Benzene Tier 1 VCCEP Submission

Appendix C

PBPK Modeling for Benzene, Interpretation of Benzene Human Biomonitoring Data, and Modeling of Benzene Concentrations in Human Milk

March 30, 2006

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1.0 Description of Project

This project has several purposes including the review of existing physiologically-based pharmacokinetic (PBPK) models for benzene, the use of PBPK models to facilitate the interpretation of the human biomonitoring data for benzene and, finally, the estimation of human blood and milk concentrations that could correlate with measured or modeled exposure data for benzene.

2.0 Literature Review for Human Pharmacokinetics and Biomonitoring of Benzene

2.1 Literature Review of Available PBPK Models

The assessment of information regarding human data on inhalation pharmacokinetics of benzene was performed using TOXLINE via National Library of Medicine, occupational health and biological monitoring literature as well as the contractor's knowledge of published work in this area. The most widely acknowledged and cited work on human PBPK modeling of benzene is that of Travis et al. (1990). The contractor has used this model previously for developing risk assessments, in a publication that was recently selected as the best paper in Toxicological Sciences, by the Society of Toxicology. The Travis et al. (1990) publication represents original work reporting the construction of a PBPK model for benzene in humans exposed by inhalation. The chemical-specific parameters of this PBPK model are given below:

Benzene partition coefficients

Blood:air	7.4
Liver:air	11.0
Fat:air	406.0
Bone marrow:air	120.0
Richly perfused tissues:air	11.0
Poorly perfused tissues:air	15.0

Benzene metabolism constants

Liver Vmax (mg/hr)	29.04
Liver Km (mg/L)	0.35
Bone marrow Vmax (mg/hr)	1.16
Bone marrow Km (mg/L)	0.35

Two other efforts of human PBPK modeling that are worth noting are those by Sherwood et al. (See Sherwood and Sinclair 1999; Sinclair et al. 1999), and by Bois et al. (See Watanabe et al. 1994; Bois et al. 1996). In the Sherwood et al. papers, the parameters of the model are not specified and as such the validity of the modeling approach is difficult to evaluate. Regarding Bois et al.'s work, their publications report the application of simple Monte Carlo and Markov-Chain Monte Carlo approaches for simulating the kinetics of benzene in populations. Given the complexity of these approaches, current state of Markov-Cain Monte

Carlo approach in risk assessment arena and the questionable nature of the parameter specification in these studies (which was based either on incomplete knowledge or on presumed distributions), these models were not further considered for the VCCEP PBPK project.

In addition to the PBPK models identified in the literature review, several controlled human volunteer studies on benzene (see Table 2.1) were identified in which benzene blood concentrations were measured following inhalation exposures. These data are potentially useful for two purposes: (1) for validating human PBPK models, and (2) to examine the relationship between blood concentration and exposure concentration in exposed individuals.

Table 2.1 Controlled human volunteer studies of Benzene pharmacokinetics

Exposure Concentration	Exposure -Sampling Duration	Reference
10 & 1.7 ppm	240- 1400 min	Pekari et al. (1992)
0.08 mg/L (25 ppm)	120 – 300 min	Sato et al. (1975)
0.08 mg/L (25 ppm)	120 – 300 min	Sato et al. (1974)
0.08 mg/L (25 ppm)	480 – 2040 min	Teisinger and Fiserova -Bergerova(1955)
0.313 mg/L (100 ppm)	90 – 390 min	Srbova et al. (1950)

2.2 Literature Review of Available Human Biomonitoring Data for Benzene

Using a similar approach to the assessment of available benzene PBPK models, a search for information regarding human biomonitoring data on benzene (limited to blood concentration measurements) was performed using TOXLINE via National Library of Medicine, occupational health and biological monitoring literature as well as the contractor's knowledge of published work in this area. A careful evaluation of all of the literature was then done in order to select the relevant data for interpretation of human biomonitoring data.

Table 2.2 summarizes, in order of publication date, the existing data on benzene blood concentrations reported in human populations. Whereas some of the publications report data on environmental concentrations as well, most of them only report the biomonitoring data without any reference to environmental concentrations.

Table 2.2 Human biomonitoring data for Benzene

Authors and year	Benzene Conc (ng/L)	Remarks
Ashley et al. (1994) Needham et al. (1995)	Mean: 130 Median: 61 95th Per: 480 5th Per: ND	• Samples from NHANES III • Reference group of individuals in US
Perbellini et al. (1988)	Mean: 184 Median: 166	Non-smokers Sampled in Italy

	Range: 49 – 438	
Perbellini et al. (1988)	Mean: 453 Median: 458 Range: 92-1136	Smokers Sampled in Italy
Brugnone et al. (1998, 1999)	Mean: 123 Median: 110 Range: 15-462	Nonsmokers Sampled in Italy
Brugnone et al. (1998, 1999)	Mean: 264 Median: 210 Range: 28-940	Smokers Sampled in Italy
Fustinoni et al. (1996)	Median: 241 Median: 365	Non-smokers Smokers
Kok and Ong (1994)	Range: 50-219 Range: 81-629	Non-smokers Smokers
Brugnone et al. (1992)	Mean: 381 Median: 291 Range: 7 – 2241 95^{th} per: 901	Smokers Sampled in Italy
Brugnone et al. (1992)	Mean: 205 Median: 163 Range: 7-924 95^{th} per: 514	Nonsmokers Sampled in Italy
Angerer et al. (1991)	Mean: 176 (nonsmokers) Range: 80-300 (nonsmokers) Mena: 211 (smokers) Range: 130 – 430 (smokers)	Sampled in Germany
Mannino et al. (1995)	Median: 290 Range: 120 - 1970	People exposed to gas fumes and auto exhaust in Albany, NY.
Goergens et al. (1991)	Mean: 239 Median: 216 Range: 83-571	Nonsmokers
Goergens et al. (1991)	Mean: 163 Median: 144 Range: 52-278	Nonsmokers
Brugnone et al. (1989)	Mean: 158 (nonsmokers) Median: 105 (nonsmokers) Mean: 256 (smokers) Median: 239 (smokers)	Blood donors, in Italy
Hajimiragha et al. (1989)	Mean: 190 (nonsmokers) Median: 218 (idem) Range: 112-455 (idem) Mean: 547 (smokers) Median: 493 (smokers) Range: 287-947 (smokers)	Sampled in Germany (Dusseldorf and surrounding areas)

After considering the above studies, the biomonitoring data from the CDC/ATSDR studies by Ashley et al. (1994) and Mannino et al. (1995) will be used for dose reconstruction (see Appendix C, Section 3.3). Only the U.S. biomonitoring studies were considered due to concerns that foreign studies are frequently conducted under local conditions (pollution levels, local allowable exposure concentrations (norms), and personal activity/habits) that are very different from the U.S.

3.0 PBPK Modeling and Interpretation

3.1 Reconstruction of Human PBPK models for Benzene

This section discusses the reconstruction of the human PBPK models for benzene (into Microsoft EXCEL® and Advanced Continuous Simulation Language (ACSL®)) and the successful reproduction of previously published simulations of benzene kinetics in humans.

The PBPK model used in this study describes the organism as a set of tissue compartments interconnected by systemic circulation and a gas-exchange lung (Figure 3.1). The compartments refer to liver, slowly perfused tissues, richly perfused tissues, adipose tissue (fat), and the bone marrow. The rate of change in the amount of benzene in each non-metabolizing tissue compartment is described as follows (Note: all abbreviations are defined in the legend for Figure 3.2):

$$V_t \frac{dC_t}{dt} = Q_t(C_a - C_{vt}) \qquad (1)$$

The rate of change in benzene concentration in liver is described as follows:

$$V_t \frac{dC_t}{dt} = [Q_t(C_a - C_{vt})] - \frac{dA_{met}}{dt} - \frac{dA_{bm}}{dt} \qquad (2)$$

In lay terms, the above equation signifies:

Rate of change in the amount of the chemical in the tissue = (blood flow x arteriovenous concentration difference) – rate of loss due to metabolism

The rate of the amount metabolized was described as a saturable process as follows:

$$\frac{dA_{met}}{dt} = \frac{V_{max} C_{vt}}{K_m + C_{vt}} \qquad (3)$$

In the benzene PBPK model, the mixed venous blood concentration has been calculated as follows:

$$C_v = \frac{\sum_t^n Q_t C_{vt}}{Q_c} \qquad (4)$$

The above equation represents the steady-state solution of the mass-balance differential equation for venous blood:

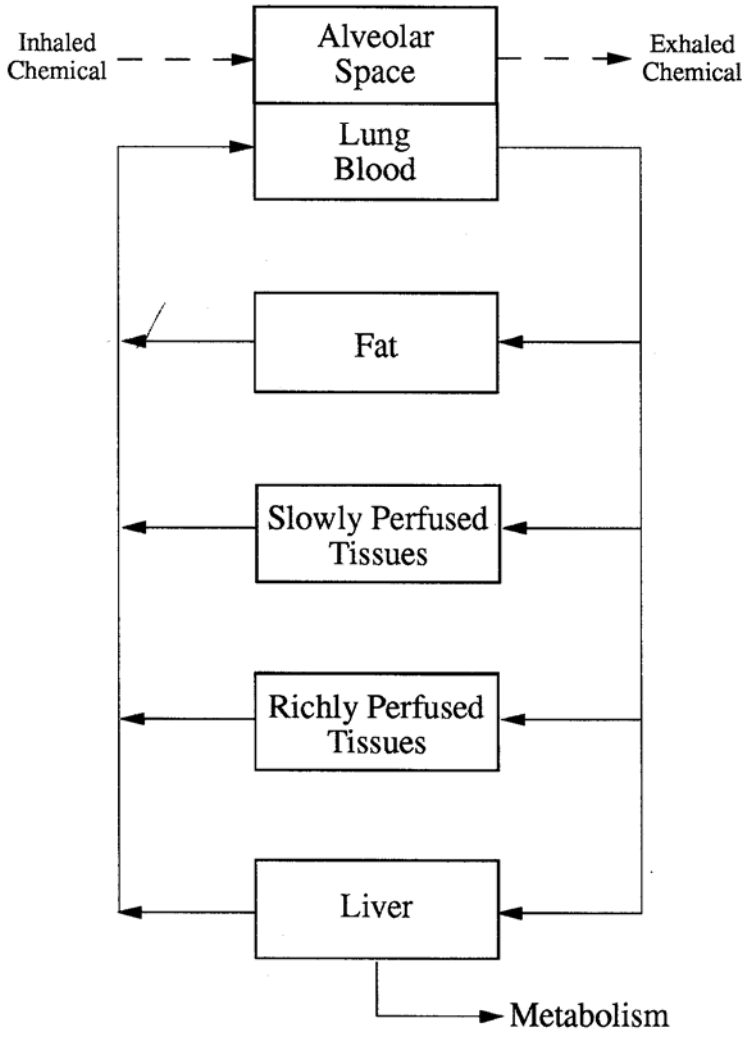
$$\left[V_b\left(dC_b / dt\right) = \sum_{t}^{n} Q_t C_{vt} - C_v Q_c \right] \quad (5)$$

The arterial blood concentration of benzene is computed with the following equation:

$$C_a = \frac{Q_p C_{inh} + Q_c C_v}{Q_c + \left(\frac{Q_p}{P_b} \right)} \quad (6)$$

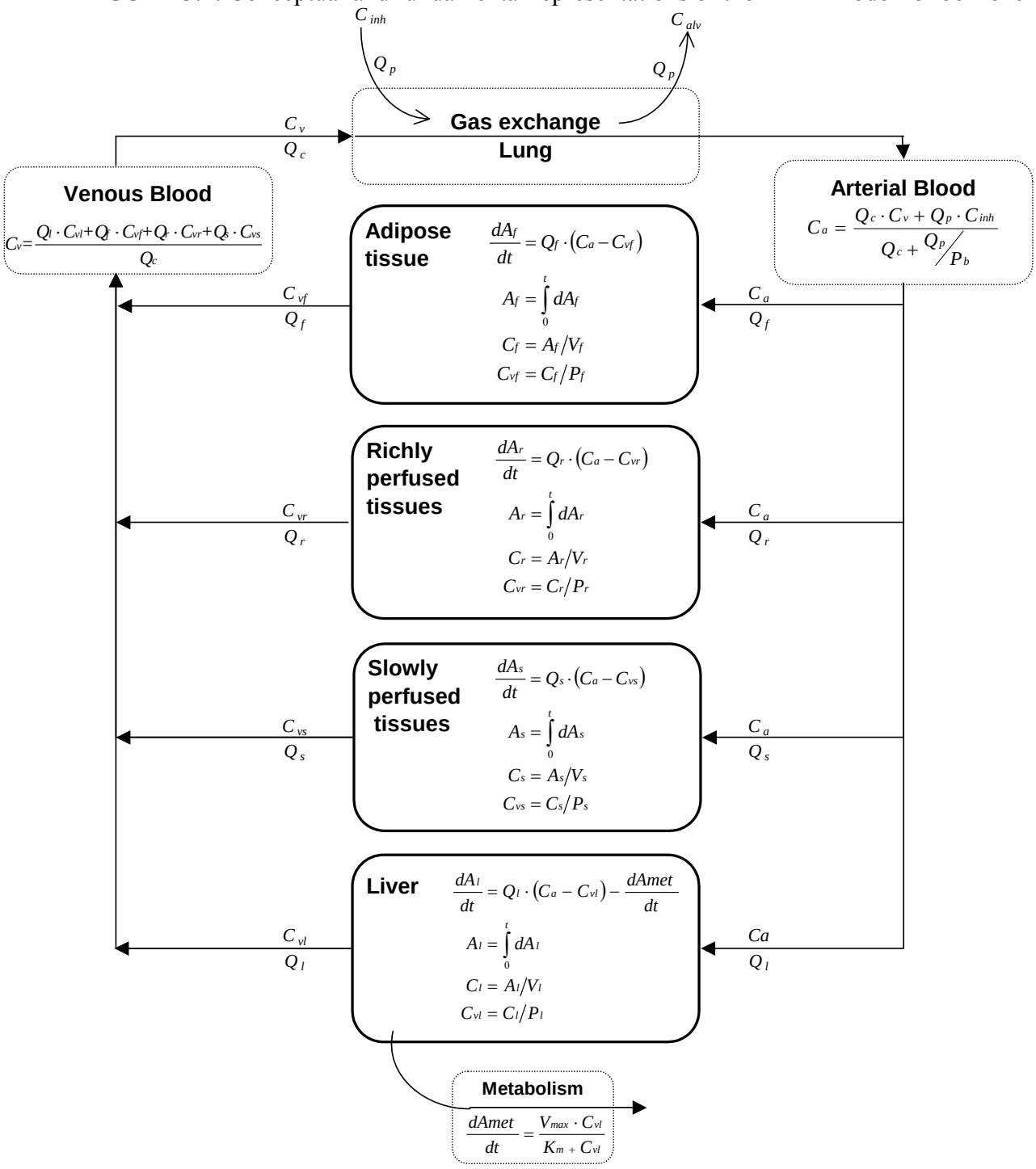
The benzene PBPK model comprises of the above equations, which are interconnected as shown in Figure 3.2.

FIGURE 3.1: Conceptual representation of the PBPK model for benzene.



Note: The bone marrow was separated out of the richly perfused tissues compartment for modeling benzene.

FIGURE 3.2: Conceptual and fundamental representations of the PBPK model for benzene



Legend: Cinh and Calv refer to inhaled and exhaled benzene concentrations. Cv and Ca refer to venous and arterial blood concentrations. Pb refers to blood:air partition coefficient. Qp and Qc refer to alveolar ventilation and cardiac output. Cv*i*, Vi, Pi, Ai and Q*i* refer to venous blood concentrations leaving tissue compartments, tissue volumes, tissue:blood partition coefficients, amount in tissues and blood flow to tissues (i.e., f: adipose tissue, s: slowly perfused tissues, r: richly perfused tissues, and l: liver). Vmax, Km and Amet refer to the maximal velocity of metabolism, Michaëlis affinity constant, and amount metabolized. dt refers to integration interaval. Please note that the bone marrow was separated out of the richly perfused tissues compartment for modeling benzene.

The numerical values of the parameters for the benzene human PBPK models are provided in Figure 3.3. These parameter values were used to solve the equations in EXCEL to generate the blood kinetics of benzene, as per the original paper (Travis et al. 1990). Using Euler algorithm for integration of differential equations, the PBPK model was solved in EXCEL spreadsheets. Accordingly, once (i) the numerical values of model parameters were provided, (ii) the equations in the first and subsequent rows of the spreadsheet entered, (iii) the time interval for integration specified, and (iv) the required number of cells chosen, the simulation begins.

The reconstructed PBPK model was used to reproduce the inhalation pharmacokinetics of benzene in humans, as per the original modeling paper. Accordingly, the PBPK model was used to simulate the blood kinetics in human volunteers exposed to 25 ppm benzene for 2 hours (original experimental data published in Travis et al. 1990 study were retrieved and used in the present study). A compact disc (CD) containing the human PBPK model codes for benzene written in Excel and ACSL was included in this project and is available upon request. These codes can be used to model the kinetics of benzene in humans using a defined inhalation exposure scenario.

In sum, these results show that the human PBPK models constructed in this study provide the same simulations as those obtained/reported by authors of the original modeling paper during the process of validation of the benzene model.

FIGURE 3.3: Values of parameters of the benzene PBPK model.

Human PBPK Model Parameters - Benzene

Tissue	Physiological		Physicochemical		Biochemical	
	Q(L/hr)	V(L)	Pi	Pb	Vmax	km
Body ©	300,00	70				
Lung (p)	372,00					
Liver (l)	75,00	1,82	1,49		29,0	0,35
Fat (f)	15,00	13,3	54,86			
Bone marrow (bm)	12,00	2,8	16,22		1,16	0,35
Richly ®	132,00	3,5	1,49			
Slowly (s)	66,00	40,6	2,03			
Blood (b)				9,5		

Exposure condition	
Cinh(ppm)	25
Cinh(mg/L)	0,079867076
Length(hr)	2
MW	78,11

Time function	
	hr
Integration interval(t)	0,005

FIGURE 3.4: Comparison of PBPK model simulations of venous blood concentrations of benzene with experimental data (symbols) obtained in humans exposed for 2 hr to 25 ppm.

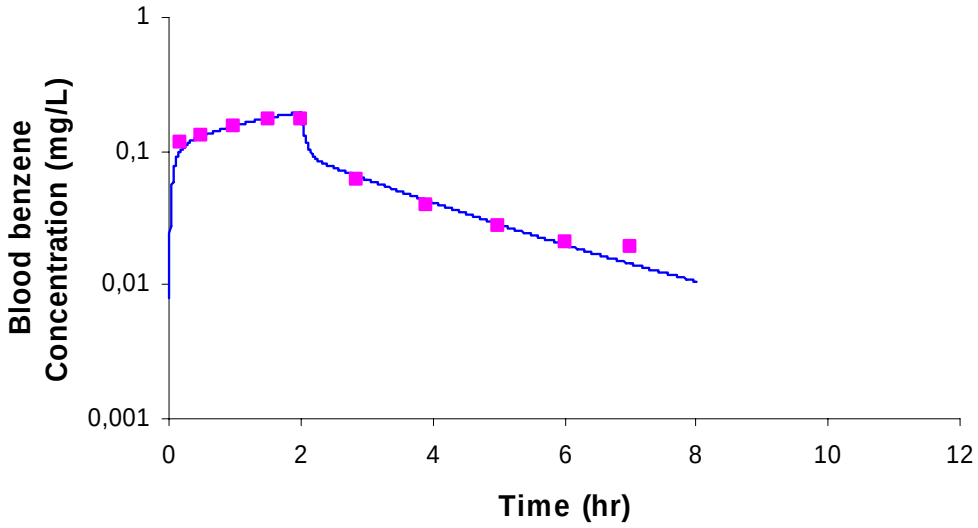
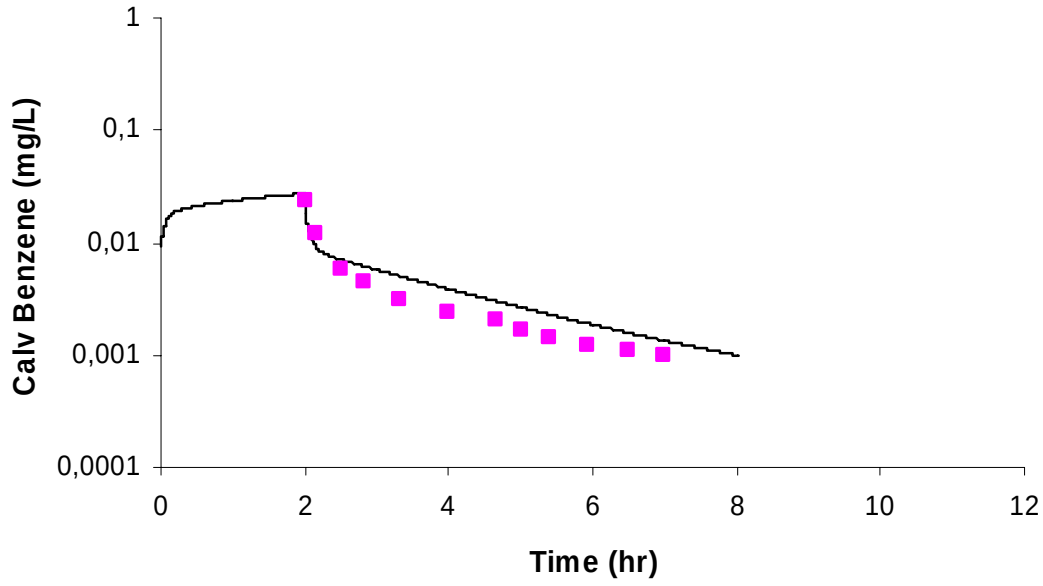


FIGURE 3.5: Comparison of PBPK model simulations of alveolar air concentrations of benzene with experimental data (symbols) obtained in humans exposed for 2 hr to 25 ppm.



3.2 Pharmacokinetic Relationship between Blood and Inhaled Air

In order to establish the relationship between the inhalation exposure concentration and blood concentration, both the duration of exposure and time of sampling should be known. However, for the environmental exposure situation, the blood concentrations of volatile chemicals such as benzene are likely to attain steady-state. The steady-state, in the present context, is defined as the situation during which the blood concentration does not change even though the exposure is continuing. During steady-state, the relationship between environmental concentration and blood concentration of benzene should be constant and is independent of time (of exposure or sampling). The relationships between the environmental and venous blood concentrations of benzene were established in the present study for exposure concentrations ranging from 0.00001 to 10 ppm. For these exposure concentrations, the resulting venous blood concentrations of benzene were calculated using the validated human PBPK model as well as using a steady-state algoriothm.

The steady-state algorithm for calculating arterial blood concentrations of volatile organic chemicals have been previously derived (Pelekis et al., 1997) and can be re-written to show that they give identical results. In the present analysis, the steady-state blood concentrations obtained with algorithms were compared with the simulations of full-fledged human PBPK model for benzene (Table 3.1). Both approaches give comparable results for exposure concentrations ranging from 0.00001 – 10 ppm.

The results reported in Table 3.1 facilitate an understanding of the quantitative relationship between the exposure concentration and venous blood concentration of benzene. The quantitative relationship can be better understood, in terms of mechanistic determinants, by examining the steady-state equation:

$$C_{vss} = \frac{Q_p \times C_i \,(1 - (QLC*E + Q_{bmc}*E_{bm}))}{(Q_p/P_b) + (QL \times E) + (Q_{bm}* E_{bm})} \qquad (7)$$

where C_{vss} = steady-state venous blood concentration (mg/L), Q_p = alveolar ventilation rate, C_i = inhaled or exposure concentration, P_b = blood:air partition coefficient, QLC = fraction of cardiac output flowing through the liver, E = hepatic extraction coefficient, Q_{bm} = blood flow rate to bone marrow, Q_{bmc} = fraction of cardiac output flowing through bone marrow, and E_{bm} = metabolic extraction ratio in bone marrow

The numerical values of the following parameters are required for establishing the relationship between blood concentration (C_{vss}) and inhaled concentration (C_i) of benzene, at steady-state: (1) Q_p, (2) P_b, (3) QL, (4) QLC, and (5) E.

All parameter values, except the extraction ratios, were obtained directly from the PBPK model. The E value was calculated as follows:

$$E = \frac{CL_{int}}{CL_{int} + QL}. \quad (8)$$

where CL_{int} = intrinsic clearance calculated as V_{max} divided by K_m for first order conditions, and QL = liver blood rate in humans.

For benzene, the E values for liver and bone marrow compartments were 0.525 and 0.216, respectively. Using these parameter values in the steady-state equation (Equation 7), the venous blood concentrations reported in Column 2 of Tables 3.1 were obtained whereas the data presented in Column 3 were obtained using the validated human PBPK model.

Table 3.1: Quantitative relationship between the steady-state venous blood concentration (C_v) and inhalation exposure concentration (C_{inh}) of benzene.

Exposure Conc. (ppm)	Steady-state algorithm (mg/L)	PBPK model simulations (mg/L)
0.00001	0.0000001	0.00000011
0.0001	0.0000011	0.0000011
0.001	0.00001	0.00001
0.01	0.00011	0.00011
0.1	0.00111	0.00110
0.5	0.00554	0.00552
1	0.01108	0.01108
2.5	0.0277	0.02793
5	0.05539	0.05667
7.5	0.08309	0.08625
10	0.11078	0.11672

Note: The above calculations were done for humans using the appropriate parameter values in a steady-state algorithm as well as using a validated PBPK model. The length of simulation was set to 200 hours to ensure the attainment of steady-state.

In sum, the data reported in Table 3.1 establish the quantitative relationship between inhaled and blood concentrations of benzene as well as confirm that the simpler steady-state equation can be used for relating blood and environmental concentrations. The appropriateness of the use of the developed algorithms depends on the existence of steady-state condition. An evaluation of the time constants indicates that exposure duration greater than 4 hours is likely to generate blood concentration data comparable to steady-state values expressed with error ranges or individual variabilities. Time constants are benchmarks that indicate the time taken to attain 50% of the steady-state concentration. These values are determined by the magnitude of the tissue:blood partition coefficients, tissue volumes and tissue blood flow rates in addition to the intrinsic clearance in the metabolizing tissues.

3.3 Use of the Human PBPK Model to Interpret Benzene Biomonitoring Data

Following the establishment of the quantitative relationships between exposure and blood concentrations, Equation 7 was re-written such that exposure concentration (C_i) can be calculated from biomarker concentrations (C_{vss}). The assumptions are that the exposure has lasted for several hours and that the blood concentration is near-steady-state. The following are the rewritten forms of Equation 7 for computing C_i from C_{vss}:

$$C_i = \frac{C_{vss}\,(Q_p/P_b + QL \times E + Q_{bm} \times E_{bm})}{Q_p \times (1 - (QLC \times E + Q_{bmc} \times E_{bm}))} \quad (8)$$

By inserting the parameter values and simplifying the above equations, we get:

$$C_i = C_{vss} \times 0.2883 \quad (9)$$

Using the above equation, the inhalation exposure concentration of benzene (C_i) can be back-calculated with information on the steady-state blood concentration (C_{vss}). The above equation provides the same results of back-calculations as the full human PBPK model, for steady-state conditions. This estimation approach assumes 100% of the blood concentrations are attributable to inhalation exposure.

The following are examples of interpretation of benzene biomonitoring data, obtained using Equation 9.

Example 1

Ashley et al. (1994) reported the blood concentrations of benzene in nonoccupationally exposed U.S. population and in groups of people suspected of exposure, as a part of the CDC NHANES III study. Their survey provided mean, median and 95th percentile values of benzene in human blood. These blood concentration results were part of the human biomonitoring data that the EPA relied upon for selection of the VCCEP pilot chemicals. Corresponding air concentrations were calculated from these blood concentrations, assuming steady-state conditions, using Equation 9. The resulting estimated air concentrations are:

Benzene:

C_i,mean = 0.13 µg/L x 0.2883 x 1000 (L/m^3) = 37.5 µg/m^3
C_i, median = 0.061 µg/L x 0.2883 x (1000 L/m^3) = 17.6 µg/m^3
C_i, 95th percentile = 0.48 µg/L x 0.2883 x (1000 L/m^3) = 138.4 µg/m^3

Example 2

Mannino et al. (1995) reported the median and range of benzene blood concentrations in people (7 smokers and 12 nonsmokers) exposed to gasoline fumes and automobile exhaust in Albany, NY. These authors also reported that the median and range of exposure concentrations of benzene, determined using vapor badges.

According to the steady-state equations developed in the present study and validated using the human PBPK model for benzene, the exposure concentration of benzene can be predicted (i.e., back-calculated). Table 3.2 presents the modeled air concentration results from the back-calculation compared to the experimental measures (using vapor badges) from the study.

Table 3.2: Comparison of Modeled Air Concentrations to Measured Air Concentrations Using Blood Samples from Mannino et al. 1995

Chemical	Measured Blood Concentration (μg/L)	Modeled Air Concentration ($\mu g/m^3$)	Measured Air Concentration ($\mu g/m^3$; vapor badge)
Benzene – Median	0.29	83.6	54
Benzene - Range	0.12 – 1.97	34.6 – 567.95	ND – 780

These results indicate that the exposure concentrations predicted from blood concentrations, using steady-state equations, appear to be within a factor of approximately 1.5 of the actual values. It should be noted that the experimental values of blood and air concentrations are median of the distribution and that each value in each distribution may not match. Further in this assessment a single value for alveolar ventilation was used, whereas the experimental data are from individuals that would likely have a range of different respiratory rates. Additional modeled results using the range of blood concentrations reported by these authors showed a similar result to that of the analysis of the median, indicating that the results are within a factor of approximately 1.5 of the measured data. The detection limit for the non-detect (ND) values was not specified in the report.

4.0 Estimation of Human Milk Concentrations

The objectives of this work were: (1) to reconstruct the PBPK model for simulating lactational transfer of benzene, and (2) to calculate benzene dose ingested by infants through the nursing exposure pathway.

4.1 Reconstruction of the PBPK model for human lactational (milk) transfer of Benzene

The simulation of breast feeding and lactational transfer of benzene was done according to a conservative schedule described by Fisher et al. (1997). All parameters for the PBPK model of benzene were obtained from Fisher et al. (1997), except the metabolic rate constants for benzene which were obtained from Tardif et al. (1995). The Fisher et al. (1997) model was reproduced successfully before using it to simulate the lactational transfer of benzene according to the defined exposure scenarios. The parameters of the model and the simulations of lactational transfer are discussed below.

These PBPK models describe the lactating mother as a set of tissue and milk compartments interconnected by systemic circulation and a gas-exchange lung. The tissue compartments refer to liver, slowly perfused tissues, richly perfused tissues and adipose tissue (fat). The rate of change in the amount of benzene in each non-metabolizing tissue compartment was described as follows (Note: all abbreviations are defined following Equation 17):

$$V_t \frac{dC_t}{dt} = Q_t(C_a - C_{vt}) \qquad (10)$$

The rate of change in benzene concentration in liver was described as follows:

$$V_t \frac{dC_t}{dt} = [Q_t(C_a - C_{vt})] - \frac{dA_{met}}{dt} \qquad (11)$$

In lay terms, the above equation signifies:

Rate of change in the amount of the chemical in the tissue = (blood flow x arteriovenous concentration difference) – rate of loss due to metabolism

The rate of the amount metabolized was described as a saturable process as follows:

$$\frac{dA_{met}}{dt} = \frac{V_{max} C_{vt}}{K_m + C_{vt}} \qquad (12)$$

The mixed venous blood concentration of benzene was calculated as follows:

$$C_v = \frac{\sum_t^n Q_t C_{vt} + Q_m C_{vm}}{Q_c} \quad (13)$$

The above equation represents the steady-state solution of the mass-balance differential equation for venous blood:

$$\left[V_b\left(dC_b / dt\right) = \sum_t^n Q_t C_{vt} + Q_m C_{vm} - C_v Q_c \right] \quad (14)$$

The arterial blood concentration of benzene was computed with the following equation:

$$C_a = \frac{Q_p C_{inh} + Q_c C_v}{Q_c + \left(\frac{Q_p}{P_b}\right)} \quad (15)$$

The equation describing the rate of change in the amount of benzene in breast milk (mg/hr) was calculated as:

$$RA_{milk} = Q_m (C_a - C_{vm}) - R_{nurse} \quad (16)$$

where,

$$R_{nurse} = C_{milk} \times V_{milk} \times Nurse \times S_{zone} \quad (17)$$

The amount of milk in the mammary tissue lumen was computed as the difference between the rate of production and rate of loss. The loss rate was set equal to the nursing rate and the volume of milk in the mammary tissue.

In the above equations, the abbreviations are as follows:

C_{inh} = inhaled concentrations of benzene
C_v = venous concentrations of benzene
C_a = arterial blood concentrations of benzene
P_b = blood:air partition coefficient
Q_p = alveolar ventilation
Q_c = cardiac output
C_{vi} = venous blood concentrations leaving tissue compartments
V_i = tissue volumes
P_i = tissue:blood partition coefficients
A_i = amount in tissues
Q_i = blood flow to tissues
V_{max} = maximal velocity of metabolism

K_m = Michaëlis affinity constant
A_{met} = amount metabolized
dt = integration interval.

Regarding the milk-related parameters, the abbreviations are as follows:

RA_{milk} = rate of change in amount of chemical in breast milk
R_{nurse} = rate of change in amount of chemical ingested by nursing infant
C_{milk} = concentration of chemical in breast milk
V_{milk} = volume of milk currently in the mammary tissue lumen
Nurse = infant nursing rate
S_{zone} = switch function to turn on or turn off the nursing over a 24-hr period
C_{vm} = venous blood leaving the milk compartment
Q_m = blood flow to the mammary tissue

4.2 Model Simulations of Human Milk Benzene Concentrations and Lactational Transfer Estimates

As mentioned before, the simulation of breast feeding and lactational transfer of benzene was done according to the schedule described by Fisher et al. (1997). In this schedule, working mothers were assumed to be exposed at the respective workplace TWA concentrations for 8 hours, on working days, and background concentrations of benzene for the remaining 16 hours of the day. Nonoccupationally exposed mothers were assumed to be exposed to background concentrations for 24 hours. Eight nursing events were assumed to occur each day, lasting 12 minutes each, with 115 mL of milk ingested per nursing event, yielding a daily milk consumption of 0.92 L. For occupationally-exposed mothers, three individual nursing events (occurring during 30-minute breaks using a milk collection devise) were assumed to occur during working hours and the remaining five nursing events were assumed to occur after working hours. The nursing events that occurred during working hours all occurred after the benzene blood concentrations had reached steady-state with the workplace exposures and occurred at 2.1, 4.1 and 7.1 hours into the workday. The remaining five nursing events occurred at 2, 5, 10, 13 and 15 hours post-work-shift. If the working day were assumed to begin at 8:00 a.m., this would amount to nursing events occurring at 2:00 a.m., 5:00 a.m., 7:00 a.m., 10:00 a.m., 12:00 p.m., 3:00 p.m., 6:00 p.m., and 9:00 p.m.

The Fisher et al. (1997) paper included modeling results for occupationally exposed mothers based on the assumption that benzene-exposed working mothers were exposed at the previous TLV of 10 ppm (8hr TWA). The current occupational exposure information presented in the Benzene VCCEP Exposure Assessment indicates that occupational exposure to benzene is considerably lower than this, so these model simulations employed the exposure values reported the Benzene VCCEP Exposure Assessment. The exposure concentrations and durations specified in the model are presented in Table 4.1.

Table 4.1. Summary of Mothers' Benzene Exposures Used in PBPK Lactation Model Simulation

Exposure Category	Exposure Concentration (μg/L)	Exposure Duration (hrs)
Rural, typical	0.0026	24
Rural, high end	0.011	24
Urban, typical	0.0026	24
Urban, high end	0.011	24
Occupational, typical	0.35	8
- Background, Urban typical	0.0026	16
Occupational, high end	1.22	8
- Background, Urban high end	0.011	16

The PBPK modeling results are presented in Table 4.2. The model predicted that the daily mass of benzene transferred to human milk in the lactating mother ranges from 0.000016 mg for the typical rural or urban exposed mother to 0.00491 mg for the high-end occupationally exposed mother. This corresponds to daily benzene doses of 0.0000023 mg/kg to 0.00068 mg/kg to the child from lactation.

Table 4.2. PBPK model predictions of amount transferred to milk in lactating mothers exposed to benzene.

Exposure Category	Modeled Human Milk Benzene Concentration (μg/L)	Mass of Benzene Consumed by Child (mg/day)	Dose (mg/kg-day)
Rural, typical	0.02	0.000016	2.3E-06
Rural, high end	0.1	0.000115	1.6E-05
Urban, typical	0.02	0.000016	2.3E-06
Urban, high end	0.1	0.000115	1.6E-05
Occupational, typical + Background, Urban typical	1.5	0.0014	1.9E-04
Occupational, high end + Background, Urban high end	5.3	0.00491	6.8E-04

5.0 References

Angerer J, Scherer G, Schaller KH and Muller J. (1991). The determination of benzene in human blood as an indicator of environmental exposure to volatile aromatic compounds. Fresenius J Anal Chem 339: 740-742.

Ashley DL, Bonin MA, Cardinali FL, McCraw JM and Wooten JV. (1994). Blood concentrations of volatile organic chemicals in a non-occupationally exposed US population and in groups with suspected exposure. Clin Chem 40: 1401-1404.

Astrand, I., Ehrner-Samuel, H., Kilbom, A. and Ovrum, P. (1972). Toluene exposure I. Concentration in alveolar air and blood at rest and during exercise. Work-Environ. Health. 9, pp. 119-130.

Astrand I, Engstrom J and Ovrum P. (1978). Exposure to xylene and ethylbenzene. I, uptake, distribution and elimination in man. Scan J Work Environ Health 4: 185-194.

Baker GL. 1969. Human adipose tissue composition and age. *American Journal of Clinical Nutrition* 22: 829-835.

Bois, F. Y., Woodruff, T. J., and Spear, R. C. (1991a). Comparison of three physiologically based pharmacokinetic models of benzene disposition. Toxicol Appl.Pharmacol. **110**, 79-88.

Bois, F. Y., Smith, M. T., and Spear, R. C. (1991b). Mechanisms of benzene carcinogenesis: application of a physiological model of benzene pharmacokinetics and metabolism. Toxicol Lett. **56**, 283-298.

Bois, F. Y., Jackson, E. T., Pekari, K., and Smith, M. T. (1996). Population toxicokinetics of benzene. Environ.Health Perspect. **104 Suppl 6**, 1405-1411.

Brugnone, F., Perbellini, L., Faccini, G.B., Pasini, F., Maranelli, G., Romeo, L., Gobbi, M. and Zedde, A. (1989a). Breath and blood levels of benzene, toluene, cumene and styrene in non-occupational exposure. *Int. Arch. Occup. Environ. Health. 61, pp. 303-311.*

Brugnone, F., Perbellini, L., Faccini, G.B., Pasini, F., Danzl, B., Maranell, G., Romeo, L., Gobbi, M., and Zedde, A. (1989b). Benzene in the Blood and Breath of Normal People and Occupationally Exposed Workers. Amer. J. Ind. Med. 16, 385-399.

Brugnone, F., Perbellini, L., Maranelli, G., Romeo, L., Guglielmi, G., and Lombardini, F. (1992). References value for Blood Benzene in the Occupationally Unexposed General Population. Int. arch. Occup. Environ. Health. 64, 179-184.

Brugnone, F., Gobbi, M., ayyad, K., Giuliari., C., Cerpelloni, M. and Perbellini, L. (1995). Blood toluene as a biological index of environmental toluene exposure in the "normal" population and in occupationally exposed workers immediately after exposure and 16 hours later. *Int. Arch. Occup. Environ. Health. 66, pp. 421-425.*

Brugnone, F., Perbellini, L., Remeo, L., Bianchin, M., Tonello, A., Pianalto, G., Zambon, D., and Zanon, G. (1998). Benzene in environmental air and human blood. Int. Arch. Environ. Health. 71, pp. 554-559.

Brugnone, F., Perbellini, L., Romeo, L., Cerpelloni, M., Bianchin, M., amd Tonello, A. (1999). Benzene in Blood as a Biomarker of low level Occupational Exposure. Sci. Total. Environ. 235, pp.247-252.

Chriske HW, Brockus A and Ewers U. (1991). Arbeitsmed. Sozialmed. Praventimed. 26: 483-485.

Fisher J, Mahle D, Bankston L, Greene R and Gearhart J. 1997. Lactational transfer of volatile chemicals in breast milk. *American Industrial Hygiene Association Journal* 58: 425-431.

Fustinoni, S., Buratti, M., Giampiccolo, R., Colombi, A. (1996). Med Lav 87 : 63-75

Gargas, M. L., Burgess, R. J., Voisard, D. E., Cason, G. H., and Andersen, M. E. (1989). Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. Toxicol Appl.Pharmacol. **98**, 87-99.

Goergens HW, Kurosinski I, Hahimiragha H, U. Ewers and A. Brockhaus. (1991). Arbeitsmed. Sozialmed. Praventimed. 26: 50-54.

Haddad, S., Tardif, R., Viau, C., and Krishnan, K. (1999). A modeling approach to account for toxicokinetic interactions in the calculation of biological hazard index for chemical mixtures. Toxicol Lett. **108**, 303-308.

Haddad, S., Beliveau, M., Tardif, R., and Krishnan, K. (2001). A PBPK modeling-based approach to account for interactions in the health risk assessment of chemical mixtures. Toxicol Sci. **63**, 125-131.

Haddad, S., Tardif, R., Charest-Tardif, 0G., and Krishnan, K. (1999). Physiological modeling of the toxicokinetic interactions in a quaternary mixture of aromatic hydrocarbons. Toxicol Appl.Pharmacol. **161**, 249-257.

Haddad, S., Charest-Tardif, G., and Krishnan, K. (2000). Physiologically based modeling of the maximal effect of metabolic interactions on the kinetics of components of complex chemical mixtures. J.Toxicol Environ.Health A **61**, 209-223.

Haddad, S., Charest-Tardif, G., Tardif, R., and Krishnan, K. (2000). Validation of a physiological modeling framework for simulating the toxicokinetics of chemicals in mixtures. Toxicol Appl.Pharmacol. **167**, 199-209.

Hajimiragha, H., Ewers, U., Brockhaus, A., and Boettger, A. (1989). Levels of Benzene and other Volatile Aromatic Compounds in the Blood of Non-Smokers and Smokers. Int. Arch. Occup. Environ. Health. 61, 513-518.

Hilderbrand, R. L., Andersen, M. E., and Jenkins, L. J., Jr. (1981). Prediction of in vivo kinetic constants for metabolism of inhaled vapors from kinetic constants measured in vitro. Fundam.Appl.Toxicol **1**, 403-409.

Hjelm, E.W., Naslund, P.H., and Wallen, M. (1998). Influence of cigarette smoking on the Toxicokinetics of Toluene in Humans. J. Toxi. Environ. Health. 25, pp.155-163.

Jang, J. Y., and Droz, P. O. (1997). Ethnic differences in biological monitoring of several organic solvents. II. A simulation study with a physiologically based pharmacokinetic model. Int.Arch.Occup.Environ.Health **70**, 41-50.

Jang J.Y., Droz, P.O. and Chung, H.K. (1999). Uncertainties in PBPK models caused by several input parameters. Int Archs Occup Environ Health 72: 247-254.

Kaneko, T., Horiuchi, J., and Sato, A. (2000). Development of a physiologically based pharmacokinetic model of organic solvent in rats. Pharmacol.Res. **42**, 465-470.
Ref ID: 120

Kok PW and Ong CN. (1994). Blood and urinary benzene determined by headspace gas chromatography with photoionization detection: application in biological monitoring of low level nonoccupational exposure. Int Archs Occup Environ Health 66: 195-201.

Konietzko, H., Keilbach, J. and Drysch, K. (1980). Cumulative effects of daily toluene exposure. Int. Arch. Occup. Environ. Health. 46, pp. 53-58.

Krewski, D., Wang, Y., Bartlett, S., and Krishnan, K. (1995). Uncertainty, variability, and sensitivity analysis in physiological pharmacokinetic models. J.Biopharm.Stat. **5**, 245-271.

Krishnan, K., and Pelekis, M. (1995). Hematotoxic interactions: occurrence, mechanisms and predictability. Toxicology **105**, 355-364.

Laparé, S., Tardif, R., and Brodeur, J. (1993). Effect of various exposure scenarios on the biological monitoring of organic solvents. I. Toluene and xylene. International Archives of Occupational and Environmental Health **64**, 569-580.

Leung HW. (1992). Use of PBPK models to establish biological exposure indices. AIHA Journal 53: 369-374.

Loizou, G. D., Jones, K., Akrill, P., Dyne, D., and Cocker, J. (1999). Estimation of the dermal absorption of m-xylene vapor in humans using breath sampling and physiologically based pharmacokinetic analysis. Toxicol Sci. **48**, 170-179.

Mannino DM, Schreiber J, Aldous K, Ashley D, Moolenar R and Almaguer D. (1995). Human exposure to volatile organic compounds – a comparison of organic vapor monitoring badge levels with blood levels. Int Archs Occup Environ Health 67: 59-64.

Needham LL, Hill RH, Ashley DL, Pirkle JL and Sampson EH. (1995) The priority toxicant reference range study interim report. Environ Health Perspect 103 (Suppl 3): 89-94.

Pekari, K., Vainiotalo, s., Heikkila, P., Palotiea., Luotamo, M., and Riihimaki, V. (1992). Biological Monitoring of Occupational Exposure to Low Levels of Benzene. Scand. J. Work. Environ. Health. 18, pp, 317-322.

Pelekis, M., and Krishnan, K. (1997). Assessing the relevance of rodent data on chemical interactions for health risk assessment purposes: a case study with dichloromethane- toluene mixture. Regul.Toxicol Pharmacol. **25**, 79-86.

Perbellini, L., Faccini, G.B., Pasini, F., Cazzoli, F., Pistoia, S., Rosellini, R., Valsecchi, M., and Brugnone, F. (1988). Environmental and Occupational exposure to Benzene by Analysis of Breath and Blood. Br. J. Ind. Med. 45, pp. 345-352.

Pierce, C.H., Dills, R.l., Morgan, M.S., Vivini, P. and Kalman, D.A. (198). Biological monitoring of controlled toluene exposure. Int. Arch. Occup. Environ. Health. 17, pp 433-444.

Price K, Haddad S and Krishnan K. 2003. Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. *Journal of Toxicology and Environmental Health* 66 A: 417-433.

Riihimaki V, Pfaffli P, Savolainen K and Pekari K. (1979a). Kinetics of m-xylene in man: general features of absorption, distribution, biotransformation and excretion in repetitive inhalation exposure. Scand J Work Environ Health 5: 217-231.

Riihimaki V, Pfaffli P and Savolainen K. (1979b). Kinetics of m-xylene in man. Influence of intermittent physical exercise and changing environmental concentrations on kinetics. Scand J Work Environ Health 5: 232-246.

Roy, A., and Georgopoulos, P. G. (1998). Reconstructing week-long exposures to volatile organic compounds using physiologically based pharmacokinetic models. J.Expo.Anal.Environ.Epidemiol. **8** , 407-422.

Sato A, Nakajima T, Fujiwara Y and Murayama N. (1974). Pharmacokinetics of toluene and benzene. Int Arch Arbeitsmed 33: 169-182.

Sato A, Nakajima T, Fujiwara Y and Murayama N. (1975). Kinetic studies on sex differences in susceptibility to chronic benzene intoxication – with special reference to body fat content. Br J Ind Med 32: 169-182.

Sherwood, R. J., and Sinclair, G. C. (1999). New PBPK model applied to old occupational exposure to benzene. Am.Ind.Hyg.Assoc.J. **60**, 259-265.

Sinclair, G. C., Gray, C. N., and Sherwood, R. J. (1999). Structure and validation of a pharmacokinetic model for benzene. Am.Ind.Hyg.Assoc.J. **60**, 249-258

Spear, R. C., and Bois, F. Y. (1994). Parameter variability and the interpretation of physiologically based pharmacokinetic modeling results. Environ.Health Perspect. **102 Suppl 11**, 61-66.

Srbova J, Teisinger J and S Skramovsky. (1950). Absorption and elimination of inhaled benzene in man. Arch Ind Hyg Occup Med 2: 1-8.

Tardif, R., and Charest-Tardif, G. (1999). The importance of measured end-points in demonstrating the occurrence of interactions: a case study with methylchloroform and m-xylene. Toxicol Sci. **49**, 312-317.

Tardif R, Charest-Tardif G, Brodeur J and Krishnan K. (1997). Physiologically based pharmacokinetic modeling of a ternary mixture of alkyl benzenes in ras and humans. Toxicol Appl Pharmacol 144: 120-134.

Tardif R, Lapare S, Charest-Tardif G, Brodeur J and Krishnan K. 1995. Physiologically based modeling of a mixture of toluene and xylene in humans. *Risk Analysis* 15: 335-342.

Tardif R, Sato A, Lapare S and Brodeur J. (1994). Ethanol induced modification of m-xylene toxicokinetics in humans. Occup Environ Med 51: 187-191.

Tardif, R., Laparé, S., Krishnan, K. and Brodeur, J. (1993). A descriptive and mechanistic study of the interaction between toluene and xylene in humans. Int. Arch. Occup. Environ. Health. 65, pp. S135-S137.

Tardif R, Laprae S, Plaa GL and Brodeur J. (1991). Effect of simultaneous exposure to toluene and xylene on their respective biological exposure indices in humans. Int Archs Occup Environ Health 63: 279-284.

Teisinger J and Fiserova-Bergerova V. (1955). Valeur comparee de la determination des sulfates et du phenol contenus dans l'urine pour l'evaluation de la concentration du benzene dans l'air. Arch Mal Prof Med Trav 16: 221-232.

Thomas, R. S., Lytle, W. E., Keefe, T. J., Constan, A. A., and Yang, R. S. (1996). Incorporating Monte Carlo simulation into physiologically based pharmacokinetic models using advanced continuous simulation language (ACSL): a computational method. Fundam.Appl.Toxicol **31** , 19-28.

Thomas, R. S., Bigelow, P. L., Keefe, T. J., and Yang, R. S. (1996). Variability in biological exposure indices using physiologically based pharmacokinetic modeling and Monte Carlo simulation. Am.Ind.Hyg.Assoc.J. **57**, 23-32.

Thrall, K. D., and Poet, T. S. (2000). Determination of biokinetic interactions in chemical mixtures using real-time breath analysis and physiologically based pharmacokinetic modeling. J.Toxicol Environ.Health A **59**, 653-670

Veulemans, H. and Masschelein, R. (1978). Experimental human exposure to Toluene. Int. Arch. Occup. Environ. Health. 42, pp. 105-117.

Travis, C. C., Quillen, J. L., and Arms, A. D. (1990). Pharmacokinetics of benzene. Toxicol Appl.Pharmacol. **102**, 400-420.

Wallace LA, Nelson WC, Pellizzari ED and Raymer JH. (1997). Uptake and decay of volatile organic compounds at environmental concentrations: application of a a four-compartment model to a chamber study of five human subjects. J Exp Anal Environ Epidemiol 7: 141-163.

Wallen M. (1986). Toxicokinetics of toluene in occupationally exposed volunteers. Scand J Work Environ Health 12: 588-593.

Wallen, M., Holm, S. and Byféalt Nordqvist, M. (1985). Coexposure to toluene and p-xylene in man: uptake and elimination. Br. J. Ind. Med. 42, pp. 111-116.

Wang, G., Maranelli, G., Perbellini, L., Guglielmi, G. and Brugnone, F. (1993). Reference values for blood toluene in the occupationally nonexposed general population. *Int. Arch. Occup. Environ. Health. 65, 201-203.*

Watanabe, K. H., Bois, F. Y., Daisey, J. M., Auslander, D. M., and Spear, R. C. (1994). Benzene toxicokinetics in humans: exposure of bone marrow to metabolites. Occup.Environ.Med. **51**, 414-420.

Watanabe, K. H., and Bois, F. Y. (1996). Interspecies extrapolation of physiological pharmacokinetic parameter distributions. Risk Anal. **16**, 741-754.

White DR, Widdowson EM, Woodard HQ and Dickerson WT. 1991. The composition of body tissues. *British Journal of Radiology* 64: 149-159.

Wigaeus-Hjelm E, Naslund PH and Wallen M. (1988). Influence of cigarette smoking on the toxicokinetics of toluene in humans. J Toxicol Environ Health 25: 155-163.

Woodruff, T. J., Bois, F. Y., Auslander, D., and Spear, R. C. (1992). Structure and parameterization of pharmacokinetic models: their impact on model predictions. Risk Anal. **12**, 189-201.