of uncertainty might suggest specific ranges of exposure concentrations and identify important parameters on which to base future experimental designs; thereby optimizing allocation of resources and increasing the output of useful results.

Overview

The remainder of this thesis consists of four chapters. Chapter two reviews of the literature necessary to establish current regulatory policies and practices and provides an

overview of both classical and physiologically based pharmacokinetics. The review will also discuss PBPK specific research for a number of chemicals, but will focus on TCE. Considerable material will be dedicated to the structure, parameters, and other characteristics of the model used for this research.

Chapter three offers a detailed description of the methodology used to achieve the research objectives. Next, chapter four will present analytical results. Finally, chapter five will discuss the significance of findings, draw conclusions, and suggest refinements for the chosen methodology and model structure.

II. *Literature Review*

Introduction

The primary purpose of this literature review is to acquaint the reader with the general field of the research, risk analysis and physiologically based pharmacokinetic (PBPK) modeling. The secondary purpose is to familiarize the reader with the specific TCE model to be used in this research. The review includes a discussion of the general risk analysis process and the historical development of that process, including risk assessment and dose-response assessment. TCE is the focus of the research, which made a review of the nature of this chemical necessary. TCE will be characterized with regard to its history, usage, carcinogenicity, and action in the body. The next topic addressed in the review is the Environmental Protection Agency's (EPA) assessment of the risk posed by TCE. This section is mainly a review of TCE's health assessment document (EPA, 1985) and its subsequent draft addendum (EPA, 1987). The development of PBPK modeling is then reviewed. Next, chemical specific research in PBPK modeling leads to a discussion of the specific model the authors will use for analysis. The structure of the model is described as it relates to the research. The following section of the review presents a general discussion of the analysis of uncertainty involved with PBPK models. The chapter is concluded with a summary of the revelations derived from the literature.

Risk Analysis

The risk analysis process attempts to gather information regarding risks, analyze that information, and use the results to understand and communicate the nature of the risk in question (Shelley, 1992). The classical steps in the analysis of risk include the identification of a hazard, risk assessment, determining the significance of the risk, and communication of risk (Cohrsses and Covello, 1989:5). PBPK modeling is used in the risk assessment phase of the overall risk analysis process.

Risk Assessment. Risk assessment is a sub-process of risk analysis which involves the strict technical assessment of the nature and magnitude of the risk (Shelley, 1992). The specific steps in the risk assessment are source/release assessment, exposure assessment, dose-response assessment, and risk characterization (Cohrsses and Covello, 1989:55). The objective of the dose-response assessment is to determine the relationship between the dose received and the magnitude of the adverse effect. The application of PBPK modeling is in the dose-response step of risk assessment, therefore this review will concentrate on that process.

Dose-Response Assessment. In conducting a dose-response assessment, one wishes to describe the relationship between the dose an exposed individual receives and the likelihood of an adverse response. The dose received can be expressed as an absorbed dose, i.e. the total amount taken up, or an effective dose, i.e. the amount which reaches a target organ. The issue of dose can be examined by considering dose-metrics. A dose-metric is a measure of a parameter which reflects the actual dose received by an individual. An example of a dose-metric is the use of the total amount of TCE metabolized, instead of the actual dose of TCE received. These dose-metrics are used whenever an understanding of the mechanism of risk points to a specific characteristic of the exposure which can be best reflected by a surrogate.

There are two possible relationships between the dose and the likelihood of an adverse effect. These relationships are the threshold and non-threshold cases. A threshold response is used when there is a dose an individual can be exposed to which will produce no likelihood of suffering an adverse effect (i.e. a threshold dose). Below this dose, an individual will not be exposed to a risk. Non-threshold response implies that as soon as an exposure to any amount of risk agent occurs, there will be some finite chance of suffering harm. This situation arises in the case of carcinogens, where it is assumed that even a minute exposure to a carcinogen poses a risk of suffering cancer. In either case, one must

attempt to quantify the relationship between dose and response in order to determine risk. This quantification requires experimentation to determine the nature of the relationship.

Studies. Two major methods are available for determining the dose-response relationship: epidemiological studies and animal bioassay studies. For most risk agents epidemiological data is not sufficient to conduct a dose-response assessment (Cohrssen and Covello, 1989:79). For this reason, most research uses data from laboratory animal studies. These studies are usually done at high dose levels. This high dose-response is extrapolated to determine a response relationship for humans. Response at high dose is then extrapolated to low dose levels commonly found in chronic exposures.

The extrapolation from a high dose-response to a low dose-response is difficult. This is especially true in the case of cancer, because little is known about the mechanism of cancer (Cohrssen and Covello, 1989:80). A general review of the literature reveals that the linearized multi-stage model is the most widely used method for extrapolation. The multi-staged model is based on the observation that it appears that cells progress through multiple stages in a carcinogenic response. In low doses, the linear terms of the multi-stage model predominate (Shelley, 1992). Therefore, the model is linearized in the low dose area and used to extrapolate from high dose to low dose. This model is based on an understanding of the cancer causing mechanism (i.e. cells progress through multiple stages toward tumor induction), it provides a simple linear relationship between dose and response, and it is conservative compared to other available models. For these reasons, this is the model of choice of the EPA (Shelley, 1992).

The authors have concentrated on reviewing the processes of classical risk analysis which are pertinent to the research topic. The contention here is that PBPK models can improve the risk analysis process by refining dose-response relationships.

Background of Trichloroethylene

The characteristics of TCE germane to this topic are its history, uses, chemical characteristics, carcinogenicity, and its action in the body, including metabolism.

History. TCE was first synthesized in 1864 and has been in commercial production for over 80 years (Steinberg and DeSesso, 1992:4). The major use of TCE in the United States, 80 to 95 percent, is for the degreasing of fabricated metal parts (EPA, 1985:3,4). There are no known natural sources of the chemical (EPA, 1985:3,5). The use of TCE has been on the decline due to increasing legislation and the availability of replacement solvents (EPA, 1985:3,5). In addition to degreasing, TCE has been used in the past as a general anesthetic, a disinfectant, in dry cleaning, as a solvent for insecticides and oils, and for spot cleaning applications (Steinberg and DeSesso, 1992:4). Chloral hydrate, a possible metabolite of TCE, is used in sleeping pills and as a sedative for children prior to medical procedures (Steinberg and DeSesso, 1992:4). The improper disposal of TCE in the past has caused the chemical to become the object of substantial remediation efforts at sites throughout the United States and especially on Air Force bases (Steinberg and DeSesso, 1992:17). The fact that TCE is receiving so much attention by regulators lends emphasis to any study to better characterize its risk causing characteristics.

Chemical Characteristics. TCE is a colorless, highly volatile hydro-carbon. It is also a highly lipophilic material (EPA, 1985:3,3). Some of the important physical properties are shown in Table 2.1. The physical parameters of the chemical control its transport in the environment and the pathways it can take. For this reason it is extremely important that they be understood.

Carcinogenicity. The EPA currently classifies TCE as a B2 carcinogen, or a probable human carcinogen (EPA, 1987:3,42). Literature reviewed by the authors indicates TCE is a weak human carcinogen, at worst (Fisher and Allen, 1993:87). The literature is also in agreement that TCE must first be metabolized to exert a carcinogenic effect, and that it is the metabolites which present the cancer causing potential. There have been very

Table 2.1

Physical Properties of TCE. (EPA, 1985:3,1)

Molecular Weight	131.39
Boiling Point	87 °C
Vapor Pressure	94 mm Hg @ 30 °C
Vapor Specific Gravity	4.55 at boiling point (air = 1)
Solubility	0.107 g percent

substantial arguments that TCE is not a human carcinogen (Steinberg and DeSesso, 1992). The argument becomes moot in light of the fact that the primary environmental regulatory agency, the EPA, considers it a carcinogen. For the purposes of this research, TCE is a human carcinogen.

Routes. The EPA considers the two most important routes of concern for exposure to TCE to be inhalation and ingestion through drinking water (EPA, 1985 1,1). The TCE health assessment document (HAD) (EPA, 1985) and its addendum (EPA, 1987) are based on these two exposure routes. The authors will evaluate the same routes in the methodology.

Metabolism. As discussed earlier, it is the metabolites of TCE that are considered to be responsible for its carcinogenicity. As such, an examination of the manner in which TCE is metabolized and of its major metabolites is extremely crucial for this research. The major metabolites of TCE are trichloroethanol (TCOH) and trichloroacetic acid (TCA) (Fisher et al., 1991:183). The metabolites are formed by the oxidation of TCE. Because TCA has been linked to liver tumors in mice, it warrants consideration as a dose-metric for TCE (Fisher et al., 1991:184). Because there are other metabolites which may also be carcinogenic, another dose-metric is the total amount of TCE metabolized. The research

will concentrate on these two dose-metrics as measures of the effective dose of TCE received.

EPA Risk Analysis

The EPA has assessed the risk posed by TCE in both its health assessment document (HAD) for TCE and the subsequent draft addendum (EPA, 1985 & 1987). The HAD reviewed both animal bioassay and epidemiological studies for TCE. For its assessment of the risk posed by ingestion of TCE in water, the EPA used rodent bioassays in which rodents were gavaged with TCE. The term 'gavage' refers to a process by which a chemical is directly injected to an animal's gastro-intestinal system through a tube inserted through the esophagus and into the stomach. The two studies used were a National Toxicology Program (NTP), 1982 study, where B6C3F1 mice were gavaged with a dose of 1000 mg/kg/day, 5 days per week for 103 weeks; and a National Cancer Institute (NCI), 1976 study, in which B6C3F1 mice were gavaged with TCE doses ranging from 700 to 2400 mg/kg/day, 5 days per week for 78 weeks (EPA, 1985:8-20 to 8-26). Results of the studies were extrapolated to humans using a body surface dose equivalence factor (EPA, 1985:8-111). The upper bound, or most conservative, of the unit risk estimate for ingestion was given as 3.2×10^{-7} per ug/L (EPA, 1985:8-143). Unit risk may be defined as the lifetime risk caused by one unit of exposure in the low dose-response region. The 1985 HAD calculated an inhalation risk based on gavage studies, however the draft addendum used newer inhalation studies to revise this risk.

In the draft addendum to the HAD, the EPA primarily utilizes a rodent bioassay by Maltoni et al. in 1986. (EPA, 1987:5-1). The study exposed Swiss and B6C3F1 mice to an inhalation of 100, 300, and 600 ppm. The addendum used a classical pharmacokinetic model to estimate the amount of TCE metabolized under exposure to TCE vapors. (EPA, 1987, 4-20). The human equivalent dose used in unit risk calculations was based on a ratio of body weights (weight of the human divided by the weight of the animal, all raised

to the one third power) multiplied by the animal metabolized dose (EPA, 1987:5-4). The unit risk for inhalation was calculated as 1.7×10^{-6} per ug/cu.m (EPA, 1987:5-11).

Both of the unit risk derivations used a linearized multi-stage model for the extrapolation from high to low dose-response. This further emphasizes the standard acceptance of this extrapolation approach as the most conservative.

A shortcoming of the EPA risk assessment methodology is expressed by Fisher and Allen as follows:

> Another shortcoming in the US EPA risk assessment methodology is that the metabolism of TCE in humans was not critically evaluated. The estimates of the metabolized dose in the rodent bioassays were adjusted by a body-surface, dose equivalence factor prior to use in the linearized multistage model. This adjustment factor was used for extrapolation of dose from rodents to humans. (Fisher and Allen, 1993:3)

An opportunity is created here for the use of PBPK modeling. This is because the PBPK model can account for differences in metabolism between species and makes the use of empirical scaling factors unnecessary.

Pharmacokinetics

Pharmacokinetics can be defined as the study of the correlation between toxic effects and an administered dose of toxic substance (Andersen, 1989:1). Biological systems are complex and often defy quantitative description; however, certain biological functions, such as metabolism, lend themselves to mathematical modeling. Analytical methods for determining dose-response correlation include classical pharmacokinetics and PBPK. This section will focus on the characteristics of each of these methods.

"Toxicokinetics, that is, the pharmacokinetics of toxic chemicals, is the study of the time course for the absorption, distribution, metabolism and elimination of a toxic substance in a biological system" (Clewell and Andersen, 1985:114). Because the

majority of literature uses the term pharmacokinetics interchangeably with toxicokinetics, the former term shall be used throughout this document when referring to toxicokinetics.

Pharmacokinetic models are used for a number of purposes including environmental risk assessment, toxicology research, clinical applications, and drug design. Anti-cancer agents, antibiotics, anesthetics, trace metals, environmental toxins, and other substances have all been subjects of pharmacokinetic modeling. In 1983, an article was published which enumerated and characterized a variety of models used to describe the time course of these agents (Gerlowski and Jain, 1983:1103-1127). Pharmacokinetic models fall into two major categories, namely classical and physiological. The next sections provide a general overview of both types.

Classical Pharmacokinetics. Classical pharmacokinetic models have served as the historical framework for describing the time dependent concentration of chemicals in the body. During the early part of this century, kinetic models, varying in sophistication, were developed to describe and predict the metabolism and concentration of anesthetics (Haggard, 1924a:753-770; Haggard, 1924b:771-781). Early models typically represented the body as a single compartment (Leung, 1991:248). So-called compartmental pharmacokinetic modeling has been widely used for the development of therapeutic drugs. It was this early use of pharmacokinetics that led to the first models sophisticated enough to describe complex biological behavior. Unfortunately, the differential equations which represented these systems did not lend themselves to closed form solutions, and computer systems necessary to perform numerical analysis had not yet been developed (Andersen, 1989:1-3). Limited analytical power necessarily forced researchers to greatly simplify models and often to assume linear dose-response relationships. These assumptions became increasingly troublesome and required modifications in order to describe the nonlinear behavior of certain dose-metrics.

Classical modeling is useful for predicting a variety of phenomena including nonlinear behavior of systems, general input-output relationships, and interpolating responses from

limited experimental data. While both toxicologists and pharmacologists have clearly established a need for classical pharmacokinetic modeling, the utility associated with such techniques is limited. Typically, numerical values for parameters associated with classical models must be determined by fitting the model to experimental data. Unfortunately, this approach does not reflect physiological realism (Clewell and Andersen, 1989:129; Leung, 1991:247). Classical, or data-based, modeling essentially depends on experimental data to determine the model's constants. These constants, however, do not provide insight about target organs. This approach is analogous to modeling a complex electrical circuit by using one or two black boxes to describe the behavior of the entire system. While it is certainly useful to know how the output behaves with respect to the input (transfer function), the input-output model does not provide information about the various sub components and their functional relationships. Like electric circuits, biological systems are composed of a large number of components. Often, one or more of these components or "target organs" are adversely effected by a toxicant and are of primary concern to toxicologists.

At least one study shows that classical models may be the appropriate choice for predicting dose-response relationships, and that over-parameterization can be detrimental to accurate predictions. Woodruff et al. used existing experimental data coupled with Monte Carlo techniques to show that both PBPK and classical models display variability across concentration scenarios. Usefulness of classical models, however, is normally limited to interpolation between data points. Use of physiologically based models is required if one requires meaningful information about internal dose-metrics. (Woodruff et al., 1992:199)

Classical approaches have also been used in the discipline of pharmacodynamics. Pharmacodynamics is the study of the time course biological response due to exposure to certain chemicals. Carcinogenicity and teratogenicity are examples of such responses. Multi-compartment classical models are developed by observing the behavior of

exponential equations which describe experimental results. (Conolly and Andersen, 1991:503-523; Clewell and Andersen, 1989:134; Andersen, 1989b:405-415)

Unfortunately, most classical models do not offer the level of detail necessary for extrapolating dose-response kinetics concerning target organs. This weakness leads to the discussion of physiologically based models.

Physiologically Based Pharmacokinetics

Unlike classical models, physiologically based models reflect a multiple compartment, mass-balance approach, modeled by ordinary differential equations, with constants based on physiological realism. Dose-metrics are predicted based on these physiological parameters, which are independent of the kinetic (experimental) data (Woodruff et al., 1992:189). By using a number of interconnected compartments to represent various organs and tissue groups, researchers are able to predict target organ response under a variety of exposure scenarios. While the benefit of such an approach may not be immediately clear to the uninitiated, the ability to quantitatively describe a variety of dose-metrics with respect to one or more target organs is a powerful tool. PBPK modeling is used in a number of research areas including dose-response, drug development, and environmental and health risk assessments. PBPK has been used to help establish threshold limit values and biological exposure indices for unusual workshifts in occupational settings (Goyal et al., 1992:109-112).

Generic Model Structure. PBPK models consist of a number of interconnected compartments which represent various organs and tissue groups. A classic graphical representation of a generic model, Figure 2.1, consists of an alveolar space, lung blood, fat tissue, muscle tissue, richly perfused tissue, and liver compartments (Clewell and Andersen, 1989:130). The liver compartment represents the primary mechanism for metabolism, governed by the Michaelis-Menten equation for TCE. This equation

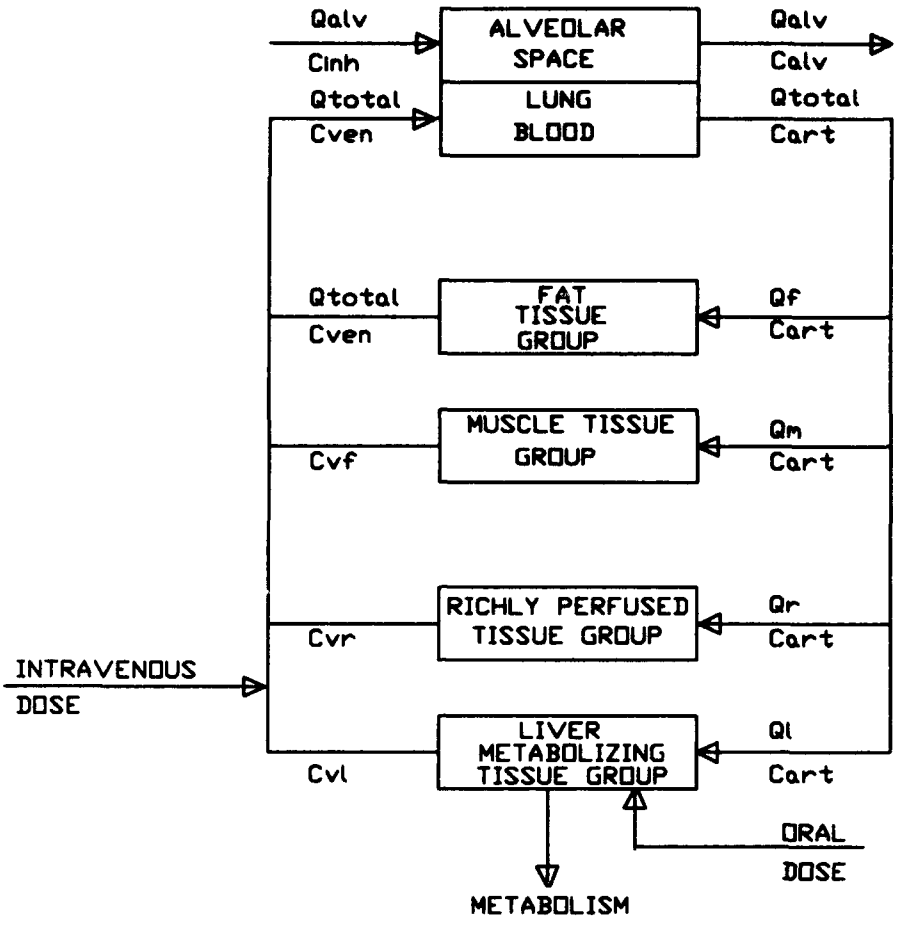


Figure 2.1.

Classical Model Structure. (Clewell and Andersen, 1989:130)

describes the metabolic rate, which is controlled by the maximum rate of metabolism (V_{max}) and Michaelis-Menten (K_m) constants. In simple terms, the liver's capacity to metabolize a chemical is limited by these constants. When a chemical's concentration rises beyond a certain point, the metabolic activity of the liver becomes saturated.

Model Development and Use. One of the unique characteristics of PBPK models is that they are largely independent of bioassay data; that is, the physiological model constants are developed based on existing knowledge of the specimen, past experimental data, or non-destructive lab techniques. After simulation, one of two results normally

occur. First, the model data may be totally unrepresentative of experimental data. In this case, the researcher is required to refine or redesign the model in order to capture the empirical behavior. In the second case, the model may generate results which are similar to the empirical data but require fitting. If fitting is required, the researcher identifies those parameters which often defy accurate measure in a laboratory setting and allows these constants to be varied by a curve fitting routine until a satisfactory fit is found. This technique can be helpful in determining the true values of certain kinetic constants. This iterative approach for model development (Figure 2.2) is useful for developing alternative explanations for chemical carcinogenicity (Clewell and Andersen, 1989:130).

PBPK Model Parameters. Parameters used in PBPK modeling can be divided into three major categories: physiological, kinetic or chemical specific, and thermodynamic. Physiological parameters include body weight, organ volumes, and flow rates. Kinetic parameters include metabolic and absorption/excretion constants. Finally, thermodynamic parameters include tissue solubility (partition coefficients) and binding coefficients (Leung, 1991:254).

Allometrics. Because a number of parameters are dependent on the mass of the biological specimen, it becomes necessary to develop models taking this dependent relationships into consideration.

> Perhaps no single factor is more dominant in constraining animal design than body size. Size-induced patterns have been identified for all aspects of animal design and function from structural dimensions, to life history characteristics, to pharmacokinetics. An animal's size is certainly among its most prominent of all distinguishing features. (Lindstede, 1987:65)

Body weight dependent parameters vary with specimen size and, therefore, must be scaled with an appropriate function which maps, or scales, the effected constants into their appropriate size range. This technique is called allometry and the mapping functions are called allometric equations. Parameters commonly scaled in this manner include

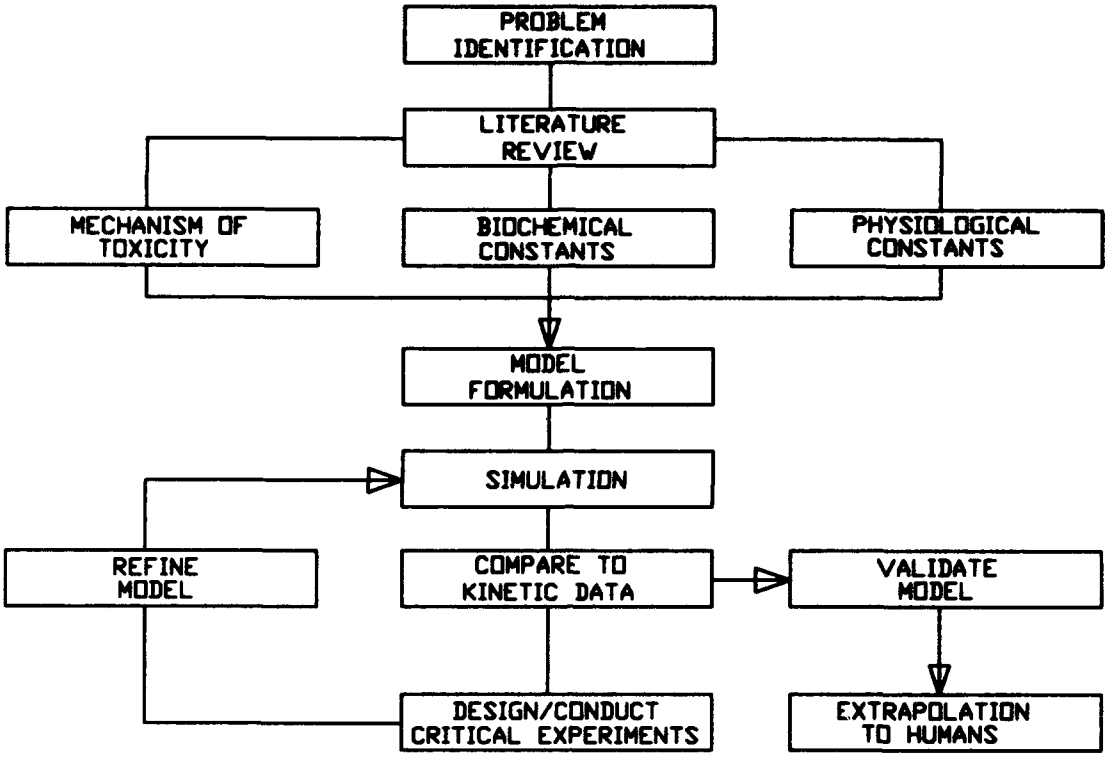


Figure 2.2. Model Development. (Clewell and Andersen, 1989:130)

ventilation rates, blood flows, volumes of distribution, maximum rates of metabolism, and organ size. Figure 2.3 shows an example of allometric relationships.

Allometric scaling provides a secondary benefit for developing computer source code in that body weight dependent parameters are automatically updated as body weight is changed. Therefore, by simply changing a single parameter, numerous others are automatically updated.

This scaling scheme has also been used from time to time as a technique for extrapolating dose-metrics from animals to humans. In one study, a lifetime human cancer risk of 1.7×10^{-6} was established for human inhalation of TCE by simply scaling up the mouse dose-metric by a ratio of mouse and human body weights (EPA, 1985).

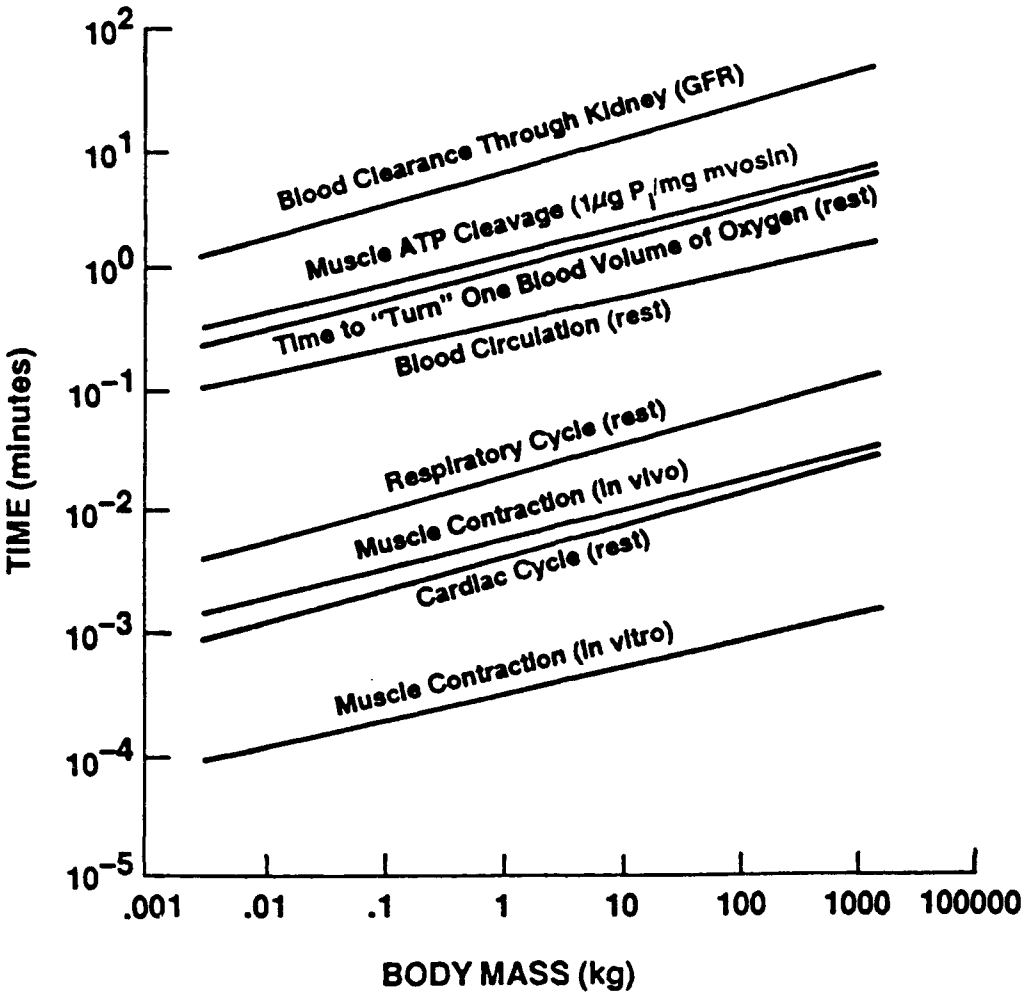


Figure 2.3 Allometric Scaling. (Lindstede, 1987:65).

This practice should only be used in the absence of other, more viable, techniques.

Dose-metrics. Dose-metrics, or dose surrogates, are the measure of interest when conducting or simulating an exposure scenario. Metrics often reflect concentrations in target organs, arterial blood, or venous blood. Other measures of interest include concentration-time products, or areas under the concentration curve. Areas under the curve are of interest because they indicate the opportunity for a toxicant to act in a specific part of the body. A metric which is almost always evaluated is amount of chemical metabolized. The amount of chemical metabolized will affect all concentrations associated with any metabolites. In many cases, the production of metabolites is of

primary concern because the metabolite, not the primary chemical, is thought be the cause of tumors. This is the case for TCE, whose major metabolites are trichloroacetic acid and dichloroacetic acid (Fisher and Allen, 1993:87)

PBPK and Hazard Evaluation. Toxicologists have increased their use of PBPK modeling techniques to refine hazard evaluation techniques. Because physiologically based models are structured around differential equations with physiological constants, simulation techniques generate data which reflect time course behavior of the chemical in target organs. This allows researchers to refine or "fit" the models to animal data, substitute human constants, and then generate human specific data. "This technique, called toxicokinetics at AAMRL, is used as an analytical tool for predicting the time-dependent uptake, distribution, metabolism, and excretion of potentially toxic chemicals and their metabolites in the body" (Clewell et al., 1988:A125). AAMRL refers to the U.S. Air Force's Armstrong Aerospace Medical Research Laboratory, now named the Occupational and Environmental Health Directorate of the Armstrong Laboratory. PBPK inter-species extrapolation, or toxicokinetics, coupled with two uncertainty analysis techniques, sensitivity analysis and Monte Carlo analysis, shall be used in this research effort to generate a Monte Carlo based risk assessment for TCE.

The number of PBPK models has grown significantly in the past decade. Of particular interest for this research effort are physiologically based pharmacokinetic models used to describe environmental toxicants. Specific to this research are models used to describe the time course behavior of TCE. The following section will describe chemical specific research efforts using PBPK models and will be followed by a section detailing TCE specific research. The specific model upon which this research is based will then be discussed.

Chemical Specific PBPK Research

Research has been carried out on the development of PBPK models for a wide range of chemicals and the use of those models to predict risk. Specifically, PBPK research has been done on 1, 4-Dioxane, chloroform, methylene chloride, diisopropylfluorophosphate, benzene, methyl chloroform, and styrene. This section will present only the most significant attributes of these research efforts.

Ramsey and Andersen, 1984. The PBPK model used in this study was for styrene. Research results of the styrene model show PBPK models can explain the relationship between blood concentration and inhalation of a chemical and that the model is useful to extrapolate from animal studies to humans. (Ramsey and Andersen, 1984:159-175)

Bogen and Hall, 1989. Bogen and Hall utilize a PBPK model to derive safe concentrations of non-carcinogens in drinking water. The research is unique in that it is the only study which dealt exclusively with threshold dose-response relationships. The chemical used in the study was methyl chloroform. (Bogen and Hall, 1989:26)

Shelley et al., 1989. Shelley et al. developed a PBPK model to examine the exposure of a nursing infant to volatile organics through its mothers occupational exposure. This unique PBPK model included a mammary compartment. The mammary compartment's output was directly connected with a gut compartment representing the infants GI tract. This interesting research showed how PBPK models can be customized to deal with a variety of situations. (Shelley et al., 1989:21-26)

Bois et al., 1990. A PBPK model was used to explore the carcinogenicity of benzene. The use of this model was significant because it did not use deterministic parameters. Instead, the model uses a range of values for the parameters. Monte Carlo simulations were used to randomly select parameter values and to calibrate the model. The Monte Carlo simulations were used to verify that, although unknown, accurate model parameter values were likely to be included in the ranges used in the study. The use of this

probabilistic determination of model parameters allowed the model to simulate a population in a more realistic manner. (Bois et al., 1990)

Gearhart et al., 1990. Gearhart et al. developed a PBPK model for the inhibition of acetylcholinesterase by Diisopropylfluorophosphate (DFP). This research demonstrated the possibility of using a PBPK model based on mammalian physiology and biochemistry to simulate in vivo data. (Gearhart et al., 1990)

Reitz et al., 1990a. Reitz et al. have developed a six compartment model for the disposition of 1, 4-Dioxane in rats, mice, and humans . The PBPK model was used to simulate an independent study. The model's predictions were "consistent" with the results of the independent study . An important aspect of this study is its use of experimentally measured partition coefficients. (Reitz et al., 1990a:41)

Reitz et al., 1990b.. A PBPK model has been developed by Reitz et al. for the prediction of liver cancer associated with human exposure to chloroform . Two different approaches were used for the hazard evaluation in the research. The first is the use of a "safety factor" based on a no observed effect level for liver tumors. The second is the calculation of lower confidence limits of exposed dose based on a non-threshold linearized multistage model. (Reitz et al., 1990b)

Clewell, 1992 and Andersen et al., 1986. Two separate studies were reviewed addressing methylene chloride. Both studies show that PBPK modeling consistently and significantly predict lower risks than those estimated by traditional risk assessment techniques. Both studies also support the use of dose-metrics linked to the mechanism of carcinogenicity to predict risk. (Clewell, 1992:129-137; Andersen et al., 1987:182-205)

Models examined for this thesis show the power of PBPK modeling in a wide variety of applications. This technique can successfully simulate experimental data, Monte Carlo analysis can provide insight into the possible range of dose-response relationships, and the technique can be used to more realistically extrapolate between animal bioassay data and

human exposure. In addition to this research, other research has been done on PBPK modeling of TCE.

TCE Specific Research Efforts Using PBPK Modeling

TCE is a common solvent once used by millions of workers on a daily basis. "It has been estimated that of 3.5 million workers believed to occupationally exposed to TCE in the U.S., at least 100,000 workers are exposed full-time, and that two-thirds of these are in work environments where there are not adequate control measures" (NIOSH, 1978). The trend in usage has been decreasing since 1978 because of increasing regulatory controls. The chemical has also been identified as a major contributing contaminant at a number of superfund sites across the country. Because of its widespread use in an occupational setting, its contribution as an environmental toxicant, and its classification as a carcinogen by the EPA, TCE has joined the ranks of numerous other chemicals attracting the attention of researchers. The following paragraphs outline several studies which use PBPK modeling techniques to evaluate the pharmacokinetics of TCE. The studies are by no means exhaustive but are representative of the subject.

Bogen, 1987. Bogen published an article wherein he discussed a PBPK approach for evaluating metabolism of TCE in humans. Bogen reviewed two previously used PBPK models for their effectiveness in determining TCE dose-metrics. The first model (Sato et al., 1977:56-63) was based on three compartments: richly perfused, poorly perfused and fatty tissues. Richly perfused tissues modeled both respiratory and metabolic elimination of the chemical. The second model (Fernandez et al., 1977:43-55) includes the same compartments as the Sato et al. model, but adds a liver and pulmonary compartment. Fernandez's model incorporates mass-balance of TCE in the compartments. Metabolism is limited by blood flow to the liver. The primary purpose of Bogen's study was to evaluate the Ramsey-Andersen PBPK model (Ramsey and Andersen, 1984:159-174) which introduces several different approaches to the pharmacokinetics of volatile organic

compounds. The addition of a Michaelis-Menten metabolism to the liver compartment established a concentration-dependent time rate change in metabolic activity. Bogen adapted the model and simulated the occupational exposure of Japanese workers to TCE, predicting urinary excretion concentrations. The results of Bogen's analysis indicated that 99.8 percent of TCE entering the blood was metabolized for workers exposed six days a week, seven hours a day. Predicted lifetime cancer risks varied between 3.9×10^{-8} and 1.1×10^{-5}. (Bogen, 1988:447-466)

Koizumi, 1989. Koizumi published an article outlining the use of the Ramsey-Andersen model to evaluate the potential of simulating TCE and Tetrachloroethylene (PERC) for a variety of exposure routes in both rats and humans. Specifically, inhalation, intravenous, drinking water for rats, and inhalation in humans were evaluated for TCE. The dose-metrics of interest for TCE were exhaled air and blood concentrations, to be used as a comparison to biologically permissible values in an occupational setting, and metabolized amounts for drinking water risk assessment. Simulation results were compared to Koizumi's experiment involving rats with free access to drinking water at a concentration 5µg/ml for 48 hours, historical occupational inhalation exposure at 100 ppm for humans (Sato and Nakajima, 1978:43-49), and 3 to 15 mg/kg intravenous exposures in rats (Withey and Collins, 1980:313-332). The drinking water simulation resulted in exhalation of 10.8% of the dose compared to an observed value of 14.5%. The inhalation simulation results are graphically presented and show similar curvilinear behavior; however, the final simulation and observed values at 14 hours are approximately .004 and .07 mg/l respectively. Finally, the intravenous simulations, again shown graphically, appear to show strong agreement with observed values. Total amount of TCE metabolized was used as an indicator for risk assessment. Using 32 ppb, the highest reported concentration of TCE for drinking water in the United States (EPA, 1975), Koizumi simulated a concentration scenario exposing humans to 32 ppb drinking water six hours per day, five days per week, for one year. The simulation resulted in total amounts

metabolized one-fifth lower than non-cancer causing levels reported in bioassays for rats exposed to 500 ppm over their lifetime (Henschler et al., 1980:237-248). No lifetime cancer risks were reported. (Koizumi, 1989:239-249)

Dallas et al., 1991. Based on limited studies involving the time-course of alveolar and blood levels during inhalation exposures to TCE, Dallas conducted a study to assess the accuracy of predictions for a PBPK inhalation model. The study assessed the PBPK model's ability to predict exhaled breath and blood concentrations for rats exposed to 50 and 500 ppm for two hours. A blood-flow limited PBPK model, implemented by Advanced Continuous Simulation Language (ACSL), was used for the simulation. Experimental results showed that TCE entered the blood in a relatively short time (1 minute). The 50 ppm exposure animals reached steady state in 25 minutes. 500 ppm exposed animals showed a steady increase in blood concentration over the entire two hour exposure. Concentration levels for the 500 ppm exposure were 25 to 30 times higher than for the 50 ppm exposures. This reflected metabolic saturation at the higher concentration exposure. PBPK simulations paralleled these findings and accurately predicted the metabolic saturation. (Dallas et al., 1991:303-314)

Fisher et al., 1991. Fisher et al. used a PBPK model to determine uptake and metabolism of TCE and the production of trichloroacetic acid (TCA), a major metabolite of TCE in mice and rats. A proportionality constant (Po) was used to link a classical one-compartment model, used to track the production and excretion of TCA, with a PBPK model for tracking TCE and TCA dose-metrics. In Vivo metabolic rate constants were determined for TCE in B6C3F1 mice; and the PBPK model was used to determine TCA yield in Fischer 344 rats and B6C3F1 mice. The model predictions for uptake of TCE in male and female rats were favorable; however, TCE clearance from the blood was less than experimental results. The model consistently over-predicted the level of TCE in mice at high concentrations (e.g. at 748 ppm, the observed and predicted values were 7.3 and 38 μg/ml respectively). The model was then used to determine, through optimization, the

range of values for Po. The results indicated that Po is much higher in rats than in mice (e.g. .18 at a concentration of 600 ppm for rats and .09 at a concentration of 889 ppm for mice). This finding is consistent with the results showing that TCA is the metabolite responsible for hepatocellular cancer formation in mice but not in rats (Herren-Freund et al., 1987:183-189). (Fisher et al., 1991:183-195)

Allen and Fisher, 1993. Allen and Fisher developed a PBPK model for TCE and its major metabolite TCA. Five published studies were used in an optimization process to determine the metabolic parameters for TCE and TCA. Other parameters were established by reviewing the appropriate literature. Reference data was derived from the five human studies (Monster et al., 1979:283-292; Monster et al., 1976:87-102; Muller et al., 1974:283; Muller et al., 1975:173; Stewart et al., 1970:64). The optimization results showed the yield of TCA from TCE (Po) to be .33 and the urinary excretion of TCA (PU) to be .93. Overall, the model's predictions were quite acceptable. Single six and four hour exposures at 100 and 140 ppm respectively showed good agreement (Figure 2.4). Simulations of repeated exposures of four hours at 70 ppm underestimated venous concentration by a factor of three. The one-compartment model for TCA adequately described the chemical's kinetics for plasma concentrations and cumulative urinary excretion, but underestimated peak TCA concentrations in one instance. Overall, the model's ability to predict TCE and TCA kinetics was excellent. (Allen and Fisher, 1993:71-86)

Uncertainty Associated With PBPK Model Based Risk Assessment

The primary purpose of this research effort is to perform a Monte Carlo based PBPK risk assessment for TCE and determine a reasonable format in which to present the results. To this point, a number of subjects related to PBPK have been discussed for the purpose of familiarization. The research objectives shall now be addressed in detail. First, in order to provide the reader with an appreciation for model uncertainty and the steps which will

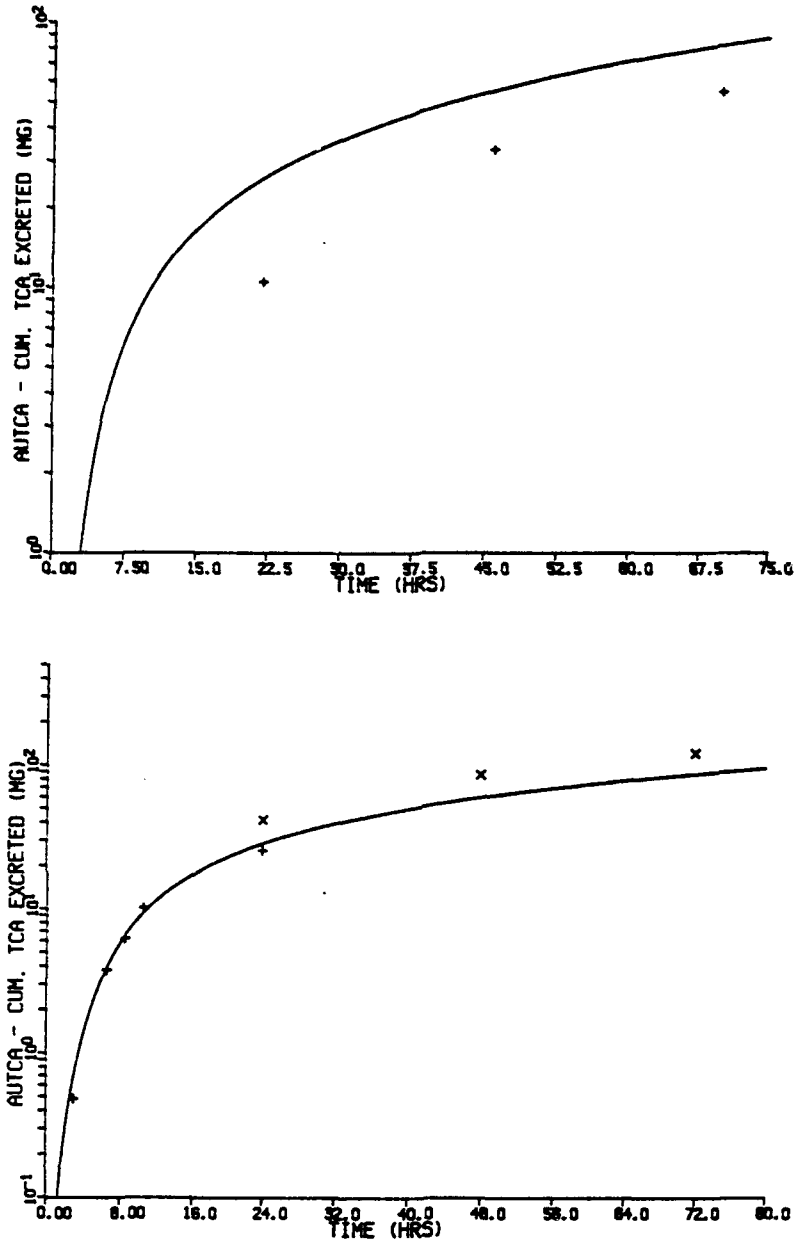


Figure 2.4. Model Predictions. (Allen and Fisher, 1993:79-80)

be discussed in the methodology section, sensitivity analysis and Monte Carlo analysis will be introduced. This introduction shall include several example studies which demonstrate the various ways researchers use these tools. Next, a deterministic PBPK study, upon which this research is based, will be discussed. This discussion will include a detailed description of the model used in the study, as well as the study's results.

Sensitivity Analysis. Sensitivity analysis can be described as the determination of how a system's output changes with respect to a small change in one or more of the input parameters.

One document (Simulation Resources, 1991) establishes two requirements for model validation; namely, the model should have enough sensitivity to reflect some change in the output no matter what the change in the input; and the model's output should not be overexcited by a small change in the input. This document defines two types of sensitivity. The first type is called point sensitivity and is defined as the change in a state variable's derivative at a single point in time with respect to a change in one or more parameters. In other words, the shape of a curve may vary with respect to a change in a parameter's value. An unreasonable change in the slope of a curve resulting from a small change in a parameter could indicate instability in a model. The second type of sensitivity is called trajectory sensitivity and reflects the change in a state variable over time with respect to a small change in a parameter. When one is interested in steady state conditions, like certain dose-metrics, this analysis probably provides more useful information than does point sensitivity. For example, if a researcher is interested in the maximum concentration levels in blood over a single exposure or steady state concentrations over long term repeated exposures, trajectory sensitivities would be of greater interest than point sensitivities. Conversely, if the dose-metric of interest is the rate of change over time, point sensitivities would be more appropriate. (Simulations Resources, 1991:47-49)

Clement International Corporation. Sensitivity analysis was used to evaluate a PBPK model for PERC. The sensitivity analysis was conducted by examining the output of the model as single parameter values were changed by one percent. In order to maintain mass-balance when a blood flow rate was changed by one percent, the entire cardiac flow rate had to be increased by one percent simultaneously. A relative percentage change in the dose-metric was recorded for each parameter change. An example of significant sensitivity was the effect the liver blood partition coefficient had on the dose-metric

AUCB (Area Under the Curve for Blood). This analysis was completed for several dose levels, routes, and species. (Clement International Corporation, 1990:VI-1, 2-10)

Bois et al., 1990. Sensitivity analysis was conducted on a PERC model by determining correlation coefficients which resulted from Monte Carlo Analysis. Examples of significant output changes were seen as a result of alveolar ventilation rates, blood-air partition coefficient, and maximum rate of metabolism. Bois et al. concluded that the multiple chamber model could be simplified by replacing the multiple chambers with a single compartment with reversible pulmonary exchange and Michaelis-Menten metabolism. (Bois et al., 1990:307-311)

Koizumi, 1989. In this study, sensitivity was determined for a PBPK model dose-metric of amount of TCE metabolized in 24 hours. This was done for inhalation exposure concentrations of 10 and 600 ppm for 6 hours in rats and 32 ppm water ingestion of two liters for humans. Koizumi's approach was to sequentially vary each parameter by 200 percent and record the corresponding change in the dose-metric value. One might question whether a change of 200 percent in each parameter is truly a sensitivity analysis; however, this approach clearly rules out those parameters whose changes have little or no impact on the output. (Koizumi, 1989:244-247)

Monte Carlo Analysis. There are few values which can be assigned to parameters with certainty. Variability in parameter value contributes considerable uncertainty to the outcome of any modeling effort. This is especially true when the end result of a study is a single, deterministic value. Not only do parameters display natural variability, they also often display some probabilistic distribution. As the number of parameters required to describe a system becomes larger, the uncertainty associated with the system's output grows. Uncertainty is precisely the reason that an analysis technique should be used to capture as much quantitative information as possible, about the uncertainty, for presentation of results. By minimizing qualitative discussion and presenting quantitative probabilistic results in a clear manner, decision makers can make more informed, rational

decisions about exposure and cleanup standards. Monte Carlo analysis can be used to provide this information.

Monte Carlo analysis for PBPK models involves repeating simulations for each member of a sample frame. Each parameter displaying variability is treated as a random variable and is randomly sampled according to its mean and distribution (Sobol, 1984:8-62). The combination of parameters can be thought of as an n-dimensional vector which changes orientation during each random sample period. Each vector represents a probabilistic combination of parameters which generates an output dose-metric. As one might imagine the output takes on its own distribution.

During the course of this literature review, two examples of probabilistic results associated with PBPK models were found.

Bois et al. Bois et al. demonstrated the use of Monte Carlo analysis in the evaluation of rate of metabolism versus human body weight, blood/air partition coefficient, maximum rate of metabolism, and affinity constant. The results (Figure 2.5) clearly demonstrate the lack of quantitative information presented by deterministic studies. That is, the distribution of parameters effects risk. This information is not represented in deterministic studies. (Bois et al., 1990:307-312)

Woodruff et al. Woodruff et al. used Monte Carlo analysis as a basis for evaluating both a three and five compartment PBPK model and a different classical compartmental model. The purpose of the study was two-fold. The first purpose was to determine how good PBPK models are for predicting dose-metrics. Secondly, output variability was to be evaluated based on the number of parameters in the models. First, Monte Carlo sampling was used to generate kinetic parameters for each model. The Monte Carlo method randomly selects parameter values based on their mean, variance, and type of distribution. The kinetic parameters were allowed to vary until each model was able to predict the results of individual experiments. The next step was to use the calibrated models to simulate two hypothetical experiments, one for gavage and one for inhalation.

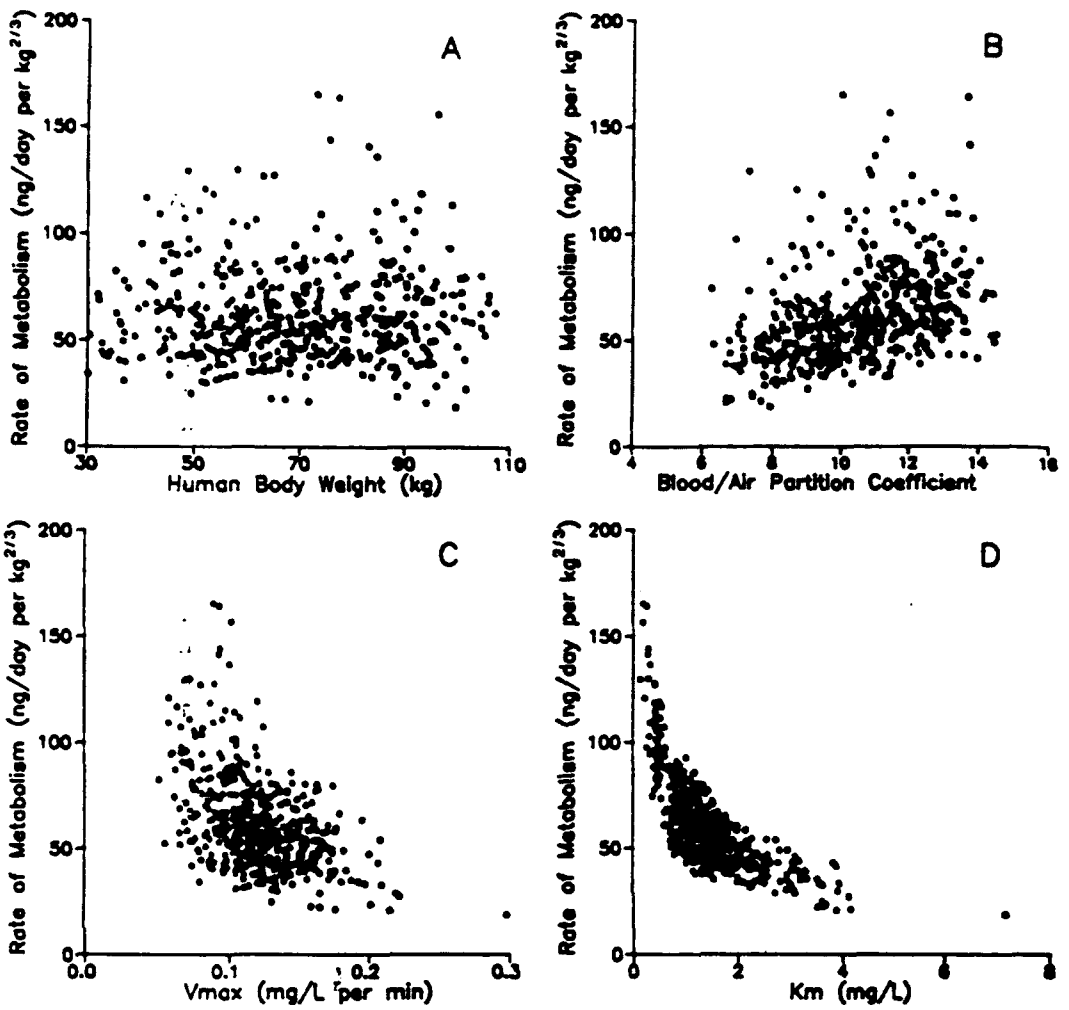


Figure 2.5. Results from Bois et al. (Bois et al., 1990)

The variability of each model was then displayed using box and whisker plots. A major result was that, even though there were differences in predictions across models, the individual model variability across exposure concentration scenarios was much greater than the inter-model differences. (Woodruff, et al., 1992:189-201)

Research Specific Study

In 1993, Fisher and Allen published an article which described the use of a PBPK model for mice to simulate inhalation and gavage experiments for specific exposure

concentrations of TCE. The dose-metrics evaluated included total amount of TCE metabolized (AMET mg/kg/day) and time factored body concentration of TCA (AUTCA mg/L/day). As the reader might remember, TCA, or trichloroacetic acid, is a major metabolite of TCE and is suspected as the cause of hepatocellular carcinomas in mice. The values of the dose-metrics were determined which corresponded to a one in one million lifetime cancer risk for mice. Once these dose-metric values were determined, a human TCE model was used to determine the exposure concentrations in air and water which correspond to the dose-metric values found in mice.

Two cancer bioassays were used as a basis for incidence data, one inhalation and one gavage study (National Cancer Institute, 1976:76-802; Maltoni et al., 1986). The model structure is shown in Figure 2.6, model differential equations in Appendix J, and model parameter values in Table 2.2.

The TCE inhalation simulation was conducted over a seven day period. Both male and female B6C3F1 mice were exposed five consecutive days per week for gavage and five consecutive days per week, seven hours per day for inhalation.

Table 2.3 shows the quantitative results for inhalation of TCE and the corresponding simulated dose-metrics for mice. Table 2.4 shows the results for gavaged mice. The AMET dose-metric clearly shows a correlation with cancer incidence. AUCTCA showed the strongest correlation with a .95 correlation coefficient for both routes of exposure in female mice. Conversely, little correspondence is seen for male mice exposed by inhalation.

Because both AMET and AUCTCA showed strong correlation with cancer incidence in mice, these dose-metrics were selected as candidates for the human simulation. Each metric's 95% lower bound was selected as a "deterministic" value and used as target values in determining human exposure concentrations. Inhalation and ingestion exposure concentrations were varied until the corresponding dose-metric values were achieved. The results indicate that, for persistent inhalation, exposures of 10-15 ppb AMET and 1

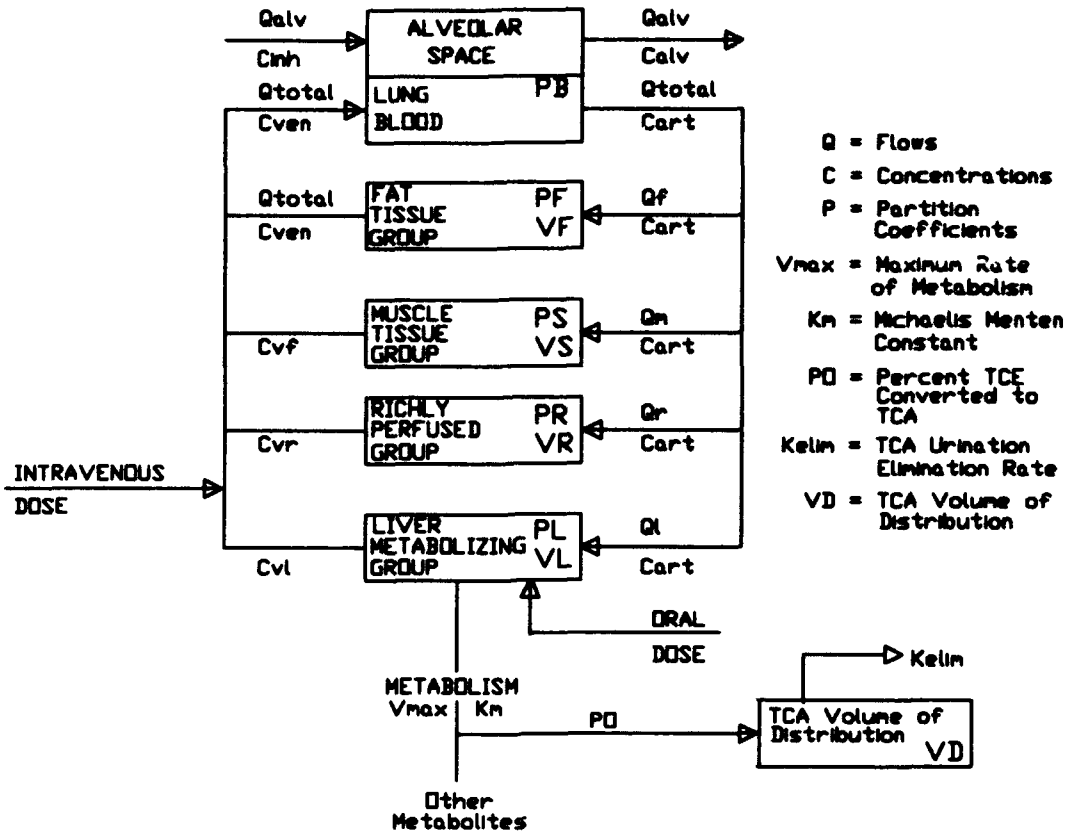


Figure 2.6. Model Structure

ppb AUCTCA; and, for intermittent inhalation, 42-69 ppb AMET and .2 ppb AUCTCA correspond to a one in one million excess risk of liver cancer in humans. Drinking water concentrations for the same risk was estimated to be 7-39 μg/L AMET and 4 ηg/L AUCTCA.

EPA standards (EPA, 1985) for this same risk level for AMET are greater than the PBPK predictions by a factor of two for ingestion of water and a factor of 71 for continuous inhalation. EPA standards for AUCTCA are greater than the PBPK results by a factor of 775 for ingestion of water and a factor of 1.4 for continuous inhalation. Based on these results, Fisher and Allen suggest that the next risk assessment for TCE should be

Table 2.2

Physiological and Kinetic Model Parameter for Mice and Humans.

(Fisher and Allen, 1993:89)

	Female Mice	Male Mice	Humans
Physiological Parameters			
Tissue Group (fraction of body wt)			
Liver	0.04	0.04	0.026
Richly Perfused	0.05	0.05	0.050
Slowly Perfused	0.72	0.78	0.620
Fat	0.10	0.04	0.190
Flow (L/hr)			
Alveolar Ventilation	$30 \cdot bW^{0.74}$	$30 \cdot bW^{0.74}$	$12.6 \cdot bW^{0.74}$
Cardiac Output (CO)	$30 \cdot bW^{0.74}$	$30 \cdot bW^{0.74}$	$14.9 \cdot bW^{0.74}$
Tissue Group (fraction of CO)			
Liver	0.24	0.24	0.26
Richly Perfused	0.52	0.52	0.44
Slowly Perfused	0.19	0.19	0.25
Fat	0.05	0.05	0.05
Kinetic Constants			
Partition Coefficients			
Liver/Blood	1.62	2.03	6.82
Richly Perfused/Blood	1.62	2.03	6.82
Slowly Perfused/Blood	0.48	1.00	2.35
Fat/Blood	31.4	41.3	73.3
Blood/Air	14.3	13.2	9.20
TCE Metabolic Rate Constants			
V_{maxc} (mg/kg/hr)[a]	23.2	32.7	14.9
K_m (mg/L)	0.25	0.25	1.5
TCA Kinetic Constants			
Inhalation			
VDC (L/kg)	0.176	0.238	0.34- 0.34·bW
K_{el} (/hr)	0.104	0.043	0.029
PO[b] (unitless)	0.18 - 0.07	0.13 - 0.07	0.0336
Gavage			
VDC (L/kg)	0.176	0.238	
K_{el}[c] (/hr)	0.062 (0.003)	0.028 (0.002)	
PO (unitless)	0.09	0.06	
$K1$[c] (/hr)	0.9 (0.110)	1.1 (0.071)	5.5

[a]Scaled as $bW^{0.74}$.

[b]PO values determined for a range of TCE exposure concentrations

[c]The value in parenthesis is the computer-generated standard deviation for the optimized parameter. K_{el} is the plasma elimination rate constant for TCA and K_1 is the "effective" TCE gastrointestinal uptake rate constant.

Table 2.3

Dose-metric Values for TCE Vapor Exposures in B6C3F1 Mice.

(Fisher and Allen, 1993:91)

		Dose-Metric		
TCE Exposure (ppm)	Liver Cancer Incidence	AMET (mg/kg/day)	FTCA (mg/kg/day)	AUCTCA (mg/L/day)
Female Mice (PO)				
600.0 (0.08)	9/87	285.7	28.4	553.0
300.0 (0.07)	4/89	249.7	21.7	422.8
100.0 (0.18)	3/90	111.5	25.0	485.5
0.0	2/90	---	---	---
Male Mice				
600.0 (0.07)	6/88	355.9	31.0	1112.7
300.0 (0.13)	3/88	301.3	58.5	1740.9
100.0 (0.11)	1/86	108.4	14.8	530.0
0.0	1/85	---	---	---

Table 2.4

Dose-metric values for B6C3F1 Mice Gavaged with TCE.

(Fisher and Allen, 1993:91)

		Dose-metric		
TCE Dose (mg/kg)	Liver Cancer Incidence	AMET (mg/kg/day)	FTCA (mg/kg/day)	AUCTCA (mg/L/day)
Male Mice				
2339.0	31/48	211.4	15.8	857.2
1169.0	26/50	176.5	13.2	715.5
0.0	1/20	---	---	---
Female Mice				
1739.0	11/47	196.2	21.0	695.6
869.0	4/50	158.7	17.7	562.7
0.0	0/20	---	---	---

based on a quantitative mechanistic or biologically based approach and not on the linearized non threshold model.

Summary

The review of the literature indicates that PBPK modeling has the capability to improve the risk analysis process for trichloroethylene. The improvement can be made because of the ability of PBPK modeling to eliminate the need for dose extrapolation and, along with the Monte Carlo Method, to quantify some of the uncertainty of the risk analysis. This research will use both sensitivity and Monte Carlo analysis to repeat the PBPK simulations performed in this study, thereby capturing the uncertainty associated with selected dose-metrics. The end result will be a probabilistic range of vapor inhalation and water ingestion concentrations which lead to a one in one million lifetime cancer risk in humans. A detailed methodology is presented in the next chapter.

III. *Methodology*

Introduction

This research effort will be carried out using a validated PBPK model for trichloroethylene (TCE). TCE is the chemical of choice because of the availability of research data and completed PBPK modeling efforts. The methodology will consist of two steps. The first step will be to conduct a sensitivity analysis of the model. The second step will be to determine the range of human TCE exposures which result in a $1x10^{-6}$ human cancer risk. The methodology will begin with the assumptions made in conducting the research. The exposure scenarios to be simulated will be described in the following section. After discussing the exposure scenarios, sensitivity analysis methods will be presented. Next, the authors will present the process which will be used to determine the range of TCE exposures which can be expected to produce a $1x10^{-6}$ risk of human cancer. The methodology will conclude with a brief summary.

Assumptions

Before the methodology is detailed, several key assumptions must be stated.

- TCE is a human carcinogen. The chemical displays non-threshold behavior, therefore the linearized multi-stage risk model will be used.
- The PBPK models are valid. The models were developed and validated for studies to simulate the effects of exposure to TCE.
- The physiological parameters are representative of male and female mice and humans. These parameters have been provided by the Toxicology Division of the Armstrong Laboratory and represent the cumulative work and experience of a number of recognized toxicokinetic researchers.
- The concentrations of dose-metrics have the same cancer causing potential

in humans as in mice. This assumption is generally accepted by both regulatory agencies and researchers.

Exposure Scenarios

In conducting the research the authors will duplicate the exposure scenarios used by Fisher et al.. These scenarios are based on the animal bioassays utilized by the EPA in their evaluation of human cancer risk due to exposure to TCE. This section will present each of the exposure scenarios to be used in the research. Description of scenarios will begin with inhalation exposures for mouse and human exposure and then proceed to the exposure scenarios for ingestion.

Inhalation Exposures. Simulated mouse inhalation exposures to TCE will be based on the Maltoni et al. TCE inhalation bioassay (Maltoni et al., 1986). The scenario will consist of an exposure to TCE for 7 hours per day, 5 days per week, with weekends off.

Human inhalation will be simulated as both an intermittent and a continuous exposure. The intermittent scenario will consist of exposure to TCE for 7 hours per day for 5 days per week. The continuous exposure will simulate human inhalation of TCE for 24 hours per day, seven days per week. The intermittent exposure is intended to replicate occupational exposure conditions while the continuous scenario is one which might be expected in a residential setting.

Ingestion Exposures. Mouse ingestion scenarios will duplicate those used by the 1976 NCI gavage bioassay dosing schedule (NCI, 1976). The NCI schedule consisted of dosing the animals for 5 days per week with weekends off. The daily exposure consisted of a single bolus dose.

Human ingestion of TCE contaminated water will simulate a residential exposure. The model will simulate drinking four one half liter ingestions. The total consumption of 2 liters will occur over 12 hours, with the one half liter ingestions occurring every three hours. Fisher and Allen note that there is no significant difference between a single bolus

dose and the approach used in this methodology (Fisher and Allen, 1993:93). The multiple ingestion approach will be used in this research, however, as it represents a more realistic scenario.

Dose-Metric Selection

Dose-metrics were selected on the basis of the 1993 Fisher and Allen study (Fisher and Allen, 1993). The dose-metrics found to be significant were the average daily lifetime amount of TCE metabolized (AMET) and the average daily lifetime area under the curve TCA (AUCTCA) (Fisher and Allen, 1993:91-93). These two dose-metrics will be the focus of this thesis.

Sensitivity Analysis

To investigate uncertainty, it will be necessary to conduct a sensitivity analysis. The analysis will provide insight about the relation of model output to changes in input parameters. A parameter which has a relatively large impact on model output, but for which the value is not reasonably known will contribute significantly to uncertainty.

The analysis will be carried out by first determining when the model reaches steady state conditions with respect to dose-metrics. This value of time will then be used for all model simulations. Actual model simulations will be conducted using Advanced Continuous Simulation Language (ACSL) (Mitchell and Gauthier Associates).

A baseline model run, using mean parameter values, will be accomplished for each population, exposure scenario, and concentration. Each physiological and kinetic parameter in Table 3.1 will be individually increased above their mean value by one percent. Subsequent model runs will then be made for each parameter. The resulting dose-metric value corresponding to the change in a particular parameter will then be compared to the dose-metric value resulting from the baseline simulations, and the

Table 3.1

Parameter Means and Distributions. (Fisher and Allen, 1993:89)

		FEMALE MICE		MALE MICE		HUMANS	
PARAMETER	**DISTRIBUTION TYPE**	**MEAN**	**S.D. (%)**	**MEAN**	**S.D. (%)**	**MEAN**	**S.D. (%)**
PHYSIOLOGICAL GROUP							
Tissue Group (fraction BW)							
Liver (VLC)	DIRICHLET	0.04	15	0.04	15	0.026	*15
Richly Perfused (VRC)	DIRICHLET	0.05	19	0.05	19	0.05	*19
Slowly Perfused (VSC)	DIRICHLET	0.72	25	0.78	25	0.62	*25
Fat (VFC)	DIRICHLET	0.1	30	0.04	30	0.19	*30
Carcass (VCARC)	DIRICHLET	0.09	10	0.09	*10		*10
Flows (L/Hr)							
Alveolar Ventilation (QPC)	NORMAL	30	56	30	56	12.6	16
Cardiac Output (QCC)	NORMAL	30	8	30	8	14.9	10
Tissue Group (Fraction of QCC)							
Liver (QLC)	DIRICHLET	0.24	*10	0.24	*10	0.26	*10
Richly Perfused (QRC)	DIRICHLET	0.52	*10	0.52	*10	0.44	*10
Slowly Perfused (QSC)	DIRICHLET	0.19	*10	0.19	*10	0.25	*10
Fat (QFC)	DIRICHLET	0.05	*10	0.05	*10	0.05	*10
KINETIC GROUP							
Partition Coefficients							
Liver/Blood (PL)	NORMAL	1.62	16	2.03	16	6.82	15
Richly Perfused/Blood (PR)	NORMAL	1.62	15	2.03	15	6.82	18
Slowly Perfused/Blood (PS)	NORMAL	0.48	15	1	15	2.35	15
Fat/Blood (PF)	NORMAL	31.4	12	41.3	12	73.3	15
Blood Air (PB)	NORMAL	14.3	16	13.2	16	9.2	20
TCE Metabolic Rate Constants							
Maximum Rate of Metabolism (Vmaxc) (mg/kg/Hr)	LOG NORMAL	23.2	30	32.7	30	14.9	30
Michaelis-Menten Constant (Km) (mg/L)	LOG NORMAL	0.25	30	0.25	30	1.5	30
TCA Kinetic Constants							
Inhalation							
Volume of Distribution for TCA (VDC) (L/Kg)	LOG NORMAL	0.176	*10	0.238	*10	.34-.0034BW	N/A
TCA Elimination Rate Constant (Kel) (1/Hr)	LOG NORMAL	0.104	*10	0.043	*10	0.029	*10
TCA Conversion Constant (PO)	LOG NORMAL	0.18 (100PPM)	*10	0.11 (100PPM)	*10	0.0336	*10
		0.07 (300PPM)	*10	0.13 (300PPM)	*10		
		.08 (600PPM)	*10	0.07 (600PPM)	*10		
Gavage							
Volume of Distribution for TCA (VDC) (L/Kg)	LOG NORMAL	0.176	*10	0.238	*10	.34-.0034BW	N/A
TCA Elimination Rate Constant (Kel) (1/Hr)	LOG NORMAL	0.062	0.3	0.028	0.2	0.029	*10
TCA Conversion Constant (PO)	LOG NORMAL	0.09	*10	0.06	*10	0.0336	*10
First Order Uptake Rate Constant (K1) (1/Hr)	NORMAL	0.9	11	1.1	7.1	5.5	*10

*Indicates an assumed distribution

percentage change determined. In this manner all parameter sensitivities can be directly compared.

Because blood flows must sum to the cardiac output, when one flow is increased the others must be decreased to compensate. The model scales the flow to richly perfused tissue according to the cardiac output and the flow to the liver. The flow to the slowly perfused tissue is scaled by the flow to the fat compartment and cardiac output. To obtain a sensitivity for these flows, the authors will increase the flow to the liver and the fat by one percent. Sensitivity results will only be presented for the liver and fat blood flows. Those sensitivities will take into account an increase in the flows to the liver and fat along with a corresponding decrease in the flows to the richly perfumed and slowly perfused tissues respectively.

The results of the sensitivity analysis will be entered into a spreadsheet and graphs will be produced for dose-metrics and exposure levels. The exposure levels used in the sensitivity analysis will correspond to the exposure bioassay for the mice and the anticipated exposure levels in human. These anticipated dose levels will be derived from the 1993 Fisher and Allen study. Table 3.2 presents the TCE exposure concentrations used in the sensitivity analysis for mice and humans.

TCE Exposure Concentration Derivation

The final part of the thesis effort will be to determine the concentrations of TCE exposure which results in $1x10^{-6}$ risk of cancer to humans. This will be done by using the PBPK_SIM program. This is a program developed for the United States Air Force for Monte Carlo based risk calculations.

The initial work in this portion of the research will consist of using the Monte Carlo algorithm in PBPK_SIM to generate random sets of animal parameters. This random sampling of parameters will be based on the mean of each parameter and the standard

Table 3.2

Bioassay Exposure Concentrations. (Fisher and Allen, 1993:91)

Inhalation(ppm)			Ingestion (ppm)		
Male Mouse (PO)	Female Mouse (PO)	Human	Male Mouse	Female Mouse	Human
600(0.07)	600(0.08)	0.1	2339	1739	0.01
300(0.13)	300(0.07)	0.01	1169	869	0.001
100(0.11)	100(0.18)	N/A	N/A	N/A	N/A

deviations from that mean. The parameters which will be varied, along with the type of distribution assumed, and their standard deviations are shown in Table 3.1. These distributions were provided to the researchers by researchers at the Armstrong Laboratory based on their experience with PBPK modeling of TCE exposure. Very little information was found in the literature review to suggest proper distribution types for these parameters. Where information on variance was available for mice, but not humans, the variance for the mouse parameter was assumed for the human. Where no information on parameter variance was available, the authors have assumed at 10% variance.

Due to time constraints, along with the need for a large sample size, the authors have chosen to generate 200 randomly selected animals for each exposure scenario. These animals will be used to predict dose-metric values, using the ACSL program.

The next step in the process will be to use the randomly generated animals to predict the dose-metric values at exposure concentrations used in the bioassays. This will result in two hundred dose-metric values for each dose level, one for each animal.

The authors will use the 200 human parameter sets selected by the Monte Carlo method to predict 200 dose-metric values for humans at differing exposure scenarios. The 200 values of the human dose-metric predicted by the model, in conjunction with the

mouse dose-metric values, and their corresponding cancer incidence rates, will be input to PBPK_SIM. This is possible based on the assumption that the same concentration of dose-metric that produces cancer in the mouse will produce cancer, at the same rate of incidence, in humans. The process will be continued until the exposure concentration of TCE relating to a $1x10^{-6}$ risk of cancer is found for the upper and lower 95% confidence interval (CI) and the mean. Thus, three concentrations of TCE will be determined for each mouse model, each dose-metric, and each exposure scenario. A schematic of the process is shown in Figure 3.1.

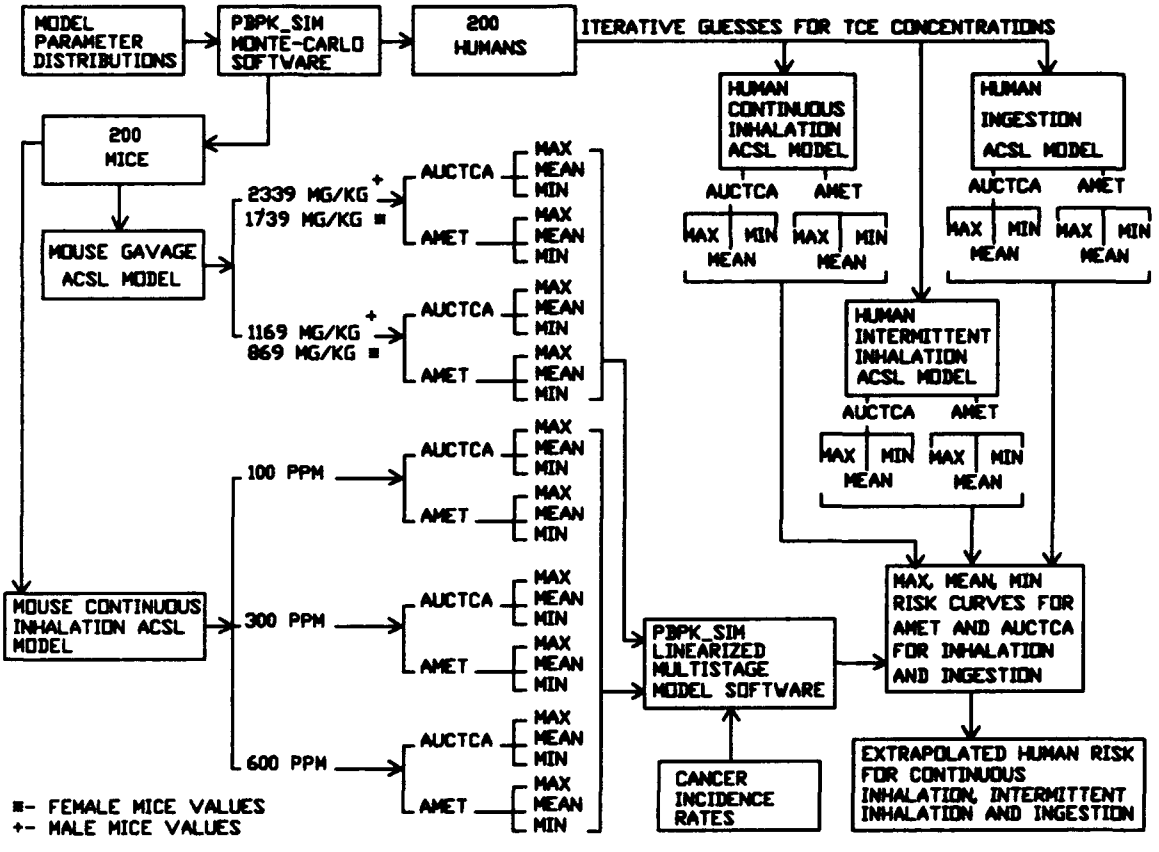


Figure 3.1. Schematic of TCE Concentration Determination Process.

The three exposure levels will correspond to an expected $1x10^{-6}$ risk of cancer. The upper 95% CI risk relates to those people who have parameter values which subject them to a higher than average risk of cancer. The risk at the upper 95% CI can be compared to the EPA standards. The lower 95% CI suggests individuals who have parameter values which make them robust to TCE exposure. The mean value would be that at which most of the population would be expected to experience a $1x10^{-6}$ risk of cancer. The three TCE concentrations, taken together, will provide information as to the uncertainty of the model. The greater the spread of TCE concentration, the more uncertainty exists. Some concept of a standard deviation in risk may be drawn from the concentrations.

The end result of this process will be a "range" of TCE exposure concentrations corresponding to a $1x10^{-6}$ cancer risk in humans. Each of the ranges will be plotted against the concentrations found by Fisher and Allen (for comparison). The ranges will finally be used to draw conclusions about uncertainty of the risk and how to present that uncertainty.

Summary

In order to quantify parameter uncertainty, the authors will conduct a sensitivity analysis along with Monte Carlo based simulations. This will identify those parameters which have the greatest impact on model output as well as quantify the impact of natural variation of parameter values has on risk.

IV. Data Description and Analysis

Introduction

This chapter will present the raw data generated during the execution of the methodology. The authors will first present the information resulting from the sensitivity analysis. The ranges of TCE exposure concentrations determined will then be presented. The chapter will conclude with a brief summary

Sensitivity Analysis Results

The results of the sensitivity analysis will be presented by route of exposure. Inhalation results for male mouse, female mouse, and human exposure will be discussed first followed by the results of the ingestion/gavage sensitivity analysis. For the purposes of this research, any sensitivity greater than 0.5% will be considered significant.

Inhalation. The results of the inhalation sensitivity analysis show a strong correlation between the male and female mouse model. This is not unexpected as the models vary only in the values of their input parameters. Table 4.1 presents the results of the analysis. As can be seen from the inhalation sensitivities, the significantly sensitive parameters for the two mouse genders are almost identical. The graphs of the sensitivities in appendices B and C also show this correlation. Overall, the most sensitive parameters are the maximum velocity of metabolism (VMC), the TCA elimination rate constant (KUPC), the alveolar ventilation rate (QPC), the cardiac output (QCC), and the volume of distribution of TCA (VDP). The AMET dose-metric in the mouse models was most significantly effected by the parameters VMC and QPC, with the female model showing sensitivity to the QCC parameter also. The AUCTCA dose-metric, on the other hand, was significantly sensitive to VMC, KUPC, QPC, and VDP, with the female model again showing sensitivity to the QCC parameter.

Table 4.1

Sensitive Parameters for Inhalation (>0.5%)

Parameters	*Male Mouse*	*Female Mouse*	*Cont. Human*	*Int. Human*
VMC	A T	A T		
KUPC	T	T	T	T
QPC	A T	A T	A T	T
QCC		A T		
BW			T	T
VDP	T	T		

A=AMET, T=AUCTC

The most significant correlation between the two models is a relatively large negative sensitivity to the parameters KUPC and VDP with respect to AUCTCA.

The human inhalation model was run under two different scenarios, intermittent and continuous. The graphs of the sensitivities for the two models are presented in appendices D and E respectively. The results of the analysis show little difference in the sensitivities of the two models, therefore they will be addressed at the same time. For AMET, the only parameter with a sensitivity of more the 0.5 percent is the alveolar ventilation rate (QPC). The blood/air partition coefficient (PB), VMC, KM, QCC, and body weight (BW) parameters all exhibit sensitivities in the 0.1-0.3 percent range. AUCTCA was most effected by the change in BW, with an increase of over 2%. The parameters KUPC and QPC also showed effects greater than 0.5%.

Overall, AMET showed a sensitivity to QPC in all of the models. This is not unexpected considering the route of exposure.

The dose-metric AUCTCA was found to be sensitive to QPC and KUPC in all models. Again, this sensitivity is not unexpected. For inhalation, the parameters which show no significant sensitivity (<0.5%) are the liver partition coefficient (PL), PB, slowly perfused partition coefficient (PS), fat partition coefficient (PF), Michaelis Menten metabolism constant (KM), volumes of all compartments, the liver blood flow (QLC), and the fat blood flow (QFC).

Ingestion. The results of the ingestion sensitivity can be found in Table 4.2. The graphs used to obtain the information in this table are in appendices F, G, and H. As with the inhalation route, the two mouse models showed almost identical responses as far as sensitivities greater than 1/2%. The mouse models parameters showing significant responses were VMC, KUPC, VDP, and the first order uptake rate constant (K1). AMET was affected by VMC and K1 in both models. The AUCTCA dose-metric was shown to be sensitive to VMC, KUPC, and VDP, with the male model also showing sensitivity to K1. As in the inhalation models, the strongest correlation between the mouse ingestion models is their identical response to changes in the parameters KUPC and VDP. The sensitivities are almost identical to those in the inhalation models.

The human ingestion model was run for a continuous exposure scenario, as discussed in the methodology. AMET was overwhelmingly affected by body weight. AUCTCA was affected by both KUPC and BW. It is readily evident that body weight is **the** parameter to be concerned with in the human ingestion model.

The parameters to which the ingestion models were not sensitive are identical to those discussed in the inhalation section.

Overall. The results of the sensitivity analysis show similar behavior across dose levels. Graphs showing this similarity are included in the appendices.

Table 4.2

Sensitive Parameters for Ingestion (>0.5%)

Parameter	Male Mouse	Female Mouse	*Human*
VMC	A T	A T	
KUPC	T	T	T
BW			A T
VDP	T	T	
KI	A T	T	

A=AMET, T=AUCTCA

Monte Carlo Analysis Results

This section will discuss the data resulting from Monte Carlo based simulations of the mouse inhalation and gavage bioassays and corresponding statistical risk analysis for human inhalation and ingestion simulations. Each dose-metric discussion will include a comparison of the Monte Carlo based simulations to the Fisher-Allen study, correlations between mouse dose-metric values and the bioassay cancer response data, and the TCE exposure ranges corresponding to 10^{-6} excess lifetime cancer risk in humans.

Mouse Dose-Metric Values and Baseline Comparison for AMET. Tables 4.3. and 4.4. present a comprehensive view of all dose-metric values associated with the mouse bioassay simulations. TCE exposure levels, liver cancer incidence, and dose-metric values are shown from left to right. For each exposure level, maximum, minimum, mean, and Fisher-Allen study results are shown. The maximum value represents animals among the sample population whose physiological make-up (i.e. their selected parameter values) was conducive to the metabolism of TCE and/or the production of TCA. Conversely, the minimum values represent the most resilient mice among the sample population.

For the dose-metric AMET, there is a strong correlation between the Monte Carlo generated mean values and the Fisher-Allen results. Tables 4.3. and 4.4. clearly demonstrate this. This isn't surprising considering the mean values published by Fisher-Allen were used to generate the mouse parameter vectors used for the Monte Carlo uncertainty analysis.

Cancer Incidence Correlations for AMET. Table 4.5. shows correlation coefficients associated with mouse TCE exposures, mouse dose-metric values, and mouse bioassay cancer incidence rates. Coefficients were generated for the maximum, minimum, and mean values associated with each exposure level. The Fisher-Allen dose-metric values are also included. Of greatest interest are the coefficients for cancer incidence vs. dose-metric value at each exposure level.

For inhalation, the maximum AMET vs. cancer coefficients are .9815 and .9254 for the female and male mouse respectively. The minimum AMET vs. cancer coefficients are somewhat less at .7997 and .8602, while the mean value coefficients are .8427 and .9009. These values indicate a strong correlation between AMET values generated by the inhalation simulations and cancer responders associated with each exposure/dose level.

Gavage maximum AMET vs. cancer coefficients are .9192 and .9999 for the female and male mouse respectively. For minimum AMET vs. cancer, the coefficients are .5734 and .9997, whereas the mean values are .8763 and .9994. The gavage coefficients for maximum and mean AMET values also show strong correlations with cancer incidence.

Human Cancer Risk Results for AMET. Human TCE exposure values corresponding to 10^{-6} risk for AMET are graphically and quantitatively reflected in Figure 4.1. The corresponding dose-metric values which yield this risk are found in Table 4.6. AMET values corresponding to 10^{-6} risk using male mouse inhalation results are 40 to 120 ppb for intermittent inhalation compared to 69 ppb in the Fisher-Allen study, and 9 to 26 ppb for male mouse continuous inhalation compared to 15 ppb in Fisher-Allen. For female

Table 4.3.

Mouse Inhalation Dose-Metric Values

TCE EXPOSURE PPM)	LIVER CANCER INCIDENCE	AMET (MG/KG/DAY)	AUCTCA (MG/L/DAY)
FEMALE MICE			
600 PPM	9/87		
MAXIMUM		789.64	1619.4
MINIMUM		143.1	271.14
MEAN		296.17	558.09
FISHER		285.7	553
300 PPM	4/89		
MAXIMUM		362.2	681.19
MINIMUM		125.68	175.5
MEAN		238.97	369.09
FISHER		249.7	422.8
100 PPM	3/90		
MAXIMUM		178.61	814.66
MINIMUM		60.892	203.74
MEAN		111.3	474.2
FISHER		111.5	485.5
0 PPM	2/90	N/A	N/A
MALE MICE			
600 PPM	6/88		
MAXIMUM		633.38	2035.2
MINIMUM		179.05	456.72
MEAN		361.66	1076.7
FISHER		355.9	1112.7
300 PPM	3/88		
MAXIMUM		471.19	2569
MINIMUM		160.59	689.16
MEAN		286.54	1576.8
FISHER		301.3	1740.9
100 PPM	1/86		
MAXIMUM		165.47	948.3
MINIMUM		59.129	242.47
MEAN		111.63	520.6
FISHER		108.4	530
0 PPM	1/85	N/A	N/A

Table 4.4.

Mouse Gavage Dose-Metric Values

TCE DOSE (MG/KG)	LIVER CANCER INCIDENCE	AMET (MG/KG/DAY)	ADCTCA (MG/L/DAY)
1739.0	11/47		
MAXIMUM		337.97	1285.4
MINIMUM		54.26	156.7
MEAN		182.4	671.97
FISHER		196.2	695.6
869.0	4/50		
MAXIMUM		235.72	853.86
MINIMUM		73.574	243.91
MEAN		143.9	533.53
FISHER		158.7	562.7
0 PPM	0/20	N/A	N/A
MALE MICE			
2339	31/48		
MAXIMUM		346.55	1492.7
MINIMUM		113.46	479.84
MEAN		209.58	841.09
FISHER		211.4	857.2
1169	26/50		
MAXIMUM		268.04	1154.4
MINIMUM		91.989	314.06
MEAN		172.61	698.39
FISHER		176.5	715.5
0 PPM	1/20	N/A	N/A

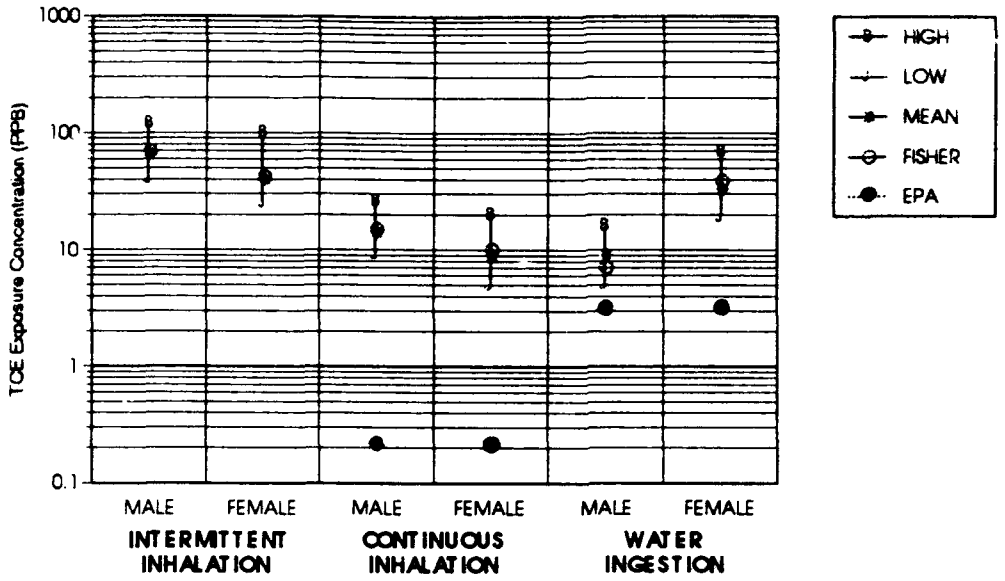
Table 4.5.

Pearson Correlations for Cancer, Dose, and Dose-Metrics

		FEMALE ORAL			MALE ORAL		
		AMET	AUCTCA	CANCER	AMET	AUCTCA	CANCER
MAXIMUM	AUCTCA	0.9993			1		
	CANCER	0.9192	0.933		0.9999	0.9999	
	DOSE	0.9749	0.9824	0.9838	0.9535	0.9535	0.9485
MINIMUM	AUCTCA	0.9945			0.9859		
	CANCER	0.5734	0.4847		0.9997	0.9895	
	DOSE	0.711	0.6337	0.9838	0.9412	0.9844	0.9485
MEAN	AUCTCA	1			1		
	CANCER	0.8763	0.8738		0.9994	0.9991	
	DOSE	0.9485	0.9468	0.9838	0.9367	0.9342	0.9485
FISHER	AUCTCA	1			1		
	CANCER	0.8662	0.8662		0.9989	0.9889	
	DOSE	0.9418	0.9418	0.9838	0.9326	0.9327	0.9485
		FEMALE INHALATION			MALE INHALATION		
		AMET	AUCTCA	CANCER	AMET	AUCTCA	CANCER
MAXIMUM	AUCTCA	0.9405			0.9059		
	CANCER	0.9815	0.9216		0.9254	0.6864	
	DOSE	0.9958	0.9078	0.9714	0.9672	0.7686	0.9859
MINIMUM	AUCTCA	0.8677			0.9119		
	CANCER	0.7997	0.7625		0.8602	0.5866	
	DOSE	0.9107	0.7969	0.9714	0.9182	0.6753	0.9859
MEAN	AUCTCA	0.8154			0.8806		
	CANCER	0.8427	0.6993		0.9009	0.613	
	DOSE	0.9399	0.7305	0.9714	0.9518	0.6969	0.9859
FISHER	AUCTCA	0.8281			0.8846		
	CANCER	0.8071	0.6609		0.8838	0.5842	
	DOSE	0.9182	0.717	0.9714	0.9364	0.6669	0.9859

mouse inhalation, AMET ranged from 25 to 100 ppb for intermittent inhalation vs. 42 ppb for Fisher-Allen, and 4.9 to 20 ppb for continuous inhalation vs. 10 ppb for Fisher-Allen.

AMET values corresponding to 10^{-6} risk using male mouse gavage simulations range from 5 to 16 ppb vs. 7 ppb in the Fisher-Allen study. For female mouse gavage simulations, AMET ranges from 19 to 69 ppb compared to the Fisher-Allen result of 39 ppb.



HIGH	120	100	26	20	16	69
LOW	40	25	9	4.9	5	19
MEAN	67	40	14	9	9	33
FISHER	69	42	15	10	7	39
EPA			0.21	0.21	3.1	3.1

Figure 4.1. TCE Exposure Concentrations for Human Excess Lifetime Cancer Risk of 1×10^{-6} for Dose-Metric AMET

Comparing AMET ranges between the two mouse sexes indicates that risks associated with female mouse inhalation are consistently higher than for male mouse inhalation. However, the opposite is true for gavage. For gavage, male mouse AMET values result in a higher human risk.

Mouse Dose-Metric Values and Baseline Comparison for AUCTCA. Like AMET, Tables 4.3. and 4.4. present the maximum, minimum, mean, and Fisher-Allen results for the dose-metric AUCTCA. AUCTCA produced mouse simulation mean values similar to Fisher-Allen. Though the Monte Carlo generated mean values are similar, they tend to be slightly lower than the Fisher-Allen study.

Cancer Incidence Correlations for AUCTCA. Table 4.5. shows that correlation coefficients generated for AUCTCA were generally lower than for AMET.

Table 4.6.

Dose-Metric Values Corresponding to 1 x 10^{-6} Excess Risk

EXPOSURE SCENARIO	AMET (MG/KG/DAY)	ADCTCA (MG/L/DAY)
MALE MOUSE/HUMAN INTERMITTENT INHALATION		
MAXIMUM	4.32E-03	2.01E-02
MINIMUM	5.29E-03	2.22E-02
MEAN	4.85E-03	1.99E-02
FISHER	5.20E-03	N/A
MALE MOUSE/HUMAN CONTINUOUS INHALATION		
MAXIMUM	4.64E-03	1.89E-02
MINIMUM	5.47E-03	2.07E-02
MEAN	4.80E-03	1.92E-02
FISHER	5.20E-03	N/A
MALE MOUSE/HUMAN INGESTION		
MAXIMUM	1.93E-04	8.41E-04
MINIMUM	2.10E-04	1.20E-03
MEAN	33E-04	7.57E-04
FISHER	1.88E-04	N/A
FEMALE MOUSE/HUMAN INTERMITTENT INHALATION		
MAXIMUM	2.77E-03	8.68E-03
MINIMUM	4.43E-03	5.03E-03
MEAN	2.90E-03	6.31E-03
FISHER	3.22E-03	8.08E-03
FEMALE MOUSE/HUMAN CONTINUOUS INHALATION		
MAXIMUM	2.61E-03	8.81E-03
MINIMUM	4.26E-03	6.13E-03
MEAN	3.13E-03	6.23E-03
FISHER	3.22E-03	8.08E-03
FEMALE MOUSE/HUMAN INGESTION		
MAXIMUM	8.25E-04	3.82E-03
MINIMUM	7.52E-04	2.02E-03
MEAN	6.76E-04	2.72E-03
FISHER	1.02E-03	3.63E-03

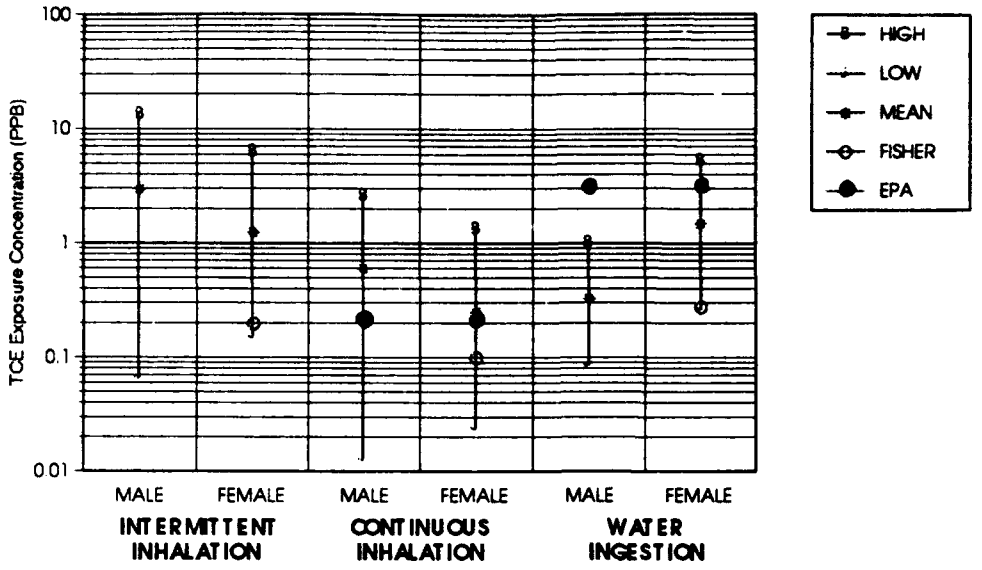
The exception was male mouse gavage. These coefficients are .9999, .9895, .9991, and .9889 for the maximum, minimum, mean, and Fisher-Allen results respectively. The next best correlations were associated with female mouse gavage with values ranging from .4847 for the minimum to .8738 for the mean dose-metric value. Both male and female

inhalation show a poor correlation between AUCTCA and cancer. Mean dose-metric correlations for female and male mouse inhalation are .6993 and .613 respectively.

Human Cancer Risk Results for AUCTCA. The TCE exposure levels corresponding to 10^{-6} risk using AUCTCA dose-metric showed little correlation with the Fisher-Allen values. Table 4.6. reflects dose-metric values associated with a 10^{-6} risk for AUCTCA, and Figure 4.2. graphically displays TCE exposure ranges resulting from the Monte Carlo simulations. Fisher-Allen did not report AUCTCA values for male mice. For female mouse simulations, the Fisher-Allen results were consistently lower then the Monte Carlo mean values. Figure 4.2. illustrates the discrepancies between mean AUCTCA values and Fisher-Allen Results.

The range of TCE exposure for human intermittent inhalation is .07 to 13.3 ppb and .161 to 6.3 ppb for male and female mice respectively. Continuous human inhalation resulted in a range of .0135 to 2.6 ppb and .025 to 6.3 ppb for male and female mice. Human ingestion simulations, which used gavage results to generate risk levels, show TCE exposure concentrations of .09 to 1 ppb for male mice and .285 to 5.28 ppb for female mice.

Unlike the dose-metric AMET, there is not a clear relationship between mouse sex and acceptable exposure levels. For AMET, the minimum, maximum, and mean values were consistently either higher or lower than the opposite sex. AUCTCA results are less clear. For example, both intermittent and continuous inhalation for the female mouse display lower maximum values than the male mouse; however, the male mouse has lower minimum values. Ingestion AUCTCA dose-metrics display consistency between sexes with the male showing smaller values.



HIGH	13.3	6.3	2.6	1.33	1	5.28
LOW	0.07	0.161	0.0135	0.025	0.09	0.285
MEAN	3	1.25	0.6	0.25	0.33	1.5
FISHER		0.2		0.1		0.28
EPA			0.21	0.21	3.1	3.1

Figure 4.2. TCE Exposure Concentrations for Human Excess Lifetime Cancer Risk of 1 x 10^{-6} for Dose-Metric AUCTCA

Worst Case Lower Bounds vs. EPA Estimates. In this section, the lower bound TCE exposure levels resulting from both mouse sexes will be used as a basis for comparison to the current EPA estimates. For the dose-metric AUCTCA, the worst case TCE exposure values are .07 ppb for human intermittent inhalation, .0135 ppb for human continuous inhalation, and .09 ppb for human water ingestion. These values are lower than the current EPA estimates of .21 ppb for continuous inhalation and 3.1 ppb for ingestion of water by factors of 3 and 34. The mean dose-metric values are 1.25 ppb, .25 ppb, and .33 ppb for intermittent inhalation, continuous inhalation, and ingestion of water respectively. The mean value for continuous inhalation is close the the EPA estimate while the mean value for ingestion is lower than the EPA estimate by a factor of 10.

The AMET worst case values are 25 ppb for human intermittent inhalation, 4.9 ppb for human continuous inhalation, and 5 ppb for human ingestion. The values for continuous inhalation and ingestion of water exceed the EPA's estimate by factors of 23 and 1.6. The worst case mean AMET values are 40 ppb, 9 ppb, and 9 ppb for intermittent inhalation, continuous inhalation, and ingestion respectively. These values exceed the EPA estimates by factors of 43 for continuous inhalation and 3 for ingestion.

Summary

The data description and analysis section has discussed the results of the sensitivity analysis. The sensitivity analysis identified those parameters to which the model shows significant response. Also presented were three aspects of the dose-metrics AMET and AUCTCA generated by the Monte Carlo uncertainty analysis. First, the mouse dose-metric values were reported and compared to the baseline Fisher-Allen study results. Next, correlation coefficients for mouse dose-metric values vs. bioassay cancer incidence were discussed. Finally, TCE exposure ranges for 10^{-6} excess risk were enumerated and discussed. The section concludes with a worst case comparison between TCE exposure levels associated with the Monte Carlo generated minimum and mean dose-metric values and the current EPA TCE exposure estimates for 10^{-6} excess lifetime cancer risk.

V. Conclusions and Recommendations

Conclusions

Physiologically Based Pharmacokinetic (PBPK) models have been successfully used to characterize human cancer risk for a number of environmental contaminants. While PBPK modeling produces results which are almost certainly more realistic than some traditional methodologies, such as extrapolation between animals and humans by scaling body surface area, parametric uncertainty remains a major area of concern. Monte Carlo simulation, coupled with parameter sensitivity analysis, provides a basis for quantifying this uncertainty. Presenting a range of contaminant exposure levels which lead to de-minimus risk, better equips decision makers to draw conclusions about acceptable exposure levels. By reducing dependency on the subjective method of qualitative uncertainty discussion, and replacing it with a quantitative range of exposures, policy makers can better justify their decisions. In light of dwindling national resources, it is imperative that environmental policies and research efforts be based on information which lends itself toward reducing, or at least quantifying, uncertainty.

This research effort provides an example of sensitivity analysis which could be used by investigators to focus research efforts on parameters which impact model output. By increasing the accuracy of measurement for sensitive parameters, risk assessments would likely reflect a corresponding improvement in accuracy. Also, sensitivity analysis results could be used as a resource allocation tool. The results of this study indicate that a number of parameters display little or no sensitivity and contribute little to the variability of dose-metrics. Examples of non-sensitive parameters include partition coefficients and certain compartmental volumes. After initially identifying and quantifying these parameters, investigators would be justified in shifting their efforts to those parameters which have a significant impact on model output.

The availability of lower bound risks resulting from Monte Carlo uncertainty analysis provides decision makers with a more concrete method for developing exposure standards than do traditional practices. This lower bound reflects de-minimus risk in light of the most sensitive sample from the population, that is, the specimen which generates the greatest dose-metric value for a given exposure scenario. Current practices include techniques whereby quantitative risk assessment results are divided bfactors of ten depending on the availability of evidence. This approach is almost certainly more conservative than necessary but is warranted in light of uncertainty. The Monte-Carlo method for PBPK modeling is based on existing bioassay data and predicts dose-metric values for the most sensitive, as well as the most resistant, specimen among the sample population. These facts reduce the need for incorporating safety factors associated with animal evidence and the most sensitive individual within a population.

Monte Carlo based uncertainty analysis could also serve as a basis for validating other studies. Data generated in other studies for the same chemical could be compared to the Monte-Carlo results to determine whether dose-metric values fall within the Monte Carlo range. Values outside the Monte Carlo range may indicate problems with research methodologies or model errors.

Study Uncertainty

As with all other research efforts, the results of this study are prone to their own uncertainty. The following topics are certainly not exhaustive but should provide the reader with a feel for the studies limitations.

Sensitivity Analysis & Non-Linearity. While the sensitivity analysis results are fairly comprehensive, one should be careful in assuming completeness.

The non-linear nature of differential equations make it difficult to predict to what extent, if at all, superposition holds true for multiple parameter variations. While a number of parameters may reflect low sensitivities on an individual basis, a combination of two or

more of these parameters might well impact the model to a greater degree than the sum of their individual contributions. It would be wise to check their combined sensitivity before drawing conclusions about their contributions to uncertainty. Also, the reader should keep in mind that tables 4.1 and 4.2 represent parameters which exceed the threshold of one-half percent change in output relative to a one percent increase in a model parameter. Appendices C through I include all sensitivities both graphically and quantitatively. Those parameters sensitivities which lie between zero and one-half percent may collectively contribute significant uncertainty to model output.

Distribution and Variance Uncertainty. This study was based, in large, on undocumented parameter distributions and variability. Dr. Fisher, Senior Scientist, Toxicology Division, Occupational and Environmental Health, Armstrong Laboratory, was able to provide variability for a number of parameters; however, the remaining variabilities were assumed. With one exception, the literature review provided no information concerning parameter distributions. Parameter distribution types were based on a single study or recommendations from Mr. Carlyle Flemming, Systems Analyst (Bio Statistition), Toxic Hazards Unit, ManTech Environmental Technology, Inc. The Monte Carlo method is, by its very nature, dependent on the types of parameter distributions and variabilities. Better characterization of distributions and variabilities could result in more realistic exposure ranges associated with de-minimus risk.

Covariation of Parameters. This study was also limited by the lack of information concerning covariation of physiological and kinetic parameters. While no data was available to support covariation assumptions, the likelihood of covariation among parameters is great. For instance, body weight is probably correlated with the size of the liver compartment.

This relationship is accounted for in the TCE model through allometric scaling; however, the liver compartment's volume constant was also allowed to vary. An increase in body weight, accompanied by a decrease in liver volume is unlikely. Although statements

cannot be made concerning the exact effects of such covariation, it is entirely possible that the ranges reported in this study could be significantly overstated. In order to quantify parameter uncertainty in a reasonable fashion, implementation of the Monte Carlo assessment process must include the integration of parameter covariation.

Recommendations

Parameter Data Base. From the beginning of this research effort, it was apparent that the greatest challenge would be determining the model parameter distributions. While intuition is a helpful tool, it cannot substitute for hard data.

The development of a centralized parameter data base would provide researchers with important information necessary to characterize parameter distributions and variabilities. Information of this nature would not only be helpful to researchers but could provide standards for future risk assessments. According to Dr. Jefferey Fisher, Senior Scientist, Toxicology Division, Occupational and Environmental Health, Armstrong Laboratory, the EPA is currently in preliminary stages of such an initiative.

Model Development. The TCE model used in this study performed well overall; however, there are several items which bear closer investigation.

Volume of Distribution. The volume of distribution for TCA in humans is defined by the following equation:

$$\mathbf{VDP = (.34 - .0034 \times BW) \times BW}$$

This equation works well for body weights up to 100 Kg but fails beyond this weight. It's apparent that, once body weight exceeds 100 Kg, the volume of distribution becomes negative. Negative volumes of distribution, in turn, create negative AUCTCA dose-metrics.

The probability for body weights to exceed 100 Kg is high; therefore, we recommend that this equation be modified to accommodate body weights that fall within at least three standard deviations of their mean value.

Numerical Problems. During this research effort, considerable time and effort was expended trying to determine the source of some numerical anomalies. After simulations were completed for the mouse bioassays, the ACSL binary files were converted to ASCII. Upon conversion, it was noted that numerous dose-metric values were negative. After spending a significant amount of time trying to determine the source of the problem, the physiologically improbable values were replaced with mean values derived from the remaining viable data. Only towards tne end of the research effort did the source of the problem become apparent. It was discovered that inconsistent dose-metric values were only being generated for high doses (300 and 600 ppm) for the inhalation scenarios in mice. Also, during the sensitivity analysis, it was noted that small perturbations of certain parameters would cause the same problem. Figures 5.1. and 5.2. display the events which lead to erroneous model output.

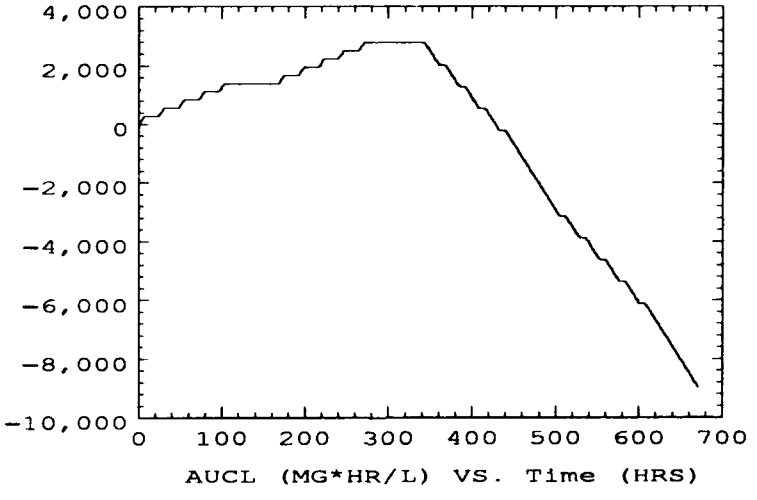


Figure 5.1.
Model Instability for Area Under the Curve Liver

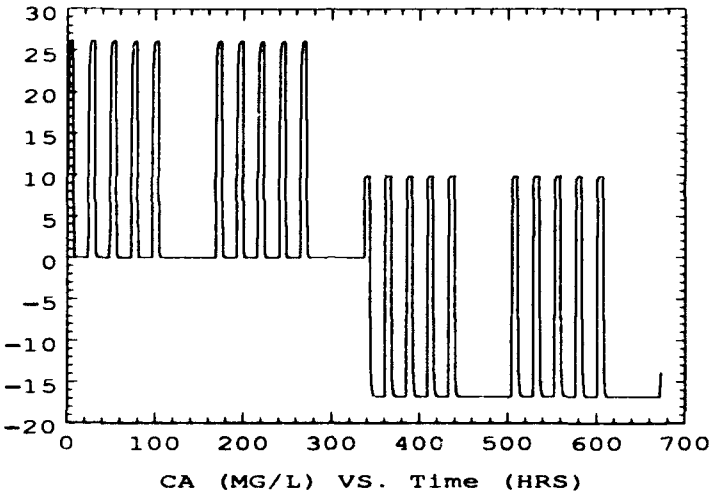


Figure 5.2.
Model Instability for Arterial Concentration

Certain parameter changes cause integrated variables to become negative. This phenomena only seems to occur at the end of a weekend and isn't necessarily associated with any particular set of parameters.

This problem can be corrected for one set of parameters by increasing the number of integration steps; however, another set of parameters then displays the same behavior. It is likely that the problem can be connected to the use of the Gear method for stiff systems for solving differential equations. During the weekend, the algorithm increases it's step size in response to zero concentration. It is possible that this larger step size is being executed at the beginning of the next exposure period, when the step size should be reduced. This results in an integration error.

Summary

While the Monte Carlo method for risk assessment isn't a panacea, PBPK modeling and Monte Carlo based uncertainty analysis, coupled with a sensitivity analysis, offers a comprehensive methodology for risk assessment.

Sensitivity analysis can be a valuable tool for researchers, enabling them to focus attention on important physiological and kinetic parameters, allocate resources, and describe variability associated with uncertainty analysis.

Monte Carlo uncertainty analysis enables the researcher to present results in a more comprehensive and physiologically realistic manner than some traditional methods. The ability to characterize risk in a more quantitative fashion reduces the amount of qualitative discussion necessary to describe uncertainty in the risk assessment process. Lower bounds on contaminant exposures offer a physiologically based conservative estimate for the most sensitive representative in a population.

Though further research in the area of parameter distribution and covariation is needed to improve the Monte Carlo risk assessment process, the method currently provide valuable tool for researchers and regulators to characterize uncertainty.

Appendix A. *Abbreviations and Acronyms*

As an aid to the reader, the authors have compiled the following list of abbreviations and acronyms that have been used in this thesis.

ACSL:	Advanced Continuous Simulation Language.
AFB:	Air Force Base.
AMET:	Amount of TCE Metabolized.
AUCB:	Area Under the Curve Blood.
AUCTCA:	Area Under The Curve TCA.
CI:	Confidence Interval.
EPA:	Environmental Protection Agency.
HAD:	Health Assessment Document.
K1:	Effective gastrointestinal uptake rate constant.
Kel:	Elimination rate constant for TCA.
KM:	Michaelis-Menten constant.
NCI:	National Cancer Institute.
PB:	Blood/Air partition coefficient.
PBPK:	Physiologically Based Pharmacokinetics.
PBPK_SIM:	PBPK simulation program.
PERC:	Perchloroethylene.
PF:	Fat/Blood partition coefficient.
PL:	Liver/Blood partition coefficient.
PO:	Percent yield of TCA from TCE.
PR:	Richly perfused tissue/Blood partition coefficient.
PS:	Slowly perfused tissue/Blood partition coefficient.
PU:	Urinary excretion rate of TCA.
QCC:	Cardiac output.
QFC:	Flow to fat tissue (fraction of cardiac output).
QLC:	Flow to liver tissue (fraction of cardiac output).
QPC:	Alveolar ventilation rate.
QRC:	Flow to richly perfused tissue (fraction of cardiac output).

QSC:	Flow to slowly perfused tissue (fraction of cardiac output).
TCA:	Trichloroacetic Acid.
TCE:	Trichloroethylene.
TCOH:	Trichloroethanol.
VCARC:	Volume of carcass (fraction of body weight).
VDC:	Volume of Distribution.
VLC:	Volume of liver (fraction of body weight).
VMAX:	Maximum Velocity of Metabolism.
VRS:	Volume of richly perfused tissue (fraction of body weight).
VSC:	Volume of slowly perfused tissue (fraction of body weight).

Appendix B. *Exposure Scenario Response*

The following diagrams illustrate the models response for mice and humans to a dose of 100ppm TCE under a specific exposure scenario. The graphs present the response of the models with respect to the rate of metabolism of TCE and the rate of prodection of TCA. The intent of the graphs is to provide the reader with a graphical representation of the exposure scenarios used in the research and to give insight as to how the model responds to those scenarios.

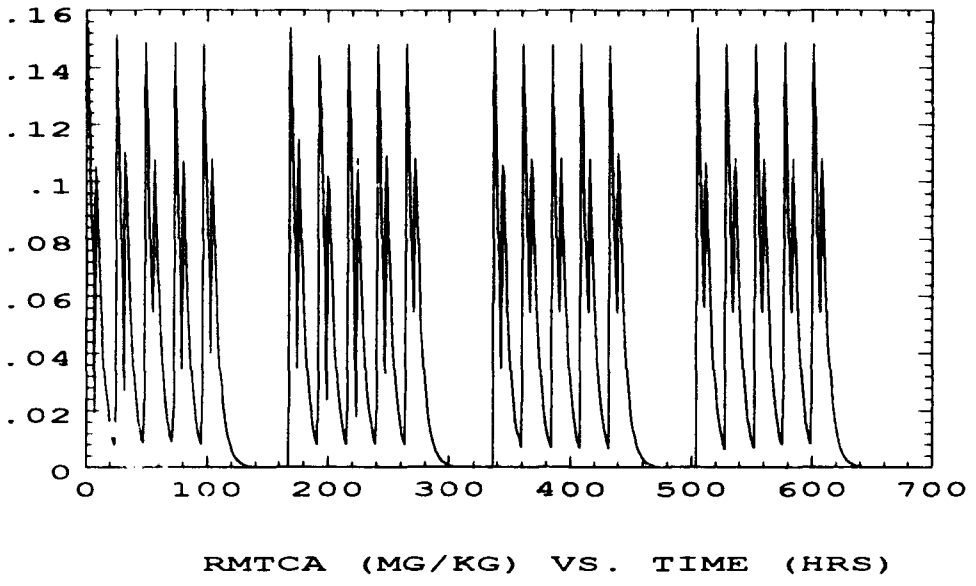


Figure B-1. Rate of TCA Production for Mouse Intermittent Inhalation @ 100 ppm.

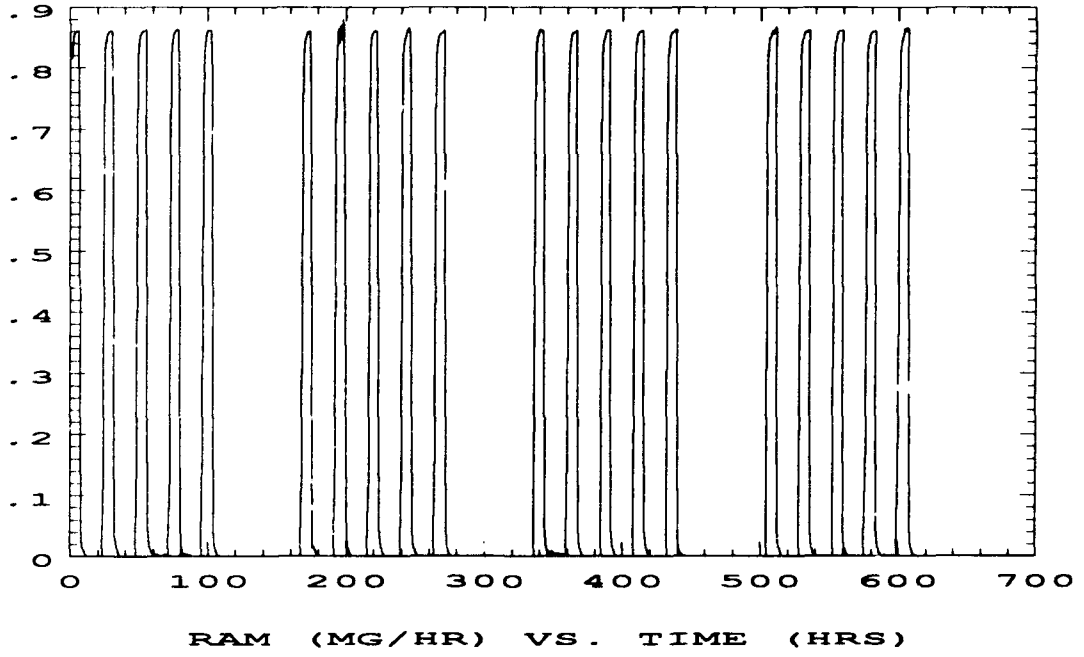


Figure B-2. Rate of Metabolism for Mouse Intermittent Inhalation @ 100 ppm.

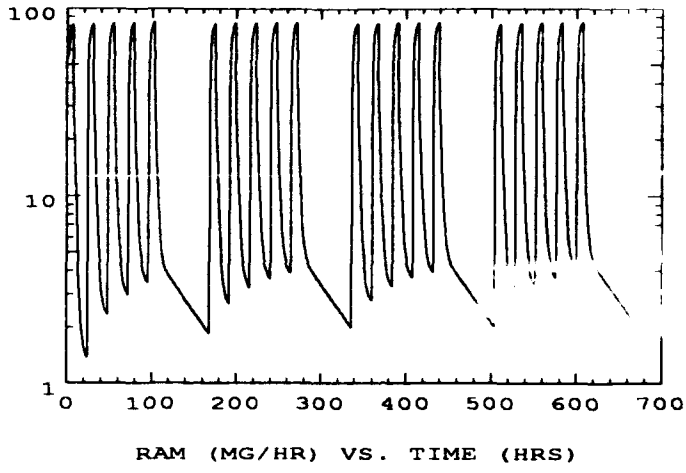


Figure B-3. Rate of Metabolism for Human Intermittent Inhalation @ 100 ppm.

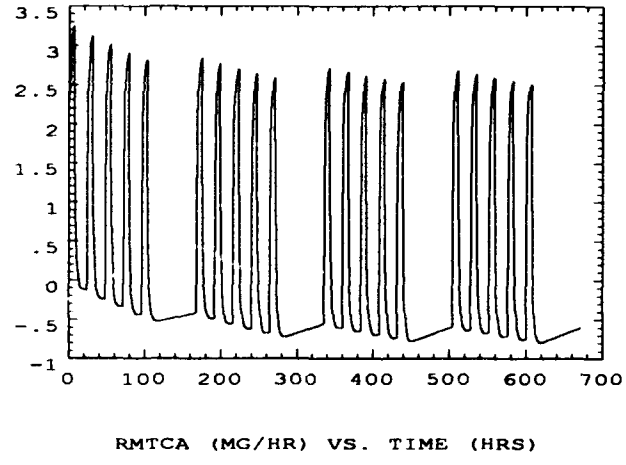


Figure B-4. Rate of TCA Production for Human Intermittent Inhalation @ 100 ppm.

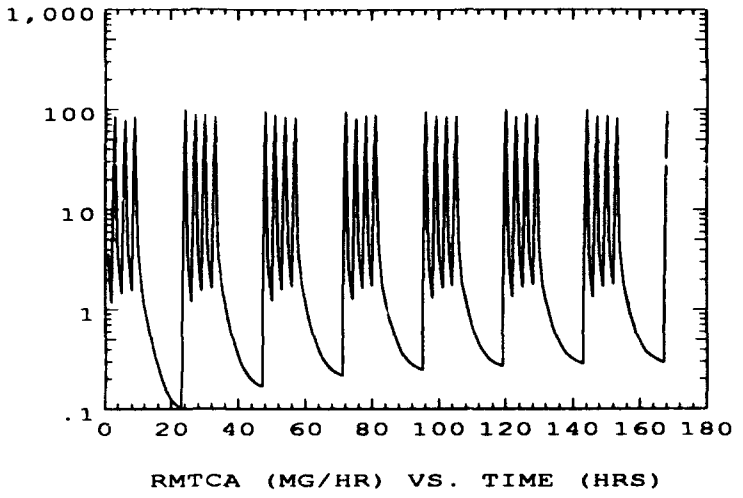


Figure B-5. Rate of Metabolism for Human Water Ingestion @ 100 ppm.

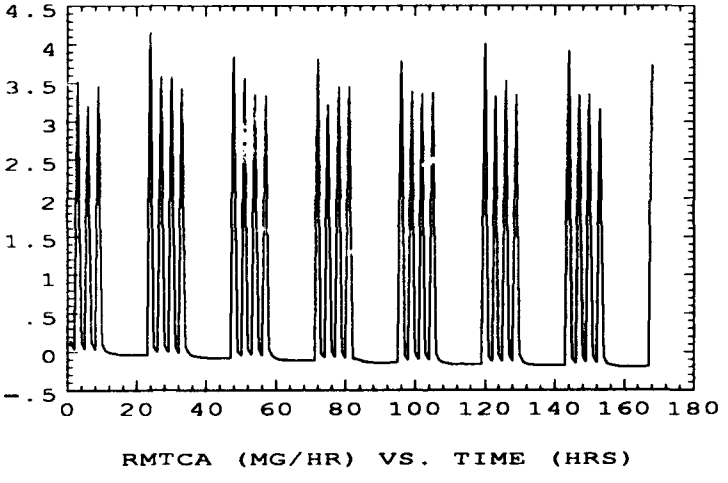


Figure B-6. Rate of TCA Production for Human Water Ingestion @ 100 ppm.

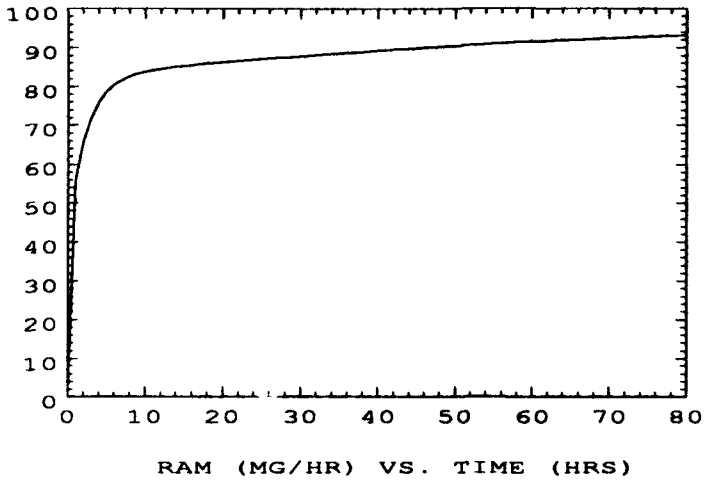


Figure B-7. Rate of Metabolism for Human Continuous Inhalation @ 100 ppm.

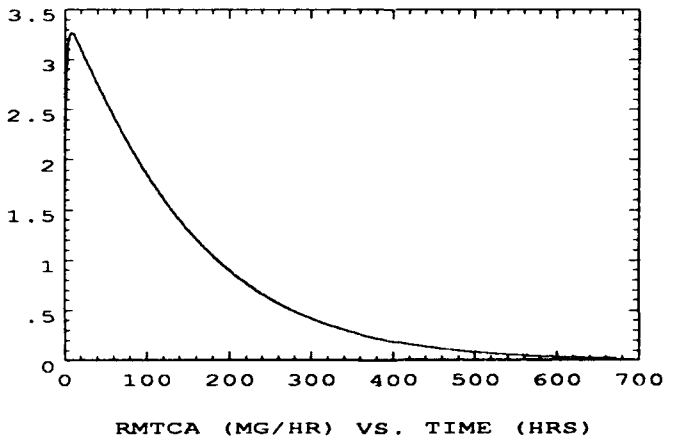


Figure B-8. Rate of TCA Production for Human Continuous Inhalation @ 100 ppm.

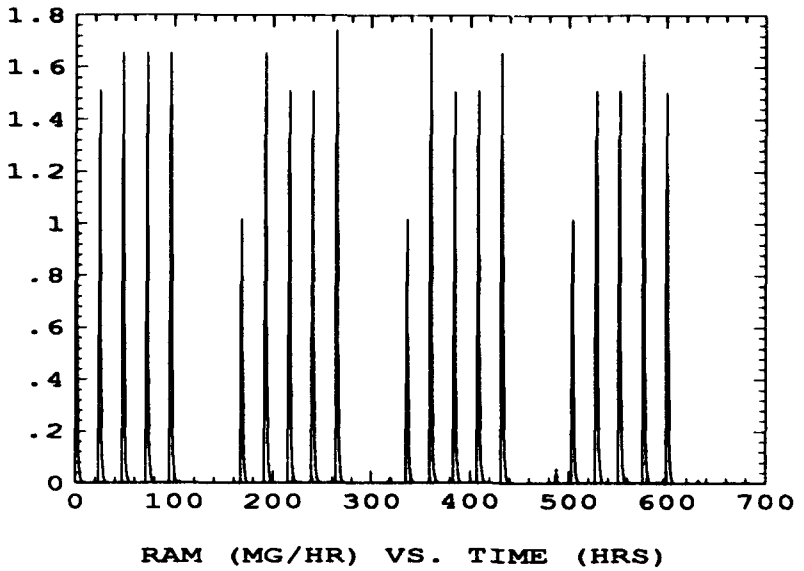


Figure B-9. Rate of Metabolism for Mouse Gavage @ 100 ppm.

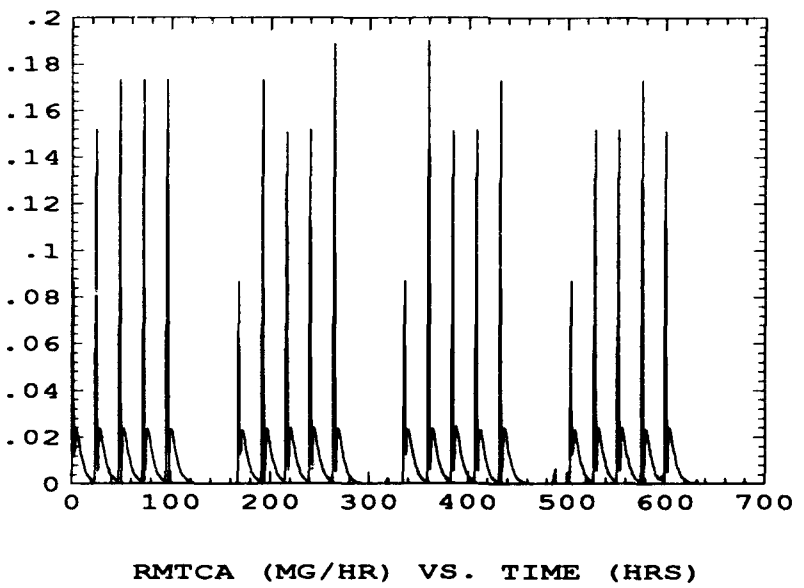


Figure B-10. Rate of TCA Production for Mouse Gavage @ 100 ppm.

Appendix C. *Male Mouse Inhalation Sensitivity*

The following graphs present the results of the male mouse inhalation sensitivity analysis. The tables of data for the analysis are first presented by exposure dose of TCE. Graphs of the sensitivity for each parameter are then presented both by dose-metric and dose level.

1% SENSITIVITY ANALYSIS
Male Mouse Inhalation

DOSE 1: 100 PPM TCE

	Model Output			Sensitivity (%)		
Parameter	AMETF	AUCTCF	AUCLF	AMETF	AUCTCF	AUCLF
PL	114.523	523.649	0.893355	0.00000	0.00019	1.00970
PR	114.523	523.65	0.88437	0.00000	0.00038	-0.00622
PS	114.523	523.648	0.884403	0.00000	0.00000	-0.00249
PF	114.522	523.645	0.884442	-0.00087	-0.00057	0.00192
PB	114.808	524.95	0.887684	0.24886	0.24864	0.36849
VMC	114.55	523.77	0.872109	0.02358	0.02330	-1.39254
KM	114.504	523.562	0.893244	-0.01659	-0.01642	0.99715
KUPC	114.523	518.463	0.884497	0.00000	-0.99017	0.00814
VLC	114.523	523.649	0.884497	0.00000	0.00019	0.00814
VRC	114.523	523.648	0.884425	0.00000	0.00000	0.00000
VSC	114.523	523.648	0.884425	0.00000	0.00000	0.00000
VFC	114.523	523.647	0.884465	0.00000	-0.00019	0.00452
QPC	115.371	527.524	0.894066	0.74046	0.74019	1.09009
QCC	114.789	524.866	0.887556	0.23227	0.23260	0.35402
BW	114.226	523.852	0.884985	-0.25934	0.03896	0.06332
VDP	114.523	518.464	0.884403	0.00000	-0.98998	-0.00249
QLC*	114.789	524.863	0.887552	0.23227	0.23203	0.35356
QFC*	114.523	523.648	0.884487	0.00000	0.00000	0.00701
MEAN	114.523	523.648	0.884425			

*QR scaled as function of QL & QC, QS scaled as function of QF & QC

DOSE 2: 300 PPM TCE

	Model Output			Sensitivity (%)		
Parameter	AMETF	AUCTCF	AUCLF	AMETF	AUCTCF	AUCLF
PL	319.598	1727.04	13.9096	0.00031	0.00058	0.99546
PR	319.598	1727.04	13.7724	0.00031	0.00058	-0.00073
PS	319.639	1727.26	13.7532	0.01314	0.01332	-0.14013
PF	319.611	1727.11	13.7665	0.00438	0.00463	-0.04357
PB	320.204	1730.31	13.9828	0.18993	0.18992	1.52696
VMC	320.903	1734.09	13.1666	0.40864	0.40879	-4.39935
KM	319.43	1726.13	13.8503	-0.05225	-0.05211	0.56489
KUPC	319.593	1709.91	13.7747	-0.00125	-0.99130	0.01597
VLC	319.597	1727.03	13.7724	0.00000	0.00000	-0.00073
VRC	319.597	1727.03	13.7725	0.00000	0.00000	0.00000
VSC	319.597	1727.03	13.7725	0.00000	0.00000	0.00000
VFC	319.615	1727.13	13.7647	0.00563	0.00579	-0.05663
QPC	320.97	1734.45	14.2568	0.42960	0.42964	3.51643
QCC	320.015	1729.29	13.9311	0.13079	0.13086	1.15157
BW	318.719	1727.43	13.7968	-0.27472	0.02316	0.17644
VDP	319.598	1709.93	13.773	0.00031	-0.99014	0.00363
QLC*	320.027	1729.36	13.9254	0.13454	0.13491	1.11018
QFC*	319.596	1727.03	13.7733	-0.00031	0.00000	0.00581
MEAN	319.597	1727.03	13.7725			

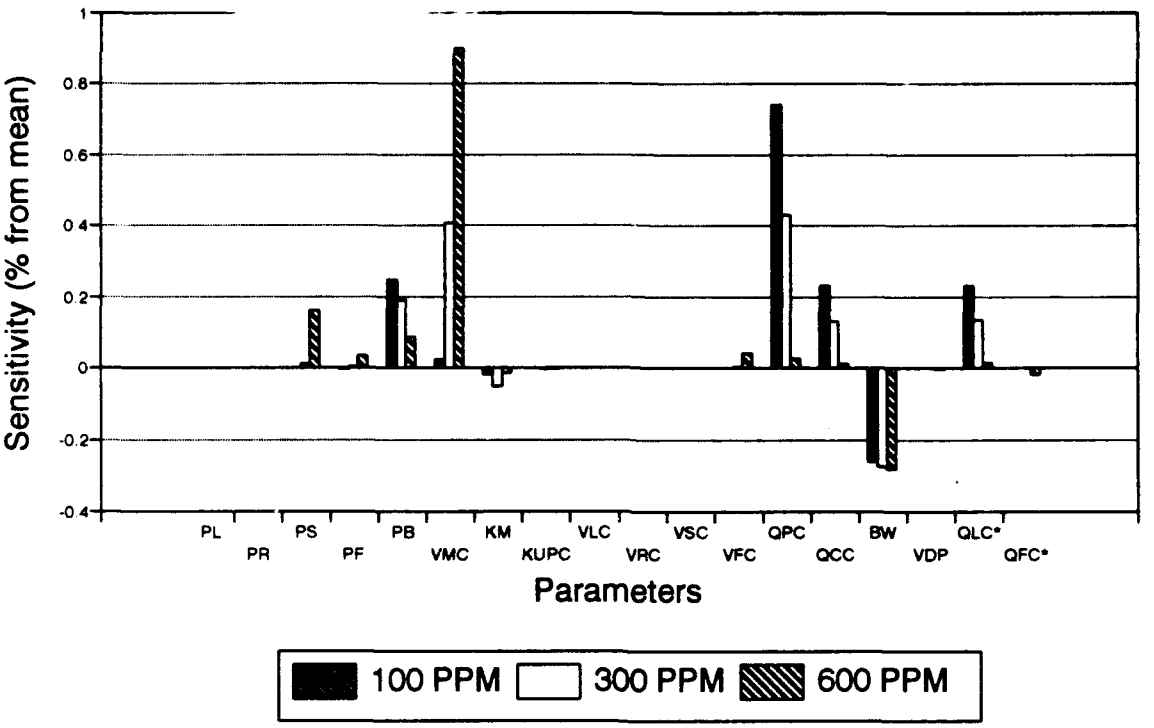
*QR scaled as function of QL & QC, QS scaled as function of QF & QC

DOSE 3: 600 PPM TCE

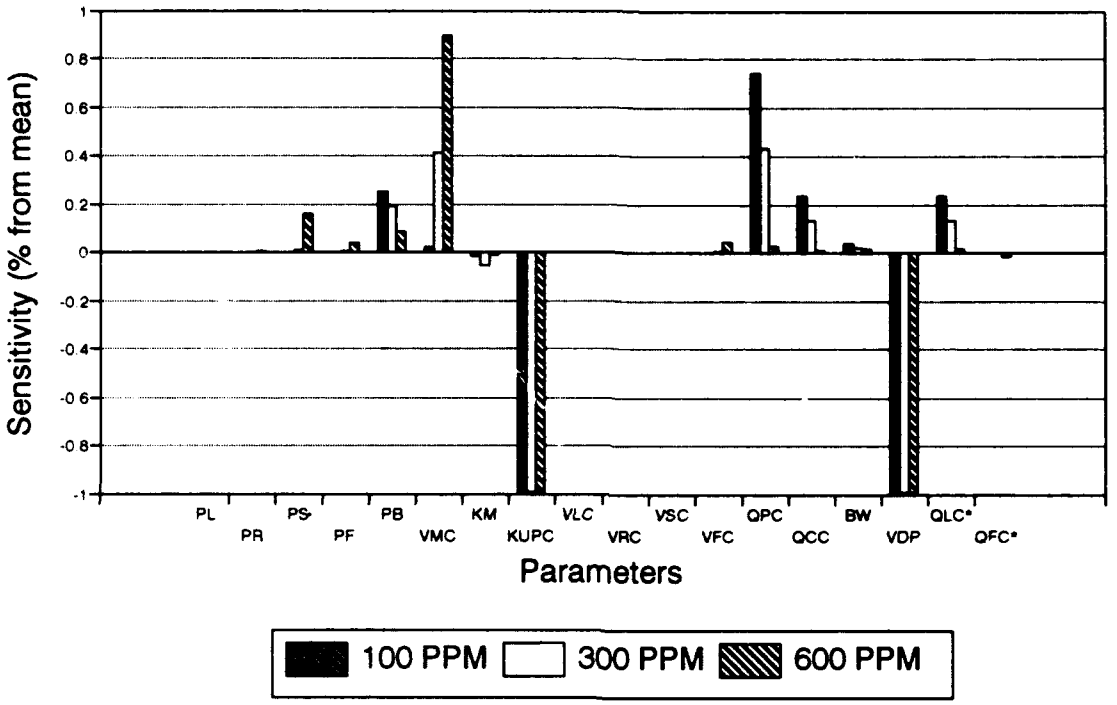
	Model Output			Sensitivity (%)		
Parameter	AMETF	AUCTCF	AUCLF	AMETF	AUCTCF	AUCLF
PL	377.941	1099.71	150.209	0.00106	0.00182	0.99917
PR	377.935	1099.69	148.722	0.00212	0.00273	-0.00269
PS	378.545	1101.46	148.442	0.16087	0.16095	-0.18894
PF	378.062	1100.06	148.663	0.03572	0.03637	-0.04236
PB	378.263	1100.64	150.479	0.08626	0.08639	1.18072
VMC	381.327	1109.56	147.151	0.89697	0.89753	-1.05700
KM	377.877	1099.52	148.749	-0.01323	-0.01273	0.01546
KUPC	377.929	1088.78	148.726	-0.00212	-0.99210	0.00202
VLC	377.932	1099.68	148.724	0.00132	0.00182	-0.00134
VRC	377.937	1099.69	148.723	0.00000	0.00000	0.00000
VSC	377.937	1099.69	148.723	0.00000	0.00000	0.00000
VFC	378.097	1100.16	148.648	0.04234	0.04274	-0.05043
QPC	378.045	1100.01	149.991	0.02858	0.02910	0.85259
QCC	377.971	1099.79	149.122	0.01164	0.01182	0.26626
BW	376.873	1099.88	148.763	-0.28153	0.01728	0.02690
VDP	377.93	1088.79	148.726	-0.00185	-0.99119	0.00202
QLC*	377.992	1099.85	149.114	0.01455	0.01455	0.26290
QFC*	377.869	1099.49	148.756	-0.01799	-0.01819	0.02219
MEAN	377.937	1099.69	148.723			

*QR scaled as function of QL & QC, QS scaled as function of QF & QC

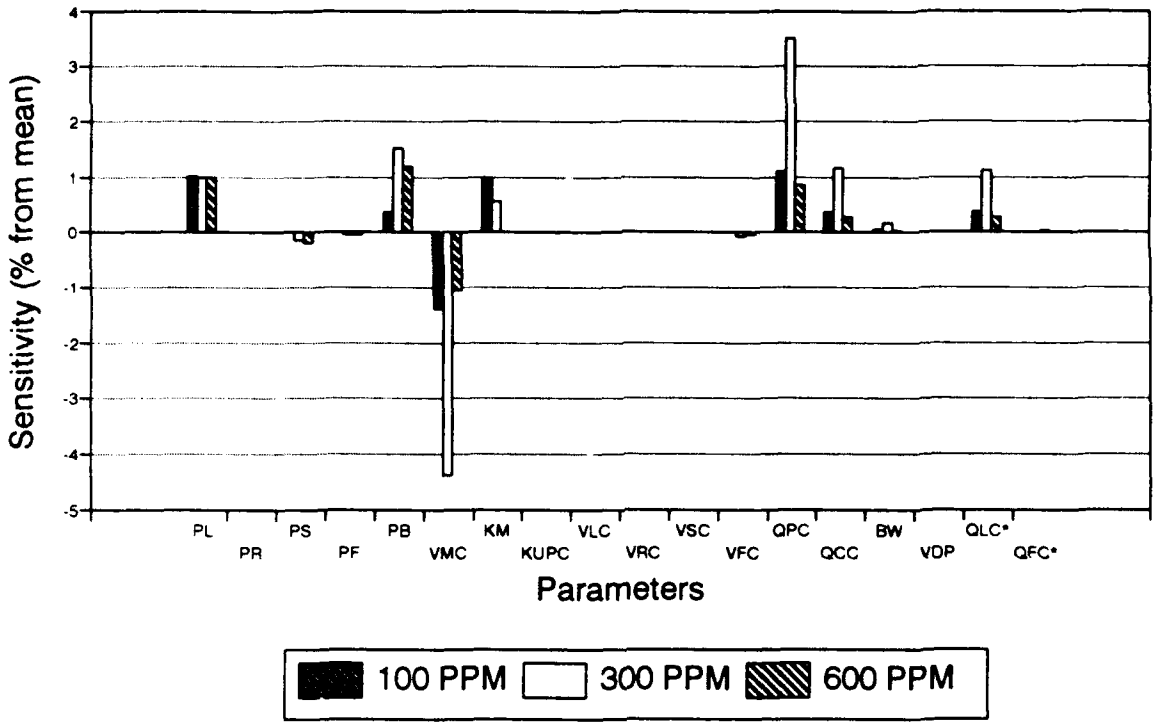
Male Mouse Inhalation 1% Sensitivity
AMET



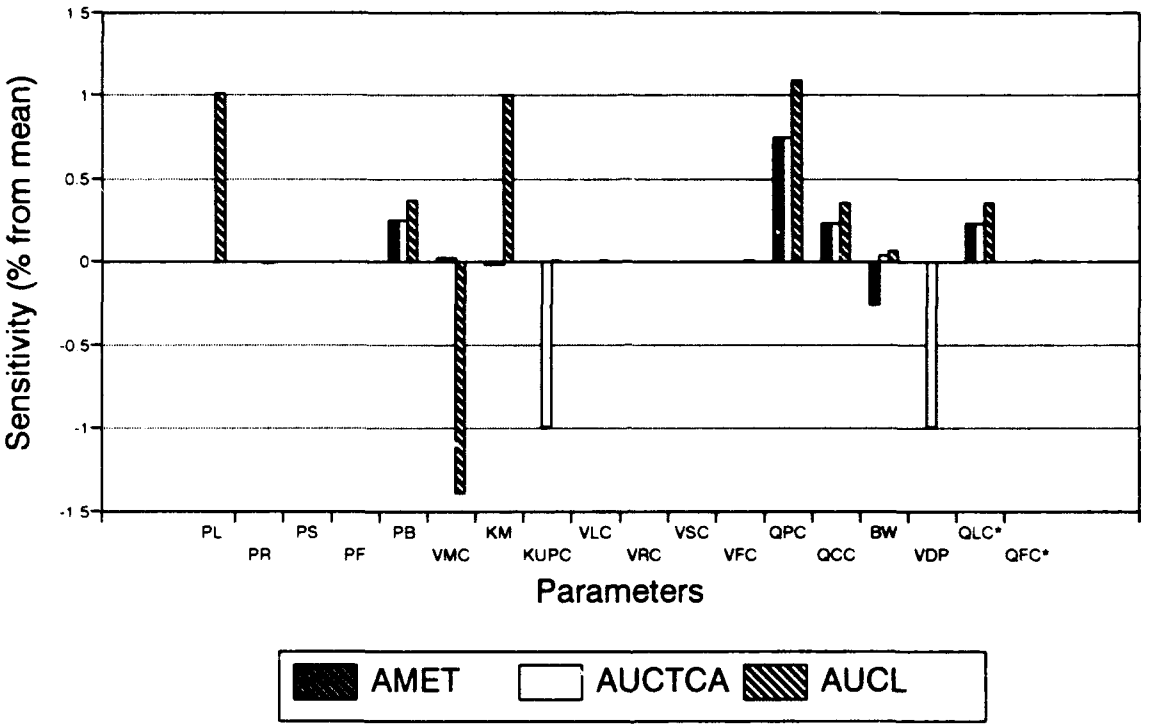
Use Inhalation 1% Sensitivity
AUCTCA



Male Mouse Inhalation 1% Sensitivity
AUCL

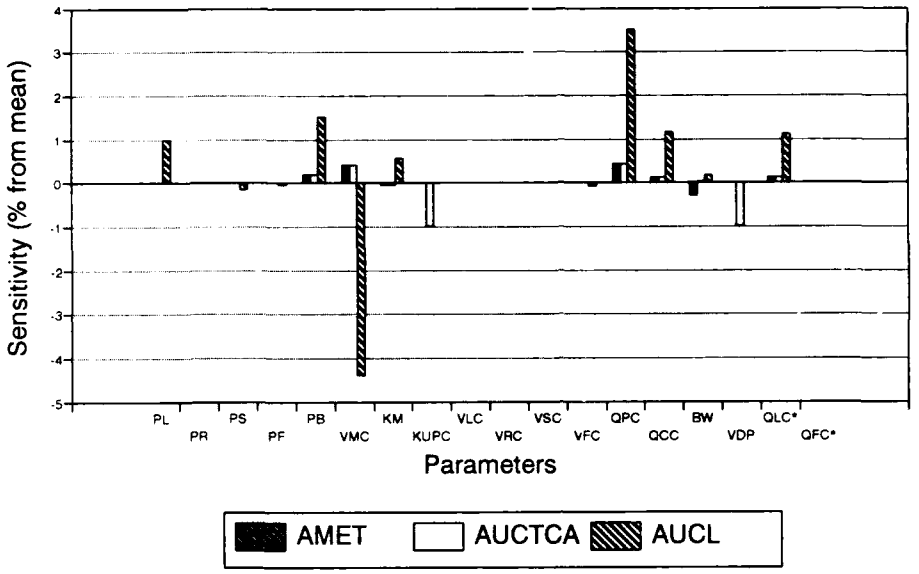


Male Mouse Inhalation 1% Sensitivity
100 PPM TCE

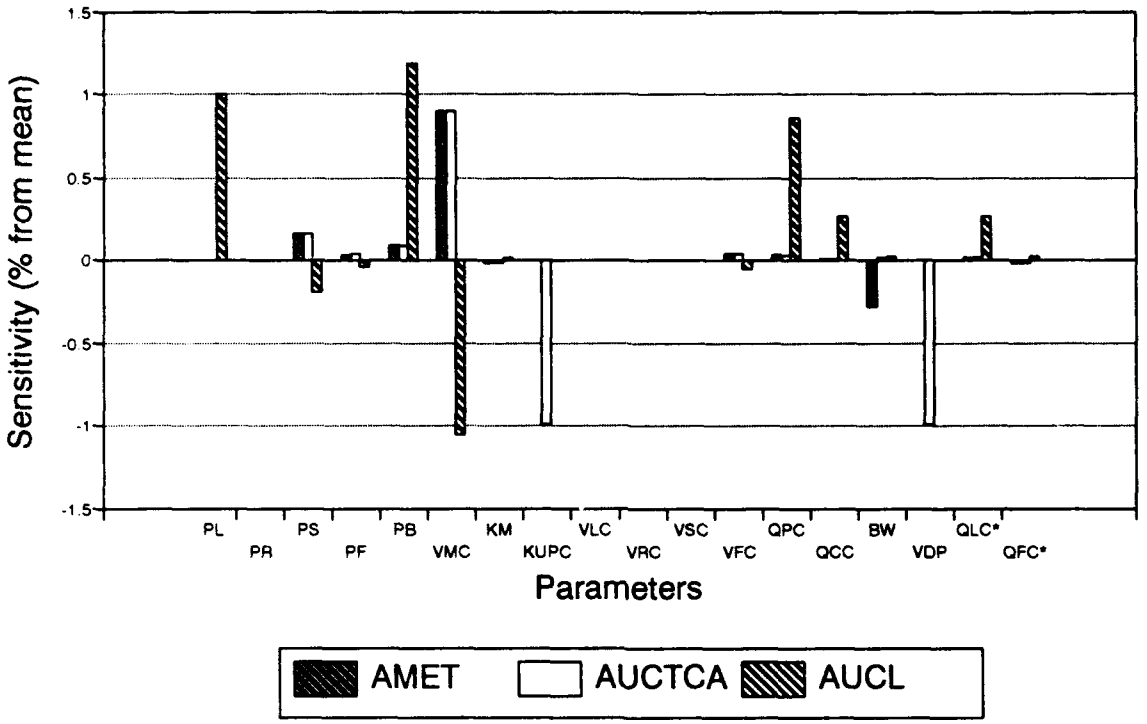


Male Mouse Inhalation 1% Sensitivity

300 PPM TCE



Male Mouse Inhalation 1% Sensitivity
600 PPM TCE



Appendix D. *Female Mouse Inhalation Sensitivity*

The following graphs present the results of the female mouse inhalation sensitivity analysis. The tables of data for the analysis are first presented by exposure dose of TCE. Graphs of the sensitivity for each parameter are then presented both by dose-metric and dose level.

1% SENSITIVITY ANALYSIS
Female Mouse Inhalation

DOSE 1: 100 PPM TCE

	Model Output			Sensitivity (%)		
Parameter	AMETF	AUCTCF	AUCLF	AMETF	AUCTCF	AUCLF
PL	115.493	483.154	1.24273	0.00000	0.00021	0.98980
PR	115.493	483.155	1.23041	0.00000	0.00041	-0.01138
PS	115.493	483.156	1.23032	0.00000	0.00062	-0.01869
PF	115.495	483.162	1.22984	0.00173	0.00186	-0.05770
PB	115.77	484.313	1.23546	0.23984	0.24009	0.39901
VMC	115.546	483.375	1.2097	0.04589	0.04595	-1.69436
KM	115.463	483.03	1.24223	-0.02598	-0.02546	0.94917
KUPC	115.493	478.372	1.23055	0.00000	-0.98954	0.00000
VLC	115.494	483.157	1.23029	0.00087	0.00083	-0.02113
VRC	115.493	483.153	1.23055	0.00000	0.00000	0.00000
VSC	115.493	483.153	1.23055	0.00000	0.00000	0.00000
VFC	115.494	483.16	1.23014	0.00087	0.00145	-0.03332
QPC	116.344	486.715	1.24671	0.73684	0.73724	1.31323
QCC	115.785	484.377	1.21721	0.25283	0.25334	-1.08407
BW	115.193	483.34	1.23102	-0.25976	0.03870	0.03819
VDP	115.493	478.357	1.23031	0.00000	-0.99265	-0.01950
QLC*	115.739	484.185	1.23528	0.21300	0.21360	0.38438
QFC*	115.493	483.156	1.23036	0.00000	0.00062	-0.01544
MEAN	115.493	483.153	1.23055			

*QS scaled as function of QF & QC, QR scaled as function of QL & QC

DOSE 2: 300 PPM TCE

	Model Output			Sensitivity (%)		
Parameter	AMETF	AUCTCF	AUCLF	AMETF	AUCTCF	AUCLF
PL	259.158	421.62	38.4405	-0.00039	-0.00024	0.99788
PR	259.162	421.626	38.0592	0.00116	0.00119	-0.00394
PS	259.169	421.637	38.0562	0.00386	0.00379	-0.01182
PF	259.276	421.812	38.0136	0.04515	0.04530	-0.12375
PB	259.451	422.096	38.5546	0.11267	0.11266	1.29766
VMC	261.22	424.975	37.2484	0.79526	0.79550	-2.13422
KM	259.068	421.473	38.0957	-0.03511	-0.03510	0.09196
KUPC	259.16	417.448	38.0596	0.00039	-0.98975	-0.00289
VLC	259.16	421.622	38.0596	0.00039	0.00024	-0.00289
VRC	259.159	421.621	38.0607	0.00000	0.00000	0.00000
VSC	259.159	421.621	38.0607	0.00000	0.00000	0.00000
VFC	259.275	421.81	38.0142	0.04476	0.04483	-0.12217
QPC	259.465	422.119	38.7224	0.11807	0.11812	1.73854
QCC	261.023	424.654	37.5552	0.71925	0.71937	-1.32814
BW	258.158	421.62	38.4405	-0.38548	0.00047	0.99815
VDP	259.16	417.435	38.0602	0.00039	-0.99283	-0.00131
QLC*	259.248	421.766	38.2531	0.03434	0.03439	0.50551
QFC*	259.155	421.615	38.0616	-0.00154	-0.00142	0.00236
MEAN	259.159	421.621	38.0607			

*QS scaled as function of QF & QC, QR scaled as function of QL & QC

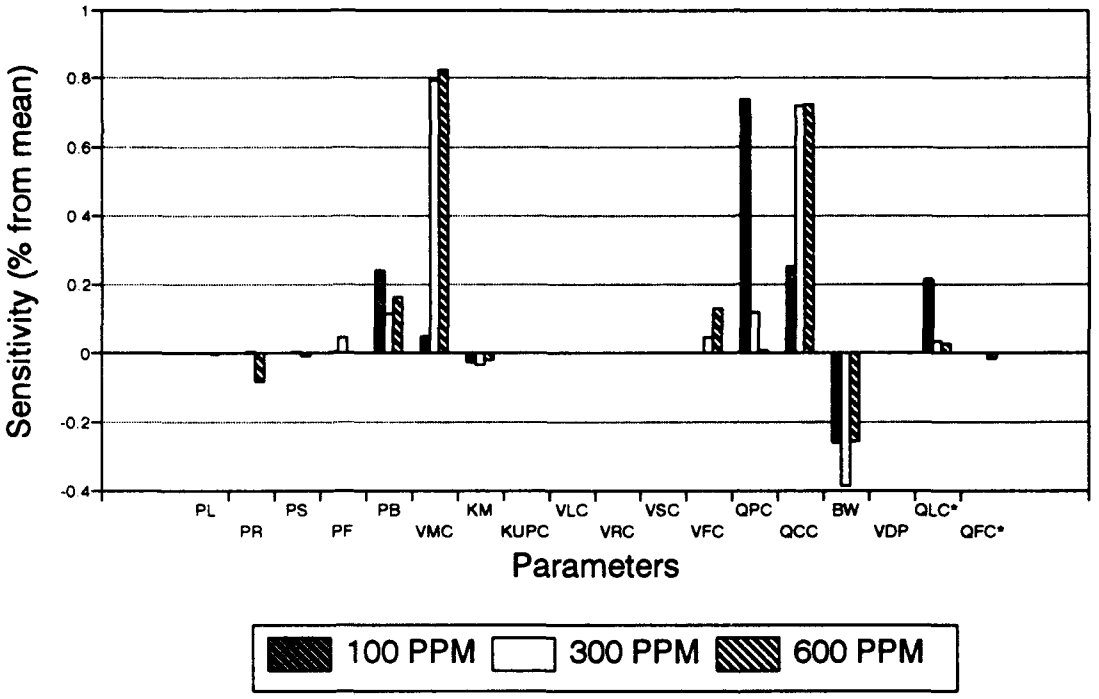
DOSE 3: 600 PPM TCE

	Model Output			Sensitivity (%)		
Parameter	AMETF	AUCTCF	AUCLF	AMETF	AUCTCF	AUCLF
PL	295.558	549.53	165.435	-0.00541	-0.00528	1.00495
PR	295.332	549.109	163.885	-0.08154	-0.08152	0.05800
PS	295.546	549.6	163.781	-0.00947	0.00746	-0.00549
PF	295.57	549.551	163.791	-0.00101	-0.00109	0.00061
PB	296.054	550.452	165.5	0.16273	0.16286	1.04402
VMC	298.002	554.11	162.826	0.82282	0.82940	-0.58917
KM	295.52	549.459	163.811	-0.01793	-0.01783	0.01282
KUPC	295.569	544.108	163.791	-0.00034	-0.99063	0.00000
VLC	295.5654	549.558	163.7861	-0.00258	0.00018	-0.00240
VRC	295.573	549.557	163.79	0.00000	0.00000	0.00000
VSC	295.573	549.557	163.79	0.00000	0.00000	0.00000
VFC	295.957	550.271	163.638	0.12992	0.12992	-0.09280
QPC	295.592	549.592	164.674	0.00744	0.00728	0.53910
QCC	297.706	553.523	163.212	0.72165	0.72167	-0.35289
BW	294.816	549.789	163.787	-0.25611	0.04222	-0.00183
VDP	295.567	544.105	163.792	-0.00101	-0.99117	0.00061
QLC	295.644	549.69	164.022	0.02504	0.02511	0.14103
QFC	295.529	549.476	163.807	-0.01489	-0.01474	0.01038
MEAN	295.573	549.557	163.79			

*QS scaled as function of QF & QC, QR scaled as function of QL & QC

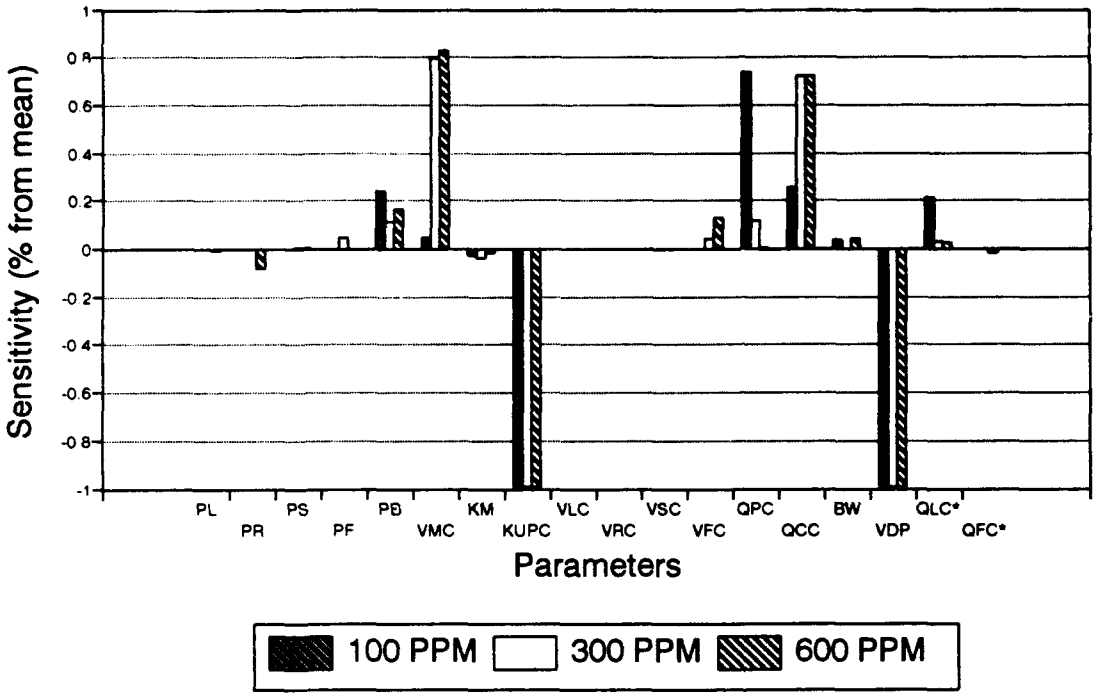
Female Mouse Inhalation 1% Sensitivity

AMET



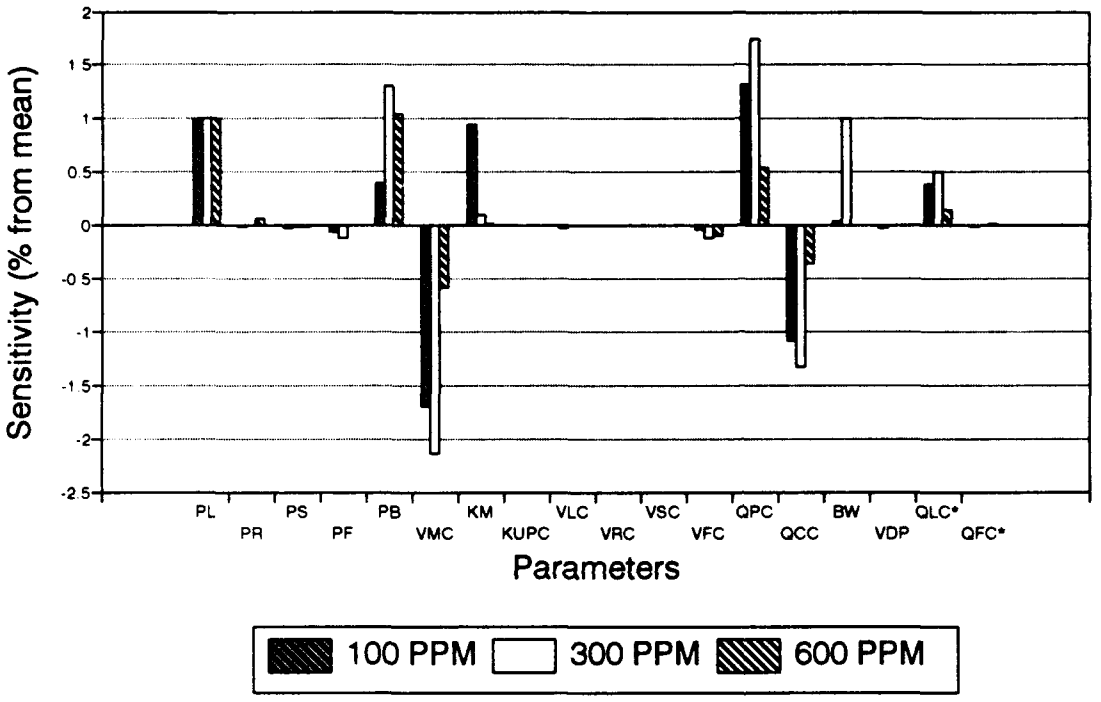
Female Mouse Inhalation 1% Sensitivity

AUCTCA



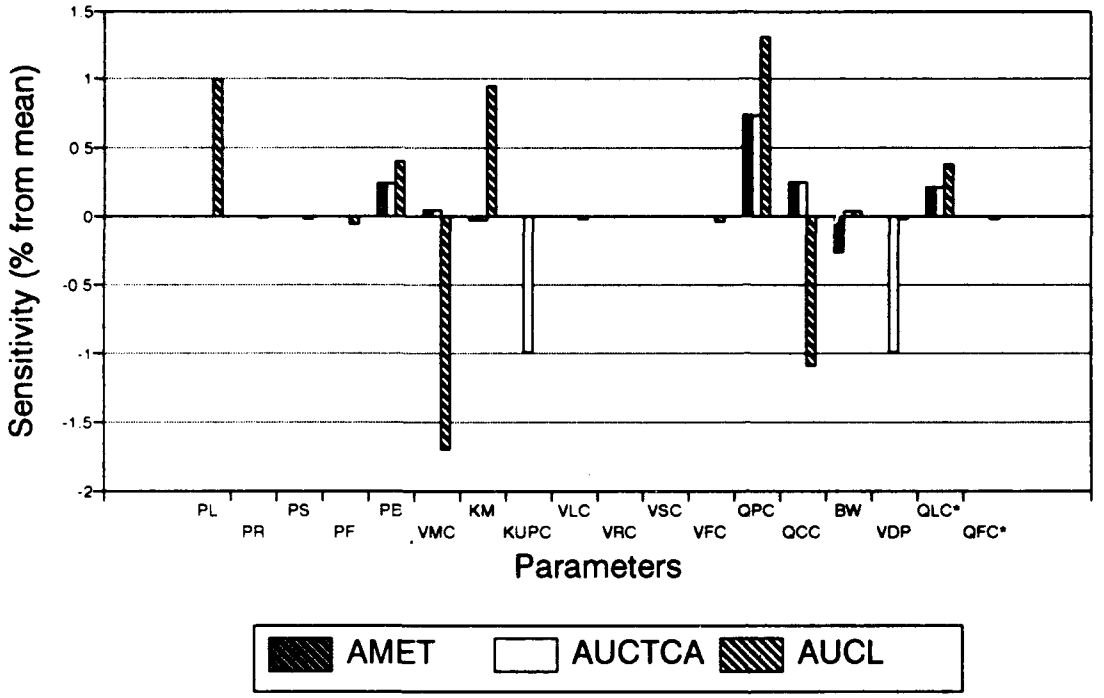
Female Mouse Inhalation 1% Sensitivity

AUCL



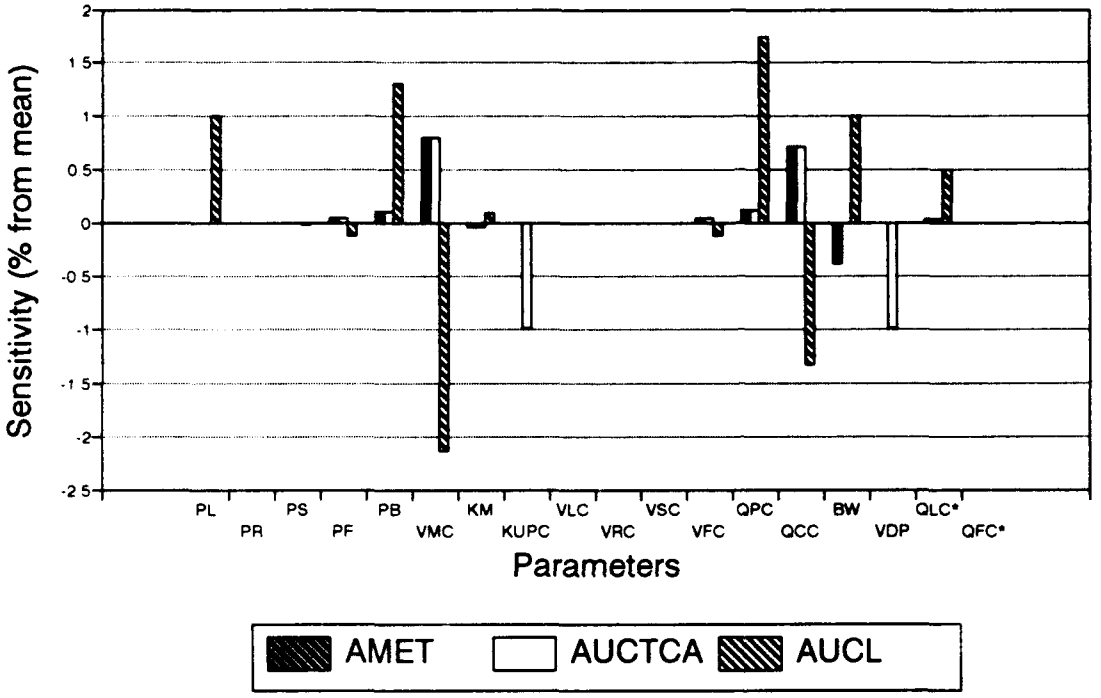
Female Mouse Inhalation 1% Sensitivity

100 PPM TCE



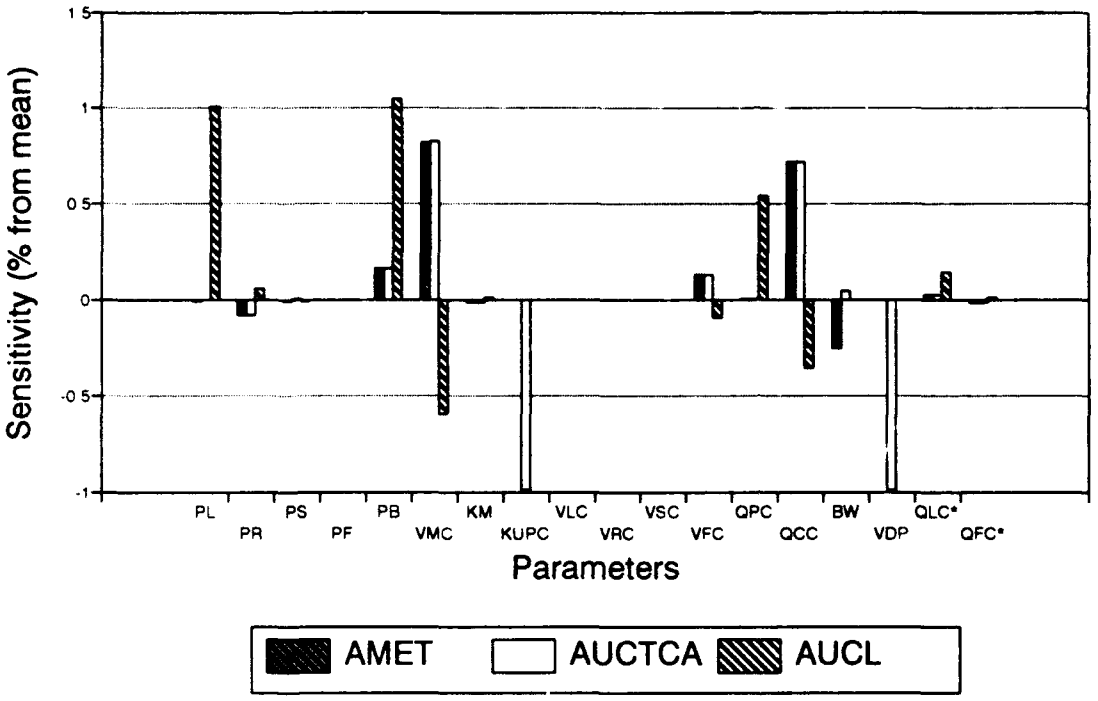
Female Mouse Inhalation 1% Sensitivity

300 PPM TCE



Female Mouse Inhalation 1% Sensitivity

600 PPM TCE



Appendix E. *Human Intermittent Inhalation Sensitivity*

The following graphs present the results of the human intermittent inhalation sensitivity analysis. The tables of data for the analysis are first presented by exposure dose of TCE. Graphs of the sensitivity for each parameter are then presented both by dose-metric and dose level.

1% SENSITIVITY ANALYSIS
Intermittent Human Inhalation

DOSE 1: 100 PPB TCE

	Model Output			Sensitivity (%)		
Parameter	AMET	AUCTCA	AUCL	AMET	AUCTCA	AUCL
PL	0.0073298	0.316913	0.0181858	-0.00409	-0.00252	0.99743
PR	0.0073300	0.316913	0.0180059	-0.00136	-0.00252	-0.00167
PS	0.0073301	0.316916	0.0180063	0.00000	-0.00158	0.00056
PF	0.0073288	0.316853	0.0180032	-0.01774	-0.02146	-0.01666
PB	0.0073545	0.317965	0.0180662	0.33287	0.32942	0.33322
VMC	0.0073378	0.317261	0.0178467	0.10505	0.10728	-0.88581
KM	0.0073220	0.316564	0.0181663	-0.11050	-0.11265	0.88914
KUPC	0.0073301	0.314436	0.0180062	0.00000	-0.78411	0.00000
VLC	0.0073302	0.316923	0.0180064	0.00136	0.00063	0.00111
VRC	0.0073301	0.316921	0.0180062	0.00000	0.00000	0.00000
VSC	0.0073301	0.316921	0.0180062	0.00000	0.00000	0.00000
VFC	0.0073289	0.316858	0.0180034	-0.01637	-0.01988	-0.01555
QPC	0.0073783	0.319015	0.0181245	0.65756	0.66073	0.65700
QCC	0.0073478	0.317683	0.0180497	0.24147	0.24044	0.24158
BW	0.0073103	0.324376	0.0180115	-0.27012	2.35232	0.02943
VDP	0.0073301	0.316921	0.0180062	0.00000	0.00000	0.00000
QLC*	0.0073474	0.317678	0.0180486	0.23601	0.23886	0.23547
QFC*	0.0073303	0.316913	0.0180067	0.00273	-0.00252	0.00278
MEAN	0.0073301	0.316921	0.0180062			

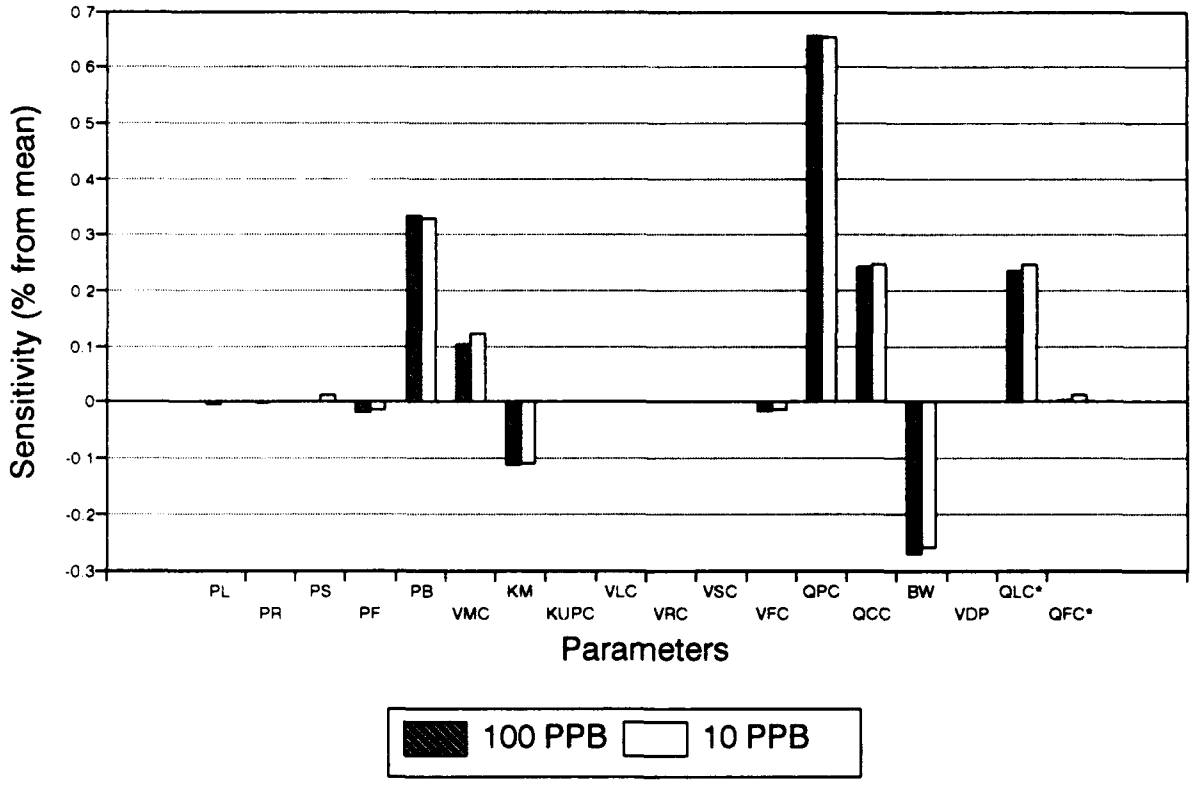
*QS scaled as function of QF & QC, QR scaled as a function of QL & QC

DOSE 2: 10 PPB TCE

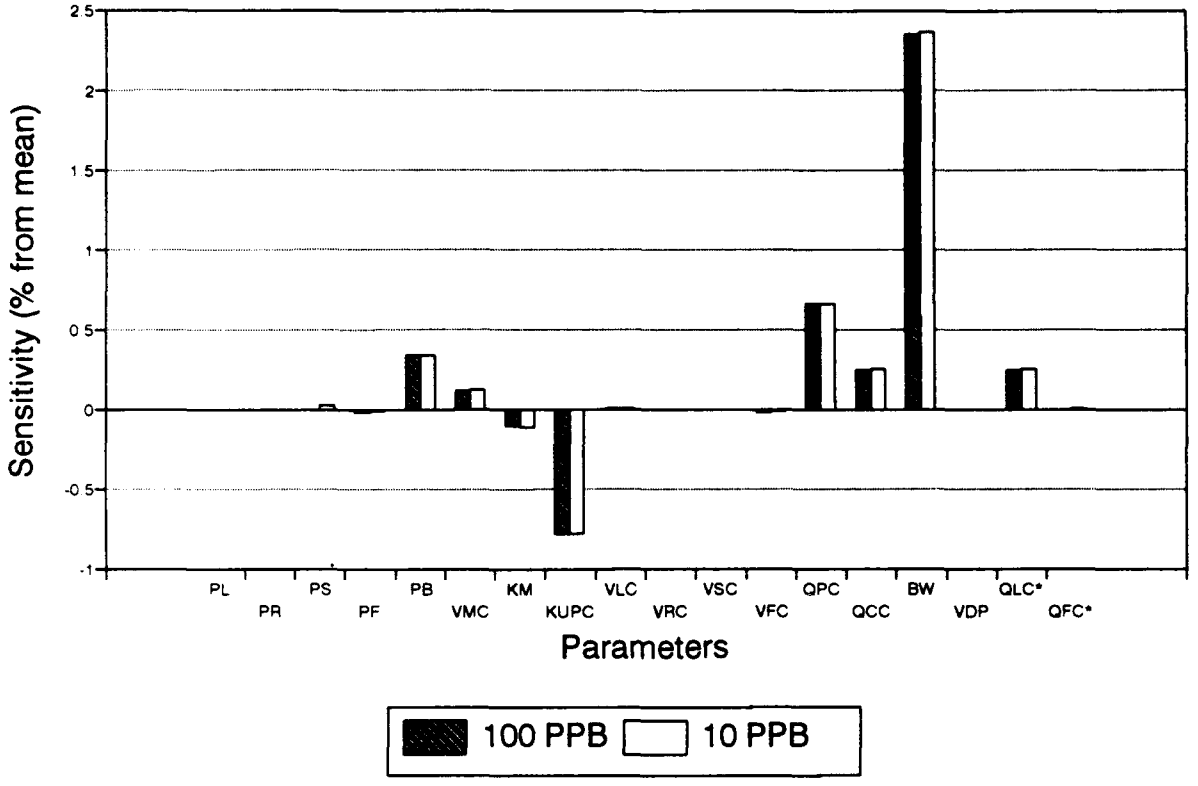
	Model Output			Sensitivity (%)		
Parameter	AMET	AUCTCA	AUCL	AMET	AUCTCA	AUCL
PL	7.3250E-04	0.0316700	1.8170E-03	0.00000	0.00000	1.00056
PR	7.3250E-04	0.0316695	1.7990E-03	0.00000	-0.00158	0.00000
PS	7.3260E-04	0.0316763	1.7994E-03	0.01365	0.01989	0.02223
PF	7.3240E-04	0.0316654	1.7988E-03	-0.01365	-0.01452	-0.01112
PB	7.3490E-04	0.0317742	1.8050E-03	0.32765	0.32902	0.33352
VMC	7.3340E-04	0.0317074	1.7833E-03	0.12287	0.11809	-0.87271
KM	7.3170E-04	0.0316331	1.8149E-03	-0.10922	0.11651	0.88382
KUPC	7.3250E-04	0.0314218	1.7990E-03	0.00000	-0.78371	0.00000
VLC	7.3250E-04	0.0316717	1.7991E-03	0.00000	0.00537	0.00556
VRC	7.3250E-04	0.0316700	1.7990E-03	0.00000	0.00000	0.00000
VSC	7.3250E-04	0.0316700	1.7990E-03	0.00000	0.00000	0.00000
VFC	7.3240E-04	0.0316650	1.7988E-03	-0.01365	-0.01579	-0.01112
QPC	7.3730E-04	0.0318789	1.8108E-03	0.65529	0.65961	0.65592
QCC	7.3430E-04	0.0317499	1.8036E-03	0.24573	0.25229	0.25570
BW	7.3060E-04	0.0324182	1.7997E-03	-0.25939	2.36249	0.03891
VDP	7.3250E-04	0.0316700	1.7990E-03	0.00000	0.00000	0.00000
QLC*	7.3430E-04	0.0317482	1.8034E-03	0.24573	0.24692	0.24458
QFC*	7.3260E-04	0.0316711	1.7992E-03	0.01365	0.00347	0.01112
MEAN	7.3250E-04	0.0316700	1.7990E-03			

*QS scaled as function of QF & QC, QR scaled as a function of QL & QC

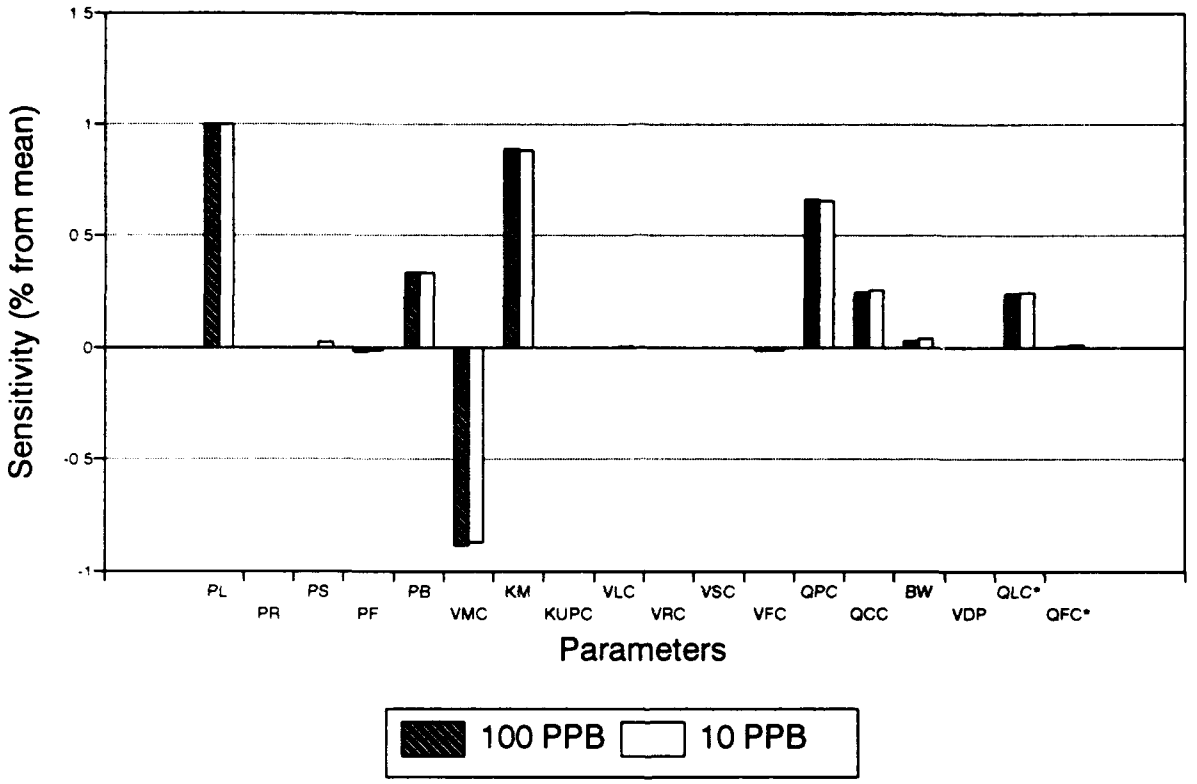
Int. Human Inhalation Sensitivity
AMET



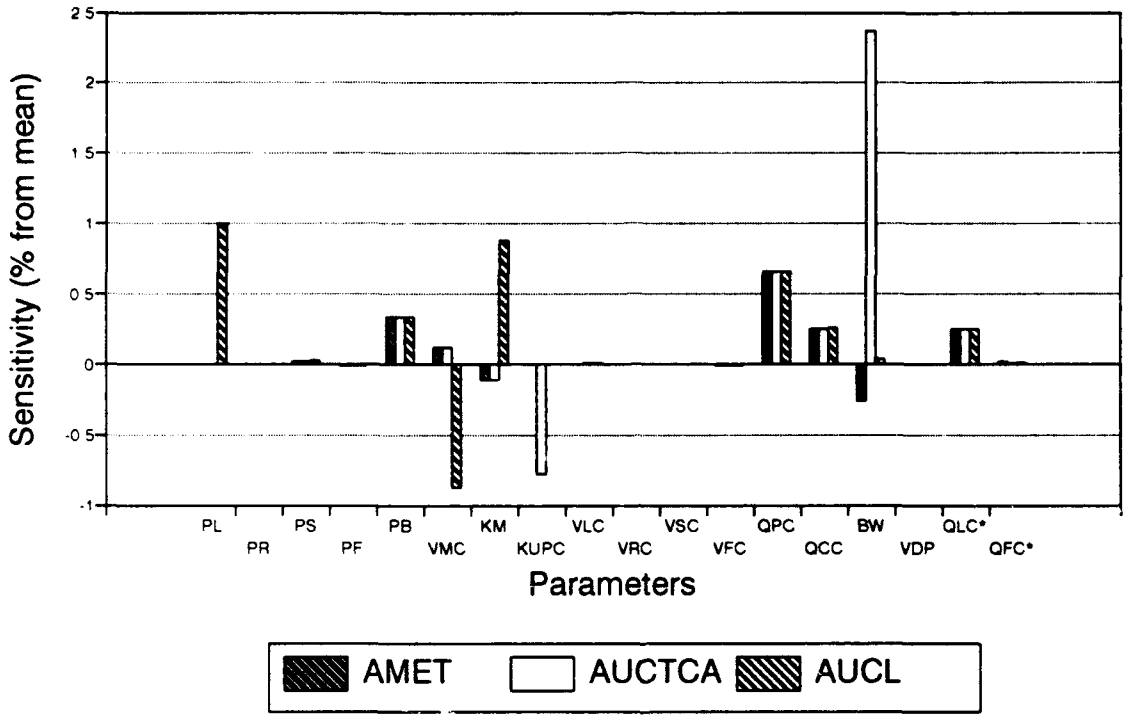
Int. Human Inhalation Sensitivity
AUCTCA



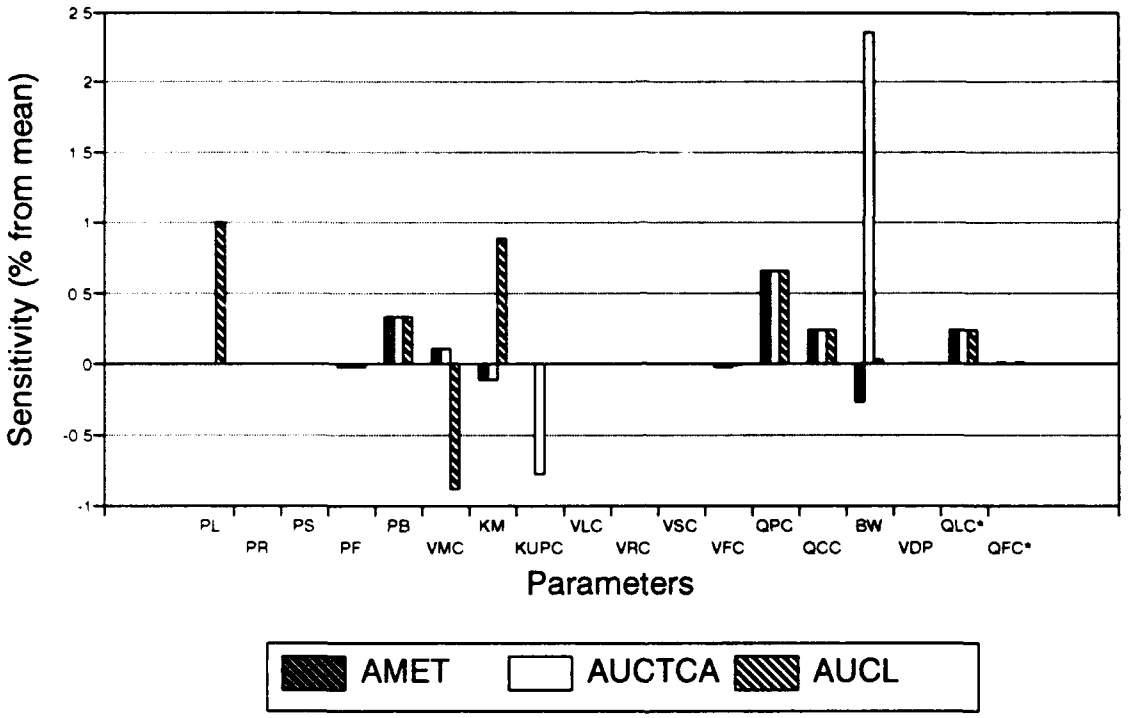
Int. Human Inhalation Sensitivity
AUCL



Int. Human Inhalation Sensitivity
10 PPB TCE



Int. Human Inhalation Sensitivity
100 PPB TCE



Appendix F. *Human Continuous Inhalation Sensitivity*

The following graphs present the results of the human continuous inhalation sensitivity analysis. The tables of data for the analysis are first presented by exposure dose of TCE. Graphs of the sensitivity for each parameter are then presented both by dose-metric and dose level.

1% SENSITIVITY ANALYSIS
Continuous Human Inhalation

DOSE 1: 100 PPB TCE

	Model Output			Sensitivity (%)		
Parameter	AMET	AUCTCA	AUCL	AMET	AUCTCA	AUCL
PL	0.0348698	1.43437	0.0865250	0.00000	0.00000	0.99990
PR	0.0348697	1.43436	0.0856681	-0.00029	-0.00070	-0.00035
PS	0.0348693	1.43434	0.0856670	-0.00143	-0.00209	-0.00163
PF	0.0348643	1.43410	0.0856548	-0.01577	-0.01882	-0.01588
PB	0.0349858	1.43912	0.0859533	0.33267	0.33116	0.33256
VMC	0.0349084	1.43597	0.0849138	0.11070	0.11155	-0.88084
KM	0.0348310	1.43276	0.0864286	-0.11127	-0.11224	0.88738
KUPC	0.0348698	1.42330	0.0856684	0.00000	-0.77177	0.00000
VLC	0.0348699	1.43437	0.0856685	0.00029	0.00000	0.00012
VRC	0.0348698	1.43437	0.0856684	0.00000	0.00000	0.00000
VSC	0.0348698	1.43437	0.0856684	0.00000	0.00000	0.00000
VFC	0.0348645	1.43411	0.0856553	-0.01520	-0.01813	-0.01529
QPC	0.0351011	1.44390	0.0862366	0.66332	0.66440	0.66326
QCC	0.0349533	1.43783	0.0858735	0.23946	0.24122	0.23941
BW	0.0347766	1.46810	0.0856947	-0.26728	2.35156	0.03070
VDP	0.0348698	1.43437	0.0856684	0.00000	0.00000	0.00000
QLC*	0.0349533	1.43783	0.0858735	0.23946	0.24122	0.23941
QFC*	0.0348698	1.43437	0.0856684	0.00000	0.00000	0.00000
MEAN	0.0348698	1.43437	0.0855684			

*QS scaled as function of QF & QC, QR scaled as a function of QL & QC

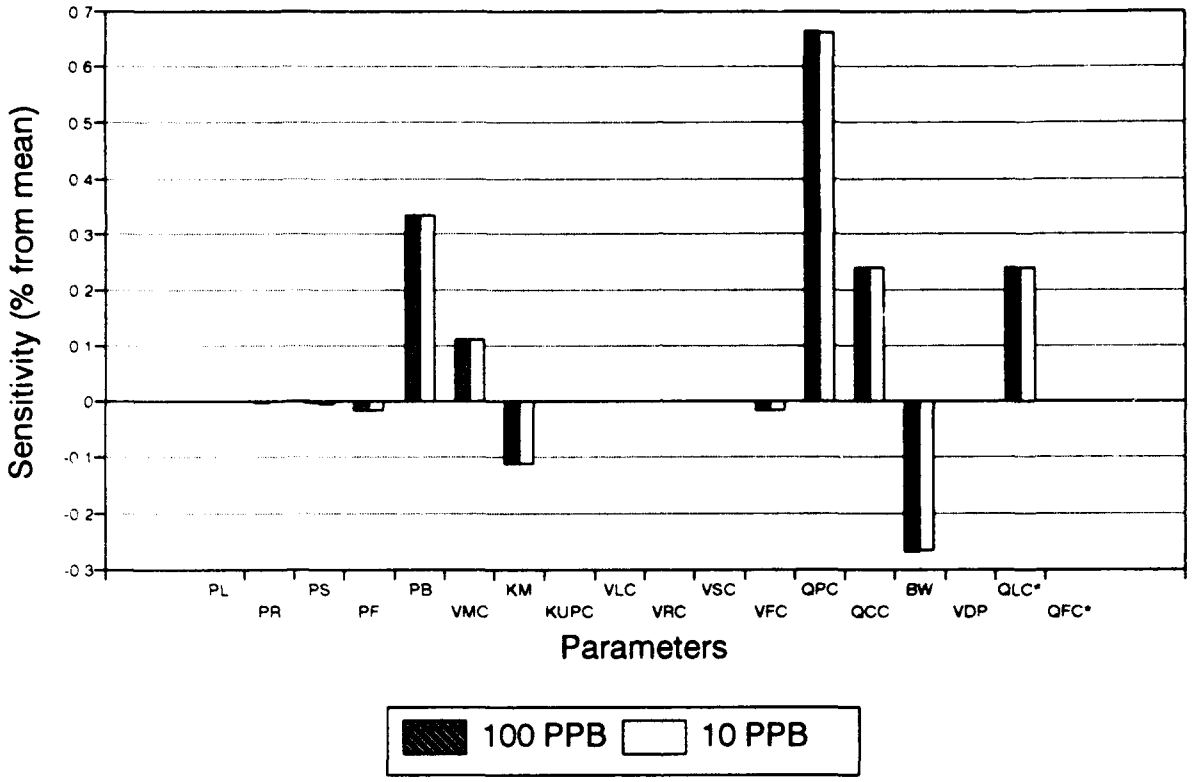
DOSE 2: 10 PPB TCE

	Model Output			Sensitivity (%)		
Parameter	AMET	AUCTCA	AUCL	AMET	AUCTCA	AUCL
PL	3.4871E-03	0.143442	8.6501E-03	0.00000	0.00000	1.00065
PR	3.4871E-03	0.143442	8.5644E-03	0.00000	0.00000	0.00000
PS	3.4870E-03	0.143439	8.5643E-03	-0.00287	-0.00209	-0.00117
PF	3.4866E-03	0.143415	8.5631E-03	-0.01434	-0.01882	-0.01518
PB	3.4987E-03	0.143917	8.5929E-03	0.33265	0.33114	0.33277
VMC	3.4910E-03	0.143602	8.4890E-03	0.11184	0.11154	-0.88039
KM	3.4832E-03	0.143281	8.6405E-03	-0.11184	-0.11224	0.88856
KUPC	3.4871E-03	0.142335	8.5644E-03	0.00000	-0.77174	0.00000
VLC	3.4871E-03	0.143442	8.5645E-03	0.00000	0.00000	0.00117
VRC	3.4871E-03	0.143442	8.5644E-03	0.00000	0.00000	0.00000
VSC	3.4871E-03	0.143442	8.5644E-03	0.00000	0.00000	0.00000
VFC	3.4866E-03	0.143416	8.5631E-03	-0.01434	-0.01813	-0.01518
QPC	3.5102E-03	0.144395	8.6212E-03	0.66244	0.66438	0.66321
QCC	3.4955E-03	0.143788	8.5849E-03	0.24089	0.24121	0.23936
BW	3.4778E-03	0.146815	8.5671E-03	-0.26670	2.35147	0.03153
VDP	3.4871E-03	0.143442	8.5644E-03	0.00000	0.00000	0.00000
QLC*	3.4955E-03	0.143788	8.5849E-03	0.24089	0.24121	0.23936
QFC*	3.4871E-03	0.143442	8.5644E-03	0.00000	0.00000	0.00000
MEAN	3.4871E-03	0.143442	8.5644E-03			

*QS scaled as function of QF & QC, QR scaled as a function of QL & QC

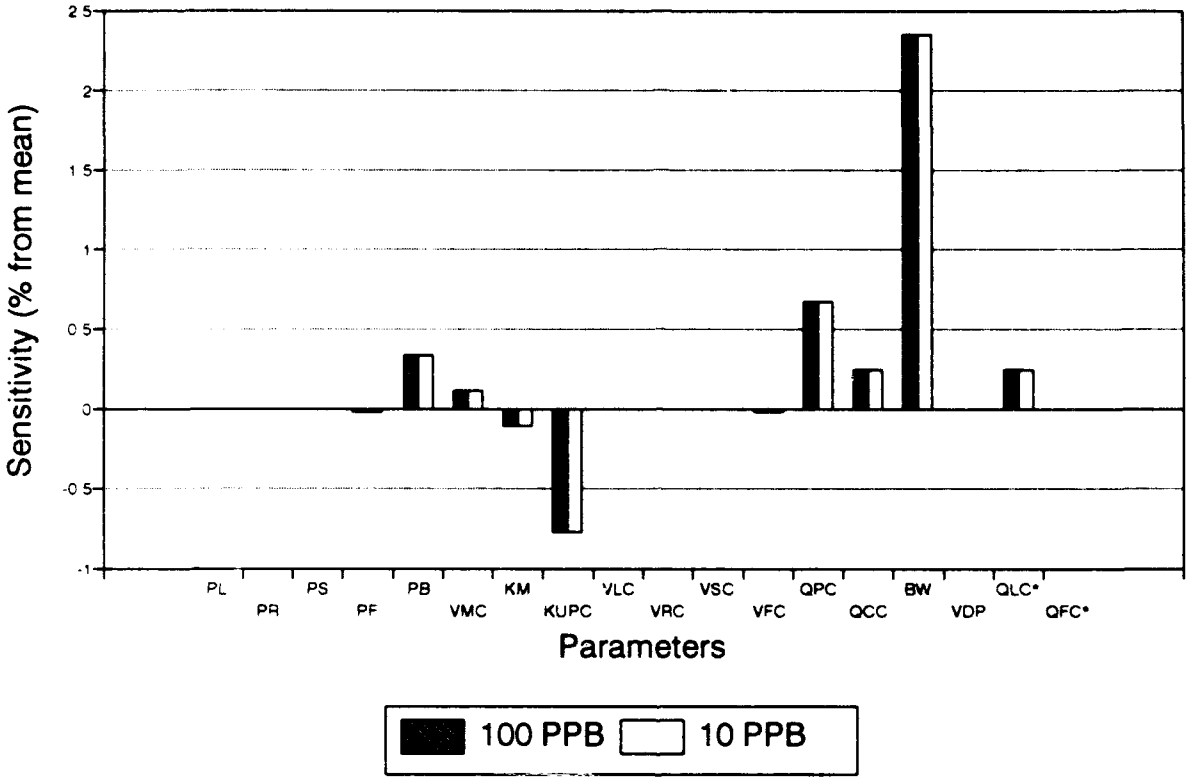
Continuous Human Inhalation Sensitivity

AMET



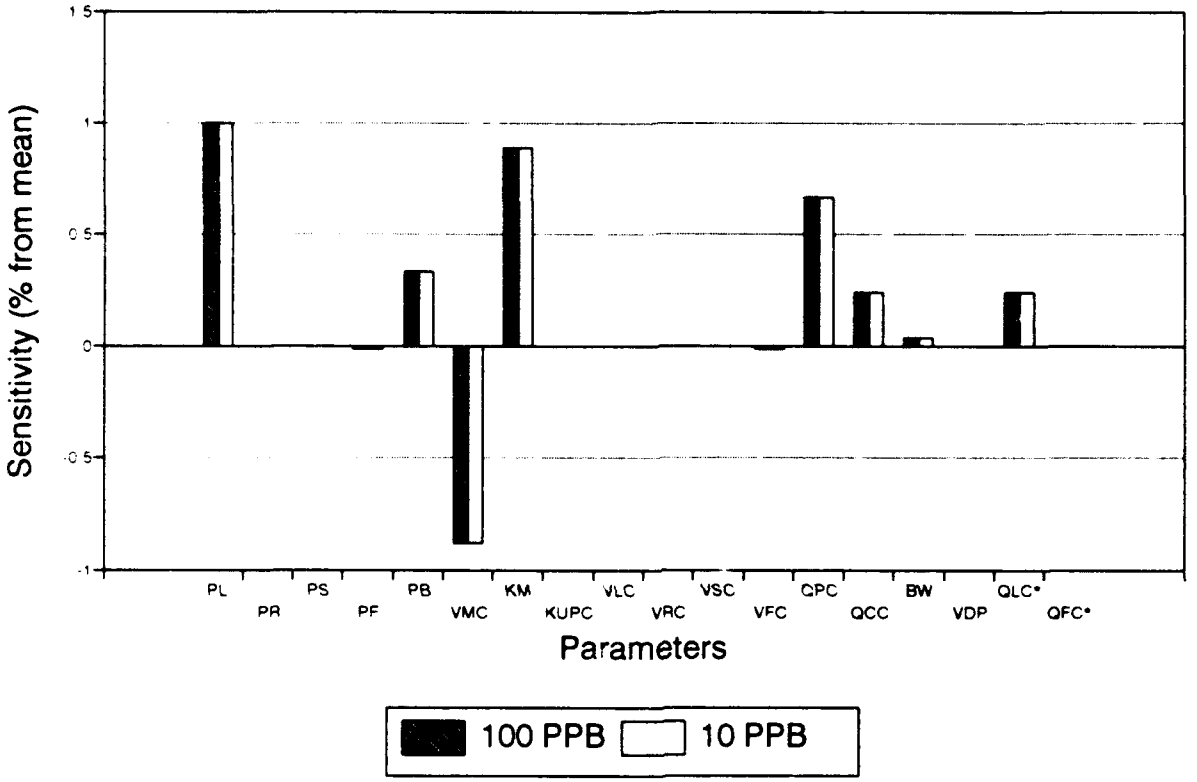
Continuous Human Inhalation Sensitivity

AUCTCA

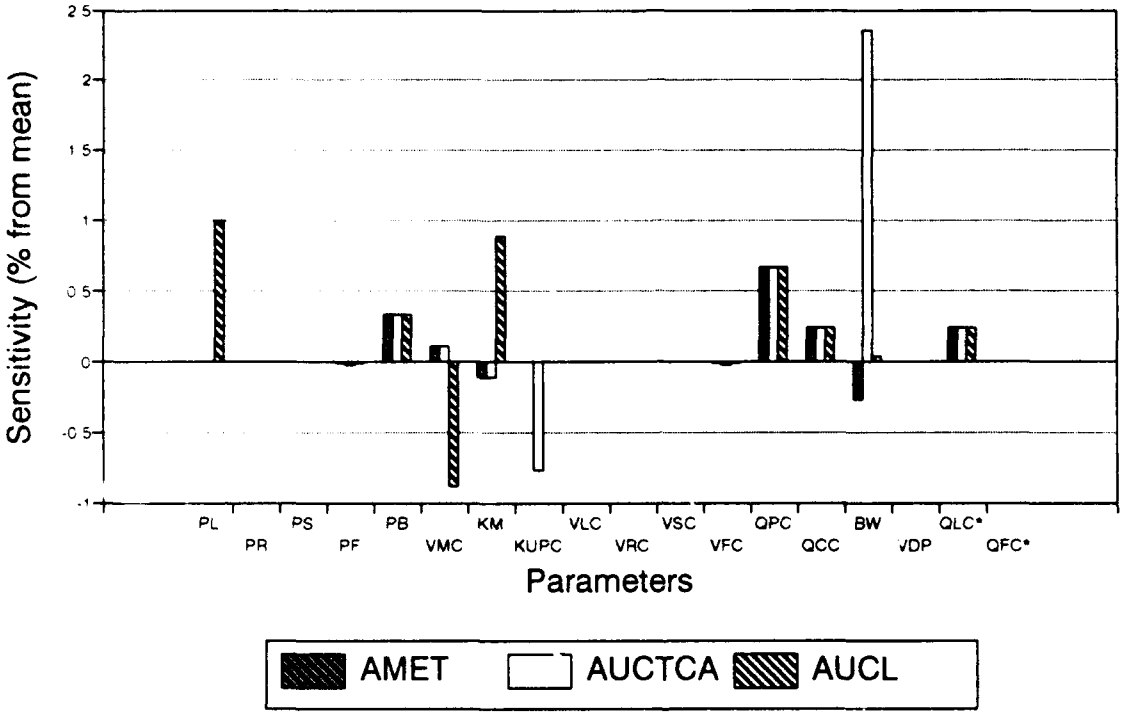


Continuous Human Inhalation Sensitivity

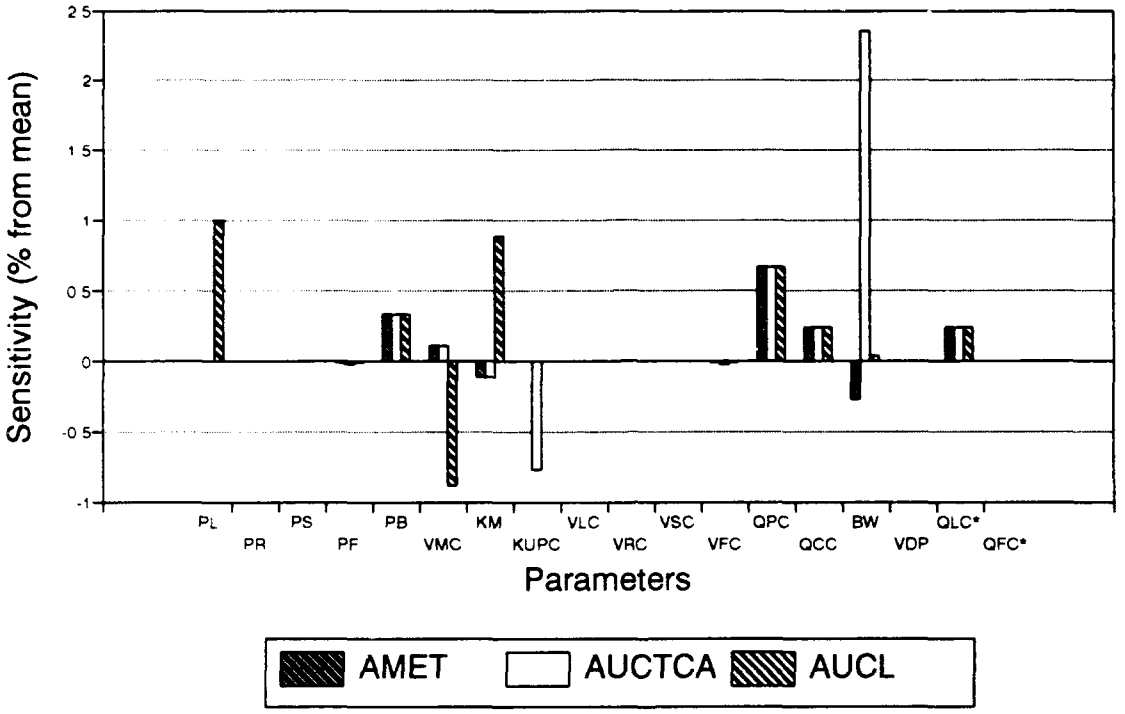
AUCL



Continuous Human Inhalation Sensitivity
10 PPB TCE



Continuous Human Inhalation Sensitivity
100 PPB TCE



Appendix G. *Male Mouse Ingestion Sensitivity*

The following graphs present the results of the male mouse ingestion sensitivity analysis. The tables of data for the analysis are first presented by exposure dose of TCE. Graphs of the sensitivity for each parameter are then presented both by dose-metric and dose level.

1% SENSITIVITY ANALYSIS
Male Mouse Ingestion

DOSE 1: 2339 PPM TCE

	Model Output			Sensitivity (%)		
Parameter	AMET	AUCTCA	AUCL	AMET	AUCTCA	AUCL
PL	218.03	834.941	484.86	0.00321	0.00299	0.99945
PR	218.029	834.934	480.06	0.00275	0.00216	-0.00042
PS	218.659	837.349	479.768	0.29171	0.29141	-0.06124
PF	218.361	836.206	479.906	0.15503	0.15451	-0.03250
PB	218.356	836.188	483.556	0.15274	0.15235	0.72782
VMC	219.704	841.349	479.283	0.77102	0.77050	-0.16227
KM	218.004	834.84	480.071	-0.00871	-0.00910	0.00187
KUPC	218.024	826.657	480.062	0.00046	-0.98920	0.00000
VLC	218.025	834.921	480.062	0.00092	0.00060	0.00000
VRC	218.023	834.916	480.062	0.00000	0.00000	0.00000
VSC	218.023	834.916	480.062	0.00000	0.00000	0.00000
VFC	218.357	836.192	479.908	0.15319	0.15283	-0.03208
QPC	217.695	833.657	476.601	-0.15044	-0.15079	-0.72095
QCC	217.927	834.547	478.967	-0.04403	-0.04420	-0.22810
BW	217.633	835.908	481.488	-0.17888	0.11881	0.29704
VDP	218.024	826.645	480.062	0.00046	-0.99064	0.00000
K1	216.783	830.166	480.638	-0.56875	-0.56892	0.11998
QLC*	218.048	835.006	478.911	0.01147	0.01078	-0.23976
QFC*	217.908	834.475	480.116	-0.05275	-0.05282	0.01125
MEAN	218.023	834.916	480.062			

*QS scaled as function of QF & QC, QR scaled as a function of QL & QC

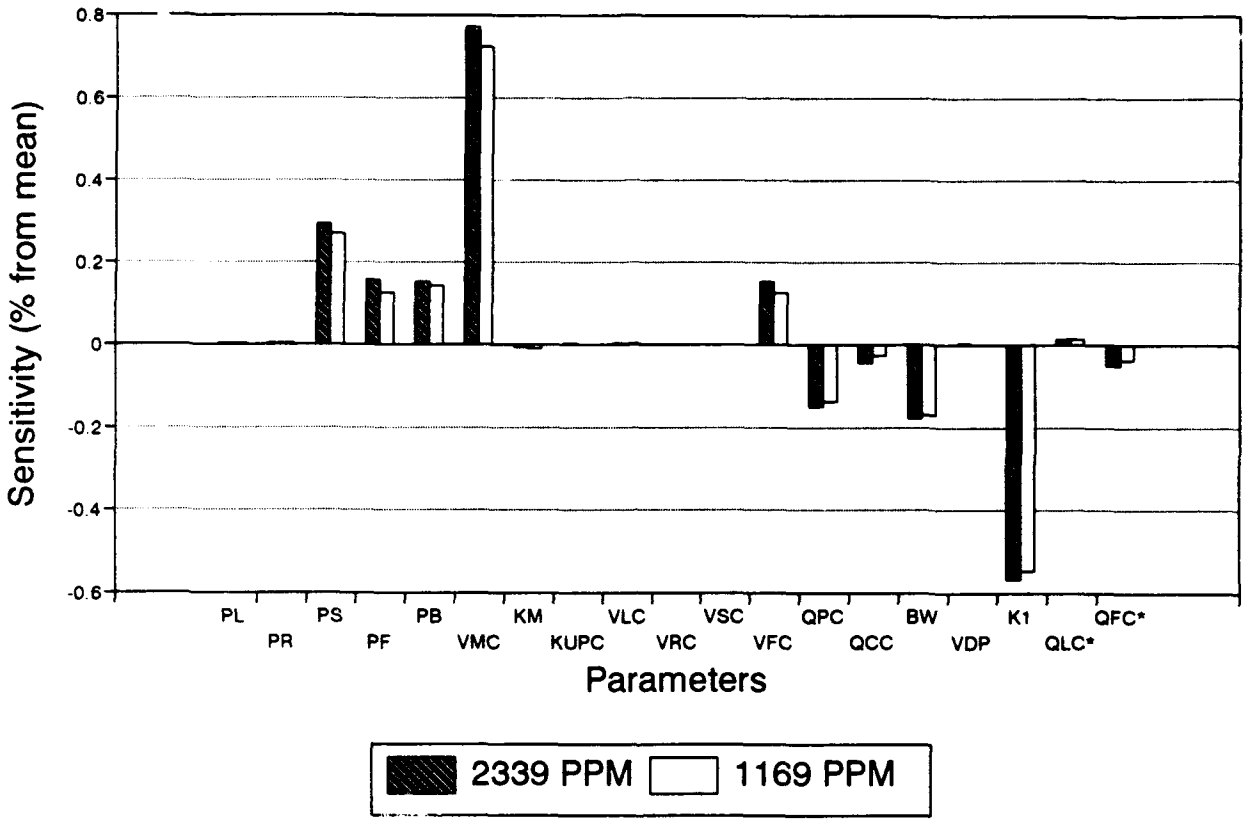
DOSE 2: 1169 PPM TCE

	Model Output			Sensitivity (%)		
Parameter	AMET	AUCTCA	AUCL	AMET	AUCTCA	AUCL
PL	181.886	696.53	208.167	0.00330	0.00316	0.99850
PR	181.884	696.522	206.107	0.00220	0.00201	-0.00097
PS	182.368	698.379	205.882	0.26831	0.26863	-0.11014
PF	182.11	697.39	206.002	0.12646	0.12663	-0.05191
PB	182.136	697.486	207.556	0.14075	0.14041	0.70206
VMC	183.19	701.522	205.501	0.72026	0.71988	-0.29499
KM	181.861	696.433	206.118	-0.01045	-0.01077	0.00437
KUPC	181.88	689.62	206.109	0.00000	-0.98893	0.00000
VLC	181.881	696.51	206.109	0.00055	0.00029	0.00000
VRC	181.88	696.508	206.109	0.00000	0.00000	0.00000
VSC	181.88	696.508	206.109	0.00000	0.00000	0.00000
VFC	182.108	697.379	206.003	0.12536	0.12505	-0.05143
QPC	181.627	695.54	204.674	-0.13910	-0.13898	-0.69623
QCC	181.829	696.315	205.643	-0.02804	-0.02771	-0.22609
BW	181.568	697.389	206.788	-0.17154	0.12649	0.32944
VDP	181.88	689.612	206.109	0.00000	-0.99008	0.00000
K1	180.884	692.697	206.571	-0.54761	-0.54716	0.22415
QLC*	181.9	696.581	205.61	0.01100	0.01048	-0.24210
QFC*	181.813	696.251	206.14	-0.03684	-0.03690	0.01504
MEAN	181.88	696.508	206.109			

*QS scaled as function of QF & QC, QR scaled as a function of QL & QC

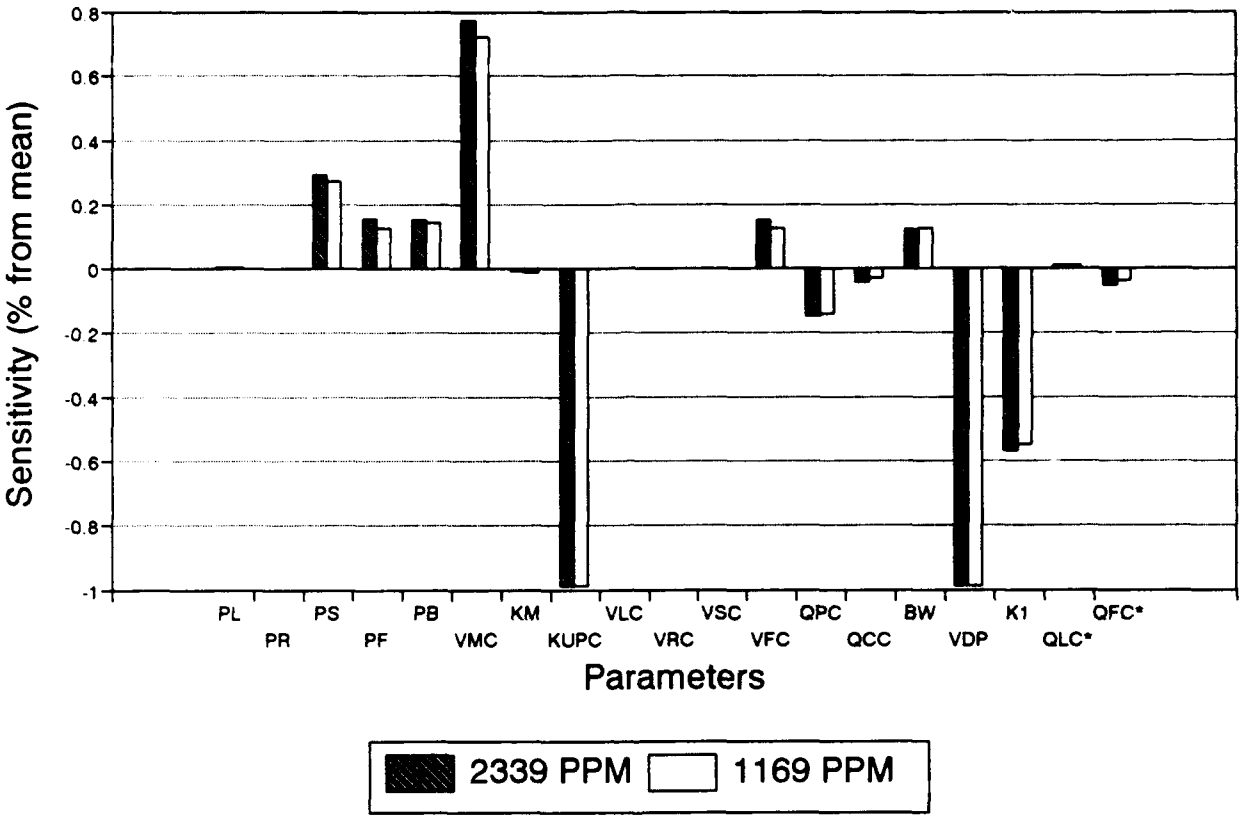
Male Mouse Ingestion 1% Sensitivity

AMET



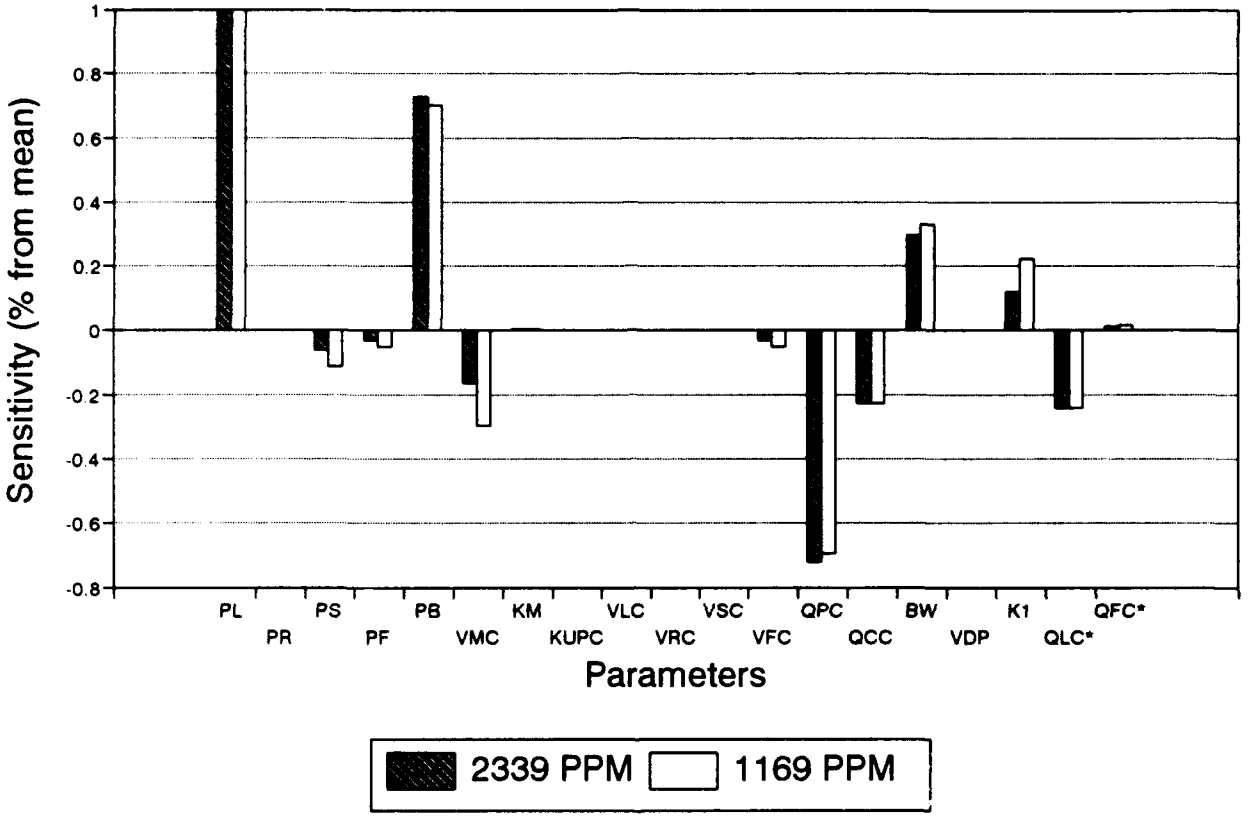
Male Mouse Ingestion 1% Sensitivity

AUCTCA

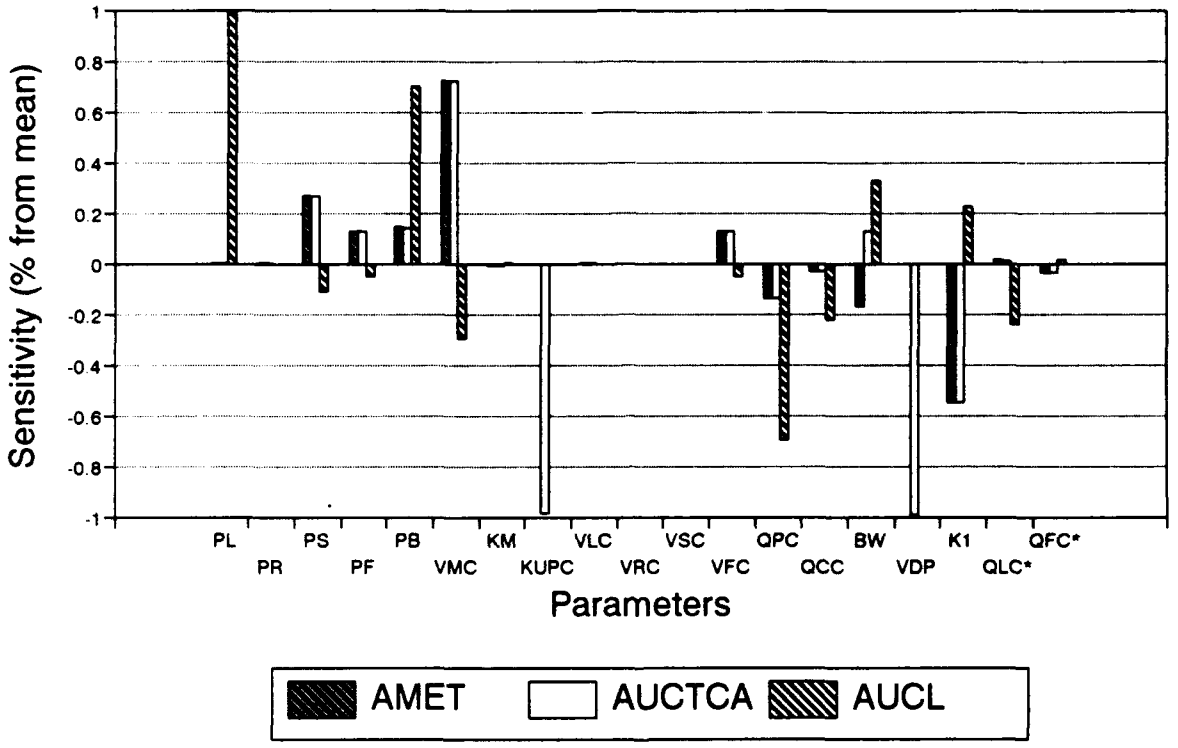


Male Mouse Ingestion 1% Sensitivity

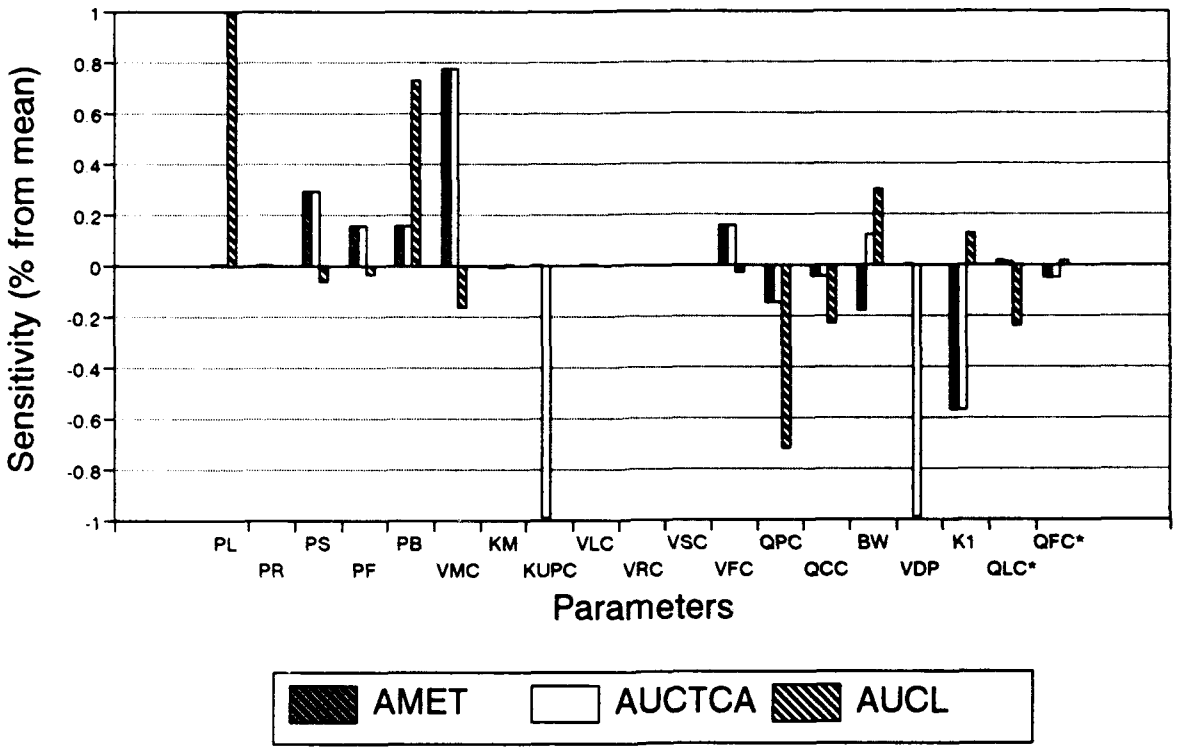
AUCL



Male Mouse Ingestion 1% Sensitivity
1169 PPM TCE



Male Mouse Ingestion 1% Sensitivity
2339 PPM TCE



Appendix H. *Female Mouse Ingestion Sensitivity*

The following graphs present the results of the female mouse ingestion sensitivity analysis. The tables of data for the analysis are first presented by exposure dose of TCE. Graphs of the sensitivity for each parameter are then presented both by dose-metric and dose level.

1% SENSITIVITY ANALYSIS
Female Mouse Ingestion

DOSE 1: 1739 PPM TCE

	Model Output			Sensitivity (%)		
Parameter	AMET	AUCTCA	AUCL	AMET	AUCTCA	AUCL
PL	196.998	691.201	292.026	0.00203	0.00217	0.99953
PR	196.998	691.201	289.134	0.00203	0.00217	-0.00069
PS	197.014	691.256	289.128	0.01015	0.01013	-0.00277
PF	197.48	692.89	288.945	0.24671	0.24653	-0.06606
PB	197.416	692.667	291.208	0.21422	0.21427	0.71662
VMC	198.444	696.273	288.565	0.73606	0.73598	-0.19748
KM	196.971	691.105	289.145	-0.01168	-0.01172	0.00311
KUPC	196.994	684.342	289.136	0.00000	-0.99018	0.00000
VLC	196.995	691.188	289.136	0.00051	0.00029	0.00000
VRC	196.994	691.186	289.136	0.00000	0.00000	0.00000
VSC	196.994	691.186	289.136	0.00000	0.00000	0.00000
VFC	197.479	692.887	288.945	0.24620	0.24610	-0.06606
QPC	196.575	689.717	287.083	-0.21270	-0.21253	-0.71005
QCC	196.876	690.773	288.536	-0.05990	-0.05975	-0.20751
BW	196.7	692.218	290.001	-0.14924	0.14931	0.29917
VDP	196.995	684.345	289.136	0.00051	-0.98975	0.00000
K1	196.091	688.018	289.491	-0.45839	-0.45834	0.12278
QLC*	197.816	694.069	288.167	0.41727	0.41711	-0.33514
QFC*	196.829	690.608	289.337	-0.08376	-0.08362	0.06952
MEAN	196.994	691.186	289.136			

*QS scaled as function of QF & QC, QR scaled as a function of QL & QC

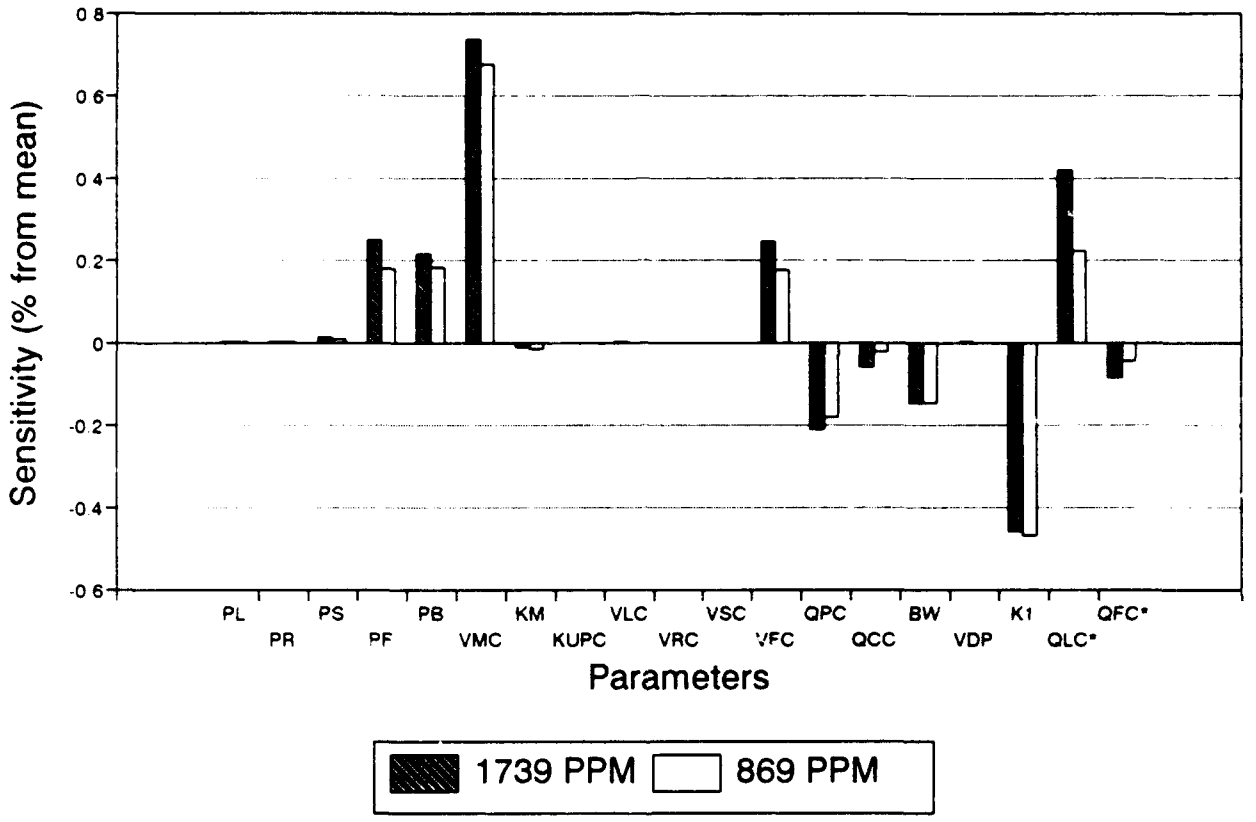
DOSE 2: 869 PPM TCE

	Model Output			Sensitivity (%)		
Parameter	AMET	AUCTCA	AUCL	AMET	AUCTCA	AUCL
PL	159.402	559.288	120.493	0.00251	0.00250	0.00000
PR	159.4	559.283	120.492	0.00125	0.00161	-0.00083
PS	159.411	559.319	120.488	0.00816	0.00805	-0.00415
PF	159.68	560.264	120.382	0.17692	0.17702	-0.09212
PB	159.689	560.296	121.31	0.18256	0.18274	0.67805
VMC	160.476	563.057	120.068	0.67629	0.67641	-0.35272
KM	159.375	559.193	120.502	-0.01443	-0.01448	0.00747
KUPC	159.398	553.739	120.492	0.00000	-0.98968	-0.00083
VLC	159.398	559.276	120.492	0.00000	0.00036	-0.00083
VRC	159.398	559.274	120.493	0.00000	0.00000	0.00000
VSC	159.398	559.274	120.493	0.00000	0.00000	0.00000
VFC	159.678	560.258	120.382	0.17566	0.17594	-0.09212
QPC	159.109	558.261	119.682	-0.18131	-0.18113	-0.67307
QCC	159.364	559.155	120.237	-0.02133	-0.02128	-0.21246
BW	159.161	560.114	120.898	-0.14868	0.15019	0.33612
VDP	159.398	553.737	120.492	0.00000	-0.99003	-0.00083
K1	158.653	556.662	120.786	-0.46738	-0.46703	0.24317
QLC*	159.749	560.505	120.086	0.22020	0.22011	-0.33778
QFC*	159.327	559.026	120.572	-0.04454	-0.04434	0.06556
MEAN	159.398	559.274	120.493			

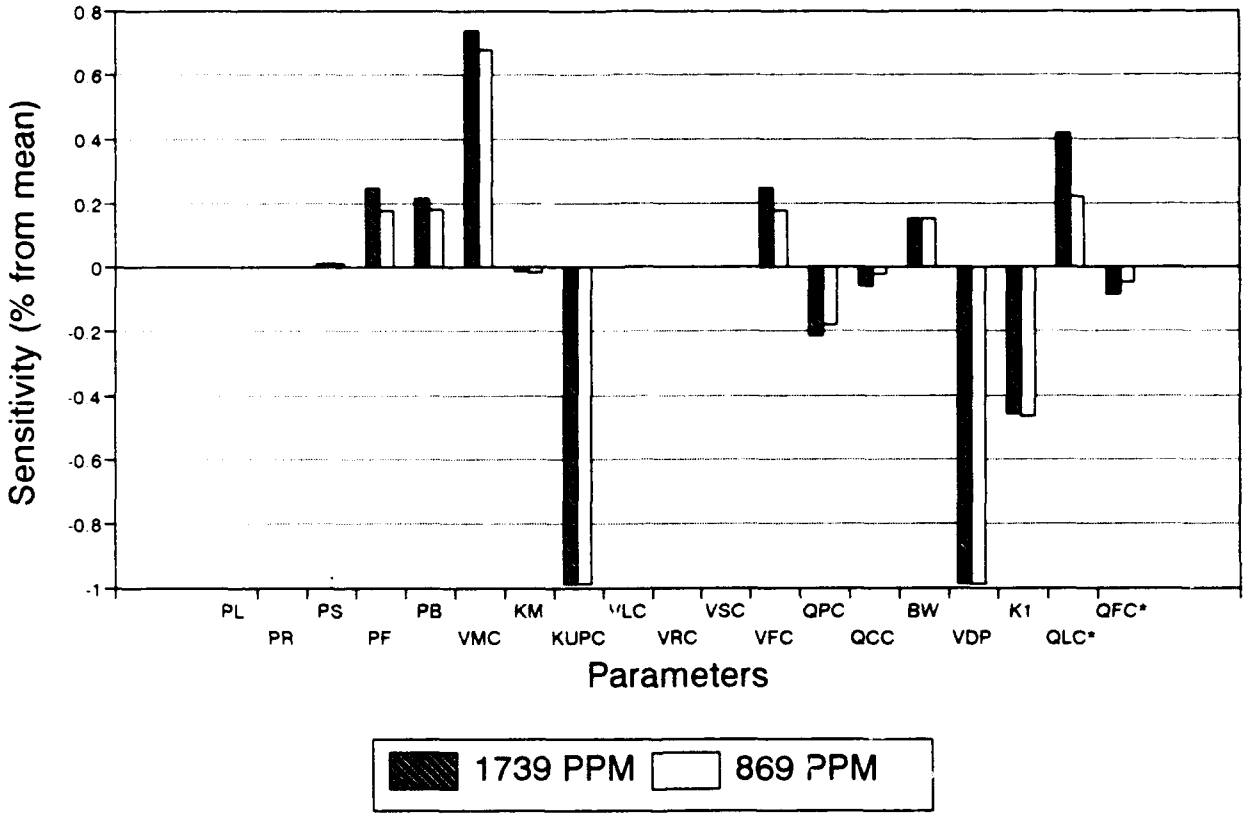
*QS scaled as function of QF & QC, QR scaled as a function of QL & QC

Female Mouse Ingestion 1% Sensitivity

AMET

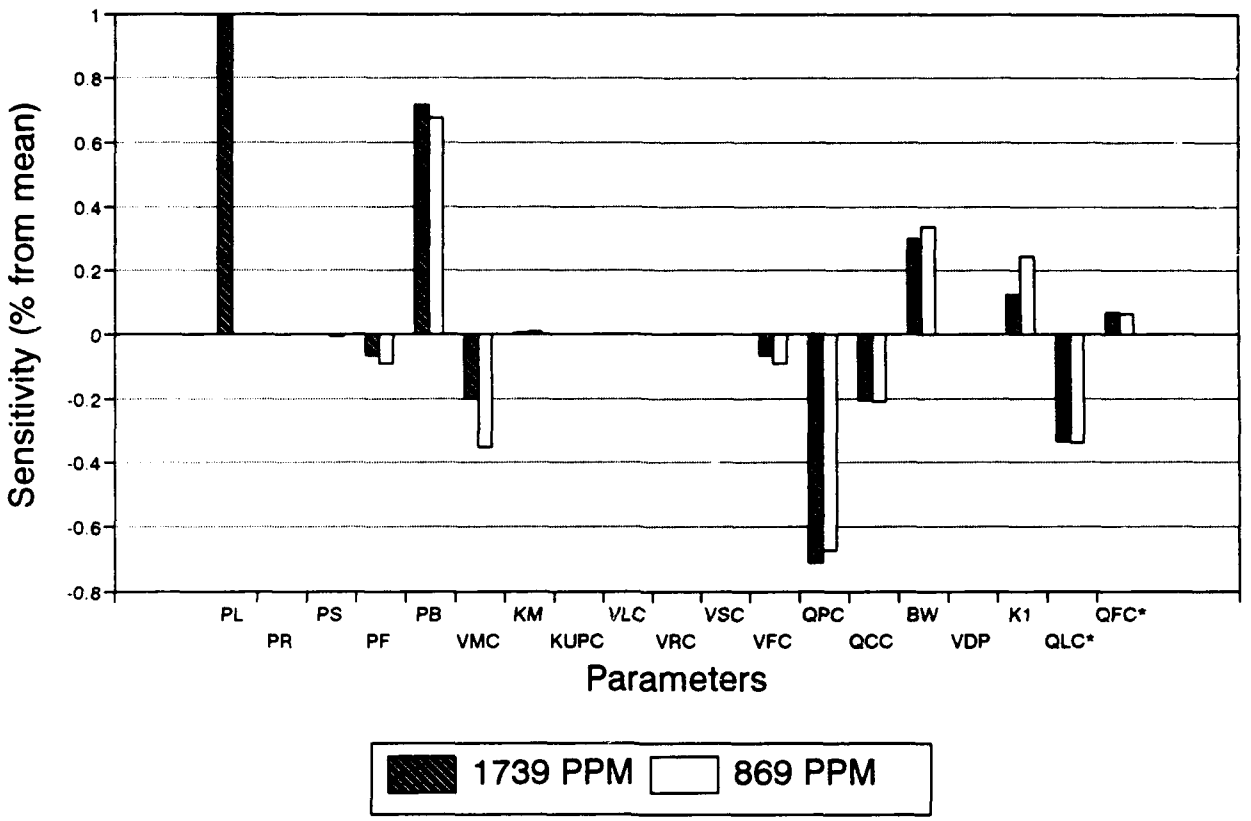


Female Mouse Ingestion 1% Sensitivity
AUCTCA

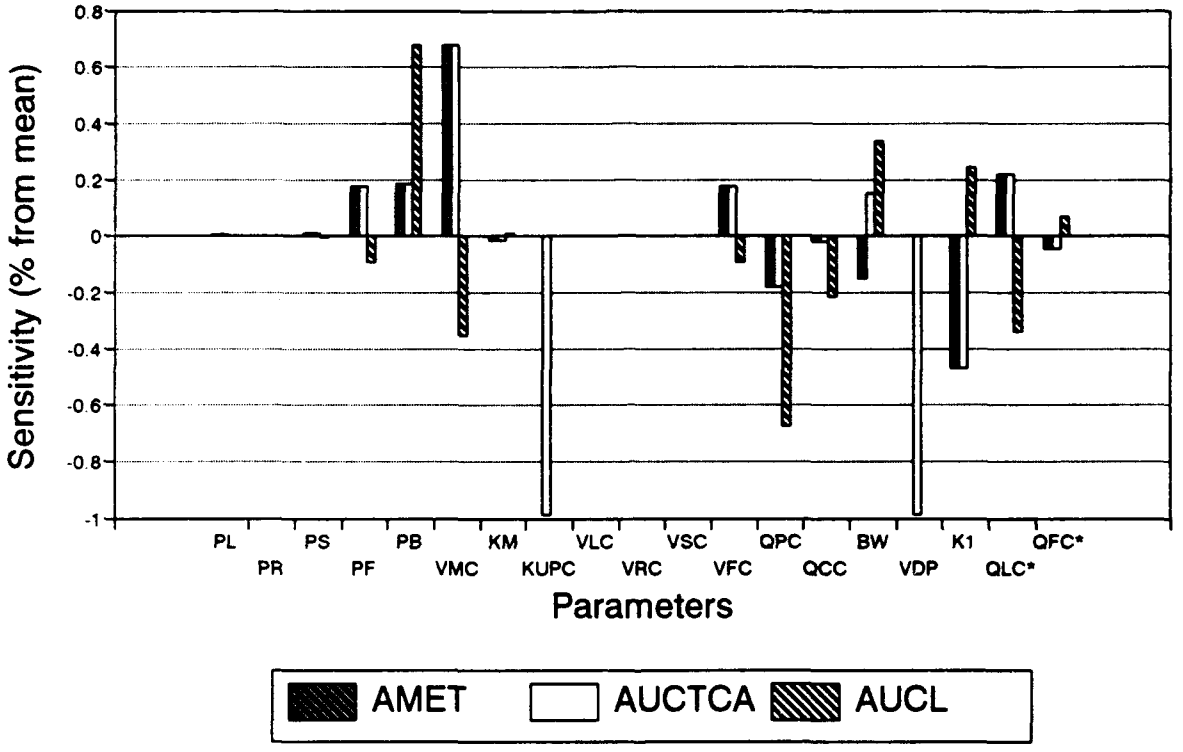


Female Mouse Ingestion 1% Sensitivity

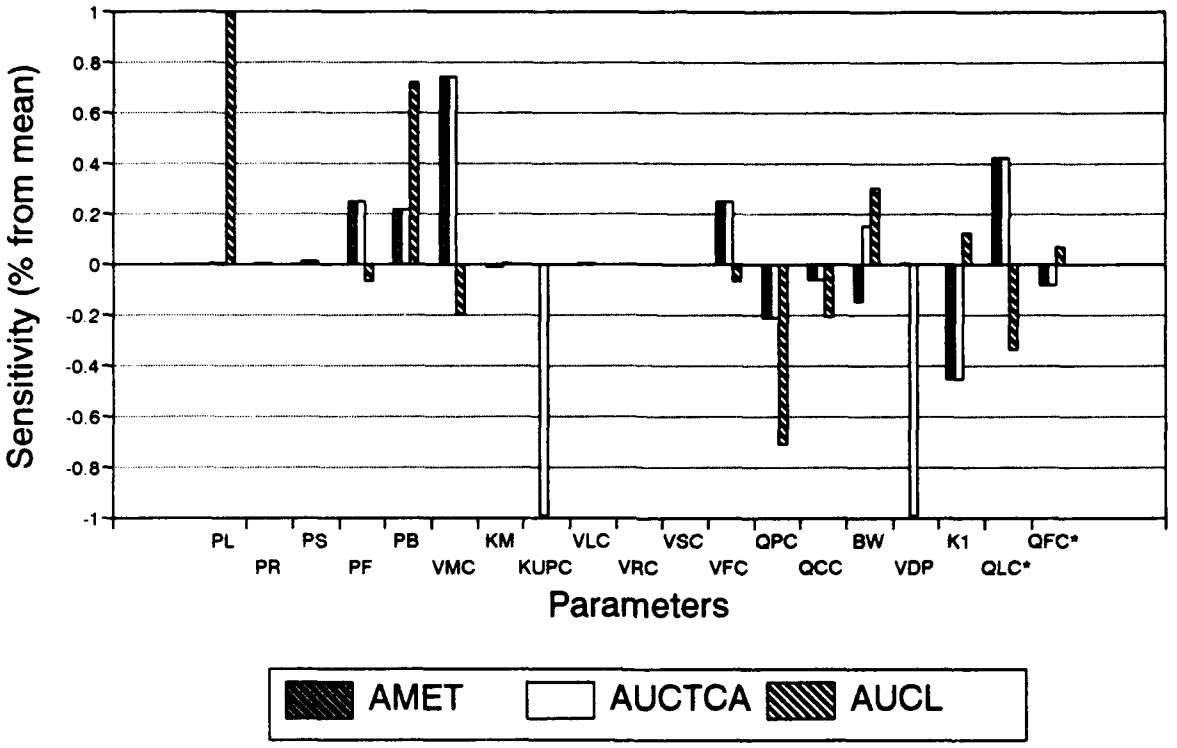
AUCL



Female Mouse Ingestion 1% Sensitivity
869 PPM TCE



Female Mouse Ingestion 1% Sensitivity
1739 PPM TCE



Appendix I. *Human Ingestion Sensitivity*

The following graphs present the results of the human ingestion sensitivity analysis. The tables of data for the analysis are first presented by exposure dose of TCE. Graphs of the sensitivity for each parameter are then presented both by dose-metric and dose level.

1% SENSITIVITY ANALYSIS
Human Ingestion

DOSE 1: 10 PPB TCE

	Model Output			Sensitivity (%)		
Parameter	AMET	AUCTCA	AUCL	AMET	AUCTCA	AUCL
PL	2.541E-04	0.0106184	6.303E-04	0.00000	-0.00094	1.00962
PR	2.541E-04	0.0106185	6.240E-04	0.00000	0.00000	0.00000
PS	2.541E-04	0.0106185	6.240E-04	0.00000	0.00000	0.00000
PF	2.541E-04	0.0106181	6.240E-04	0.00000	-0.00377	0.00000
PB	2.543E-04	0.0106266	6.245E-04	0.07871	0.07628	0.08013
VMC	2.544E-04	0.0106299	6.185E-04	0.11806	0.10736	-0.88141
KM	2.538E-04	0.0106066	6.296E-04	-0.11806	-0.11207	0.89744
KUPC	2.541E-04	0.010536	6.240E-04	0.00000	-0.77695	0.00000
VLC	2.541E-04	0.0106185	6.240E-04	0.00000	0.00000	0.00000
VRC	2.541E-04	0.0106185	6.240E-04	0.00000	0.00000	0.00000
VSC	2.541E-04	0.0106185	6.240E-04	0.00000	0.00000	0.00000
VFC	2.541E-04	0.0106181	6.240E-04	0.00000	-0.00377	0.00000
QPC	2.539E-04	0.0106104	6.235E-04	-0.07871	-0.07628	-0.08013
QCC	2.540E-04	0.0106152	6.238E-04	-0.03935	-0.03108	-0.03205
BW	2.515E-04	0.0107891	6.197E-04	-1.02322	1.60663	-0.68910
VDP	2.541E-04	0.0106185	6.240E-04	0.00000	0.00000	0.00000
K1	2.541E-04	0.0106181	6.240E-04	0.00000	-0.00377	0.00000
QLC*	2.540E-04	0.0106153	6.238E-04	-0.03935	-0.03014	-0.03205
QFC*	2.541E-04	0.0106185	6.240E-04	0.00000	0.00000	0.00000
MEAN	2.541E-04	0.0106185	6.240E-04			

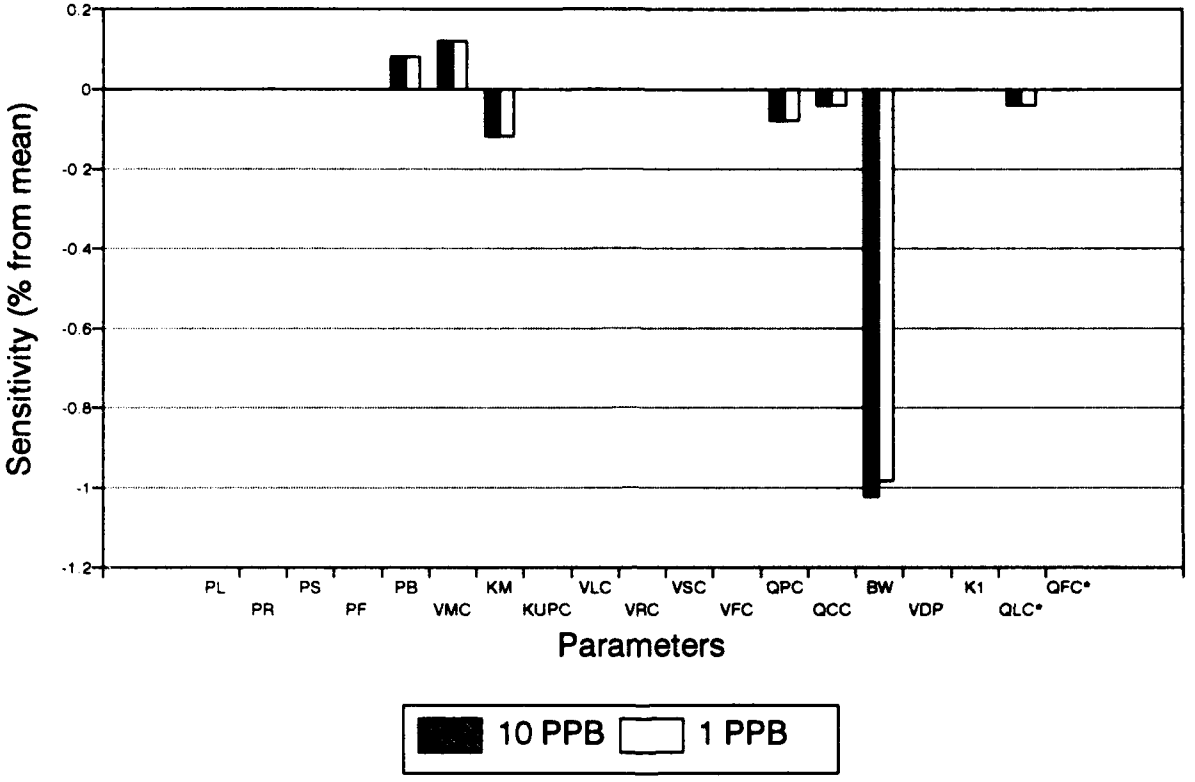
*QS scaled as function of QF & QC, QR scaled as a function of QL & QC

DOSE 2: 1 PPB TCE

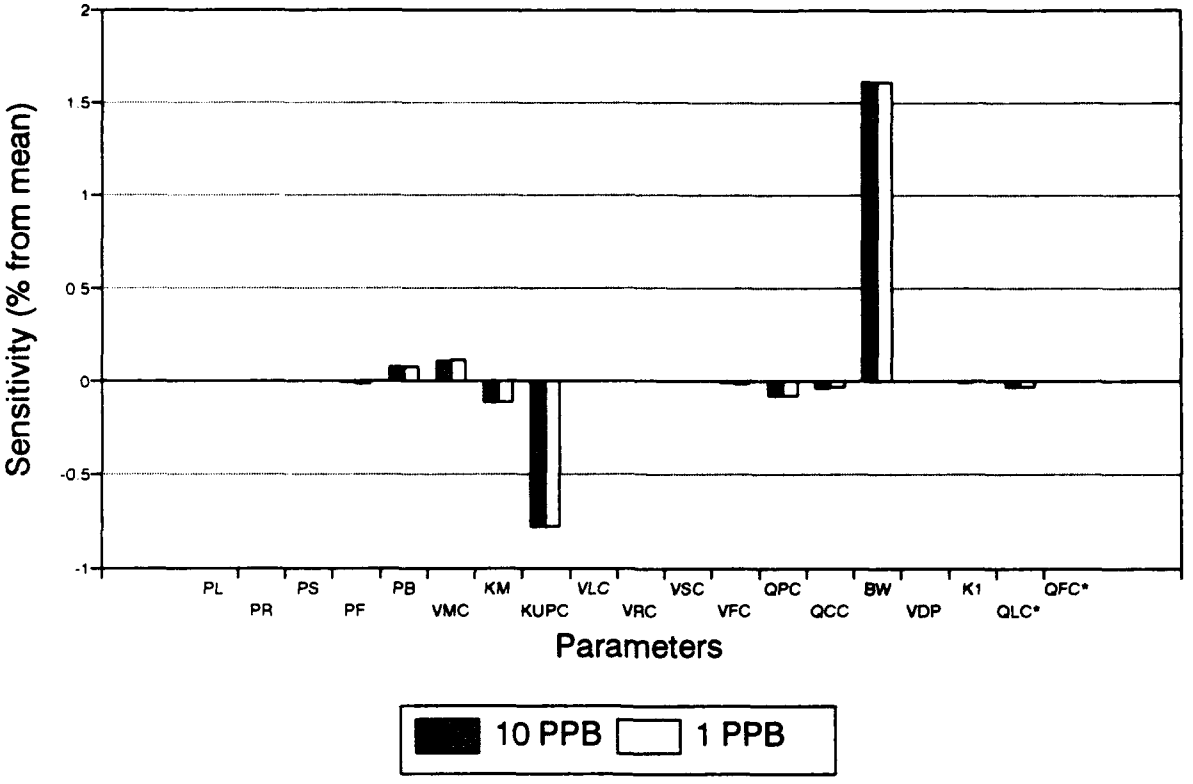
	Model Output			Sensitivity (%)		
Parameter	AMET	AUCTCA	AUCL	AMET	AUCTCA	AUCL
PL	2.541E-05	0.0010624	6.303E-05	0.00000	0.00000	1.00962
PR	2.541E-05	0.0010624	6.240E-05	0.00000	0.00000	0.00000
PS	2.541E-05	0.0010624	6.240E-05	0.00000	0.00000	0.00000
PF	2.541E-05	0.0010623	6.240E-05	0.00000	-0.00941	0.00000
PB	2.543E-05	0.0010632	6.245E-05	0.07871	0.07530	0.08013
VMC	2.544E-05	0.0010636	6.185E-05	0.11806	0.11295	-0.88141
KM	2.538E-05	0.0010612	6.296E-05	-0.11806	-0.11295	0.89744
KUPC	2.541E-05	0.0010541	6.240E-05	0.00000	-0.78125	0.00000
VLC	2.541E-05	0.0010624	6.240E-05	0.00000	0.00000	0.00000
VRC	2.541E-05	0.0010624	6.240E-05	0.00000	0.00000	0.00000
VSC	2.541E-05	0.0010624	6.240E-05	0.00000	0.00000	0.00000
VFC	2.541E-05	0.0010623	6.240E-05	0.00000	-0.00941	0.00000
QPC	2.539E-05	0.0010616	6.235E-05	-0.07871	-0.07530	-0.08013
QCC	2.540E-05	0.0010621	6.238E-05	-0.03935	-0.02824	-0.03205
BW	2.516E-05	0.0010795	6.196E-05	-0.98386	1.60956	-0.70513
VDP	2.541E-05	0.0010624	6.240E-05	0.00000	0.00000	0.00000
K1	2.541E-05	0.0010624	6.240E-05	0.00000	0.00000	0.00000
QLC*	2.540E-05	0.0010621	6.238E-05	-0.03935	-0.02824	-0.03205
QFC*	2.541E-05	0.0010624	6.240E-05	0.00000	0.00000	0.00000
MEAN	2.541E-05	0.0010624	6.240E-05			

*QS scaled as function of QF & QC, QR scaled as a function of QL & QC

Human Ingestion 1% Sensitivity
AMET

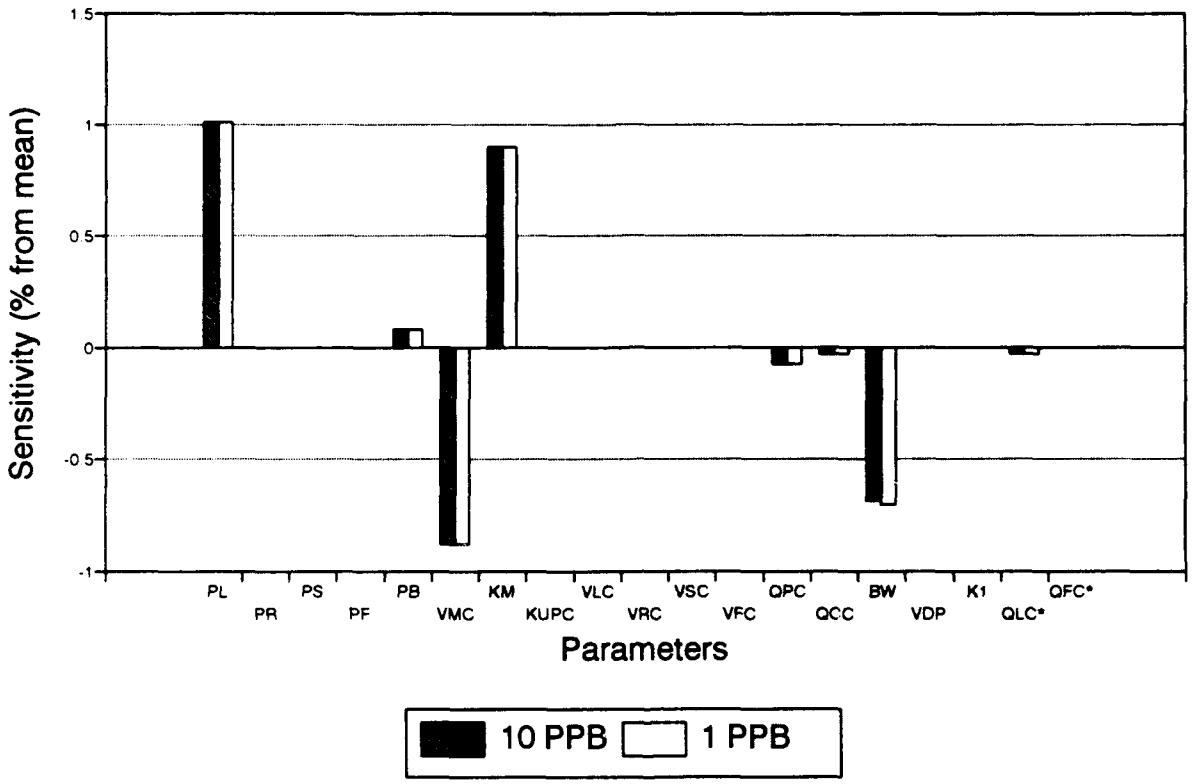


Human Ingestion 1% Sensitivity
AUCTCA

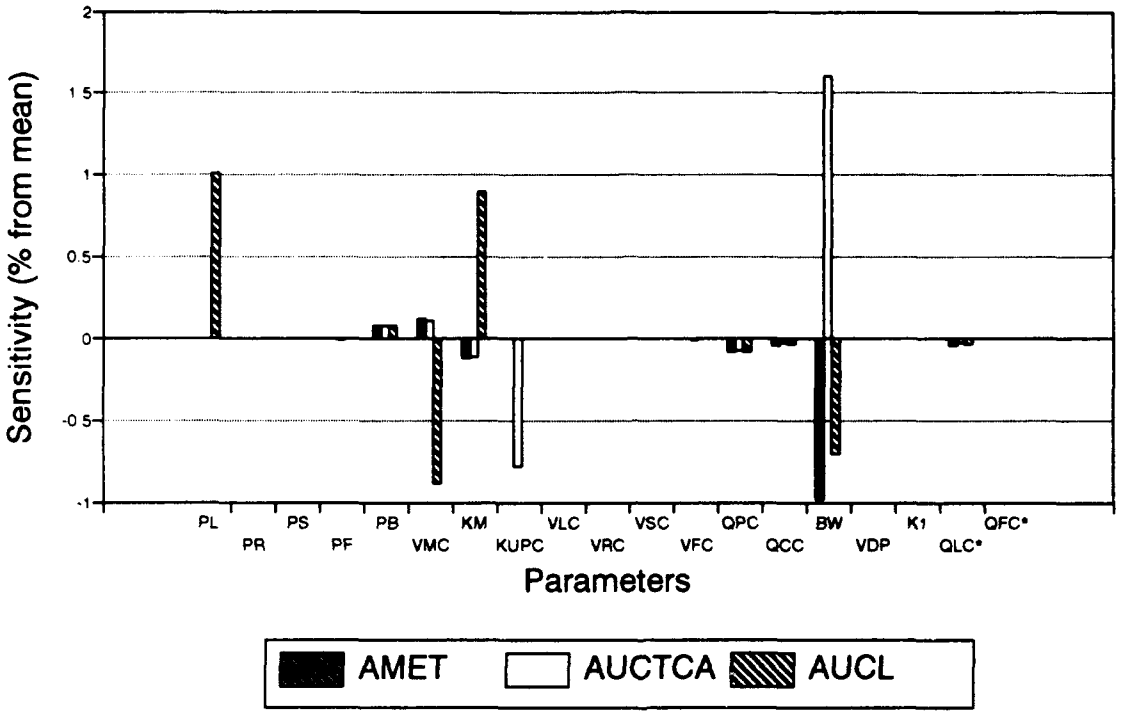


Human Ingestion 1% Sensitivity

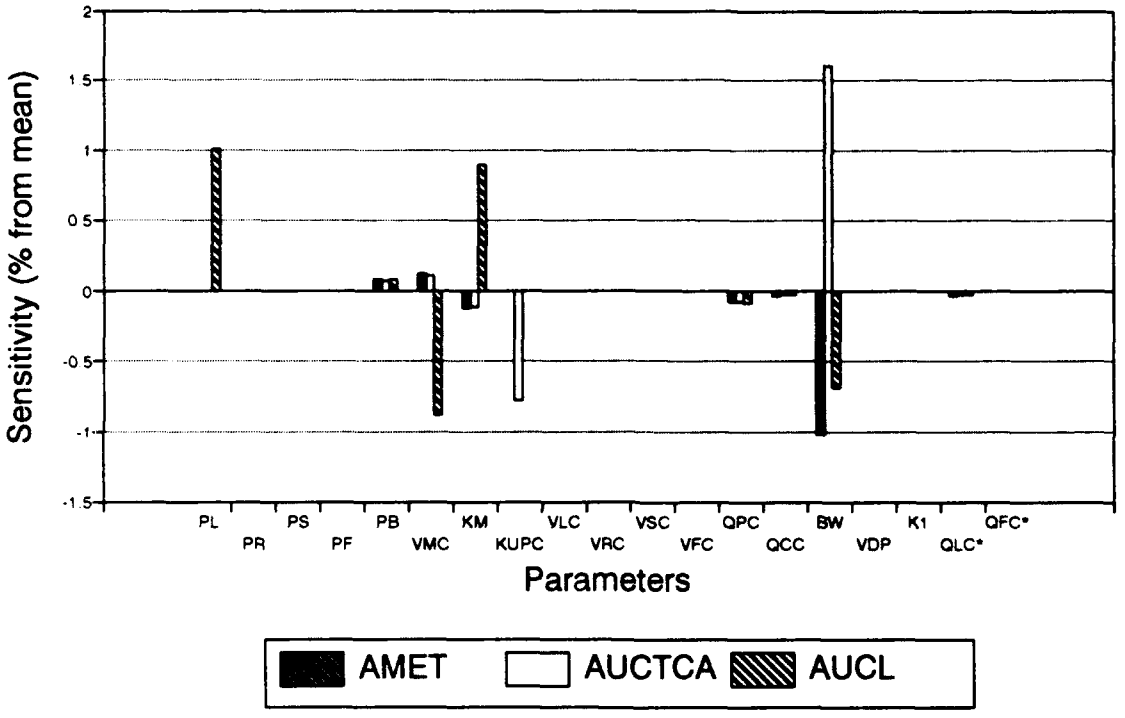
AUCL



Human Ingestion 1% Sensitivity
1 PPB TCE



Human Ingestion 1% Sensitivity
10 PPB TCE



Appendix J. *Model Code*

The following is the Advanced Continuous Simulation Language (ACSL) code for the model used in the research. The authors intent was to use the original model provided by Armstrong Lab. This was not possible as the original model was not designed to be used with the type of methodology employed. The compartments and the differential equations for those compartments and between compartments are exactly the same as the original model. The main change was to incorporate logical operators in the model to allow switching between animals and exposure scenarios. A discrete analysis block was also added to evaluate and average dose-metrics.

```
PROGRAM TCE.CSL                          $"#TCE ORAL/INHALATION MODEL"

INITIAL

LOGICAL MALE                             $"#SWITCH FOR SETTING SET VALUES"
LOGICAL CONT                             $"#SWITCH FOR CONT/INTERMITTENT EXP"
LOGICAL INGEST                           $"#SWITCH FOR INGESTION/INHALATION"

"#CAT- SIMULATION LENGTH CONTROL"
 CONSTANT TSTOP=672                      $"#LENGTH OF EXPERIMENT (HRS)"
 CONSTANT POINTS=1.0                     $"#NUMBER OF PLOT POINTS"
 CONSTANT H=500000.0                     $"#NUMBER OF INTEGRATION STEPS"

"#ENDCAT"

"#CAT- EXPOSURE TIMING/ACCUMULATION CONTROL"
 CONSTANT WDAYS =5.0                     $"#NUMBER OF WEEKDAYS"
 CONSTANT WEDAYS=2.0                     $"#NUMBER OF WEEKEND DAYS"
 CONSTANT WIDTH =1.0                     $"#INGESTION EXPOSURE LENGTH"
 CONSTANT DAYS  =7.0                     $"#NUMBER OF DAYS IN WEEK"
 CONSTANT TIMER =0.0                     $"#INGESTION INCREMENT TIMER"
 CONSTANT TOTAL =0.0                     $"#STOMACH CONTENTS ACCUMULATOR"

'#CAT- INGESTION CONSTANTS"
 CONSTANT AST=0.0                        $"#AMOUNT REMAINING IN STOMACH"
 CONSTANT KI = 1.1                       $"#TCE TRANSPORT CONSTANT (I/HR)"
"#ENDCAT"

"#CAT- DOSING INFORMATION"
 CONSTANT PDOSE=0.0                      $"#ORAL DOSE (MG/KG)...(PPB) HUMAN
 CONSTANT IVDOSE=0.0                     $"#IV DOSE (MG/KG)"
 CONSTANT TINF=0.0                       $"#LENGTH OF IV INFUSION (HRS)"
 CONSTANT CONC =600.0                    $"#INHALATION CONCENTRATION (PPM)"
"#ENDCAT"

"#CAT- FLOW RATES"
 CONSTANT QPC=30.0                       $"#ALVEOLAR VENT RATE (L/HR/KG)"
 CONSTANT QCC=30.0                       $"#CARDIAC ART OUTPUT (L/HR/KG)"
"#ENDCAT"

"#CAT- TISSUE FLOW RATES"
 CONSTANT QLC=0.24                       $"# % OF QCC FLOW TO LIVER
 CONSTANT QFC=0.05                       $"# % OF QCC FLOW TO FAT "
 CONSTANT QRC=0.52                       $"# % OF QCC FLOW RICH TISSUE
 CONSTANT QSC=0.19                       $"# % OF QCC FLOW SLOW TISSUE"
"#ENDCAT"

"#CAT- BODYWEIGHT"
 CONSTANT BW=.028                        $"#BODY WEIGHT (KG)"
"#ENDCAT"

"#CAT- FRACTIONAL VOLUME OF TISSUES"
 CONSTANT VLC=0.04                       $"# % LIVER TISSUE"
 CONSTANT VFC=0.04                       $"# % FAT TISSUE"
```

```
 CONSTANT  VSC=0.78                    $ # % SLOWLY PERF TISSUE"
 CONSTANT  VRC=0.05                    $"# % RICHLY PERF TISSUE"
 CONSTANT  VDPC=.238
 CONSTANT  VCARC=.09                   $# % CARCASS TISSUE"
"#ENDCAT"

"#CAT- PARTITION COEFFICIENTS"
 CONSTANT  PL=2.03                     $"#LIVER/BLOOD PARTITION COEFFICIENT"
 CONSTANT  PF=41.3                     $"#FAT/BLOOD PARTITION COEFFICIENT"
 CONSTANT  PS=1.0                      $"#SLOWLY PERFUSED PART COEFFICIENT"
 CONSTANT  PR=2.03                     $"#RICHLY PERFUSED PART COEFFICIENT"
 CONSTANT  PB=13.2                     $"#BLOOD/AIR PART COEFFICIENT"
"#ENDCAT"

"#CAT- CHEMICAL DATA"
 CONSTANT  MW=131.4                    $"#MOLECULAR WEIGHT TCE (G/MOL)"
 CONSTANT  MWTCA=163.4                 $"#MOLECULAR WEIGHT TCA (G/MOL)"
"#ENDCAT"

"#CAT- KINETIC CONSTANTS"
 CONSTANT  VMAXC=32.7                  $"#MAX VEL OF METABOLISM (MG/KG/HR)"
 CONSTANT  KM  =0.25                   $"#MICHAELIS-MENTEN CONSTANT (MG/L)"
 CONSTANT  KFC  =0.0                   $"#FIRST ORDER MET RATE CONSTANT"
 CONSTANT  KUPC =0.043                 $"#1ST ORDER TCA ELIM CONST (l/HR)"
"#ENDCAT"

"#SCAT- MALE?"                         $"#LOGICAL FOR ASSIGNING PCTA"
 CONSTANT  MALE=.TRUE.
"#ENDCAT"

"#SCAT- CONTINUOUS?"                   $"#LOGICAL FOR ASSIGNING INH/ING"
 CONSTANT  CONT=.TRUE.
"#ENDCAT"

"#SCAT- INGESTION?"                    $"#LOGICAL FOR ASSIGNING INH/ING"
 CONSTANT INGEST=.FALSE.
"#ENDCAT"

"SCALED PARAMETERS"
 CINT=TSTOP/POINTS                     $"#COMMUNICATION INTERVAL"
 NSTP=CINT*H+1                         $"#NUMBER OF INTEGRATION STEPS"
 QC  =QCC*BW**0.74                     $"#SCALED CARDIAC OUTPUT (L/HR)"
 QP  =QPC*BW**0.74                     $"#SCALED ALVEOLAR VENT RATE (L/HR)"
 QL  =QLC*QC                           $"#SCALED FLOW TO LIVER (L/HR)"
 QF  =QFC*QC                           $"#SCALED FLOW TO FAT (L/HR)"
 QS  =0.30*QC-QF                       $"#SCALED FLOW TO SLOW PERF (L/HR)"
 QR  =0.70*QC-QL                       $"#SCALED FLOW TO RICH PERF (L/HR)"
 VL  =VLC*BW                           $"#SCALED % LIVER (KG)"
 VF  =VFC*BW                           $"#SCALED % FAT (KG)"
 VS  =0.81*BW-VF                       $"#SCALED % SLOW PERF (KG)"
 VR  =0.076*BW-VL                      $"#SCALED % RICH PERF (KG)"
 VMAX=VMAXC*BW**0.7                    $"#SCALED MAX VEL OF MET (MG/HR)"
 KF  =KFC/BW**0.3                      $"#SCALED IST ORDER MET RATE"
 KUP =KUPC/BW**0.3                     $"#1ST ORDER TCA ELIM RATE (l/HR)"
```

```
 IF(BW.LT.1.0) DOSE=PDOSE*BW        $"#AMOUNT TCE INGESTED/MOUSE  (MG/KG)"
 IF(BW.GT.1.0) DOSE=(PDOSE*2)/4     $"#AMOUNT TCE INGESTED..HUMAN (UG)"
 IF(BW .GT. 1.0) GOTO HUMAN         $"#HUMAN ASSIGNS HUMAN VALUES"
 IF(BW .LT. 1.0) GOTO FMOUSE        $"#FMOUSE ASSIGNS FEMALE SETTINGS"

FMOUSE.. CONTINUE                   $"#FMOUSE ASSIGNS FEMALE SETTINGS"

 LIFE=.75
 DTIME=24.0
 VDP=VDPC*BW
 IF (MALE) GOTO MMOUSE
 IF(CONC .EQ. 100) PCTCA=.18
 IF(CONC .EQ. 300) PCTCA=.07
 IF(CONC .EQ. 600) PCTCA=.08
 IF(INGEST) PCTCA=.09

GOTO FINAL

MMOUSE.. CONTINUE                   $"#MALE SETTINGS

 LIFE=.75
 DTIME=24.0
 VDP=VDPC*BW
 IF(CONC .EQ. 100) PCTCA=.11
 IFICONC .EQ. 300) PCTCA=.13
 IF(CONC .EQ. 600) PCTCA=.07
 IF(INGEST) PCTCA=.06

GOTO FINAL

HUMAN.  CONTINUE                    $"#HUMAN SETTINGS"

 LIFE=1.0
 DTIME=3.0
 PCTCA=.0336
 VDP=(.04-.0034*BW)*BW

FINAL.. CONTINUE                    $"#END SETTINGS"

 IF (CONT) GOTO NO                  $"#CONTINUOUS/INTERMITTENT SETTINGS"
 AV=.142857
 TCHNG=7
 TI=TSTOP-168

GOTO OK

NO.. CONTINUE
 TI=TSTOP-24
 AV=1
 TCHNG=21

OK..CONTINUE                        $"#END OF CONTINUOUS/INTERMITTENT"
```

```
END                                    $"#END OF INITIALIZATION"

DYNAMIC

ALGORITHM IALOG=2                      $"#GEAR METHOD FOR EQUATIONS"

DERIVATIVE

PROCEDURAL                             $"#INHALATION TIMING CODE'

 IF(CONT) GOTO NOBRK
 BREAK=PULSE(0.0,(WDAYS+WEDAYS)*24,WDAYS*24)

GOTO WEOFF

NOBRK..CONTINUE

  BREAK=PULSE(0.0,(WDAYS+WEDAYS)*24.0,(WDAYS+WDAYS)*24)

WEOFF..CONTINUE

PFLAG=PULSE(0.0,24.0,TCHNG)*BREAK
PFLAG=PFLAG*PULSE(0.0,DAYS*24.0,DAYS*24.0)
CI=CONC*PFLAG*MW/24450.0

END

PROCEDURAL                             $"#MOUSE INGESTION TIMING CODE

EXPOS=PULSE(0.0,24.0,WIDTH)*PULSE(0.0,(WDAYS+WEDAYS)*24.0,...
(WDAYS+WEDAYS)*24.0)
EXPOS=EXPOS*PULSE(0.0,DAYS*24.0,DAYS*24.0)*BREAK

END                                    $"#END OF INGESTION TIMING CODE"

PROCEDURAL                             $"#HUMAN INGESTION TIMING CODE"

SIP=PULSE(0.0,3.0,WIDTH)*PULSE(0.0,24.0,12.0)*PULSE(0.0,...
(WDAYS+WEDAYS)*24.0,(WDAYS+WEDAYS)*24.0)*PULSE(0.0,...
DAYS*24.0,DAYS*24.0)

END                                    $"#END OF INGESTION TIMING CODE

PROCEDURAL                             $"#INGESTION DOSE TIMING CODE

 IF (T.LT.TIMER) GO TO BYPASS
 IF(BW .GT. 1.0) TOTAL=TOTAL+SIP*DOSE
 IF(BW .LT. 1.0) TOTAL=TOTAL+EXPOS*DOSE
 TIMER=TIMER+DTIME

BYPASS.. CONTINUE

END                                    $"#END OF DOSE TIMING CODE
```

```
"#INGESTION CODE"

RST=KI*AST                                  $"#RATE TO LIV (MG/HR)"
AST=TOTAL-INTEG(RST,0.0)                    $"#REMAIN IN STOM (MG)"
AO =INTEG(RST,0.0)                          $"#ENTERING LIVER (MG)"

"#ARTERIAL CONCENTRATION"

CA =(QC*CV+QP*CI)/(QC+(QP/PB))              $"#CON IN BLOOD (MG/L)"
AUCB =INTEG(CA.0.0)                         $"#AUC BLOOD (MG*HR/L)"


"#EXHALED AIR"

 CX=CA/PB                                   $"#CON EXHALED (MG/L)"
 CXPPM=(0.7*CX+0.3*CI)*24450.0/MW           $"#CON EXHALED (PPM)"
 RAX=QP*CX                                  $"#RATE EXHALED (MG/HR)"
 AX=INTEG(RAX,0.0)                          $"#AMOUNT EXHALED (MG)"

"#SLOWLY PERFUSED TISSUES"

 RAS=QS*(CA-CVS)                            $"#RATE ENTER (MG/HR)"
 AS=INTEG(RAS,0.0)                          $"#AMOUNT (MG)"
 CVS=AS/(VS*PS)                             $"#VENOUS (MG/L)"
 CS=AS/VS                                   $"#CONC (MG/KG)"

"#RAPIDLY PERFUSED TISSUES"

 RAR=QR*(CA-CVR)                            $"#RATE ENTER (MG/HR)"
 AR=INTEG(RAR,0.0)                          $"#AMOUNT (MG)"
 CVR=AR/(VR*PR)                             $"#VENOUS (MG/L)"
 CR=AR/VR                                   $"#CONC (MG/KG)"

"#FAT TISSUE"

 RAF=QF*(CA-CVF)                            $"#RATE ENTER (MG/HR)"
 AF=INTEG(RAF,0.0)                          $"#AMOUNT (MG)"
 CVF=AF/(VF*PF)                             $"#VENOUS (MG/L)"
 CF=AF/VF                                   $"#CONC (MG/KG)"

"#LIVER TISSUE"

 RAL=QL*(CA-CVL)-RAM+RST                    $"#RATE ENTER (MG/HR)"
 AL=INTEG(RAL,0.0)                          $"#AMOUNT (MG)"
 CVL=AL/(VL*PL)                             $"#VENOUS (MG/L)"
 CL=AL/VL                                   $"#CONC (MG/KG)"
 AUCL=INTEG(CL,0.0)                         $"#AUC LIV (MG*HR/KG)

"#AMOUNT METABOLIZED"

 RAM=(VMAX*CVL)/(KM+CVL)+KF*CVL*VL          $"#RATE OF MET (MG/HR)"
 AM=INTEG(RAM,0.0)                          $"#AMT MET (MG)"
```

```
 TAM=AM/BW                                                    $"#AMT MET (MG/KG)"

"#AMOUNT TCA PRODUCED"

 RMTCA=PCTCA*(VMAX*CVL)/(KM+CVL)*(163.4/131.4)-RKU            $"#+-RATE (MG/HR)'
 RTCAL=PCTCA*(VMAX*CVL)/(KM+CVL)*(163.3/131.4)                $"#+ RATE (MG/HR)"
 TOTTCA=INTEG(RTCAL,0.0)                                      $"#TOTAL TCA (MG)"
 BWTCA=TOTTCA/BW                                              $"#TOTAL (MG/KG)"
 AMTCA=INTEG(RMTCA,0.0)                                       $"#AMT REM (MG)"

#AREA UNDER THE CURVE TCA"

 CTCA=AMTCA/VDP                                               $"#TCA (MG/L)"
 TCAAUC=INTEG(CTCA,0.0)                                       $"#AUC TCA (MG*HR/L)"

#ELIMINATION RATE FOR TCA"

 RKU=KUP*VDP*CTCA                                             $"#TCA ELIM (MG/HR

"#MIXED VENOUS BLOOD CONCENTRATION"

 CV=(QF*CVF+QL*CVL+QS*CVS+QR*CVR)/QC                          $"#VENOUS CONC (MG/L)"

"#MASS BALANCE EQUATION"

 TMASS=AF+AL+AS+AR+AM+AX                                      $"#TOT MASS (MG)"
 RINH=QP*CI                                                   $"#RATE INHALED (MG/HR)"
 AINH=INTEG(RINH,0.0)                                         $"#TOT INHALED (MG)"
 BAL=TMASS-AINH                                               $"#BALANCE (MG)"
 RCV=QC*CV                                                    $"#VEN RATE (MG/HR)"
 ACV=INTEG(RCV,0.0)                                           $"#VEN AMT (MG)"
 RCA=QC*CA                                                    $"#ART RATE (MG/HR)"
 ACA=INTEG(RCA,0.0)                                           $"#ART AMT (MG)"

TERMT(T.GE.TSTOP)                                             $"#TERMINATE SIMULATION"

END                                                           $"#END DERIVATIVE"

DISCRETE SAMPLE                                               $"#SAMPLE @ TI"

 INTERVAL DTSAMP=504
 AMETI=TAM
 AUCLI=AUCL
 AUCTCI=TCAAUC

END                                                           $"#END SAMPLE



END                                                           $"#END DYNAMIC"

TERMINAL                                                      $"#FINAL VALUES"
```

```
  AUCTCF=(TCAAUC-AUCTCI)*AV*LIFE                $"#LIFETIME AVERAGES"
  AMETF=(TAM-AMETI)*AV*LIFE                     $"#LIFETIME AVERAGES"
  AUCLF=(AUCL-AUCLI)*AV*LIFE                    $"#LIFETIME AVERAGES"
  IF(AUCLF.LT.0.0) AUCLF=AUCLI*LIFE/21          $"#DOSE-METRIC ERROR
  IF(AUCLF.LT.0.0) AUCTCF=AUCTCI*LIFE/21        $"#CORRECTION FOR"
  IF(AUCLF.LT.0.0) AMETF=AMETI*LIFE/21          $"#INCORRECT DATA"
  IF(AUCTCF.LT.0.0) AUCTCF=AUCTCI*LIFE/21       $"#GENERATION"
  IF(AUCTCF.LT.0.0) AUCTCF=0.0
  IF(AMETF.LT.0.0) AMETF=AMETI*LIFE/21
  TIMER=0.0                                     $"#RESET TIMER"
  TOTAL=0.0                                     $"#RESET TOTAL"
  X=X+ 1                                        $"#SAMPLE COUNTER"

END                                             $"#END TERMINAL"

END                                             $"#END PROGRAM"
```

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Vita

William J. Cronin was born on 20 September 1960 in Portsmouth,Virginia. He graduated from Clay Center High School in Clay Center, Nebraska in 1978 and enlisted in the U.S. Air Force in March 1979. After basic training, Captain Cronin was assigned to Little Rock AFB, Arkansas as a security specialist for the 308 Strategic Missle Wing. Between 1982 and 1985, Captain Cronin attended the University of Central Arkansas and the University of Arkansas. In 1985, Captain Cronin was selected for the Airman's Education Commissioning Program and began undergraduate engineering studies at the University of Nebraska, Lincoln. In 1988, he received his Bachelor of Science degree in Electrical Engineering and was immediately assigned to the Air Force Officer's Training School where he received his commission. After being commissioned, Captain Cronin was assigned to the 67th Civil Engineering Squadron, Bergstrom AFB, Texas where he performed duties as a Military Construction Project Programmer. The majority of his Bergstrom assignment was spent as the unit's Prime Base Engineering Emergency Forces (BEEF) officer. In 1990, he was assigned to the 7276th Air Base Group, Iraklion Air Station, Crete, Greece where he performed duties as the Chief of Quality Assurance responsible for quality assurance operations associated with civil engineering operations at Iraklion A.S. and several mainland Greece geographically seperated units. During his assignment at Iraklion, Captain Cronin was selected as the civil engineering Technical Team Chief for the rewrite, source selection, and award of the Greece Base Maintenance Contract. In 1991, he was selected to attend the School of Engineering, Air Force Institute of Technology where he commenced studies in May 1992.

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Vita

Captain Eric J. Oswald was born on 28 October 1965 in Battle Creek, Michigan. He graduated from Lakeview High School in Battle Creek, Michigan in 1983 and attended Michigan State University, graduating with a Bachelor of Science in Civil Engineering in March 1988. Upon graduation, he received a commission in the USAF and served his first tour of duty at Loring AFB, Maine. He began as an environmental engineer, managing the base's hazardous waste management program and other various environmental programs until January 1991. He then was assigned as chief of the environmental compliance section for the base. At this position, he was responsible for the entire environmental compliance program. His responsibilities included polychlorinated biphenyl (PCB) removal, hazardous waste management, environmental impact analysis (EIAP), the environmental compliance assessment and management program (ECAMP), regulatory liaison, and emergency spill response. Captain Oswald remained at this position until entering the School of Engineering, Air Force Institute of Technology, in May 1992.

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REPORT DOCUMENTATION PAGE

September 1993	Master's Thesis

A REFINEMENT OF RISK ANALYSIS PROCEDURES FOR TRICHLOROETHYLENE THROUGH THE USE OF THE MONTE CARLO METHOD IN CONJUNCTION WITH PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING

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This study refines risk analysis procedures for trichloroethylene (TCE) using a physiologically based pharmacokinetic (PBPK) model in conjunction with the Monte Carlo method. The Monte Carlo method is used to generate random sets of model parameters, based on the mean, variance, and distribution types. The procedure generates a range of exposure values for human excess lifetime cancer risk of $1x10^{-6}$, based on the upper and lower bounds and the mean of a 95% confidence interval. Risk ranges were produced for both ingestion and inhalation exposures. Results are presented in a graphical format to reduce reliance on qualitative discussions of uncertainty. A sensitivity analysis of the model was also performed.

This method produced acceptable TCE exposures, for total amount TCE metabolized, greater than the Environmental Protection Agency's (EPA) by a factor of 23 for inhalation and a factor of 1.6 for ingestion. Sensitive parameters identified were the elimination rate constant, alveolar ventilation rate, and cardiac output.

This procedure quantifies the uncertainty related to natural variations in parameter values. Its incorporation into risk assessment could be used to promulgate, and better present, more realistic standards.

Risk analysis, physiologically based pharmacokinetics, pbpk, trichloroethylene, monte carlo method. 162

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