# Xylenes Tier 1 VCCEP Submission

# Appendix C

# PBPK Modeling for Xylenes, Interpretation of Xylenes Human Biomonitoring Data, and Modeling of Xylenes Concentrations in Blood and Human Milk

**October 6, 2005**

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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 xylenes, the use of PBPK models to facilitate the interpretation of the human biomonitoring data for xylenes and, finally, the estimation of human blood and milk concentrations that could correlate with measured or modeled exposure data for xylenes.

## 2.0 Literature Review for Human Pharmacokinetics and Biomonitoring of Xylenes

### 2.1 Literature Review of Available PBPK Models

The assessment of information regarding human data on inhalation pharmacokinetics of xylenes 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.

There have basically been three human PBPK modeling efforts for xylenes: (1) Tardif et al., (2) Droz et al. and (3) Loizou et al. Whereas the modeling work of Tardif et al. (1993, 1995) simulated the kinetics of not only m-xylene but also that of the other isomers, Droz and Loizou uniquely simulated the kinetics of m-xylene. Jang and Droz (1997), in their human model for m-xylene, described metabolism using constants generated by Tardif et al. (1993). Loizou et al. (1999) also derived the Km for metabolism from a previous University of Montreal study (Lapare et al. 1993). However, Loizou et al. (1999) assessed Vmax by fitting to experimental data collected in human volunteers, and the model lacked validation of its ability to simulate the inhalation pharmacokinetics of xylenes in humans. Based on analysis of these facts, the PBPK model of Tardif et al. (1995) will be used in further efforts in this project. The chemical-specific parameters of this PBPK model are as follows:

**Xylenes - partition coefficients**

| | |
|---|---|
| Blood:air | 26.4 |
| Liver:air | 90.9 |
| Fat:air | 1859.0 |
| Richly perfused tissues:air | 90.9 |
| Poorly perfused tissues:air | 41.9 |

**Xylenes - metabolism constants**

| | |
|---|---|
| Liver Vmax (mg/hr/kg) | 8.4 |
| Liver Km (mg/L) | 0.22 |

Table 2.1 presents a summary of the human pharmacokinetic studies, in which xylenes blood concentrations were measured following inhalation exposures. These data are potentially useful for two purposes: (1) for validating the 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 Xylenes BTX pharmacokinetics**

| Exposure Concentration | Exposure -Sampling Duration | Reference |
|---|---|---|
| 25 & 50 ppm | 7 h/d, 3 days | Lapare et al. (1993) |
| 100 & 400 ppm | 2 - 4 hr | Tardif et al. (1994) |
| 33 ppm | 7 hr | Tardif et al. (1997) |
| 40 ppm | 7 hr | Tardif et al. (1991) |
| 80 ppm | 4 hr | Tardif et al. (1991) |
| 87 – 200 ppm | 6 h/day, 5 days | Riihimaki et al. (1979b) |
| 435 – 780 mg/$m^3$ | 30 – 90 min | Astrand et al. (1978) |
| 3.6 – 8.2 mmol/$m^3$ | 6 h/d, 5 days | Riihimaki et al. (1979a) |

## 2.2 Literature Review of Available Human Biomonitoring Data for Xylenes

Using a similar approach to the assessment of available xylenes PBPK models, a search for information regarding human biomonitoring data on xylenes (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 xylenes 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 Xylenes**

| Authors and year | Xylenes Conc (ng/L | Remarks |
|---|---|---|
| Hajimiragha et al. (1989) | Mean : 1580<br>Median : 1094<br>Range : 584-5602 | Nonsmokers (meta and para isomers) |
| Hajimiragha et al. (1989) | Mean : 1705<br>Median : 1490<br>Range : 916 – 3008 | Smokers (meta and para isomers) |
| Hajimiragha et al. (1989) | Mean : 409<br>Median : 324<br>Range : 129 – 1472 | Nonsmokers (ortho isomer) |

| Authors and year | Xylenes Conc (ng/L | Remarks |
|---|---|---|
| Hajimiragha et al. (1989) | Mean : 463<br>Median : 352<br>Range : 236 – 1130 | Smokers (ortho isomer) |
| Chriske et al. (1991) | Mean : 590<br>Range : 339 – 940 | Nonsmokers (meta and para isomers) |
| Chriske et al. (1991) | Mean : 191<br>Range : 106 – 291 | Nonsmokers (ortho isomer) |
| Goergens et al. (1991) | Mean : 687<br>Median : 378<br>Range : 170 – 2287 | Nonsmokers (meta and para isomers) |
| Goergens et al. (1991) | Mean : 223<br>Median : 134<br>Range : 46 – 872 | Nonsmokers (ortho isomer) |
| Ashley et al. (1994) | Mean : 140<br>Median : 110<br>$5^{th}$ perc: 44<br>95th perc : 300 | NHANES III (ortho isomer) |
| Ashley et al. (1994)<br>Needham et al. (1995) | Mean : 370<br>Median : 190<br>$5^{th}$ perc: 74<br>95th perc : 780 | NHANES III (meta and para isomers) |
| Mannino et al. (1995) | Median: 320<br>Range: 70 – 2010 | People exposed to gasoline fumes and auto exhausts in Albany NY (ortho isomer) |
| Mannino et al. (1995) | Median: 950<br>Range: 160 – 9780 | People exposed to gasoline fumes and auto exhausts in Albany NY (meta and para isomers) |
| Fustinoni et al. (1996) | Mean : 604 (nonsmokers)<br>Mean: 794 (smokers) | Total xylenes |

After considering the above studies, the biomonitoring data from the following CDC/ATSDR studies (Ashley (1994), Mannino (1995), and Needham (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 than the U.S.

## 3.0 PBPK Modeling and Interpretation

### 3.1 Reconstruction of Human PBPK models for Xylenes

This section discusses the reconstruction of the human PBPK models for xylenes (into Microsoft EXCEL® and Advanced Continuous Simulation Language (ACSL®)) and the successful reproduction of previously published simulations of xylenes 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 and adipose tissue (fat). The rate of change in the amount of xylenes in each non-metabolizing tissue compartment is described as follows (Note: all abbreviations are defined in the legend for Figure 3.2):

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

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

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

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:

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

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

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

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 xylenes 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 xylenes PBPK model is comprised of the above equations, which are interconnected as shown in Figure 3.2.

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

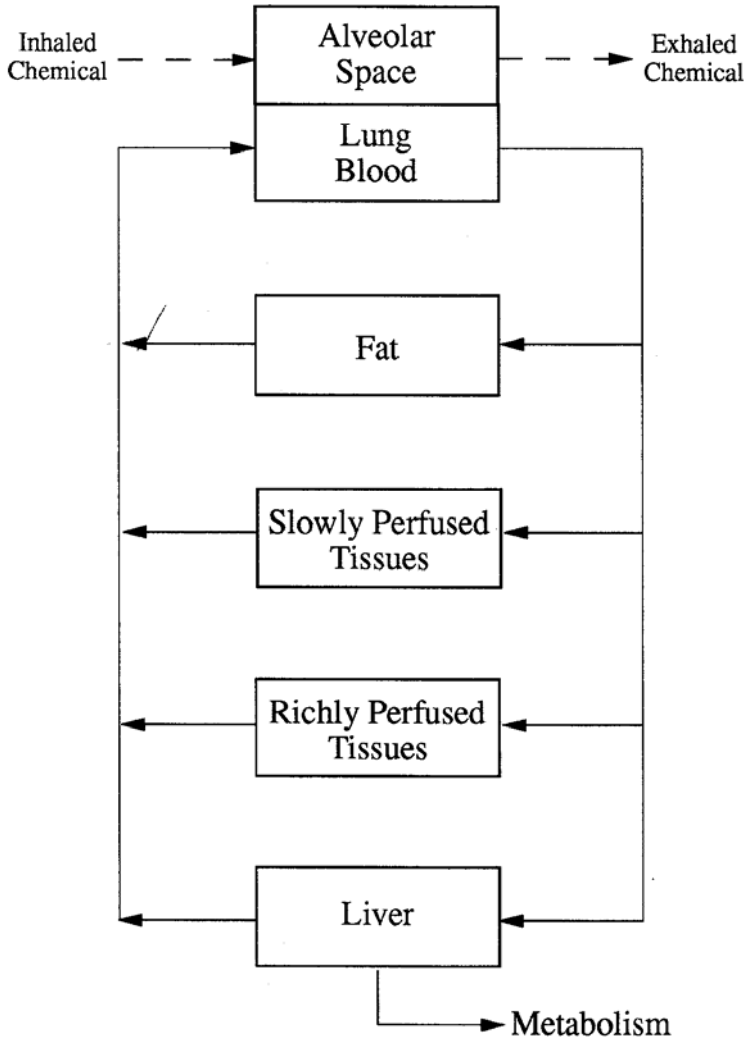
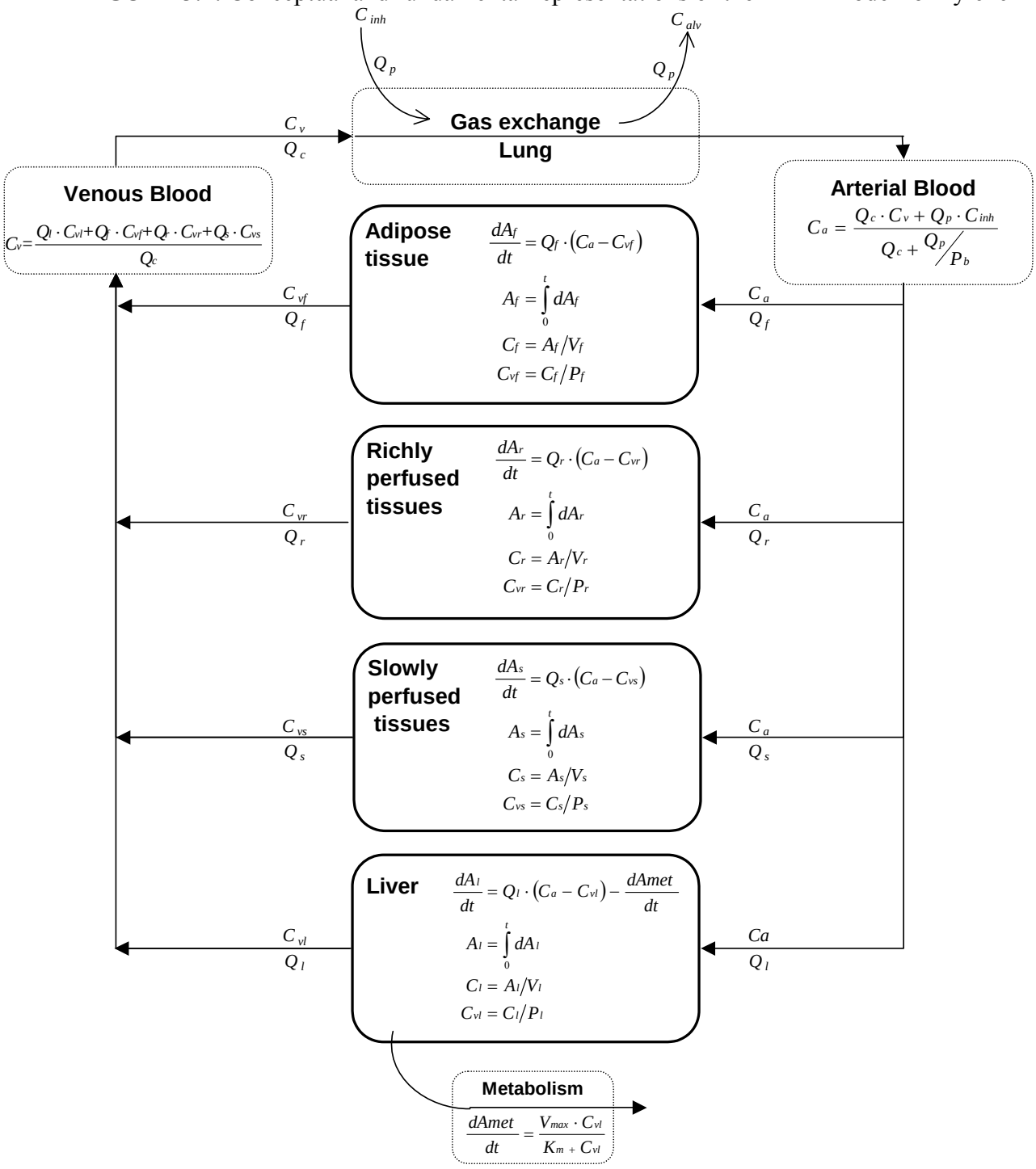


FIGURE 3.2: Conceptual and fundamental representations of the PBPK model for xylene




**Legend**: $C_{inh}$ and $C_{alv}$ refer to inhaled and exhaled xylenes concentrations. $C_v$ and $C_a$ refer to venous and arterial blood concentrations. Pb refers to blood:air partition coefficient. $Q_p$ and $Q_c$ refer to alveolar ventilation and cardiac output. $C_{vi}$, $V_i$, $P_i$, $A_i$ 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 interval.

The numerical values of the parameters of human m-xylene PBPK model are provided in Figure 3.3. These parameter values were used to solve the equations in Excel to generate the blood kinetics of xylenes, as per the original papers (Tardif et al. (1995). 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 was conducted.

The reconstructed PBPK model was used to reproduce the inhalation pharmacokinetics of xylenes in humans, as per the original modeling papers. Accordingly, the PBPK model was used to simulate the blood kinetics in human volunteers exposed to 33 ppm m-xylene for 7 hours (original experimental data published in Tardif et al. 1997, were retrieved and used in the present study). A compact disc (CD) containing the human PBPK model codes for xylenes written in Excel and ACSL was included in this project and is available upon request. These codes can be used on most computers to simulate the kinetics of xylenes in humans, provided the inhalation exposure scenario is defined.

Figure 3.4 presents the comparisons of the model predictions with the experimental data on the venous blood concentrations in human volunteers exposed to 33 ppm m-xylene for 7 hours. Figure 3.5 presents the PBPK model predictions of the alveolar air concentrations in humans exposed to 33 ppm m-xylene for 7 hours under controlled conditions (experimental data from Tardif et al. 1997).

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 papers during the process of validation of the xylene model.

FIGURE 3.3: Values of parameters of the m-xylene PBPK model.

| ***Human PBPK Model - m-Xylene*** | | | | | | |
|---|---|---|---|---|---|---|
| Tissue | Physiological | | Physicochemical | | Biochemical | |
| | Q(L/hr) | V(L) | Pi | Pb | Vmax | km |
| Body © | 352.24 | 70 | | | | |
| Lung (p) | 352.24 | | | | | |
| Liver (l) | 91.58 | 1.82 | 3.02 | | 203.3 | 0.2 |
| Fat (f) | 17.61 | 13.3 | 77.8 | | | |
| Richly ® | 154.99 | 3.5 | 4.42 | | | |
| Slowly (s) | 88.06 | 43.4 | 3.0 | | | |
| Blood (b) | | | | 26.4 | | |

| Exposure condition | |
|---|---|
| Cinh(ppm) | 32.9 |
| Cinh(mg/L) | 0.14 |
| Length(hr) | 7 |
| MW | 106.17 |

| Time function | |
|---|---|
| | hr |
| Integration interval(t) | 0.005 |

FIGURE 3.4 Comparison of PBPK model simulations of venous blood concentrations of m-xylene with experimental data (symbols) obtained in humans exposed for 7 hours to 33 ppm of this solvent.

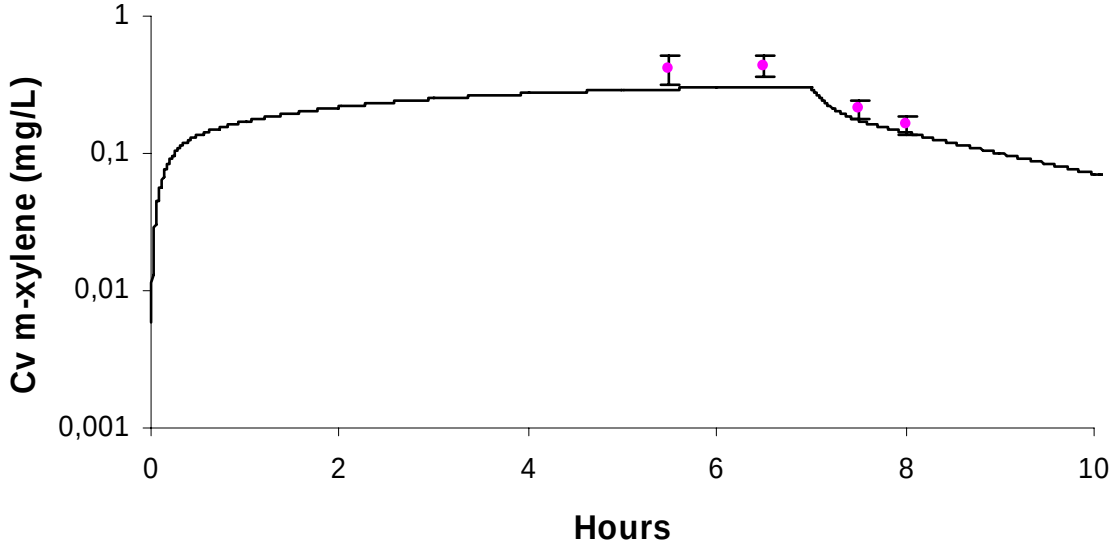
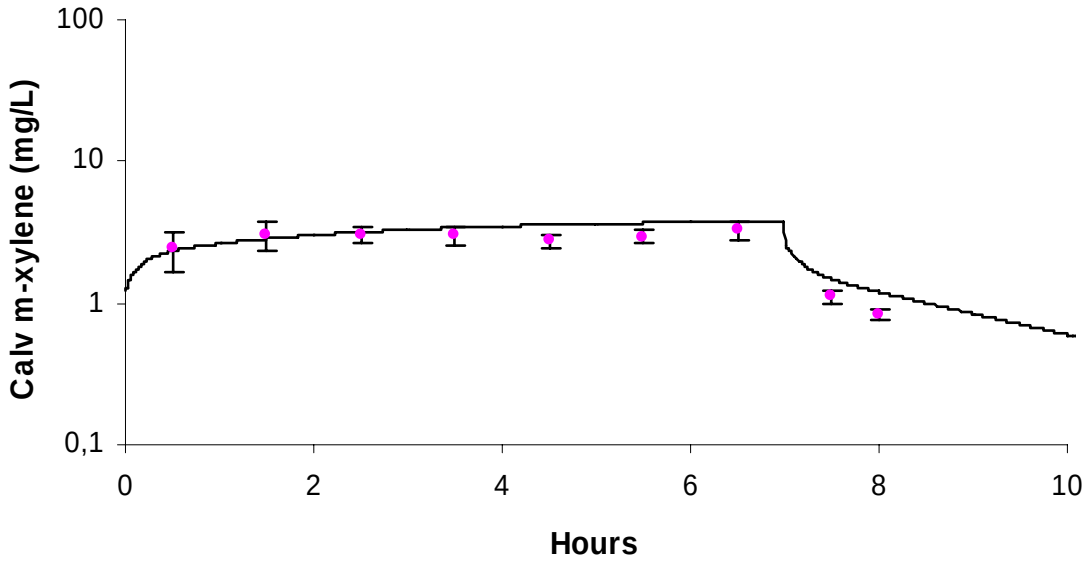



FIGURE 3.5 Comparison of PBPK model simulations of alveolar air concentrations of m-xylene with experimental data (symbols) obtained in humans exposed for 7 hours to 33 ppm of this solvent.



## 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 xylenes 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 xylenes should be constant and is independent of time (of exposure or sampling). The relationships between the environmental and venous blood concentrations of xylenes 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 xylenes 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 derived by Andersen (1981) (Toxicology and Applied Pharmacology 60: 509-526), Pelekis et al. (1997) (Toxicology Methods 7: 205-225) and Filser and Csanady (2001) (Archives of Toxicology 74: 663-672). The steady-state algorithms developed by these three groups can be re-written to show that they give identical results. In the present study, the steady-state blood concentrations obtained with algorithms were compared with the simulations of full-fledged human PBPK model for xylene (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 xylenes. The quantitative relationship can be better understood, in terms of mechanistic determinants, by examining the steady-state equation:

$$Cvss = \frac{Qp \times Ci\,(1 - QLC*E)}{(Qp/Pb) + (QL \times E)} \qquad (7)$$

where Cvss = steady-state venous blood concentration (mg/L), Qp = alveolar ventilation rate, Ci = inhaled or exposure concentration, Pb = blood:air partition coefficient, QLC = fraction of cardiac output flowing through liver, and E = hepatic extraction coefficient.

The numerical values of the following parameters are required for establishing the relationship between blood concentration (Cass) and inhaled concentration (Ci) of xylenes, at steady-state: (1) Qp, (2) Pb, (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:

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

where CLint = intrinsic clearance calculated as Vmax divided by Km for first order conditions, and QL = liver blood rate in humans.

Using Equation 8, the E value obtained in this study for m-xylene was 0.914. Using this parameter value in the steady-state equation (Equation 7), the venous blood concentrations reported in Column 2 of Table 3.1 were obtained whereas the data presented in Column 3 were obtained using the validated human PBPK models.

**Table 3.1: Quantitative relationship between the steady-state venous blood concentration (Cv) and inhalation exposure concentration (Cinh) of m-xylene.**

| Exposure Conc. (ppm) | Steady-state algorithm (mg/L) | PBPK model simulations (mg/L) |
|---|---|---|
| 0.00001 | 0.00000012 | 0.00000012 |
| 0.0001 | 0.00000120 | 0.00000119 |
| 0.001 | 0.00001201 | 0.00001192 |
| 0.01 | 0.0001201 | 0.00011918 |
| 0.1 | 0.00120144 | 0.0011919 |
| 0.5 | 0.006007 | 0.005961 |
| 1 | 0.01201 | 0.01193 |
| 2.5 | 0.03003597 | 0.029847 |
| 5 | 0.06007 | 0.059799 |
| 7.5 | 0.0901 | 0.08986 |
| 10 | 0.1201 | 0.1200 |

**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 equal to 230 h 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 xylenes as well as confirm that the simpler steady-state equation can be used for relating blood and environmental concentrations.

### 3.3 Use of the Human PBPK Model to Interpret Xylenes Biomonitoring Data

Following the establishment of the quantitative relationships between exposure and blood concentrations, Equation 7 was re-written such that exposure concentration (Ci) can be calculated from biomarker concentrations (Cvss). 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 Ci from Cvss:

Ci for Xylenes:

$$Ci = \frac{Cvss\ (Qp/Pb + QL \times E)}{Qp \times (1 - QLC \times E)} \quad (9)$$

By inserting the parameter values and simplifying the above equation, we get the following Ci equation for xylenes:

$$Ci = Cvss \times 0.3614 \quad (10)$$

Using the above equation, the inhalation exposure concentration of xylenes (Ci) can be back-calculated with information on the steady-state blood concentration (Cvss). The above equation provides the same results of back-calculations as full-fledged human PBPK models, for steady-state conditions.

The following are examples of interpretation of xylenes biomonitoring data, obtained using Equation 10.

**Example 1**

Ashley et al. (1994, Clinical Chemistry 40: 1401-1407) reported the blood concentrations of xylenes in nonoccupationally exposed US population and in groups of people suspected of exposure, as a part of NHANES III. Their survey indicated that the mean, median, 5$^{th}$ percentile and 95$^{th}$ percentile values of xylenes which were interpreted in the present study in terms of exposure concentrations, on the basis of pharmacokinetic principles and steady-state algorithms. Accordingly,

*m*- and *p*- Xylene:

Ci, mean = 0.37 µg/L x 0.3614 x 1000 (L/m$^3$) = 133.7 µg/m$^3$
Ci, median = 0.19 µg/L x 0.3614 x (1000 L/m$^3$) = 68.7 µg/m$^3$
Ci, 5$^{th}$ percentile = 0.074 µg/L x 0.3614 x (1000 L/m$^3$) = 26.7 µg/m$^3$
Ci, 95$^{th}$ percentile = 0.78 µg/L x 0.3614 x (1000 L/m$^3$) = 281.9 µg/m$^3$

*o*-Xylene:

Ci, mean = 0.14 µg/L x 0.3614 x 1000 (L/m$^3$) = 50.6 µg/m$^3$
Ci, median = 0.11 µg/L x 0.3614 x (1000 L/m$^3$) = 39.8 µg/m$^3$
Ci, 5$^{th}$ percentile = 0.044 µg/L x 0.3614 x (1000 L/m$^3$) = 15.9 µg/m$^3$
Ci, 95$^{th}$ percentile = 0.3 µg/L x 0.3614 x (1000 L/m$^3$) = 108.4 µg/m$^3$

**Example 2**

Mannino et al. (1995; International Archives of Occupational and Environmental Health 67: 59-64) reported the median and range of xylenes blood concentrations in people (7 smokers and 12 nonskmokers) exposed to gasoline fumes and automobile exhaust in Albany, NY. These authors also reported that the median and range of exposure concentrations of xylenes, determined using vapor badges.

According to the steady-state equations developed in the present study and validated using the human PBPK model for xylenes, the exposure concentration of xylenes can be predicted (i.e., back-calculated). The following table presents the results of back-calculations which are compared to experimental measures (vapor badge measures). These results indicate that the exposure concentrations predicted from blood concentrations, using steady-state equations, are within a factor of 2 of the actual values. It should be noted that the experimental values of blood and environmental concentrations are median of the distribution and that each value in each distribution may not match. Further in the present study we have used a single value for alveolar ventilation, whereas the experimental data are from individuals with a variety of respiratory rates.

| Chemical | Blood conc (μg/L) | Exposure conc (μg/m$^3$, back-calculated) | Exposure conc. (μg/m$^3$; vapor badge measures) |
|---|---|---|---|
| o-Xylene | 0.32 | 115.6 | 61 |
| m- and p- Xylene | 0.95 | 343.3 | 230 |

Additional back-calculations were done using the range of blood concentrations reported by these authors. The predicted range of exposure concentrations (Column 3) are compared to experimental range (Column 4). The ND values correspond to 16 μg/m$^3$ for xylene.

| Chemical | Blood conc (μg/L) | Exposure conc (μg/m$^3$, back-calculated) | Exposure conc. (μg/m$^3$; vapor badge measures) |
|---|---|---|---|
| o-Xylene | 0.07 – 2.01 | 25.3 – 726.4 | ND – 350 |
| m- and p- Xylene | 0.16 – 9.78 | 57.8 – 3534.5 | ND – 2400 |

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 (See Section 4.0).

In sum, the steady-state algorithms facilitate xylenes dose reconstruction from knowledge of human blood concentration and such predictions are within a factor of 2 of the available, approximate experimental measures.

## 4.0 Estimation of Human Blood and Milk Concentrations

Work under this phase involved the use of exposure data, from the Xylene VCCEP Exposure Assessment, to develop estimates of resulting blood concentrations for xylene. The blood concentrations associated with exposure concentrations of xylenes were obtained using a human PBPK model. Further, the anticipated blood concentrations were also computed using the steady-state algorithm (which gives the same results as the PBPK model, but with fewer parameters). The results of this algorithm and PBPK model were comparable provided the exposure lasts for at least 10 hours. In this phase, the exposure duration that leads to the onset of steady-state condition in normal human populations was also defined for xylene.

### 4.1 PBPK Modeling for Human Blood Concentrations

In order to establish the blood concentrations associated with inhalation exposure to xylenes, the contractor used the validated PBPK model as described in Section 3.1. The PBPK model describes humans as a set of tissue compartments interconnected by systemic circulation and a gas-exchange lung. The compartments refer to liver, slowly perfused tissues, richly perfused tissues and adipose tissue (fat). The rate of change in the amount of xylenes in each non-metabolizing tissue compartment was described as follows (Note: all abbreviations are defined following Equation 6):

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

The rate of change in BTX concentration in liver was 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 xylene PBPK model, the mixed venous blood concentration was calculated as follows:

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

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 xylene was computed with the following equation:

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

In Equations (1) – (6), Cinh, Cv and Ca refer to inhaled, venous and arterial blood concentrations of xylenes. 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 (t) . Vmax, Km and Amet refer to the maximal velocity of metabolism, Michaëlis affinity constant, and amount metabolized. dt refers to integration interval.

PBPK models were constructed for five different age groups, namely 9 months old, 3 year old, 10 year old and 16 year old children, as well as for adult females. These sub-groups of population correspond to the mean of the age brackets for which exposure data were provided in the Xylenes VCCEP Exposure Assessment.

Of the model parameters, the age-specific physiological parameters were obtained from Fisher et al. (1997) (adult females) and Price et al. (2003) (children of all age groups). The blood:air and tissue:air partition coefficients were obtained from Tardif et al. (1995). There is no evidence that the blood:air partition coefficients or the tissue:air coefficients differ as a function of age (White et al. 1981; Price et al. 2003). However, the adipose tissue:air partition coefficient for the 9 month and 3-year old children were lower than that of the adults by a factor of 0.761 and 0.853, respectively, as defined by the differences in lipid content (Baker, 1969). Whereas the Michaelis affinity constant for metabolism was kept constant across age groups, the maximal velocity for metabolism (Vmax) was calculated using the body weight (BW) of the age groups in the following equation:

$$Vmax = Vmaxc * BW^{0.75} \quad (7)$$

where Vmaxc = maximal velocity normalized to 1 kg (mg/hr/kg).

The current data on CYP2E1 suggest that the protein concentration relating to this metabolizing enzyme is similar in children greater 90 days old and adults (Johnsrud et al. 2003). Therefore, there is a possibility of significantly lower metabolic clearance in groups of children aged less than 3 months. Since the current study used exposure data for 9 months and older children, the metabolic clearance based on body weight$^{0.75}$ scaling is considered appropriate, based on the current state of knowledge.

The PBPK models were used to simulate the blood concentration profiles of xylenes in children and adults as per the exposure concentration and duration provided in the Xylenes VCCEP Exposure Assessment for the various categories (rural vs. urban, school day vs. non-school day, indoor vs. outdoor, upper bound vs. typical). All model parameters, as well as simulations of blood kinetics in children (9 months, 3-year old, 10-year old and 16-year old) and adult females, are provided in Appendix X (attachment to Appendix C of the Xylenes Tier 1 VCCEP Submission).

Table 4.1 summarizes the PBPK model predictions of venous blood concentrations of xylenes at the end of exposure for the various age groups, according to the exposure category. The model predictions indicate that the blood concentration is somewhat higher in children compared to adults (approximately by a factor of 1.5), for the same exposure concentration. These results suggest that, in interpreting biomarker data, either the adult-children difference may be neglected (conservative approach) or the data in children may be used to infer lower exposure concentrations (as shown in this study).

**Table 4.1. PBPK model predictions of venous blood concentrations (ng/L) of m-xylene in the subpopulations exposed to same ambient concentrations for varying lengths of time**

| Exposure category | Child 9 month | Child 3 yr | Child 10 yr | Child 16 yr | Adult |
|---|---|---|---|---|---|
| In school, typical | NA | 7.01 | 7.56 | 6.55 | NA |
| In school, upper bound | NA | 64.68 | 69.86 | 60.42 | NA |
| In vehicle | 26.17 | 19.46 | 15.98 | 16.97 | 16.34 |
| Rural, typical, in home, non-school day | 6.84 | 6.17 | 5.79 | 4.90 | 4.60 |
| Urban, typical, in home, non-school day | 20.93 | 18.88 | 17.70 | 14.98 | 14.07 |
| Rural, upper bound, in home, non-school day | 17.71 | 15.97 | 14.98 | 12.68 | 11.91 |
| Urban, upper bound, in home, non-school day | 61.99 | 55.90 | 52.43 | 44.37 | 41.69 |
| Rural, typical, outdoor, non-school day | 2.26 | 2.30 | 1.91 | 1.55 | 1.38 |
| Urban, typical, outdoor, non-school day | 7.01 | 7.13 | 5.90 | 4.69 | 4.28 |

| Exposure category | Child 9 month | Child 3 yr | Child 10 yr | Child 16 yr | Adult |
|---|---|---|---|---|---|
| Rural, upper bound, outdoor, non-school day | 5.93 | 6.03 | 4.99 | 4.05 | 3.62 |
| Urban, upper bound, outdoor, non-school day | 20.75 | 21.11 | 17.47 | 14.18 | 12.68 |
| Rural, typical, in home, school day | NA | 6.09 | 5.56 | 4.70 | NA |
| Urban, typical, in home, school day | NA | 18.61 | 17.00 | 14.39 | NA |
| Rural, typical, in home, school day | NA | 15.75 | 14.38 | 12.17 | NA |
| Urban, typical, in home, school day | NA | 55.12 | 50.34 | 42.61 | NA |
| Rural, typical, outdoor, school day | NA | 2.05 | 1.84 | 1.55 | NA |
| Urban, typical, outdoor, school day | NA | 6.33 | 5.69 | 4.05 | NA |
| Rural, upper bound, outdoor, school day | NA | 5.36 | 4.82 | 4.79 | NA |
| Urban, upper bound, outdoor, school day | NA | 18.75 | 16.86 | 14.18 | NA |
| Occupational, typical | NA | NA | NA | NA | 37.35 |
| Occupational, upper bound | NA | NA | NA | NA | 84.65 |

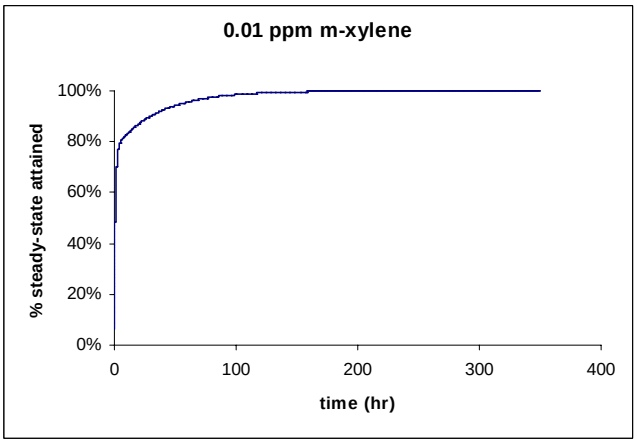
NA: not available & not applicable.

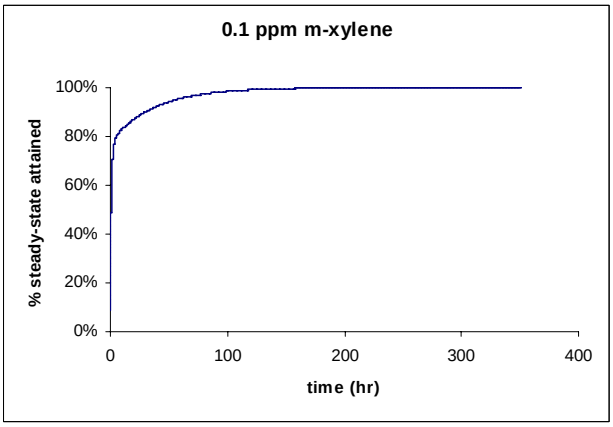
## 4.2 Establishment of blood concentrations associated with xylenes exposures using steady-state algorithms

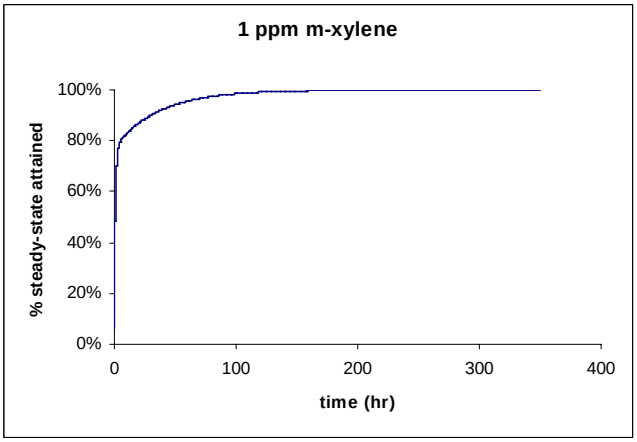
During continuous inhalation exposures, the blood concentrations of volatile chemicals such as xylenes will attain steady-state. The steady-state, in the present context, is defined as the situation during which the blood concentration does not change even though the exposure is continuing.

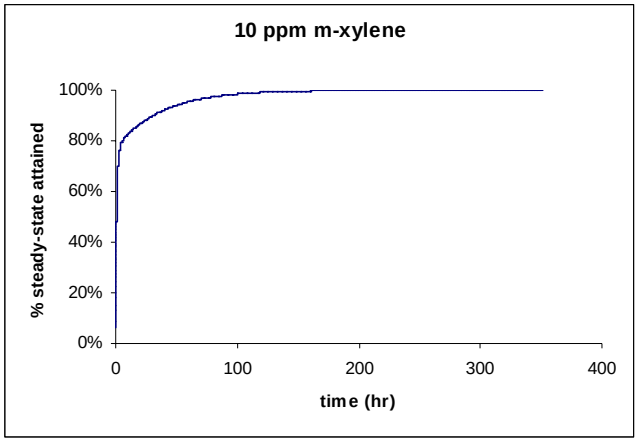
For xylenes, 57% of the steady-state concentration is attained after 1 hour of exposure. After 10 hours of continued exposure, the blood concentration of xylenes is about 83% of the steady-state level, whereas at 350 hours near-steady-state concentrations are achieved for all four dose levels (0.01, 0.1, 1, 10 ppm)(Figure 4.1).

Figure 4.1. Percent steady-state m-xylene blood concentration attained during exposure in adult humans.



During steady-state, the relationship between environmental concentration and blood concentration of xylenes is constant and independent of the time (of exposure and sampling). Based on the results of the present study (see Figure 4.1), any inhalation exposure exceeding 10 hours can be expected to lead to blood concentrations that are close to the steady-state levels. In reality, at about 10 hours, only 80% of the steady-state concentration would have been achieved. But given the simplicity of the calculation, if a 20% deviation is considered acceptable, then steady-state equations can be used to estimate blood concentrations that should be about 80% of the values predicted with the full-fledged PBPK models.

The following steady-state equation (from phase-II work and associated references) was used to estimate venous blood concentration (Cvss, mg/L) from inhaled concentration (Ci, mg/L):

$$Cvss = \frac{Qp \times Ci\ (1 - QLC*E)}{(Qp/Pb) + (QL \times E)} \qquad (8)$$

where Qp = alveolar ventilation rate, Pb = blood:air partition coefficient, QLC = fraction of cardiac output flowing through liver, and E = hepatic extraction coefficient.

The numerical values of the following parameters, for each age group, were required for determing the venous blood concentration (Cvss) associated with a given inhaled concentration (Ci) of xylenes, at steady-state: (1) Qp, (2) Pb, (3) QL, (4) QLC, and (5) E.

For adult females, the Qp, Pb, Ql, Qlc, and E values were 496.64, 26.4, 90.02, 0.29 and 0.9096, respectively (Fisher et al., 1997; Tardif et al. 1995).

For 16-year old children, the Qp, Pb, Ql, Qlc, and E values were 325.77, 26.4, 67.93, 0.16 and 0.9274, respectively (Tardif et al. 1995, Price et al. 2003).

For 10-year old children, the Qp, Pb, Ql, Qlc, and E values were 218.53, 26.4, 39.9, 0.12 and 0.9345, respectively (Tardif et al. 1995, Price et al. 2003).

For 3-year old children, the Qp, Pb, Ql, Qlc, and E values were 93.41, 26.4, 14.68, 0.1 and 0.9626, respectively (Tardif et al. 1995, Price et al. 2003).

For 9-month old children, the Qp, Pb, Ql, Qlc, and E values were 53.19, 26.4, 7.31, 0.1 and 0.9652, respectively (Tardif et al. 1995, Price et al. 2003).

By inserting the above adult female age-specific parameter values and simplifying Equation 8, we get: the following steady-state equation for calculating blood concentration of xylene from its exposure concentration:

***Venous blood concentration of xylenes (µg/L) =***
***Exposure concentration of xylenes (µg/L) x 3.6312*** (Eq. 10)

The constant in the above equation is equal to 3.6874, 4.2674, 4.8038 and 5.3047, respectively, for 16-year olds, 10-year olds, 3-year olds and 9-month old children.

Using Equations (10), the steady-state blood concentrations of xylenes were calculated using 24-hr time weighted average (TWA) exposure concentrations derived from the exposure estimates in the Exposure Assessment. These steady-state modeled results are presented on Table 4.2. Similar to the PBPK results, the steady-state results indicated blood concentrations approximately 1.5 times higher in young children as compare to adults. In general, the steady state results were 2-3 times higher than the PBPK results.

**Table 4.2: Predicted Average Blood Concentrations in Children Based on VCCEP Exposure Estimates**

| Age | Mixed xylenes 24-hour TWA ($\mu g/m^3$) | | Mixed xylenes blood concentration (µg/L) | |
|---|---|---|---|---|
| | Typical | High-End | Typical | High-End |
| 9 months | 8.9 | 35 | 0.047 | 0.19 |
| 3 years | 8.4 | 33 | 0.040 | 0.16 |
| 10 years | 8.6 | 34 | 0.037 | 0.15 |
| 16 years | 8.6 | 34 | 0.032 | 0.12 |
| Adult females | 8.8 | 35 | 0.032 | 0.13 |

In summary, the steady-state algorithms (Equation 10) facilitate a faster calculation of blood concentrations of xylenes associated with continuous inhalation exposures. The steady-state calculations, which are very simple, provide results that are 50 to 80% of the measured values if the exposure lasts between 1 and 10 hours. Overall, the PBPK models and steady-state algorithms produced during this phase should be useful for generating the biomarker concentrations (i.e., blood concentrations of parent chemicals in this case) in adults as well as children of various age groups (9 months, 3 years, 10 years, and 16 years.

## 4.3 PBPK modeling of human lactational (milk) transfer of Xylenes

The objectives of this work were: (1) to reconstruct the PBPK model for simulating lactational transfer of xylene, and (2) to calculate xylenes dose ingested by infants through the nursing exposure pathway. The milk concentrations and infant exposure dose associated with maternal exposure to xylene were obtained using human PBPK models. The maternal exposure data used in this modeling is from the Xylenes VCCEP Exposure Assessment (Section 7).

The PBPK model was used to simulate the lactational transfer of xylene based on information on maternal exposure from Xylenes VCCEP Exposure Assessment (Section 7). The exposure concentration and duration specified in the model are presented in Table 4.3.

The simulation of breast feeding and lactational transfer of xylene was done according to a conservative schedule described by Fisher et al. (1997). Accordingly, during the workday, the mother was assumed to be exposed at the respective workplace TWA concentrations for 8 hours and background concentrations of xylene for the remainder of the day. 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. Three individual nursing events were assumed to occur during working hours and the remainder five nursing events were assumed to occur after working hours. The nursing events that occurred during working hours all occurred after the xylene 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 exposure concentration and duration specified in the model are presented in Table 4.3.

**Table 4.3. Summary of Mothers' Xylene Exposures**

| Exposure Category | Exposure Concentration (μg/L) | Exposure Duration (hrs) |
|---|---|---|
| Rural, typical | 0.00170 | 24 |
| Rural, Upper Bound | 0.00440 | 24 |
| Urban, typical | 0.00520 | 24 |
| Urban, Upper Bound | 0.01540 | 24 |
| Occupational, Typical | 15.00000 | 8 |
| - Background, Urban typical | 0.00520 | 16 |
| Occupational, Upper Bound | 34.00000 | 8 |
| - Background, Urban Upper Bound | 0.01540 | 16 |

All parameters for the PBPK model of xylene were obtained from Fisher et al. (1997), except the metabolic rate constants for xylene 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 xylene according to the defined exposure scenarios. The parameters of the model and the simulations of lactational transfer are included in Section 4.4. Additionally, all model codes in MS Excel® and modeling results are recorded in the Compact Disc accompanying this report.

The PBPK modeling results are presented in Table 4.4. The model predicted that the mass of xylenes transferred to human milk in the lactating mother ranges from

**Table 4.4. PBPK model predictions of amount transferred to milk in lactating mothers exposed to xylene.**

| Exposure Category | Mass of Xylene Consumed by Child (mg/day) | Dose mg/kg-d |
|---|---|---|
| Rural, typical | 0.000024 | 3.3E-06 |
| Rural, Upper Bound | 0.000063 | 8.8E-06 |
| Urban, typical | 0.00013 | 1.8E-05 |
| Urban, Upper Bound | 0.000513 | 7.1E-05 |
| Occupational, Typical Background, Urban typical | 0.0027 | 3.8E-04 |
| Occupational, Upper Bound Background, Urban Upper Bound | 0.1896 | 2.6E-02 |

## 4.4 Model Parameters and the Simulations of Lactational Transfer

In order to establish the amount transferred via milk following inhalation exposure to xylene, the contractor used the PBPK model and the parameters published by Fisher et al. (1997). 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 xylene in each non-metabolizing tissue compartment was described as follows (Note: all abbreviations are defined following Equation 16):

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

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

$$V_t \frac{dC_t}{dt} = [Q_t(C_a - C_{vt})] - \frac{dA_{met}}{dt} \qquad (12)$$

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:

$$\frac{dA_{met}}{dt} = \frac{V_{max} C_{vt}}{K_m + C_{vt}} \quad (13)$$

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

$$C_v = \frac{\sum_t^n Q_t C_{vt} + Q_m C_{vm}}{Q_c} \quad (14)$$

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

$$\left[ V_b (dC_b / dt) = \sum_t^n Q_t C_{vt} + Q_m C_{vm} - C_v Q_c \right] \quad (15)$$

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

$$C_a = \frac{Q_p C_{inh} + Q_c C_v}{Q_c + \left( \frac{Q_p}{P_b} \right)} \quad (16)$$

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

$$RAmilk = Qm (Ca – Cvm) – Rnurse \quad (17)$$

where,

$$Rnurse = Cmilk \times Vmilk \times Nurse \times Szone \quad (18)$$

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, Cinh, Cv and Ca refer to inhaled, venous and arterial blood concentrations of xylene. 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 (t) . Vmax, Km and Amet refer to the maximal velocity of metabolism, Michaëlis affinity constant, and amount metabolized. dt refers to integration interval.

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

RAmilk = rate of change in amount of chemical in breast milk
Rnurse = rate of change in amount of chemical ingested by nursing infant
Cmilk = concentration of chemical in breast milk
Vmilk = volume of milk currently in the mammary tissue lumen
Nurse = infant nursing rate
Szone = switch function to turn on or turn off the nursing over a 24-hr period
Cvm = venous blood leaving the milk compartment
Qm = blood flow to the mammary tissue

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