Modeling of the Internal Kinetics of Benzo(a)pyrene and 3-Hydroxybenzo(a)pyrene Biomarker from Rat Data

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Measurements of 3-hydroxybenzo(a)pyrene (3-OHBaP) in urine has been proposed for the biomonitoring of exposure to benzo(a)pyrene (BaP) in workers. To allow a better understanding of the toxicokinetics of BaP and its key biomarker, a multi-compartment model was developed based on rat data previously obtained by this group. According to the model, iv injected BaP is rapidly distributed from blood to tissues ($t_{1/2} = 3.65 \text{ h}$), with particular affinity for tissue lipid components and liver and lung proteins. BaP is then rapidly distributed to lungs, where significant tissue uptake occurs, followed by the skin, liver, and adipose tissues. Once in liver, BaP is readily metabolized, and 3-OHBaP is formed with a $t_{1/2}$ of 3.32 h. Lung metabolism of BaP was also accounted for, but its contribution to the whole kinetics was found to be negligible. Once formed, 3-OHBaP is distributed from blood to the various organs almost as fast as the parent compound ($t_{1/2} = 2.26 \text{ h}$). In kidneys, 3-OHBaP builds up as a result of the smaller rate of 3-OHBaP urinary excretion ($t_{1/2} = 4.52 \text{ h}$) as compared with its transfer rate from blood to kidneys ($t_{1/2} = 27.8 \text{ min}$). However, overall clearance of 3-OHBaP from the body is driven by its biliary transfer from liver to the gastrointestinal tract ($t_{1/2} = 3.81 \text{ h}$). The model provides a great fit to independent sets of published data on 3-OHBaP urinary excretion time course ($\chi^2 = 0.019$). This model proves useful in establishing the main biological determinants of the overall kinetics of these compounds.

Key Words: benzo(a)pyrene; 3-hydroxybenzo(a)pyrene; toxicokinetic model; rat.

Biomonitoring is recognized as a privileged approach to assess health risks associated with occupational exposure to polycyclic aromatic hydrocarbons (PAHs) (Angerer et al., 2007; Jacob and Seidel, 2002). Measurements of 1-hydroxypyrene (1-OHP), a metabolite of pyrene, have been proposed for the biomonitoring of exposure to PAHs (Jongeneelen et al., 1987). Numerous biomonitoring studies in workers of different settings (aluminum plant, coke plant, wood treatment plant, etc.) have shown the usefulness of this biomarker to identify exposed groups of workers (Bouchard and Viau, 1999; Maitre et al., 2008). However, given that pyrene is not a carcinogenic PAH, measurement of its metabolite 1-OHP for the biomonitoring of exposure to carcinogenic PAH has been questioned. Some authors have thus developed methods to identify other biomarkers of exposure to carcinogenic PAH (Bouchard et al., 2009; Gendre et al., 2002, 2004). This includes the development of sensitive methods allowing the measurement of urinary 3-hydroxybenzo(a)pyrene (3-OHBaP) as an indicator of occupational exposure to BaP (see Fig. 1 for chemical structures), one of the most studied and abundant carcinogenic PAH in workplaces (Barbeau et al., 2011; Forster et al., 2008; Gendre et al., 2002, 2004; Lafontaine et al., 2004; Simon et al., 2000; Straif et al., 2005). However, in the study of Gendre et al. (2004) in workers occupationally exposed to PAH, it was shown that 3-OHBaP urinary excretion was delayed compared with that of 1-OHP, with maximum excretion lagging by about 15 h that of 1-OHP.

To help interpret biomonitoring data on 3-OHBaP such as the ones published by Gendre et al. (2004), the detailed time profiles of BaP and its biomarker 3-OHBaP have recently been documented in blood, tissues, and excreta of iv exposed rats (Marie et al., 2010). The latter data complemented previously published urinary time course data of 3-OHBaP in rats iv injected with BaP under the same exposure conditions or with 3-OHBaP metabolite (Bouchard and Viau, 1996). The atypical urinary time course data of 3-OHBaP previously reported by Bouchard and Viau (1996) following iv injection with an initial increase in urinary excretion rates in the hours post-dosing followed by a gradual elimination matched kidney time course data of 3-OHBaP recently documented by Marie et al. (2010). Ramesh et al. (2001) also determined the time courses of the parent compound BaP and total aqueous and organic metabolites in blood, tissues, and excreta of rats following...
a very high oral dose of BaP (400 μmol/kg), along with the relative distribution of specific BaP metabolites including 3-OHBaP; nonetheless, the time courses of the different metabolites were not reported. Other researchers have performed mass-balance studies following administration of labeled BaP (¹⁴C-BaP or ³⁵⁸BaP) but the kinetics of specific metabolites to be used as biomarkers were not quantified (Mitchell, 1982; Moir et al., 1998; Uziel and Haglund, 1988; Withey et al., 1993).

Although animal kinetic data are now available for 3-OHBaP (Bouchard and Viau, 1996; Lee et al., 2003; Marie et al., 2010), the main biological processes governing the overall kinetics of 3-OHBaP in blood, tissues, and excreta have not yet been fully elucidated. Knowledge of the kinetics of 3-OHBaP is needed for efficient use of 3-OHBaP as a potential biomarker of exposure to BaP. To better understand the biological determinants of the kinetic behavior of 3-OHBaP, the objective of this study was to develop a biomathematical model describing the toxicokinetics of BaP and 3-OHBaP specifically.

The modeling approach consisted of developing a biologically based kinetic model in rats that captures essential biological determinants of the kinetics of BaP and its key biomarker of exposure, 3-OHBaP, for later extension to humans. It emphasizes on mass-balance and uses available in vivo time courses of BaP and 3-OHBaP in blood, key tissues, and excreta for the determination of model parameters, without the need for detailed determination of anatomical or in vitro-based physiological parameters. In the determination of model parameter values, extensive use is made of the different time scales over which the various biological processes occur. Regrouping tissues according to these specific time scales simplified the determination of the key parameters governing the overall model kinetics.

**MATERIALS AND METHODS**

**General modeling approach.** A toxicokinetic model has been developed where BaP and 3-OHBaP in blood, tissues, and excreta were represented as compartments. The conceptual representation of the model was based on the analysis of the kinetic behavior experimentally observed from data collected in our laboratory (Bouchard and Viau, 1996; Marie et al., 2010). The time evolution of BaP and 3-OHBaP amounts in each compartment of the model was then mathematically described by a system of coupled differential equations.

**Experimental data used for model development.** The data of Marie et al. (2010) on the time profiles of BaP and 3-OHBaP in blood, tissues (liver, kidney, lung, adipose tissues, and skin), and excreta (urine and feces) of male Sprague-Dawley rats over a 72-h period following an iv injection of 40 μmol/kg bw of BaP (collection times = 2, 4, 8, 16, 24, 33, 48, and 72 h postinjection) served as a basis for the development of the toxicokinetic model for BaP and 3-OHBaP.

**Model representation.** Figure 2 illustrates the conceptual model of the kinetics of BaP and its 3-OHBaP biomarker in blood, tissues, and excreta. Symbols and abbreviations are described in Table 1. In the model, blood and key organs contributing significantly to the absorption, distribution, and retention of BaP or 3-OHBaP, or otherwise to the metabolism of BaP into 3-OHBaP or other metabolites, were represented as compartments as well as urinary and fecal elimination routes. The rates of change in the amounts of BaP or 3-OHBaP (dXᵢ/dt) in a given compartment (on a molar basis) were then expressed by the difference between incoming and outgoing rates from the compartment. Transfer rates between compartments represented either the physical transfer of BaP or 3-OHBaP from one organ to the other or the absorption rate at a site-of-entry compartment or otherwise the biotransformation rate of BaP into 3-OHBaP or into other metabolites in a metabolizing organ (on a molar basis). Resolution of the differential equations simulating the kinetics of BaP and 3-OHBaP in the body generated the mathematical functions (Xᵢ(t)) describing the time profile of these molecules in the different model compartments.

The model was built while ensuring conservation of mass (in moles), hence at all times, the dose was equal to the sum of burdens in the different compartments (parent compound and metabolites) as well as those accumulated in excreta since exposure.

**Determination of model parameter values.** The parameters of the kinetic model were the intercompartment transfer rates of BaP and 3-OHBaP, the metabolism rates of BaP into 3-OHBaP and other metabolites, the elimination rates of BaP and 3-OHBaP, and their fractional partition in tissues in equilibrium with blood (see Table 1 for the different model parameters). Parameter values were established by best-fit adjustments of the analytical solution of the differential equations to the experimental kinetic data of Marie et al. (2010) on the time profiles of BaP and 3-OHBaP in blood, tissues, and excreta following iv injection of BaP. The software Mathematica 7.0 from Wolfram Research, Inc. (Champaign, IL) was used to analytically solve the system of differential equations. It is important to note that several procedures exist to best-fit general analytical functions to data sets. For fitting, the algorithm FindFit (included in Mathematica) was used, which essentially reproduces a least-square minimization.

To simplify differential equations and allow a first estimate of parameter values, extensive use was made of the different time scales during which the various biological processes occur (e.g., time of absorption, distribution, metabolism, and excretion). These different time scales render the use of the quasi steady state approximation (QSSA) possible (Segel, 1988; Segel and Slemrod, 1989). Briefly, QSSA predicts that a compartment X reaches a kinetic equilibrium with its feeding compartment when the output rate is significantly more rapid than the time variations in its feeding source. In these conditions, burden of compartment X is always proportional to that of the source compartment. Therefore, if the burden of the feeding compartment varies as a function of time, that of the compartment X will vary such that the ratio of burdens in compartment X to that of the feeding compartment remains constant. This phenomenon is very frequent in kinetics of drugs and toxic substances.

With these considerations, over specific time periods, the experimental data can be expressed by a single exponential function. For example, in the case of BaP blood compartment, between 6 and 24 h post-dosing, blood time course of BaP is determined by distribution of BaP from blood to tissues; at times > 24 h, all the BaP has been distributed to tissues, hence, blood time course of BaP is simply driven by the slow feeding from storage tissues, hence lung and adipose tissues.

As another example of the application of the QSSA, the time course of BaP in lung (LU(t)) can be considered. The analytical solution describing this time

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**FIG. 1.** Chemical structures of BaP and its 3-OHBaP metabolite.
course is proportional to (see Table 1 for the description of symbols and abbreviations):

\[ LU(t) = \frac{2DK_{BLU}}{\sqrt{(K_{LGI} + K_{KU} + K_{BAT} + K_{BLU} + K_{LUB})^2 - 4K_{LUB}(K_{LGI} + K_{KU} + K_{BAT})}} \left( e^{[K_{LGI} + K_{KU} + K_{BAT} + K_{BLU} + K_{LUB}]t} - 4K_{LUB} \left( K_{LGI} + K_{KU} + K_{BAT} \right) \right) \]

however, at times > 24 h, this function reduces to the single exponential:

\[ LU(t) \approx \frac{2DK_{BLU}}{\sqrt{(K_{LGI} + K_{KU} + K_{BAT} + K_{BLU} + K_{LUB})^2}} \left( e^{-K_{LUB}t} - 1 \right) \]

With those initial values fixed, an iteration procedure allowing a one-by-one readjustment of each parameter values for several cycles (at least 100 for each parameter) was then performed. As a first step, parameters describing the kinetics of BaP were fixed and, as a second step, an optimization of parameter values related to 3-OHBaP was performed. The algorithm started by fitting the critical transfer rates governing the kinetics, whereas the other values were kept constant. Once an iteration cycle was completed, another fitting cycle was carried out for several cycles until the resulting variations in the fitted values were negligible. Each of these best fittings was based on the least-square minimization method included in Mathematica and on common linear regression (for an extensive explanation on the algorithm, please see Weisstein, 2010).

**Model simulations.** The analytical resolution of the systems of equations, described in previous section, was a required step to fix transfer rate values of the toxicokinetic model, by fitting experimental data of Marie et al. (2010). Once the parameter values of the model were determined in Mathematica, the system of differential equations representing the complete model of the kinetics of BaP and 3-OHBaP was numerically solved in MathCad 14 (Parametric Technology Corporation, Needham, MA) using the Adams-Bashforth-Moulton method in order to reproduce the experimental data of Marie et al. (2010), Bouchard and Viau (1996), Lee et al. (2003), and Lafontaine et al. (2004).

**Experimental data used for model validation.** The data of Bouchard and Viau (1996) on the time course of 3-OHBaP in the urine of male Sprague-Dawley rats over a 90-h period following an iv injection of 40 μmol/kg bw of BaP were used for model validation (collection times = 2, 4, 6, 8, 10, 12, 18, 24, 30, 42, 48, 54, 66, 72, 78, and 90 h postinjection of BaP). In order to define the goodness of fit of our model, the Pearson’s chi-square test was used. The data of Lee et al. (2003) on the time course of 3-OHBaP in the urine of adult male Sprague-Dawley rats over a 96-h period following an ip injection of 20 mg/kg bw of BaP were also used for model validation (at 4- to 12-h interval postinjection of BaP).

As a preliminary assessment of the use of the modeling approach for human biomonitoring, the available published human data of Lafontaine et al. (2004) on the time course of 3-OHBaP in the urine of a worker over a 48-h period following an occupational respiratory exposure to BaP were further used. These authors collected all urine voided starting with pre-shift urine sample on the first day of a workweek (following at least a 36-h period without occupational exposure) and ending with beginning-of-shift urine sample of the third working day. For this modeling, parameter values were kept as determined in animals except those governing the overall excretion kinetics of the key biomarker of exposure 3-OHBaP.
## TABLE 1
Variables and Parameters of the Toxicokinetic Model for BaP and 3-OHBaP

<table>
<thead>
<tr>
<th>Variables and parameters</th>
<th>Symbols or abbreviations</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td><strong>Variables</strong></td>
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</tr>
<tr>
<td>$B(t)$</td>
<td>Burden of BaP (mol) in blood as a function of time (h)</td>
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<tr>
<td>$L(t)$</td>
<td>Burden of BaP (mol) in liver as a function of time (h)</td>
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</tr>
<tr>
<td>$S(t)$</td>
<td>Burden of BaP (mol) in skin as a function of time (h)</td>
<td></td>
</tr>
<tr>
<td>$K(t)$</td>
<td>Burden of BaP (mol) in kidney as a function of time (h)</td>
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</tr>
<tr>
<td>$LU(t)$</td>
<td>Burden of BaP (mol) in lung as a function of time (h)</td>
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<tr>
<td>$AT(t)$</td>
<td>Burden of BaP (mol) in adipose tissues as a function of time (h)</td>
<td></td>
</tr>
<tr>
<td>$GI_1(t)$</td>
<td>Burden of BaP (mol) in the first segment of the GI tract as a function of time (h)</td>
<td></td>
</tr>
<tr>
<td>$GI_2(t)$</td>
<td>Burden of BaP (mol) in the second segment of the GI tract as a function of time (h)</td>
<td></td>
</tr>
<tr>
<td>$U(t)$</td>
<td>Cumulative urinary amounts of BaP as a function of time (h)</td>
<td></td>
</tr>
<tr>
<td>$F(t)$</td>
<td>Cumulative fecal amounts of BaP as a function of time (h)</td>
<td></td>
</tr>
<tr>
<td>$OLU(t)$</td>
<td>Burden of metabolites of BaP other than 3-OHBaP (mol) resulting from lung metabolism as a function of time (h)</td>
<td></td>
</tr>
<tr>
<td>$OL(t)$</td>
<td>Burden of metabolites of BaP other than 3-OHBaP (mol) resulting from liver metabolism as a function of time (h)</td>
<td></td>
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<tr>
<td>$b(t)$</td>
<td>Burden of 3-OHBaP (mol) in blood as a function of time (h)</td>
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<tr>
<td>$l(t)$</td>
<td>Burden of 3-OHBaP (mol) in liver as a function of time (h)</td>
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<tr>
<td>$s(t)$</td>
<td>Burden of 3-OHBaP (mol) in skin as a function of time (h)</td>
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<tr>
<td>$k(t)$</td>
<td>Burden of 3-OHBaP (mol) in kidney as a function of time (h)</td>
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<tr>
<td>$lu(t)$</td>
<td>Burden of 3-OHBaP (mol) in lung as a function of time (h)</td>
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<tr>
<td>$at(t)$</td>
<td>Burden of 3-OHBaP (mol) in adipose tissues as a function of time (h)</td>
<td></td>
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<tr>
<td>$g_{i1}(t)$</td>
<td>Burden of 3-OHBaP (mol) in the first segment of the GI tract as a function of time (h)</td>
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<tr>
<td>$g_{i2}(t)$</td>
<td>Burden of 3-OHBaP (mol) in the second segment of the GI tract as a function of time (h)</td>
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<tr>
<td>$u(t)$</td>
<td>Cumulative urinary amounts of 3-OHBaP as a function of time (h)</td>
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<tr>
<td>$f(t)$</td>
<td>Cumulative fecal amounts of 3-OHBaP as a function of time (h)</td>
<td></td>
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<tr>
<td>$ol(t)$</td>
<td>Burden of metabolites of 3-OHBaP (mol) resulting from liver metabolism as a function of time (h)</td>
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<tr>
<td><strong>Parameters</strong></td>
<td></td>
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<tr>
<td>$f_B$</td>
<td>Fraction of BaP in blood compared with total amounts in blood and tissues in equilibrium with blood (%)</td>
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<tr>
<td>$f_L$</td>
<td>Fraction of BaP in liver compared with total amounts in blood and tissues in equilibrium with blood (%)</td>
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<tr>
<td>$f_S$</td>
<td>Fraction of BaP in skin compared with total amounts in blood and tissues in equilibrium with blood (%)</td>
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<tr>
<td>$f_K$</td>
<td>Fraction of BaP in kidney compared with total amounts in blood and tissues in equilibrium with blood (%)</td>
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<tr>
<td>$f_N$</td>
<td>Fraction of BaP in non-observed organs compared with total amounts in blood and tissues in equilibrium with blood (%)</td>
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<tr>
<td>$K_{BL}$</td>
<td>Transfer rate of BaP from blood to liver (per h)</td>
<td></td>
</tr>
<tr>
<td>$K_{BS}$</td>
<td>Transfer rate of BaP from blood to skin (per h)</td>
<td></td>
</tr>
<tr>
<td>$K_{BK}$</td>
<td>Transfer rate of BaP from blood to kidney (per h)</td>
<td></td>
</tr>
<tr>
<td>$K_{BLU}$</td>
<td>Transfer rate of BaP from blood to lung (per h)</td>
<td></td>
</tr>
<tr>
<td>$K_{BAT}$</td>
<td>Transfer rate of BaP from blood to adipose tissues (per h)</td>
<td></td>
</tr>
<tr>
<td>$K_{BN}$</td>
<td>Transfer rate of BaP from blood to non-observed tissues in equilibrium with blood (per h)</td>
<td></td>
</tr>
<tr>
<td>$K_{LB}$</td>
<td>Transfer rate of BaP from liver to blood (per h)</td>
<td></td>
</tr>
<tr>
<td>$K_{SB}$</td>
<td>Transfer rate of BaP from skin to blood (per h)</td>
<td></td>
</tr>
<tr>
<td>$K_{KB}$</td>
<td>Transfer rate of BaP from kidney to blood (per h)</td>
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<tr>
<td>$K_{LUB}$</td>
<td>Transfer rate of BaP from lung to blood (per h)</td>
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</tr>
<tr>
<td>$K_{ATB}$</td>
<td>Transfer rate of BaP from adipose tissues to blood (per h)</td>
<td></td>
</tr>
<tr>
<td>$K_{NB}$</td>
<td>Transfer rate of BaP from non-observed tissues to blood (per h)</td>
<td></td>
</tr>
<tr>
<td>$K_{GGLI}$</td>
<td>Transfer rate of BaP from liver to the GI tract (per h)</td>
<td></td>
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<tr>
<td>$K_{GIGI}$</td>
<td>Transfer rate of BaP from the first segment of the GI tract to the second segment (per h)</td>
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</tr>
<tr>
<td>$K_{GIF}$</td>
<td>Transfer rate of BaP from the second segment of the GI tract to feces (per h)</td>
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</tr>
<tr>
<td>$f_o$</td>
<td>Fraction of 3-OHBaP in blood compared with total amounts in blood and tissues in equilibrium with blood (%)</td>
<td></td>
</tr>
<tr>
<td>$f_i$</td>
<td>Fraction of 3-OHBaP in liver compared with total amounts in blood and tissues in equilibrium with blood (%)</td>
<td></td>
</tr>
<tr>
<td>$f_s$</td>
<td>Fraction of 3-OHBaP in skin compared with total amounts in blood and tissues in equilibrium with blood (%)</td>
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components, and finally distribution to the lungs (see Fig. 2).

intestinal (GI) tract, renal clearance, distribution to lipidic compartments for BaP and to a certain extent for 3-OHBaP, (5) organs for BaP, (4) lipidic components in organs act as storage compartments for BaP and 3-OHBaP in blood, tissues, urine, and feces of rats exposed iv to BaP.

The excellent correspondence of our mathematical model with experimental data has corroborated our principal hypotheses about BaP and 3-OHBaP kinetics, namely: (1) BaP and 3-OHBaP are rapidly distributed (see Figs. 3–5), (2) most organs monitored are in kinetic equilibrium with blood (see Figs. 3B and 3C), (3) liver and lungs are two biotransformation organs for BaP, (4) lipidic components in organs act as storage compartments for BaP and to a certain extent for 3-OHBaP, (5) a buildup of 3-OHBaP is found in the kidneys (see Fig. 4C), and (6) 3-OHBaP is only a quantitatively minor metabolite of BaP (see Fig. 5).

Overall elimination half-lives of BaP and 3-OHBaP from blood and tissues in equilibrium with blood were found to be 3.65 and 2.26 h, respectively, which represents a rapid tissue distribution, biotransformation, and elimination of both molecules (see Table 2). Five different processes contribute to this total elimination rate of BaP and 3-OHBaP: biotransformation in the liver, elimination through the gastrointestinal (GI) tract, renal clearance, distribution to lipidic components, and finally distribution to the lungs (see Fig. 2).

Of all the preceding mechanisms, the most important contribution to the overall elimination rate of BaP from blood is liver biotransformation (72.6%). On the other hand, elimination of 3-OHBaP through the gastrointestinal tract contributes mainly to 3-OHBaP overall clearance from blood (62.9%).

Because distribution of BaP and 3-OHBaP in the body is very rapid, some tissues are found to reach a kinetic equilibrium with blood. In the case of BaP, this assumption was also very well corroborated by our model in which blood, skin, liver, kidneys, and other non-observed tissue were described by a fraction of the analytical function $f_{S}(t)$, which represents the sum of BaP levels in blood and in tissues in equilibrium with blood, those showing highest partitioning of BaP (see Table 2). Consequently, to obtain the function describing BaP present in blood, liver, skin, and kidneys, this function simply has to be multiplied by the fractional partition for the compartment of interest: $B(t) = f_{B}B(t)$, $L(t) = f_{L}B(t)$, $S(t) = f_{S}B(t)$, and $K(t) = f_{K}B(t)$. The blood compartment for BaP only accounts for 0.914% of the total of BaP found in blood and all tissues in equilibrium with blood. In a similar manner, kidneys just receive 0.935% of total BaP in blood and tissues in equilibrium with blood. Among the studied tissues in equilibrium with blood, those showing highest partitioning of BaP were the skin (6.27% of total BaP in blood and tissues in equilibrium with blood) and the liver (12.8%). On the other hand, for 3-OHBaP, only the skin and the liver were found to be in equilibrium with the blood compartment with corresponding partitioning of 2.67% for blood, 4.12% for skin, and 5.07%

### RESULTS

Table 2 presents parameter values of the model, and Table 3 depicts blood and tissue half-lives calculated from model parameters; this single set of parameters was used in all data simulations. Figures 3–5 show that the model reproduced closely the data of Marie et al. (2010) on the time courses of BaP and 3-OHBaP in blood, tissues, urine, and feces of rats exposed iv to BaP.

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<tbody>
<tr>
<td>$f_{w}$</td>
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</tr>
<tr>
<td>$k_{bl}$</td>
<td>Transfer rate of 3-OHBaP from blood to liver (per h)</td>
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<tr>
<td>$k_{bub}$</td>
<td>Transfer rate of 3-OHBaP from blood to adipose tissues (per h)</td>
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<tr>
<td>$k_{lb}$</td>
<td>Transfer rate of 3-OHBaP from liver to blood (per h)</td>
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<tr>
<td>$k_{lub}$</td>
<td>Transfer rate of 3-OHBaP from liver to the GI tract (per h)</td>
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<tr>
<td>$k_{atb}$</td>
<td>Transfer rate of 3-OHBaP from adipose tissues to blood (per h)</td>
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<tr>
<td>$k_{bat}$</td>
<td>Transfer rate of 3-OHBaP from blood to adipose tissues (per h)</td>
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<tr>
<td>$k_{egi}$</td>
<td>Transfer rate of 3-OHBaP from the first segment of the GI tract back to liver (per h)</td>
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<tr>
<td>$k_{gif}$</td>
<td>Transfer rate of 3-OHBaP from the second segment of the GI tract to feces (per h)</td>
<td></td>
</tr>
<tr>
<td>$k_{kub}$</td>
<td>Transfer rate of 3-OHBaP from kidney to blood (per h)</td>
<td></td>
</tr>
<tr>
<td>$k_{kb}$</td>
<td>Transfer rate of 3-OHBaP from blood to kidney (per h)</td>
<td></td>
</tr>
<tr>
<td>$k_{sb}$</td>
<td>Transfer rate of 3-OHBaP from skin to blood (per h)</td>
<td></td>
</tr>
<tr>
<td>$k_{bs}$</td>
<td>Transfer rate of 3-OHBaP from blood to skin (per h)</td>
<td></td>
</tr>
<tr>
<td>$k_{lb}$</td>
<td>Transfer rate of 3-OHBaP from liver to blood (per h)</td>
<td></td>
</tr>
<tr>
<td>$k_{bl}$</td>
<td>Transfer rate of 3-OHBaP from blood to liver (per h)</td>
<td></td>
</tr>
<tr>
<td>$L_{LU}$</td>
<td>Metabolism rate of BaP into 3-OHBaP in lung (per h)</td>
<td></td>
</tr>
<tr>
<td>$L_{L}$</td>
<td>Metabolism rate of BaP into 3-OHBaP in liver (per h)</td>
<td></td>
</tr>
<tr>
<td>$L_{GLO}$</td>
<td>Metabolism rate of BaP into metabolites other than 3-OHBaP in lung (per h)</td>
<td></td>
</tr>
<tr>
<td>$L_{LO}$</td>
<td>Metabolism rate of BaP into metabolites other than 3-OHBaP in liver (per h)</td>
<td></td>
</tr>
<tr>
<td>$L_{U}$</td>
<td>Metabolism rate of 3-OHBaP into other metabolites in liver (per h)</td>
<td></td>
</tr>
</tbody>
</table>
for liver. The distribution is more uniform for 3-OHBaP, given that it is less lipophilic than its parent compound.

Conversely, at initial times (< 36 h), BaP in lungs and adipose tissues were not observed to be in equilibrium with BaP in blood; these tissues presented the highest levels of BaP. First, relatively rapid BaP uptakes in lungs and adipose tissues are evident from the measured time profiles (t1/2 = 9.72 min for lungs and t1/2 = 43.7 min for adipose tissues). As shown in Figure 2, these uptakes are represented by the transfer rate of BaP from the blood compartment to the lungs or adipose tissues, KBL and KBAT, respectively. These parameters, at first order, simply have the control over the rate of uptake and total amounts of BaP found in lungs or adipose tissues.

Subsequently, the parallel time course of BaP in the lungs and adipose tissues indicate a similar retention mechanism once BaP reaches both these tissues (t1/2 = 29.3 h for lungs and t1/2 = 27.2 h for adipose tissues). The slopes of the time course curves of 3-OHBaP in these tissues are also similar to those of BaP, which suggests that the same biological determinants govern the kinetics. However, 3-OHBaP is much less retained in lungs because the amounts of 3-OHBaP observed were significantly lower than those of adipose tissues. Two hours postinjection, 2.47% of BaP were found in adipose tissues and 17.0% of BaP in lungs, whereas only 0.0230 and 0.0241% of 3-OHBaP were observed in both lungs and adipose tissues, respectively. The elimination rate of BaP from the lungs (KLU = 2.55 × 10^-2/h) and the transfer rates of BaP from adipose tissues to blood (KATB = 2.37 × 10^-4/h) are the parameters influencing the most the whole kinetics, for if they are too large, storage will no longer occur. Conversely, if they are too small, the first phase of rapid distribution of BaP from blood to lungs or adipose tissues will simply consist of an endless accumulation of BaP and the kinetics would no longer be driven by the parent compound.

Concerning the kidney, in the BaP case, it responds as any other organ in kinetic equilibrium with blood. However, this is not the case for 3-OHBaP for which a small built-up in the kidney is observed. Our toxicokinetic model established a smaller elimination rate of 3-OHBaP from the kidneys (either by excretion to urine, kuru, or by reabsorption into blood, kkk [t1/2 = 4.52 h] compared with the rate of transfer from blood to kidney, kbk [t1/2 = 28.8 min]). The values of the

**TABLE 2**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>f_b</td>
<td>9.14 × 10^-1</td>
</tr>
<tr>
<td>f_L</td>
<td>12.80</td>
</tr>
<tr>
<td>f_S</td>
<td>6.27</td>
</tr>
<tr>
<td>f_K</td>
<td>9.35 × 10^-1</td>
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<tr>
<td>f_N</td>
<td>79.08</td>
</tr>
<tr>
<td>f_o</td>
<td>2.67</td>
</tr>
<tr>
<td>f_r</td>
<td>5.07</td>
</tr>
<tr>
<td>f_s</td>
<td>4.12</td>
</tr>
<tr>
<td>f_n</td>
<td>88.14</td>
</tr>
</tbody>
</table>

Transfer rates (per h)

| K_BLU      | 4.28 |
| K_BAT      | 0.952 |
| K_LUB      | 3.19 × 10^-3 |
| K_ATB      | 2.55 × 10^-2 |
| K_LGI      | 5.89 × 10^-3 |
| K_GGIGI    | 4.96 × 10^-2 |
| K_GGIF     | 1.25 × 10^-1 |
| K_KLU      | 3.68 × 10^-1 |
| k_bb       | 1.50 |
| k_bu       | 1.63 × 10^-2 |
| k_bu       | 1.47 × 10^-1 |
| k_bb       | 1.44 × 10^-1 |
| k_bu       | 4.58 |
| k_ab       | 9.76 × 10^-2 |
| k_bb       | 3.81 |
| k_ggig      | 7.39 × 10^-2 |
| k_gg       | 1.77 × 10^-2 |
| k_gg       | 1.25 × 10^-1 |
| k_ku       | 9.11 × 10^-3 |
| K_LU       | 3.91 × 10^-3 |
| K_LI       | 2.09 × 10^-1 |
| K_LUOLU    | 1.66 × 10^-2 |
| K_LOL      | 8.68 × 10^-1 |
| k_kb       | 3.70 |

These fractions are defined as follows: f_X = BaP in compartment X / BaP in all tissues in equilibrium with blood.

**TABLE 3**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Parameter</th>
<th>Value</th>
<th>(\chi^2)</th>
<th>Parameter</th>
<th>Value</th>
<th>(\chi^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>t1/2BAT</td>
<td>0.728</td>
<td>0.456</td>
<td>t1/2BAT</td>
<td>4.76</td>
<td>0.014</td>
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<tr>
<td></td>
<td>t1/2BLU</td>
<td>0.162</td>
<td></td>
<td>t1/2BLU</td>
<td>42.7</td>
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<tr>
<td></td>
<td>t1/2BU</td>
<td>0.463</td>
<td></td>
<td>t1/2BU</td>
<td>0.463</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>t1/2L1</td>
<td>3.32</td>
<td>15.8</td>
<td>t1/2L1</td>
<td>0.182</td>
<td>0.809</td>
</tr>
<tr>
<td></td>
<td>t1/2L2</td>
<td>118</td>
<td></td>
<td>t1/2L2</td>
<td>0.505</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t1/2L2OL</td>
<td>0.799</td>
<td></td>
<td>t1/2L2OL</td>
<td>0.799</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>t1/2K1</td>
<td>1.89</td>
<td>0.410</td>
<td>t1/2K1</td>
<td>76.1</td>
<td>0.063</td>
</tr>
<tr>
<td>Skin</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Faeces</td>
<td>t1/2F1</td>
<td>14.0</td>
<td>0.324</td>
<td>t1/2F1</td>
<td>9.38</td>
<td>19.4</td>
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<td></td>
<td>t1/2F2</td>
<td>5.55</td>
<td></td>
<td>t1/2F2</td>
<td>5.55</td>
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</tr>
<tr>
<td>Urine</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.139</td>
</tr>
<tr>
<td>Adipose tissues</td>
<td>t1/2AT</td>
<td>27.2</td>
<td>5.88</td>
<td>t1/2AT</td>
<td>7.10</td>
<td>0.132</td>
</tr>
<tr>
<td>Lung</td>
<td>t1/2LUB</td>
<td>217</td>
<td>0.304</td>
<td>t1/2LUB</td>
<td>0.151</td>
<td>0.122</td>
</tr>
<tr>
<td></td>
<td>t1/2L2UB</td>
<td>41.9</td>
<td></td>
<td>t1/2L2UB</td>
<td>41.9</td>
<td></td>
</tr>
</tbody>
</table>

\(\chi^2\) is the coefficient of goodness of fit defined by: \(\chi^2 = \sum_{i=1}^{N} \frac{(y_{\text{exp}} - y_{\text{theo}})^2}{\sigma^2}\), where the exponent “exp” refers to experimental data, “theo” is related to model prediction of data points at time i, and \(\sigma\) represents the SD from the corresponding data set.
FIG. 3. Comparison of model simulations (solid and dotted lines) with experimental data (symbols) of Marie et al. (2010) on the time profiles of BaP in blood and tissues of male Sprague-Dawley rats following an iv injection of 40 μmol/kg bw of BaP. Each symbol represents experimental means, and vertical bars are SDs (n = 4). (A) ▲, lung; ●, adipose tissues; (C) ●, liver; *, blood; (B) ▼, skin; ■, kidney.

FIG. 4. Comparison of model simulations (solid and dotted lines) with experimental data (symbols) of Marie et al. (2010) on the time profiles of 3-OHBaP in blood and tissues of male Sprague-Dawley rats following an iv injection of 40 μmol/kg bw of BaP. Each symbol represents experimental means, and vertical bars are SDs (n = 4). (A) ▲, lung; ●, adipose tissues; (C) ●, liver; *, blood; (B) ▼, skin; ■, kidney.
parameters $k_{bk}$, $k_{br}$ and $k_{ra}$ determine maximum levels of 3-OHBaP in the kidneys and time to peak levels, which was observed at around 8 h. The later value, $k_{br}$, is also fixed by the fraction of injected BaP recovered overall in urine as 3-OHBaP.

Regarding biotransformation, our model accounts for liver and lung metabolism. In the liver and lung, total elimination of BaP occurs mainly through metabolism (99.5% of BaP reaching the liver and 86.5% of lung BaP being metabolized). According to our model, 3-OHBaP itself is also partly metabolized (26.5% of 3-OHBaP reaching the liver being metabolized). Liver metabolism of BaP to 3-OHBaP and other metabolites as well as biotransformation of 3-OHBaP itself had to be considered relatively fast to provide a close approximation to the set of data. Lung metabolism of BaP into 3-OHBaP and other metabolites was found to be slow ($K_{LUB} + K_{LOLU} = 2.05 \times 10^{-2}/h$) compared with that of the liver, but it was faster than the transfer of BaP from lung to the blood stream ($K_{LUB} = 3.19 \times 10^{-3}/h$). There are thus two different parameters representing biotransformation process in both liver and lung. In the lung compartment, BaP is converted into 3-OHBaP at a low $K_{LUB}$ rate of $3.91 \times 10^{-3}/h$ ($t_{1/2} = 7.39$ days) and into other metabolites at the $K_{LOLU}$ rate of $1.66 \times 10^{-2}/h$ ($t_{1/2} = 41.8$ h). In the liver, the corresponding rates are much more rapid than those of the lungs with $K_{LUB}$ rate of $2.09 \times 10^{-1}/h$ ($t_{1/2} = 3.32$ h) and $K_{LOLU}$ rate of $8.68 \times 10^{-1}/h$ ($t_{1/2} = 47.9$ min).

It was then verified, with the parameters described previously, that the model was able to closely reproduce the cumulative excretions in urine and feces for both substances (see coefficients of goodness of fit in Table 3), as expected given the direct link with metabolites levels in biological matrices measured for a given internal BaP dose. Simulations were in excellent accordance with the observed cumulative excretion time courses, especially considering the fact that measurements are not “real” cumulative excretions from a single rat but are rather collections from different rats sacrificed at different time points.

In the particular case of the GI tract, it has been modeled as separate subcompartments to describe the kinetics of both BaP and 3-OHBAp. The first segment ($G_{1}$ for BaP and $g_{i1}$ for 3-OHBAp) is the one that effectively represents the kinetics of BaP and 3-OHBAp in the GI tract and therefore receiving liver burdens and representing possible enterohepatic recycling of 3-OHBAp. By definition, the first segments describe different kinetics for BaP and 3-OHBAp. Transfer rate values from liver to the $G_{1}$ segment and from the $G_{1}$ to $G_{2}$ segments are $K_{L_G1} = 5.89 \times 10^{-3}/h$ and $K_{G1G2} = 4.96 \times 10^{-2}/h$ for the BaP compartment and $K_{g_{i1}} = 3.81/h$ and $K_{g_{i2}} = 7.39 \times 10^{-2}/h$ for 3-OHBAp compartment, whereas enterohepatic recycling of 3-OHBAp from the GI back to the liver is represented by the $K_{g_{il}}$ rate of $1.77 \times 10^{-2}/h$. The second segment ($G_{2}$ for BaP and $g_{2}$ for 3-OHBAp) simply represents a compartment which delays fecal excretion with a half-life of 5.55 h. This segment acts as a transition delay to account for the GI tract transit time. For this reason, elimination rate values associated with this second segment ($K_{G_{il}}$ and $K_{g_{il}}$) were set to be equal for BaP and 3-OHBAp.

With the parameter values determined from the rat data of Marie et al. (2010), the model was found to provide an excellent approximation ($\chi^2 = 0.323$) of the experimental data of Bouchard and Viau (1996) on the excretion time course of 3-OHBAp in the urine of rats iv exposed to BaP (Fig. 6), with no a posteriori adjustment of parameters. The $p$ value corresponding to 5 degrees of freedom in the experimental data (the data set reported in Bouchard and Viau, 1996 was obtained from five rats) was calculated to be very small, hence 0.00281.

The model also reproduced closely the experimental data of Lee et al. (2003) (Fig. 7). However, this is based on a visual assessment given that these authors did not report the standard deviation of their experimental data such that the corresponding chi-square value could not be computed. Furthermore, because the experimental data of Lee et al. (2003) were obtained in rats exposed ip, simulation was performed with a delay of 10.5 h to account for differences between the iv and ip routes of exposure.

On the basis of the rat model and animal-to-human adjustment of parameter values governing the overall excretion kinetics of the key biomarker of exposure 3-OHBAp, the time course of 3-OHBAp in the urine of a worker over a 48-h period following an occupational respiratory exposure to BaP was simulated. A good approximation of the available published human time course data was obtained by keeping unchanged all the parameters established from rata data except for the transfer rates of both BaP and 3-OHBAp from adipose tissues or lung to blood (Fig. 8). The latter transfer rates were shown to determine the excretion kinetics. Animal-to-human extrapolation of the transfer rates of both BaP and 3-OHBAp
KINETIC MODEL FOR BAP AND 3-OHBAP BIOMARKER

from adipose tissues or lungs to blood was conducted using the following factor:

\[
K_{ATB}^{human} = \frac{V_{fat}^{human} \times Q_{fat}^{human} \times P_{fat}^{human}}{V_{fat}^{fat} \times Q_{fat}^{fat}} K_{ATB}^{rat}
\]

(3)

\[
K_{LUB}^{human} = \frac{V_{lungs}^{human} \times Q_{lungs}^{human} \times P_{lungs}^{human}}{V_{lungs}^{fat} \times Q_{lungs}^{fat}} K_{LUB}^{rat}
\]

(4)

where \(K_{ATB}^{human}\) and \(K_{ATB}^{rat}\) are the transfer rates of BaP from adipose tissues to blood in humans and rats, respectively. \(Q_{fat}^{human}\) and \(Q_{fat}^{rat}\) represent the regional blood flow rates in humans and rats, respectively. \(V_{fat}^{human}\) and \(V_{fat}^{rat}\) are the volumes of adipose tissues in humans and rats, respectively, and \(P_{fat}\) is the adipose tissue-blood partition coefficient, which is taken to be equal in humans and rats. \(K_{LUB}^{human}\) and \(K_{LUB}^{rat}\) are the transfer rates of BaP from lung to blood in humans and rats, respectively. \(Q_{lungs}^{human}\) and \(Q_{lungs}^{rat}\) represent the regional blood flow rate in humans and rats, respectively. \(V_{lungs}^{human}\) and \(V_{lungs}^{rat}\) are the volumes of lung tissues in humans and rats, respectively, and \(P_{lungs}\) is the lung tissue-blood partition coefficient, which is also taken to be equal in humans and rats. The same type of extrapolation was done for the corresponding 3-OHBaP parameter values. Simulation conducted with these adjusted human model parameters was very accurate without the need for adjustment of any other parameter (including enterohepatic recycling).

DISCUSSION

This study allowed a better understanding of the kinetics of 3-OHBaP, a potential key biomarker of exposure to BaP. It enabled to relate an internal dose of BaP to its time course in key tissues and accessible biological fluids (such as blood and urine) along with that of its biomarker 3-OHBaP. The model provided a very good approximation of the experimental time course data of Marie et al. (2010), Bouchard and Viau (1996), Lee et al. (2003), and Lafontaine et al. (2004).

FIG. 7. Comparison of model simulations (solid line) with experimental data (symbols) of Lee et al. (2003) on the cumulative excretion time courses of 3-OHBaP in the urine of male Sprague-Dawley rats following an ip injection of 20 mg/kg bw of BaP (supposing an average animal weight of 200 g and a constant 25 mL of urine excreted per rat per period). Each symbol represents experimental means (n = 5).

FIG. 6. Comparison of model simulations (solid line) with experimental data (symbols) of Bouchard and Viau (1996) on the cumulative excretion time courses of 3-OHBaP in the urine of male Sprague-Dawley rats following an iv injection of 40 pmol/kg bw of BaP. Each symbol represents experimental means, and vertical bars are SDs (n = 5).

FIG. 8. Comparison of model simulations with the human data (symbols) of Lafontaine et al. (2004) on the excretion time courses of 3-OHBaP in the urine of a worker of a carbon disk factory following an occupational exposure to BaP. The occupational scenario (gray bars) was obtained from Lafontaine et al. (2004) with two shifts of 6.75 h and 4.75 h. The worker’s simulation considered an atmospheric concentration of 1514 and 3028 ng/m³, a ventilation rate of 1.20 m³/h, and an absorption fraction of 7.98%. Dashed line represents simulation based on the rat model parameter values, whereas the solid line shows simulation with animal-to-human extrapolated parameter values.
Kinetics of BaP and Model Simulations

According to model simulations, the main biological processes governing the kinetics of BaP are its uptake in the lipidic components of the lung (such as membrane lipids) and in adipose tissues combined to its slow metabolism in the lung compared with the liver as confirmed by experimental data (Mitchell, 1983; Prough et al., 1979). The lipophilic properties of BaP and slow lung metabolism thus appear to explain in large part its uptake in lipidic components and parallel slow elimination time course in lungs and adipose tissues compared with other tissues, such as the liver. In the lung, this is combined to the fact that, following iv injection, it is the first organ, after the heart, to receive total iv dose. After an oral administration of BaP in rats, Ramesh et al. (2001) found no such accumulation in lung; in the latter study, BaP levels were similar in lung and liver, whereas plasma was more concentrated in BaP.

In the liver, skin and kidney compartments of the model, levels of BaP are in kinetic equilibrium with those of BaP in the blood compartment, such that they evolve in parallel, as experimentally observed. The more rapid elimination rate of BaP in blood and these tissues reflects renal clearance and biliary elimination rate of rapidly distributed and metabolized BaP, whereas the slower elimination phase is driven by the rate-limiting elimination of BaP stored in lung components and adipose tissues. This second stage of slower elimination drives the whole kinetics as soon as initial tissue distribution and uptake are completed.

The current toxicokinetic model for BaP suggests that total amounts of BaP in the lung as a function of time is determined not only by the lipid affinity but also by some other storage process, otherwise adipose tissues would have presented larger amounts of BaP. The affinity difference between these two compartments could be regarded as a rough indicator of protein-binding contribution (about 77.8%) in the lungs, supposing that lipid components of the lungs are comparable to those of adipose tissues measured, as follows:

\[
\text{ProteinBinding} = \frac{K_{\text{BAT}} - K_{\text{BLU}}}{K_{\text{BLU}}}
\]

In the liver, BaP affinity for proteins is accounted for by its large partition coefficient \(f_L = 12.8\%\) compared with those of the other organs, which are in kinetic equilibrium with blood. This suggests again that there is an affinity mechanism that leads to the higher BaP levels in the liver compared with other organs. These results are all in accordance with published in vitro studies showing significant binding of BaP to Ah receptors along with P450 1A1 induction in liver and lung (Shimada et al., 2002).

With regard to metabolism of BaP, as mentioned previously, model also suggests that liver metabolism of BaP was significantly higher than lung metabolism \(t_{1/2,L} = 3.32\) h and \(t_{1/2,L\_\text{met}} = 7.34\) day, respectively. This is in complete agreement with experimentally observed slow disappearance of BaP from the lungs compared with the rapid disappearance from the liver. Furthermore, the model assessed that liver and lung metabolism of BaP into 3-OHBaP is much less important than biotransformation to other BaP metabolites.

Also according to model simulations, no saturation in lung and liver metabolism of BaP was apparent at the 40 \(\mu\text{mol/kg iv dose administered to rats. BaP time profile in the lung, where highest BaP concentrations were observed, was very similar to that of adipose tissues, whose levels were an order of magnitude smaller. This kind of kinetics is an indication that no saturation mechanism is present at the injected dose.}

Kinetics of 3-OHBaP and Model Simulations

With regard to 3-OHBaP kinetics, the model suggests that the terminal elimination phase in blood, liver, skin, and lung is driven in large part by the elimination rate of BaP stored in lung, adipose tissues, or other body lipidic components. There is also definitely a certain contribution of 3-OHBaP itself stored in adipose tissues and tissue lipid components. As observed for BaP, there must be an affinity of the metabolite 3-OHBaP for lipids. The accumulation of 3-OHBaP in adipose tissues hence reflects the lipophilicity of the metabolite itself even after monohydroxylation of the parent compound.

In the toxicokinetic model, 3-OHBaP and BaP in the GI tract have further been represented by two subcompartments. As mentioned in the “Results” section, the first segment represents the actual kinetics of the GI tract, whereas the second simply delays appearance in feces with a half-life of 5.55 h for both molecules. Differences are apparent in the overall kinetics of BaP and 3-OHBaP. First, the liver transfers only very small amounts of BaP to the GI tract with a half-life of 4.91 day, whereas large amounts of 3-OHBaP are secreted with a \(t_{1/2}\) of 10.9 min. In addition to this difference in the GI tract uptake of the two compounds (three orders of magnitude), elimination from the GI also differs. In the case of BaP, fecal excretion is limited with a \(t_{1/2}\) of 14.0 h. In the case of 3-OHBaP, fecal excretion is substantial; overall elimination of 3-OHBaP from the GI was found to occur with a \(t_{1/2}\) of 7.57 h.

The model also accounted for enterohepatic recycling of 3-OHBaP by including a transfer of 3-OHBaP \(k_{\text{gill}}\) from the first segment of the GI tract back to the liver compartment. Significant enterohepatic recycling of BaP metabolites has also been described in a study in cannulated rats (Chipman et al., 1981). The same type of recycling can also occur for BaP (Chipman et al., 1981); nevertheless, contribution of this process to the overall excretion kinetics is negligible, given the very small fraction of internal BaP dose observed as BaP in feces (0.397%) compared with that of 3-OHBaP (12.9%) (i.e., two orders of magnitude apart). Our mathematical model showed that this recycling mechanism of 3-OHBaP has a noticeable impact on the kinetics of distribution and elimination of this metabolite and therefore contributes significantly to the overall cumulative amounts of 3-OHBaP.
observed in urine (19.3% of the available 3-OHBaP is reabsorbed according to the model).

As for the atypical time course of 3-OHBaP in urine experimentally documented by Bouchard and Viau (1996) and Lee et al. (2003), it is determined by its kidney uptake and slow output rate according to the model. Uptake of 3-OHBaP in most tissues (i.e., liver, blood, skin, lungs, and adipose tissues) is governed by the fast metabolism rate of BaP in the liver \((t_{1/2} = 3.32 \text{ h})\). However, in kidney, maximum levels of 3-OHBaP are reached only around 8 h after injection as compared with 4 h post-dosing in the liver, although large amounts of metabolites are found in kidney compared with other organs in equilibrium with blood. Such kidney profile can exist only if there is a relatively rapid uptake in kidneys \((t_{1/2} = 27.8 \text{ min})\) but a slower elimination from this tissue \((t_{1/2} = 4.52 \text{ h})\), either by excretion of 3-OHBaP in urine or its transfer back to blood. This built-up phenomenon of this metabolite in this organ may be explained by a delayed active tubular secretion of the conjugated form of this metabolite in proximal tubules (Gosselin et al., 2005).

### Comparison with Other Published Models

Roth and Vinegar (1990) developed a PBPK model to describe the kinetics of the parent compound BaP following intraarterial injection. Metabolism was considered to occur in both the lung and the liver, and binding was incorporated in the liver and lung compartments. Parametric values are, however, not reported in this study.

Furthermore, Bevan and Weyand (1988) developed a compartmental model of the distribution of total radioactivity in male Sprague-Dawley rats following intratracheal instillation of \(^3\text{H}\)-BaP. Compartments included blood, liver, lung, intestine (including its contents), and carcasses (the latter of which were modeled as the sum of two compartments) along with undefined additional compartments introduced in the model to account for a delay in urinary excretion of radioactivity or in the transfer of radioactivity from liver to the intestinal compartments through biliary secretion. The delay in urinary excretion of blood radioactivity introduced in the model of Bevan and Weyand (1988) is compatible with the 3-OHBaP kidney buildup considered in our model. As in our model, these authors also accounted for a significant enterohepatic recycling of BaP metabolites to provide an adequate fit to experimental data. Although these models provided valuable information on the kinetics of BaP and total radioactivity, they did not focus on the kinetic modeling of 3-OHBaP as a potential key biomarker of exposure to BaP.

Overall, this study succeeded in developing a toxicokinetic model for BaP and 3-OHBaP, which provided a close match to a large set of experimental time course data in several biological matrices of exposed rats. This modeling provided new insights into the mechanistic determinants of 3-OHBaP kinetics that can serve for a better understanding and use of 3-OHBaP biomonitoring data.

### FUNDING

Agence nationale de sécurité sanitaire de l’alimentation, de l’environnement et du travail (ANSES, 2009_CRD_24).

### APPENDIX

#### Linear differential equations for each model compartment

The mathematical representation of Figure 1 is given by the following system of differential equations (see Table 1 for definitions of symbols and abbreviations):

### Kinetics of BaP

\[
\frac{\partial c(t)}{\partial t} + \left[ \left( K_{KL} + K_{LO} \right) f_{IL} + \left( K_{KH} + K_{KL} \right) f_{IL} \right]c(t) = Dc(t) + K_{AT}c(t) + K_{LU}c(t) + K_{LU}c(t)
\]

\[
\frac{\partial c(t)}{\partial t} + K_{AT}c(t) = K_{AT}f_{B}(t)
\]

\[
\frac{\partial c(t)}{\partial t} + K_{LU}c(t) = K_{LU}f_{B}(t)
\]

### Kinetics of 3-OHBaP metabolites

\[
\frac{\partial g_1(t)}{\partial t} + \left( k_{ge} + k_{gh} \right) g_1(t) + k_{gh}g_1(t) = k_{gh}g_1(t) + k_{gh}g_1(t)
\]

\[
\frac{\partial a(t)}{\partial t} + k_{ah}a(t) = k_{ah}a(t) + k_{ah}a(t)
\]

\[
\frac{\partial u(t)}{\partial t} + k_{uh}u(t) = k_{uh}u(t) + K_{LU}u(t)
\]

\[
\frac{\partial g_1(t)}{\partial t} + \left( k_{ge} + k_{gh} \right) g_1(t) = k_{gh}g_1(t)
\]
\[
\frac{\partial g_{12}(t)}{\partial t} + k_{j10}g_{12}(t) = k_{g1}g_{11}(t)
\]  
(20)

\[
\frac{\partial g(t)}{\partial t} = k_{g1}g_{11}(t)
\]  
(21)

\[
\frac{\partial k(t)}{\partial t} + (k_{w1} + k_{d1})k(t) = k_{d1}\delta(t)
\]  
(22)

\[
\frac{\partial a(t)}{\partial t} = k_{u1}a(t)
\]  
(23)

\[
b(t) = f a(t)
\]  
(24)

\[
l(t) = f b(t)
\]  
(25)

\[
S(t) = f b(t)
\]  
(26)

REFERENCES


