THE INFLUENCES OF EXPOSURE PATTERN AND DURATION ON ELIMINATION KINETICS AND EXPOSURE ASSESSMENT OF TETRACHLOROETHYLENE IN HUMANS

By :

YEH-CHUNG CHIEN

A Dissertation submitted to the

Graduate School - New Brunswick

Rutgers, The State University of New Jersey

and

UMDNJ, Robert Wood Johnson Medical School

in partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

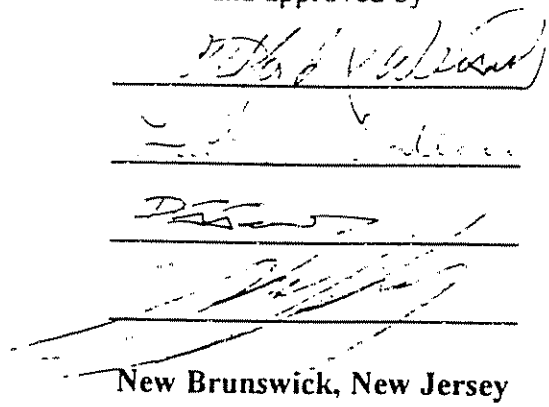
Graduate Program in Environmental Sciences and in Public Health

Option in Human Exposure Measurement and Assessment

Written under the direction of

Professor Clifford P Weisel

and approved by



New Brunswick, New Jersey

January, 1997

ABSTRACT OF THE DISSERTATION

The Influences of Exposure Pattern and Duration on Elimination Kinetics and Exposure Assessment of Tetrachloroethylene in Humans

By

YEH-CHUNG CHIEN

Dissertation Director: Professor Clifford P. Weisel

The influence of exposure duration and pattern on body's kinetics during and after environmental tetrachloroethylene exposure were examined using exhaled breath analysis. A subject was exposed to tetrachloroethylene in dry-cleaning stores for three durations, during which personal exposure (breathing-zone air) and alveolar breath samples were collected concurrently, and in a controlled environment facility for different exposure duration and pattern combinations, all of which had the same total exposures. Three dynamic processes that affect elimination were examined: absorption, distribution and elimination. Absorption kinetics were examined using the relationships between the exposure air and exhaled breath concentrations measured simultaneously. It was found that shorter exposure duration resulted in higher percent absorption and therefore higher internal dose. The distributions of Perc within the body were studied using both compartment and pharmacokinetic models. Shorter exposure duration or

higher exposure intensity resulted in higher Perc body burden, higher peak brain concentration and higher short-term metabolic burden than a longer exposure with lower exposure concentration. A shorter exposure (30-minute) also resulted in greater percent amounts expired, larger area under the postexposure curve and longer first elimination half-lives than longer (90-minute) exposures. The exposure pattern did not have a large influence on the elimination kinetics for 30-minute exposures, but showed significant effects for the longer, 90 minutes, exposures. These findings support the current hypothesis that exposure conditions affect body elimination kinetics. These measurements were also used to evaluate a pharmacokinetic model for tetrachloroethylene. The use of an integrated air concentration as the model input resulted in similar prediction as the actual exposure profiles. The pharmacokinetic model with optimized parameters effectively predicted the postexposure breath concentrations of short-term exposures with small fluctuation in exposure concentration, but is insufficient to predict under extreme exposure scenarios, especially for longer exposure duration. The results also indicated that the assumption of instantaneous exchange between alveolar air and blood may not be valid. The percent Perc metabolized in the liver was predicted to be 36% for the current exposure levels

ACKNOWLEDGMENT

I want to express my sincere appreciation to the dissertation director, Dr. C. Weisel, for his support and guidance throughout the study. Gratitude also goes to all other committee members, Dr. P. Georgopolus, Dr. P. Lioy and Dr. L. Wallace, for their keen suggestions and comments.

I would like to thank Mr. C. Mohan for the assistance in sampling, Mr. T. Waiman for operating the controlled facility, and Dr. A. Roy for both technical assistance and conceptual inspiration of the modeling work.

I am also grateful to the staffs at EOSHI, Ms. J. Bowman, Mrs. A. Bicknell, Mrs. R. Salinger, Mrs. S. Wund for the administrative supports, and to my fellow graduate students, Mr. C. Lee, Mr. H. Kim, Drs. Y. Yu, Mrs. P. Tsai, Mr. S. Wang, Mr. T. Shin, Mr. L. Yiin, for their help, sharing and inspiration.

And, I would like to dedicate this work to my family for love, encouragement and supports.

TABLE OF CONTENTS

ABSTRACT ii

ACKNOWLEDGMENT iv

TABLE OF CONTENTS v

LIST OF TABLES xi

LIST OF FIGURES xiii

ABBREVIATIONS xvii

1. INTRODUCTION 1

1.1. Background 1

1.2. Hypotheses 4

1.3. Specific Aims 4

1.4. Approaches 5

1.5. Literature Review 7

1.5.1 The Sources and Uses of Perc 7

1.5.2. Tetrachloroethylene Exposures 8

1.5.2.1. Occupational Exposure 8

1.5.2.2. Environmental Exposure 9

1.5.3 Biomarker of Exposure 12

1.5.3.1 Exhaled Perc 12

1.5.3.2 Perc in Blood 13

1.5.3.3 Perc Metabolites in Urine 13

1.5.3.4. Perc in Urine 14

1.5.4. Toxicity and Health Effects 15

1.5.4.1. Respiratory Irritation 15
1.5.4.2. Neurological Effect 15
1.5.4.3. Hepatic Effects 16
1.5.4.4. Renal Effects 16
1.5.4.5. Reproductive Effects 17
1.5.4.6. Cancer 17
1.5.4.7. Death 18
1.5.5. Pharmacokinetics and Toxicokinetics 18
1.5.5.1. Absorption 18
1.5.5.2. Distribution 20
1.5.5.3. Metabolism and Excretion 21
1.5.6. Pharmacokinetic Modeling 24
1.5.6.1. Definition of A PBPK Model 24
1.5.6.2. The Usages and Advantages 24
1.5.6.3. The Shortcomings 25
2. MATERIALS AND METHODS 26
2.1. Subject Selection 26
2.2. Sample Collection 27
2.2.1. Sampling Tubes 27
2.2.2. Preparation of New Sampling Tubes 27
2.2.3. Reconditioning Sampling Tubes 27
2.2.4. Sampling Pumps 28
2.2.5. Alveolar-Breath Sampling Device 28
2.2.6. Collection Efficiency of The Breath Sampling Device 29

2.2.7. Contamination of Breath Samples 30

2.2.8. Breathing-Zone Air Samples during Exposure 31

2.2.9. Breakthrough of Sampling Tubes 31

2.2.10. Cleanning Breath Sampling Device 32

2.2.11. Study Protocols 32

2.2.11.1. Field Study 32

2.2.11.2. Controlled-Environment Facility (CEF) Study 33

2.2.12. Analytical Method 35

2.2.12.1. Method Detection Limit 35

2.2.12.2. Thermal Desorption System 35

2.2.12.3. Gas Chromatography/Mass Spectrometer Condition 36

2.2.12.4. External Standard Solution and Standard Curve 36

2.2.12.5. Identification and Quantification of Perc 37

2.2.13. Quality Assurance/Control 38

2.2.13.1. Sample Quality Assurance 38

2.2.13.2. Analytic Quality Assurance 38

2.2.14. Statistical Analysis 39

2.2.14.1. Calculation of the Absorbed or Internal Dose 39

2.2.14.2. Calculation of the Amount of Perc Expired 39

2.2.14.3. Calculation of the Elimination Half-lives 39

2.2.14.4. Test Statistics 40

2.2.15. PBPK Model Evaluation 40

2.2.15.1. Model Selection 40

2.2.15.2. The Mathematical Equations Defining The PBPK Model 41

2.2.15.3. Data and Model Modification 44

2.2.15.4. Simulation Language 45

2.2.15.5. Model Evaluation Criteria and Statistics 45

2.2.15.6. The Algorithm For Estimating Model Parameters 46

2.2.15.7. Simulation Procedure 47

3. RESULTS 49

3.1. Quality Assurance 49

3.1.1. Sample Quality Assurance 49

3.1.2. Analytical Quality Assurance 50

3.2. Field Study 50

3.2.1. Perc Air Concentrations Inside Laundromats 50

3.2.2. Perc Concentration in The Exhaled Breath During Exposure 51

3.2.3. Percentage of Perc Absorbed 51

3.2.4. Amount of Perc Absorbed or Internal Dose 51

3.2.5. The Relationship between Exposure and Internal Dose 52

3.2.6. The Relationship between Estimated Internal Dose and The Postexposure Breath Concentration 52

3.2.7 The Elimination of Perc After Exposure 52

3.2.8 Amount and Percentage of Perc Expired after Exposure 53

3.3. Controlled-Environment Facility Study 53

3.3.1. Percent Absorption and Dose Index 53

3.3.2. The Elimination of Perc after Exposure 53

3.3.3. Empirical Modeling of Postexposure Breath Concentrations 54

3.3.4. Elimination Half-lives 54

3.3.5. The Area under the Postexposure Breath Curve 55

3.4. PBPK Model Evaluation 55

3.4.1. Evaluation of a PBPK Model Using Field Data 55

3.4.1.1. Total Exposure Calculation 55

3.4.1.2. Initial Evaluation 56

3.4.1.2.1. The Model Predictions with Constant versus Variable Exposure 56

3.4.1.2.2. Model Predictions During and After Exposure 56

3.4.1.2.3. Model Modification 56

3.4.1.2.4 Optimization of Model Parameters 57

3.4.2. Comparison of The Model Predictions using CEF Data 58

3.4.2.1. Sensitivity Analysis 58

3.4.2.2. Optimization of Parameters 58

3.4.2.3. The Model Predictions Using Constant and Variable Inputs 59

3.4.2.4 The Prediction of Tissue Concentrations for The Different Exposures 59

4 DISCUSSION 98

4.1 The Body Absorption Kinetics and Percent Absorption During Exposures 100

4.2. The Effects From Exposure Duration on Internal Dose Estimation 103

4.3. Elimination Half Lives of Perc in the Exhaled Breath 105

4.4. The Elimination of Perc Via Exhalation After Exposure 109

4.5. The Influences from Exposure Duration and Pattern on Elimination Kinetics 111

4.5.1 The Effects from The Exposure Duration 111

4.5.2. The Effects from The Exposure Pattern 113

4.6 The Exposures from Visiting a Perc-Contaminated Facility 115

4.7 Pharmacokinetic Model Evaluation 117

4.7.1 Field Exposure Data with Temporal Variability 118

4.7.1.1. The Model Predictions During and After Exposure 118

4.7.1.2. The Nature of the Data 120

4.7.1.3. The Uncertainty in Model Parameters 120

4.7.1.4. Optimization of Model Parameters 121

4.7.1.5. Comparisons of the Fit with Different Exposure Inputs 122

4.7.2. Controlled Exposure Data of Constant and Variable Exposures 124

4.7.2.1. Sensitivity of the Model Prediction to Selected Parameters. 124

4.7.2.2. Comparison of the Model Predictions for the Two Exposure Inputs 125

4.7.2.3. Comparisons of The Model Prediction Between The Compartment and PBPK Model 128

4.7.2.4. Intra-individual Differences in Tissue Concentration for the Different Exposures 128

5. CONCLUSIONS AND IMPLICATIONS 137

6. FUTURE RESEARCH 139

7. REFERENCES 140

APPENDIX I Description of the Controlled Environment Facility 149

APPENDIX II The PBPK Model Parameter Values 151

APPENDIX II The Experimental Data 152

CURRICULUM VITA 159

LIST OF TABLES

Section	Content	Page
2.1	The demographic information of the studied subject	26
2.2	The experiment design for the controlled-environment facility study	34
3.1	The Perc air concentrations inside three laundromats that also contain dry-cleaning operation	60
3.2	The experimental data of Perc air and breath concentrations, air/breath ratios and percent absorption during exposure for three field exposure experiments with different exposure durations	61
3.3	The exposure durations, average exposure concentrations and percent absorption of Perc during exposure for the field exposure experiments	62
3.4	The exposure, calculated internal dose, postexposure breath concentrations, and amount and percent Perc expired for the experiments of the field exposure study	63
3.5	The percent absorption, calculated internal dose and dose index for the two constant exposures with different exposure durations of the CEF study	64
3.6	Values of the coefficients and their standard deviation of the estimate for the tri-exponential decay of postexposure Perc breath concentrations from the experiments of the CEF study	65
3.7	The average exposure concentrations and the three elimination half-lives of the tri-phasic exponential decay for the experiments of the CEF study	66
3.8	The area under the postexposure breath Perc concentration curve for the experiments of the CEF study with different exposure scenarios	67
3.9	The PBPK model simulation results of the fit of the model to the experimental data of the field Perc exposure study for two different types of exposure input, using the original model parameters, and corrected for method-specific inhaled air contribution	68

3.10 The PBPK model simulation results of the fit of the model to experimental data of the field Perc exposure study for two different types of exposure input, using the original model parameters, but not corrected for method-specific inhaled air contribution 69

3.11 Summary of the PBPK model simulation results of the fit of the model to the postexposure data of the field Perc exposure study before and after parameter optimization forconstant and variable exposure inputs 70

3.12 The percent prediction errors between the model prediction and postexposure data using variable exposure input and default model parameters for two field Perc exposure experiments 71

3.13 The PBPK model simulation results of the fit of the model to experimental data of the controlled Perc exposure study between two different types of exposure input using the optimized model 72

4.1 Summary of Perc kinetic studies, data format and the resulting elimination half-lives 132

LIST OF FIGURES

Section	Content	Page
3.1	The exposure air and exhaled breath concentration during a 60-minute field exposure (experiment p1020) at the front counter of a dry-cleaning store. Linear concentration change between two adjacent data points was assumed	73
3.2	The exposure air and exhaled breath concentration during a 45-minute field exposure (experiment p1208) at the front counter of a dry-cleaning store. Linear concentration change between two adjacent data points was assumed	74
3.3	The exposure air and exhaled breath concentration during a 30-minute field exposure (experiment p1220) at the front counter of a dry-cleaning store. Linear concentration change between two adjacent data points was assumed	75
3.4.a	The correlation and regression plot between the internal doses calculated from the field Perc exposure study and the total exposures estimated from the area under the temporal short-term air measurements	76
3.4.b	The correlation and regression plot between the internal doses calculated from the field Perc exposure study and the total exposures calculated from the product of average exposure air concentration and exposure duration	77
3.5	The scatter plot between the internal dose and breath concentration measured at 6-minute postexposure for the field Perc exposure study	78
3.6	The correlation and regression plot between the internal doses and breath concentrations measured at 6-minute postexposure for the field Perc exposure study, with the three different exposure durations indicated	79
3.7	The Breath Perc concentrations during and after exposure for three field exposure experiments with different exposure duration and resulting internal dose. Time zero is the end of the exposure	80

3.8 The box plot of the percent dose expired for up to 6 minutes post exposure for the Perc field exposure experiments for three exposure durations. The dotted lines inside the boxes represent the group means ... 81

3.9 The postexposure breath Perc levels for the 30- and 90-minute constant exposures of the controlled exposure study. Alveolar breath concentrations (C_{alv}) were normalized to the exposure by dividing it by the product of average exposure air concentration and exposure duration. Vertical bars indicate standard deviations of the means of triplicate experiments ... 82

3.10 The postexposure breath Perc levels for the two different exposure patterns of the 30 minutes exposure of the controlled exposure study. Alveolar breath concentrations (C_{alv}) were normalized to the exposure by dividing it by the product of average exposure air concentration and exposure duration. Vertical bars indicate standard deviations of the means of triplicate experiments ... 83

3.11 The postexposure breath Perc levels for the two different exposure patterns of the 90 minutes exposure of the controlled exposure study. Alveolar breath concentrations (C_{alv}) were normalized to the exposure by dividing it by the product of average exposure air concentration and exposure duration. Vertical bars indicate standard deviations of the means of triplicate experiments ... 84

3.12 The experiment data and PBPK model prediction of breath concentrations during exposure for a 30-minute Perc field exposure experiment (p0301) using the default PBPK model and an integrated exposure air concentration as the model exposure input (constant) ... 85

3.13 The experiment data and PBPK model prediction of breath concentrations during exposure for a 30-minute Perc field exposure experiment (p0301) using the default PBPK model and temporal short-term air measurements as the model exposure input (variable) ... 86

3.14 The experiment data and PBPK model prediction during and after exposure for a 30-minute field exposure experiment (p0301) with a small V/A ratio (1.10) using variable exposure input and optimized parameters ... 87

3.15 The experiment data and PBPK model prediction during and after exposure for a 45-minute field exposure experiment (p1214) with a large V/A ratio (1.35) using variable exposure input and optimized parameters 88

3.16 Sensitivity analysis - the experimental data and PBPK model predictions using default parameters and optimized blood/air partition coefficient (PB) during and after exposure for a 90-minute constant exposure experiment (p01106) of the controlled exposure study 89

3.17 Sensitivity analysis - the experimental data and PBPK model predictions using default parameters and optimized slow perfused tissue-to-blood partition coefficient (PS) and metabolic constants (Vmax and Km) during and after exposure for a 90-minute constant exposure experiment (p01106) of the controlled exposure study 90

3.18 The experimental data and PBPK model predictions of during-exposure breath concentration using variable exposure input and the optimized PBPK model for a 30-minute variable exposure experiment (p0319) of the controlled Perc exposure study 91

3.19 The experimental data and PBPK model predictions of during-exposure breath concentration using variable exposure input and the optimized PBPK model for a 90-minute variable exposure experiment (p0529) of the controlled Perc exposure study 92

3.20 The experimental data and PBPK model predictions of postexposure breath concentration for the two exposure inputs using the optimized PBPK model for a 30-minute variable exposure experiment (p03196) of the controlled Perc exposure study 93

3.21 The experimental data and PBPK model predictions of postexposure breath concentration for the two exposure inputs using the optimized PBPK model for a 90-minute variable exposure experiment (p06206) of the controlled Perc exposure study 94

3.22 The prediction of brain Perc concentration during and after exposure using the optimized PBPK model for the two constant exposures of the controlled Perc exposure study with the same total exposures [297 (mg/m^3) × minutes] 95

3.23 The prediction of brain Perc concentration during and after exposure using the optimized PBPK model for the two different exposure patterns of 30-minute (upper) and 90-minute (lower) exposures of the controlled exposure study with the same total exposures [297 (mg/m^3) × minutes] 96

3.24 The prediction of cumulative amounts of Perc metabolized in the liver using the optimized PBPK model for the two constant exposures of the controlled exposure study with the same total exposures [297 (mg/m^3) × minutes] 97

4.1 The prediction of postexposure breath concentrations of the 30-minute controlled constant exposures from both the compartment and the optimized PBPK model. Two model predictions were made for experiment p02146 only. The other two experimental data shown are parts of the triplicate experiment, with slightly different exposure intensity, of the 30-minute constant exposure scenario 133

4.2 The prediction of postexposure breath concentrations of the 90-minute controlled constant exposures from both the compartment and the optimized PBPK model. Two model predictions were made for experiment p01166 only. The other two experimental data shown are parts of the triplicate experiment, with slightly different exposure intensity, of the 90-minute constant exposure scenario 134

4.3 The prediction of postexposure breath concentrations of the 30-minute controlled variable exposures from both the compartment and the optimized PBPK model. Two model predictions were made for experiment p03196 only. The other two experimental data shown are parts of the triplicate experiment, with different exposure intensity, of the 30-minute variable exposure scenario 135

4.4 The prediction of postexposure breath concentrations of the 90-minute controlled variable exposures from both the compartment and the optimized PBPK model. Two model predictions were made for experiment p06206 only. The other two experimental data shown are parts of the triplicate experiment, with different exposure intensity, of the 90-minute variable exposure scenario 136

ABBREVIATIONS

A/B	Air to breath concentration ratio
AUC_A	The area under the air concentration curve
AUC_B	The area under the breath concentration curve
CEF	Controlled environment facility
CNS	Central nervous system
GC/MS	Gas chromatography/mass spectrometry
GC/FID	Gas chromatography/flame ionization detector
Km	Michaelis constant
LIF	Log likelihood function
MAPE	Mean absolute percent error
MDL	Method detection limit
PB	Blood Partition Coefficient
PERC	Perchloroethylene, tetrachloroethylene
PBPK	Physiologically-based pharmacokinetic
PBR	Brain Partition Coefficient
PF	Fat Partition Coefficient
PL	Liver Partition Coefficient
PR	Rapid-Perfused Tissue Partition Coefficient
PS	Slow-Perfused Tissue Partition Coefficient
PSK	Skin Partition Coefficient
RSD	Relative standard deviation
TCA	Trichloroacetic acid
TCE	Trichloroethanol

TTC	Total tri-chlorocompounds
TWA	Time-weighted average
UHP	Ultra high purity
V/A	Variable to average exposure ratio
Vmax	Michaelis-Menten maximum metabolic rate
VOC	Volatile organic compound

1. INTRODUCTION

1.1. Background

Tetrachloroethylene (Perc) is a volatile organic solvent that has been used extensively for a number of industrial purposes such as metal degreasing and textile dry cleaning operations. Consequently, the potential for occupational exposure exists, through inhalation of vapor and dermal contact. In addition, environmental exposures of people living near or visiting these establishments can also occur. Considerable attention has been directed to the measurements of occupational exposures that are associated with the dry cleaning practices (Aggazzotti *et al.* 1994; Jang *et al.* 1993; Petreas *et al.* 1992; Solet *et al.* 1990; Materna. 1985), but the magnitude of environmental exposures, such as staying in a commercial laundromat that also houses dry-cleaning operations have not been well characterized.

Perc is a anesthetic agent and can cause death at a sufficiently high dose (Levine *et al.* 1981). Its acute toxic effects are primarily central nervous system (CNS) related, such as dizziness, nausea, headache and confusion, but effects on the liver and kidney after severe exposures have also been documented (WHO, 1987; EPA, 1985). Exposure to Perc has been shown to be associated with infertility (Eskenazi *et al.* 1991b) and spontaneous abortion (Eskenazi *et al.* 1991a; Lindbohm *et al.* 1992) among dry cleaning workers. Perc was found to be carcinogenic for some rodents (NCI, 1977) and therefore is classified as a possible human carcinogen (IARC, 1987). The current occupational exposure level of 100 ppm (8-hour TWA) was set to protect workers from

non-carcinogenic adverse health effects (OSHA, 1995). The effects of chronic low-level environmental exposures on the public health (including cancers) are unknown.

The uptake and elimination of Perc following inhalation exposure under either controlled conditions and in occupational settings have been relatively well studied (Stewart *et al.* 1970; Ogata *et al.* 1971; Fernandez *et al* 1976; Monster *et al* 1979 & 1983; Ludwig *et al.* 1983; Solet *et al.* 1990). Postexposure exhaled breath is highly correlated to time-weighted air concentration during exposure, and is therefore considered a simple and effective biomarker for assessing exposure (Petreas *et al.* 1992; Thomas *et al.* 1992; Solet *et al.* 1990; Monster & Smolders, 1984). It is not certain that the relationship established between exhaled Perc breath concentrations and controlled, constant exposures in the mid- to high-ppm range will be manifested at lower, fluctuating concentrations in the low-ppm to ppb range for the shorter durations encountered during environmental exposures In addition, the effects of fluctuations in the exposure concentration and of different exposure durations on body absorption and elimination kinetics have not been elucidated. Further, exhaled breath has not been validated as a surrogate of exposure or absorbed dose following short-term environmental exposures.

Physiologically based pharmacokinetic (PBPK) modeling has been used to predict concentrations of drugs, environmental contaminants and their metabolites in target tissues or biological medium in equilibrium with tissue concentrations, such as exhaled breath concentration following known exposures and dose (Rowland and Tucker, 1986). PBPK modeling is based on equilibrium exchange among body fluid and 'compartments' of body tissues, utilizing the actual physiological parameters to

describe the distribution of xenobiotics in the body (Ward *et al.* 1988). A validated PBPK model is useful for predicting tissue and biological medium concentrations/doses from known exposures and, inversely, reconstructing a previous exposure or total dose based on the concentrations in a tissue or biological fluid, such as postexposure breath concentrations (Georgopoulos *et al.* 1994). PBPK modeling is also used to extrapolate between different dose levels, routes of exposure and across different species. Unfortunately, most PBPK model simulations are performed on a theoretical basis (Sato *et al.* 1991; Fiserova-Bergerova, 1985) or validated using literature data from controlled exposure studies which are not necessarily designed for evaluating PBPK models (Ward *et al.* 1988; Andersen *et al.* 1991; Rao & Brown, 1993), for short-term fluctuating environmental exposure conditions. Evaluations of PBPK models which consider the influences from the fluctuations in exposure condition on the model output are very limited in number (Lapare *et al.* 1993 & 1995). Therefore, it is uncertain whether currently available PBPK models, which was evaluated using the data from long-term controlled exposures of high-ppm levels, can effectively predict biomonitoring values and target tissue concentrations for fluctuating environmental exposures.

The current investigation was designed and conducted to examine the body kinetic behaviors, by means of breath analysis, under various exposure conditions to test the validity of using exhaled breath as a biological marker of exposure and absorbed dose following environmental exposures, and to evaluate and utilize an existing PBPK model under various exposure conditions for improving exposure/risk characterization.

1.2. Hypotheses

The absorption and subsequent elimination of Perc after inhalation exposure depends on its body kinetics. Any factor that affects pulmonary absorption, distribution, metabolism, or elimination will change this dynamic process. The hypotheses for the current investigation are:

1. **Changes in exposure patterns and durations during short term environmental Perc exposures alter the body elimination kinetics, more than the variability that exist for a single person on different days.**

2. **An integrated air concentration can be used as an exposure input for an optimized physiologically based pharmacokinetic (PBPK) model for Perc to predict the postexposure breath concentrations after fluctuating exposures.**

1.3. Specific Aims

These hypotheses were tested using breath and exposure data collected during a field study and from a controlled-environment facility study. The measurements obtained were used to evaluate a PBPK model. The specific aims used to test the hypotheses are:

a) to examine the body kinetics, by means of breath analysis, during and after rapid-changing environmental exposure situations.

b) to assess the potential exposures within laundromats that also house a dry-cleaning operation resulting in Perc concentrations in the low-ppm range.

c) to estimate the amount of Perc absorbed (internal dose) during exposure, and correlate this dose with postexposure breath concentrations and other body kinetic variables.

d) to examine the differences in the body kinetics, specifically absorption and elimination rate of Perc, for different exposure durations and patterns.

e) to determine how exposure duration and pattern influence postexposure breath concentrations, and the feasibility of utilizing breath concentration as a biomarker for environmental exposures.

f) to evaluate and optimize a previously validated physiologically-based pharmacokinetic (PBPK) model for Perc for the study subject, and to determine the differences in model prediction using either an integrated air concentration or temporal air measurements as the model inputs.

g) to examine the differences between predicted and observed breath values, as well as other tissue concentrations, for different exposure situations with the same total exposures using an optimized PBPK model.

1.4. Approaches

This study examined the potential influences from exposure pattern and duration on body kinetics, which includes the process of pulmonary absorption, distribution within the body, disposition in various body tissues, metabolism and elimination. These kinetic behaviors were studied using the breath data collected from a field and a controlled exposure study, and the simulation results from a Perc PBPK model. The field exposure study was conducted first in selected dry-cleaning stores to examine the body's

responses, such as the changes in absorption, to the environmental changes, and to study the potential effects from exposure durations on body absorption and elimination kinetics. The exposure levels and patterns during these field exposure varied over time but the exposure durations (30, 45 or 60 minutes) were pre-determined. The exposure duration was found to affect the body absorption and elimination kinetics for these low-ppm environmental exposures. However, this finding may have been confounded by the fluctuations in exposure levels (exposure pattern), since large variations in air concentrations during exposures were also observed. Therefore, a study design which employed well-controlled exposure conditions was developed in order to unambiguously examine and determine the potential influences from both exposure duration and pattern. In the controlled exposure study, subject was exposed to Perc in a controlled-environment facility to a combination of two exposure durations (30 and 90 minutes) and two exposure patterns (constant and variable). These two time periods represent the time people might stay in a laundromat. Constant exposures of 30- or 90-minute were used to test the effects from exposure duration while two exposure scenarios for each duration were used to examine the effects from exposure pattern. The variable exposure scenario was used to simulate an activity pattern that might occur in the laundromats. The exposure levels, temperature and humidity were based on the values measured in laundromats. The breath measurements from both studies were used to examine the effects from exposure duration and pattern on body elimination kinetics, and to evaluate the feasibility of using breath concentrations as an indicator of environmental exposures and absorbed (internal) dose associated with these exposures.

The breath measurements were used to evaluate an existing PBPK model for Perc. PBPK model was selected because it allows for the extrapolation of results between subjects, across different species and across different routes of exposure. Thus these models provide a method for applying the current experimental findings to the general population. The PBPK model was also used to study the distribution of Perc within the body, particularly brain Perc concentration and amounts metabolized in the liver, for different exposure pattern and duration. The experimental data were also used to examine the differences in model predictions between the two exposure inputs: an integrated air concentration; and temporal short-term air measurements, to evaluate whether an integrated air concentration was adequate to predict the exhaled breath levels during and after real-world fluctuating environmental exposures.

1.5. Literature Review

1.5.1. The Sources and Uses of Perc

Tetrachloroethylene is a clear, colorless, relatively water insoluble, nonflammable synthetic organic solvent with a chloroform-like sweet odor. It is a commercially important chlorinated hydrocarbon solvent and chemical intermediate. It is not known to occur as a natural product (IARC, 1979), and is synthesized through three different processes: 1) reaction between acetylene and chlorine; 2) direct chlorination of certain hydrocarbons, such as methane;, and 3) oxychlorination of ethylene via ethylene dichloride (ATSDR, 1995). It has been used as a dry cleaning and textile-processing solvent due to its lipophilic properties and relatively high boiling point, and for vapor degreasing in metal-cleaning operations because of its ability to dissolve many organic

and inorganic compounds. Perc is also an intermediate for the synthesis of fluorocarbon 113, 114, 115 and 116 which account for about 55% of the annual Perc use. Dry-cleaning and textile-processing, and metal degreasing industries consume about 15% and 25% of total Perc uses, respectively (Chemical Profile 1995). Other miscellaneous uses include: as a carrier solvent for rubber coatings, printing inks, adhesives, lubricants, etc.; as rug and upholstery cleaner; and as heat transfer medium. Perc has also been used as an anthelmintic in the treatment of hookworm and some nematode infestations, but it has been replaced by other less toxic drugs (HSDB, 1994).

1.5.2. Tetrachloroethylene Exposures

1.5.2.1. Occupational Exposure

The National Occupational Exposure Survey (NOES), conducted by NIOSH from 1981 to 1983, estimated that 688,110 workers employed at 49,025 plant sites were potentially exposed to Perc in the United States during that period (NOES, 1990). It was found that the air concentration of Perc at dry cleaning establishments varied widely, ranging from near zero to above 100 ppm (Aggazzotti *et al.* 1994; Jang *et al.* 1993; Petreas *et al.* 1992; Solet *et al.* 1990; Materna, 1985; Ludwig *et al.* 1983). Differences in Perc air concentration among various dry cleaning stores were predominantly determined by the store's (or machine's) ventilation and the process types (Petreas *et al.* 1992; Solet *et al.* 1990; Materna, 1985; Shipman and Whim, 1980). Stores that utilize a transfer-type of operation, which requires transferring loads from washers to dryers, usually have higher Perc concentration than stores that use fully automatic dry-to-dry (a closed system) process. The mean air concentrations for dry-to-dry stores for two studies were found to

be 8.05 ppm (Petreas *et al.* 1992) and 7.09 ppm (Solet *et al.* 1990), whereas the mean air levels in transfer-type facilities were 14.2 ppm (Petreas *et al.* 1992) and 29.5 ppm (Solet *et al.* 1990). Five-minute peak concentrations measured in samples taken during clothing transfer demonstrated significant exposure levels ranging from 11.3 to 533.8 ppm (Materna, 1985). A lower mean peak exposure (25.3 ppm) was found for stores with local exhaust ventilation having the recommended rate (0.5 m/s) than for stores with poorly or unventilated cleaning machines (159.7 ppm) (Materna, 1985). The Perc concentrations in the stores were also higher when the doors and windows were closed during the winter months (Solet *et al* 1990). Two studies identified job title (task) as an additional factor that affected the potential for exposure. Machine operators generally had higher exposures than garment pressors, seamstresses and front-counter clerks, with geometric mean of Perc exposures by task of 22.0, 3.3, 3.0 and 3.1 ppm, respectively (Ludwig *et al.* 1983), and 46.5, 14.8, 19.0 and 11.7 ppm, respectively (Solet *et al.* 1990). However, a third study found no differences between two principal tasks, operator vs. presser, on workers' exposures (Petreas *et al.* 1992).

1.5.2.2. Environmental Exposure

The general population is exposed to low but detectable levels of Perc from ambient air (low-ppt to low-ppb), foods (few µg/kg), and drinking water (sub- to low-ppb). Inhalation of Perc in the ambient air and ingestion of contaminated drinking water are considered the most important routes of exposure for the public (ATSDR, 1995). The results from the US EPA's TEAM (Total Exposure Assessment Methodology) study, which measured the personal exposures of 600 people in several cities in the US,

showed that the indoor air was a more significant exposure source of Perc than outdoor air, even near chemical plants (Wallace, 1986; Wallace *et al.* 1986). The personal exposure levels, primarily indoor, of Perc in the TEAM study were in the ranges of a few to nearly a hundred $\mu g/m^3$ and exceeded outdoor air values, by ratios of 2 to 5. Different segments of the general population, however, can be exposed to Perc at significantly higher concentrations than background levels. For example, the geometrical mean of indoor Perc concentrations in homes across the street, from two houses adjacent to, one house adjacent to and an apartment above a dry cleaning store were <0.1, 0.22, 1.0 and 4.9 mg/m^3, respectively, consistent with air concentrations decreasing with distance from a Perc source (Verberk and Scheffers, 1980). People who live near dry-cleaning shops may have elevated blood Perc and urinary trichloroacetic acid levels, depending on the floor and the construction type of the building where they live (Popp *et al.* 1992). Certain sub-population, specifically youngsters and elderly, who live near Perc emission sources (e.g., factories and waste dumps) can have exposures higher than the background (Monster & Smolders, 1984). People who have their clothes dry-cleaned increase the potential for exposures, not only because they visit these establishments (Wallace *et al.* 1989) but also from Perc off-gassing from the dry-cleaned clothes (Weber, 1992; Kawauchi & Nishiyama, 1989; Verberk & Scheffers, 1980). A car transporting a freshly dry cleaned down jacket had air concentration inside the cabin as high as 24.8 mg/m^3, after 108 minutes (Gulyas & Hemmerling, 1990). Storage of newly dry-cleaned garments in a residential closet resulted in Perc levels of 2.9 mg/m^3 in the closet after 1 day, followed by a rapid decline to 0.5 mg/m^3 which persisted for several days (Tichenor *et al.* 1990). People who utilize coin-operated dry

cleaning machines have exposure levels as high as 351 mg/m^3 (Gulyas & Hemmerling, 1990). The US EPA's TEAM also determined, using stepwise regression method, that increased Perc exposures were associated with certain activities/occupations that were related to Perc sources, such as visiting a dry-cleaner and working at a textile plant (Wallace, 1986).

Family members of workers exposed to Perc have higher exposures since they can be exposed to moderate levels (0.025-9.6 mg/m^3) of Perc at homes when Perc is released in the expired breath and from the clothes of the occupational-exposed workers (Aggazzotti *et al.* 1994). A simulation study predicted that air concentrations inside the house of these workers are mainly dependent upon the household ventilation conditions (Thompson and Evans, 1993).

Additional exposure concerns have been focused on contaminated groundwaters (well waters) because Perc has been identified in the water samples from at least 771 of the 1416 proposed National Priorities List hazardous waste sites (HAZDAT, 1995). People can be exposed to Perc if these polluted waters are used for drinking and bathing/showering (Wallace *et al.* 1986a; Kido *et al.* 1989; Rao & Brown, 1993; Terttu *et al.* 1993) and if the contaminated groundwaters either infiltrate the structure or release Perc, as part of the soil gas, into the basement of a house (Kliest *et al* 1989; Hodgson *et al.* 1992).

In addition to the above exposure pathways, infants can also be exposed to Perc via the breast milk of exposed mothers (Bagnell and Ellenberger, 1977; Pellizzari *et al.* 1982).

The current study estimated the exposures to individuals visiting a coin-operated laundromat that also contain dry-cleaning facility. Coin-operated conventional washing

machines are sometimes located in a room that also houses or is adjacent to dry cleaning stores. This arrangement results in elevated Perc indoor air concentrations due to the releases from dry cleaning process. As a result, people who utilize these facilities can be exposed to sub- or even low- occupational levels (hundreds of ppb to a few ppm) of Perc for a prolonged period (1-2 hours) when washing (waiting for) their clothes in these stores.

1.5.3. Biomarker of Exposure

1.5.3.1. Exhaled Perc

Measurements of Perc in exhaled breath is a simple and effective method for assessing both occupational and environmental exposure, with the advantages of being noninvasive and specific (Petreas *et al.* 1992; Solet *et al.* 1990; Wallace *et al.* 1989; Monster & Smolders, 1984; Monster *et al.* 1983; Stewart and Dodd, 1964). The presence of Perc in breath indicates that exposure has occurred (Wallace *et al.* 1986) and provides a measure of the internal dose or body burden because exogenous compounds in the breath originally come from the exposures (Raymer *et al.* 1992). The US EPA's TEAM study found that the breath Perc levels of the 355 New Jersey residents correlated significantly with personal air exposures, indicating exhaled breath concentrations can be used to assess environmental Perc exposures (Wallace *et al.* 1985; Wallace, 1986; Wallace *et al.* 1986). In occupational exposure studies, measured 8-hour time-weighted average (TWA) Perc exposure concentrations are highly correlated with the breath Perc concentration measured right after a shift, with the correlation coefficients from these studies from 0.75 (Aggazzotti *et al.* 1994; Solet *et al.*

1990), 0.89 (Petreas *et al.* 1992) to 0.93 (Monster *et al.* 1983). The TWA also correlated with the Perc breath levels measured the next morning, though the correlation coefficients were slightly lower. These exposure/response relationships confirm that postexposure exhaled breath can be used as a reliable biomarker of past exposure. The partition coefficient of Perc between venous blood and alveolar breath after exposure remained nearly constant at 16, indicating that exhaled breath is also a good indicator for the Perc body burden (Monster *et al.* 1979). It was also found that Perc in the breath was highly correlated with Perc in the blood (r^2=0.94) (Petreas *et al.* 1992) which again indicates that Perc in the breath was a very reliable indicator of Perc body burden.

1.5.3.2. Perc in Blood

Increased Perc levels in blood have been measured in individuals who drank water contaminated with Perc at levels above 120 mg/L (Kido *et al.* 1989). Blood Perc levels measured at the end of a Friday workshift correlated well (r^2=0.95) with weekly TWA (Monster *et al.* 1983). The Perc blood concentrations measured in the morning following a 4-day shift correlated best (r^2=0.89) with the average TWA of the 1st and 2nd work day (Petreas *et al.* 1992). However, the usefulness of this marker is hindered by its methodological limitations (invasiveness).

1.5.3.3. Perc Metabolites in Urine

Attempts to determine the best biomarker for Perc exposure have included measuring its principal metabolites, trichloroacetic acid (TCA) and trichloroethanol (TCE), in the

urine. Two occupational exposure studies (Monster *et al.* 1983; Jang *et al.* 1993) found that urinary TCA concentrations in samples collected at the end of the Friday shift were highly correlated (r^2=0.91 and 0.95, respectively) with weekly TWA, indicating that urinary TCA levels are useful in estimating past exposures. This approach, however, should be used with caution not only because these metabolites are not specific for Perc *i.e.*, they also result from the metabolism of trichloroethylene and trichloroethane (Monster, 1988), but also because the enzyme system becomes saturated at high exposures. Urinary excretion of TCE and TCA in workers increased linearly with Perc concentration in the air of up to either 50 ppm (Ikeda *et al.* 1972) or 100 ppm (Ohtsuki *et al.* 1983), and the total trichloro-compounds (TTC) excreted in the urine increased linearly up to air concentrations of either 112 ppm (Seiji, 1989) or 100 ppm (Ohtsuki *et al.* 1983). Blood TCA level was found to continue to increase until about 20 hours after the end of a 4-hour exposure, therefore, the peak urinary TCA levels should exhibit a lag of several hours following the end of an exposure that does not saturate the enzyme system (Monster *et al.* 1983).

1.5.3.4. Perc in Urine

A study examining 40 workers exposed to Perc at various plants found a direct correlation (r^2=0.87) between levels monitored in the personal breathing zone (4-hour TWA up to 500 mg/m^3) and levels of unmetabolized Perc in the urine collected at the end of the shift (Ghittori *et al.* 1987). Another study also found that Perc concentration in the urine, collected right after exposure, was correlated with the TWA air concentrations of individuals who were occupational exposed (n=55) or voluntarily

exposed in a controlled chamber (n=15) (Imbriani *et al* 1988). This study also found that the total uptakes correlated with urinary Perc (r^2=0.67) or TWA Perc air concentrations.

1.5.4. Toxicity and Health Effects

1.5.4.1. Respiratory Irritation

Human volunteers self-reported upper respiratory tract irritation after exposure to Perc at 216 ppm for 45-minute to 2-hour (Rowe *et al*. 1952). Respiratory irritations were also experienced by metal-degreasing workers exposed to Perc vapors at concentrations of 232 to 385 ppm (Coler and Rossmiller, 1953). But no effects on the pulmonary function tests were identified after sequentially exposed to 0, 20, 100 or 150 ppm of Perc vapor for 7.5-hour/day, 5-day/week, one week exposures at each vapor concentration (Stewart *et al*. 1981).

1.5.4.2. Neurological Effects

Neurological symptoms of dizziness and drowsiness occurred at exposures of 216 ppm for 45-minute to 2-hour, whereas loss of motor coordination occurred at exposures to 280 ppm for 2 hours and 600 ppm for 10 minutes (Rowe *et al*. 1952). Exposure to Perc concentrations of 100 ppm for 7 hours produced minor symptoms, such as headache, dizziness, difficulty in speaking, and sleepiness (Stewart *et al* 1970). Impairments in perceptual function, attention and intellectual function were found, by means of psychological tests and questionnaires, in dry-cleaning workers exposed to a TWA of 12 and 54 ppm, but no dose-response relationship was identified (Seeber, 1989).

Electroencephalogram (EEG) pattern changes, which are similar to that observed in healthy adults during drowsiness, light sleep and the first stages of anesthesia, were found in seven out of the nine subjects exposed to 100 ppm of Perc (Stewart *et al.* 1981). The neurotoxic effects in humans may be associated with the uptake of this lipophilic compound by the brain membranes (ATSDR, 1995).

1.5.4.3. Hepatic Effects

Cellular damage in liver was found in a case report of a woman occupationally exposed to Perc fumes over 2.5 months (Meckler and Phelps, 1966). Diffuse fatty liver was also reported in a dry cleaner who died shortly following exposure to a high level (not specified) of Perc fumes (Levine *et al.* 1981). On the other hand, serum alkaline phosphatase, SGOT and SGPT levels were not increased among workers exposed to Perc at a mean TWA of 20 ppm for between 1-120 months (Cai *et al* 1991), and serum SGPT levels were not increased in 22 dry cleaning workers exposed to a TWA of 21 ppm of Perc over an average of 6 years (Lauwerys *et al.* 1983). No difference in clinical chemistry tests, which included: serum alkaline phosphatase, SGOT, SGPT and bilirubin levels, from background values were observed in 20 human volunteers sequentially exposed to 0, 20, 100 or 150 ppm for 4 weeks (Stewart *et al.* 1981).

1.5.4.4. Renal Effects

Renal dysfunction, including proteinuria and hematuria, have been associated with accidental exposure to Perc at anesthetic levels (Hake and Stewart, 1977). Urinary lysozyme activity increased in workers exposed to Perc at an average of 23 ppm for

about nine years (Vyskocil *et al.* 1990). However, no difference in urinalysis and blood urea nitrogen from baseline were observed for 20 human volunteers sequentially exposed to 0, 20, 100 or 150 ppm for 4 weeks (Stewart *et al.* 1981), and no effects on blood urea nitrogen or serum creatinine were found in workers exposed to a mean concentration of 20 ppm between 1-20 months (Cai *et al.* 1991).

1.5.4.5. Reproductive Effects

An exploratory study reported menstrual disorders (Zielhuis *et al.* 1989), and two case-control studies have found an increased risk of spontaneous abortion (Ahlborg, 1990; Kyyronen *et al.* 1989) among female dry cleaning workers. However, no increase in spontaneous abortion rate was found among laundry and dry-cleaning workers in a cross-sectional study (McDonald *et al.* 1986).

A study of semen quality showed no difference in the percentage of abnormal sperm and sperm counts between dry cleaners and controls (Eskenazi *et al.* 1991a). However, it was reported that it may take slightly longer for the wives of dry cleaners to become pregnant and that they seek help for infertility problem more often than the general population (Eskenazi *et al.* 1991b).

1.5.4.6. Cancer

An excess risk of primary liver cancer in male workers in laundry and dry-cleaning industries was reported in a case-control study, after controlling for alcohol consumption and smoking, however, the solvents type and exposure levels were not specified (Stemhagen *et al.* 1983). Increased mortality resulting from kidney, lung,

skin, cervix, colon and hematopoietic/lymphatic system cancers for laundry and dry-cleaning workers have also been reported (Blair *et al.* 1979 & 1990; Duh and Asal, 1984; Katz and Jowett, 1981), as have a small excess risk of liver cancer among female workers in laundry and dry-cleaning operations (Lynge and Thygesen, 1990). However, attributing the adverse health effect to Perc exposure is confounded by various factors, including exposure to other solvents, smoking and alcohol consumption, socioeconomic status, and by a low occurrence of the disease (small number of observed cases). One retrospective study, which examined 615 workers exposed only to Perc, failed to find any excess in cancer risk at any site (Brown and Kaplan, 1987).

1.5.4.7. Death

The 4-hour inhalation LC_{50} derived from an animal study was 5,200 ppm (Friberg *et al.* 1953). Human deaths caused by inhaling high levels of Perc vapor associated with dry cleaning operations have been reported (Levine *et al* 1981; Lukaszewski, 1979), though the exposure levels were not specified. These deaths are presumed to result from either excessive depression of the respiratory center caused by an anesthetic effect, or the onset of a fatal cardiac arrhythmia induced by epinephrine sensitization (ATSDR, 1995). Significantly increased total mortality in workers occupationally exposed to Perc compared to controls have not been reported (Blair *et al* 1990; Brown and kaplan, 1987; Katz and Jowett, 1981; Spirtas *et al.* 1991).

1.5.5. Pharmacokinetics and Toxicokinetics

1.5.5.1. Absorption

Perc is readily absorbed by humans through the lungs into the blood stream. therefore. the primary route of entry for Perc is via the lung during inhalation exposure (Monster *et al.* 1979; Hake & Stewart. 1977). Perc was also rapidly and readily absorbed across the GI tract following ingestion (Koppel *et al.* 1985; Pegg *et al.* 1979). but not across the skin following dermal exposure (Stewart and Dodd. 1964).

The rate of pulmonary absorption of solvents increase with increasing blood/air partition coefficient and lipid solubility following a single breath exposure (Morgan. *et al.* 1970). Postexposure breath concentrations were higher for an individual exposed to 150 ppm of Perc while exercising than at rest. confirming that an increase in ventilation rate results in an increase in Perc body burden. Elevated breath concentrations (body burden) were also observed when exposures occur on sequential days. Pulmonary uptake of Perc is proportional to the exposure concentration. ventilation rate and exposure duration. Co-consumption of alcohol increased the uptake of Perc at 25 ppm exposure level. while it decreased the uptake at higher exposure level (100 ppm) (Hake and Stewart. 1977).

Subjects exposed to 72. 142 and 144 ppm for 4 hours showed that the total uptake was influenced more by the amount of lean body mass than by respiratory minute volume or the amount of adipose tissue (Monster. 1979). The rate of uptake decreased during the course of exposure due to a decrease in retention as tissues became saturated. The uptake was about 25% higher during the first hour of exposure than during the last hour for a subject at rest. The mean uptake at an exposure of 144 ppm at rest was 2.1 times higher than at 72 ppm when the individual was at rest which implies a proportionality between uptake and exposure level. The total uptake was increased 40% when two 0.5

hour-100W exercise period were included in the 4-hour exposure, compared to similar exposures to sedentary individuals. The level of activities during exposure has been shown to have a great impact on the resulting internal dose following VOC exposures (Raymer *et al.* 1992).

In a study of 24 subjects exposed to 100, 150 and 200 ppm for 1 to 8 hours, the rise of concentration in alveolar breath was very rapid during the first few minutes and then slowed until the end of exposure, thus the uptake (retention) initially declined rapidly followed by a slower decline, as the exposure continued (Fernandez *et al.* 1976). It has been speculated that the retention of Perc through the lungs could be reduced at a more intensive exposure (Ohtsuki, *et al.* 1983). If the ventilation rate increased, the concentration in alveolar air would also increase, but not proportionally, whereas, an increase in cardiac output would increased the retention of Perc and thus reduce the concentration in alveolar air (Fernandez *et al.* 1976).

In another study (Pezzagno *et al.* 1988), 15 volunteers were exposed to Perc during periods of rest or during periods of rest alternated with periods of exercise. It was found that the retention decreased with increasing ventilation at rest for different subjects and during exercise.

1.5.5.2. Distribution

Only limited information is available describing the distribution of Perc after different routes of exposures. The distribution of Perc in the human body following a high dose, inhalation exposures was studied in two fatality cases. One found that the Perc concentration in the brain was 120 times higher than that in the lung (Lukaszewski,

1979) while the second detected that Perc concentration in the liver was 8. 3.4 and 3.5 times higher than in the lung, kidney and brain, respectively (Levine *et al.* 1981). A four-compartment PBPK model developed by Guberan and Fernandez (1974) predicted that approximately half of the body burden would be distributed to the fat tissue after an 8-hour exposure to 100 ppm of Perc. Perc was found to distribute primarily to adipose tissues, especially perirenal fat, in Sprague-Dawley rats 17-hour after the fourth daily exposure in a five-day 200 ppm exposure. Perc concentrations measured in the fat were 145 times higher than in blood (Savolainen *et al.* 1977). Perc crosses the placenta and is distributed to the fetus and amniotic fluid following 10 minutes and 1 hour inhalation exposures of pregnant mice to radioactive Perc (Ghantous *et al.* 1986). High levels of radioactivity were found in various maternal tissues, such as body fat, brain, blood, liver, kidneys and lungs. Evidence of kidney damage following gavage administration of 1500 mg/kg/day for 42 days were found in male rats. This study also indicated that Perc was distributed to the liver and kidneys (Green *et al.* 1990).

1.5.5.3. Metabolism and Excretion

Stewart and coworkers (1970) found that there was a slight, but definite accumulation of Perc within the subjects exposed to Perc for 5 consecutive days, with the uptake never achieving a steady state. They also found that individuals with greater body mass had a higher alveolar breath Perc concentration during the postexposure monitoring period, and this trend became stronger with time.

Another study also showed that 67 hours after a 3-hour exposure to Perc vapors, the excretion of TCA in the urine of volunteers was about 1.8% of the estimated Perc retained (Ogata *et al.* 1971).

Monster and coworkers (1979) calculated that 80-100% of the absorbed Perc was excreted unchanged via the lung by 162 hours after the exposure. The concentration of TCA in blood continued to increase until 20 hours after exposure and decreased exponentially from about 60 hours after exposure, with a half life of 75-80 hours. Only a part of the total TCA, approximately 1% of the uptake, was excreted in urine until 70 hours after the start of exposure. The remaining TCA, which also was about 1% of the uptake, accumulated in the body (Monster *et al.* 1979). Approximately 15% of the absorbed dose was excreted in breath within an hour after a single breath exposure to Cl^{38}-labelled Perc (Morgan *et al.* 1970).

The concentration of Perc in alveolar air during the first hours of the postexposure period decreased rapidly following an exponential decay. A time period of more than two weeks was necessary to completely eliminate the Perc retained after 8 hours-100 ppm exposure. The shape of the expiration curve depends on the exposure concentration, blood-to-air and tissue-to-blood partition coefficients and physiological parameters, such as alveolar ventilation and cardiac output. There was no direct proportionality between the exposure length and the alveolar breath concentration. TCE was not found in two subjects exposed to Perc at 150 ppm for 8 hours, and about 1.85 % of the uptake was metabolized as TCA and eliminated in urine within 72 hours after exposure (Fernandez *et al.* 1976).

The total trichloro compounds (TTC) in urine was not found to be proportional to the exposure concentration when the latter exceeded 100 ppm (Ohtsuki *et al.* 1983; Ikeda, 1977). It was calculated that, at the end of an 8-hour shift with exposure to Perc at 50 ppm (TWA), 38% of the Perc absorbed would be exhaled unchanged and less than 2% would be metabolized and excreted in the urine (Ohtsuki *et al.* 1983).

A linear relationship (r=0.87, n=40) was found between the TWA exposure concentration and urinary Perc concentration after a 4-hour occupational exposure (Pezzagno *et al.* 1988).

The alteration of metabolism due to co-exposure to other chemicals has been studied. Seiji and co-workers (1989) found that a linear correlation existed between the urinary TTC levels collected at the end of shift and the 8-hour TWA Perc concentrations of up to 112 ppm for subjects of both genders. However, urinary metabolite levels were markedly lower in the second exposure group that were exposed to both Perc and trichloroethylene, as compared to exposed to trichloroethylene alone. This finding suggested that the metabolism of trichloroethylene was suppressed by the co-exposure to Perc in humans. The results also indicated the presence of ethnic and gender differences in Perc metabolism (Jang *et al.* 1993; Seiji *et al.* 1989). Differences in elimination kinetics resulting from the variations in metabolism between male and female was also suggested (Raymer *et al.* 1992).

1.5.6. Pharmacokinetic Modeling

1.5.6.1. Definition of A PBPK Model

Pharmacokinetics is the study of time course of drugs (xenobiotics) or their metabolites within the body which includes the process of absorption/uptake, transport, disposition and elimination (Bischoff and Brown, 1966; Wagner, 1968). A physiological pharmacokinetic (or toxicokinetic) model attempts to utilize basic anatomical (e.g., organ and tissue sizes), physiological (e.g., blood flow rates, membrane permeabilities and enzyme reactions) and biochemical (e.g., drug-protein binding isotherms) information in conjunction with the chemical kinetic principals (e.g., mass balance) and chemical-specific properties (e.g., blood/air and tissue/blood partition coefficients) to construct a mathematical relationship to describe the pharmacokinetics of a chemical (Rowland and Tucker, 1986). In a PBPK model, tissues/organs with similar physiological properties are lumped as a "compartment" within which the concentration of a drug is assumed to be uniform. Differential mass balance equations are written for each compartment to describe the inflow, outflow, accumulation and metabolism of the drug (Himmelstein and Lutz, 1979).

1.5.6.2. The Usage and Advantages

PBPK models were developed because they can 1) provide a rational basis for correlation of data; 2) reduce data to a number of meaningful parameters; and 3) indicate areas in which data are insufficient (Wagner, 1968; Himmelstein and Lutz, 1979; Rowland and Tucker, 1986). PBPK mathematical models incorporate the mechanism of transport, disposition and elimination etc., permitting the extrapolation of

results between different subjects and different species can be accommodated within the context of the physiological or anatomical parameters (Himmelstein and Lutz, 1979). The PBPK models were originally used to predict the concentrations of therapeutic drugs (or their effective derivatives) in the blood and at the sites of action (Hladky, 1990). Recently, PBPK models have also been used to derive internal doses (Andersen *et al.* 1987; Reitz *et al.* 1990) and the total amount of metabolites in the liver (Gearhart *et al.* 1993), to predict the concentrations/amounts of compounds in the target organs (Farrar *et al.* 1989; Smith, 1992; Rao and Brown, 1993), and to re-construct past exposures (Georgopoulos *et al.* 1994).

1.5.6.3. The Shortcomings of PBPK Model

A typical PBPK models usually consists of 30-40 parameters, which are difficult to obtain, especially for humans. Parameters obtained from *in vitro* human and animal studies introduce an unknown degree of uncertainties to the model (Gearhart *et al.* 1993). Secondly, large number of data are necessary to verify the model. These data are often unavailable since it is almost impossible to analyze various tissues for chemical content in humans (Rowland and Tucker, 1986)

2. MATERIALS AND METHODS

2.1. Subject Selection

One healthy male subject with no known occupational exposure to Perc and not on any medications participated throughout the study to control for the possible inter-individual variability. The subject consented to participate in both field and controlled exposure study. The subject avoided consuming alcohol for 48 hours preceding exposure, and during the experimental period. The participant also recorded the personal activities 24 hours prior to and after each exposure to confirm no activities known to contribute to Perc exposure occurred during these time period. The intervals between experiments were at least one week to minimize any carryover effect from the previous exposure. The total ventilation rate (l/min) for the subject was measured using a Cosmed Pony Spirometer (Cosmed Srl, Italy), and a mean value of 8.3 l/min was obtained from 15 measurements taken at the laboratory. The alveolar ventilation rate was assumed to be approximately 67% of the resting total ventilation rate (EPA, 1991). The demographic information of the subject is listed in Table 2.1.

Table 2.1 - The demographic information of the studied subject.

Gender	Age	Weight (Kg)	Height (cm)	Race
Male	32	70	177	Asian

2.2 Sample Collection

2.2.1. Sampling Tubes

Perc in the air and breath were collected onto stainless steel adsorbent traps (Perkin Elmer Inc.) measuring 0.25 inch i.d. x 3.5 inch and filled with 0.30 g of graphitized carbon black (Carbotrap®, Supelco Inc.) using a personal sampling pump. The Carbotrap adsorbent has excellent adsorbing and desorbing properties for various VOCs including Perc.

2.2.2. Preparation of New Sampling Tubes

A stainless steel gauze (Perkin Elmer Inc.) was first placed at one end (sampling side) of the tube and then small amount of glass wool was inserted to prevent the leakage of the adsorbent through the gauze. The tube was filled with 0.30 g of Carbotrap, and glass wool and gauze were placed on the other end of the tube, which was secured with a retaining spring (Perkin Elmer Inc.). Each newly packed tube was placed into a rack which was connected with steady flow of ultra high purity (UHP) grade nitrogen gas (Air Products and Chemicals, Inc.) and housed inside an oven. The new tubes were then heated at 340 ^{o}C for 6 hours while continuously flushing with nitrogen gas at the flow rate of 500 ml per minute. The conditioned tubes were sealed with Teflon caps and stored in a desiccator. The sampling tubes were re-packed with new adsorbent after 15 uses.

2.2.3. Reconditioning Sampling Tubes

After analysis or storage of tubes for more than two weeks, sampling tubes were reconditioned to remove contaminant residues in the adsorbent. Sampling tubes are reconditioned by continuously flushing with UHP grade nitrogen gas while being heated

at 340° C for 3 hours. One tube out of each newly reconditioned batch (15 tubes) was analyzed to verify that the Perc residue was below the detection limit. The date of each tube conditioning was recorded in a log note book.

2.2.4. Sampling Pumps

The alveolar breath and breathing-zone air samples were pulled through the adsorbent traps using constant flow pumps. An Aircheck sampler (Model 224-43XR, SKC Inc.) was used for the breath sampling while personal sampling pumps (Model P4LA, Du Pont Co.; and Model MG-4, Ametek-Mansfield & Green Division) were used for the air sampling. Each pump was fully charged before use. The flow rate of the pumps were calibrated before and after each sampling day using an electronic bubble flow meter (Buck Calibrator, A.P. Buck Inc.) with a sampling tube in line.

2.2.5. Alveolar-Breath Sampling Device

Alveolar air (also called end-exhaled breath) of the subject was sampled by the method modified from Raymer *et al* (1990). This portable breath sampling device consists of an inhalation air source which can be purified when connected to a NIOSH-certified activated charcoal cartridge filters, a one-way valve (Laerdal Valve IV) which directs the air flow to and from subject's mouth, a disposable mouth piece into which the subject breathes, a Teflon connector, a temporary storage tubing (1/2 inch I.D. × 300 inch) and sampling tube connected to a personal sampling pump. The dimensions of the storage tubing were chosen to minimize mixing of the alveolar air with deadspace air and to provide a sufficient volume (about 1.0 liter) to contain the alveolar breath. The air of the

exposed environment was inhaled, while wearing a noseclip, through the one-way respiratory valve into the subject's mouth. The expired air closes the one-way valve, directing the breath to the Teflon connector and tubing. One end (sampling side) of the breath sampling adsorbent tube was connected to the sideport of the Teflon connector while the other end was connected to a personal sampling pump. The sampling rates was set to between 0.7-1.3 l/min, and the sampling duration during and shortly after exposure was 30 seconds for the field study and 15 seconds for the controlled exposure study. A sampling time of 2 minutes was used for background breath samples and samples collected 7-8 hour after the exposure to reduce the analytical uncertainty. All breath samples were collected while the subject was standing and at rest.

2.2.6. Collection Efficiency of The Breath Sampling Device

The collection efficiency of the breath sampling device was assessed using a simulated breath source. Two known concentrations of Perc were prepared in the Tedlar bags (10 × 10.5 inch, SKC Inc.). This was done by first injecting known amounts of Perc into a 2-liter static dilution glass bottle (Corning Inc.) for initial dilution. A portion (1-2 ml) of air inside the bottle was then pulled, using a gas-tight syringe (Hamilton Co.) and injected into a glass sampling bulb (kept at 37 ^{o}C), through which a predetermined volume of nitrogen gas flowed into the bags. Each Tedlar bag was connected to the one-way respiratory valve to serve as the exhaled breath source, and was periodically pressed manually to simulate the exhalation cycles during the sampling. Each press lasted about 3 seconds and exhausted ~500 ml in volume. The air inside the bags was then collected and

analyzed by the same method as for the normal exhaled breath. But this method does not consider the dilution of exhaled breath from the air of the anatomic dead space.

2.2.7. Contamination of Breath Samples

Two controlled studies were performed to determine whether the breath samples were contaminated. The first study determined whether low level breath samples were contaminated when surrounding air containing high Perc levels passed through the tubing. This test was particularly important for the first samples collected during the beginning of exposure and postexposure period when greater changes in air/breath concentration occurs. This was accomplished by collecting samples through the breath device immediately after entering the controlled environment facility containing ~1.5 ppm of Perc using a simulated breath source. The simulated breath source (Tedlar bag, 12 × 16 inch, SKC Inc.) contained UHP-grade nitrogen to which water vapor was added at 90% saturation at ~38 °C, and was connected to the breathing valve. During this experiment the tubing initially contained clean air as during the actual exposure experiments. The bag was pressed 3 times, each press exhausted ~500 ml air and lasted about 3 seconds to simulate actual breaths, before turning on the sampling pump. The sample tube was then connected to the sampling port. Then bag was pressed again to simulate breathing and a 15 second sample, using a flow rate of ~0.8 l/min., was collected. The second study examined the possible contamination from the carryover of previous breathes, *i.e.*, the absorption/desorption processes on the surface of the temporary storage tubing. The breath sampling device was placed inside the controlled facility for 10 minutes, and was filled with the surrounding Perc-containing air (~1.5

ppm) by pulling the air through it. The breath source was then connected and the procedure outlined above, pressing the bag three times, and then collecting a 15-second sample at ~0.8 l/min., was followed. Both tests were run in duplicate.

2.2.8. Breathing-Zone Air Samples during Exposure

The personal exposure (breathing-zone air) samples were collected concurrent with breath samples, at 2 to 7 minutes time intervals, during the exposure period. The sampling tubes were placed adjacent to the inlet of the breath sampler. The sampling rates were adjusted to between 0.2-0.3 l/min. and the sampling durations were the same as that of the coinciding breath samples. In addition, one integrated breathing-zone air sample (sampling rate of ~3 ml/min) was also collected for the entire exposure period. An additional air sample was taken where postexposure breath samples were collected in the field study to confirm a low Perc background level. The sampling tube was connected to the pump via a Cajon Ultra-Torr stainless steel fitting (Cajon Co.) and Tygon tubing (1/4 inch I.D. × 20 inch, Norton Performance Plastics).

2.2.9. Breakthrough of Sampling Tubes

To determine if Perc breakthrough occurred, a controlled test was performed. Three different Perc air concentrations, which represented low, moderate and high levels found in the field study, were prepared in the Tedlar bags and were sampled using two adsorbent tubes connected in series operated at the maximum sampling rates and durations used during the field study. The Perc present in the second tubes were compared to that in the blank tubes to determine the degree of breakthrough.

2.2.10. Cleaning the Breath Sampling Device

The breath sampling device was cleaned thoroughly after each sampling day to eliminate possible absorption and subsequent release of Perc residues from the tubing. The sampler was first dissembled, rinsed with tap water and washed with soap water, and flushed with copious amounts of tap water. The breathing valve and connector were then soaked in methanol for 30 minutes, and the tubing was rinsed twice with methanol. Finally, all parts were rinsed twice with de-ionized water and dried with charcoal-filtered air.

2.2.11. Study Protocols

2.2.11.1. Field Study:

The subject was exposed to Perc in two selected dry cleaning stores for 30, 45 or 60 minutes. Seven (8 for 30-minute exposures) experiments were performed at each exposure duration. The experimental equipment was set up by an assistant before the experiments began. Simultaneous personal exposure (breathing-zone air) and alveolar breath samples were taken, at front counter, as soon as the subject entered the store and at time intervals of between 3 and 7 minutes thereafter until the end of the predetermined exposure period. The subject left the store immediately after the last sample was taken. After the exposure ceased, 5 to 6 postexposure breath samples were taken at a nearby outdoor area at time intervals of between 3 to 10 minutes in order to measure the rapidly changing concentrations at the beginning of the elimination phase. The stay in the outdoor area also facilitated the release of Perc residue from the subject's clothes which may have resulted in additional exposure. Breath samples were taken at 15 to 120 minutes intervals upon returning to the laboratory until about 8-12 hours after the visit.

Subject breathes 2 to 3 times through the breathing valve before the samples were collected to minimize the chance of Perc contamination from desorbing/residue in the tubing. Samples were stored in a plastic box which was placed in a desiccator at room temperature for up to two days prior to analysis.

2.2.11.2. Controlled-Environment Facility (CEF) Study:

A preliminary performance test of the instantaneous mixing and clearance of the chamber for Perc was done by measuring changes in its concentration with time, using adsorbent tube/GC-MS, on site GC/FID (Model 3700, Varian Inc.) and total organic carbon analyzer (Model 23-500, Gow-Mac Instrument Co.). Both the temporal and spacial distribution within the CEF were evaluated to ensure that complete mixing occurred and the concentration was within ± 10% of the target value. The study of Perc uptake and elimination was based on a 2×2 factorial experimental design with 3 replications for each setting (Table 2.2). The first factor examines the effects of exposure duration (30 and 90 minutes) while the second factor tests the influences from exposure pattern (constant and variable). All of these experiments were designed to have the same total exposure, 297 [$(mg/m^3) \times$ minute]. To achieve this the constant exposures were 3.3 mg/m^3 for 90 minutes and 9.9 mg/m^3 for 30 minutes. The exposure pattern for the 30-minute variable exposures was 19.8 mg/m^3 for the time periods 0-5 and 10-15 minutes, no exposure for 5-10 and 15-20 minutes and 9.9 mg/m^3 for 20-30 minutes, whereas for the 90-minute variable exposures it was 4.95 mg/m^3 for the time periods 0-10, 25-65 and 80-90, and no exposure for 10-25 and 65-80 minutes. The temperature and relative humidity were set at 70 °F and 30%, respectively. The description of the controlled-

environment facility and the solvent delivery system are provided in Appendix I. The exposure concentrations selected were within the range measured in local laundromats, and the two exposure durations (30 and 90 minutes) represented different time periods that people might spend in the laundromats. The temperature and relative humidity were also commonly found in the field study. The interaction between exposure duration and pattern on body absorption and elimination kinetics was assumed to be negligible and therefore not addressed in this study. Each experiment was implemented randomly and separated by at least one week. The subject entered the facility after the Perc concentration reached the desired level, wearing a laboratory coat to minimize Perc contamination of the subject's clothes. Air and breath samples were taken simultaneously during the exposure, by the method described earlier, at 2 to 5 minutes time intervals for the first 10 minutes and 7 to 15 minutes time intervals thereafter. The postexposure breath samples were collected in a room with no known Perc sources, using a sampling sequence similar to that used in the field study. A questionnaire was used to ascertain potential exposure to solvents that may have occurred during the previous 24 hours prior to CEF exposure and during the postexposure sample collection period. Pre-exposure breath samples were collected to verify a low Perc body burden before the experiment began.

Table 2.2 - The experiment design for the controlled-environment facility study

Exposure Duration	Exposure Pattern	
	Variable	Constant
30 minute	3[a]	3
90 minute	3	3

a represents 3 replicate measurements

2.2.12. Analytical Method

2.2.12.1. Method Detection Limit

The method detection limit (MDL) is defined as the minimum amount of a substance that can be measured and reported with a 99% confidence that the analyte concentration is greater than zero. This was determined following the EPA guidelines published in the Chapter 1 of the Code of Federal Regulation 40 (EPA, 1995). Seven Perc external standards were prepared in methanol at about 3 times the amount of the estimated MDL, and analyzed as described below. The MDL was calculated as follows:

$$MDL = t_{(n-1, 0.99)} \times S$$

where:

$t_{(n-1, 0.99)}$ = the student's t value appropriate for a 99% confident level and a standard deviation estimate with n-1 degrees of freedom.

S = the standard deviation of the replicate analyses.

2.2.12.2. Thermal Desorption System

Perc collected in the charcoal sampling tube was desorbed using an automated thermal desorption unit (Model ATD 400, Perkin Elmer Inc.). Each sample was sequentially loaded, tested for leakage, and heated to 330 °C for 6 minutes to release Perc from the adsorbent into a stream of inert gas (zero grade Helium, Air Products and Chemicals, Inc.). The released chemicals were then concentrated on a quartz cold (kept at -30 °C) trap (1/16 inches ID, Perkin Elmer Inc.), containing 0.2 mg of Tenax TA®. The cold trap was finally heated rapidly (at 40 °C/second) to sweep the absorbed chemicals to the GC column via a heated (at 220 °C), fused silica capillary transfer line.

2.2.12.3. Gas Chromatography\Mass Spectrometer Conditions

Air and breath samples were analyzed by gas chromatography (Model 5890A, Hewlett Packard Co.) coupled with detection by mass spectrometry (Model 5970 series Mass Selective Detector, Hewlett Packard Co.). A 60-meter capillary column (DB-5) with a 1 μm of film thickness (JW Scientific Inc.) was used for GC separation, and zero grade helium (Air Products and Chemicals, Inc.) was used as a carrier gas. The GC temperature program was an initial temperature of 35 °C for 5.5 minutes, followed by a temperature ramp of 10.0 °C/minute to a temperature of 170 °C, which was held for 1.0 minutes, then a second temperature ramp of 50.0 °C/minute with a final temperature of 225 °C, which was held for 5.0 minutes. The response of the mass detector was checked each day by analyzing 50 ng of 2-bromofluorobenzene (BFB) adsorbed onto a sampling tube. The relative responses (normalized to m/e=95) of the seven main ion fragments of BFB were compared to the pre-established criteria. A QC chart for the BFB peak area was prepared, and the response for each day were recorded. A tune checking program was run when the BFB peak area was beyond a ±20% of the average area or when the BFB response criteria were not met. The parameters of the mass detector were sometimes adjusted to optimize the instrument's performance. The ion source of the detector was cleaned when the BFB response was not satisfactory. An autotune check program and BFB tune check were run after any major changes to the system.

2.2.12.4. External Standard Solutions and Standard Curve

Perc external standard solutions, ranged from 20 to 1000 ng/μl, were prepared for quantitation. The calibration solutions were made by dilution of HPLC-grade

Tetrachloroethylene (Aldrich Chemical Co.) with methanol (A.C.S., Fisher Scientific), and stored at 5 °C in 5-ml glass vials capped with mininert valves. These calibration standards were transferred to clean sampling tubes using a flash evaporation system (see below). One micro liter of each standard solution was injected into a 125-ml glass gas sampling bulb (Supelco Inc.), maintained between 220 and 240 °C using heating tape. One end of the sampling bulb was connected to a nitrogen gas stream (UHP grade, Air Products and Chemicals) maintained at a flow rate of 150 ml/minute which transferred the injected standards to the sampling tube within 5 minutes. The sampling tube was connected to the second end of the sampling bulb with a quick release Cajon connector. The sampling tubes spiked with external standards were analyzed using the same method as the samples. The external standard curve was calculated from at least 6 different concentrations using the linear regression technique. A correlation coefficient greater than 0.995 was required for an acceptable calibration curve.

2.2.12.5. Identification and Quantification of Perc

Perc was identified based on its chromatographic retention time and confirmed with the compound-specific mass ion fragment pattern ratios. The retention time of Perc was approximately 19.4 minutes for the specified GC conditions, and the four main ion fragments examined were 166, 164, 129 and 131 amu. Perc were quantified in the samples by comparing the peak areas of the target ion (m/e=166) fragment to the response of Perc in external standards calculated using a linear regression equation, as described in the previous section.

2.2.13. Quality Assurance/Control:

Quality control/assurance in the sample collection and analysis was an integral part of the current study as described in the following section.

2.2.13.1. Sample Quality Assurance

The breath collection system was thoroughly cleaned and disinfected before and after each use to minimize the chance of contamination. The sampling pumps were calibrated at the beginning and end of each sampling day to confirm a constant flow rate. Measurements were made to evaluate the precision and accuracy of the data. The precision of the method was estimated from the analysis of daily duplicate samples. One air and one breath duplicate samples were collected for each set of data. The accuracy was assessed by analyzing samples spiked with Perc at amounts similar to that collected in breath and air samples. Two blank sampling tubes for each data set were analyzed to determine whether the tube or the analytical system were contaminated. One background breath sample was collected each sampling day before the exposure began to ensure a sufficiently low level. The Perc levels inside the CEF were monitored on-site continuously by a total organic carbon analyzer and periodically by a GC/FID. These results were compared with those obtained from the charcoal tubes/GC-MS analysis to ensure the desired exposure scenarios were actually achieved

2.2.13.2. Analytic Quality Assurance

The status of the GC/MS system was check daily before use to ensure the response of the instrument was within a pre-established criteria. Perc external standards were analyzed

before the analysis of samples and after every 10th sample to verify that the instrument's response was stable to within an ±20%.

2.2.14. Statistical Analysis

2.2.14.1. Calculation of The Absorbed or Internal Dose

The absorbed dose was calculated from the difference between the area under the curve of inhaled air (AUC_A) and that of alveolar breath concentration (AUC_B) during the entire exposure period. The AUC_A and AUC_B for field study were calculated, assuming a linear change between two adjacent data points. The difference between AUC_A and AUC_B was multiplied by the subject's average alveolar ventilation rate (5.6 l/minute) to determine the amount of Perc absorbed through the lung, *i.e.*, internal dose.

2.2.14.2. Calculation of The Amount of Perc Expired

The amount of Perc expired after exposure which was calculated from the AUC_B, using the trapezoidal rule within Sigmaplot Scientific Graph System (Jandel Scientific Corp.). The resulting areas, expressed as [($\mu g/m^3$) × minute], were multiplied by the subject's average alveolar ventilation rate (5.6 l/min.) to obtain the total amount expired.

2.2.14.3. Calculation of The Elimination Half-lives

The Perc in the postexposure breath was empirically modeled, assuming a tri-phase exponential decay, using the SimuSolv® program (Dow Chemical Co.). The mathematical equation defining the three compartmental decay process is shown in Equation 2-1. The six parameters in the equation were obtained through the program's

statistical optimization process, which utilizes the principle of maximum log likelihood function (section 2.2.14.5) to maximize the fit of the equation to the experimental data.

$$Y = A \times e^{(-Bt)} + C \times e^{(-Dt)} + E \times e^{(-Ft)} \quad \text{----------Equation 2}$$

where

Y	=	Breath concentration in mg/m^3
A, C, E	=	Amplitude of 1st, 2nd and 3rd exponential, respectively
B, D, F	=	Rate constant of 1st, 2nd and 3rd exponential, respectively
e	=	Natural antilogarithm
t	=	Elapsed time from the end of exposure

The values of rate constant, *i.e.*, B, D and F, were used to determine the half lives of Perc in each corresponding compartment, using equation 2-2.

$$t_{\frac{1}{2},i} = \frac{Ln\ 2}{R_i} = \frac{0.693}{R_i} \quad \text{---------- Equation 2-2}$$

Where

$t_{1/2,i}$	=	Elimination half lives of the ith compartment
Ln	=	Natural logarithm
R_i	=	Rate constant of the ith compartment

2.2.14.4. Test Statistics

The student's *t* statistic and analysis of variance (ANOVA) were used to test the significance of the differences in kinetic variables such as percent absorption. The α level was set at 0.05.

2.2.15. PBPK Model Evaluation

2.2.15.1. Model Selection

The human PBPK model formulation for Perc, described by Rao and Brown (1993), was used to code the computer model evaluated by the exhaled breath measurements. It is a six-compartment model which includes the liver, fat, rapidly-perfused tissues, slowly-perfused tissues, brain and skin. These physiologically realistic compartments are connected by arterial and venous blood flow pathways. The liver was assumed to be the only organ that metabolizes Perc and follows a saturable pathway, known as the Michaelis-Menten kinetics. The model was originally used to assess the potential neurological effects from the bathing and showering using water from a private well contaminated with Perc, and was evaluated using literature data from various controlled human exposure studies. The potential dermal absorption of vapor phase Perc was not addressed in the current study because neither the air/skin partition coefficient nor the exposed skin area is expected to significantly contribute to the overall uptake by the body. The model did not consider the potential dilution of alveolar breath by inhaled air, rather literature breath concentrations were assumed to be solely alveolar air concentrations.

2.2.15.2. The Mathematical Equations Defining The PBPK Model

The mass balance differential and algebraic equations defining the PBPK model are as following:

1. For the Brain compartment

Amount changes with respect to time or rate of accumulation (mg/min):

$$\delta A_B/\delta t = Q_B \times (C_{ART} - C_{VB})$$

Concentration of Brain (mg/L):

$$C_B = A_B/V_B$$

Concentration of venous blood from brain:

$$C_{VB} = C_B/PBR$$

2. For the Rapidly-Perfused Tissue compartment

Amount changes with respect to time or rate of accumulation (mg/min):

$$\delta A_R/\delta t = Q_R \times (C_{ART} - C_{VR})$$

Concentration of Rapidly-Perfused Tissue (mg/l):

$$C_R = A_R/V_R$$

Concentration of venous blood from Rapidly-Perfused Tissue (mg/l):

$$C_{VR} = C_R/PR$$

3. For the Slowly-Perfused Tissue compartment

Amount changes with respect to time or rate of accumulation (mg/min):

$$\delta A_S/\delta t = Q_S \times (C_{ART} - C_{VS})$$

Concentration of Slowly-Perfused Tissue (mg/L):

$$C_S = A_S/V_S$$

Concentration of venous blood from Slowly-Perfused Tissue (mg/l) :

$$C_{VS} = C_S/PS$$

4. For the Fat compartment

Amount changes with respect to time or the rate of accumulation (mg/min):

$$\delta A_F\delta t = Q_F \times (C_{ART} - C_{VF})$$

Concentration of Fat (mg/L):

$$C_F = A_F/V_F$$

Concentration of venous blood from Fat (mg/l):

$$C_{VF} = C_F/PF$$

5. For the Skin compartment

Amount changes with respect to time from perfusin (mg/min):

$$\delta A_{SK}/\delta t = Q_{SK} \times (C_{ART} - C_{VSK})$$

Concentration of Skin (mg/L):

$$C_{SK} = A_{SK}/V_{SK}$$

Concentration of venous blood from Skin (mg/l):

$$C_{VSK} = C_{SK}/PSK$$

6. For the Liver compartment

Amount changes with respect to time or rate of accumulation (mg/min):

$$\delta A_L/\delta t = Q_L \times (C_{ART} - C_{VL}) - (Vmax \times C_{VL})/(Km + C_{VL})$$

Concentration of Liver (mg/L):

$$C_L = A_L/V_L$$

Concentration of venous blood from the Liver:

$$C_{VL} = C_L/PL$$

7. The concentration of Perc in the Arterial Blood

$$C_{ART} = (Q_C \times C_{VEN} + Q_{ALV} \times C_{INH}) / [Q_C + (Q_{ALV}/PB)]$$

8. The concentration of Perc in the Venous Blood

$$C_{VEN} = (Q_L \times C_{VL} + Q_F \times C_{VF} + Q_R \times C_{VR} + Q_S \times C_{VS} + Q_B \times C_{VB} + Q_{SK} \times C_{VSK})/Q_C$$

9. The concentration of Perc in the Measured Breath

$$C_{BRE} = C_{ART}/PB$$

where

Q_B, Q_R, Q_S, Q_F, Q_{SK}, Q_L and Q_C = the blood flow rates of brain, rapidly-perfused tissues, slowly-perfused tissues, fat, skin, liver and whole body, respectively.

V_B, V_R, V_S, V_F, V_{SK}, and V_L = the tissue volumes of brain, rapidly-perfused tissues, slowly-perfused tissues, fat, skin, liver, respectively.

PBR, PR, PS, PF, PSK and PL = brain, rapidly-perfused tissues, slowly-perfused tissues, fat, skin, liver to blood partition coefficient, respectively.

PB = blood to air partition coefficient.

Q_{ALV} = alveolar ventilation rate (l/min).

C_{INH} = concentration of the inhaled air (mg/l).

Vmax = Michaelis-Menten maximum metabolic rate (mg/min).

Km = Michaelis constant (mg/l).

The model parameter values used in this evaluation are provided in Appendix II.

2.2.15.3. Data and Model Modification

Both air and alveolar breath in this study were collected as average, short-term samples of 30 to 120 seconds, rather than as instantaneous grab samples. The geometric mean of the sampling duration was added to the starting time at which samples were collected to estimate the actual time points that these breath samples represent, since the changes of breath concentration after exposure follow an exponential function. Some of the air in the deadspace of the respiratory tract is inevitably collected with alveolar air under the current sampling protocol. Therefore, an approximate 5% contribution from deadspace (inhaled) air to the measured breath was incorporated into the model. All data were

logarithmically transformed to minimize the differences in analytical uncertainties across different concentration ranges.

2.2.15.4. Simulation Language

The model used in the present study was coded and the simulations were performed under the UNIX operating system using the SimuSolv® program which is a multifunctional computational package. The program consists of two languages: Advanced Continuous Simulation Language (ACSL), used to code a model definition program containing the mathematical model under study; and a runtime language, used to invoke and implement the program and to perform statistical optimization routines for model parameters.

2.2.15.5. Model Evaluation Criteria and Statistics

The criterion used to determine the agreement ("goodness of fit") of the model predictions to the breath measurements was the "Mean Absolute Percent Error (MAPE)" which is defined as follows:

$$\text{MAPE} = \frac{\sum_{i=1}^{k} 100 \times |(P_i - O_i) / O_i|}{N}$$

Where

P_i = Model predicted values
O_i = Observed values
N = Numbers of data point within specified time frames

This expression represents the average deviation (error) of model predictions from observed values, independent of whether the predictions are over- or under-estimations. A

non-parametric statistic, sign test (Daniel, 1991), was used to test the significance of the differences in MAPEs for all six data sets between two different types of exposure inputs as well as before and after an intervention, such as optimization of parameters. The α level was set at 0.1.

2.2.15.6. The Algorithm For Estimating Model Parameters

The Simusolv program utilizes the principle of likelihood function (log likelihood function for mathematical convenience) in parameters estimation. The log likelihood function are written as follows for the univariate case using a normal probability density function:

$$LIF = \log(LF) = -n \times \log(\sigma\sqrt{2\pi}) - \sum_{r=1}^{n}\left(\frac{(Zr-\mu)^2}{2\sigma^2}\right)$$

where

n = total number of measurements
σ = true standard deviation of the measurements
Zr = measured value for the ith measurement
μ = true value for the ith measurement

This specific function is based on the assumptions that the errors in the measurements are independent and normally distributed. By maximizing the log likelihood function, or the joint probability density function, the parameter values obtained are most likely to be correct.

2.2.15.7. Simulation Procedure

The PBPK simulations for the field data were first performed utilizing the same parameters as provided by Rao & Brown (1993). Two exposure inputs into the model were examined: the measured integrated air concentration (referred to as constant exposure) and a combination of the linear step functions constructed from all the short term air samples measured periodically during exposure (referred to as variable exposure). The simulations were also done without correcting for the method-specific inhaled air contribution in order to examine its influences on the model predictions. Four of the model parameters [the blood/air (PB), liver/blood (PL), rapid perfused tissue/blood (PR) and slow perfused tissue/blood (PS) partition coefficients] were calibrated using the data from a single experiment (p0301) to obtain the best fit for the individual under consideration through a statistical parameter optimization process. The optimized parameters were then used to predict breath concentration for all other exposures, and the agreement between the model predictions and the measured breath concentrations, expressed via MAPE, for the two types of exposure input were compared.

To interpret the results of CEF study, a sensitivity analysis was first performed for the seven key parameters [PB, PR, PL, PS, fat/blood partition coefficient (PF), Michaelis-Menten maximum metabolism rate (Vmax) and Michaelis constant (Km)] to examine their effects on the model output. Four parameters (PB, PS, Vmax and Km) were statistically optimized for the pooled data, which consists of all six experiments collected from the constant exposure settings. These optimized parameters values were then used in all data sets, and the differences of the model fit for the two types of exposure input as

well as some other outputs, such as the concentration-time profiles within the brain, were compared.

3. RESULTS

3.1. Quality Assurance

3.1.1. Sample Quality Assurance

The method detection limit (MDL) for Perc was calculated to be 14.9 ng/tube. This corresponds to a MDL for typical air samples of 0.4 liters of 38 $\mu g/m^3$ and the breath samples of 1.5 liters of 10 $\mu g/m^3$. Different sampling rates and sampling durations alter the overall MDL. One breath and one air sample for every experiment were collected as duplicates. An overall relative standard deviation (RSD) of 9.60% and 10.1% for the air and breath samples were obtained, respectively. One background breath sample was collected for each experiment to determine the Perc residual body burden of the subject. The mean background Perc concentrations in the breath were 14.7 and 20.7 $\mu g/m^3$ for the field and CEF study, respectively. Two sampling tubes were analyzed as the field blank for each experiment. Perc was below the MDL in all of the blank sampling tubes.

The mean collection efficiency for the breath system (section 2.2.6) was determined to be 93% and 103% for Perc concentrations of 122 $\mu g/m^3$ and 4868 $\mu g/m^3$, respectively. No breakthrough (section 2.2.9) of Perc at 2820, 5640 and 11280 $\mu g/m^3$ was identified based on the amount of Perc present in the second of the two sequential sampling tubes being identical to the amount present on the blank tubes which are below MDL.

The results from the two contamination tests (section 2.2.7) showed that no contamination of breath samples from the surrounding air were found at exposure level of 10220 $\mu g/m^3$. The amounts collected in all of the samples were below the MDL. The

second test also found no contamination of breath samples from absorption/desorption processes of previous samples could be identified at exposure level of 10220 $\mu g/m^3$ based on the finding that samples collected from the simulated breath source were identical to the sample blanks, which were directly collected from the simulated breath source.

The potential dilution of breath concentrations from the reserve air in the lungs were considered minimal since all breath samples were collected at rest and under normal breathing, therefore, the chances of using large volume of reserve air was small.

3.1.1. Analytical Quality Assurance

The stability of the instrument's response was checked by analyzing Perc external standards each day when samples were analyzed with a predetermined criteria of ±20% required for samples to be valid.

3.2. Field Study

3.2.1. Perc Air Concentrations inside Laundromats

Breathing-zone short-term (30 or 60 seconds) air samples were taken, near the conventional wash/dry machines, from three laundromats that also contain dry-cleaning operations to assess the possible exposures. The results (Table 3.1) showed that the Perc air concentrations inside selected laundromats ranged from 0.11 to 7.84 mg/m^3.

3.2.2. Perc Concentration in The Exhaled Breath During Exposure

The breath samples during the exposures were collected concurrently with air samples to examine the body's responses (pulmonary absorption) to the fluctuating exposure conditions. The air and corresponding breath levels during exposures from three representative exposure days are shown in Figure 3.1, 3.2 and 3.3.

3.2.3. Percentage of Perc Absorbed

The percentage of Perc that was absorbed between any two sequential samples was calculated from the difference of AUC_A and AUC_B between adjacent data points, divided by AUC_A. The percentage of Perc absorbed for three experiments are shown in Table 3.2. The percent absorption at the beginning of the exposure was consistently higher than during the later part of the exposure. The mean percent absorption for the experiments p1208, p1220 and p1020 were 0.69±0.03, 0.66±0.07 and 0.29±0.42, respectively. The overall mean absorption coefficients for 30, 45 and 60 minute exposures were 0.69, 0.61 and 0.60, respectively (Table 3.3).

3.2.4. Amount of Perc Absorbed or Internal Dose

The present study was designed to provide an estimate of the internal dose by simultaneously collecting air and breath samples during the exposure. A linear response (change) between two adjacent data points was assumed when calculating the external and internal dose. The AUC_A is the external (or potential) dose, while the AUC_B is what was expired, *i.e.*, not absorbed. Therefore, the summation of the difference between air and breath concentration during the entire exposure period multiplied by the

estimated subject's alveolar ventilation rate, 5.6 l/min, represents the dose absorbed or internal dose. The calculated internal doses are shown in Table 3.4.

3.2.5. The Relationship Between Exposure and Internal Dose

Exposure is commonly defined as exposure concentration times exposure duration. A strong correlation, with the coefficient of 0.99 ($p<.05$), between the calculated internal dose and the total exposures calculated from the area under the short-term air measurements was found (Fig. 3.4.A). A correlation coefficient ($r^2=0.97$, $p<.05$) between the calculated internal dose and the total exposures calculated from the product of the average exposure air concentration was also found (Fig. 3.4.b).

3.2.6. The Relationship Between Estimated Internal Dose and The Postexposure Breath Concentration

A strong correlation ($r^2=.98$, $p<.05$) was found between internal dose and 6-minute postexposure breath concentrations (Fig. 3.5). However, statistically significant ($p<.05$) difference in slopes was also identified for the three different exposure durations (Fig. 3.6).

3.2.7. The Elimination of Perc after Exposure

Figure 3.7 shows postexposure breath elimination curves of three experiments with different exposure durations and absorbed doses. The breath concentrations measured right after the cessation of exposure are greatly influenced by the air concentration right before the exposure ceased, followed by breath concentrations decreasing gradually.

3.2.8. Amount and Percentage of Perc Expired after Exposure

The total amount of Perc expired for up to 6- and 400-minute post exposure were obtained from the product of the average ventilation rate and the AUC_B. The percent dose expired was calculated by dividing the amount expired by the internal dose. These results are listed in Table 3.4. The means of the amount and percent dose expired up to 6 minutes postexposure among the three different exposure durations were statistically significantly different ($p<.05$), as shown in Figure 3.8 for the percent dose expired.

3.3. Controlled-Environment Facility Study

3.3.1. Percent Absorption and Dose Index

The percentage of Perc absorbed during constant exposure were calculated from the difference of AUC_A and AUC_B, divided by AUC_A. The internal dose are calculated using the method described in the section 2.2.13.1. The internal doses are normalized to total exposure to obtain dose index. These results are listed in Table 3.5.

3.3.2. The Elimination of Perc After Exposure

Tri-phasic exponential decay was found for the postexposure exhaled breath, same as that found in the field study. The means and standard deviation of the postexposure breath, normalized total exposure (average concentration times duration), for the three replicates done at four different exposure settings are shown in Figure 3.9, 3.10 and 3.11.

3.3.3. Empirical Modeling of Postexposure Breath Concentrations

Perc in the postexposure breaths were mathematically modeled, assuming tri-phasic decays. The calculated values of the six coefficients and their standard deviations for the tri-exponential decay equation (Equation 3-1) are shown in Table 3.6. The values of A, C and E define the amplitude of 1st, 2nd and 3rd exponential, respectively, which are dependent on the exposure concentration. Therefore, all of the coefficients were determined after normalizing the postexposure breaths to the average exposure air concentration. The values of B, D and F, the rate constant of the first, second and third exponential, respectively, determine the residence time of Perc in the body, and were used to calculate the half-lives for each of the corresponding body compartment.

3.3.4. Elimination Half-lives

The elimination half-lives of Perc were calculated using the Equation 3-2 and the calculated coefficients (Table 3.6), and are shown in Table 3.7. The overall mean half-lives for the first, second and third compartment are 3.68, 31.3 and 229 minutes, respectively. The means of the first half life for the 30-minute constant exposures are statistically significantly longer ($p<.05$) than that of 90-minute constant exposures. The mean of the half lives for the 30-minute variable exposures were not statistically significantly different ($p<.05$) from their constant exposure counterparts due to a larger within-group variations.

3.3.5. The Area under The Postexposure Breath Curve

The AUC_B for the three time intervals, *i.e.*, 0-12, 0-73 and 0-373 minutes postexposure are shown in Table 3.8. The means of AUC_B for the three time intervals for the 30-minute constant exposures are statistically significantly lower ($p<.05$) than that of 90-minute constant exposures. The means of AUC_B for the three time intervals between 30-minute constant and 30-minute variable exposure groups were not statistically significantly different ($p>.05$). For the 90-minute exposures, the means of AUC_B for the three time intervals between constant and variable exposure groups are also not statistically significantly different ($p>.05$).

3.4. PBPK Model Evaluation

3.4.1. Evaluation of A PBPK Model Using Field Data

3.4.1.1. Total Exposure Calculation

The total exposure for each experiment was obtained either by multiplying the average air concentration and the exposure duration, or by calculating the area under the curve of the short term air concentrations during exposure, assuming a linear concentration change between adjacent data points. The ratio between the two exposure calculations, *i.e.*, variable to average (V/A) exposure, is therefore an indicator of how well the exposures, extrapolated from the temporal air measurements, represent the actual total exposure. Differences could exist because of the rapidly fluctuating air concentration within the dry cleaning establishments. A ratio greater than one indicates that the total exposure was over-estimated when using the area under the temporal air concentration curve, while a value less than one indicates an underestimation. The variable to constant

exposure ratios are listed in Table 3.9. Six experiments, which have V/A ratios less than 1.35 and have distinctive exponential decay pattern in the postexposure breath (visually determined), were used for the current evaluation.

3.4.1.2. Initial Evaluation

3.4.1.2.1. The Model Predictions with Constant Versus Variable Exposure

The goodness of fit of model predictions to each set of breath measurements was first determined using each complete data set, *i.e.*, breath data collected during and after the exposure. It was found that the averages of the goodness of fit ranged from 3.85 to 5.24 for the constant exposure input and from 2.84 to 3.99 for the variable exposure input (Table 3.9, Column 7 & 8). The fits of the model for during and after exposure are discussed in the following section.

3.4.1.2.2. Model Predictions During or After Exposure

An example of air and breath concentrations measured during exposure, along with the model predictions, are presented in Figure 3.12 and 3.13.

The mean MAPE for model predictions for the postexposure breath data was 2.53 (ranged from 1.94 to 2.91) for constant exposure, and was 3.14 (ranged from 1.87 to 4.00) for variable exposure input (Table 3.9, Column 6).

3.4.1.2.3. Model Modification

The simulation results using the original parameters but without correcting for the inhaled air contribution are shown in Table 3.10. The degradation in the goodness of fit