A Physiological Toxicokinetic Model for Inhaled Propylene Oxide in Rat and Human with Special Emphasis on the Nose

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Chronic exposure to high concentrations of PO induced inflammation in the respiratory nasal mucosa (RNM) of rodents and, for concentrations $\geq$ 300 ppm, caused nasal tumors. Considering the nose to be the most relevant target organ for PO-induced tumorigenicity, we developed a physiological toxicokinetic model for PO in rats and humans. It includes compartments for arterial, venous, and pulmonary blood, liver, muscle, fat, richly perfused tissues, lung, and nose. It simulates inhalation of PO, its distribution into tissues by blood flow, and its elimination by exhalation and metabolism. In nose, lung, and liver of rats, PO conjugation with glutathione (GSH), PO-induced GSH depletion, and formation of PO adducts to DNA are described. Also modeled are PO adducts to hemoglobin of rats and humans. Required partition coefficients and metabolic parameters were derived experimentally or from publications. In rats, simulated PO concentrations in blood and GSH levels in tissues agreed with measured data. If compared with reported values, levels of adducts with hemoglobin were underpredicted up to a factor of about 2. Adducts with DNA differed up to a factor of 3. Hemoglobin adducts predicted for PO-exposed workers were 1.5–1.9 times higher than the reported ones. Considering identical conditions of PO exposure, similar PO concentrations in RNM were modeled for rats and humans. Also, PO concentrations in blood, about 1/30th of those in RNM, were similar in both species. Since the model was evaluated on all available data in rats and humans, we consider it to be useful for estimating the risk from inhalation exposure to PO.

Key Words: physiological toxicokinetic model; propylene oxide; glutathione; hemoglobin and DNA adducts; respiratory nasal mucosa; rat; human.

Previous work has shown that inhaled vaporous PO was rapidly biotransformed by rats. Only a small fraction (3%) was exhaled unchanged (Golka et al., 1989). Metabolism of PO was also studied in microsomes and cytosol. PO hydrolysis is catalyzed by epoxide hydrolase (EH, Faller et al., 2001; Guengerich and Manson, 1980) and its glutathione (GSH) conjugation by glutathione S-transferase (GST, Faller et al., 2001; Fjellstedt et al., 1973). The epoxide PO binds directly to macromolecules forming hydroxypropyl adducts with hemoglobin and DNA. In rats and mice, PO adducts to DNA have been detected in all tissues investigated (Osterman-Golkar et al., 2003; Ríos-Blanco et al., 1997, 2003a; Segerbäck et al., 1994, 1998; Snyder and Solomon, 1993; Svensson et al., 1991). N7-(2-hydroxypropyl)guanine (N7-HPG) showed by far the highest adduct levels as also demonstrated in vitro (Solomon et al., 1988). In humans exposed occupationally to PO, DNA adducts have been reported too (Czéne et al., 2002). PO adducts to amino residues of hemoglobin have been detected in PO-exposed rodents and humans (Bailey et al., 1987; Boogaard et al., 1999; Czéne et al., 2002; Farmer et al., 1982; Högestedt et al., 1990; Jones et al., 2005; Osterman-Golkar et al., 2003; Pero et al., 1985; Ríos-Blanco et al., 2002; Segerbäck et al., 1994; Svensson et al., 1991). PO was mutagenic in Drosophila, yeast, fungi, and bacteria. It was clastogenic in mammalian cells in vitro (reviewed in IARC, 1994; Lee et al., 2005) and at very high doses (300 and 450 mg/kg, intraperitoneal administration) in mice in vivo (Bootman et al., 1979; Farooqi et al., 1993). However, after oral PO administration of very high PO doses to mice, neither micronucleus formation nor dominant lethal mutations had been detected (Bootman et al., 1979). Also, an inhalation study in rats did not result in dominant lethal mutations (Hardin et al., 1983). Furthermore, there were no increases in the sister chromatid exchanges nor in chromosomal aberrations in Cynomolgous monkeys exposed via inhalation for over 2 years up to 300 ppm PO (Lynch et al., 1984b). In vitro studies demonstrated that PO was a much less potent genotoxicant than ethylene oxide (EO) or epichlorohydrin (Agurell et al., 1991; Kolman and Dusinska, 1995; Kolman et al., 1997).

The colorless liquid propylene oxide (PO) is a highly volatile industrial chemical used primarily in the synthesis of polyether polyols, propylene glycols, and propylene glycol ethers. It is also applied as package fumigant for certain foodstuffs (summarized in IARC, 1994).
In long-term studies with rats and mice, PO induced tumors at the application site. In some rat studies, also an increase in tumors has been detected in few glands in which tumors developed spontaneously. In inhalation studies with B6C3F1 mice (Renne et al., 1986) and Fischer 344 rats (Lynch et al., 1984a; Renne et al., 1986), the most striking findings were exposure-related increases in the incidences of inflammatory lesions and hyperplastic changes in the nasal mucosa and, in the high exposure groups ($\geq 300$ ppm), nasal tumor formation. In a long-term inhalation study with Wistar rats, increased degenerative and hyperplastic changes in the nasal mucosa occurred in all treatment groups. There was one ameloblastic fibrosarcoma and one squamous cell carcinoma in the nasal cavity of a male animal at the low dose of 30 ppm. In the high-dose group (300 ppm), one squamous cell carcinoma was found in the nasal cavity of a male animal. Additionally, in four males of the high-dose group, a carcinoma was detected in the larynx or pharynx, trachea, or lungs (Kuper et al., 1988). Following repeated PO inhalation exposures, induction of cell proliferation was observed in respiratory nasal mucosa (RNM) of rats (Eldridge et al., 1995; Ríos-Blanco et al., 2003b). Also, by far the highest levels of PO adducts to DNA were found in RNM, with up to 25-fold higher than in other, systemically exposed tissues (Osterman-Golkar et al., 2003; Ríos-Blanco et al., 1997, 2003a; Segerbäck et al., 1998). Considering these findings together with experimental data on the GSH status in RNM and other tissues of PO-exposed Fischer 344 rats, Lee et al. (2005) concluded that continuous and severe perturbation of GSH in RNM of rats exposed repeatedly to high PO concentrations resulted in inflammatory lesions and cell proliferation. The authors hypothesized these steps to be critical on the path toward nasal tumorigenicity of PO and its genotoxicity alone to be too weak for inducing tumors. Consequently, a scientifically founded estimation of the risk arising from PO exposure requires not only knowledge of the PO burdens in RNM and other tissues but also of the corresponding GSH levels.

Up to now, toxicokinetics of inhaled PO have been described by means of a one-compartment model that enabled calculating the average body burden of PO in rats (Golka et al., 1989). No physiological toxicokinetic (PT) model has been developed allowing to calculate tissue-specific PO concentrations and the resulting GSH levels. In order to fill this gap, we present in the following a PT model for rats and humans by considering the RNM as the relevant target tissue for PO-induced tumorigenicity. Prerequisites for the development of this model were kinetic data obtained from PO-exposed rats (Lee et al., 2005; Schmidbauer, 1997) and partition coefficients tissue: blood and air: blood (Schmidbauer, 1997), metabolic parameters of PO in cell fractions from liver, lung, and RNM (Faller et al., 2001), as well as GSH measurements in PO-exposed rats (Lee et al., 2005). Published adduct levels of PO to DNA in rats and to hemoglobin in rats and humans together with GSH depletion data in rats served as dose surrogates for PO in tissues.

### MATERIALS AND METHODS

#### PT Model

The PT model is based on our previous model for styrene (Csáňady et al., 2003). The pulmonary compartments were revised, and new compartments for the nose were incorporated (Fig. 1). The figure shows three major modules corresponding to nose, lung, and residual body along the path by which inhaled PO enters the body. The modules consist of compartments representing nasal and pulmonary air spaces and tissues, pulmonary blood, liver, muscle, fat, and the richly perfused tissue group. All tissues are connected to the blood circuit represented by the arterial and the venous blood. A perfusion-limited process describes the uptake of PO from the arterial blood into the tissue compartments. PO is metabolized in liver, lung, nose, richly perfused tissue group, and blood. All equations describing the model are presented in the Appendix. The species-specific physiological parameters were taken from Arms and Travis (1988) if not indicated otherwise and are summarized in Tables 1 and 2. Partition coefficients and diffusion coefficients of PO are given in Table 3. Biochemical parameters are compiled in Tables 4 and 5. For rodents and humans lacking standard body weights, alveolar ventilation, and cardiac output were scaled to body surface ($BW^{1/3}$, Filser, 1992).

#### Nose Module

The nose module (Fig. 1) is modeled according to Morris et al. (1993). The inhaled air stream is divided into two paths. One passes over the lateral-ventral and the other one over two successive dorso-medial regions of the nose. PO vapors are transported in the airflow through the corresponding air compartments by breathing (convection) and from there by mass transfer into the associated well-stirred tissue compartments (diffusion) with each consisting of mucus, epithelium, and submucosa. The lateral-ventral tissue compartment contains respiratory mucosa; one dorsal-medial compartment corresponds to respiratory and the other one to olfactory mucosa. The air paths leaving the lateral-ventral respiratory and the dorsal-medial olfactory air compartments join again and the combined air stream enters the lung.

The PO concentration in each nasal tissue compartment is governed by the mass transfer between air and tissue compartment, by the blood flow through the compartment, and by its metabolic activity towards PO catalyzed by EH and GST. Kinetics of PO hydrolysis is modeled according to Michaelis-Menten; conjugation of PO with GSH is described by a sequential ordered ping-pong mechanism (see liver).

The inhalation and exhalation processes are simulated to occur simultaneously. Diffusion processes are described to occur at steady state across a thin film according to Fick’s law (e.g., Cussler, 1984). Physiological parameters such as the specific surface area of respiratory and olfactory mucosae were taken from the literature (Table 1). For rats, we determined by own measurements that the portion of the lateral-ventral respiratory tissue is 72% of the whole respiratory tissue. The volumes of the air phases located above the lateral-ventral respiratory mucosa, dorsal-medial respiratory mucosa, and olfactory mucosa were calculated using the areas and the average weights of these mucosae measured in our laboratory in 80 rats that had been thoroughly engrafted before nasal tissue preparation. These weights (means ± SDs) were 52 ± 5 mg and 65 ± 6 mg for respiratory and olfactory mucosae, respectively. The nasal blood flow for rats was modeled to be the average (0.59 ml/min) of the values published (0.5% of $Q_{pa}$ corresponding to 0.41 ml/min, Andersen et al., 1999; 1% of $Q_{pa}$ corresponding to 0.83 ml/min, Bush et al., 1998; 1% of $Q_{pa}$ corresponding to 0.83 ml/min, Frederik et al., 1998; 0.19 ml/min, Morris et al., 1993; 0.22 ml/min, Plowchalk et al., 1997; and 0.91 ml/min, Stott and McKenna, 1984). The total nasal blood flow passing through the respiratory and olfactory regions was split according to the corresponding tissue weights. Consequently, 44% of the nasal blood flow passes through the respiratory mucosa. We assumed the fraction of the blood flow passing through...
the lateral-ventral part of the respiratory mucosa to equal its mass fraction (0.72).

For humans, the weights of olfactory and respiratory tissues were calculated using published values on the surface areas and the corresponding tissue thicknesses (Table 1). These weights were used to calculate the blood flows through the corresponding mucosae. The fraction of the blood flow passing through the lateral-ventral respiratory mucosa was assumed to equal its surface fraction, which was obtained from Frederick et al. (2001). This surface parallels the mass of this mucosa because a constant tissue thickness was assumed. Mucosal blood flow in humans was assumed to be 60 ml/min/100 g mucosa, which is the average value of measured data (93 ml/min/100 g, Aust et al., 1978; 33 ml/min/100 g and 29 ml/min/100 g, Bende, 1983; 0.28 µl/cm²/s = 135 ml/min/100 g, Drettner and Aust, 1975; 57 ml/min/100 g, Holmberg et al., 1989; 35 ml/min/100 g, Olsson, 1986; 32.8 ml/min/100 g, Ozdem and Ercan, 1984; 40 ml/min/100 g, Paulsson et al., 1985; and 81 ml/min/100 g, Riechelmann and Krause, 1994). Considering the surface of mucosa (135.6 cm²), the mucosal thickness of 125 µm (Drettner and Aust, 1975) and a tissue density of 1 g/ml, the average mucosal blood flow is 61 ml/h (135.6 × 100 × 125 × 10⁻⁶ × 100 × 60) corresponding to a very small fraction (61/1000/372 = 0.000164) of the cardiac output (372 l/h). Physiological parameters used for the nasal compartments of a 0.25-kg rat and a 70-kg human are summarized in Table 2.

The average of the partition coefficients of PO determined in lungs of mice, rats, and humans (Schmidbauer, 1997) was used as surrogate for nasal tissues (Table 3). This value was chosen because it was the largest of all the partition coefficients tissue:blood given in Table 3 (except for fat), yielding the highest...
### TABLE 1

Physiological Parameters Used in the PT Model

<table>
<thead>
<tr>
<th>Physiological parameters</th>
<th>Rat</th>
<th>Human</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>0.25</td>
<td>70</td>
<td>kg</td>
</tr>
<tr>
<td>Pulmonary ventilation</td>
<td>10.44</td>
<td>450</td>
<td>l/h</td>
</tr>
<tr>
<td>Cardiac output</td>
<td>4.98</td>
<td>372</td>
<td>l/h</td>
</tr>
<tr>
<td>Specific surface area of tracheobronchiolar region (conducting zone)</td>
<td>0.71</td>
<td>0.61</td>
<td>dm²/kg</td>
</tr>
<tr>
<td>Specific surface area of alveolar endothelium</td>
<td>112</td>
<td>122</td>
<td>dm²/kg</td>
</tr>
<tr>
<td>Specific surface area of capillary endothelium</td>
<td>126</td>
<td>115</td>
<td>dm²/kg</td>
</tr>
<tr>
<td>Specific surface area of olfactory mucosa</td>
<td>0.25</td>
<td>0.0019</td>
<td>dm²/kg</td>
</tr>
<tr>
<td>Specific surface area of respiratory mucosa</td>
<td>0.22</td>
<td>0.018</td>
<td>dm²/kg</td>
</tr>
<tr>
<td>Air volume of the lung</td>
<td>0.02</td>
<td>0.043</td>
<td>l/kg</td>
</tr>
<tr>
<td>Air volume of the nose</td>
<td>0.001</td>
<td>1.4·10⁻⁴</td>
<td>l/kg</td>
</tr>
<tr>
<td>Fraction of the lung accounting for the conducting zone</td>
<td>1/3</td>
<td>1/3</td>
<td>—</td>
</tr>
<tr>
<td>Fraction of inhaled PO following the dorsal-medial path in the nose</td>
<td>0.13</td>
<td>0.083</td>
<td>—</td>
</tr>
<tr>
<td>Tissue thickness air-to-plasma, harmonic mean</td>
<td>4.05·10⁻⁶</td>
<td>7.45·10⁻⁶</td>
<td>dm</td>
</tr>
<tr>
<td>Thickness of the epithelium layer in the conducting zone</td>
<td>7.5·10⁻⁴</td>
<td>1.25·10⁻³</td>
<td>dm</td>
</tr>
<tr>
<td>Thickness of respiratory mucosa</td>
<td>7·10⁻⁴</td>
<td>1.25·10⁻³</td>
<td>dm</td>
</tr>
<tr>
<td>Thickness of olfactory mucosa</td>
<td>8·10⁻⁴</td>
<td>1.25·10⁻³</td>
<td>dm</td>
</tr>
<tr>
<td>Blood flows as fraction of cardiac output</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conducting zone</td>
<td>0.01”</td>
<td>0.01”</td>
<td>—</td>
</tr>
<tr>
<td>Fatty tissue</td>
<td>0.017”</td>
<td>0.05</td>
<td>—</td>
</tr>
<tr>
<td>Liver</td>
<td>0.25</td>
<td>0.26</td>
<td>—</td>
</tr>
<tr>
<td>Nose</td>
<td>0.007”</td>
<td>0.00017”</td>
<td>—</td>
</tr>
<tr>
<td>VRG</td>
<td>0.51</td>
<td>0.44</td>
<td>—</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.206</td>
<td>0.24</td>
<td>—</td>
</tr>
<tr>
<td>Compartment volumes as fractions of body weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial blood</td>
<td>0.0167”</td>
<td>0.0178”</td>
<td>—</td>
</tr>
<tr>
<td>Venous blood</td>
<td>0.050”</td>
<td>0.0533”</td>
<td>—</td>
</tr>
<tr>
<td>Lung blood</td>
<td>0.0074”</td>
<td>0.0076”</td>
<td>—</td>
</tr>
<tr>
<td>Fat</td>
<td>0.06”</td>
<td>0.19</td>
<td>—</td>
</tr>
<tr>
<td>Lung</td>
<td>0.005”</td>
<td>0.0076”</td>
<td>—</td>
</tr>
<tr>
<td>Liver</td>
<td>0.04</td>
<td>0.026</td>
<td>—</td>
</tr>
<tr>
<td>VRG</td>
<td>0.045</td>
<td>0.042</td>
<td>—</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.676</td>
<td>0.541</td>
<td>—</td>
</tr>
</tbody>
</table>

Physiological parameters were taken from Arms and Travis (1988), if not indicated otherwise.

In rats, pulmonary ventilation depends on the exposure concentration (see Fig. given in supplementary data).

Brown et al. (2005).

Crapp et al. (1983).

Average values of Morris et al. (1993) and Stott and McKenna (1984).

Frederick et al. (2001).

The area of lateral-ventral respiratory mucosa was calculated by multiplying the surface area of respiratory mucosa with 0.72. This value reflects the portion of the lateral-ventral respiratory mucosa of the whole respiratory mucosa (own measurements).

Gross et al. (1982).

Considering the air volume over the respiratory and olfactory mucosae (Frederick et al., 2001).

Average values of Andersen and Sarangapani (1999), Andersen et al. (1999), Morris et al. (1993), and Plowchalk et al. (1997).

Average values of Andersen et al. (2000), Frederick et al. (2001), and Sarangapani et al. (2004).

The value was set to the average thickness of respiratory and olfactory mucosae.

Values were calculated by dividing the areas of the respiratory and olfactory mucosae by the average respiratory (52 mg) and olfactory (65 mg) mucosa weights measured by us in 80 rats.

Drettner and Aust (1975); Loring and Tenney (1973).

Tsujino et al. (2005).

Csanady et al. (2003).

See Materials and Methods.

The volumetric ratio of the arterial to the venous compartment was set 1/3 (Brown et al., 1997; Menzel et al., 1987).

The volume of pulmonary blood in relation to body weight was published for humans by Snyder et al. (1975). For rodents, the same ratio was used.

According to Brown et al. (1997), values are between 0.05 and 0.07.

Brown et al. (1997).

Value equals that of the “vessel-rich group” (Arms and Travis, 1988) minus lung volume.

Value equals that of the “slowly perfused tissue group” of Arms and Travis (1988) minus blood volume.
TABLE 2
Physiological Parameters for the Nasal Compartments
Given for a 0.25-kg Rat and a 70-kg Human

<table>
<thead>
<tr>
<th>Physiological parameters</th>
<th>Rat</th>
<th>Human</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal airspace</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Over lateral-ventral RNM</td>
<td>8.7 × 10^{-5}</td>
<td>0.0087</td>
<td>l/h</td>
</tr>
<tr>
<td>Over dorsal-medial RNM</td>
<td>3.3 × 10^{-5}</td>
<td>7.4 × 10^{-4}</td>
<td>l/h</td>
</tr>
<tr>
<td>Over olfactory nasal mucosa</td>
<td>1.4 × 10^{-4}</td>
<td>5.6 × 10^{-4}</td>
<td>l/h</td>
</tr>
<tr>
<td>Surface area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral-ventral RNM</td>
<td>0.04</td>
<td>1.14</td>
<td>dm²</td>
</tr>
<tr>
<td>Dorsal-medial RNM</td>
<td>0.015</td>
<td>0.1</td>
<td>dm²</td>
</tr>
<tr>
<td>Olfactory nasal mucosa</td>
<td>0.063</td>
<td>0.13</td>
<td>dm²</td>
</tr>
<tr>
<td>Tissue weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral-ventral RNM</td>
<td>3.7 × 10^{-5}</td>
<td>1.4 × 10^{-4}</td>
<td>kg</td>
</tr>
<tr>
<td>Dorsal-medial RNM</td>
<td>1.5 × 10^{-5}</td>
<td>1.2 × 10^{-5}</td>
<td>kg</td>
</tr>
<tr>
<td>Olfactory nasal mucosa</td>
<td>6.5 × 10^{-5}</td>
<td>1.6 × 10^{-5}</td>
<td>kg</td>
</tr>
<tr>
<td>Blood flow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral-ventral RNM</td>
<td>0.011</td>
<td>0.052</td>
<td>l/h</td>
</tr>
<tr>
<td>Dorsal-medial RNM</td>
<td>0.0043</td>
<td>0.0045</td>
<td>l/h</td>
</tr>
<tr>
<td>Olfactory nasal mucosa</td>
<td>0.020</td>
<td>0.006</td>
<td>l/h</td>
</tr>
<tr>
<td>Air flow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral-ventral RNM</td>
<td>9.08</td>
<td>412.6</td>
<td>l/h</td>
</tr>
<tr>
<td>Dorsal-medial RNM</td>
<td>1.36</td>
<td>37.4</td>
<td>l/h</td>
</tr>
<tr>
<td>Olfactory nasal mucosa</td>
<td>1.36</td>
<td>37.4</td>
<td>l/h</td>
</tr>
</tbody>
</table>

TABLE 3
Physicochemical Parameters Used in the PT Model

<table>
<thead>
<tr>
<th>Physicochemical parameters</th>
<th>Rat</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood-air</td>
<td>65.7</td>
<td></td>
</tr>
<tr>
<td>Fat-blood</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td>Liver-blood</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>Lung-blood</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Lung-air</td>
<td>63.7</td>
<td></td>
</tr>
<tr>
<td>Nasal mucosa: blood</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Nasal mucosa: air</td>
<td>63.7</td>
<td></td>
</tr>
<tr>
<td>Muscle: blood</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>VRG: blood</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Diffusion coefficients of PO in gas phase (dm²/h)</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Diffusion coefficients of PO in water phase (dm²/h)</td>
<td>5.8 × 10^{-4}</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 4
Biochemical Parameters and Rate Constants Used for the Respiratory and Olfactory Nasal Mucosa in the PT Model

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Parameter</th>
<th>Rat</th>
<th>Human</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olfactory EH</td>
<td>Vmax_{EH,olf}</td>
<td>0.013</td>
<td>0</td>
<td>mmol/h/g tissue</td>
</tr>
<tr>
<td>K_EH_{olf}</td>
<td>0.76</td>
<td>mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olfactory GST</td>
<td>Vmax_{GST,olf}</td>
<td>0.37</td>
<td>0</td>
<td>mmol/h/g tissue</td>
</tr>
<tr>
<td>K_GST_{olf}</td>
<td>0.16</td>
<td>mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal GSH</td>
<td>C_{GSH,olf}</td>
<td>5.3</td>
<td>mmol/l</td>
<td></td>
</tr>
<tr>
<td>K_{GSH,olf}</td>
<td>3.7</td>
<td>mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSH turnover rate</td>
<td>K_{GST,olf}</td>
<td>2.2</td>
<td>h⁻¹</td>
<td></td>
</tr>
<tr>
<td>Respiratory EH</td>
<td>Vmax_{EH,resp}</td>
<td>0.012</td>
<td>0</td>
<td>mmol/h/g tissue</td>
</tr>
<tr>
<td>K_EH_{resp}</td>
<td>2.2</td>
<td>mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory GST</td>
<td>Vmax_{GST,resp}</td>
<td>0.14</td>
<td>0</td>
<td>mmol/h/g tissue</td>
</tr>
<tr>
<td>K_GST_{resp}</td>
<td>0.16</td>
<td>mmol/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal GSH</td>
<td>C_{GSH,resp}</td>
<td>3.7</td>
<td>mmol/l</td>
<td></td>
</tr>
<tr>
<td>K_{GSH,resp}</td>
<td>2.2</td>
<td>mmol/l</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aValues are valid for single and repeated exposure conditions. For the calculations of metabolic parameters see Materials and Methods.
*bNasal K_{GSH} and GSH turnover rate was assumed to be the same as in liver.
*cSee Lee et al. (2005). The GSH concentration in the olfactory mucosa was assumed to be the same as in the respiratory mucosa.
*dThe same rate for GSH turnover in olfactory mucosa was assumed as in the respiratory mucosa.
*eParameter was obtained from fitting in vivo measurements.

*Partition coefficients were calculated as described in Materials and Methods.

*The measured lung volume was used.

*Average value of those measured in brain, kidney, liver, and spleen.

*Estimated according to Fuller (given in Cussler, 1984).

*Estimated by the Wilke-Chang method (Reid et al., 1977).
In vitro derived clearance values were converted for in vivo conditions considering average microsomal and cytosolic protein contents of 3.3 and 28.6 mg/g lung, respectively (Faller, 1998). For rats, Vmax for PO conjugation with GSH (Table 5) was estimated, since in vitro only a clearance had been determined. The value of KmPO (see legend of Table 5) was modeled to equal the highest PO concentration (100 mmol/l) used in the experiments of (Faller et al., 2001). For GST-mediated metabolism in human lung, both KmGSHlung and VmaxGSTlung (see Table 5) were determined by (Faller et al., 2001). The latter was converted to in vivo conditions in lung cytosol as described for the clearance values. The turnover of the cytosolic GSH was described by zero-order production and first-order elimination processes. The initial average GSH concentrations in lungs of naive rats and humans (CSGSHlung) are given in Table 5. For modeling human exposure to PO, the same GSH turnover rate constant (kGSHlung) Table 5) was used as earlier (Csányi et al., 2003).

### In vivo Measurements

The parameter for repeated exposures was set to 0.1 (mmol/l) for both species (Csányi et al., 2003).

### Metabolic Capacities

Metabolic capacities were divided according to the mass ratios of the two compartments respecting that experimentally determined metabolic parameters were obtained in cell fractions of the whole lung (Faller et al., 2001). The

### Biochemical Parameters and Rate Constants Used for Liver and Lung in the PT Model

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Parameter</th>
<th>Rate</th>
<th>Human</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic EH</td>
<td>Vmax&lt;sub&gt;EH&lt;/sub&gt;</td>
<td>0.023</td>
<td>0.083</td>
<td>mmol/h/g liver</td>
</tr>
<tr>
<td></td>
<td>Km&lt;sub&gt;EH&lt;/sub&gt;</td>
<td>2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5</td>
<td>mmol/l</td>
</tr>
<tr>
<td>Hepatic GST</td>
<td>Vmax&lt;sub&gt;GST&lt;/sub&gt;</td>
<td>7.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.12</td>
<td>mmol/h/g liver</td>
</tr>
<tr>
<td></td>
<td>Km&lt;sub&gt;GSH&lt;/sub&gt;</td>
<td>0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>mmol/l</td>
</tr>
<tr>
<td></td>
<td>Km&lt;sub&gt;PO&lt;/sub&gt;</td>
<td>32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8</td>
<td>mmol/l</td>
</tr>
<tr>
<td></td>
<td>C&lt;sub&gt;GSH&lt;/sub&gt;</td>
<td>6.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>mmol/l</td>
</tr>
<tr>
<td></td>
<td>k&lt;sub&gt;lung&lt;/sub&gt;</td>
<td>0.2&lt;sup&gt;f&lt;/sup&gt;, 0.46&lt;sup&gt;d,f&lt;/sup&gt;</td>
<td>0.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>h&lt;sup&gt;−1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pulmonary EH</td>
<td>C&lt;sub&gt;EH&lt;/sub&gt;</td>
<td>0.0011&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.0063</td>
<td>l/h/g lung</td>
</tr>
<tr>
<td>Pulmonary GST</td>
<td>Vmax&lt;sub&gt;GST&lt;/sub&gt;</td>
<td>2.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.11</td>
<td>mmol/h/g lung</td>
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<tr>
<td></td>
<td>Km&lt;sub&gt;GSH&lt;/sub&gt;</td>
<td>1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>mmol/l</td>
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<td>3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>mmol/l</td>
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<td>1.7&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.95&lt;sup&gt;d&lt;/sup&gt;</td>
<td>mmol/l</td>
</tr>
<tr>
<td></td>
<td>kδ&amp;&lt;sub&gt;PO&lt;/sub&gt;</td>
<td>14&lt;sup&gt;e&lt;/sup&gt;, 3.2&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>h&lt;sup&gt;−1&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Intraportal injection k&delta;&<sub>PO</sub> | 250<sup>x</sup> | — | h<sup>−1</sup> |

### Liver

Hydrolysis of PO in the liver compartment is modeled to follow kinetics according to Michaelis-Menten. PO conjugation with GSH is described by the two-substrate ordered sequential ping-pong mechanism (according to Johanson and Filsers, 1993, reviewed in Mannervik, 1985). This mechanism is increasingly used in physiological models to describe GST-mediated GSH conjugation with epoxides (e.g., Csányi et al., 1994, 2003; Fennell and Brown, 2001; Sarangapani et al., 2004; Sweeney et al., 1997). It describes better than the also used less physiological second-order kinetics the well-known observation that the rate of GST-mediated metabolic elimination collapses only when the GSH pool is almost depleted. The required Km toward GST (Km<sub>GSH</sub>) of 0.1 mmol/l was derived from literature values (see Johanson and Filsers, 1993). This value was used in our PT models dealing with GST-mediated GSH conjugation by Csányi et al. (1994, 2003) and Johanson and Filsers (1993), also by Fennell and Brown (2001) and Sweeney et al. (1997), and presumably by Sarangapani et al. (2004). For EH of both species, the in vitro derived Vmax values (Faller et al., 2001) were converted for in vivo conditions considering the average microsomal contents of 30 mg/g liver (Kreuzer et al., 1991). For GST, the apparent Michaelis constant for GSH (Km<sub>GSH</sub>) was set to 0.1 (mmol/l) for both species (Csányi et al., 1994). The clearance value determined in rat liver cytosol (Faller et al., 2001) was converted for in vivo conditions considering an average cytosolic protein content of 95 mg/g liver (Kreuzer et al., 1991). For rats, Vmax for PO conjugation with GSH (Table 5) was estimated, since in vitro only a clearance had been determined. The value of KmPO (see legend of Table 5) was modeled to equal the highest PO concentration (100 mmol/l) used in the experiments of Faller et al. (2001). For GST-mediated metabolism in human liver, both KmPOlung and VmaxGSTlung (see Table 5) were determined experimentally in vitro by Faller et al. (2001). The latter was converted to in vivo conditions as described for the clearance value.

For describing the cytosolic GSH pool, an earlier published procedure (Csányi et al., 1994; Johanson and Filsers, 1993) was used. It was assumed that
only cytosolic GSH, representing 90% of the total hepatocellular GSH content, is involved in the conjugation reaction, whereas the mitochondrial GSH (about 10%); see e.g., Fernandez-Checa and Kaplowitz, 2005) cannot be depleted.

The turnover of the cytosolic GSH was described by zero-order production and first-order elimination processes. The initial average GSH concentrations are given for livers of naive rats and humans ($C_{\text{GSH}}^{\text{liver0}}$) in Table 5. For single exposure experiments of rats and for human exposure, the same GSH turnover rate constants were used as in Csánády et al. (2003).

**Vessel-rich tissue group.** From closed-chamber gas uptake data in male rats, the metabolic clearance of PO, related to its average concentration in the body of a rat of 250 g, was calculated using a two-compartment model (Fisler, 1992) to be 2.13 l/h for atmospheric PO concentrations below 100 ppm (Schmidbauer, 1997). In order to relate this clearance to the blood concentration, it is multiplied with the partition coefficient blood to average body (60/51 = 1.18, calculated from the partition coefficient blood:air [60] and from the partition coefficient body:air [51], both measured by Schmidbauer [1997]). The obtained clearance is 2.5 l/h. The rate of blood flow through the liver of a rat of 250 g is 1.25 l/h (Arms and Travis, 1988), which represents the highest possible hepatic metabolic clearance. Comparing the two values it becomes obvious that there is additional PO elimination in total extrahepatic tissues, which is comparable to that in the liver. Because the corresponding clearance of 1.25 l/h in total extrahepatic tissues cannot be explained by spontaneous hydrolysis, which is rather slow at pH 7.4 and 37°C (Schmidbauer, 1997). In order to relate this clearance to the compound concentrations in blood. A comparison with the compound concentrations in blood and first-order elimination processes. The initial average GSH concentrations are given for livers of naive rats and humans ($C_{\text{GSH}}^{\text{liver0}}$) in Table 5. For single exposure experiments of rats and for human exposure, the same GSH turnover rate constants were used as in Csánády et al. (2003).

**Sensitivity analysis**

Model predictions of PO concentrations are affected by uncertainties associated with physiological, physicochemical, and biochemical parameters used in the calculations. Quantitative information on the influences of these parameters on predicted PO concentrations can be obtained by sensitivity analysis (e.g., Frank, 1976; Rabitz, 1981). The “log-normalized sensitivity coefficient” ($S$) gives the relative change in a predicted variable (e.g., venous blood concentration of PO) for a given change of a selected model parameter (e.g., $V_{\text{max}^{\text{EH}}}$):

$$S = \frac{\partial \ln C_{\text{PO}_{\text{VenBlood}}}^{\text{EH}}}{\partial \ln V_{\text{max}^{\text{EH}}}} = \frac{\partial C_{\text{PO}_{\text{VenBlood}}}^{\text{EH}}}{\partial V_{\text{max}^{\text{EH}}}} \approx \frac{\Delta C_{\text{PO}_{\text{VenBlood}}}^{\text{EH}}}{\Delta V_{\text{max}^{\text{EH}}}} \frac{V_{\text{max}^{\text{EH}}}}{C_{\text{PO}_{\text{VenBlood}}}^{\text{EH}}}$$

For example, a log-normalized sensitivity coefficient of 2.0 describes the contribution of $V_{\text{max}^{\text{EH}}}$ to the PO concentration in venous blood, indicates that a 1% increase in the value of $V_{\text{max}^{\text{EH}}}$ results in a 2% increase in predicted PO concentrations. Generally, the parameters should not be changed

---

**TABLE 6**

<table>
<thead>
<tr>
<th>Rate constant</th>
<th>Rat</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formation of HOPR-Val (l/h·g Hb)</td>
<td>0.22·10^{-4}</td>
<td>0.25·10^{-4}</td>
</tr>
<tr>
<td>Formation of N7-HPG (l/h·g DNA)</td>
<td>0.25·10^{-4}</td>
<td>0.25·10^{-4}</td>
</tr>
<tr>
<td>Depurination in nasal mucosa (h^{-1})</td>
<td>0.0053³</td>
<td>0.0053³</td>
</tr>
<tr>
<td>Depurination in lung (h^{-1})</td>
<td>0.0050³</td>
<td>0.0050³</td>
</tr>
<tr>
<td>Depurination in liver (h^{-1})</td>
<td>0.0044³</td>
<td>0.0044³</td>
</tr>
<tr>
<td>Life span of erythrocytes (h)</td>
<td>1440⁴</td>
<td>3024⁴</td>
</tr>
<tr>
<td>First-order rate constant of PO elimination from blood (h^{-1})</td>
<td>0.69⁵</td>
<td>1.3⁵</td>
</tr>
</tbody>
</table>

³Segerbäck et al. (1994).
⁴Osterman-Golkar et al. (2003).
⁵For humans, rat value was used for simulations.
⁷Lee et al. (2005).
⁸Unpublished data (C. Hutzler).
more than 1% when carrying out sensitivity analysis since the first derivatives are discretely approximated.

The influences of all model parameters on the PO concentrations in venous blood and in RNM were investigated in rats and humans. The log-normalized sensitivity coefficients were approximated using the above equation for steady-state exposures to 10 ppm PO (both species) and to 300 ppm (rats) by introducing 1% changes in the corresponding parameter value.

Simulations

The PT model is described by a series of mass balance differential equations. The model was solved numerically on an Apple G5 quad 2.5 GHz Power Mac computer (Apple Computer Inc., CA, USA) using the program Mathematica (5.2, Wolfram Research, Inc., IL, USA).

MODEL CALIBRATION AND VALIDATION

Single Exposure to PO

Male Fischer 344 rats were single exposed in a series of experiments to atmospheric PO (10, 25, 50, 100, 150, 300, 500, 625, or 750 ppm) for 6 h. Immediately thereafter, PO in blood and NPSH in RNM, lung, and liver were determined (Lee et al., 2005). In order to describe the measured PO concentrations by the PT model, the Km values governing the hepatic elimination of PO were adjusted to the data given in Figure 3A and 3B. The obtained Km values are given in Table 5. The modeled solid line in Figure 2A, describing the PO concentration in blood versus the exposure concentration in the atmosphere, agrees excellently with the measured blood concentrations up to 300 ppm PO. At 460 ppm, there is a modeled concentration jump followed by a further, almost linear increase up to 760 ppm PO. At both highest measured PO concentrations in blood (at 650 and 760 ppm), the model overpredicts somewhat the measured values. When presenting the original data, a simple regression line was calculated through all the values (Lee et al., 2005). However, a close look on the data shows indeed that they are better described by two straight lines, one with a flatter slope up to 300 ppm and one with a steeper slope through the three highest PO concentrations. The break in the modeled curve results from complete loss of GSH in the liver, modeled to occur at 460 ppm (Fig. 2B). This leads to a distinct loss of metabolic PO elimination yielding to an increase in PO blood and tissue concentrations. In our model, there is noticeable extrahepatic metabolism in the vessel-rich tissue group (required as explained above). This metabolism parallels that in the liver. The overprediction of the two highest measured PO concentrations in blood of single exposed rats might result from a less drastic loss of PO metabolism in this tissue group.
than the modeled one. However, there are not enough reliable data enabling a well-founded more complex model for single PO exposure.

The turnover rate constant \( k_{D,\text{h}} = 0.2 \text{ h}^{-1} \), required for describing GSH concentrations in livers of single PO-exposed rats (Fig. 2B, Lee et al., 2005), was taken from our previous styrene model (Csanády et al., 2003). The apparent Michaelis constant for the substrate affinity to GST \((K_m^{\text{PO}})\) was obtained by fitting the solid line describing the GSH depletion in this organ in dependence of the PO exposure concentration.

No model parameters were adjusted for the description of the GSH depletion in lung given by the dashed line (in Fig. 2C, Lee et al., 2005). It represents a model prediction obtained with the GSH turnover rate constant \( k_{D,\text{lung}} = 1.8 \text{ h}^{-1} \), the value used in our styrene model (Csanády et al., 2003). Although the predicted dependence of the GSH concentrations in the lung from the PO exposure concentrations agrees fairly well with the measured data, we lowered the turnover rate constant somewhat to \( k_{D,\text{lung}} = 1.4 \text{ h}^{-1} \), yielding a very nice description of the measured data (solid line in Fig. 2C).

In RNM, NPSH levels decreased with increasing PO concentration until a constant base level was reached (Fig. 2D, Lee et al., 2005). According to Potter et al. (1995), the mean sum of GSH plus cystein—the latter possibly formed from GSH during the surgical procedure—was 76% of the NPSH level in RNM of rats. Considering this, we modeled 70 or 85% of the NPSH in RNM to represent cytosolic GSH that could conjugate with PO. The simulation (solid line in Fig. 2D) was obtained by fitting the turnover rate in the RNM to the measured data \((k_{D,\text{resp}} = 2.2 \text{ h}^{-1})\). Similar to the data, the model simulates NPSH levels in RNM to become depleted to 15% of the control level at exposure concentrations above 125 ppm PO. (According to the above given model structure, derived from the GSH and NPSH data of Potter et al. [1995], cytosolic GSH is completely depleted at a residual NPSH level in RNM in the range between 15 and 30% of the NPSH control level.)
In order to investigate the time course of GSH depletion in RNM of Fischer 344 rats, short-term exposure experiments at 300 ppm were carried out using the methodology of Lee et al. (2005). The measured NPSH levels decreased rapidly with increasing time, yielding a nonlinear curve (Fig. 3A). The modeled curve agrees well with the measured data down to a nondepletable NPSH level of 30% of the control level. (According to the above given model structure, derived from the GSH and NPSH data of Potter et al. [1995], cytosolic GSH is completely depleted at a residual NPSH level in RNM in the range between 15 and 30% of the NPSH control level.)

Using the closed-chamber technique described by Filser (1992), Schmidbauer (1997) investigated the toxicokinetics of PO in male Fischer 344 rats. Inhalation experiments (Fig. 3B) were started by injecting defined amounts of gaseous PO into closed all-glass chambers of 6.4 l each of which contained two rats. Due to its uptake by the animals, PO disappeared from the chamber atmospheres in a time-dependent manner. The semilogarithmic plot depicts almost linear declines for the three lowest initial concentrations with almost identical slopes. At the highest initial concentration (10,000 ppm), the concentration-time course could be monitored for 3 h only. This experiment had to be terminated because of dramatic signs of toxicity. At the second highest exposure concentration, the initial slope was flatter than at lower concentrations but become steeper with decreasing PO concentrations in the chamber. The model explained this concentration-time course by GSH depletion in the high concentration range. No model parameters were adjusted to describe this data set. The model predictions shown as continuous lines are in agreement with the measured data.

Schmidbauer (1997) investigated also the concentration-time course of atmospheric PO in closed chambers each containing one male Fischer 344 rat treated intraperitoneally with a dose of 0.59 mmol PO/kg (Fig. 3C). Exhaled PO appeared almost immediately in the chamber air. Maximum concentrations were reached within 20 min. Thereafter, PO was rapidly eliminated from the atmosphere. Model predictions were obtained after adjusting a single model parameter, the first-order absorption rate for intraperitoneally administered PO ($k_{ap} = 250 \text{ h}^{-1}$), with all other model parameters held constant. The modeled curve is in good agreement with the measured data.

Uptake of inspired PO into the upper respiratory tract of Fischer rats was investigated in dependence of the time of exposure (Morris et al., 2004). A urethane-anesthetized rat with its surgically isolated upper respiratory tract was placed in a nose-only chamber containing vaposolar PO of 300 ppm. While the residual rat inspired room air through an incised trachea, chamber air was drawn through the isolated upper respiratory tract at constant unidirectional flows of 50 or 200 ml/min. The efficiency of PO uptake was calculated from the difference between the atmospheric PO concentrations entering and leaving the upper respiratory tract. For modeling this uptake, Equations 4, 7, 10, and 13 (see Appendix) were changed in order to reflect the nonphysiological unidirectional flow (Equations 27–31). No model parameters were adjusted to describe this data set (Fig. 3D). For the high flow (physiological conditions), the model predicts uptake efficiencies between 11.7 and 12.4%, which agrees excellently with the measured value of 12 ± 2%. For the low flow of 50 ml/min, representing only 1/3 of the pulmonary ventilation of a 200-g rat (150 ml/min, Morris, 1999), the model overpredicts the measured data by a factor of 1.3. Both the measured data and the predicted curve demonstrate that steady-state distribution between PO in air and in mucosa is reached almost immediately.

Morris et al. (2004) also investigated the uptake efficiency of the upper respiratory tract toward PO in dependence of exposure concentration (25–300 ppm) using two different unidirectional flows (see Table in the supplemental information provided online). The data vary independently of the PO concentration between 21 and 26% at the low flow and between 11 and 12% at the high flow. At a flow of 200 ml/min (physiological condition), the model predicts an average uptake efficiency of 12.3%, which agrees excellently with the measured data. At the fourfold smaller flow the model overpredicts the measured data by a factor of 1.3. Furthermore, the uptake efficiency is predicted to decrease somewhat at both flows—at the high one from 12.6% (25 ppm) to 11.7% (300 ppm)—an effect, resulting from saturation kinetics of nasal PO metabolism. Obviously, the uptake efficiency is almost independent on the exposure concentration (at least up to 300 ppm) but highly dependent on the flow.

Repeated Exposures to PO

Lee et al. (2005) investigated PO concentrations in blood and NPSH levels in various tissues of Fischer 344 rats exposed for 3 days or 4 weeks (6 h/d, 5 d/wk) to PO concentrations of 5, 25, 50, 300, and 500 ppm (Fig. 4). Figure 4A shows also the PO concentrations determined in blood following another 4-week exposure of male Fischer 344 rats (6 h/d, 5 d/wk) to PO (Osterman-Golkar et al., 1999). After repeated PO exposures, PO concentrations in blood were linearly related to the exposure concentrations of up to 300 ppm. At 500 ppm, the concentrations in blood were somewhat overproportional if compared to the values at the lower exposure concentrations. Furthermore, there were no differences between 3 days or 4 weeks of exposure (Fig. 4A). After increasing the hepatic turnover rate constant to $k_{Dh} = 0.46 \text{ h}^{-1}$ in order to cope with the reduced GSH loss if compared with the single exposure, the modeled curve to the NPSH data given in Figure 4B was obtained. The higher value of $k_{Dh}$ reflects a raised GSH synthesis answering the greater GSH consumption by PO. The augmented GSH availability in the liver results in an efficient GST-mediated PO elimination in this organ even at the high PO concentrations, thereby preventing the sudden increase in the PO blood concentration seen in Figure 2A. In contrast to single
exposures, the model shows an almost linear relation between PO in air and PO in blood, not reaching at the highest exposure concentration of 500 ppm the mean PO blood concentration measured (Fig. 4A), in spite of the predicted less decreased pulmonary ventilation for repeated exposures to high PO concentrations (see Fig. 2). The difference between measured and predicted PO blood concentrations at 500 ppm could be explained assuming a divergence between GSH production and GSH consumption. Both rates are modeled to be identical in nonexposed rats. However, we abstained to revise the model accordingly for lack of sufficient data justifying such a procedure.

To describe GSH depletion in the lung (Fig. 4C, Lee et al., 2005), the turnover rate constant for pulmonary GSH was changed in the same proportion as in the liver to $k_{D_{\text{lunge}}} = 3.22$ h$^{-1}$. The modeled curve agrees with the measured data. It is evident that increases in $k_{D_{\text{g}}} \text{values should be dependent on the PO concentration. However, corresponding functions could not be deduced from the available data.}

In RNM (Fig. 4D), the modeled NPSH simulation (solid line) was obtained using the same GSH turnover rate constant ($k_{D_{\text{in}}}=2.2$ h$^{-1}$) as was used to describe NPSH levels in the single exposures. No fitting to the measured data was done. According to the model simulation, NPSH becomes depleted in the RNM at exposure concentrations of above 125 ppm at which NPSH levels of 30% of the control value are reached. (According to the above given model structure, derived from the GSH and NPSH data of Potter et al. [1995], cytosolic GSH is completely depleted at a residual NPSH level in RNM in the range between 15 and 30% of the NPSH control level.)

**Adducts**

Using *in vitro* determined rate constants for adduct formation and elimination, adducts with hemoglobin and DNA can be predicted based on the modeled concentration-time courses of PO in blood. The quality of the adduct predictions is strongly influenced by the accuracy of the published rate constants.

Formation of 2-hydroxyproplylvaline (HOPR-Val) in hemoglobin and N7-HPG in DNA of RNM was determined in Fischer 344 rats following repeated (6 h/d, 5 d/wk, 4 wk) exposures to PO. The model was used to simulate the concentration-time courses of HOPR-Val (Fig. 5A) and N7- HP2 (Fig. 5C) in rats exposed to 500 ppm PO. The simulations demonstrated that DNA adducts reached almost steady state after 2 weeks of exposure, whereas hemoglobin adducts required more than 4 weeks. Such concentration-time courses were used to predict the dose-response curves for formation of...
hemoglobin adducts (Fig. 5B) and of DNA adducts in RNM (Fig. 5D). Concerning HOPR-Val adducts, model predictions deviate from measured values with increasing PO concentration (Fig. 5B). This deviation is negligible at PO concentrations up to 50 ppm. At the highest exposure concentration (500 ppm), the model predicts an adduct level of 47 pmol HOPR-Val/mg globin, whereas the measured values, showing a two-fold variation, were 77.7 ± 4.7 (Osterman-Golkar et al., 1999), 90.2 ± 11.4 (Ríos-Blanco et al., 2000), 130 ± 5 (Osterman-Golkar et al., 2003), and 159.1 ± 9.9 (Ríos-Blanco et al., 2002). For N7-HPG adducts in RNM, the predicted adduct levels agreed with the measured values at low exposure concentrations, up to 50 ppm (Fig. 5D). In contrast to the underpredicted HOPR-Val adducts at 300 and 500 ppm, the N7-HPG adducts in RNM were overpredicted at both PO exposure concentrations. Table 7 shows N7-HPG adducts measured in diverse tissues of Fischer 344 rats exposed to 500 ppm PO. The model overpredicted the DNA adducts in RNM and lung but underpredicted those in liver. The data measured in the same tissue by different laboratories showed up to 1.7-fold variation. At least up to an exposure concentration of 300 ppm, the difference between predicted and measured hemoglobin adducts cannot result from incorrectly modeled PO blood concentrations as evidenced by Figure 4A. Most probably, the underprediction of the hemoglobin adducts demonstrated in Figure 5B results primarily from an incorrect rate constant for adduct formation. The same considerations hold for the difference between predicted and measured DNA adduct formation. These arguments are discussed in more detail in Discussion.

An overview of how data sets were used for model calibration and validation are presented in Table 8.

Sensitivity Analysis

Log-normalized sensitivity coefficients were calculated for steady-state exposures of rats and humans to 10 ppm PO as well as for rats to 300 ppm PO (Fig. 6). The influences of all the model parameters given in Tables 1, 2, 3, 4, and 5 were investigated on the PO concentration in RNM (Fig. 6A) and in blood (Fig. 6B) of rats and humans. The metabolic parameters chosen were those obtained after repeated exposures. Sensitivity coefficients are only presented if they exceeded a value of 0.05.
In rats, the sensitivity coefficients are almost identical at both exposure concentrations. In both species, the most sensitive model parameter for determining PO burden in RNM is the partition coefficient nose:air (Fig. 6A). Other parameters as pulmonary ventilation, cardiac output, and partition coefficients nose:blood and lung:air exhibited a much smaller influence. In both species, the most significant determinant of the PO blood concentration is the pulmonary ventilation (Fig. 6B). Predicted PO concentrations in blood increase in both species as Km values or blood flows increase and decrease when Vmax, liver volume, or partition coefficients tissue:blood increase (Fig. 6B). Apart from the pulmonary ventilation all model parameters show sensitivity coefficients less than 0.5.

**DISCUSSION**

**Model Development**

The PT model represents primarily an extension of our previous model for styrene and styrene-7,8-oxide by the nose. This former model has already been discussed extensively (Csándy et al., 2003). Therefore, the discussion focuses only on new features.

**Nose Module**

The arrangement of the nasal compartments in both species followed the structure presented earlier (Morris et al., 1993). A similar model structure was used for vinyl acetate (Plowchalk et al., 1997) and methyl methacrylate (Andersen et al., 1999). For rats, other authors used even more compartments (e.g., Bogdanffy et al., 1999; Bush et al., 1998; Frederick et al., 1998).

In these models, the mucosa of a specific nasal region was subdivided into stacks, with each stack representing a distinct compartment corresponding to, for example, mucus, epithelial cell layers, and submucosa. In our model, the mucosa of a specific nasal region was not modeled as a stack but as a single compartment. From a scientific point of view, such a model is superior to the more complex stack structure because there is only information on average tissue concentrations (adducts and GSH levels) but there are no measured data in any of the layers within a given nasal tissue stack. Additionally, the values of the parameters describing the layers are unknown. Very recently, Schroeter et al. (2006), who modeled the nasal extraction of inhaled hydrogen sulfide, came to the same conclusion that a simple compartment nasal tissue model was sufficient, and a more elaborate one was deemed unnecessary. The straightforward model structure used by us allowed to reduce assumptions about quantitative values of anatomical, physiological, and biochemical parameters to a minimum. The model relies on measured pulmonary ventilation and on values for the surface areas of respiratory and olfactory mucosae, and for mucosal thickness (Table 1), which were derived from measured weights of mucosa as determined in 80 Fischer 344 rats. The obtained thickness of respiratory mucosa was 70 μm compared to the 90 and 100 μm used by Morris et al. (1993) and Frederick et al. (1998), respectively. For the olfactory mucosa, a value of 80 μm was obtained, which is less than the 130 μm used by Frederick et al. (1998) and the total depth of 210 μm (Morris et al., 1993). The values used by Morris and Frederick represent mixtures of “assumptions,” “personal communications,” and measured data, while our values rely only on measured data.

For rats and humans, the species-specific values representing the blood flow through the nasal mucosa are averages of published values. The mean of measured flows through human mucosa is only 0.0164% of the cardiac output. This value is 60 times smaller than the value used for physiological toxicokinetic modeling by previous authors (Andersen et al., 2000; Frederick et al., 1998). Unfortunately, the difference cannot be elucidated because the source of the value used by these authors was only “estimated” (Frederick et al., 1998).

**Rat Model**

No measured PO concentrations are available in RNM of PO-exposed rats to prove the soundness of our model. However, evidence for the validity of the model is provided using the experimental determination of NPSH levels in RNM in combination with data simulated by the PT model. Since PO reacts with GSH by forming conjugates, the tissue level of GSH in RNM is influenced by the activities of EH and GST toward PO. The same approach used for estimating styrene-7,8-oxide concentrations in lungs of styrene-exposed rats and mice (Csándy et al., 2003) was later validated by styrene-7,8-oxide measurements in styrene vapor–exposed ventilated and perfused lungs of both species (Hofmann et al., 2006). As evident from Figures 3B and 6B, the present PT model describes fairly well the PO-dependent depletion of GSH in RNM.

**TABLE 7**

Levels of N7-HPG (pmol/mg DNA) in DNA Measured in Tissues of Fischer 344 Rats Exposed to 500 ppm PO (6 h/d, 5 d/wk, 4 wk)

<table>
<thead>
<tr>
<th></th>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>RNM</td>
<td>358</td>
<td>485</td>
<td>281.9 ± 18.9</td>
<td>302 ± 10.5</td>
</tr>
<tr>
<td>Lung</td>
<td>60</td>
<td>56</td>
<td>45 ± 2.6</td>
<td>50.1 ± 7.6</td>
</tr>
<tr>
<td>Liver</td>
<td>17</td>
<td>22</td>
<td>24.6 ± 4.7</td>
<td>18.9 ± 3.5</td>
</tr>
</tbody>
</table>

*a Adduct levels given in pmol/mmol guanine were converted using the average guanine content of 0.8 mmol/g DNA.
modeled turnover rate of GSH in RNM (2.2 h⁻¹) compares well to that calculated for lung. It is 14 times faster than that of 0.16 h⁻¹ published by Potter et al. (1995) who calculated it from the appearance of ³⁵S-GSH in RNM of Fischer 344 rats following intravenous injection of ³⁵S-cysteine. However, when using the small value of these authors, the model simulated an almost immediate GSH depletion in RNM in contrast to the much slower degree that was actually observed (Fig. 3A). This figure shows that the model predicts the time course of the NPSH depletion, measured in rats single exposed to 300 ppm PO, reasonably well. Morris et al. (2004) measured also the time course in the RNM of anesthetized rats exposed to 300 ppm PO in the above-described semi-in vivo assay. The authors detected the drastic loss of NPSH only after 15 min of exposure. Because their NPSH data were not related to tissue weight, we could not model them.

A straightforward procedure enables to evaluate the maximum range covering reasonable predictions of PO concentrations in RNM: The highest possible nasal PO concentration can be calculated considering only the thermodynamic partition coefficient RNM:air. Multiplying the exposure concentration (ppm expresses as ml/m³) with the partition coefficient of 63.7 and converting the resulting concentration into mmol/l by dividing it with the molar volume of an ideal gas (25,130 ml/mol), the upper curve in Figure 7A was obtained. Considering the mucosa to be exposed only during the inhalation...

### TABLE 8
Overview of Data Sets Used for Model Calibration and Validation

<table>
<thead>
<tr>
<th>Study description</th>
<th>Available data</th>
<th>Parameters derived</th>
<th>Use in modeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open chamber inhalation. Single 6 h exposures. Concentration range: 10–750 ppm (Lee et al., 2005)</td>
<td>Concentrations of PO measured in blood at end of exposures (Fig. 2A)</td>
<td>$K_m$ for EH in liver</td>
<td>Calibrate PT model</td>
</tr>
<tr>
<td></td>
<td>Concentrations of NPSH measured in RNM at end of exposures (Fig. 2D)</td>
<td>$k_{D,exp}$ for GSH turnover in RNM</td>
<td>Calibrate PT model</td>
</tr>
<tr>
<td></td>
<td>Concentrations of NPSH measured in lung at end of exposures (Fig. 2C)</td>
<td>$k_{D,exp}$ for GSH turnover in lung</td>
<td>Calibrate PT model</td>
</tr>
<tr>
<td></td>
<td>Concentrations of NPSH measured in liver at end of exposures (Fig. 2B)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Concentration-time courses of PO measured in the atmosphere (Fig. 3C)</td>
<td>First-order rate constant for the absorption of PO ($k_{PO}^{0}$)</td>
<td>Calibrate PT model</td>
</tr>
<tr>
<td>Closed-chamber uptake. Intrauterine administration: 0.59 mmol/kg (Schmidbauer, 1997)</td>
<td>Concentrations of NPSH measured in liver at end of exposures (Fig. 4B)</td>
<td>$k_{D,b}$ for GSH turnover in liver</td>
<td>Calibrate PT model</td>
</tr>
<tr>
<td></td>
<td>Concentrations of NPSH measured in lung at end of exposures (Fig. 4C)</td>
<td>$k_{D,b}$ was predicted from $k_{D,b}$</td>
<td>Calibrate PT model</td>
</tr>
<tr>
<td></td>
<td>Concentrations of NPSH measured in RNM at end of exposures (Fig. 4D)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Concentration-time courses of PO measured in the atmosphere (Fig. 3B)</td>
<td>None</td>
<td>Validate PT model</td>
</tr>
<tr>
<td>Open chamber inhalation. Single exposures up to 6 h, 300 ppm (unpublished data D. Klein)</td>
<td>Time course of NPSH measured in RNM (Fig. 3A)</td>
<td></td>
<td>Validate PT model</td>
</tr>
<tr>
<td>Rat upper respiratory tract studies with PO in dependence of time. Concentration: 300 ppm (Morris et al., 2004)</td>
<td>Uptake efficiency of PO in dependence of time (Fig. 3D)</td>
<td></td>
<td>Validate PT model</td>
</tr>
<tr>
<td>Rat upper respiratory tract studies with PO in dependence of exposure concentration. Concentration range: 25–300 ppm (Morris et al., 2004)</td>
<td>Uptake efficiency of PO in dependence of exposure concentration (Table in the supplemental information provided online)</td>
<td></td>
<td>Validate PT model</td>
</tr>
<tr>
<td>Measurements of PO adduct to hemoglobin and DNA of rats following repeated (6 h/d, 5 d/wk) exposures to PO. Concentration range: 5–500 ppm (Osterman-Golkar et al., 1999, 2003; Rios-Blanco et al., 2000, 2002; Segerbäck et al., 1998)</td>
<td>Hydroxypropyl adducts to hemoglobin and DNA (Fig. 5 and Table 7)</td>
<td></td>
<td>Validate PT model</td>
</tr>
</tbody>
</table>
is switched off in the PT model by setting $V_{\text{superposed}}$ by the other one). If metabolic elimination in RNM line and dashed blue line in Figure 7A; one line almost bracket predictions obtained by the present PT model (solid red line). This calculation was done assuming that inhalation and exhalation equally contribute noticeably to the PO concentration in RNM, as can be concluded from the small difference between both colored curves and the lowest curve in Figure 7A. Obviously, the PO concentration in the breathing air represents the driving force for the PO concentration in RNM. Fortunately, the uncertainty of the nasal physiological parameters has only limited impact on the PO concentration in RNM. Nasal metabolism does not influence the PO burden in venous blood as evidenced by the scarcely existing difference between both colored lines in Figure 7B (solid red line with and dashed blue line without metabolism). However, knowledge of the nasal PO metabolism is an essential prerequisite to describe the GSH status in RNM.

The rat model was also thoroughly elaborated and evaluated using PO concentrations measured in blood and air, as well as NPSH levels determined in various tissues of PO-exposed rats (Figs. 2, 3, and 4). Maples and Dahl (1993) published a PO concentration of 2.7 ng/g blood (47 nmol/l) determined in Fischer 344 rats exposed for 60 min to 14 ppm PO. For this exposure condition, the PT model predicts an almost 15-fold higher PO blood concentration. There was probably loss of PO during blood collection and the subsequent analytical procedures (cryogenic distillation), resulting in a lower reported blood concentration.

The thermodynamic distribution coefficient, whole body to air ($K_{\text{eq}}$, Filser, 1992), represents the highest possible enrichment of PO in the organism. It can only be reached if no metabolic elimination takes place. Considering such a condition, the model predicts this value to be 57, indicating that PO concentrations will be 57 times higher in an average tissue than in air, provided that no PO metabolism occurs in the rat. This value agrees excellently with that of 51 ± 2 obtained by Schmidbauer (1997) and is somewhat larger than the value of 45, calculated earlier by Golka et al. (1989). Due to the extensive PO metabolism in the body, much lower average body concentrations are achieved. The concentration ratio, whole body to air, realized under steady-state conditions is given by the bioaccumulation factor ($K_{\text{St}}$, Filser, 1992). For exposure concentrations below 100 ppm, the present PT model predicts a $K_{\text{St}}$ value of only 1.5, indicating a highly effective metabolic elimination. Based on a two-compartment model, similar values were derived for Fischer 344 rats (1.9, Schmidbauer, 1997) and for Sprague-Dawley rats (1.2, Golka et al., 1989). For example, for a rat exposed at steady state to 50 ppm PO, the PT model calculates an average tissue concentration of about 0.003 mmol PO/l (1.5 × 50/25,130). This value matches the measured blood concentration of 0.0028 mmol/l since PO distributes almost equally among the systemic tissues due to its amphiphilic nature. The PT model predicts a metabolic rate of 0.17 µmol PO/h in a rat of 0.25 kg exposed at steady state to 1 ppm PO (0.04 µmol PO/l air). The ratio of the metabolic rate to the atmospheric exposure concentration (in µmol/l air) gives the clearance of metabolism related to the atmospheric PO concentration at 25°C (Filsler, 1992). The value of 4.25 l/h calculated with the PT model agrees excellently with those obtained previously from closed-chamber gas uptake studies (4.3, Golka et al., 1989; 4.0, Schmidbauer, 1997).
Human Model

The human model reflects worst-case considerations. Since no metabolic data were available for PO in human nasal tissues, the rate of metabolic elimination in the nasal mucosa was set to zero. Also, the vessel-rich tissue group did not include metabolic elimination (in contrast to rats). The smallest PO elimination rates determined by Faller et al. (2001) in human liver and lung were used to calculate metabolism in these organs. Because of the lack of in vivo data, human hepatic $K_m$ values were taken as determined in vitro (Faller et al., 2001). This procedure differed from that used for rats where $K_m$ values were obtained by adjusting to in vivo data. In agreement with the worst-case scenario, only nasal breathing was considered. To simulate workplace exposures under light physical workload of 50 W, the pulmonary ventilation rate and cardiac output were augmented from their values at rest (given in Table 1) to 1490 and 590 l/h, respectively (Åstrand, 1983). A model validation on PO concentrations in blood of exposed workers was not possible because we did not find any publication that provided such data. Therefore, we used reported HOPR-Val adducts as surrogates for PO blood concentrations. The model predicts the AUC (area under the curve) of PO in blood of workers exposed for 8 h to 1 ppm PO to be 0.0014 mmol h/l. Using this AUC, the daily adduct increment is calculated to be 35 pmol HOPR-Val/g Hb (0.0014 mmol h/l $\times$ 0.25 $\times$ 10$^{-4}$ l/h/g Hb $\times$ 10$^9$ pmol/mmol) by considering the rate constant (0.25 $\times$ 10$^{-4}$ l/h/g Hb; see Table 6). The accumulation of hemoglobin adducts during repeated daily exposures is a net result of daily increments and daily losses due to the removal of erythrocytes from the circulation (Osterman-Golkar et al., 1976). Steady-state adduct levels are reached as soon as the exposure period covers the life span of erythrocytes (126 d). The steady-state adduct level resulting from an exposure to 1 ppm PO (8 h/d, 5 d/wk, at least 126 d) is calculated to be 1.6 nmol/g Hb (35 pmol HOPR-Val/g Hb $\times$ 126/2 $\times$ 5/7 $\times$ 0.001 nmol/pmol). Using data measured in workers (Högstedt et al., 1990), a three times lower value of 0.5 nmol HOPR-Val/g Hb was calculated for the same exposure condition of 1 ppm PO for 40 h/wk (Kautiainen and Törnqvist, 1991).

FIG. 7. Impact of breathing, blood flow, and metabolism in RNM on the PO concentration in RNM (A) and impact of metabolism in RNM on the PO concentration in blood (B) of rats as well as PO concentrations in RNM (C) and blood (D) of humans. (A) Predicted PO concentrations at steady state in RNM of a male Fischer 344 rat (250 g) exposed to PO in dependence of exposure concentration. The top curve represents the highest possible nasal PO concentration considering thermodynamic equilibrium between RNM and the PO concentration in inhaled air. The bottom curve represents the average PO concentration in RNM during a breathing cycle considering no PO exhalation from the alveoli, no metabolism in RNM, and no PO exchange with blood. The colored lines represent PT model simulations with PO metabolism in RNM (dashed blue line) and without PO metabolism in RNM (solid red line). (B) Predicted PO concentrations at steady state in venous blood of a male Fischer 344 rat (250 g) exposed to PO. The colored lines represent PT model simulations with PO metabolism in RNM (dashed blue line) and without PO metabolism in RNM (solid red line). (C) Predicted PO concentrations at steady state in RNM of a human (BW = 70 kg) exposed to PO at rest. (D) Predicted PO concentrations at steady state in venous blood of a human (BW = 70 kg) exposed to PO at rest.
From the HOPR-Val adduct levels measured in 28 workers exposed to PO, a daily adduct increment of 84.4 pmol HOPR-Val/g globin was calculated for a PO exposure concentration of 4.15 ppm given as a 8-h time weighted average concentration (Boogaard et al., 1999). For an exposure concentration of 1 ppm, the corresponding daily increment is 20.3 pmol HOPR-Val/g globin, which compares to the value of 35 predicted by the PT model. Czéne et al. (2002) reported an average value of 2.7 nmol/g globin for steady-state adduct levels measured in eight workers exposed to an estimated PO concentration of 2.3 ppm. For an exposure concentration of 1 ppm, the corresponding steady-state value is 1.17 nmol HOPR-Val/g globin, which matches with the predicted value of 1.6 nmol HOPR-Val/g globin. In a recent publication (Jones et al., 2005), a correlation was derived based on human biomonitoring data resulting in 10 pmol HOPR-Val/g globin adducts related to an average airborne exposure concentration of 0.008 ppm PO. According to these authors, exposure at steady state to 1 ppm PO should lead to 1.25 nmol HOPR-Val/g globin, matching the model predicted value of 1.6 nmol HOPR-Val/g globin. Not considering the data of Kauttainen and Törnvist (1991), the model prediction for workplace conditions is 1.5 times higher than published.

For the following model simulations, pulmonary ventilation was set to its physiological value of 450 l/h at rest (Arms and Travis, 1988) given in Table 1. The PT model predicts the PO concentrations in human RNM to increase linearly with the exposure concentration at steady state (Fig. 7C). In the RNM of equally exposed rats and humans, the expected PO concentration is almost identical. For example, the PO concentration expected at an exposure concentration of 50 ppm is about 0.074 and 0.076 mmol/l in RNM of rats and humans, respectively.

The PO concentration in venous blood at steady state is modeled to increase linearly with the exposure concentration up to 50 ppm (Fig. 7D). For example, the blood concentration of PO in humans exposed at steady state to 50 ppm PO is about 0.0026 mmol/l, which is very similar to the value measured in equally exposed rats (0.0028 mmol/l). The thermodynamic distribution coefficient, whole body:air, and the bioaccumulation factor are predicted to be 59 and 1.3, respectively. These values are similar to the corresponding rat values. The model predicts a rate of metabolism of 7.2 mmol PO/h in a 70-kg human exposed to 1 ppm PO. The clearance of metabolism related to the atmospheric concentration is predicted to be 181 l/h. From the corresponding clearance of metabolism observed in rats (Schmidbauer, 1997), an allometrically (Filser, 1992) derived value of 171 l/h (4.0 × (70/0.25)2/3) is calculated comparing well with the PT model prediction. In summary, at least up to 50 ppm, the toxicokinetic behavior of PO is similar in humans and rats.

Adducts

The inconsistency between predicted and measured adduct levels in the rat might be related to the adduct measurements but might also arise from some uncertainty related to the rate constants describing adduct formation or elimination. Concerning hemoglobin adduct measurement, it has been shown in ring tests that detected adduct levels varied distinctly between laboratories (Törnvist et al., 1992; Pauwels et al., 1997). This demonstrates the problems in the determination of the true value. The rate constant for the reaction between the N-terminal valine in human hemoglobin and PO was estimated to be 0.25 × 10⁻⁴ l/g Hb/h (Segerbäck et al., 1994). An almost two times lower value was reported later (1 l/mol Hb/h corresponding to 0.14 × 10⁻⁴ l/g Hb/h; Pauwels and Veulemans, 1998). The difference might be related to the probably too short incubation times used for adduct monitoring (0.5–2 h), when compared to the expected half-life of adduct formation (about 170–310 h). The rate constant of the adduct formation of PO with guanine N7 in calf thymus DNA was 0.25 × 10⁻⁴ l/g DNA/h (Segerbäck et al., 1994), whereas in lymphocytes of human blood, a lower value was reported (5.1 × 10⁻³ l/mol DNA nucleotide per hour corresponding to 0.16 × 10⁻⁴ l/g DNA/h; Pauwels and Veulemans, 1998). This difference might also indicate a lower accessibility of cellular DNA, compared to calf thymus DNA. A lower rate constant would certainly increase the precision of the adduct predictions in RNM and lung. In addition, the half-lives of DNA adducts used in the model influence the predicted adduct levels. The half-lives of N7-HPG used in the PT model was determined in tissues of Fischer 344 rats exposed to PO to be between 5.5 and 6.5 days (Table 6, Osterman-Golkar et al., 2003). These values agree well with the half-life of spontaneous depurination, determined in vitro (120 h at neutral pH and 37°C, Koskinen and Pihlã, 2000). Furthermore, dynamic changes not incorporated into the PT model as prolonged, organ-specific cell cycles or apoptosis and cell proliferation might influence adduct levels.

Sensitivity Analysis

The log-normalized sensitivity coefficients calculated for a steady-state exposure to 10 ppm PO (Fig. 6) support the species-specific discussions presented. Sensitivity analysis indicated that PO concentrations in RNM and in blood are not sensitive to optimized parameters but mostly to the pulmonary ventilation and partition coefficients. Considering the PO concentration in RNM, the only major determinant was the partition coefficient nose:air. In the human model, the partition coefficient lung:air influenced somewhat the nasal PO burden (reciprocally proportional), indicating that the air leaving the lung during exhalation had a small impact too. No physiological parameter of the nose (surface area, air flows, blood flows, and tissue thickness) exerted a significant influence on the PO concentration (Fig. 6A) because these parameters determine mainly how fast steady state is reached. Consequently, the result can be interpreted as a hint for a possible reduction of the nasal model structure. This conclusion is also supported by our findings that in both species metabolism in RNM is not relevant concerning the PO burden in
this tissue. Sensitivity coefficients calculated for blood showed
that uncertainty in the pulmonary ventilation would have the
largest influence on the predicted uncertainty of the PO burden in
blood (Fig. 6B). Although the sensitivity analysis was conducted
for an exposure to 10 ppm, its results are valid in both species at an
exposure to 1 ppm PO, too, because all modeled processes are
linear in this range. For the same reason sensitivity coefficients do
not differ in rats at an exposure to 300 ppm PO (Fig. 6).

CONCLUSION

In order to establish the PT model on a solid and reliable
database, a too complex model structure was avoided. The model
for inhalation exposure to PO was thoroughly de-
veloped, evaluated, and validated on all available data in rats
and humans. Using the here presented toxicokinetic model
for rats and humans and the rat nasal tumor data, the 95% lower
confidence limit on the concentration producing a 10% in-
crease in additional tumor risk (LED10) in rats and
humans was estimated to be 69 and 66 ppm, respectively,
in both species at an exposure to 1 ppm PO, too, because all modeled processes are
linear in this range. For the same reason sensitivity coefficients do
not differ in rats at an exposure to 300 ppm PO (Fig. 6).

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.
oxfordjournals.org/.

APPENDIX

The PT model is formulated by a series of mass balance
differential equations. For each compartment, an equation
describes the uptake and elimination of PO. In order to
integrate the differential equations, the initial concentrations
of PO in the body compartments are set to zero. Equations
describing adduct formations are given elsewhere (Csánády
et al., 2000). The equations below describe the actual changes
in the amount over time in an organism.

Exposure Chamber

PO is inhaled by the nose. Its concentration in chamber air is
denoted by $C_{PO}^{Ch}$. Here, the air stream splits into two paths:
a lateral-ventral one (with the PO concentration $C_{PO}^{RV,air}$) that
leads over ventral respiratory mucosa and a dorsal-medial one
($C_{RD,air}$) leading subsequently over dorsal respiratory and
olfactory mucosa. The fraction of air stream following the
dorsal-medial path is denoted by $f_{DR}$. Consequently, the differen-
tial equation describing mass change over time in the
chamber atmosphere is given as follows:

$$V_{Ch} \times \frac{dC_{PO}^{Ch}}{dt} = -N \times Q_{pulm} \times \left( C_{PO}^{Ch} - f_{DR} \times C_{RD,air} + (1 - f_{DR}) \times C_{RV,air} \right).$$

The number of rats exposed simultaneously is denoted by $N$.
The initial chamber concentration at $t = 0$ is represented by $C_{PO}^{Ch,0}$.

Nasal Compartments

The nasal tissue is separated in olfactory mucosa (PO
concentration $C_{PO}^{Diff,tissue}$) and respiratory mucosa that is further
divided into a lateral-ventral ($C_{RV,tissue}$) and a dorsal-medial
region ($C_{RD,tissue}$). Each of these tissue compartments is con-
ected to a corresponding air compartment through which PO
vapors are transfered by the airflow. Two processes take place
each nasal air compartment: transport of PO through the
compartment by breathing (convection) and mass transfer of
PO to the corresponding tissue compartment (diffusion).
The simplest way to calculate the diffusion driven mass transfer
of PO is using the film theory (see e.g., Cussler, 1984), so the steady-state flux ($J$) across a thin film can be expressed by
the mass transport coefficient ($k$) or by the diffusion coefficient
($D$):

$$J = k \times (C_1 - C_0) = \frac{D}{\tau} \times (C_1 - C_0).$$

The film thickness is denoted with $d$ and the concentration
difference with the term $(C_1 - C_0)$. Since no measured value
for the film thickness is available, it is approximated by the
mucosal tissue thickness $t$ that was determined by us in nasal respiratory
and olfactory mucosa. Consequently, mass transport through
the mucosa of the area $A$ can be formulated as:

$$\frac{dN}{dt} = J \times A = k \times A \times (C_1 - C_0) \approx \frac{D}{t} \times A \times (C_1 - C_0).$$

The volumes of the air phases located above the lateral-
ventral respiratory mucosa, dorsal-medial respiratory mucosa,
and olfactory mucosa are divided in the ratios 0.34 ($f_{RV,air}$),
0.13 ($f_{RD,air}$), and 0.53 ($1 - f_{RV,air} - f_{RD,air}$) times the whole
nasal air space corresponding to their tissue surfaces.

In the following, equations corresponding to the lateral-ventral
respiratory mucosa are discussed in detail. The change per time
unit of the amount of PO in the air phase located above the
lateral-ventral respiratory mucosa is governed by the uptake from
the inhaled air ($C_{PO}^{Ch}$), by the uptake from the exhaled air coming
from the tracheobronchial (conducting) zone ($C_{PO}^{lungC,air}$), by
the air leaving the compartment ($C_{RV,air}$), and by the diffusion
driven mass transfer of PO into the tissue compartment:

$$V_{nose} \times f_{RV,air} \times \frac{dC_{PO}^{RV,air}}{dt} = Q_{pulm} \times \left( 1 - f_{DR} \right) \times \left( C_{PO}^{Ch} + C_{PO}^{lungC,air} - 2 \times C_{PO}^{RV,air} \right) - D_{g} \times P_{nose,air}.$$

The gas phase diffusion coefficient of PO is denoted by $D_{g}$.
The change per time unit of the amount of PO in the tissue

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compartment corresponding to the lateral-ventral respiratory mucosa is governed by the mass transfer, by the blood flow, and by the metabolic elimination of PO catalyzed by EH and GST. The conjugation of GSH with PO is modeled by a ping-pong mechanism (see liver). The conversion factor of 1000 was introduced to calculate the metabolic capacity in units of mmol/h.

\[
V_{tissue}^{RV} \times \frac{dc_{PO}^{RV,tissue}}{dt} = D_{g}^{PO} \times \frac{A_{Resp}}{\tau_{Resp}} \times \left( \frac{c_{PO,air}^{C_{RV,air}} - c_{PO,air}^{C_{RV,tissue}}}{P_{nose,air}} \right) \\
+ Q_{RV} \times \left( c_{PO,ArtBlood}^{C_{PO,ArtBlood}} - c_{PO,air}^{C_{RV,tissue}} \right) \\
- V_{tissue}^{RV} \times 1000 \times V_{max}^{GSH,resp} \times c_{PO,resp}^{C_{PO,resp}} \times c_{GSH,resp}^{C_{GSH,resp}} \\
- \frac{V_{tissue}^{RV}}{K_{m}^{D_{g}^{PO},resp}} \times \frac{c_{PO,resp}^{C_{PO,resp}} + c_{GSH,resp}^{C_{GSH,resp}}}{(K_{m}^{D_{g}^{PO},resp} + c_{GSH,resp}^{C_{GSH,resp}})} \\
+ Q_{RD} \times \left( \frac{c_{PO,ArtBlood}^{C_{RD,ArtBlood}} - c_{PO,air}^{C_{RD,tissue}}}{P_{nose,air}} \right) \\
- \frac{V_{tissue}^{RD} \times 1000 \times V_{max}^{GSH,resp} \times c_{PO,resp}^{C_{RD,resp}} \times c_{GSH,resp}^{C_{RD,resp}}}{K_{m}^{D_{g}^{PO},resp}} \\
- \frac{V_{tissue}^{RD} \times 1000 \times V_{max}^{GSH,resp} \times c_{PO,resp}^{C_{RD,resp}} \times c_{GSH,resp}^{C_{RD,resp}}}{K_{m}^{D_{g}^{PO},resp}} \times \frac{c_{PO,resp}^{C_{RD,resp}} + c_{GSH,resp}^{C_{RD,resp}}}{(K_{m}^{D_{g}^{PO},resp} + c_{GSH,resp}^{C_{RD,resp}})} \\
\]

Cytosolic GSH in the dorsal-medial respiratory mucosa:

\[
V_{tissue}^{RD} \times \frac{dc_{GSH,RD,tissue}}{dt} = V_{tissue}^{RD} \times \frac{A_{Resp}}{\tau_{Resp}} \times \left( c_{GSH,resp}^{C_{GSH,resp}} - c_{GSH,RD,tissue}^{C_{GSH,RD,tissue}} \right) \\
- \frac{V_{tissue}^{RD} \times 1000 \times V_{max}^{GSH,resp} \times c_{PO,resp}^{C_{GSH,resp}} \times c_{GSH,resp}^{C_{GSH,resp}}}{K_{m}^{D_{g}^{PO},resp}} \\
- \frac{V_{tissue}^{RD} \times 1000 \times V_{max}^{GSH,resp} \times c_{PO,resp}^{C_{GSH,resp}} \times c_{GSH,resp}^{C_{GSH,resp}}}{K_{m}^{D_{g}^{PO},resp}} \times \frac{c_{PO,resp}^{C_{RD,resp}} + c_{GSH,resp}^{C_{GSH,resp}}}{(K_{m}^{D_{g}^{PO},resp} + c_{GSH,resp}^{C_{RD,resp}})} \\
\]

Air phase above the olfactory mucosa:

\[
V_{air}^{nose} \times f_{RD,air} \times \frac{dc_{RD,air}^{PO}}{dt} = Q_{palm} \times f_{DR} \\
\times \left( c_{PO,air}^{C_{RD,air}} + 2 \times c_{PO,air}^{C_{RD,air}} \right) \\
- D_{g}^{PO} \times \frac{A_{Resp}}{\tau_{Resp}} \times \left( c_{PO,air}^{C_{RD,air}} - c_{PO,air}^{C_{RD,tissue}} \right) \\
\]

Cytosolic GSH in the olfactory mucosa:

\[
V_{tissue}^{Off} \times \frac{dc_{GSH,Off,tissue}}{dt} = V_{tissue}^{Off} \times \frac{A_{Off}}{\tau_{Off}} \times \left( c_{GSH,Off,tissue}^{C_{GSH,Off,tissue}} - c_{GSH,Off,tissue}^{C_{GSH,Off,tissue}} \right) \\
- \frac{V_{tissue}^{Off} \times 1000 \times V_{max}^{GSH,Off,tissue} \times c_{PO,Off,tissue}^{C_{GSH,Off,tissue}} \times c_{GSH,Off,tissue}^{C_{GSH,Off,tissue}}}{K_{m}^{D_{g}^{PO},Off,tissue}} \\
- \frac{V_{tissue}^{Off} \times 1000 \times V_{max}^{GSH,Off,tissue} \times c_{PO,Off,tissue}^{C_{GSH,Off,tissue}} \times c_{GSH,Off,tissue}^{C_{GSH,Off,tissue}}}{K_{m}^{D_{g}^{PO},Off,tissue}} \times \frac{c_{PO,Off,tissue}^{C_{RD,Off,tissue}} + c_{GSH,Off,tissue}^{C_{GSH,Off,tissue}}}{(K_{m}^{D_{g}^{PO},Off,tissue} + c_{GSH,Off,tissue}^{C_{RD,Off,tissue}})} \\
\]

The average concentration of \( C^X \) (\( X = \) PO or GSH) in RNM (\( C_{RNM,tissue} \)) is calculated by using the corresponding amount
of the substance in the lateral-ventral and dorsal-medial
respiratory mucosae:

\[
C_{\text{RNM, tissue}}^{X} = \frac{(V_{\text{R}}^{\text{tissue}} \times C_{\text{R, tissue}}^{X}) + (V_{\text{RD}}^{\text{tissue}} \times C_{\text{RD, tissue}}^{X})}{V_{\text{R}} + V_{\text{RD}}}.
\] (12a)

### Pulmonary Compartments

The combined air streams from olfactory and lateral-ventral
respiratory mucosae enters into the tracheobronchial tract
(conducting zone). Both the air space and tissue volumes
of this zone are modeled to represent 1/3 \((f_{i})\) of the corresponding
volumes of the whole lung. The change per time unit of the
amount of PO in the conducting zone is governed by the uptake
from the air leaving the nose, by the uptake from the exhaled
air coming from the alveolar zone, by the exhaled air leaving
the conducting zone, and by the PO transfer from air phase to
the tissue phase.

\[
V_{\text{air}} \times f_{i} \times \frac{dC_{\text{LungC, tissue}}}{dt} = Q_{\text{pulm}} \times (f_{\text{DR}} \times C_{\text{Off, air}} + (1 - f_{\text{DR}}) \times C_{\text{R, tissue}}^{\text{PO}}) - D_{\text{f}} \times \frac{A_{\text{LungC}}}{\tau_{\text{LungC}}} \times (C_{\text{LungC, tissue}}^{\text{PO}} - C_{\text{LungC, tissue}}^{\text{PO}}) \times \left(\frac{C_{\text{P, tissue}}^{\text{PO}} - C_{\text{LungC, tissue}}^{\text{PO}}}{P_{\text{lung, tissue}}}\right).
\] (13)

\(V_{\text{air}}\) is the air volume in the lung, \(A_{\text{LungC}}\) denotes the surface
area of the conducting zone, and \(\tau_{\text{LungC}}\) is the thickness of the
epithelium layer film. For this variable, the average thickness
of the nasal epithelia \((7.5 \times 10^{-4}\ \text{dm})\) is used.

The change per time unit of the amount of PO in the
conducting zone depends on diffusion, blood flow through the
tissue, and metabolism catalyzed by EH and GST.

\[
V_{\text{tissue}} \times f_{i} \times \frac{dC_{\text{LungC, tissue}}}{dt} = D_{\text{f}} \times \frac{A_{\text{LungC}}}{\tau_{\text{LungA}}} \times \left(C_{\text{LungC, tissue}}^{\text{PO}} - C_{\text{LungC, tissue}}^{\text{PO}}\right) - Q_{\text{pulm}} \times \left(C_{\text{PO, tissue}}^{\text{PO}} - C_{\text{LungC, tissue}}^{\text{PO}}\right) \times \frac{C_{\text{P, tissue}}^{\text{PO}} - C_{\text{LungC, tissue}}^{\text{PO}}}{P_{\text{lung, tissue}}} - V_{\text{tissue}} \times f_{i} \times 1000 \times C_{\text{LungC, tissue}}^{\text{PO}} \times \left(C_{\text{LungC, tissue}}^{\text{GSH}} + V_{\text{max, GST}} \times C_{\text{LungC, tissue}}^{\text{GSH}}\right) \times \left(K_{\text{m, GST}} \times C_{\text{LungC, tissue}}^{\text{GSH}} + \frac{C_{\text{LungC, tissue}}^{\text{GSH}}}{V_{\text{max, GST}} \times C_{\text{LungC, tissue}}^{\text{GSH}}} \right).
\] (14)

The conjugation of GSH with PO is modeled by a ping-pong
mechanism:

\[
V_{\text{tissue}}^{\text{PO}} \times f_{i} \times \frac{dC_{\text{LungC, tissue}}^{\text{PO}}}{dt} = V_{\text{tissue}}^{\text{PO}} \times f_{i} \times k_{\text{D, LungC}} \times \left(C_{\text{LungC, tissue}}^{\text{GSH}} - C_{\text{LungC, tissue}}^{\text{GSH}}\right) \times \left(\frac{C_{\text{PO, tissue}}^{\text{PO}} \times C_{\text{LungC, tissue}}^{\text{GSH}}}{\left(K_{\text{m, GST}} \times C_{\text{LungC, tissue}}^{\text{GSH}} + \frac{C_{\text{LungC, tissue}}^{\text{GSH}}}{V_{\text{max, GST}} \times C_{\text{LungC, tissue}}^{\text{GSH}}} \right)}\right).
\] (15)

The exchange of PO in the alveolar compartment is governed
by the uptake of PO from the conducting zone, by the
exhalation of PO from the alveolar compartment, and by the
PO transfer from air phase to the tissue phase.

\[
V_{\text{air}} \times (1 - f_{i}) \times \frac{dC_{\text{LungA, tissue}}^{\text{PO}}}{dt} = Q_{\text{pulm}} \times (c_{\text{PO, LungC, tissue}}^{\text{PO}} - c_{\text{PO, LungA, tissue}}^{\text{PO}}) - D_{\text{f}} \times \frac{A_{\text{LungA}}}{\tau_{\text{LungA}}} \times \left(c_{\text{PO, LungA, tissue}}^{\text{PO}} - c_{\text{PO, LungA, tissue}}^{\text{PO}}\right) \times \frac{c_{\text{PO, LungA, tissue}}^{\text{PO}}}{P_{\text{lung, tissue}}}.
\] (16)

\(A_{\text{LungA}}\) denotes the surface area of the alveolar epithelium and
\(\tau_{\text{LungA}}\) the harmonic mean of the thickness of the “air-
to-plasma” alveolar tissue.

The amount of PO taken up into the alveolar tissue
is described by the PO exchanges between air phase and
tissue phase, and between tissue phase and pulmonary
blood, as well as by metabolism catalyzed by EH and GST.

\[
V_{\text{tissue}}^{\text{PO}} \times f_{i} \times \frac{dC_{\text{LungA, tissue}}^{\text{PO}}}{dt} = D_{\text{f}} \times \frac{A_{\text{LungA}}}{\tau_{\text{LungA}}} \times \left(C_{\text{PO, LungA, tissue}}^{\text{PO}} - C_{\text{PO, LungA, tissue}}^{\text{PO}}\right) - D_{\text{f}} \times \frac{A_{\text{cap}}}{\tau_{\text{LungA}}} \times \left(C_{\text{PO, LungA, tissue}}^{\text{PO}} - C_{\text{PO, LungA, tissue}}^{\text{PO}}\right) - V_{\text{tissue}}^{\text{PO}} \times (1 - f_{i}) \times 1000 \times C_{\text{LungA, tissue}}^{\text{PO}} \times \left(C_{\text{LungC, tissue}}^{\text{GSH}} + V_{\text{max, GST}} \times C_{\text{LungC, tissue}}^{\text{GSH}}\right) \times \left(K_{\text{m, GST}} \times C_{\text{LungC, tissue}}^{\text{GSH}} + \frac{C_{\text{LungC, tissue}}^{\text{GSH}}}{V_{\text{max, GST}} \times C_{\text{LungC, tissue}}^{\text{GSH}}} \right).
\] (17)

The term describing the PO transfer from tissue phase to
the pulmonary blood includes the fluid phase diffusion
coefficient of PO \((D_{\text{f}}^{\text{PO}})\), the surface of capillary endothelium
\((A_{\text{cap}})\), and the harmonic mean of the thickness of the
tissue air-to-plasma \((\tau_{\text{LungA}})\) assuming it to be identical with
the thickness of the tissue-to-blood tissue. The cytosolic GSH
turnover in the alveolar tissue compartment is
described by zero-order production and first-order elimination.
The conjugation of GSH with PO is modeled by a ping-pong mechanism:

$$V_{\text{tissue}} \times (1-f_i) \times \frac{dGSH}{dt} = V_{\text{tissue}} \times (1-f_i) \times k_{D,\text{tissue}} \times (C_{\text{GSH, tissue}} - C_{\text{GSH, tissue}})$$

$$- \frac{V_{\text{tissue}} \times (1-f_i) \times 1000 \times V_{\text{max}}_{\text{GST}}}{K_{m,\text{GST}} \times C_{\text{PO, tissue}} \times C_{\text{GSH, tissue}}} = \frac{K_{m,\text{GST}} \times C_{\text{PO, tissue}} + C_{\text{GSH, tissue}}}{K_{m,\text{GST}} + C_{\text{GSH, tissue}}} \times \left( \frac{C_{\text{PO, tissue}} + C_{\text{GSH, tissue}}}{K_{m,\text{GST}} + C_{\text{GSH, tissue}}} \right).$$

(18)

The average pulmonary concentration of $X$ ($X = \text{PO or GSH}$) is calculated as the weighted sum of the concentrations in the conducting and alveolar zone:

$$C_{\text{Lang, tissue}} = f_c \times C_{\text{Lang, tissue}} + (1-f_i) \times C_{\text{Lang, tissue}}.$$

(19)

**Pulmonary Blood**

The mass balance equation for the compartment representing the pulmonary blood is described by three terms. The first one represents the net amount of chemical taken up per time unit from the alveolar zone, the second one describes mass changes per time unit due to the blood flow entering and leaving the compartment, and the third one gives the first-order elimination of PO from blood due to metabolism with the elimination constant $k_{\text{blood}}$:

$$V_{\text{PulmBlood}} \times \frac{dC_{\text{PO, PulmBlood}}}{dt} = D_{\text{PO}} \times A_{\text{ve}} \times \left( \frac{C_{\text{PO, Lang, tissue}}}{P_{\text{Lang, blood}}} - C_{\text{PO, PulmBlood}} \right)$$

$$+ Q_{\text{ve}} \times \left( C_{\text{PO, PulmBlood}} - C_{\text{PO, PulmBlood}} \right) - V_{\text{PulmBlood}} \times k_{\text{blood}} \times C_{\text{PO, PulmBlood}}.$$

(20)

**Arterial Blood**

The blood leaving the lung reaches the arterial blood compartment. The change per time unit of the amount of chemical in this compartment depends on the cardiac output and on the first-order elimination of PO.

$$V_{\text{ArterBlood}} \times \frac{dC_{\text{PO, ArterBlood}}}{dt} = Q_{\text{sh}} \times \left( C_{\text{PO, PulmBlood}} - C_{\text{ArterBlood}} \right) - V_{\text{ArterBlood}} \times k_{\text{blood}} \times C_{\text{PO, ArterBlood}}.$$

(21)

**Nonmetabolizing Organs and Tissues**

The change per time unit of the amount of chemical in a nonmetabolizing compartment $i$ is calculated as given below. The subscript $i$ refers to the compartments fat and muscle.

$$V_i \times \frac{dC_{\text{PO, ip}}}{dt} = Q_i \times \left( C_{\text{PO, ArterBlood}} - C_{\text{PO, th}} \right).$$

(22)

**Liver**

The intraperitoneal administration of PO is described by first-order kinetics using a rate constant $k_{\text{ip}}$. The administered dose ($D_{\text{PO}}$) is modeled to enter the liver directly. In all other cases, PO is inhaled and enters the liver by the arterial blood flow. Metabolism of PO in the liver is catalyzed by EH following Michaelis-Menten kinetics. The conjugation of PO with cytosolic GSH is described by a ping-pong mechanism among GSH, PO, and GST (according to Johanson and Filser [1993]). The conversion factor of 1000 is introduced to calculate the metabolic capacity in units of mmol/h.

The change per time unit of PO in the liver is given by:

$$V_h \times \frac{dC_{\text{PO, h}}}{dt} = Q_h \times \left( \frac{C_{\text{PO, ArterBlood}}}{P_{\text{ArterBlood}}} - C_{\text{h}} \right) + D_{\text{PO}} \times k_{\text{PO, h}} \times e^{-k_{\text{PO, h}}},$$

$$- \frac{V_h \times 1000 \times V_{\text{max}}_{\text{EH}}}{K_{m,\text{EH}} + C_{\text{h}}} \times C_{\text{PO, h}}$$

$$- \frac{V_h \times 1000 \times V_{\text{max}}_{\text{GST}}}{K_{m,\text{GST}} + C_{\text{h}}} \times C_{\text{PO, h}} \times C_{\text{GSH, h}}.$$

(23)

Cytosolic GSH turnover is described by zero-order production and first-order elimination with the rate constant $k_{D,\text{h}}$ and considering the PO conjugation with GSH:

$$V_h \times \frac{dC_{\text{GSH, h}}}{dt} = V_h \times k_{D,\text{h}} \times \left( C_{\text{GSH, h}} - C_{\text{GSH, h}} \right)$$

$$- \frac{V_h \times 1000 \times V_{\text{max}}_{\text{GST}}}{K_{m,\text{GST}} + C_{\text{h}}} \times C_{\text{GSH, h}} \times C_{\text{GSH, h}} \times \left( K_{m,\text{GST}} + C_{\text{h}} \right).$$

(24)

**Vessel-Rich Tissue Group**

PO mass exchange in this group is characterized by blood flow and PO metabolism. From closed-chamber gas uptake studies, it became evident that the metabolic clearance of PO was as about twice as high as the liver blood flow (Schmidbauer, 1997). Consequently, the PO clearance by the liver was also incorporated into this tissue group.

$$V_{\text{veg}} \times \frac{dC_{\text{PO, veg}}}{dt} = Q_{\text{veg}} \times \left( \frac{C_{\text{PO, ArterBlood}}}{P_{\text{ArterBlood}}} - C_{\text{veg}} \right)$$

$$- \frac{C_{\text{veg}} \times \left( V_h \times 1000 \times V_{\text{max}}_{\text{EH}} \right)}{K_{m,\text{EH}} + C_{\text{h}}} \times C_{\text{PO, veg}}$$

$$+ \frac{V_h \times 1000 \times V_{\text{max}}_{\text{GST}} \times C_{\text{GSH, h}}}{K_{m,\text{GST}} + C_{\text{h}}} \times \left( K_{m,\text{GST}} + C_{\text{h}} \right).$$

(25)

**Venous Blood**

The change per time unit of the amount of PO in venous blood is calculated as the sum of all amounts leaving the tissue compartments minus the amounts eliminated from venous blood minus that leaving the venous compartment by entering the pulmonary blood compartment. The subscript $i$ refers to the compartments conducting tissue zone, fat, muscle, liver, vessel-rich tissue group, and nasal tissue compartments.
In order to model the uptake efficiency data measured in the upper respiratory tract of Fischer 344 rats using constant unidirectional flows (Morris et al., 2004), the Equations 4, 7, 10, and 13 were changed to describe the nose for these special conditions.

In the air compartment of the lateral-ventral respiratory mucosa no reuptake from the olfactory air compartment occurs due to the unidirectional flow. PO can leave this compartment by flow only in one direction:

\[
V_{\text{VenBlood}} \frac{dC_{\text{PO VenBlood}}}{dt} = \sum_i Q_i \left( \frac{C_i^{\text{PO}}}{P_{i \text{Blood}}} \right) - Q_{\text{air}} \times C_{\text{PO VenBlood}} - V_{\text{VenBlood}} \times k_{\text{Blood}} \times C_{\text{PO VenBlood}}^2. \tag{26}
\]

**Changes in the Model**

In order to model the uptake efficiency data measured in the upper respiratory tract of Fischer 344 rats using constant unidirectional flows (Morris et al., 2004), the Equations 4, 7, 10, and 13 were modified since compartment volumes were changing over time:

\[
\frac{dt}{dt} \left( V_i \times C_i \right) = V_i \times \frac{d(C_i)}{dt} + C_i \times \frac{d(V_i)}{dt}. \tag{32}
\]

In addition, other physiological parameters as blood flows and ventilation were also treated as linear functions of time.

**Symbols**

- \( A_{\text{cap}} \) = surface of capillary endothelium (dm²)
- \( A_{\text{LangA}} \) = surface of alveolar epithelium (dm²)
- \( A_{\text{LangC}} \) = surface of conducting zone (dm²)
- \( A_{\text{Olf}} \) = surface of olfactory mucosa (dm²)
- \( A_{\text{RD Resp}} \) = surface of dorsal-medial respiratory mucosa (dm²)
- \( A_{\text{RV Resp}} \) = surface of lateral-ventral respiratory mucosa (dm²)
- \( C_{\text{PO Blood}} \) = concentration of PO in arterial blood (mmol/l)
- \( C_{\text{PO Cam}} \) = concentration of PO in chamber air (mmol/l)
- \( C_{\text{PO Endotrach}} \) = concentration of PO in endotracheal tube
- \( C_{\text{GSH LimOx0}} \) = initial concentration of cytosolic GSH in compartment i (mmol/l)
- \( C_{\text{PO LangA tissue}} \) = concentration of PO in alveolar zone (mmol/l)
- \( C_{\text{PO LangA air}} \) = concentration of PO in air space of alveolar zone (mmol/l)
- \( C_{\text{PO LangC tissue}} \) = concentration of PO in conducting zone (mmol/l)
- \( C_{\text{PO LangC air}} \) = concentration of PO in air space of conducting zone (mmol/l)
- \( C_{\text{X Lang tissue}} \) = average concentration of PO or GSH in whole lung (mmol/l)
- \( C_{\text{PO Off tissue}} \) = concentration of PO in olfactory mucosa (mmol/l)
- \( C_{\text{PO Off air}} \) = concentration of PO in air space over olfactory mucosa (mmol/l)
- \( C_{\text{X RNM tissue}} \) = average concentration of PO or GSH in whole RNM (mmol/l)
- \( C_{\text{PalmBlood}} \) = concentration of PO in pulmonary blood (mmol/l)
- \( C_{\text{PO RD tissue}} \) = concentration of PO in dorsal-medial respiratory mucosa (mmol/l)
- \( C_{\text{PO RD air}} \) = concentration of PO in air space over dorsal-medial respiratory mucosa (mmol/l)
- \( C_{\text{PO RV tissue}} \) = concentration of PO in lateral-ventral respiratory mucosa (mmol/l)
- \( C_{\text{PO RV air}} \) = concentration of PO in air space over lateral-ventral respiratory mucosa (mmol/l)
- \( C_{\text{PO VenBlood}} \) = concentration of PO in venous blood (mmol/l)
- \( C_{\text{Cl EH}} \) = clearance of PO elimination catalyzed by epoxide hydrolase in lung (l/h/g lung)

\( D_{\text{g PO}}, D_{\text{f PO}} \) = diffusion coefficient of PO in the gas and fluid phase (dm²/h)

\( f_{\text{DR}} \) = fraction of inhaled PO following the dorsal-medial path in the nose

For simulations, the chamber volume in Equation 1 was set to a large value (10 m³) and the PO concentration measured at the endotracheal tube (\( C_{\text{PO Endotrach}} \)) was calculated as:

\[
C_{\text{PO Endotrach}} = f_{\text{DR}} \times C_{\text{PO Off air}} + (1 - f_{\text{DR}}) \times C_{\text{PO RV air}}. \tag{31}
\]
\[ f_{\text{RD,air}} = \text{fraction of nasal air space representing the air phase located above the dorsal-medial respiratory mucosa} \]

\[ f_{\text{RV,air}} = \text{fraction of nasal air space representing the air phase located above the lateral-ventral respiratory mucosa} \]

\[ f_s = \text{fraction of the lung accounting for the conducting zone} \]

\[ k_{\text{blood}} = \text{first-order elimination rate constant of PO in blood (h\(^{-1}\))} \]

\[ k_{\text{GSH}} = \text{first-order elimination rate constant for GSH turnover in tissue compartment i (h\(^{-1}\))} \]

\[ k_{\text{IP}} = \text{first-order rate constant for the absorption of PO from the peritoneum (h\(^{-1}\))} \]

\[ K_{\text{m,PO}} = \text{apparent Michaelis constant of PO hydrolysis in tissue compartment i (mmol/l)} \]

\[ K_{\text{m,GSH}} = \text{apparent Michaelis constant of GSH with GST in tissue compartment i (mmol/l)} \]

\[ Met = \text{metabolic elimination} \]

\[ N = \text{number of rats exposed simultaneously in the closed chamber} \]

\[ P_{\text{PO, blood}} = \text{tissue:blood partition coefficient of PO for a tissue compartment i} \]

\[ P_{\text{PO, air}} = \text{tissue:air partition coefficient of PO for a tissue compartment i} \]

\[ Q_i = \text{blood flow through a tissue compartment i (l/h)} \]

\[ Q_{\text{pulm}} = \text{pulmonary minute volume (l/h)} \]

\[ Q_{\text{car}} = \text{cardiac output (l/h)} \]

\[ \tau_{\text{LangA}} = \text{harmonic mean of the thickness of the tissue “air-to-plasma” (dm)} \]

\[ \tau_{\text{LangC}} = \text{thickness of the epithelium layer in the conducting zone (dm)} \]

\[ \tau_{\text{Off}} = \text{tissue thickness of olfactory mucosa (dm)} \]

\[ \tau_{\text{Resp}} = \text{tissue thickness of respiratory mucosa (dm)} \]

\[ V_{\text{Ch}} = \text{volume of closed chamber (l)} \]

\[ V_i = \text{volume of a compartment i (l)} \]

\[ V_{\text{max} \text{GSH}} = \text{maximum metabolic elimination rate of PO in tissue compartment i catalyzed by epoxide hydrolase (mmol/h/g tissue)} \]

\[ V_{\text{max} \text{GST}} = \text{maximum metabolic elimination rate of PO in tissue compartment i catalyzed by glutathione S-transferase (mmol/h/g tissue)} \]

**References**


