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1 **Stochasticity in physiologically-based kinetics models: Implications for cancer risk**  
2 **assessment**

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1 **ABSTRACT**

2 In case of low dose exposure to a substance, its concentration in cells is likely to be stochastic.  
3 Assessing the consequences of this stochasticity in toxicological risk assessment requires the  
4 coupling of macroscopic dynamics models describing whole body kinetics with microscopic  
5 tools designed to simulate stochasticity. In this paper, we propose an approach to approximate  
6 stochastic cell concentration of butadiene in the cells of diverse organs. We adapted the  
7 dynamics equations of a physiologically-based pharmacokinetic (PBPK) model and used a  
8 stochastic simulator for the system of equations we derived. We then coupled kinetics  
9 simulations with a deterministic hockey stick model of carcinogenicity. Stochasticity induced  
10 substantial modifications relative to dose-response curve, compared to the deterministic  
11 situation. In particular, there was non-linearity in the response and stochastic apparent  
12 threshold was lower than the deterministic one. The approach we developed could easily be  
13 extended to other biological studies to assess the influence at macroscopic scale of  
14 stochasticity for compounds dynamics at cell level.

15

16 **KEY WORDS:** PBPK, butadiene, systems biology, stochasticity, cancer

## 1. INTRODUCTION

The dynamics of chemical species at the level of the cell are rather discrete and stochastic than continuous and deterministic, in so far as they are determined by the action of only a few molecules (Rao and Arkin, 2003). Stochastic resonance in biology is a well known phenomenon, able to enhance detection and improve the transmission efficiency of weak information in nonlinear systems (Hänggi, 2002). In particular, it explains why deterministic threshold can be exceeded, with a rate in relation to the intensity of the signal, even if the mean value of this signal throughout time is below the threshold. The concepts apply to toxicology: For low exposures to a chemical, stochasticity is likely to play a role in the occurrence of toxic effects at the cell level, even if the mean cellular concentration is below some deterministic threshold, inferred for instance, through mechanistic considerations (Lovell, 2000). Studying stochasticity in toxicant cellular concentration is particularly relevant for carcinogenesis which might be induced at very low doses of exposure. In this paper, we present a modelling framework to simulate stochastic concentrations at cell level and to derive consequences for cancer risk assessment. Simulations are performed based on actual exposure levels and a physiologically-based toxicokinetic model for butadiene.

1,3-Butadiene is a highly volatile four-carbon chemical mostly made from the processing of petroleum. It can be detected in urban air pollution, cigarette smoke and gasoline vapors. Butadiene is an established carcinogen, as a DNA-reactive chemical leading to production of DNA adducts in rodents in liver, lung and tissue (Preston, 2007). It is hypothesized that butadiene carcinogenicity is a consequence of the genotoxicity of its metabolites (Albertini et al., 2003). It is not clear whether or not there is a threshold for effects in humans (Preston 2007).

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Physiologically based toxicokinetic (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 characterized by a set of parameters of physiological relevance (e.g., volume or blood perfusion rate) which play a crucial role in explaining the behavior of chemical substances in the body, and represent invariants across substances. A three-compartment physiologically based pharmacokinetics (PBPK) model has been proposed to describe the distribution of butadiene and the production of its first metabolite following oxidation, 1,2-epoxy-3-butene (Brochot and Bois, 2005). We use here an extension of this model with 23 compartments. The model parameters (organ volumes etc.) correspond to those of an adult man.

These macroscopic models can describe the distribution of chemicals in the different organs but are unable to capture the stochasticity at cell level. In contrast, a recent discipline, systems biology, aims at studying the dynamics of the components of a cell, and tools to study the influence of stochasticity at cell level have being developed. The main objective of this paper is to develop a methodology for coupling microscopic and macroscopic dynamics models to assess the consequences at organ level of stochastic chemical concentration and effects at cell level. Coupling PBPK models and systems biology is a step forward to develop integrated approaches able to relate information obtained at cell level, like for instance “omics” data, and effects on health.

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**2. MATERIALS AND METHODS**

**2.1. PBPK model for butadiene**

Our PBPK model contains 23 compartments (See Figure 1). Compartments are mainly connected by blood circulation, by air exchange at the lung level, excretion to urine and feces, and metabolism. Concentrations  $C$ , in  $\mu\text{g/L}$ , are obtained at any time by dividing the quantity of butadiene,  $Q$  (in  $\mu\text{g}$ ) by the compartment volume (supposed constant in time). Volumes are in L, time in min, flows and rates in L/min.  $Q$  and  $C$  depend on time but we omit the time argument when possible for simpler notation. A list of parameters and their values can be found in Tables I-IV. We assume that butadiene is only eliminated through metabolism or exhalation, and that intake only occurs through inhalation.

For adipose tissue, adrenals, bone marrow, brain, breast, heart, kidneys, muscles, other organs and tissues, pancreas, skin, spleen, testes and thyroid, the differential equation giving the rate of change for the quantity of BD is:

$$\frac{dQ}{dt} = F_i \left( C_{art} - \frac{C_i}{P_i} \right) \tag{1}$$

where  $F_i$  are blood flows (values given in Table 2), and  $P_i$  are tissue over blood partition coefficients. These partition coefficients were calculated by multiplying the partition coefficients for fat (Table III) by fat content of each organ (Table IV). Indeed, for highly lipophilic organic chemicals,  $P_i$  values can be approximated by the ratio of lipids in adipose tissues and blood (Haddad et al., 2000).

1 For upper respiratory tract:

2 
$$\frac{Q_{ur}}{V} = \frac{F_{pul} C_{pul} + F_{alv} C_{alv}}{V} \quad (2)$$

3 where  $F_{pul}$  designates the pulmonary ventilation rate (9 L/min for an average human) and  $F_{alv}$   
 4 the alveolar ventilation rate (6 L/min) (ICRP, 2002).

5 For the lung:

6 
$$\frac{Q_{lu}}{V} = \frac{F_{total} C_{total}}{V} \quad (3)$$

7 where  $F_{total}$  designates the sum of the  $F$  values for the following organs or tissues: adipose  
 8 tissue, adrenals, bone marrow, brain, breast, heart, kidneys, liver, muscles, other organs and  
 9 tissues, skin, testes and thyroid.

10 For the liver, blood comes from the arterial pool, spleen, pancreas, stomach and gut, and there  
 11 is metabolism occurring in the liver (see further):

12 
$$\frac{Q_{li}}{V} = \frac{F_{spleen} C_{spleen} + F_{pancreas} C_{pancreas} + F_{stomach} C_{stomach} + F_{gut} C_{gut}}{V} \quad (4)$$

13 where  $F_{liver}$ , is:

14 
$$F_{liver} = F_{spleen} + F_{pancreas} + F_{stomach} + F_{gut} \quad (5)$$

15 For arterial blood:

16 
$$\frac{Q_{ar}}{V} = \frac{F_{total} C_{total}}{V} \quad (6)$$

17 For venous blood:

18 
$$\frac{Q_{ve}}{V} = \frac{F_{total} C_{total}}{V} \quad (7)$$

1 where  $i$  designates the adipose tissue, adrenals, bone marrow, brain, breast, heart, kidneys,  
2 liver, muscles, other organs and tissues, skin, testes and thyroid.

3 For epoxy metabolite (EB) in the liver:

4  (8)

5 For simplicity, the possibility of EB molecules to come back to the liver through blood  
6 circulation has been neglected, assuming substantial elimination through secondary  
7 metabolism and exhalation. To obtain more relevance, incorporating EB kinetics may be  
8 proposed later, based on the PBPK model for butadiene and major metabolites proposed by  
9 Brochet et al. (2007).

10

## 11 **2.2. Exposure and epidemiological data**

12 We used data from Higashino et al. (2007) on human exposures to 1,3-butadiene in Japan.  
13 The average concentration in the general environment is  $0.25 \mu\text{g}/\text{m}^3$ , with a background  
14 concentration  $0.06 \mu\text{g}/\text{m}^3$  in unpolluted areas. Exposure concentrations above  $0.8 \mu\text{g}/\text{m}^3$  are  
15 only found in vicinity of industrial activities. Lifetime excess cancer risk level is estimated at  
16  $10^{-5}$  for an exposure concentration of  $1.7 \mu\text{g}/\text{m}^3$  (Higashino et al., 2007). In Japan, 0.03% of  
17 the total population (that is 36 000 persons) are exposed to concentrations exceeding this  
18 value. With a molar mass of 54.09 g/mol for butadiene,  $1.7 \mu\text{g}/\text{m}^3$  corresponds to  $18.7 \cdot 10^{12}$   
19 molecules/L.

20

## 21 **2.3. Simulation Software used**

22 We used the Systems Biology Workbench (SBW) (Sauro et al., 2003), version 2.0.39. We  
23 implemented our PBPK model in Jdesigner. The model was then converted to an SBML file

1 and input in the Dizzy 1.11.4 software (Ramsey et al., 2005), which is able to perform  
2 stochastic simulations of chemical kinetics. We used the Gillespie stochastic algorithm  
3 (Gillespie, 1977) for stochastic simulations. It is an algorithm for modeling the kinetics of a  
4 set of coupled chemical reactions, taking into account stochastic effects from low copy  
5 numbers of the chemical species. With the same differential equation, the deterministic  
6 approach regards the time evolution as continuous, whereas the stochastic approach regards  
7 the time evolution as a kind of random-walk process. In Gillespie's approach, chemical  
8 reaction kinetics are modelled as a Markov process in which reactions occur at specific times  
9 separated by Poisson-distributed intervals. The mean interval is recomputed each reaction  
10 time. At each reaction time, a specific chemical reaction occurs, randomly selected from the  
11 set of all possible reactions with a probability given by the individual reaction rates.

12

#### 13 **2.4. Stochastic simulations at steady state**

14 Exact stochastic simulators track the actual number of molecules involved in a set of reaction  
15 in a given portion of space. It is impossible for them to handle as many molecules as can be  
16 found in an entire organ. We first computed the steady state concentration values in each  
17 organ using the deterministic (ordinary differential equation) simulator of the SBW, with  
18 continuous inhalation exposure concentrations of 0.25 and 1.7 mg/m<sup>3</sup> of BD. Initial condition  
19 for the number of molecules in a cell was set to the deterministic value from the PBPK model.  
20 We consider that a human has typically about 10<sup>14</sup> cells. In our PBPK model, the total volume  
21 of a man is 75 L, which results in a mean density of 1.37 10<sup>12</sup> cells/L.

22

23 Except for liver, the uptake of butadiene is performed from the arterial blood, in which  
24 butadiene concentration was set at steady state deterministic value from PBPK model. The

1 kinetic equation for one cell is similar to that of the organ to which it belongs except for the  
 2 flux value:

$$3 \quad \frac{dQ_{cell}}{dt} = F_{cell} \left( \frac{C_{art}}{P_{org}} - \frac{C_{cell}}{P_{org}} \right) \quad (9)$$

4 with  $\frac{F_{organ}}{F_{cell}} = \frac{C_{cell}}{C_{art}} \frac{V_{organ}}{V_{cell}}$

5 This equation cannot be computed directly in the softwares we use because of rounding errors  
 6 due to the extremely small value of  $F_{cell}$  (less than  $10^{-13}$  for most organs). We reformulate  
 7 equation (9):

$$8 \quad \frac{d(N_{cell})}{dt} = \frac{F_{organ}}{V_{organ}} \left( \frac{\aleph \times C_{art}}{Cell\_density \times M_{butadiene}} - \frac{N_{cell}}{P_{organ}} \right) \quad (10)$$

9 where  $\aleph$  is the Avogadro's number,  $M_{butadiene}$  is the molar weight of butadiene and  $N_{cell}$  the  
 10 number of molecules in the cell. This equation can be studied with Jdesigner and Dizzy with a  
 11 fixed  $C_{art}$  value. Equation (4) was reformulated in a similar way.

12

13 We performed simulations with five organs: spleen, which has the lowest partition coefficient  
 14 over blood (0.77), fat with the highest one (22), marrow, which has a low scaled flux value  
 15 ( $0.0786 \text{ min}^{-1}$ ), kidney with a high scaled flux value ( $3.9838 \text{ min}^{-1}$ ) and liver, for which we  
 16 studied the concentrations of both butadiene and its first metabolite.

17 That choice of organs is also relevant relative to cancer risk of butadiene. Mutagenicity has  
 18 been shown to occur for spleen and marrow of rodents (Preston, 2007). The same author  
 19 reported butadiene-induced lymphomas so as lung and liver tumours in mice.

1 We ran 100 simulations at steady state. For spleen, kidney and liver, we reported the number  
2 of molecules at time 100 min, which is large compared to the time needed to reach  
3 equilibrium in the deterministic model for these organs. For marrow and fat, we reported this  
4 number at 500 min and 10000 min respectively due to a longer time to reach equilibrium.

5

## 6 **2.5. Coupling with cancer PD model**

7 To assess how dose-response curves are affected by stochasticity at the cellular level, we  
8 coupled the stochastic PBPK model described above to a linear model with a threshold for  
9 effect, which belongs to the family of the “hockey stick models” introduced into carcinogenic  
10 risk assessment by Cornfield (1977). For a given cell at a given time, the probability  $R$  of  
11 carcinogenesis is supposed to be proportional to  $\max(N(t)-N_0, 0)$  where  $N(t)$  is the number of  
12 molecules of BD metabolites per cell at time  $t$ ,  $N_0$  the threshold number of molecules to get an  
13 effect. We chose 1 as the threshold number of molecules per cell able to initiate liver  
14 carcinogenesis with a non-zero probability. This is the minimum reasonable number and it  
15 corresponds to approximately twice the general environment exposure in Japan (Higashino et  
16 al., 2007). This is just a choice for simulations. It is not based on particular knowledge about  
17 butadiene mechanisms of carcinogenicity. It is also worth noting that a purely linear dose-  
18 response would not be affected by stochasticity. We adapted the proportionality factor  
19 between  $R$  and excess number of molecules so that the lifetime excess cancer risk level  
20 estimated for a continuous exposure to  $1.7 \mu\text{g}/\text{m}^3$  BD is  $10^{-5}$ , as in the study by Higashino et  
21 al. (2007).

22 We performed simulations for exposure concentrations from 0 to  $1.7 \mu\text{g}/\text{m}^3$ , with a step of  $0.1$   
23  $\mu\text{g}/\text{m}^3$ . For each concentration, we simulated, at steady state, the number of BD metabolites  
24 molecules in 100 cells exposed during 100 minutes.

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## 2.6. Stochastic simulations in time-varying conditions

To study the system in a non steady-state situation (*e. g.* for a time-varying change in the exposure level) the exposure scenario simulated 9 hours a day to a BD concentration of 1.7  $\mu\text{g}/\text{m}^3$ , followed by 15 hours a day to a BD concentration of 0.25  $\mu\text{g}/\text{m}^3$ . This is typically what a factory worker would be exposed to in Japan (Higashino et al., 2007). For simplicity, holidays and week-ends were not accounted for.

We added a component (accounting for a liver cell) in parallel to the liver in the PBPK model. It was not possible to implement time-varying deterministic concentrations in Jdesigner and Dizzy and use the same methods as in section 2.4. Therefore, we had to simulate with Dizzy simultaneously the dynamics in all organs and in a cell. However, we could not track all the molecules in the body even for an environmental exposure level of BD. Therefore, we had to adapt the PBPK model equations implemented in Jdesigner and Dizzy, so that simulated organ concentrations were approximately the deterministic ones and so that Gillespie algorithm was unchanged for the cell.

Equation (10) is formally equivalent, relative to dynamics at the cell level, to the following equation:

$$\frac{dN_{cell}}{dt} = \left( \frac{100 \times \kappa}{Cell\_density \times M_{butadiene}} \right) N_{cell} \quad (13)$$

Consequently, if, in the PBPK model equations, we multiply the concentrations in all compartments but the liver cell by  $\left( \frac{100 \times \kappa}{Cell\_density \times M_{butadiene}} \right)$ , fix at 0.01 the volume of the liver cell (both operations largely minimise stochasticity at organ level so as the contribution

1 of the liver cell to the whole system) and divide  $F_{organ}$  by 100, we have approximately the  
2 same kinetics at the organs level, without affecting the kinetics for  $Q_{cell}$ . The Gillespie  
3 algorithm is unchanged, because the reaction probability density function is unchanged. We  
4 indeed checked with Jdesigner and Dizzy that, once the model was adapted, the stochastic  
5 values of all organ concentrations differed by less than 5 % from their deterministic values  
6 during the simulations we performed. Using 10 and 0.1 instead of 100 and 0.01 in equation  
7 (13) would have led a large difference between deterministic values and stochastic ones. In  
8 contrast, using 1000 and 0.001 would have led to huge calculation times for Dizzy.  
9 We focused here only on EB in the liver. We performed simulations for 20 cells during the 15  
10 hours in the general environment and reported cell concentration. The concentrations at time 0  
11 (time at which the subject is just leaving industrial vicinity) are extracted from simulations  
12 with Jdesigner over 50 days. For the last 10 days, the values at time 0 only differed by 0.1 %,  
13 i.e. dynamics steady state was reached. Cancer risk was computed at each time step (every  
14 minute).

15

### 16 **3. RESULTS**

#### 17 **3.1. Stochastic simulations at steady state**

18 The mean number of molecules in a cell, the standard deviation and coefficient of variation  
19 per organ for exposure concentration  $1.7 \mu\text{g}/\text{m}^3$  are presented in Table V. Stochastic mean  
20 values equal deterministic ones.

21 The stochastic variability is mainly influenced by the partition coefficient (which determines  
22 the mean number of molecules at steady state). The fact that marrow and kidney have  
23 comparable standard deviations but very different mean number of molecules suggests that  
24 low blood flow to the cell also tends to decrease variability relative to the mean value. The

1 results obtained for metabolites in the liver are presented in Figure 2. There is a large  
2 variability of the number of metabolites in a liver cell (mean 2.91, standard deviation 1.71  
3 resulting in coefficient of variation of 0.588). Simulations for exposure concentration 0.25  
4  $\mu\text{g}/\text{m}^3$  led to the same conclusions relative to organ characteristics and coefficient of variation  
5 (data not shown).

6

### 7 **3.2. Coupling with cancer model**

8 Figure 3 shows that accounting for stochasticity when coupling the PBPK model and cancer  
9 model increased the excess of risk even below the deterministic threshold of  $0.6 \mu\text{g}/\text{m}^3$ . The  
10 dose-response curve appears to have two distinct regimes: a “quadratic” looking region and a  
11 “linear” looking one, closed to the deterministic response.

12

### 13 **3.3. Stochastic simulations in time-varying conditions**

14 Figure 4 shows the results at dynamics steady state for metabolites in one liver cell for 15  
15 hours at exposure concentration  $0.25 \mu\text{g}/\text{m}^3$  after 9 hours at exposure concentration  $1.7 \mu\text{g}/\text{m}^3$ .  
16 The metabolite mean concentration quickly reaches low levels (half of the initial value is  
17 reached after 13 minutes), at which stochasticity is high and kinetics is slow.

18 The cancer model was used to assess the instantaneous excess risk of liver cancer during this  
19 period. There was no increased risk for times over 65 minutes, according to the deterministic  
20 approach. The sum over time of instantaneous excess risk values during this period outside  
21 the industrial vicinity corresponded to 12 minutes at steady state exposure at the high  
22 concentration, which is low compared to the 9 hours exposure. In contrast, when accounting  
23 for stochasticity, the mean sum of excess risk corresponded to 85 minutes of high exposure.

24

#### 1        4. DISCUSSION

2  
3    The simple algorithms outlined in the works by Gillespie (Gillespie, 1977) permit the  
4    modeling of microscopic stochastic phenomena (Haseltine and Rawlings, 2002). Gillespie's  
5    algorithms can be so expensive computationally that alternatives have been proposed to  
6    approximate exact simulations (Haseltine and Rawlings, 2002 ; Rathinam et al., 2003). For  
7    the present work, we chose to use the exact algorithm, for simulations could be performed in a  
8    reasonable amount of time with modern calculation stations, thanks to the progress that  
9    computer technology has still made in the past few years.

10   Coupling our PBPK model to a model of excess risk of cancer affected dose response curve. It  
11   looks curvilinear, with a breakpoint dose lower than the theoretical one. Mutagenesis data  
12   from Elhajouji et al. (1997) showed experimentally comparable profile, with a slight increase  
13   in mutagenicity, then a highly significant steep increase for compounds likely to have a  
14   threshold for effects. We do not pretend to fully assess cancer risk associated to exposure to  
15   butadiene. Our study is a simulation study aiming at assessing whether or not stochasticity  
16   should be considered when assessing risk. Therefore, at least in the case of exposure to low  
17   doses of compounds having a threshold for carcinogenicity, stochasticity in kinetics should  
18   not be neglected.

19   In a paper on dose-response and threshold-mediated mechanisms in mutagenesis, Lovell  
20   (2000) points that "absolute" threshold are difficult to estimate from toxicity data due to  
21   background noise as a consequence of stochasticity. The concept of absolute threshold may  
22   therefore make no physical sense at all. Lovell is in favour of "pragmatic" thresholds,  
23   constructed through dose-response models (in particular biology-based dose-response  
24   (BBDR) models) coupled with knowledge about what level of response is biologically

1 important, and what level is not. We believe that dose-response models construction should  
2 seriously consider stochasticity to get the best accurate representation of reality at low doses.  
3 BBDR models would permit to derive a deterministic approximate threshold, then  
4 stochasticity studies would permit to derive a realistic one based on stochastic simulations  
5 with BBDR models coupled with PBPK models.

6 To our knowledge, this study is the first time that the dynamics of compounds at the cell level  
7 is studied in parallel to the dynamics at the body level. Coupling microscopic and  
8 macroscopic dynamics is a real challenge, because of the very large scale difference between  
9 numbers of molecules in cell and in organ. Here, we achieved the coupling of PBPK models  
10 with tools developed in the framework of systems biology through two different approaches.

11 In case of steady state for organs, concentrations in PBPK compartments were fixed and exact  
12 dynamic equations for butadiene in the cell was derived. In case of time varying  
13 concentrations in the organs, we adapted the equations to get an approximate simulation of the  
14 dynamics at the cell level. We were then able to assess the influence of stochasticity relative  
15 to dose-response in toxicant risk assessment, with constant or time-varying exposure  
16 concentration. This coupling is not limited to toxicology. It can be immediately generalized to  
17 the study of stochastic concentration of many compounds in the cell, like for instance  
18 pharmaceuticals and hormones which may be effective at extremely low concentrations  
19 (Gurevich et al., 2003).

20

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1 Table I. Values of the organ or tissue volumes (in L). These constants were calculated for a  
 2 standard man of 1.76 m and 73 kg, using the organ weights given by the ICRP 2002 Pub 89  
 3 (p.18(T 2.8) p.19(T 2.9)). Density for the organs is supposed equal to 1 excepted for adipose  
 4 tissues (density 0.9) and bones (density 2)

Tissue / organ	Symbol	Value
Adipose	$V_{adip}$	18.8
Adrenals	$V_{adrenal}$	0.014
Arterial blood	$V_{art}$	1.40
Venous blood	$V_{ven}$	4.20
Bone	$V_{bone}$	2.75
Brain	$V_{brain}$	1.45
Breast	$V_{breast}$	0.025
Gut	$V_{gut}$	1.02
Gut lumen	$V_{gut\_lumen}$	0.65
Heart	$V_{heart}$	0.33
Kidney	$V_{kidney}$	0.31
Liver	$V_{liver}$	1.80
Lung	$V_{lung}$	0.50
Upper respiratory tract	$V_{urt}$	0.15
Bone marrow	$V_{marrow}$	3.65
Muscles	$V_{muscle}$	29.0
Others	$V_{other}$	7.06
Pancreas	$V_{pancreas}$	0.14
Skin	$V_{skin}$	3.30
Spleen	$V_{spleen}$	0.15
Stomach	$V_{stomach}$	0.15
Stomach lumen	$V_{stom\_lumen}$	0.25
Testes	$V_{testes}$	0.056
Thyroid	$V_{thyroid}$	0.019

5

1 Table II. Blood flows for the various organs or tissues (Unit L/min). These have been  
 2 computed using cardiac output, percent blood flows per tissue mass and organ weights given  
 3 in ICRP 2002 Pub 89 [14] (Table 2.8 p18-19, Table 2.39 p28, Table 2.40 p29) or provided  
 4 by William & Leggett [15].

Tissue or organ	Symbol	Value
Adipose	$F_{adip}$	0.564
Adrenals	$F_{adrenal}$	0.02
Brain	$F_{brain}$	0.78
Breast	$F_{breast}$	0.00
Gut	$F_{gut}$	0.98
Heart	$F_{heart}$	0.35
Kidney	$F_{kidney}$	1.23
Liver	$F_{eport}$	0.45
Lung	$F_{total}$	6.72
Bone marrow	$F_{marrow}$	0.29
Muscles	$F_{muscle}$	1.11
Others	$F_{other}$	0.19
Pancreas	$F_{pancreas}$	0.065
Skin	$F_{skin}$	0.33
Spleen	$F_{spleen}$	0.19
Stomach	$F_{stomach}$	0.065
Testes	$F_{testes}$	0.004
Thyroid	$F_{thyroid}$	0.094

5

1

2 Table III. BD and EB-specific parameters. These have been taken from Brochot et al. (2005)

3 for BD parameters and Brochot et al. (2007) for EB parameter.

Parameter	Symbol	Value	Unit
BD fat over blood partition coefficient		22	–
BD lung over air partition coefficient	$P_{lung\_over\_air}$	0.653	–
EB liver over blood partition coefficient	$P_{liver\_EB}$	0.59	–
Metabolisation rate for BD into EB	$K_{met}$	0.3	L/min

4

5 Table IV. Fat content for the various organs or tissues. These have been taken from Fiserova-

6 Bergerova (1983) and Van der Molen (1996). Default value is 0.049, which corresponds to

7 “remaining organs” in Van der Molen (1996).

Tissue or organ	Value
Adipose	0.859
Adrenals	default
Brain	0.11
Breast	default
Gut	0.065
Heart	0.083
Kidney	0.052
Liver	0.049
Lung	0.017
Bone marrow	0.186
Muscles	0.064
Others	default
Pancreas	0.105
Skin	0.15
Spleen	0.03
Stomach	default
Gonads	default
Thyroid	default

8

1 Table V. Mean number of molecules in a cell, standard deviation and coefficient of variation  
2 per organ at steady state at time 100 (5000 for fat, 500 for marrow) for 100 simulations  
3 performed with Dizzy. Exposure concentration is  $1.7 \mu\text{g}/\text{m}^3$ .

Organ	Mean number of molecules in a cell	Standard deviation	Coefficient of variation
Fat	379.3	10.0	0.026
Marrow	82.3	4.49	0.0545
Kidney	22.9	4.52	0.197
Liver	15.2	3.2	0.211
Spleen	13.6	3.86	0.284

4

1 **Figure legends**

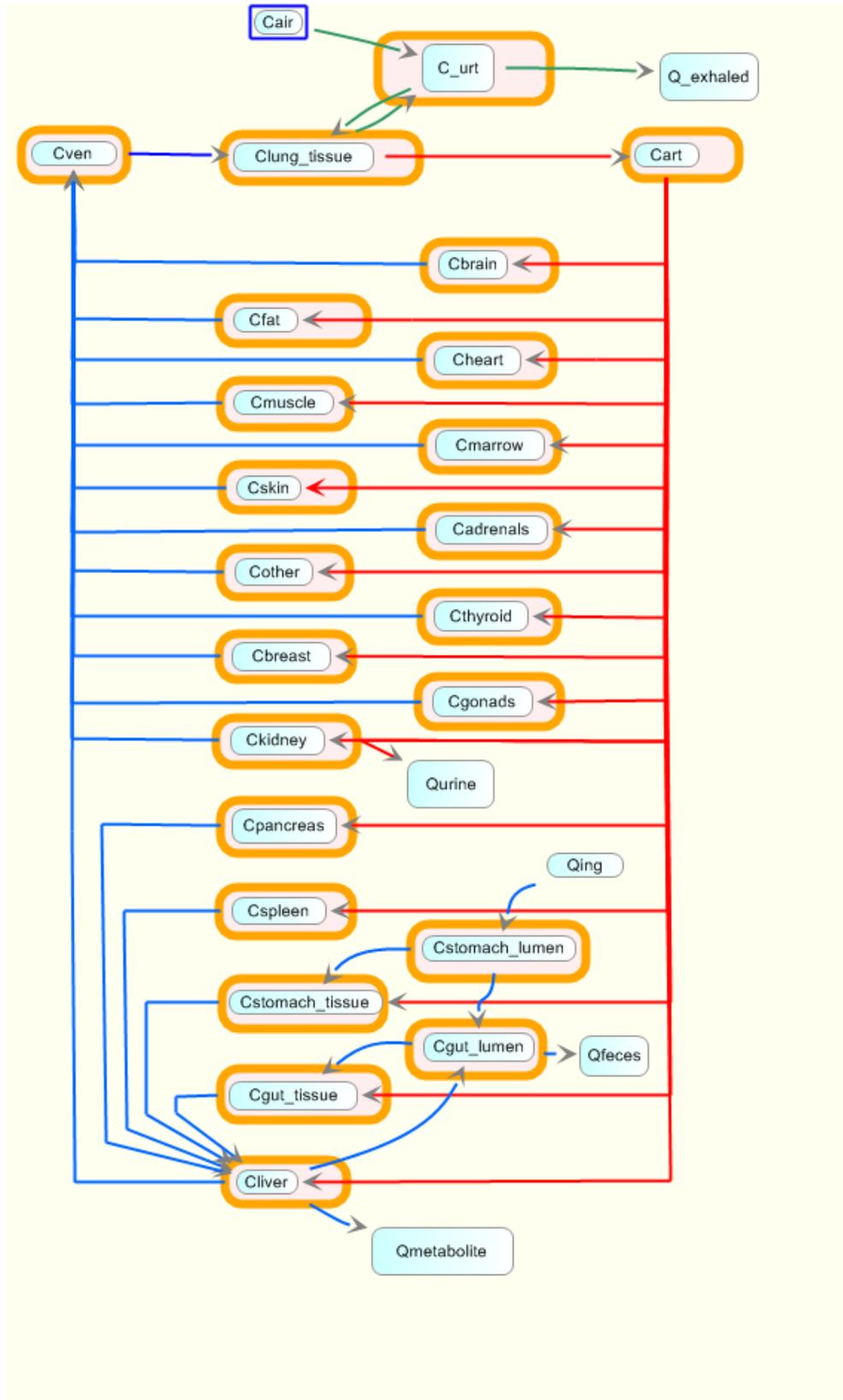
2

3 Figure 1. PBPK model for butadiene, as implemented in Jdesigner.

4 Figure 2. Distribution of BD metabolites number per cell in a liver cell at steady state at time  
5 100 for 100 simulations performed with Dizzy. Exposure concentration is  $1.7 \mu\text{g}/\text{m}^3$ .

6 Figure 3. Excess liver cancer risk in relation to exposure concentration. The deterministic  
7 probability is represented by the plain line. The points are the mean values obtained by  
8 stochastic simulations for 100 cells.

9 Figure 4. Kinetics of butadiene metabolites in one liver cell for a man leaving industrial  
10 vicinity to general environment. Plain line is the deterministic model and points are stochastic  
11 predictions for different time points (the time step is one minute).

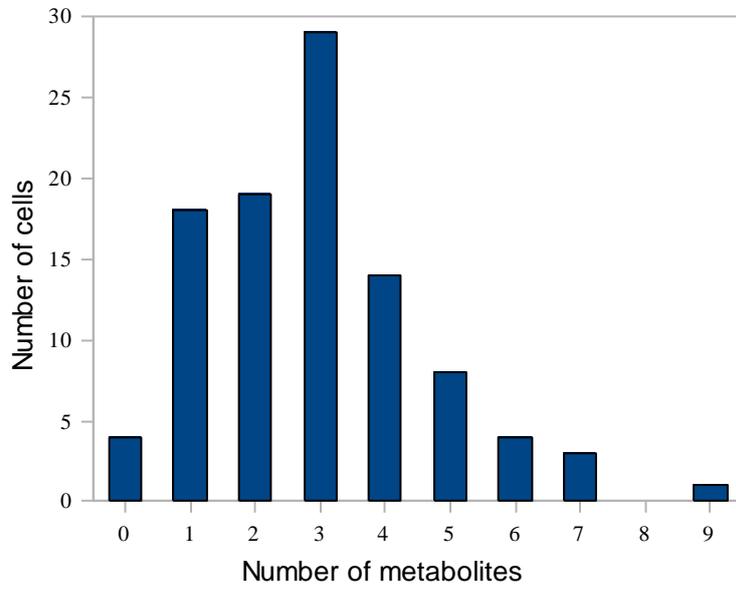


1 Figure 1.

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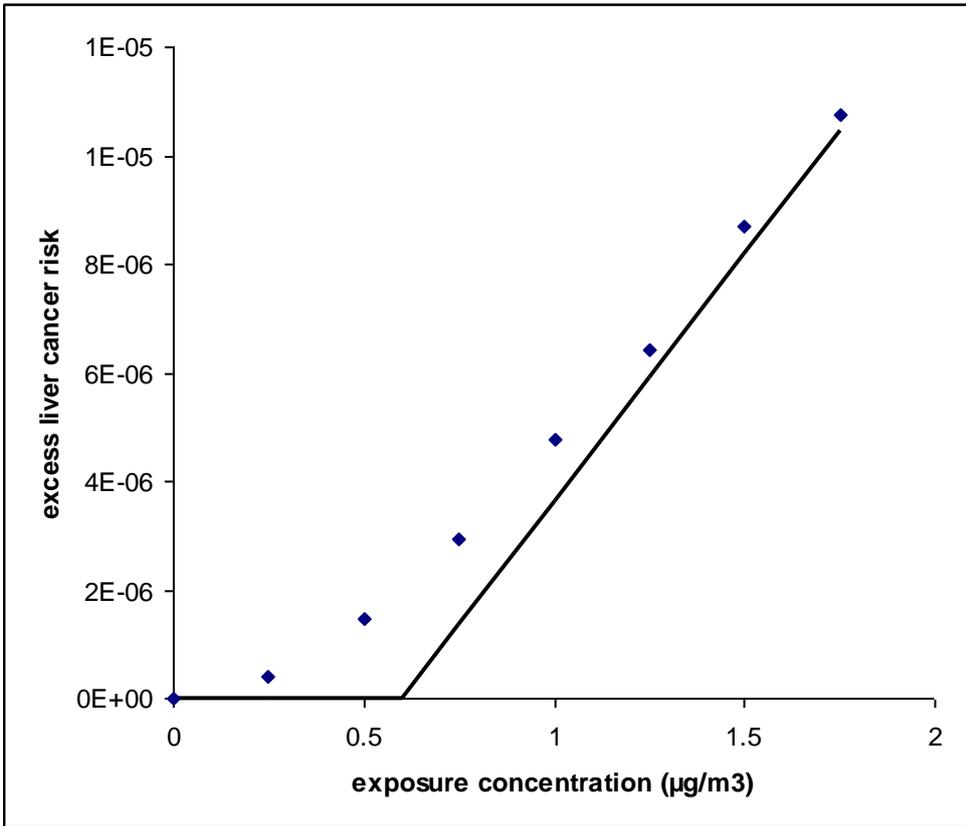


4

5 Figure 2.

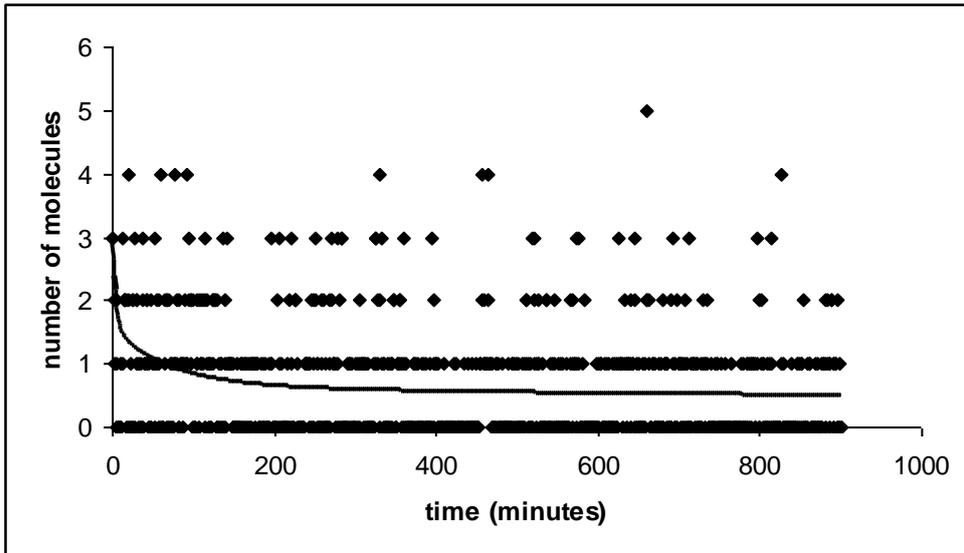
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3 Figure 3.

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3 Figure 4.

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