Styrene-7,8-Oxide Burden in Ventilated, Perfused Lungs of Mice and Rats Exposed to Vaporous Styrene

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Received July 1, 2005; accepted November 12, 2005

INTRODUCTION

Styrene (ST) is an important industrial chemical. In long-term inhalation studies, ST-induced lung tumors in mice but not in rats. To test the hypothesis that the lung burden by the reactive metabolite styrene-7,8-oxide (SO) would be most relevant for the species-specific tumorigenicity, we investigated the SO burden in isolated lungs of male Sprague-Dawley rats and in-situ prepared lungs of male B6C3F1 mice ventilated with air containing vaporous ST and perfused with a modified Krebs-Henseleit buffer (37°C). Styrene vapor concentrations were determined in air samples collected in the immediate vicinity of the trachea. They were almost constant during each experiment. Styrene exposures ranged from 50 to 980 ppm (rats) and from 40 to 410 ppm (mice). SO was quantified from the effluent perfusate. Lungs of both species metabolized ST to SO. After a mathematical translation of the ex-vivo data to ventilation and perfusion conditions as they are occurring in vivo, a species comparison was carried out. At ST concentrations of up to 410 ppm, mean SO levels in mouse lungs ranged up to 0.45 nmol/g lung, about 2 times higher than in rat lungs at equal conditions of ST exposure. We conclude that the species difference in the SO lung burden is too small to consider the genotoxicity of SO as sufficient for explaining the fact that only mice developed lung tumors when exposed to ST. Another cause is considered as driving force for lung tumor development in the mouse.

Key Words: styrene; styrene-7,8-oxide; mouse; rat; ventilated, perfused lung; inhalation; mode of action; glutathione.

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lungs of Kessler et al. (1990). The limit of detection was about 30 nmol/l perfusat at an injection volume of 25 μl and a signal-to-noise ratio of 3:1.

The GC/MSD determination by capillary gas chromatography was done according to the method of Bitzenhofer (1993) on a gas chromatograph HP 5890 series II equipped with a mass selective detector (HP 5970; both Agilent, Waldbronn, Germany. 1 μl of the concentrated n-hexane extract was manually injected onto a “fused silica” pre-column (length 10 m, ID 0.53 mm; from Agilent, Waldbronn, Germany) using the “Gerstel-KaltAufgabeSystem KAS 3” from Gerstel, Mülheim an der Ruhr, Germany. The pre-column was connected to the separation column (HP-1 MS, film 0.33 μm, length 25 m, ID 0.2 mm; from Agilent, Waldbronn, Germany) by a “Miccon” press fit connector. Helium (0.8 ml/min) was used as carrier gas. Column temperature was maintained at 35°C for 0.25 min during the injection process. Then, it was heated up to 220°C with a rate of 30°C/min. The temperature of the transfer line to the MSD was 250°C. The electron ionization potential of the MSD was at 70 eV. Retention times were 7.7 min (SO) and 8.1 min (PPO). Both substances were detected in the single ion-monitoring mode at m/z 89 and 119 (SO) and 89 and 133 (PPO) and were quantified using the m/z 89 according to Langvardt and Nolan (1991). The detection limit for SO was about 15 nmol/l perfusat at an injection volume of 1 μl and a signal-to-noise ratio of 3:1.

Calibration curves (signal area of SO to signal area of PPO versus the concentration of SO) were constructed over a concentration range from 0.1 μmol/l to 10 μmol/l using five different SO concentrations. Analysis of linear regression through the origin revealed correlation coefficients of at least 0.999 (both GC/FID and GC/MS) between peak areas and SO concentrations. At each ST perfusion experiment, a three-point calibration curve through the origin was constructed.

Animals and Surgery

Male Spague-Dawley rats and male B6C3F1 mice were obtained from Charles River Wiga GmbH, Sulzfeld, Germany. All experimental procedures with animals were performed in conformity with the “Guide for the Care and Use of Laboratory Animals” (7th edition, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press Washington, D.C., 1996) under the surveillance of the authorized representative for animal welfare of GSF. Up to 4 weeks before use, two rats or five mice each were housed in the GSF-Institute of Toxicology in a Macrolon type III cage placed in an IVC top-flow system (Tecniplast, Buguggiate, Italy). This system provided the animals with filtered room air. A constant 12-h light/dark cycle was maintained in the chamber room. Animals had free access to standard chow (Nr. 1324 from Altromin; Lage, Germany) and tap water. When using the animals, body weights were in the range of 230–360 g (rats) and 25–34 g (mice).

Lungs were prepared as described in Uhlig and Wollin (1994) for rats and in von Bethmann et al. (1998) for mice with the exceptions that different narcotics were used instead of pentobarbital sodium. To rats, a mixture of 0.8 ml/kg ketamine and 1 ml/kg Rompun was administered. Per kg body weight, mice received a mixture of 1.4 ml ketamine, 0.4 ml Rompun, and 6.3 ml of a 0.9% saline. Immediately before intraperitoneal injection of this mixture, animals were anesthetized by isoflurane inhalation.

In summary, animals were intubated and ventilated by positive pressure (rats: 80 breath/min, tidal volume approximately 2 ml; mice: 90 breath/min, tidal volume approximately 200 μl) using the ventilation–perfusion systems for rat and mouse lungs “Isolierte perfundierte Lunge Größe 2” (rat) and “Isolierte perfundierte Lunge Größe 1” (mouse) manufactured by Hugo Sachs Elektronik (HSE), March-Hugstetten, Germany. After laparotomy, they received intracardial injections of Liquemin® N25000 (0.1 ml per rat and 0.05 ml per mouse). Thereafter, the diaphragms were removed and the animals were exsanguinated. A ligature was placed around both the pulmonary artery and the aorta. The cannula for the influent perfusate was inserted into the pulmonary artery and fixed by the ligature thereby closing the aorta. Immediately, the apex cordis was opened by a cut. Then the cannula for the effluent perfusate was inserted into the left ventricle and also fixed by a ligature. The rat lung together with the heart were carefully isolated and suspended by the trachea in the humidified and
to perfusate of SO. SO lung burdens were calculated by means of the partition coefficient lung-extrapolated to the species-specific effluent perfusate. Thereafter, the perfusion and ventilation parameters were vapors of defined concentrations in the inhaled air, and SO was measured in the The once-through perfused lungs were ventilated by negative pressure with ST vapors. For safety reasons, all exposure experiments were carried out under the hood. The once-through perfused lungs were ventilated by negative pressure with ST vapors of defined concentrations in the inhaled air, and SO was measured in the effluent perfusate. Thereafter, the perfusion and ventilation parameters were extrapolated to the species-specific in vivo condition, and the corresponding SO lung burdens were calculated by means of the partition coefficient lung-to-perfusate of SO.

**Experimental Procedure**

For ventilation–perfusion of rat and mouse lungs. Both above-described exposure systems from HSE, described in Uhlig and Wollin (1994) and Uhlig and Heiny (1995) for the rat as well as in von Bethmann et al. (1998) for the mouse, were modified to enable inhalation exposure to a gas of constant concentration.

**Modifications of the rat system for inhalation exposure.** Figure 1 presents a scheme of the mechanical parts of the isolated perfused lung system for rats including the following modifications: A T-formed hollow tube (T-tube) made in-house out of brass was cold-welded to the upper end of the pneumotachograph tube and connected via a Tygon® tubing to the ST-vapor–transporting glass pipe. A constant flow of ST vapor from the “ST-stock desiccator” through the glass pipe and the horizontal duct of the T-tube was established (flow of ST vapor containing air: 15 ml/min) by means of the roller pump I that contained Tygon tubing. The lung took its breathing air from the T-tube via the pneumotachograph tube. In order to enable the determination of ST vapor concentrations immediately in front of the trachea, a custom-made “Kel-F” hub needle (Hamilton, Bonaduz, Switzerland) was directed through a small pipe in the lid of the artificial thorax into a lateral outlet of the trachea attachment and affixed gas-tight by polytetrafluoroethylene (PTFE; Teflon) and silicone tubing. The upper end of this cannula possession a Teflon luer lock usually closed by a plug (item 5, Fig. 1). For measuring ST concentrations in inhaled air, the plug was exchanged by a Hamilton syringe (100 μl series 1710 TLL, obtained from Machery-Nagel, Düren, Germany), and a gas sample of 50 μl was collected and immediately injected on the column of the GC-8A.

**Modifications of the mouse system for inhalation exposure.** Figure 2 presents a scheme of the mechanical parts of the isolated perfused lung system for mice, including the modifications enabling inhalation exposure. The original system is equipped with a moistener for inhaled air (item 11, Fig. 2). This vial (volume about 2 cm³), which is covered by a PTFE plug, contains two openings, one to the external air and the other to the pneumotachograph (item 8, Fig. 2). After drilling a hole through the PTFE plug, a small PTFE hose was inserted tightly. The ST vapor was transferred from the ST-stock desiccator by the roller pump I (11.3 ml/min) into the air space of the moistener. The ventilated lung inhaled the ST vapor from the air mixture passing through the modified moistener. To determine the actual inhaled ST concentration, the 51-mm-long needle of a Hamilton syringe (100 μl series 1710 TLL) was inserted into the trachea through the opening of the moistener to the room air (item 5, Fig. 2), and an air sample of 50 μl was collected and immediately injected on the column of the GC-8A.

**Exposure conditions.** Rat lungs (n = 13) were ventilated in the negative chamber pressure mode as described in Uhlig and Wollin (1994) and in Uhlig and Heiny (1995), with the exception that inspired air was neither moistened nor warmed above room temperature. Instead of applying the recirculating buffer method of Uhlig and Wollin (1994) the less elaborate once-through perfusion technique was used. Inhaled air contained mean ST concentrations between 49 and 984 ppm (see Table 2). The exposures lasted between 32 and 43 min. At each exposure start, background SO in blood was measured, but it was in no case detectable. The experimental conditions (mean ± SD) were as follows: perfusion flow 18 ± 1.4 ml/min (manually adjusted); tidal volume 2.2 ± 1.3 ml; breaths/min 80 (fixed) with a deep breath at every 5 min to prevent atelectasis (see Uhlig and Wollin 1994); pulmonary resistance (the flow resistive forces in the airways) 0.46 ± 0.14 cm H₂O s/ml; dynamic compliance (an index of the functional stiffness of the lung) 0.20 ± 1.3 ml/cm H₂O.

In all mouse experiments (n = 12), lungs were ventilated in the negative chamber pressure mode and were perfused as described in von Bethmann et al. (1998), with the exceptions that inhaled air was neither moistened nor warmed.
above room temperature. This air contained average ST concentrations between 39 and 407 ppm (see Table 3). The exposures lasted between 55 and 68 min. At each exposure start, background SO in blood was measured, but it was in no case detectable. The experimental conditions (mean ± SD) were: breaths/min: 90 (fixed) with a deep breath at every 5 min, tidal volume 210 ± 45 ml, and perfusion flow 1.0 ± 0.24 ml/min. The dynamic compliance was 0.027 ± 0.005 ml/cm H₂O.

The values of these parameters were controlled and monitored by a computer running the software (“Pulmodyn W” from HSE) of the ventilation–perfusion system that possessed the “PLUGSYS” unit (from HSE) to amplify the raw data.

To test for the integrity of the alveolar membranes, lactate dehydrogenase (LDH) release into the perfusate was measured using the test kit “LDH IFCC-Methode 37/C176/C” (Rolf Greiner BioChemica, Flacht, Germany). Mean activity (±S.D.) in the influent perfusates (background controls) was 7.1 ± 4.3 U/l (n = 25). In the effluent perfusates it was 6.7 ± 4.3 U/l (n = 93). The maximum values in the influent and effluent perfusate were identical: 17.6 U/l. Consequently, the lungs were considered to be physiologically and biochemically intact.

**Partition coefficient lung-to-perfusate of SO (P_{L/P}).** The thermodynamic partition coefficient P_{L/P} depends on the temperature and on physicochemical properties of SO, lung tissue, and perfusate. For tissue and perfusate, water and lipid contents are most relevant. P_{L/P} had to be determined (at 37°C) because it enabled calculation of the actual SO concentration in the cellular tissue (C_{LSOc}) of the ST-inhaling lung at steady state from measurements of SO in the vasculature (the effluent perfusate; C_{PSO}). Under this condition, C_{LSOc} is in thermodynamic equilibrium with the perfusate and can therefore be calculated by the relationship:

\[ C_{LSOc} \approx P_{L/P} C_{PSO}. \]  

About 30 min before preparation of the rat lungs for determination of P_{L/P}, the animals were administered intraperitoneally diethyl maleate (0.6 ml/kg body weight i.p.), a glutathione-depleting compound (see Plummer et al., 1981). Thereafter, rats were narcotized and the lungs were positively ventilated and perfused *in situ* (see Animals and Surgery). Perfusion was carried out with the above-described perfusion medium for 10 min with a flow of 20 ml/min in order to remove blood completely from the lung. Because only one ventilation-and-perfusion system was available and three rat lungs were required for one experiment, the lungs had to be stored before use. Therefore, they were flash frozen in liquid nitrogen and stored in a freezer at −80°C.

After cutting the freshly thawed lungs into halves, each half was placed in a glass beaker (10 ml) together with enough physiological saline to cover it completely. Then, the beakers were placed for 5 min in a boiling water bath to denature the SO-metabolizing enzymes. Thereafter, lung-halves were taken from the beakers, carefully dabbed with a tissue, weighed, and cut in small slices. Each sliced lung-half was placed in a glass culture tube (7 ml; with a PTFE covered screw cab; K&K Laborbedarf, München, Germany) to which 1 ml of perfusion medium was added to a defined SO concentration (1 or 10 μmol/l). The tubes were sealed and immediately incubated in a shaking water bath (37°C). Parallel SO incubations were carried out in perfusion medium alone to determine loss of SO by non-enzymic hydrolysis. Aliquots of 0.5 ml perfusion medium (one vial containing one lung-half represented one time point) were taken at selected time points, PPO (5 μmol/l) was added and the SO content in the liquid phase was determined by GC/MSD as described above.

From the concentration-time courses of SO obtained in the incubations with or without lung, the P_{L/P} was calculated by means of a two-compartment model describing distribution between compartment one (medium) and compartment two (lung) and elimination from compartment one by hydrolysis. The partition coefficient was calculated using the following equation:

\[ P_{L/P} = \frac{k_{1} V_{1}}{k_{2} V_{2}}; \]
The elimination rate $k_{el}$ was determined from SO incubations with perfusion 

these constants, together with $k_{el}$ (the elimination rate constant describing the 

Partition Coefficient Lung-to-Perfusate of SO ($P_{LP}$) 

The elimination rate $k_d$ was determined from SO incubations with perfusion 

for Macintosh (GraphPad Software, San Diego, CA, USA).

Results

Partition Coefficient Lung-to-Perfusate of SO ($P_{LP}$) 

Partition coefficient lung-to-perfusate ($P_{LP}$) was calculated 

where 

$P_{LP} = \frac{C_{SOLV}}{C_{SOP}} \left( \frac{Q_{av}}{Q_{av}}\right) \left( \frac{Q_{Lev}}{Q_{Liv}} \right)$, 

$C_{SOLV}$ = SO concentration in lung *in vivo* [nmol/g]; 

$C_{SOP}$ = SO concentration in effluent perfusate (ex vivo) [nmol/ml]; 

$Q_{av}$ = alveolar ventilation *in vivo* (mouse: 25; rat: 117 [ml/min])*; 

$Q_{Lev}$ = lung perfusion *ex vivo* [ml/min]; 

$Q_{Liv}$ = lung perfusion *in vivo* (mouse: 17; rat: 83 [ml/min])**; 

* = determined at 37°C; 

** = from Arms and Travis (1988); 

The number "2/3" represents the conversion factor from pulmonary to 

alveolar ventilation (e.g., Arms and Travis (1988)).
Figures 4 and 5 depict concentration–time courses that are characteristic for the inhalation studies with ventilated, perfused lungs of rats (Fig. 4) and mice (Fig. 5). Atmospheric ST concentrations in the air entering the lungs were maintained nearly constant during the exposure time (Figs. 4A and 5A). Concentrations of metabolically produced SO in the effluent perfusate remained nearly constant from about 5 min after starting ST exposures until the end of the experiments (Figs. 4B and 5B). In both figures, the straight lines represent the mean concentrations of the measured data from about 5 min after starting the respective exposure to its end.

The experimentally determined mean SO concentrations in effluent perfusate (Tables 2 and 3) were mathematically translated from the experimental to the species-specific in vivo conditions of lung perfusion and ventilation (see Eq. 6). These translated (“measured”) lung levels of SO are depicted in Figure 6 (both species) versus the corresponding ST concentrations in inhaled air. Over the whole demonstrated concentration range of ST, the “measured” pulmonary SO burden is about 2 times lower in rat than in mouse. At ST concentrations of 100 ppm and above, the SO levels in rat lungs are higher than in mouse lungs at 40 ppm. Additionally, the SO levels in rat lungs at 350 ppm ST and above are similar to those in mouse lungs at 160 ppm ST.

DISCUSSION

The pulmonary SO levels resulting solely from ST metabolism in the lung (Fig. 6), are at least 5 times (mice) and 10 times smaller than the SO blood levels that were reached during inhalation exposure of rats and mice to equal ST concentrations of up to 250 ppm. From the blood data in rats and mice measured (Kessler et al., 1992; shown also in IARC, 1994) and using the partition coefficient lung/blood of SO (1.9; Csánády et al., 2003), a maximum pulmonary SO level of about 2.4 nmol/g can be extrapolated for a steady-state exposure of rats or mice to 160 ppm ST by neglecting production as well as metabolism of SO in the lung. Interestingly, this value is not far from the pulmonary SO levels of about 1.5 and 2.5 nmol/g predicted for both species by a physiological toxicokinetic model that contained pulmonary ST metabolism to SO and metabolic SO elimination in the lung (Csánády et al., 2003). Considering the low SO burdens obtained in the ventilated, perfused lungs, it has to be concluded that pulmonary ST metabolism is only of minor relevance with respect to the SO lung burden in vivo. For in vivo exposure, it follows that the

### TABLE 1

Values of the Parameters Describing the Concentration-Time Courses of SO Plotted in Figure 3 (pH 7.4; 37°C; perfusate volume $V_1 = 1$ ml) and Hereof Calculated $P_{LP}$ Values

<table>
<thead>
<tr>
<th>Number of experiment</th>
<th>$C_0$ [µmol/l]</th>
<th>$k_{el}$ [min$^{-1}$]</th>
<th>Number of experiment</th>
<th>$C_0$ [µmol/l]</th>
<th>$k_{el}$ [min$^{-1}$]</th>
<th>Mean $k_{el} \pm SD$ [min$^{-1}$]; $n = 4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10.0</td>
<td>0.0022</td>
<td>3</td>
<td>2.27</td>
<td>0.0021</td>
<td>$0.0023 \pm 0.0002$</td>
</tr>
<tr>
<td>2</td>
<td>4.83</td>
<td>0.0025</td>
<td>4</td>
<td>0.55</td>
<td>0.0025</td>
<td>$0.0002$</td>
</tr>
</tbody>
</table>

Solid curves: $C_{SO} = C_1 e^{a t} + C_2 e^{b t}$

<table>
<thead>
<tr>
<th>Number of experiment</th>
<th>$C_1$ [µmol/l]</th>
<th>$C_2$ [µmol/l]</th>
<th>$a$ [min$^{-1}$]</th>
<th>$b$ [min$^{-1}$]</th>
<th>$V_2$ [ml]</th>
<th>$P_{LP}^*$ (calculated using $k_{el}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>4.03</td>
<td>6.00</td>
<td>-0.096</td>
<td>-0.0026</td>
<td>0.51</td>
<td>1.34</td>
</tr>
<tr>
<td>6</td>
<td>0.42</td>
<td>0.59</td>
<td>-0.033</td>
<td>-0.0021</td>
<td>0.53</td>
<td>1.30</td>
</tr>
<tr>
<td>7</td>
<td>0.40</td>
<td>0.60</td>
<td>-0.188</td>
<td>-0.0019</td>
<td>0.53</td>
<td>1.24</td>
</tr>
</tbody>
</table>

Mean $P_{LP} \pm SD$ ($n = 3$): 1.29 ± 0.05.

Curves 1, 2, 3, and 4 (dashed lines with filled symbols in Figure 3) were recorded to determine the rate constant $k_{el}$ of the SO hydrolysis in perfusate. Curves 5, 6, and 7 (straight lines with open symbols in Figure 3) were obtained from measuring SO in perfusate containing heat-inactivated lung tissue probes of volumes $V_2$ (mean values of each experiment). The values of $C_0$, $C_1$, $C_2$, $k_{el}$, $a$, and $b$ were obtained by curve fitting.

For calculation of $P_{LP}$ from these values, see Equations 2–5.

SO Burden in ST Inhaling Lungs

Figures 4 and 5 depict concentration–time courses that are characteristic for the inhalation studies with ventilated, perfused lungs of rats (Fig. 4) and mice (Fig. 5). Atmospheric ST concentrations in the air entering the lungs were maintained nearly constant during the exposure time (Figs. 4A and 5A). Concentrations of metabolically produced SO in the effluent perfusate remained nearly constant from about 5 min after starting ST exposures until the end of the experiments (Figs. 4B and 5B). In both figures, the straight lines represent the mean concentrations of the measured data from about 5 min after starting the respective exposure to its end.

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pulmonary SO levels are predominantly dependent on the actual SO blood levels, and that not only blood but also lung levels by the alkylating SO are similar in both rodent species below 250 ppm ST. One might speculate that not the SO burden of the whole lung but that of the terminal bronchioles, the actual target of the pulmonary ST toxicity (e.g., Cruzan et al., 2001), should be considered as relevant. However, considering a 6-h exposure to 160 ppm ST, the model of Csanaédy et al. (2003) predicts for the bronchioles of rat lungs an SO concentration of almost 2 nmol/g (similar to the SO concentration of the whole lung) and for mice an SO concentration of almost 3 nmol/g (not much higher than in the whole lung in this species). For equal exposure conditions, the physiologically based pharmacokinetic model of Sarangapani et al. (2002) predicts for the terminal bronchioles of rats almost the same SO concentration as does the Csanaédy model, but for the terminal bronchioles of mice almost 8 nmol/g are expected. In a third recently published physiologically based pharmacokinetic model concerning ST and its metabolite SO, the physiological description of the respiratory pathways did not enable distinguishing between whole lung and transitional bronchiolar region (Cohen et al., 2002). The small species difference in the SO burden of whole lungs and bronchioles predicted by our model (Csanaédy et al., 2003) are in agreement with studies on DNA binding of metabolites of 14C-labeled ST carried out in rats and mice that were exposed by inhalation to 160 ppm ST over 6 h (Boogaard et al., 2000). In the lungs of the animals sacrificed immediately at the end of exposure, about the same extremely low N7-(hydroxyphenylethyl)guanine adduct level of 14C-labeled SO of about 1 adduct/10^8 nucleotides was quantified in both species; adduct levels in mouse Clara cells, most rich in

![FIG. 5. Concentration–time courses in three experiments with ventilated, perfused mouse lungs, each at a different ST concentration in inhaled air. Styrene concentrations in inhaled air are given in 4A (filled symbols) and corresponding, metabolically formed SO concentrations, determined in effluent perfusate, are presented in 4B (open symbols).](https://academic.oup.com/toxsci/article-abstract/90/1/39/1692231)

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Mean Concentrations of Styrene (ST) in Inhaled Air and of Styrene-7,8-Oxide (SO) in Effluent Perfusate as Well as Tidal Volumes and Perfusion Flows Measured in the Experiments with Ventilated, Perfused Rat Lungs</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST (ppm)</td>
<td>SO (µmol/l)</td>
</tr>
<tr>
<td>49 ± 3^a</td>
<td>0.063 ± 0.021^a</td>
</tr>
<tr>
<td>113 ± 10</td>
<td>0.11 ± 0.028</td>
</tr>
<tr>
<td>160 ± 16</td>
<td>0.50 ± 0.046</td>
</tr>
<tr>
<td>167 ± 14</td>
<td>0.44 ± 0.11</td>
</tr>
<tr>
<td>261 ± 40^a</td>
<td>0.61 ± 0.040^a</td>
</tr>
<tr>
<td>265 ± 49</td>
<td>0.26 ± 0.058</td>
</tr>
<tr>
<td>335 ± 52</td>
<td>0.48 ± 0.10</td>
</tr>
<tr>
<td>378 ± 48</td>
<td>0.29 ± 0.017</td>
</tr>
<tr>
<td>454 ± 32^a</td>
<td>1.10 ± 0.10^a</td>
</tr>
<tr>
<td>494 ± 67</td>
<td>0.69 ± 0.091</td>
</tr>
<tr>
<td>477 ± 52</td>
<td>1.40 ± 0.11</td>
</tr>
<tr>
<td>631 ± 85</td>
<td>0.78 ± 0.054</td>
</tr>
<tr>
<td>984 ± 308</td>
<td>2.05 ± 0.10</td>
</tr>
</tbody>
</table>

Means ± SD represent at least five concentration measurements per exposure experiment (compare Fig. 4).

^aMean values of the data presented in Figure 4.

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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>ST (ppm)</td>
<td>SO (µmol/l)</td>
</tr>
<tr>
<td>39 ± 5</td>
<td>0.26 ± 0.044</td>
</tr>
<tr>
<td>42 ± 5^a</td>
<td>0.25 ± 0.033^a</td>
</tr>
<tr>
<td>80 ± 4^a</td>
<td>0.64 ± 0.077^a</td>
</tr>
<tr>
<td>158 ± 10</td>
<td>2.66 ± 0.53</td>
</tr>
<tr>
<td>169 ± 19</td>
<td>1.00 ± 0.28</td>
</tr>
<tr>
<td>172 ± 15</td>
<td>1.23 ± 0.08</td>
</tr>
<tr>
<td>202 ± 16</td>
<td>2.42 ± 0.18</td>
</tr>
<tr>
<td>255 ± 28</td>
<td>1.27 ± 0.31</td>
</tr>
<tr>
<td>257 ± 14^a</td>
<td>2.22 ± 0.19^a</td>
</tr>
<tr>
<td>271 ± 21</td>
<td>1.30 ± 0.25</td>
</tr>
<tr>
<td>374 ± 19</td>
<td>2.66 ± 0.33</td>
</tr>
<tr>
<td>407 ± 41^a</td>
<td>3.16 ± 0.39^a</td>
</tr>
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</table>

Means ± SD represent at least 6 concentration measurements per exposure experiment (compare Fig. 5).

^aMean values of the data presented in Figure 5.
CYP450 enzymes (reviewed in Gram, 1997), were similar as in the total lung. For the whole lung of mice exposed to about 170 ppm over 6 h, a comparable guanine adduct level of 1.3 adducts/10^8 nucleotides was also reported by another group (Vodicka et al., 2001). “Covalent binding indices” calculated by Boogaard et al. (2000) in livers and lungs of the ^14^C-ST exposed rodents were also very small and similar in rats and mice. The authors concluded “that DNA adduct formation does not play an important role in styrene tumorigenicity in chronically exposed mice”.

Considering that in a long-term inhalation study (Cruzan et al., 2001) with mice exposed to concentrations of up to 160 ppm the frequency of lung tumors had increased at ST concentrations ≥ 20 ppm, and that no tumors had been found in lungs of rats exposed up to 1000 ppm ST under comparative conditions (Cruzan et al., 1998), one has to conclude that the species-specific lung tumorigenicity of ST in mice does not result directly from the lung burden by the genotoxic metabolite SO. As already concluded from the results delivered by our ST model, it is highly probable that a non-genotoxic glutathione-dependent mechanism is involved (Filser et al., 2002).

Based on modeled tissue concentrations of SO and on kinetic interactions of the glutathione turnover with the SO forming and eliminating enzyme activities that had been measured in vitro (Oberste-Frielinghaus et al., 1999), our physiological toxico kinetic model (Csanády et al., 2003) predicted a drastic glutathione decrease of 40% in the bronchiolar region of mice and only a marginal one in that of rats following a 6-h inhalation exposure to 20 ppm ST. Although model predictions for the whole lung agreed with measured data (Dhawan-Robl et al., 2000; Filser et al., 2002), it cannot be ruled out that other ST metabolites might also contribute to the glutathione depletion in mouse lungs. For instance, 4-vinylphenol, a putative ST metabolite in mice (Carlson et al., 2001) and a minor one detected in urine of rats (Bakke and Scheline, 1970; Manini et al., 2002; Pantarotto et al., 1978) and humans (Manini et al., 2002; Pfäffli et al., 1981), resulted in a certain glutathione depletion in the lungs of mice, following intraperitoneal administration (Turner et al., 2005). 4-Vinylphenol was hepatotoxic and pneumotoxic in rodents due to the formation of metabolites, probably ring-opened products (Carlson et al., 2001; Carlson 2002).

Repeated administration of mouse Clara cell toxicants as coumarin, naphthalene, or 4-ipomeanol can result in a certain tolerance to these chemicals, probably as a result of upregulation of the detoxifying glutathione (Boyd et al., 1981; Born et al., 1999; Vassallo et al., 2004; West et al., 2000). However, after intermittence of daily injections of naphthalene for 4 days, elevated glutathione levels in the terminal airways of tolerant mice had declined to control levels and mice were again susceptible to naphthalene-induced acute Clara cell toxicity. They described a broad heterogeneity of loss of cellular glutathione and other sulfhydryls in the cell populations of the minimally susceptible lobar and proximal bronchi. However, in the most susceptible distal bronchioles, high loss of glutathione was detected in all cells. The authors concluded that at least 50% of the intracellular glutathione pool must be lost before cell organelle changes become apparent and that a loss of at least 75% is required before toxic cellular changes become irreversible. The observed cytotoxic effects should result at least directly from the glutathione depletion and not from a reactive naphthalene metabolite, because Phimister et al. (2005) demonstrated that abrupt glutathione depletion in mice resulted in Clara cell toxicity similar to naphthalene treatment with respect to Clara cell swelling, plasma membrane blebs, and actin cytoskeleton disruptions.

Furthermore, it is well known that severe changes in the glutathione homeostasis can lead to apoptosis and cell proliferation (reviewed in, e.g., Rahman et al., 1999). Together with a depletion of glutathione in lung homogenate, such effects have been detected in Clara cells of mice repeatedly exposed to ST (Gamer et al., 2004). Following repeated SO or 4-vinylphenol administration to mice, cell proliferation, histomorphological changes, and apoptosis in bronchi and terminal bronchioles were observed. It was suggested that Clara cells were primary target cells (Kaufman et al., 2005).

Considering this information, we propose the following hypothesis to explain lung tumor formation in ST exposed mice: glutathione conjugation with the ST metabolite SO results in perturbation of the glutathione pathway in Clara cells of the terminal bronchioles because of insufficient glutathione turnover. This effect leads to cell death and regenerative proliferation. Together with the cell burdens by reactive ST metabolites as SO and possibly ring-oxidized derivatives, the finally observed tumorigenesis in mouse lungs becomes
comprehensible. In the rat lung, no such effects are to be expected, because ST-induced loss of glutathione is minimal in this species (see Filser et al., 2002; Csámady et al., 2003).

ACKNOWLEDGMENTS

We thank Prof. Dr. Albrecht Wendel, Biochemical Pharmacology, University of Konstanz, Germany, and Prof. Dr. Stefan Uhlig, Division of Pulmonary Pharmacology, Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Borstel, Germany, for friendly advice concerning the perfused, ventilated lung system. Financial support by the Styrene Steering Committee (SSC) of the European Chemical Industry Council (CEFIC) is gratefully acknowledged.

REFERENCES


