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– A LIFE-TIME PBPk MODEL FOR HUMANS DESCRIBING METABOLIC INTERACTIONS BETWEEN SUBSTANCES –

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Section 1 – INTRODUCTION

Physiologically based pharmacokinetic (PBPK) models propose a realistic even if simplified description of the mechanisms of absorption, distribution, metabolism and elimination of chemicals in the body. In these models, the body is subdivided into various compartments representing specific organs or homogeneous groups of tissues linked and irrigated by blood vessels. Compartments are characterised by a set of parameters of physiological relevance (*e.g.*, volume or blood perfusion rate) which play a crucial role in explaining the behaviour of chemical substances in the body, and represent invariants across substances. Physiologically based pharmacokinetic (PBPK) models offer great flexibility. In particular, they provide a parametric framework suitable for dealing with extrapolations between species, routes or dose levels (Gerlowski *et al.*, 1983; Reddy *et al.*, 2005; Chiu *et al.*, 2007).

A human lifespan is a long period compared to the typical residence time of chemicals in the body, and parameters are therefore usually assumed to be constant. Notable exceptions arise for persisting substances, such as 2,3,7,8-tetrachlorodibenzodioxin or lead, for which the elimination half-life may span years or decades (Hryhorczuk *et al.*, 1985; Kerger *et al.*, 2006). Parameters extrapolation between generation is an issue for all compounds, whatever their elimination rate. Considerable progress has been made in adult PBPK models for a variety of drugs. However, large gaps remain for newborns and infants, for which biometry and metabolism differ from adults (Yang *et al.*, 2006). The factors influencing the pharmacokinetics of chemicals are physiological (*e.g.*, tissue volumes and blood flow rates), physicochemical (*e.g.*, tissue:blood partition coefficients) and biochemical (*e.g.*, rates of chemical metabolism). The combination of the age-dependent changes in these factors may lead to adult-children differences in pharmacokinetics and tissue dose of chemicals. Some of these factors (*e.g.*, tissue volumes) are known to vary continually throughout the growth and development of the child. PBPK models may be used to integrate the available information on the age-dependent changes in the physiological, physicochemical and biochemical factors, and then evaluate their influence on the pharmacokinetics of chemicals (Yang *et al.*, 2006).

Another issue is kinetics during pregnancy and gestation, during which the pace of organogenesis and remodeling can be measured in days and becomes commensurable with the scale of persistence of many chemicals in the body. The pharmacokinetics of drugs or toxic chemicals during pregnancy are particularly difficult to study, for obvious reasons of risks to the foetus. A solution to this problem is to model the kinetics of substances in foetus and in the pregnant woman with PBPK models taking into account the time evolution of the foetus physiology and anatomy and, for the woman, the changes related to her pregnancy. Such models were adapted from the model developed by Luecke *et al.* (1994)

The deliverable is divided into 3 parts: description of a life-time PBPK model for humans, including man, woman, children and foetus, parameterization of the model, and applications. The example concerns a mixture of four VOCs (Benzene, Toluene, Ethylbenzene and Xylenes) and the assessment of the effects of the co-exposition on the individual pharmacokinetic profiles.



Section 2 – DESCRIPTION OF THE GENERAL PBPK MODEL

Two sections are introduced. We first present the structure of the PBPK model and then describe the models proposed for metabolism.

2.1. BASIC STRUCTURE OF THE PBPK MODEL

2.1.1. Man and non-pregnant woman

The model is presented in Figure 1. It subdivides the human body into 22 compartments: adipose tissues, adrenal glands, blood (arterial and venous), bones, brain, breast, gut, lumen in gut, heart, kidney, liver, lung, marrow, muscles, pancreas, sexual organs (testes, epididymes and prostate for man, and ovaries, fallopian tubes and uterus for woman), skin, spleen, stomach, lumen in stomach, thyroid, urinary tract (bladder, ureters, urethra). That structure is identical for man and woman.

That model yields to a set of ordinary differential equations. The differential equations were established for the quantity, Q , of chemical in each compartment. Concentrations, C , are obtained at any time by dividing Q by the compartment volume. Let introduce the notations used in the following: $Card$ is the cardiac output, F_i the blood flow entering in compartment i , PC_i the tissue i : blood partition coefficient, $PC_{lung:air}$ the partition coefficient of lung over air, C_{art} and C_{ven} the concentration in arterial and venous blood, Ka_i a diffusion parameter, Ke_i an excretion constant, K_{ij} a parameter describing exchanges between compartments i and j . All the parameters are subject to be time-dependent. For the clarity of the reading, we chose not to indicate that dependence in the parameter notations. Only the state variables are indicated as being time-dependent.

Units are: volumes in L, time in min, flows in L/min, concentrations in mM, masses in kg, perfusion rates in L/min/kg of tissue.

All the organs/tissues are considered as well-mixed compartments, and most of them are blood flow limited. Compartments with no input, elimination and metabolism, that are adipose tissues, adrenals, bone, brain, breast, heart, marrow, muscles, pancreas, sexual organs, skin, spleen, thyroid and urinary tract, are described by the following differential equation:

$$\frac{dQ_i(t)}{dt} = F_i \times \left(C_{art}(t) - \frac{C_i(t)}{PC_i} \right) \quad (1)$$

In kidneys, chemicals may be excreted via urine:

$$\frac{dQ_{kidney}(t)}{dt} = F_{kidney} \times \left(C_{art}(t) - \frac{C_{kidney}(t)}{PC_{kidney}} \right) - Ke_{renal} \times C_{kidney}(t) \quad (2)$$

The tractus gastro-intestinal is modelled with four compartments: gut, stomach and their respective lumens. In the stomach lumen, the parameter $Ka_{stomach}$ governs the diffusion of chemicals in the stomach, and then in the systemic circulation:

$$\frac{dQ_{stom_lumen}(t)}{dt} = RateIng - (F_{stom_lumen} + Ka_{stomach}) \times C_{stom_lumen}(t) \quad (3)$$



$$\frac{dQ_{stomach}(t)}{dt} = Ka_{stomach} \times C_{stom_lumen}(t) + F_{stomach} \times \left(C_{art}(t) + \frac{C_{stomach}(t)}{PC_{stomach}} \right) \quad (4)$$

$$\begin{aligned} \frac{dQ_{gut_lumen}(t)}{dt} = & F_{stom_lumen} \times C_{stom_lumen}(t) + Ke_{bile} \times C_{liver}(t) \\ & - (Ka_{gut} + F_{gut_lumen}) \times C_{gut_lumen}(t) \end{aligned} \quad (5)$$

$$\frac{dQ_{gut}(t)}{dt} = Ka_{gut} \times C_{gut_lumen}(t) + F_{gut} \times \left(C_{art}(t) - \frac{C_{gut}(t)}{PC_{gut}} \right) - Km_{gut} \times C_{gut}(t) \quad (6)$$

Outputs of spleen, pancreas, stomach and gut feed liver, as well as an arterial entry. In liver, chemicals can be eliminated via bile (Ke_{bile}) or be metabolised (Km_{liver}).

$$\begin{aligned} \frac{\partial Q_{liver}(t)}{\partial t} = & F_{liver_art} \times C_{art}(t) + F_{spleen} \times \frac{C_{spleen}(t)}{PC_{spleen}} + F_{pancreas} \times \frac{C_{pancreas}(t)}{PC_{pancreas}} + F_{gut} \times \frac{C_{gut}(t)}{PC_{gut}} \\ & + F_{stomach} \times \frac{C_{stomach}(t)}{PC_{stomach}} - F_{liver} \times \frac{C_{liver}(t)}{PC_{liver}} - (Ke_{bile} + Km_{liver}) \times C_{liver}(t) \end{aligned} \quad (7)$$

The sorting blood flow in liver is then given by:

$$F_{liver} = F_{liver_art} + F_{gut} + F_{pancreas} + F_{spleen} + F_{stomach} \quad (8)$$

and the total blood flow by:

$$\begin{aligned} F_{blood} = & F_{adip} + F_{adrenal} + F_{bone} + F_{brain} + F_{breast} + F_{heart} + F_{kidney} + F_{liver} \\ & + F_{marrow} + F_{muscle} + F_{organ_sex} + F_{skin} + F_{thyroid} + F_{urin_tract} \end{aligned} \quad (9)$$

In lungs, inhalation and metabolism may occur:

$$\frac{dQ_{lung}(t)}{dt} = F_{blood} \times \left(C_{ven}(t) - \frac{C_{lung}(t)}{PC_{lung}} \right) + F_{alv} \times \left(C_{inh}(t) - \frac{C_{lung}(t)}{PC_{lung:air}} \right) - Km_{lung} \times C_{lung}(t) \quad (10)$$

Differential equations for arterial and venous blood are:

$$\frac{dQ_{art}(t)}{dt} = F_{total} \times \left(\frac{C_{lung}(t)}{PC_{lung}} - C_{art}(t) \right) \quad (11)$$

$$\frac{dQ_{ven}(t)}{dt} = \sum_i \left[F_i \times \frac{C_i(t)}{PC_i} \right] - F_{total} \times C_{ven}(t) \quad (12)$$

where i designate the following compartments: adipose, adrenal, bone, brain, breast, heart, kidney, liver, marrow, muscle, sexual organs, skin, thyroid, and urinary tract.



2.1.2. Modelling pregnancy, foetus and post-pregnancy (including lactation)

Twenty compartments are added to the general PBPK model to describe pregnancy. They correspond to the placenta, the amniotic fluid and the foetus. Figure 2 presents the description of the model for the pregnant woman including the foetus.

Pregnancy

Pregnancy has an impact on four compartments: adipose tissues, breast, sexual organs and blood. However, the pregnancy does not change the structure of the general PBPK model except for the compartment "sexual organs". During pregnancy, equal diffusion flow from uterus to placenta and vice-versa is assumed. We also assume that the weight of the ovaries and the fallopian tubes is negligible in the compartment "sexual organs" compared to the weight of the uterus, and that exchanges with the foetus occurred via the "sexual organs" compartment. The differential equation for that compartment then becomes:

$$\frac{dQ_{sex_organs}(t)}{dt} = F_{sex_organs} \times \left(C_{art}(t) - \frac{C_{sex_organs}(t)}{PC_{sex_organs}} \right) - K_{uter2plac} \times (C_{placenta}(t) - C_{sex_organs}(t)) \quad (13)$$

Equations for the placenta and the amniotic fluid are the following ones:

$$\begin{aligned} \frac{dQ_{placenta}(t)}{dt} = & K_{uter2pla} \times (C_{placenta}(t) - C_{sex_organs}(t)) + F_{placenta} \times \left(C_{art_foetus}(t) - \frac{C_{placenta}(t)}{PC_{placenta}} \right) \\ & - K_{pla2amniot} \times \left(C_{placenta}(t) - C_{amniot}(t) \frac{PC_{placenta}}{PC_{amniot}} \right) - Km_{placenta} \times C_{placenta}(t) \end{aligned} \quad (14)$$

$$\begin{aligned} \frac{dQ_{amniot}(t)}{dt} = & K_{pla2amniot} \times \left(C_{placenta}(t) - C_{amniot}(t) \frac{PC_{placenta}}{PC_{amniot}} \right) + Ke_{gut_foetus} \times C_{gut_foetus}(t) \\ & + Ke_{bile_foetus} \times C_{liver_foetus}(t) - Ka_{amniot_foetus} \times C_{amniot}(t) \end{aligned} \quad (15)$$

Foetus

Eighteen compartments describe the foetus. Adipose tissue, adrenal glands, brain, breast, heart, kidneys, lungs, marrow, muscles, the compartment "other tissues", pancreas, skin, spleen, thymus, and thyroid are supposed to be well-mixed and blood flow limited, and are described by Equation (1).

In gut and liver, the exchanges of materials are given by:

$$\frac{dQ_{gut}(t)}{dt} = F_{gut} \times \left(C_{art}(t) - \frac{C_{gut}(t)}{PC_{gut}} \right) + Ka_{amniot} \times C_{amniot}(t) - Ke_{gut} \times C_{gut}(t) \quad (16)$$

$$\begin{aligned} \frac{dQ_{liver}(t)}{dt} = & F_{liver_art} \times C_{art}(t) + F_{spleen} \times \frac{C_{spleen}(t)}{PC_{spleen}} + F_{pancreas} \times \frac{C_{pancreas}(t)}{PC_{pancreas}} - \\ & F_{liver} \times \frac{C_{liver}(t)}{PC_{liver}} - (Ke_{bile} + Km_{liver}) \times C_{liver}(t) \end{aligned} \quad (17)$$

where the liver blood flow is:



$$F_{liver} = F_{liver_art} + F_{spleen} + F_{pancreas} \quad (18)$$

The quantity of substance in the foetus venous blood is obtained by:

$$\frac{dQ_{ven}(t)}{dt} = \sum_i \left[F_i \times \frac{C_i(t)}{PC_i} \right] - F_{total} \times C_{ven}(t) + F_{placenta} \times \frac{C_{placenta}(t)}{PC_{placenta}} \quad (19)$$

where i designate the following organs: adipose tissues, adrenal glands, brain, gut, heart, kidney, liver, marrow, muscle, "other tissues", skin, thymus and thyroid.

There is no need for a differential equation for arterial blood as the concentration of chemical can be obtained at any time using:

$$C_{art}(t) = \left(1 - \frac{F_{lung}}{F_{total}} \right) \times C_{ven}(t) + \frac{F_{lung}}{F_{total}} \times \frac{C_{lung}(t)}{PC_{lung}} \quad (20)$$

In the foetus, lung is irrigated by a bifurcation and its blood flow does not enter the calculation for the total blood flow:

$$F_{blood} = F_{adip} + F_{adrenal} + F_{brain} + F_{gut} + F_{heart} + F_{kidney} + F_{liver} + F_{marrow} + F_{muscle} + F_{other} + F_{placenta} + F_{skin} + F_{thymus} + F_{thyroid} \quad (21)$$

Lactation

In case of lactation, an elimination occurs in breast, via milk production:

$$\frac{dQ_{breast}(t)}{dt} = F_{breast} \times \left(C_{art}(t) - \frac{C_{breast}(t)}{PC_{breast}} \right) - F_{milk} \times \frac{C_{breast}(t) \times PC_{milk}}{PC_{breast}} \quad (22)$$

2.2. Modelling metabolism and metabolic interactions

During co-exposures of chemicals, interactions at the metabolic level occur when one chemical induce or inhibit the metabolism of another chemical in the mixture. Metabolic inhibition can be 'competitive' when chemicals compete as substrates for the same site on an enzyme. In 'noncompetitive inhibition', the inhibitor binds to the enzyme, causing a change in the stereochemical arrangement of the enzyme such that the substrate cannot bind. If the chemicals have different enzymatic binding sites, and the substrate must first bind to the enzyme before the inhibitor can, the interaction is termed 'uncompetitive inhibition'. In other cases, some chemicals can also increase the number of binding sites through an induction process leading to an induction of hepatic metabolism.

To model such phenomena, PBPK models accounting for metabolic interactions can be used. For example, in binary mixtures, a PBPK model is developed for each chemical, and the PBPK models are interconnected at the level of the tissue compartment where the interaction is supposed to occur (Krishnan *et al.*, 2002). An equation or a set of equations is used to model that interconnection. Here, we proposed two kinds of model: the modelling of enzyme kinetics and the Michaelis-Menten relationship.

2.2.1. Modelling the enzymatic kinetics

The reaction of a substrate S with an enzyme E is given by the following scheme:



where ES is the complex Enzyme-Substrate, P the product of the reaction, and k_i the reaction rates. That reaction can be modelled by the following set of differential equations:

$$\begin{cases} \frac{d[S]}{dt} = k_{-s} \cdot [ES] - k_s \cdot [E] \cdot [S] \\ \frac{d[P]}{dt} = k_c \cdot [ES] \\ \frac{d[E]}{dt} = (k_c + k_{-s}) \cdot [ES] - k_s [S] \cdot [E] \\ \frac{d[ES]}{dt} = k_s \cdot [E] \cdot [S] - (k_{-s} + k_c) \cdot [ES] \end{cases} \quad (23)$$

In case of co-exposures, that model becomes for the substrate i and the enzyme j (mixture with n substrates and m enzymes):

$$\begin{cases} \frac{d[S_i]}{dt} = \sum_{j=1}^m (k_{-E_j S_i} \cdot [E_j S_i] - k_{E_j S_i} \cdot [E_j] \cdot [S_i]) \\ \frac{d[P_i]}{dt} = \sum_{j=1}^m (k_{C_j S_i} \cdot [E_j S_i]) \\ \frac{d[E_j]}{dt} = \sum_{j=1}^m \left(\sum_{i=1}^n [E_j S_i] \cdot (k_{C_j S_i} + k_{-E_j S_i}) - [E_j] \cdot \sum_{i=1}^n k_{E_j S_i} [S_i] \right) \\ \frac{d[E_j S_i]}{dt} = \sum_{j=1}^m (k_{E_j S_i} \cdot [E_j] \cdot [S_i] - (k_{-E_j S_i} + k_{C_j S_i}) \cdot [E_j S_i]) \end{cases} \quad (24)$$

where $k_{E_j S_i}$ is the rate of the reaction $S_i + E_j \rightarrow E_j S_i$, and $k_{C_j S_i}$ the rate of the reaction $E_j S_i \rightarrow E_j + P_i$.

2.2.2. Michealis-Menten model

Typically chemical metabolism is described by the classical Michaelis-Menten relationship. The rate of metabolism is then given by:

$$RAM = \frac{Vmax \times C}{C + Km} \quad (25)$$

where $Vmax$ is the maximal velocity of metabolism, Km the Michaelis affinity constant, and C the venous blood concentrations of the chemical leaving the site of metabolism. When metabolic interactions occur, the rate of metabolism of each chemical can be calculated using a classical Michaelis-Menten equation modified by a modulation factor reflecting the effect of interaction. The resulting change in the metabolism rate for chemical j (RAM_j) can be expressed as a function of the following variables: Its maximal velocity of metabolism ($Vmax_j$), its Michaelis affinity constant (Km_j), the venous blood concentrations of the chemical leaving the site of metabolism (C_{vt}^j), the venous blood concentrations of the competing chemicals at the site of metabolism (C_{vt}^a) and the inhibition constant (Ki_{aj}). Table 1 presents the formulae used for the different kinds of interaction.



Section 3 – Parameterisation of the PBPK model

A literature search was performed to obtain data on modifications of the physiology of human related to age. That search was focused on the time evolution of the bodyweight, the relative tissue weight, the total or relative tissue blood flows, the elimination capabilities (metabolism and excretion), the parameters driving the entering of chemicals in the body (*i.e.*, inhalation or ingestion). Two sources were used (Altman *et al.*, 1962; International Commission on Radiological Protection, 2002). We supposed that the parameters' values proposed for adults in the ICRP report were valid for humans from the age of 20 years old.

3.1. Physiological and anatomical parameters

3.1.1. Body mass

Data on age-related changes in human bodyweight were obtained from Altman and Dittmer (1962) for children (0 up to 18 years old) and in the ICRP report (International Commission on Radiological Protection, 2002) for adults. Using a biology-based model (in particular Von Bertalanffy equation), as suggested by some authors (Kooijman, 2000) to fit human growth data, did not provide a relevant fitting. A polynomial function was thus chosen to fit the data for males and females, where Age is in years:

MALES:

if Age < 20 then

$$BDW = -1.2252E-03 \times Age^4 + 4.9976 E-02 \times Age^3 - 5.8012 E-01 \times Age^2 + 4.7834 \times Age + 4.8104 \quad (26)$$

if Age ≥ 20 then BDW =73.

FEMALES:

if Age < 20 then

$$BDW = 2.2334E-04 \times Age^5 - 1.2150E-02 \times Age^4 + 2.2912E-01 \times Age^3 - 1.7317 \times Age^2 + 7.2401 \times Age + 3.7721 \quad (27)$$

if Age ≥ 20 then BDW =60.

3.1.2. Tissue volumes

We used the data collected by Haddad *et al.* (2001b) and ICRP (International Commission on Radiological Protection, 2002) on the time evolution of organ weights during childhood to set values to the tissue volumes. Instead of dealing with tissue volumes, we used the relative tissue volumes (fraction of bodyweight). The relative volume was constant during life time for 2 compartments: bone and pancreas. The volume for adipose tissues was modelled as 96% of body mass minus the volume of other tissues. Separable connective tissues and certain lymphatic tissues account for most of the remaining 4% of body mass.

Three different models were fitted to the collected data. Model 1 is the following exponential function:

$$Parameter = y_{inf} + (y_0 - y_{inf}) \times \exp(-\lambda \times Age) \quad (28)$$



where y_{inf} is the value of parameter in infinity (set to the adult values), and y_0 the parameter's value at birth. y_0 and λ are estimated. Model 2 corresponds to:

$$Parameter = \frac{Age + a}{b + c \times Age} \quad (29)$$

Parameters a , b and c are estimated. The third model is a polynomial function. The first two functions were preferred to the polynomial function to describe the time evolution of some physiological and anatomical parameters since these models have an asymptote in infinity. For few parameters, it has not been possible to define a function for the lifetime, so two functions were defined on two different time ranges. Table 2 and 3 sum up the equations used for the relative volumes for man and woman respectively. Figure 3 presents the evolution of few relative organs' volumes with age for males and females.

3.1.3. Cardiac output

We used the data proposed in the ICRP report to fit an exponential relationship. The cardiac output for males and females is respectively given by:

$$Card = 6.5 + (0.385 - 6.5) \times \exp(-0.144 \times Age) \quad (30)$$

$$Card = 5.9 + (0.362 - 5.9) \times \exp(-0.171 \times Age) \quad (31)$$

3.1.4. Blood flows

Due to the lack of data on blood flow changes with age, regional blood flows were assumed to change proportionally with tissue volumes, as proposed by Clewell et al. (2004). The adult fractional tissue blood flows referenced by ICRP (International Commission on Radiological Protection, 2002) were used along with the age-specific tissue volumes and the adult tissue volumes. To maintain mass balance for the blood flows, the age-specific fractional blood flows were normalized to always sum to 0.95 (sum of the relative blood flows of the organs and tissues described in the model). The age-specific fractional blood flows were obtained with:

$$sc_F_i = \frac{sc_V_i}{sc_V_{i_adult}} \times sc_F_{i_adult} \quad (32)$$

$$F_i = \frac{sc_F_i}{\sum_i F_i} \times Card \quad (33)$$

$$\frac{1}{0.95}$$

where sc_F_i and sc_V_i correspond to the relative organ blood flow and weight. The values for the blood flow scaling factor in adult are given in Table 4.

3.1.5. Respiratory parameters and transits

Three parameters were used to describe the lungs: tidal volume, respiratory frequency and dead space volume. Table 5 presents the relationships fitted to reference values (International Commission on Radiological Protection, 2002). These parameters were used to calculate the ventilation minute and the alveolar flow rate as following:



$$F_{pul} = V_{tidal} \times FreqBreath \quad (34)$$

$$F_{alv} = (V_{tidal} - V_{ds}) \times FreqBreath \quad (35)$$

The time evolution for transits are also presented Table 5.

3.1.6. Parameterisation of the foetus

We used the models developed by Luecke *et al.* (1994) to parameterise the foetus. The foetal weight (in grams) is given by:

$$BDW_{foetus} = 0.00137 \times \exp\left(\frac{0.1974 \times (1 - \exp(-0.01306 \times GestAge))}{0.01306}\right) \quad (36)$$

where *GestAge* is the gestational age computed in days.

The volumes of foetal compartment are assumed to be proportional to foetal weight and are described by the following relationship:

$$V_{i_{foetus}} = a_i \times \exp\left((b_i + c_i \times \ln(BDW_{foetus})) \times \ln(BDW_{foetus})\right) \quad (37)$$

The proportionality constants are given in Table 6 for each compartment. Figure 4 presents the time evolution of the foetal bodyweight and of some organs' volumes.

The relative blood flows for the foetus were obtained with:

$$sc - F_{i_{foetus}} = \frac{sc - V_{i_{foetus}}}{sc - V_{i_{newborn}}} \times sc - F_{i_{newborn}} \quad (38)$$

The cardiac output was obtained by adding all the blood flows.

3.1.7. Parameters changing with pregnancy

During pregnancy and the postpartum period, volumes and blood flows of four maternal compartments are modified: adipose tissues, blood, breast and sexual organs. As proposed by Luecke *et al.* (1994), the increase in the volume was modelled by equation (19), as well as the volume of the placenta and the amniotic fluid. Parameters' values are listed in Table 6. The blood flow of these compartments was a function of the volume and the blood flow of the organ before pregnancy and the organ's volume at the considered moment (Gentry *et al.*, 2002):

$$F_{i_{pregnancy}} = F_i \times \frac{V_i}{V_{i_{pregnancy}}} \quad (39)$$

Due to the lack of data on decreases in tissue volumes after pregnancy and / or during lactation, we made several assumptions. The tissue volume for adipose was modelled as a linear decrease over 6 months to the pre-pregnancy value (Gentry *et al.*, 2003b). The tissue volumes for blood and sexual organs were modelled as a linear decrease over 1 week to the pre-pregnancy value. In case of lactation, it was assumed that maximum increases in breast volume would be achieved approximately two weeks postpartum and sustained throughout lactation. Increases in breast volume, compared to tissue volume prior to lactation, are based on information from ICRP. In case of not a lactation period, breast volume was modelled as a linear decrease over 1 week to the pre-pregnancy value.



3.2. Substance specific parameters

Parameters specific to the substance include partition coefficients, elimination constant, or diffusion. Such parameters are typically obtained in the literature from previous experiments or with QSAR models. If no information is available for partition coefficients, we propose to calculate them by multiplying the fraction of fat in the corresponding tissue by the reference ("pure fat" over blood) partition coefficient, PC_{ref} :

$$PC_i = f_{fat_i} \times PC_{ref} \quad (40)$$

Table 7 gives the fraction of fat in human organs.

Section 4 – APPLICATIONS: THE BTEX MIXTURE

In this section, we propose to model the toxicokinetics of the BTEX mixture. To treat this example, and for brevity, we use a simplified version of our general PBPK model. The time evolutions of organs are not modelled since we are only interested here in short exposures (few hours). We lumped together some compartments of the model to describe only compartments of particular toxicological interest. The model used here is described below.

The PBPK model for a generic mixture of chemicals is represented as a combination of "single chemical" models interconnected at level of hepatic metabolism where the effect of the interaction is evaluated according to the potential mechanism of action (competitive, non-competitive, and uncompetitive metabolic inhibitions). The latter, in the case of a BTEX mixture, is assumed to be of "competitive inhibition" since the four VOC's considered are known substrates for the same cytochrome P450 isozyme (CYP2E1). This was confirmed by the analysis of the kinetic data from all binary exposure studies relevant to the BTEX mixture (Haddad *et al.*, 1999).

4.1. Models for BTEX

The models for toluene, ethylbenzene and all the family of xylenes are all four-compartment models encompassing richly perfused tissues (RPT), poorly perfused tissues (PPT), adipose tissues (FAT), and liver (metabolising tissue), interconnected by systemic circulation and a gas exchange lung. The model for benzene is a six-compartment model. The six tissue groups include: liver (main metabolic tissue); adipose tissue (FAT); richly perfused tissues (RTP); poorly perfused tissues (PPT); bone marrow (the main target organ for benzene toxicity) and the kidney; each one interconnected to the others by systemic circulation and a gas-exchange lung. The bone marrow was included because it is recognised as the main site manifesting benzene toxicity (i.e., leukaemia) and because it is, together with the kidney, a potential site benzene metabolism. The liver was further subdivided into three equal volume sub-compartments according to the zonal distribution of enzymes that mediate benzene metabolism (Cole *et al.*, 2002). Metabolism mediated by CYP2E1 is assumed to occur by and large in "zone 3" of the liver; sulfation takes place primarily in "zone 1"; non-enzymatic metabolism occurs in all the three compartments as well as in all tissues. The overall conceptual representation of the PBPK model for a mixture of BTEX is shown in Figure 5.

The rate of change in the concentration of chemical in each non-metabolizing tissue (t) is described by ordinary differential equations of the following general type:

$$Vol_t \cdot \left(\frac{dC_t^j}{dt} \right) = Q_t \cdot (C_a^j - C_{vt}^j) \quad (41)$$



where V_{vt} is the tissue volume; C_t^j the concentration of chemical j in tissue t ; Q_t is the blood flow to tissues t ; C_a^j the arterial blood concentration of chemical j and C_{vt}^j the venous blood concentrations of chemical j leaving tissue compartments.

The concentration of chemical i in venous blood leaving tissue t is described by:

$$C_{vt}^j = \frac{C_t^j}{P_t^j} \quad (42)$$

where P_t^j is the tissue (t): blood partition coefficient for chemical j

The arterial blood concentration is:

$$C_a^j = \frac{Q_{ven} C_{inh}^j + Q_c C_v^j}{Q_c + \left(\frac{Q_{ven}}{P_{b.a}^j}\right)} \quad (43)$$

where Q_{ven} is the alveolar ventilation; C_{inh}^j the concentration of chemical j inhaled, Q_c is the cardiac output; C_v^j is the venous blood concentration of chemical j and $P_{b.a}^j$ the partition coefficient of blood:air for chemical j .

To account for differences in the physiological and metabolic parameters due to varying body weights, the following allometric equations were used:

$$Q_{TOT} = Q_{car} \cdot BW^{0.75} \quad (44)$$

$$Q_{ven} = Q_{venc} \cdot BW^{0.75} \quad (45)$$

$$V_{max} = V_{max_c} \cdot BW^{0.75} \quad (46)$$

where Q_{tot} is the cardiac output, Q_{ven} is the alveolar ventilation rate and BW is the Body Weight and V_{max} is the maximal velocity for metabolism.

For tissues site of metabolism, a Michaelis-Menten term describing the metabolic clearance is added. For them, the general differential equation assumes the following form:

$$Vol_t \cdot \left(\frac{dC_t^j}{dt} \right) = Q_t \cdot (C_a^j - C_{vt}^j) - \frac{V_{max_j} \cdot C_{vt}^j}{Km_j \cdot \left(1 + \frac{C_{vt}^a}{Ki_{aj}} + \frac{C_{vt}^b}{Ki_{bj}} + \frac{C_{vt}^c}{Ki_{cj}} \right) + C_{vt}^j} \quad (47)$$

where the last term on the right is the already described term modulating the rate of metabolism of chemical j considering the interaction with the other chemical.

The high potential toxicity of benzene metabolites associated to the leukaemia risk in humans, suggested taking into account more in detail the metabolic chain from benzene to its key metabolites through a more refined PBPK model for that chemical. The whole metabolic chain of benzene (see Figure 6) was modelled starting from previously developed PBPK models for benzene metabolism in mice (Cole *et al.*, 2002) and its extrapolation to humans (Yokley *et al.*, 2006). The model, shown in Figure 7, evaluates tissue levels of benzene, benzene oxide (BO), phenol (PH), and hydroquinone



(HQ), as well as the total amounts of muconic acid (MA), phenylmercapturic acid (PMA), phenol conjugates, hydroquinone conjugates, and total catechol produced. For benzene oxide, phenol, and hydroquinone, the body is divided into five compartments: kidney; liver; fat; rapidly perfused tissues (RTP), and slowly perfused tissues (PPT). As for the benzene model the liver is subdivided into three compartments of equal volume according to the specific enzymatic distribution. The further metabolism of BO, PH and HQ is supposed to occur in the liver (main metabolism organ) and to a lesser extent in the kidney.

More in detail the followings metabolic transformations (mediated by CYP2E1) are supposed to occur in zone 3 of the liver as well as in the kidney:

benzene → benzene oxide

phenol → hydroquinone

phenol → catechol

hydroquinone → trihydroxy benzene

The equations describing these metabolic reactions are given hereafter. All the abbreviations and symbols used in the PBPK model are given in Appendix.

CYP2E1 activity in the liver (zone 3 of the liver):

$$RM_{BO,Liver3}^{BZ} = k_1 \frac{V_{2E1} C_{Liver3}^{BZ}}{D_L} C^{MP} \frac{T_L}{3} \quad \text{Benzene to Benzene Oxide}$$

$$RM_{HQ,Liver3}^{PH} = k_5 \frac{V_{2E1} C_{Liver3}^{PH}}{D_L} C^{MP} \frac{T_L}{3} \quad \text{Phenol to Hydroquinone}$$

$$RM_{CAT,Liver3}^{PH} = k_6 \frac{V_{2E1} C_{Liver3}^{PH}}{D_L} C^{MP} \frac{T_L}{3} \quad \text{Phenol to Catechol}$$

$$RM_{THB,Liver3}^{HQ} = k_7 \frac{V_{2E1} C_{Liver3}^{HQ}}{D_L} C^{MP} \frac{T_L}{3} \quad \text{Hydroquinone to Trihydroxy benzene}$$

where:

$$D_L = 1 + A^{BZ} C_{Liver3}^{BZ} + A^{PH} C_{Liver3}^{PH} + A^{HQ} C_{Liver3}^{HQ}$$

Since the kidney contains approximately 10% of the concentration of CYP2E1 found in the liver (Dewaziers *et al.*, 1990), it is assumed that relative to the metabolism in the liver, 10% of the metabolism mediated by CYP2E1 is in the kidney. The V2E1, that is the CYP2E1 specific activity as determined by the oxidation of p-nitrophenol to p-nitrocatechol, is scaled by 10% to give us the rate equations for the metabolisms using CYP2E1 in the kidney:

CYP2E1 activity in the kidney:

$$RM_{BO,Kidney}^{BZ} = k_1 \frac{V_{2E1} C_K^{BZ}}{10D_K} C^{MP} T_K \quad \text{Benzene to Benzene Oxide}$$

$$RM_{HQ,Kidney}^{PH} = k_5 \frac{V_{2E1} C_K^{PH}}{10D_K} C^{MP} T_K \quad \text{Phenol to Hydroquinone}$$



$$RM_{CAT,Kidney}^{PH} = k_6 \frac{V_{2E1} C_K^{PH}}{10D_K} C^{MP} T_K \quad \text{Phenol to Catechol}$$

$$RM_{THB,Kidney}^{HQ} = k_7 \frac{V_{2E1} C_K^{HQ}}{D_K} C^{MP} T_K \quad \text{Hydroquinone to Trihydroxy benzene}$$

where:

$$D_K = 1 + A^{BZ} C_{Kidney}^{BZ} + A^{PH} C_{Kidney}^{PH} + A^{HQ} C_{Kidney}^{HQ}$$

and T_L and T_K are respectively the total mass of the liver and the total mass of the kidney. Assuming that tissue has the same density as water, we obtain:

$$T_j = V_j \times \frac{10^3 \text{ g}}{1L}$$

where j could be the liver (L) or the kidney (K)

Since epoxide hydrolase, which mediates the metabolism of benzene oxide to muconic acid, is found in the centrilobular region (zone 3) of the liver (Parkinson, 1996), this metabolism is also supposed to occur in the zone 3 of the liver and it can be described by the first-order equation:

$$RM_{MA,Liver3}^{BO} = k_4 C_{Liver3}^{BO} \frac{V_L}{3} \quad \text{Benzene Oxide to Muconic Acid}$$

The metabolism of hydroquinone to its conjugates is assumed occurs in zone 3 since the glucuronidation capacity is greater in this region (Medinsky *et al.*, 1996). It can be represented by the equation for glucuronidation from (Seaton *et al.*, 1995):

$$RM_{Conj,Liver3}^{HQ} = \frac{V_{HQ} C_{Liver3}^{HQ}}{K_m^{HQ} + C_{Liver3}^{HQ}} C^{MP} \frac{T_L}{3} \quad \text{Hydroquinone to its conjugates}$$

Since the sulfation takes place primarily in zone 1 of the liver (Seaton *et al.*, 1995), the conjugation of phenol was simulated occurring only in zone 1 and, according to (Seaton *et al.*, 1995), it is represented by the following equation:

$$RM_{Conj,Liver1}^{PH} = \left(\frac{V_{PH1} C_{Liver1}^{PH}}{K_{m,1}^{PH} + C_{Liver1}^{PH}} + \frac{V_{PH2} C_{Liver1}^{PH}}{K_{m,2}^{PH} + C_{Liver1}^{PH}} \right) C^{CP} \frac{T_L}{3} \quad \text{Phenol to its conjugates}$$

The metabolism of benzene oxide to phenol is nonenzymatic, so we assumed that this metabolism occurs in all compartments. This process is described by the first-order equation:

$$RM_{PH,j}^{BO} = k_2 C_j^{BO} V_j \quad \text{Benzene Oxide to Phenol}$$

where j is the compartment index.

Finally, glutathione S-transferase, which is required for the metabolism of benzene oxide to phenylmercapturic acid, is found in tissue such as the liver, kidney, muscle, and heart (Lee, 1984). Within the liver, glutathione S-transferase is found primarily in the plate limiting hepatocytes of the central vein (Mainwaring *et al.*, 1996). Thus, we consider first-order metabolism to occur in the slowly and rapidly perfused tissues, the fat, the kidney, the blood, and the third zone of the liver according to the following equation:



$$RM_{PMA,j}^{BO} = k_3 C_j^{BO} V_j$$

Benzene Oxide to Phenylmercapturic acid

4.2. Parameters' values

The numerical values of the inhibition constant are given in Table 8. The scaling coefficients Q_{car} , Q_{venc} and V_{max_c} are reported in Table 9 and they were determined from reference values given in (Haddad *et al.*, 2001a). The numerical values of the physiological, physicochemical and biochemical parameters are given in Table 9 along with their reference source. The partition coefficients for metabolites used are summarised in Table 10 while the numerical values of the parameters related to benzene metabolism are given in Table 11.

Section 5 – DISCUSSION

5.1. The general PBPK model

We developed a lifetime PBPK model for humans, including man, woman, children and foetus. It also models pregnancy and lactation. Several physiological models have been proposed in the literature to describe the life-time period (Clewel *et al.*, 2004) or the gestation/lactation period (Luecke *et al.*, 1994; Corley *et al.*, 2003; Gentry *et al.*, 2003a; Gentry *et al.*, 2003b). Here we propose a PBPK model that includes these two periods. This general model was parameterised using data from the literature (Altman *et al.*, 1962; International Commission on Radiological Protection, 2002). The anatomical or physiological changes in humans have been previously described by several authors (Haddad *et al.*, 2001b; Price *et al.*, 2003; Clewel *et al.*, 2004). However, in this work, we have developed new mathematical functions for modelling those changes since most of the functions proposed in the literature were developed only for children and do not include adulthood. The specificity of the functions we proposed is that they are continuous during the lifetime.

However, our model has some limitations that just reflect some lacks of physiological data. For instance, it does not take into account the changes related to the age for adults. During aging, physiological and disease-induced changes occur which might affect pharmacokinetics of many chemicals (Klotz, 1998). Moreover, elderly patients represent an increasing part of our population who consume disproportionately high amounts of drugs. Assessing distributions and interactions of drugs and chemicals present in the environment is particularly meaningful for this population.

Our general PBPK model is quite detailed and proposes an intensive list of organs. In some cases, due to a relatively small amount of data, or because it is relevant to focus only on some specific organs, it would be interesting to reduce the model by aggregating some of the organs. There are some general principles for lumping correctly PBPK models (Nestorov *et al.*, 1998). An alternative for simplification could be to express all organ-specific partition coefficients as a function of a two generic partition coefficients, for fat and lean tissues for instance (Bjorkman, 2003). Partition coefficients for organs would then be derived in accordance with the percentage of these two tissues in the organs. Organs of particular pharmacological or toxicological interest should of course be investigated separately as needed.

To conclude, our PBPK model is quite general and can be easily adapted to every particular situation.



5.2. The interaction models

Two methods were developed to study metabolic interactions. They all assumed that interactions occur at the metabolic level. The first one is based on the modelling of metabolism with the Michaelis-Menten relationship and the second one on the modelling of the enzyme kinetics. Our next step would be to compare behaviours and predictions of these two models. Data for a mixture of four VOCs (Benzene, Toluene, Ethylbenzene and Xylenes) should permit this comparison.

Calibrating the interaction models will make necessary an extensive bibliographic search on enzyme in humans (sites and levels in the different tissues for humans of all ages). Then, for a given substance, a collection of the metabolic pathways together with analysis of experimental data would permit to parameterise the model and extrapolate kinetics data for different doses and different ages.



Section 6 – REFERENCES

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Section 7 – TABLES

Table 1: Mathematical descriptions of the metabolic interactions for different kinds of inhibition. In these models, the substance 1 is the substrate and the substance 2 the inhibitor.

Table 2: Equations describing the change in the relative organ weights as a function of age (year) for males.

Table 3: Equations describing the change in the relative organ weights as a function of age (year) for females.

Table 4: Relative blood flow (as percentage of the cardiac output) for human adults.

Table 5: Time evolution of some physiological parameters for humans.

Table 6: Values for parameters (a , b , c) of equations describing the change in the relative organ weights as a function of the gestational age (day) for the foetus and the pregnant woman.

Table 7: Fraction of fat in organ or tissue.

Table 8: Inhibition constants (K_i) for the BTEX mixture (Haddad *et al.*, 1999).

Table 9: Human physiological, physicochemical and biochemical parameters used in PBPK model for BTEX.

Table 10: Partition coefficient for benzene metabolites (Cole *et al.*, 2002)

Table 11: Human metabolic parameters used in the benzene section of the PBPK model.



Table 1: Mathematical descriptions of the metabolic interactions for different kinds of inhibition. In these models, the substance 1 is the substrate and the substance 2 the inhibitor.

Mechanism of inhibition	Model
No inhibition	$\frac{V_{max,1} \times C_{F,1}}{K_{m,1} + C_{F,1}}$
Competitive	$\frac{V_{max,1} \times C_{F,1}}{K_{m,1} \times \left(1 + \frac{C_{F,2}}{K_{i,2}}\right) + C_{F,1}}$
Uncompetitive	$\frac{V_{max,1} \times C_{F,1}}{(K_{m,1} + C_{F,1}) \times \left(1 + \frac{C_{F,2}}{K_{i,2}}\right)}$
Non-competitive	$\frac{V_{max,1} \times C_{F,1}}{K_{m,1} + C_{F,1} \times \left(1 + \frac{C_{F,2}}{K_{i,2}}\right)}$



Table 2: Equations describing the change in the relative organ weights as a function of age (year) for males.

Organs	Equation	
Adrenals ⁽¹⁾	$0.0002 + (0.00171 - 0.0002) \times \exp(-2.02 \times \text{Age})$	
Blood ⁽¹⁾	$(0.0500 - 0.0771) \times \text{Age} + 0.0771$	If Age < 1
	$(\text{Age} - 0.6939) / (-6.8933 + 13.016 \times \text{Age})$	if Age ≥ 1
Brain ⁽²⁾	$0.0199 + (0.117 - 0.0199) \times \exp(-0.163 \times \text{Age})$	
Breast ⁽¹⁾	0	If Age ≤ 10
	$(\text{Age} - 10.001) / (2747.8 \times \text{Age} - 23464)$	if Age > 10
Gut ⁽¹⁾	$-0.000082562 \times \text{Age}^2 + 0.0013523 \times \text{Age} + 0.01293$	If Age ≤ 16
	0.0140	if Age > 16
Gut lumen ⁽¹⁾	$0.0089 + (0.0276 - 0.0089) \times \exp(-0.574 \times \text{Age})$	
Heart ⁽²⁾	$-4.0837\text{E-}07 \times \text{Age}^3 + 1.9982\text{E-}05 \times \text{Age}^2 - 2.6369\text{E-}04 \times \text{Age} + 5.5982\text{E-}03$	If Age ≤ 18
	$2\text{E-}4 \times \text{Age} + 8.5\text{E-}3$	if Age ≤ 20
	0.0045	if Age > 20
Kidneys ⁽²⁾	$0.0042 + (0.00767 - 0.0042) \times \exp(-0.206 \times \text{Age})$	
Liver ⁽²⁾	$0.0247 + (0.0409 - 0.0247) \times \exp(-0.218 \times \text{Age})$	
Lung ⁽¹⁾	$1.4770\text{E-}05 \times \text{Age}^2 - 3.7583\text{E-}04 \times \text{Age} + 8.4216\text{E-}03$	If Age ≤ 20
	0.0068	if Age > 20
Marrow ⁽¹⁾	$0.05 + (0.0138 - 0.05) \times \exp(-0.112 \times \text{Age})$	
Muscle ⁽¹⁾	$0.3973 + (0.201 - 0.3973) \times \exp(-0.141 \times \text{Age})$	
Sexual organs ⁽¹⁾	$-1.5156\text{E-}07 \times \text{Age}^3 + 9.3351\text{E-}06 \times \text{Age}^2 - 1.1177\text{E-}04 \times \text{Age} + 4.7966\text{E-}04$	If Age ≤ 20
	0.0008	if Age > 20
Skin ⁽¹⁾	$-1.1706\text{E-}05 \times \text{Age}^3 + 5.4130\text{E-}04 \times \text{Age}^2 - 6.1966\text{E-}03 \times \text{Age} + 4.6231\text{E-}02$	If Age ≤ 20
	0.0452	if Age > 20
Spleen ⁽²⁾	$2.8337\text{E-}08 \times \text{Age}^5 - 1.6264\text{E-}06 \times \text{Age}^4 + 3.3436\text{E-}05 \times \text{Age}^3 - 2.9124\text{E-}04 \times \text{Age}^2 +$	If Age ≤ 20
	$9.0793\text{E-}04 \times \text{Age} + 2.5047\text{E-}03$	
	0.0021	if Age > 20
Stomach ⁽¹⁾	$-1.1901\text{E-}07 \times \text{Age}^3 - 9.2180\text{E-}06 \times \text{Age}^2 + 1.7879\text{E-}04 \times \text{Age} + 1.9319\text{E-}03$	If Age ≤ 15
	0.0021	if Age > 15
Stomach lumen ⁽¹⁾	$0.0034 + (0.0105 - 0.0034) \times \exp(-0.522 \times \text{Age})$	
Urinary tract ⁽¹⁾	$0.001041 + (0.00145 - 0.001041) \times \exp(-0.348 \times \text{Age})$	

(1): (International Commission on Radiological Protection, 2002)

(2): (Altman *et al.*, 1962)



Table 3: Equations describing the change in the relative organ weights as a function of age (year) for females.

Organs	Equation	
Adrenals ⁽¹⁾	$0.0002+(0.00171-0.0002) \times \exp(-2.02 \times \text{Age})$	
Blood ⁽¹⁾	$(0.0498-0.0771) \times \text{Age}+0.0771$	If Age \leq 1
	$3.2850\text{E-}05 \times \text{Age}^3 - 1.2097\text{E-}03 \times \text{Age}^2 + 1.2367\text{E-}02 \times \text{Age} + 3.8613\text{E-}02$	if Age $>$ 1
Brain ⁽²⁾	$0.0217+(0.114-0.0217) \times \exp(-0.182 \times \text{Age})$	
Breast ⁽¹⁾	0	If Age \leq 10
	$(\text{Age} - 10.024)/(104.95 \times \text{Age} - 687.75)$	if Age $>$ 10
Gut ⁽¹⁾	$-7.4206\text{E-}05 \times \text{Age}^2 + 1.2759\text{E-}03 \times \text{Age} + 1.2983\text{E-}02$	If Age $<$ 16
	0.0160	if Age \geq 16
Gut lumen ⁽¹⁾	$0.0100+(0.0294-0.0100) \times \exp(-0.932 \times \text{Age})$	
Heart ⁽²⁾	$0.0042+(0.0053-0.0042) \times \exp(-0.0817 \times \text{Age})$	
Kidneys ⁽²⁾	$0.0046+(0.00709-0.0046) \times \exp(-0.221 \times \text{Age})$	
Liver ⁽²⁾	$0.0233+(0.038-0.0233) \times \exp(-0.122 \times \text{Age})$	
Lung ⁽¹⁾	$0.000016766 \times \text{Age}^2 - 0.00041745 \times \text{Age} + 0.0084805$	If Age \leq 20
	0.0070	if Age $>$ 20
Marrow ⁽¹⁾	$0.045+(0.0138-0.045) \times \exp(-0.136 \times \text{Age})$	
Muscle ⁽¹⁾	$0.2917+(0.207-0.2917) \times \exp(-0.339 \times \text{Age})$	
Sexual organs ⁽¹⁾	$-1.064\text{E-}03 \times \text{Age} + 1.338\text{E-}03$	If Age \leq 1
	$2.6380\text{E-}07 \times \text{Age}^3 - 1.7943\text{E-}06 \times \text{Age}^2 - 5.6465\text{E-}06 \times \text{Age} + 2.8105\text{E-}04$	if Age $>$ 20
	0.001552	if Age \geq 20
Skin ⁽¹⁾	$-7.8882\text{E-}06 \times \text{Age}^3 + 4.0224\text{E-}04 \times \text{Age}^2 - 5.2146\text{E-}03 \times \text{Age} + 4.5605\text{E-}02$	If Age $<$ 20
	0.0383	if Age \geq 20
Spleen ⁽²⁾	$-6.8784\text{E-}09 \times \text{Age}^6 + 4.3253\text{E-}07 \times \text{Age}^5 - 1.0546\text{E-}05 \times \text{Age}^4 + 1.2530\text{E-}04 \times \text{Age}^3$	If Age $<$ 20
	$- 7.3946\text{E-}04 \times \text{Age}^2 + 1.8530\text{E-}03 \times \text{Age} + 1.8922\text{E-}03$	
	0.0022	if Age \geq 20
Stomach ⁽¹⁾	$3.5831\text{E-}08 \times \text{Age}^3 - 1.1503\text{E-}05 \times \text{Age}^2 + 1.8645\text{E-}04 \times \text{Age} + 1.9297\text{E-}03$	If Age \leq 15
	0.0023	if Age $>$ 15
Stomach lumen ⁽¹⁾	$0.0038+(0.0113-0.0038) \times \exp(-0.882 \times \text{Age})$	
Urinary tract ⁽¹⁾	$0.001+(0.0014-0.001) \times \exp(-0.735 \times \text{Age})$	

(1): (International Commission on Radiological Protection, 2002)

(2): (Altman *et al.*, 1962)



Table 4: Relative blood flow (as percentage of the cardiac output) for human adults.

Organ / tissue	Value		Organ / tissue	Value	
	Man	Woman		Man	Woman
Adipose tissue	5	8.5	Marrow	3	3
Adrenal	0.3	0.3	Muscle	17	12
Bone	2	2	Pancreas	1	1
Brain	12	12	Sexual organs	0.05	0.4
Breast	0.02	0.4	Skin	5	5
Gut	14	16	Spleen	3	3
Heart	4	5	Stomach	1	1
Kidney	19	17	Thyroid	1.5	1.5
Liver arterial	6.5	6.5	Urinary tract	0.06	0.06



Table 5: Equations describing the change in males respiratory parameters as a function of age (year).

Equation	
Respiratory parameters	
Man	
V_{tidal}	$(Age < 20 ? 0.0337 \times Age + 0.0407 : 0.75)$
$FreqBreath$	$12 + (38.9 - 12) \times \exp(-0.176 \times Age)$
V_{ds}	$(Age < 16 ? 0.0076 \times Age + 0.0101 : 0.15)$
Woman	
V_{tidal}	$0.46 + (0.0392 - 0.46) \times \exp(-0.127 \times Age)$
$FreqBreath$	$12 + (38.9 - 12) \times \exp(-0.176 \times Age)$
V_{ds}	$0.12 + (0.0107 - 0.12) \times \exp(-0.0986 \times Age)$
Transits	
Man	
$F_{stom2gut}$	$0.005556 + (0.000637 - 0.005556) \times \exp(-0.0789 \times Age)$
$F_{gut2faeces}$	$0.0004688 + (0.0000852 - 0.0004688) \times \exp(-0.0671 \times Age)$
Ke_{renal}	$(Age < 20 ? 0.00004 \times Age + 0.0002 : 1.111E-3)$
Woman	
$F_{stom2gut}$	$0.003833 + (0.000604 - 0.003833) \times \exp(-0.159 \times Age)$
$F_{gut2faeces}$	$0.000375 + (0.0000856 - 0.000375) \times \exp(-0.0931 \times Age)$
Ke_{renal}	$0.0008333 + (0.000208 - 0.0008333) \times \exp(-0.0857 \times Age)$



Table 6: Values for parameters (a , b , c) of equations describing the change in the relative organ weights as a function of the gestational age (day) for the foetus and the pregnant woman.

Parameter	a	b	c
Foetal organs or tissues			
adipose tissue	0.0001803		
adrenals	0.000007467	0.01425	
blood ^l	0.00006796	0.9729	
brain	0.0001871	0.9585	
gut	0.0000163		
heart ^l	0.00001012	0.9489	
kidney	0.000004203	1.255	-0.02127
liver	0.00006050	0.9737	
lung ^l	0.00009351		
marrow	0.00001425	0.9943	
muscle	0.00002668	1.234	
other	0.0001575		
pancreas	0.0001883	0.3854	
skin	0.0000514		
spleen	0.0000001302	1.204	0.02909
thymus	0.000001218	1.093	
thyroid	0.0006470	1.023	
Maternal organs or tissues			
adipose tissue	0.0009911	2.142	-0.1405
blood	0.000004712	3.295	-0.2164
breast	0.01274	0.608	-0.02209
uterus	0.0002057		
amniotic fluid	0.001		
placenta	0.0002		



Table 7: Fraction of fat in organ or tissue.

Organ / tissue	Value	Organ / tissue	Value
Adipose tissue ⁽¹⁾	0.859	Marrow ⁽¹⁾	0.186
Adrenal ⁽²⁾	0.049	Muscle ⁽¹⁾	0.064
Amniotic fluid ⁽³⁾	0.0052	Other ⁽²⁾	0.049
Blood ⁽¹⁾	0.0052	Pancreas ⁽⁴⁾	0.105
Brain ⁽⁴⁾	0.11	Placenta ⁽²⁾	0.049
Breast ⁽²⁾	0.049	Skin ⁽⁴⁾	0.150
Gut ⁽⁴⁾	0.065	Spleen ⁽⁴⁾	0.030
Gut Lumen ⁽²⁾	0.049	Stomach ⁽²⁾	0.049
Heart ⁽⁴⁾	0.083	Stomach Lumen ⁽²⁾	0.049
Kidney ⁽⁴⁾	0.052	Thymus ⁽²⁾	0.049
Liver ⁽¹⁾	0.049	Thyroid ⁽²⁾	0.049
Lung ⁽⁴⁾	0.017	Uterus ⁽²⁾	0.049

⁽¹⁾ (Van der Mollen *et al.*, 1996)

⁽²⁾ Default value corresponding to the "remaining organs" in (Van der Mollen *et al.*, 1996).

⁽³⁾ Same value as blood.

⁽⁴⁾ (Fiserova-Bergerova, 1983)



Table 8: Inhibition constants (K_i) for the BTEX mixture (Haddad *et al.*, 1999).

Symbol	Inhibitor	Substrate	K_i value ($\mu\text{mol/l}$)
$K_{i_{BT}}$	Benzene	Toluene	2.85
$K_{i_{BE}}$	Benzene	Ethylbenzene	8.01
$K_{i_{BX}}$	Benzene	Xylene	2.89
$K_{i_{TB}}$	Toluene	Benzene	1.56
$K_{i_{TE}}$	Toluene	Ethylbenzene	10.29
$K_{i_{TX}}$	Toluene	Xylene	3.87
$K_{i_{EB}}$	Ethylbenzene	Benzene	2.41
$K_{i_{ET}}$	Ethylbenzene	Toluene	1.58
$K_{i_{EX}}$	Ethylbenzene	Xylene	4.76
$K_{i_{XB}}$	Xylene	Benzene	2.03
$K_{i_{XT}}$	Xylene	Toluene	3.09
$K_{i_{XE}}$	Xylene	Ethylbenzene	15.70



Table 9: Human physiological, physicochemical and biochemical parameters used in PBPK model for BTEX.

Parameters				
Body weight (kg)	70			
Cardiac output (l/h/kg)	18			
Alveolar ventilation rate (l/h/kg)	18			
	Benzene	Toluene	Xylene	Ethylbenzene
Blood flow rate (% of cardiac output)				
Fat	0.0425	0.0425	0.0425	0.0425
Poorly perfused tissues	0.1327	0.1717	0.1717	0.1717
Richly perfused tissues	0.3461	0.5488	0.5488	0.5488
Bone Marrow	0.039			
Liver	0.237	0.237	0.237	0.237
Kidney	0.2027			
Volumes (% of body weight)				
Fat	0.1429	0.1429	0.1429	0.1429
Poorly perfused tissues	0.694	0.734	0.734	0.734
Richly perfused tissues	0.040	0.044	0.044	0.044
Bone Marrow	0.040			
Liver	0.025	0.025	0.025	0.025
Kidney	0.004			
Partition coefficients				
Blood/air	7.80	15.60	26.40	28.00
Fat/blood	54.50	65.45 ⁽¹⁾	70.42 ⁽¹⁾	55.57 ⁽¹⁾
Poorly perfused tissues/blood	2.05	1.78 ⁽¹⁾	1.59 ⁽¹⁾	0.93 ⁽¹⁾
Richly perfused tissues/blood	1.92	5.36 ⁽¹⁾	3.44 ⁽¹⁾	2.15 ⁽¹⁾
Bone Marrow/blood	16.22 ⁽²⁾			
Liver/blood	1.486 ⁽¹⁾	5.36 ⁽¹⁾	3.44 ⁽¹⁾	2.99 ⁽¹⁾
Kidney/blood	1.920			
Metabolic parameters				
V _{max} (μmol/h/kg)	15.36 ⁽²⁾	37.33 ⁽¹⁾	61.13 ⁽¹⁾	60.19 ⁽¹⁾
K _m (μmol/l)	4.48 ⁽²⁾	1.14 ⁽¹⁾	4.24 ⁽¹⁾	9.80 ⁽¹⁾

⁽¹⁾: (Haddad *et al.*, 1999)

⁽²⁾: (Travis *et al.*, 1990)



Table 10: Partition coefficient for benzene metabolites (Cole *et al.*, 2002)

	Benzene	Phenol	Hydroquinon
Fat/blood	54.5	27.63	4.06
Poorly perfused tissues/blood	2.05	1.22	0.94
Richly perfused tissues/blood	1.92	2.17	1.04
Liver/blood	2.95	2.17	1.04
Kidney/blood	1.92	2.17	1.04



Table 11: Human metabolic parameters used in the benzene section of the PBPK model.

Symbol	Meaning	Values
C^{CP}	Concentration of microsomal protein per gram of tissue in the liver (mg/g)	14.5
C^{MP}	Concentration of cytosolic protein per gram of tissue in the liver (mg/g)	58
$K_{m,1}^{PH}$	Concentrations at half-saturation of PH by two sulfate transferases ($\mu\text{mol/L}$)	1.4
$K_{m,2}^{PH}$	Concentrations at half-saturation of PH by two sulfate transferases ($\mu\text{mol/L}$)	220
K_m^{HQ}	Concentration at half-saturation for HQ ($\mu\text{mol/L}$)	746
A^{BZ}	Affinity parameter for CYP2E1 for substrate of benzene ($\text{L}/\mu\text{mol}$)	0.0397
A^{PH}	Affinity parameter for CYP2E1 for substrate of PH ($\text{L}/\mu\text{mol}$)	1.3×10^{-2}
A^{HQ}	Affinity parameter for CYP2E1 for substrate of HQ ($\text{L}/\mu\text{mol}$)	10^{-7}
k_1	Efficiencies of CYP2E1 for specific oxidation relative to V2E1 ($\text{L}/\mu\text{mol}$)	4.2×10^{-2}
k_2	Rate constant for the metabolism of benzene oxide to PH (1/hr)	32.16
k_3	Rate constant for the metabolism of benzene oxide to MA (1/hr)	0.51156
k_4	Rate constant for the metabolism of benzene oxide to PMA (1/hr)	12.409
k_5	Efficiencies of CYP2E1 for specific oxidation relative to V2E1 ($\text{L}/\mu\text{mol}$)	4.0×10^{-2}
k_6	Efficiencies of CYP2E1 for specific oxidation relative to V2E1 ($\text{L}/\mu\text{mol}$)	2.13×10^{-3}
k_7	Efficiencies of CYP2E1 for specific oxidation relative to V2E1 ($\text{L}/\mu\text{mol}$)	2.03×10^{-4}
k_9	Binding coefficients for PH to tissue (1/hr)	0.1163
k_{10}	Binding coefficients for HQ to tissue (1/hr)	0.1443
V_{2E1}	CYP2E1 specific activity as determined by the oxidation of p-nitrophenol to p-nitrocatechol ($\mu\text{mol}/\text{mg}/\text{hr}$)	0.01803
V_{PH1}	Maximum rates of metabolism of PH by two sulfate transferases ($\mu\text{mol}/\text{mg}/\text{hr}$)	1574.09
V_{PH2}	Maximum rates of metabolism of PH by two sulfate transferases ($\mu\text{mol}/\text{mg}/\text{hr}$)	116.746
V_{HQ}	Maximum rate of metabolism for HQ ($\mu\text{mol}/\text{mg}/\text{hr}$)	1.0021



Section 8 – FIGURES

Figure 1: Schematic representation of the physiologically based pharmacokinetic model for humans. Twenty-two compartments describe the human body. Possible administration routes are by inhalation (via lungs) or by ingestion (in stomach lumen). Metabolism is assumed to occur in lungs, gut and liver. The substance may also be eliminated via gut lumen, kidneys and lungs.

Figure 2: Schematic representation of the physiologically based pharmacokinetic model for human pregnancy. The maternal body and foetus are subdivided into compartments describing organs or diffuse tissues. In gray: organs or tissues whose volume changes during pregnancy. Chemical substances are transported or transferred through the compartments. Thick arrows: potential routes of input and outputs for chemical substances. Thin arrows: transport paths through blood flows. Dotted arrows: transfers and diffusions paths.

Figure 3: Evolution of few relative organs' volumes with age for males and females.

Figure 4: Time evolution of the foetal bodyweight and of some organs' volumes.

Figure 5: Conceptual representation of the PBPK model for a mixture of BTEX

Figure 6: Metabolic chain of benzene to its primary metabolites

Figure 7: Schematic representation of the benzene section of the PBPK compartmental model

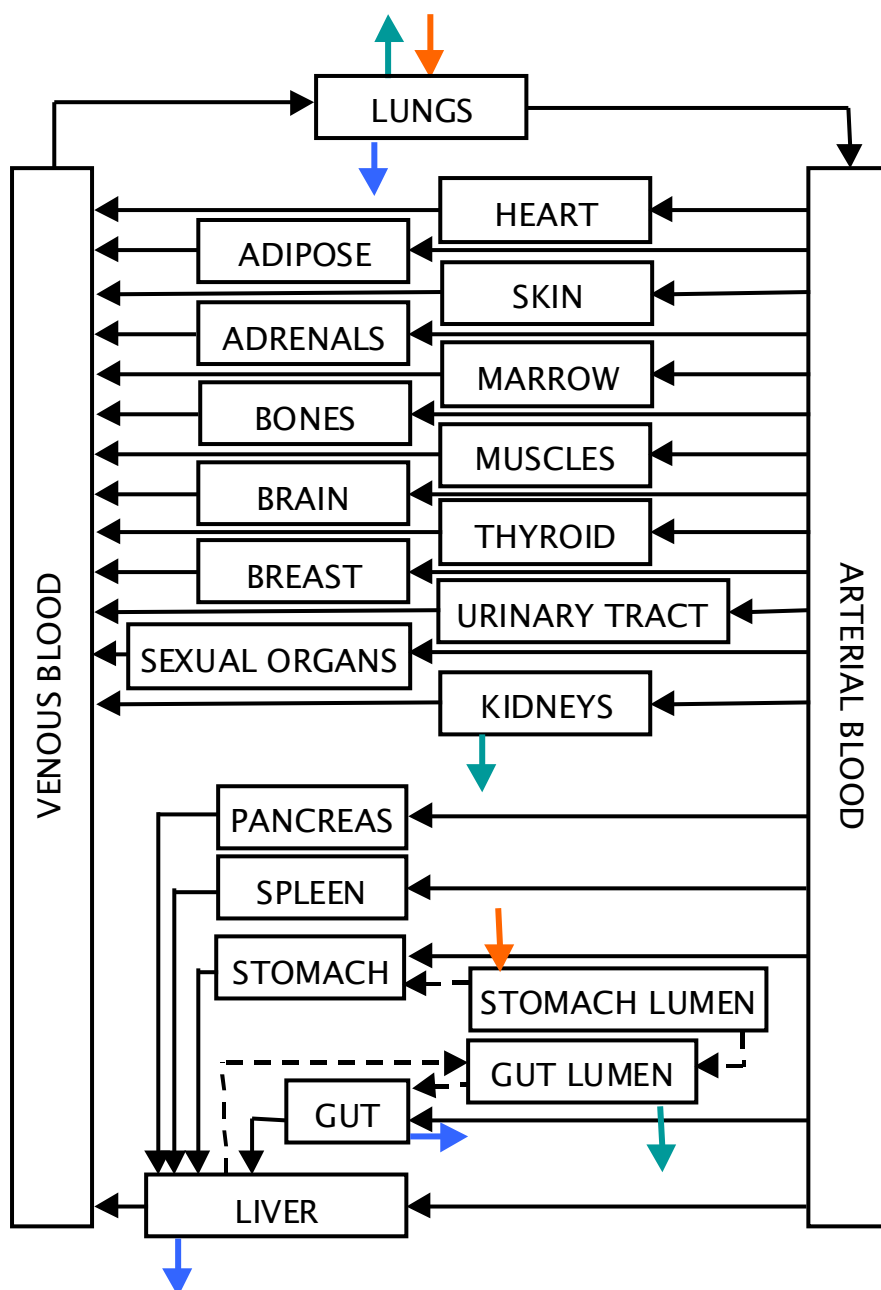


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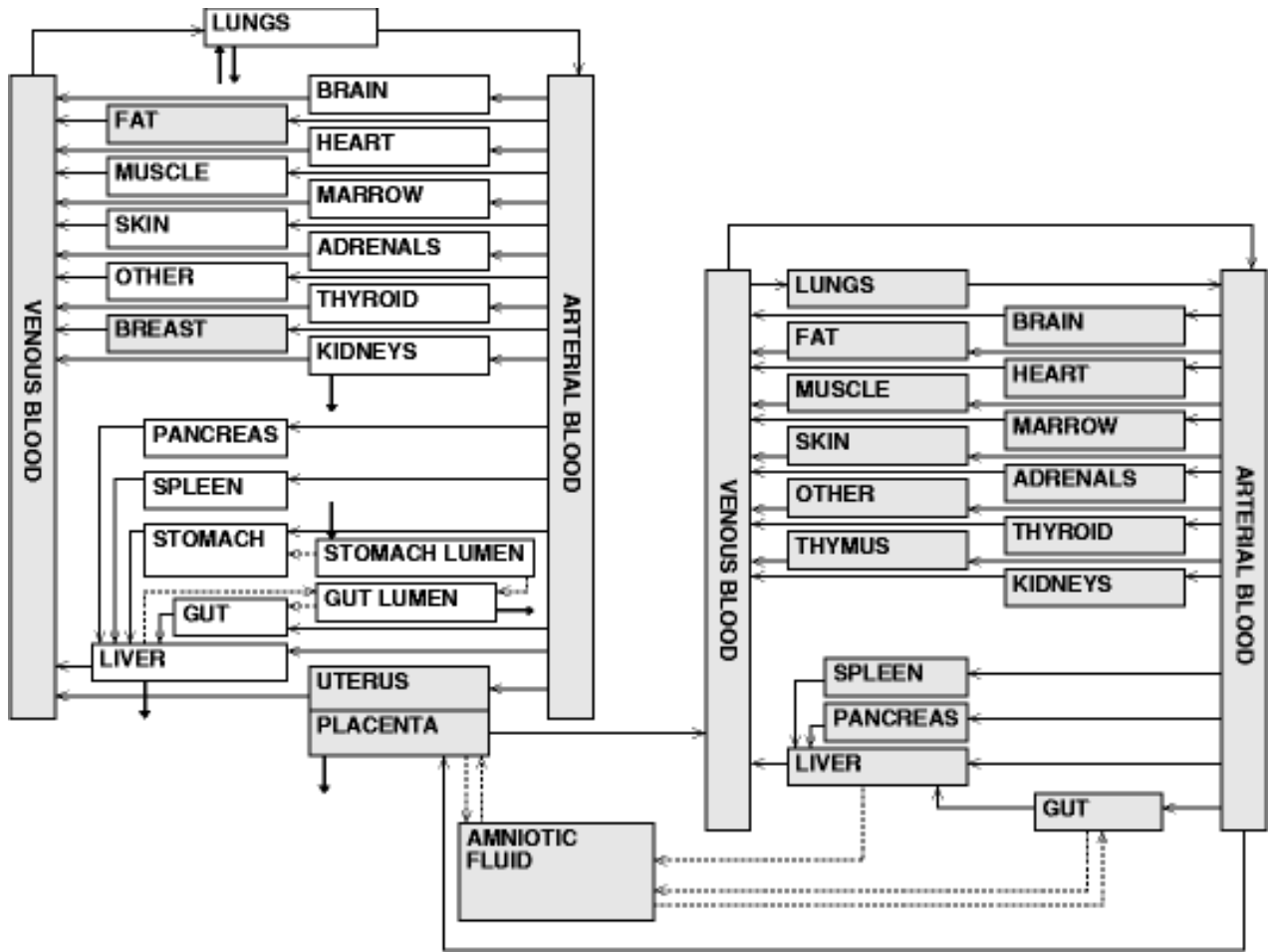


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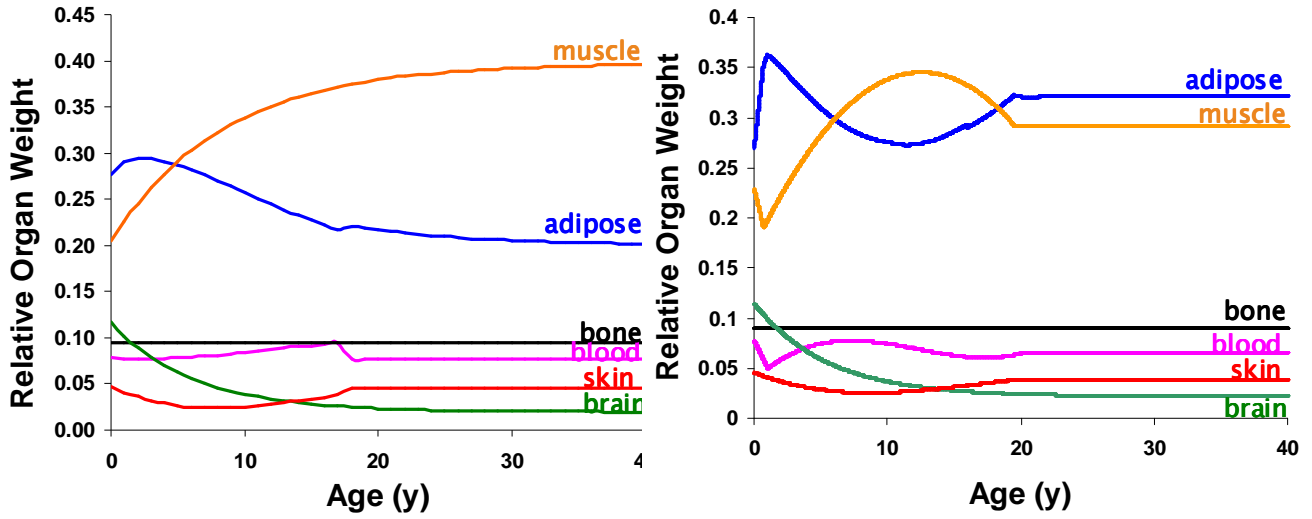


Figure 3: Evolution of few relative organs' volumes with age for males (left panel) and females (right panel).

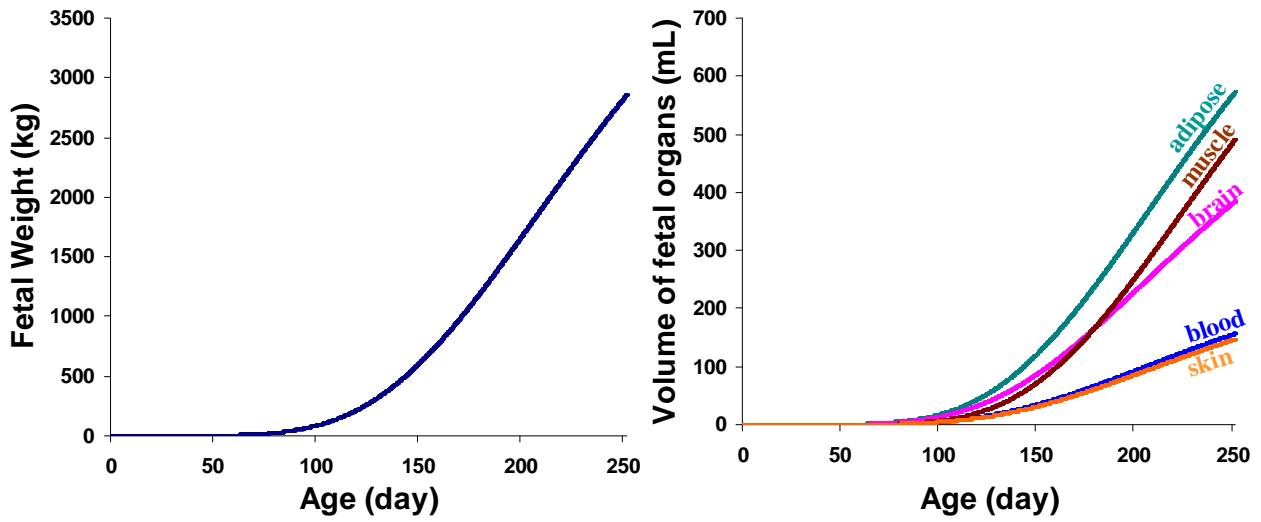


Figure 4: Time evolution of the foetal bodyweight and of some organs' volumes.

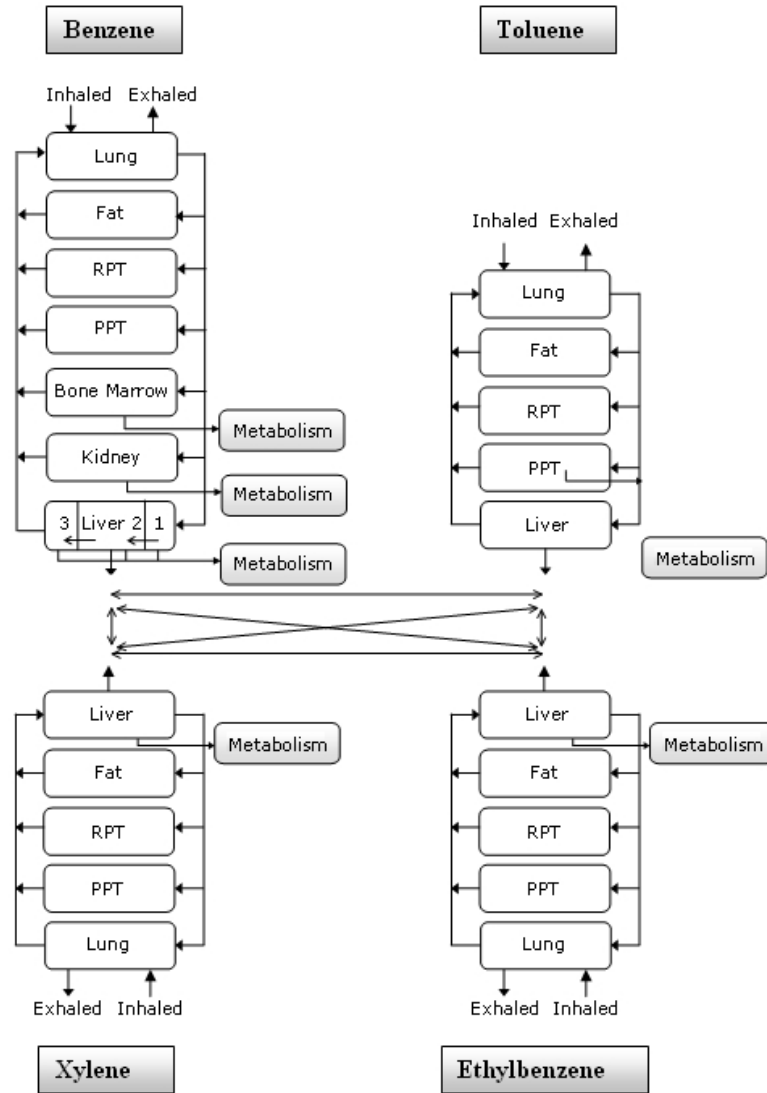


Figure 5: Conceptual representation of the PBPK model for a mixture of BTEX

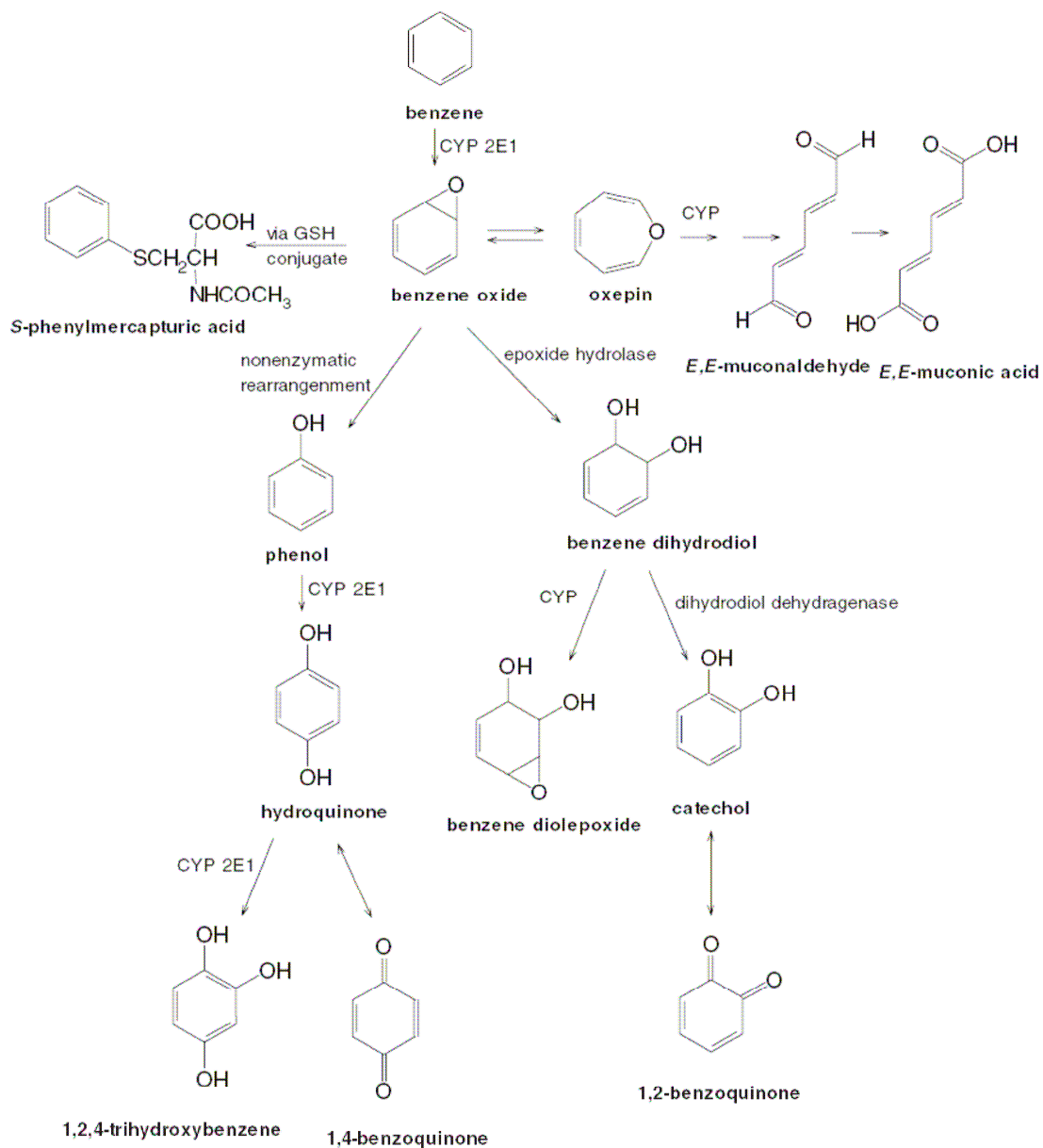


Figure 6: Metabolic chain of benzene to its primary metabolites

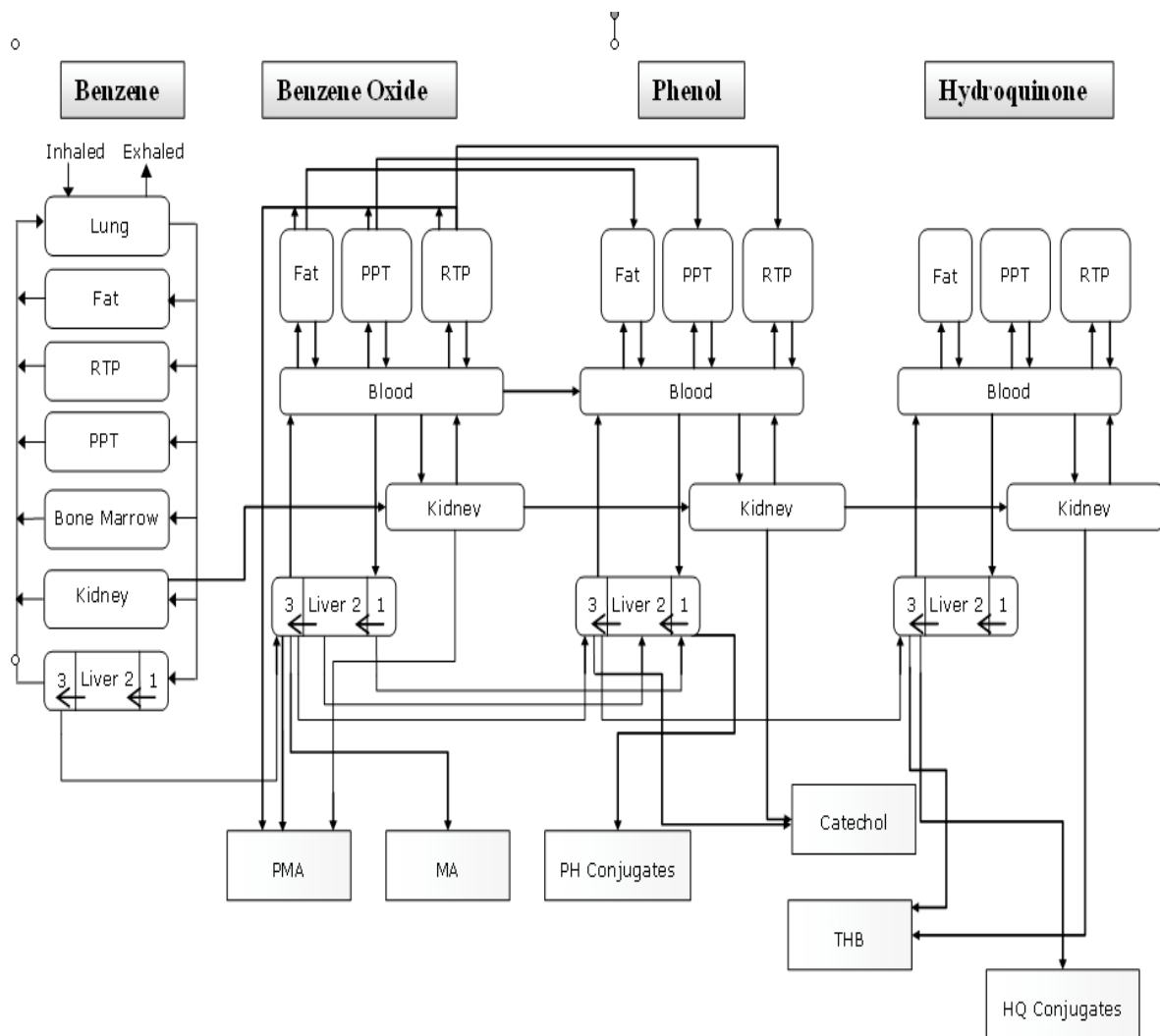


Figure 7: Schematic representation of the benzene section of the PBPK compartmental model.