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Uptake of Styrene in the Upper Respiratory Tract of the CD Mouse and Sprague-Dawley Rat

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Inspired styrene is an olfactory toxicant in the mouse and rat. To provide nasal dosimetric information, upper respiratory tract (URT) uptake efficiency (UE) of styrene was measured in the surgically isolated URT of the urethane-anesthetized CD mouse and Sprague Dawley rat throughout a 45-min exposure. In the first studies, the effect of inspiratory flow rate on styrene UE was examined. At flows of 12-, 24-, or 70-ml/min average UE of 17, 9.8, and 4.1%, respectively, were observed in the mouse. For the rat, UE averaged 14, 9.1 and 5.7% at flow rates of 70, 150, and 400 ml/min, respectively. In the second study, UE was measured at inspired concentrations of 5, 10, 25, 50, 100, or 200 ppm at a flow rate of 12 ml/min in the mouse and 70 ml/min in the rat in both naïve and metyrapone (150 mg/kg sc) pretreated animals. In the rat, steady state UE decreased with increasing exposure concentration, averaging between 24 and 10% efficiency at 5 to 200 ppm ($p < 0.0001$). Metyrapone pretreatment resulted in statistically significant reductions in UE with steady-state UE averaging 10–14% at 5–200 ppm. Metyrapone pretreatment abolished the concentration dependence. In naïve mice, styrene UE did not maintain a steady state, but steadily declined during exposure. The mechanisms of the non-steady state behavior are not known, but they appear to be due to a styrene metabolite, as evidenced by the fact that steady-state UE was observed in metyrapone-pretreated mice. In the mouse, UE averaged between 42 and 10% efficiency at 5 to 200 ppm ($p < 0.0001$). Metyrapone pretreatment resulted in statistically significant reductions in UE, with steady state UE averaging 20–10% at 5–200 ppm. As in the rat, metyrapone pretreatment abolished the concentration dependence. In toto, these data provide strong evidence that inspired styrene is metabolized in nasal tissues in the rat and mouse and that a metabolic basis exists for the observed inspired concentration dependence of UE.

Key Words: **metyrapone pretreatment; uptake efficiency (UE); concentration dependence; styrene metabolism.**

Styrene is a clear pungent liquid that is used extensively in the polymer industry. Annual production of styrene in the U.S. in 1993 was estimated to be in excess of 5 million tons (Miller *et al*., 1994). The current ACGIH TLV is 20 ppm. The OSHA PEL is 100 ppm; however, the styrene industry and OSHA have adopted a voluntary standard of 50 ppm. The U.S. Environmental Protection Agency (EPA) RfC is 1 mg/m3 (0.24 ppm) and is based upon neuropsychological effects. Styrene is a sensory irritant. RD50 values in mice of 157, 586, and 980 ppm have been reported (Alarie, 1973, 1981; deCeaurriz *et al*., 1981).

Recent rodent inhalation toxicity studies have revealed that styrene is an olfactory toxicant. In both the rat and mouse, subchronic exposure leads to olfactory mucosal degeneration with the lesions being most severe in the dorsal medial portions of the nasal cavity (Cruzan *et al*., 1997, 1998). Subchronic (13-week) inhalation studies provided a NOEL of 200 ppm and a LOEL of 500 ppm for styrene-induced olfactory degeneration in the rat. In the mouse, a 13-week exposure to 50-ppm results in olfactory degeneration, suggesting the mouse olfactory mucosa is more sensitive to this vapor than is the rat′s. It is noted, however, that 1- or 2-year exposures to 50-ppm styrene result in olfactory degeneration in the rat (Cruzan *et al*., 1998). The mechanism(s) responsible for styrene-induced nasal injury or the apparent heightened sensitivity of the mouse is not known.

Styrene is a CYP450 substrate (Sumner and Fennell, 1994). Styrene oxide is the initial product of the metabolic pathway. The nose expresses high levels of CYP450 (Thornton-Manning and Dahl, 1997), suggesting that nasal tissues might be capable of metabolizing styrene. Specific data on nasal mucosal-sytrene metabolism are not currently available, however. The role of metabolism in olfactory injury is unknown (Cruzan *et al*., 1998). However, the pattern of injury—olfactory mucosal damage in the absence of marked respiratory mucosal injury—is typical of that observed for toxicants metabolically activated in olfactory tissue.

Quantitative inhalation risk-assessment for styrene requires knowledge of the nasal dosimetry of this vapor. The proposed study was designed to provide such information. Toward these ends, the effect of inspiratory flow rate on nasal styrene-uptake efficiency was examined. Such data are useful in the formulation of mathematical UE simulation models (Morris *et al*., 1993; Frederick *et al*., 1998; Plowchalk *et al*., 1997; U.S. EPA, 1984). In addition, styrene UE was measured over a wide range of inspired concentrations (5–200 ppm) in normal animals and

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in animals pretreated with the CYP450 inhibitor, metyrapone. These studies were performed to provide insights into the metabolism of inspired styrene in nasal tissues over a range of inspired concentration. Our previous studies have shown that this inhibitor reduces nasal UE of two structurally similar CYP450 substrate vapors—xylene and bromobenzene (Morris, 1993). All studies were performed in both the Sprague-Dawley rat and CD-1 mouse to provide comparative information between the two species, which have been most recently utilized in styrene inhalation toxicity testing.

MATERIALS AND METHODS

General design. This study contained 2 phases. In the first phase, the effect of inspiratory flow rate on upper respiratory tract (URT) UE was examined. The URT is defined as all regions of the respiratory tract anterior to and including the larynx. These studies were performed at flow rates equivalent to 50, 100, and 275% of the predicted minute ventilation of each species. These flows have been used in our previous studies (Morris, 1997). Only one exposure concentration was used—50 ppm, corresponding to the voluntary occupational standard for styrene. In the second-phase studies, UE efficiency over a wide range of concentrations was examined (5, 10, 25, 50, 100, or 200 ppm). In addition, the effect of the CYP inhibitor, metyrapone, on UE was examined at each of these concentrations. Only one flow rate was used for these studies. Because only moderate UE were anticipated, a flow rate of 50% of the minute ventilation was selected to maximize the observed values. Data from animals in the low-flow rate group of the first phase studies were included in statistical analysis of the second phase to enhance statistical power. Metyrapone was administered sc at a dose of 150 mg/kg (25 mg/ml in distilled water) 30 min prior to UE measurement. The dosing regimen was used in our previous studies on URT UE of CYP450 substrate vapors (Morris, 1993).

Animals and reagents. Specific pathogen-free male Sprague-Dawley rats (Crl:CDBR, 125–150 g on arrival) or CD-1 mice (Crl:CD-1 (ICR) BR, 19–21 g on arrival) were obtained from Charles River laboratories (Wilmington, MA). Animals were housed over hardwood bedding in filter-top cages in animal rooms maintained at 22–25°C with a 12-h light-dark cycle (lights on 6:30 AM). Animals were acclimated for 1 week prior to use and were used within 3 weeks of arrival. At the time of use, body weights averaged approximately 30 g and 220 g for mice and rats, respectively. Styrene (99% pure) and metyrapone were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were obtained from local suppliers and were the highest purity available.

Exposure protocols. For exposure the URT was isolated by the method used previously in this laboratory (Morris, 1990). All procedures were performed after the onset of urethane anesthesia (1.3 g/kg ip). The URT was isolated by insertion of a polyethylene tube in the trachea in an anterior direction such that its tip lay at the larynx. The animal was then placed in a nose-only chamber and chamber air was drawn through the isolated URT for 45 min under the flow conditions described below. Immediately after the end of exposure, each animal was killed by exsanguination.

UE measurement. To measure URT UE, vapor concentration was measured in chamber air (i.e., air entering the URT, Cin) and in air that had been drawn through the URT (Cex, exiting air concentration). UE was calculated from the difference between these two concentrations by the formula (Cin-Cex)/Cin and expressed as a percent. Exiting air concentrations were measured every 3 min during the 45-min exposure. Values obtained between 15 and 45 min were used to assess UE. In our previous studies, steady UE values were not observed until 15 min of exposure; therefore values obtained at earlier exposure times were not used to estimate UE (Morris, 1999). It is recognized that this is an arbitrary decision, but the results are not altered if the data from 9–45 or 12–45 min are utilized.

Measurements were made under unidirectional inspiratory flow conditions at flow rates of 70, 150, or 400 ml/min in the rat and 12, 24, or 70 ml/min in the mouse. These flows have been used previously in our laboratory (Morris, 1997) and correspond to approximately 50, 100, and 275% of the predicted minute ventilation of each species (Guyton, 1947).

For each animal, Cin was measured immediately before and again immediately after Cex determination, using the same flow conditions as used for Cex determination. The average value of these chamber concentrations was used to calculate UE. The ratio of the "before" and "after" chamber concentrations provides an indirect index of the steadiness of the chamber vapor concentrations during the exposure. This ratio averaged 98.0 ± 6.6% (mean ± SD) for the mouse studies and 99.4 ± 6.5% (mean ± SD) for the rat studies. Since the before and after samples were separated by 45 min, these ratios suggest that chamber air levels changed by 0.05% or less per min during the exposure (assuming a linear change during the 45-min exposure period).

Air sampling and analysis. A schematic of the air-sampling system is provided in Morris, 1999. The sampling system used for drawing air samples consisted of stainless-steel tubing with a stainless-steel T. Polyethylene tubing was used to connect the sampling system to either the chamber sampling port (inspired air analysis) or the animal endotracheal tube (exiting air analysis). Air was drawn off one arm of the T and through an 8-port 2-loop (0.5 ml) gas sampling valve (connected to a gas chromatograph) via heated tubing at a flow rate of 7 ml/min. The other arm of the sample line T was connected to a vacuum source to control total airflow rates at the desired level (e.g., 12, 24, or 70 ml/min in the mouse). Airflow rates were controlled with rotameters, which were calibrated in the sample line with a bubble meter. Air samples were injected into the chromatograph from the gas-sampling valve every 3.0 min, to provide continuous sampling.

Airborne styrene concentrations were measured in a Varian model 3600 gas chromatograph equipped with a flame ionization detector. A 15-m DB-Wax megabore column (J&W Scientific, Folsom, CA) was used with a column oven temperature of 100°C and a carrier gas (N2) flow rate of 30 ml/min. Styrene peaks eluted 0.45 min after injection onto the column. Peak heights (Varian model 4290 integrator) were converted to concentrations based on a standard curve that was prepared for each vapor. For this purpose, 4 μl aliquots of styrene standard (dissolved in methanol) were injected into teflon gas sampling bags (Cole-Parmer, Niles, IL), which were then filled with 0.8 l of clean air. After at least 1 h to allow for evaporation, air was drawn from the bags through the sample train used for UE measurement and into the GC gas-sampling valve for analysis.

Chamber conditions and vapor generation. Total airflow rates through the 1.2-l stainless steel Jaeger-NYU directed flow, nose-only inhalation chamber (CH Technologies, Westwood NJ) were maintained at 10 l/min with clean filtered, heated, humidified air. Chamber air temperature averaged 40°C and airborne water content exceeded 30 mg/l, corresponding to greater than 70% relative humidity at 37°C. Chamber walls, inlet tubing, and sample tubing were heated to prevent condensation. A hot-air gun was used to warm the animal and also to minimize condensation in the endotracheal tubing.

Chamber atmospheres were generated with a syringe pump system (Model 355, Sage Instruments). Pure styrene was fed into a J tube maintained at 80°C. Air (0.6 l/min) was passed through the tubing and into the chamber diluting airline. Chamber concentrations were controlled by changing the styrene delivery rate. The chamber was operated for at least 45 min prior to measurement of deposition to allow for equilibration.

Nominal styrene concentrations for these studies were 5, 10, 25, 50, 100, and 200 ppm. The measured concentrations were (mean ± SD) 5.8 ± 0.9, 10.9 ± 0.9, 25.5 ± 2.3, 52.0 ± 5.1, 104 ± 10, and 202 ± 25 ppm.

Mathematical analysis. All data are presented as mean ± SD unless otherwise indicated. Linear relationships were assessed and compared by linear regression analysis. Groups of data were analyzed by *t*-test, single-, or multifactorial analysis of variance followed by Newman-Keuls test. Statistical tests were performed with Statistica software (Statsoft, Inc., Tulsa OK).

TABLE 1
Effect of Inspiratory Flow Rate on Upper Respiratory Tract Styrene Uptake Efficiency in the Mouse and Rat

	Inspiratory flow rate		
	Low	Mid	High
Mouse	17.2 ± 4.5[a] (5)	9.8 ± 1.9[b] (5)	4.1 ± 4.2[c] (5)
Rat	14.1 ± 4.8[a] (5)	9.1 ± 4.0[b] (5)	5.7 ± 0.7[c] (5)

Note. Average uptake efficiency between 15 and 45 min is expressed as percent. Numbers in parentheses refer to the numbers of animals (*n*). Data are reported as mean ± SD. The low-, mid-, and high-flow rates correspond to approximately 50, 100, and 275% of the predicted minute ventilation for each species. The actual flows for the rats and mice were 70, 150, and 400 ml/min and 12, 24, and 70 ml/min, respectively. Data were analyzed by 2-factor ANOVA, which detected a significant effect of flow rate ($p = 0.00005$), no difference between species ($p = 0.60$) and no interaction between flow rate and species ($p = 0.34$). Each group was then compared by the Newman-Keuls test; groups with differing superscripts differ significantly ($p < 0.05$) from each other.

RESULTS

Flow Rate Study

The measured exposure concentration was 54 ± 6.5 ppm. UE tended to decline somewhat during exposure in the rat and mouse, with the rate of decline being greater in the latter species. Linear regression analysis was performed on the relationship between UE and exposure time for each animal to calculate the slope, i.e., the rate of change in UE during 15 and 45 min of exposure. A slope of zero would indicate the maintenance of a steady state. In the mouse, the average slope values were negative at all flow rates; however, for no flow rate were the average values significantly different from zero ($p > 0.05$, *t*-test). Among all mice (regardless of flow), the values averaged −0.116 ± 0.23%/min ($n = 15$). In the rats, both negative and positive slopes were observed, the overall average being −0.03 ± 0.23%/min ($n = 15$), a value not different from zero ($p > 0.05$, *t*-test).

An average UE efficiency was calculated for each animal by averaging the UE efficiencies obtained between 15 and 45 min of exposure. These values were then compared by 2-factor ANOVA with the factors being flow rate and species (Table 1). A significant effect of flow rate was observed, no difference between species was detected, and no interaction between flow rate and species was detected. Newman-Keuls test revealed that UE efficiency was significantly different among all three flow rates, with UE efficiency decreasing as flow rate increased.

Concentration Dependence Study

In this study, UE was measured in naïve (not pretreated) or metyrapone pretreated rats or mice at inspired concentrations of 5, 10, 25, 50, 100, or 200 ppm.

Mice. Shown in Figure 1 is the average UE during the exposure in naïve and metyrapone-pretreated mice at an inspired concentration of 5 ppm. UE efficiency steadily diminished during 15 to 45 min of exposure in the naïve mice but remained steady in the metyrapone-pretreated mice. This pattern was observed at all exposure concentrations.

As in the flow rate study, linear regression analysis was performed on the UE-versus-time relationship for each animal, and the resulting slope values for the animals in each group were averaged to statistically evaluate steady state UE behavior. The average slope values in every naïve exposure group were negative, ranging between −0.44 and −0.10%/min, depending on the concentration. In contrast, in the naïve mice, average slope values were both negative and positive and ranged between −0.02 and +0.09, except for the 200-ppm group in which the slope averaged −0.175 ± 0.16 ($n = 5$). This latter value was not statistically different from zero.

Slope data among groups were compared by 2-factor ANOVA with the factors being concentration and metyrapone pretreatment. This analysis detected a significant effect of metyrapone ($p = 0.002$), no effect of styrene concentration ($p = 0.31$), and no interaction between styrene and metyrapone ($p = 0.34$). Since a significant effect of concentration was not detected, data from all groups were averaged. In the 32 pretreated mice, the slopes averaged 0.014 ± 0.212% per min, a value not different from zero. In the 35 naïve mice, the slopes averaged 0.224 ± 0.272% per min, a value significantly different from zero ($p = 0.0005$, *t*-test). In toto, these results indicate that steady state UE of styrene was maintained from 15 to 45 min of exposure in the metyrapone-pretreated, but not in the naïve, mice.

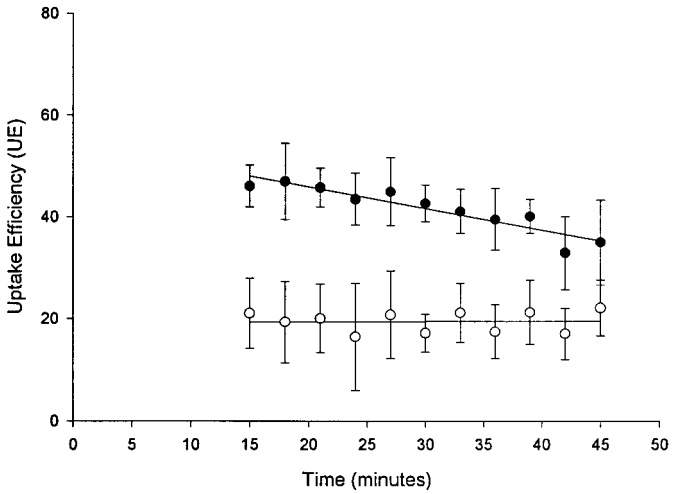

FIG. 1. Shown is the average uptake efficiency (expressed as a percent) obtained at each time point of the naïve (closed circles) and metyrapone-pretreated (open circles) mice. The exposure concentration averaged 6.3 ± 0.8 (mean ± SD) among the 6 naïve and 6 metyrapone-pretreated mice. Data are presented as mea ± SD. The linear regression lines (uptake efficiency versus time) are shown.

TABLE 2
Upper Respiratory Tract Styrene Uptake Efficiency in the Mouse

	Inspired styrene concentration (ppm)					
	5	10	25	50	100	200
Naïve	41.7 ± 4.0[a] (6)	29.7 ± 4.4[b] (6)	23.7 ± 8.3[b,c] (6)	17.8 ± 4.4[c] (6)	12.5 ± 7.3[d] (5)	9.6 ± 6.6[d] (6)
Metyrapone	19.4 ± 5.6[c,d] (6)	18.8 ± 2.7[c,d] (6)	16.7 ± 5.8[c,d] (5)	16.1 ± 4.8[c,d] (5)	9.8 ± 4.5[d] (5)	13.2 ± 4.3[d] (5)

Note. Average uptake efficiency between 15 and 45 min expressed as percent. Numbers in parentheses refer to numbers of animals (*n*). Data are reported as mean ± SD; naïve animals received no pretreatment, metyrapone animals received 150 mg/kg metyrapone, sc, 30–60 prior to exposure. Data were analyzed by 2-factor ANOVA, which detected a significant effect of metyrapone ($p = 0.00005$), of styrene concentration ($p = 0.00001$) and an interaction between metyrapone and styrene concentration ($p = 0.000002$). Each group was then compared by a Newman-Keuls test; groups with differing superscripts differ significantly ($p < 0.05$) from each other. Measured exposure concentrations for the mouse studies were: 6.3 ± 0.9, 10.5 ± 1.0, 23.8 ± 2.1, 51.6 ± 2.5, 106 ± 11, and 205 ± 30 ppm (mean ± SD).

The average UE between 15 and 45 min of exposure are shown in Table 2. These values were compared among the groups. It should be noted, however, that since the naïve mice did not maintain steady state, the precise relationships among groups are time-dependent. For this reason only generalized conclusions are drawn. Data were compared by 2-factor ANOVA with the factors being styrene concentration and metyrapone pretreatment. A significant effect of exposure concentration, metyrapone pretreatment, and a significant interaction were detected. The interaction demonstrates that metyrapone had statistically differing effects at the different exposure concentrations. Groups were then compared by Newman-Keuls test. In the naïve animals, UE efficiencies at exposure concentrations of 5, 10, 25, or 50 ppm were significantly higher than at 100 or 200 ppm. In contrast, UE were similar at all exposure concentrations in the metyrapone-pretreated animals. Among the 32 metyrapone-pretreated mice, UE averaged 15.9 ± 5.5%. Thus, metyrapone pretreatment abolished the inspired concentration dependence of UE. The UE in all metyrapone-pretreated animal groups were similar to those observed at the high concentrations (100 or 200 ppm) in the naïve animals. At the two lowest exposure concentrations (5 or 10 ppm) UE averaged significantly higher in the naïve than metyrapone-pretreated animals. Direct comparisons between these groups should be made with caution because the metyrapone-pretreated animals demonstrated steady state UE and the naïve animals did not; however, these results indicate a generalized diminished UE of metyrapone-pretreated animals (see also Fig. 2).

The average uptake rate (μg/min) can be calculated from the inspired concentration, flow rate, and UE. In naïve animals, the uptake rate ranged from approximately 0.1 to 1.0 μg/min at inspired concentrations of 5 to 200 ppm, respectively.

Rats. Shown in Figure 2 is the average UE during the exposure in naïve and metyrapone-pretreated rats at an inspired concentration of 5 ppm. UE diminished slightly during 15 to 45 min of exposure in both the naïve and metyrapone-pretreated rats, but the decline was not statistically different from zero. Linear regression analysis was performed on the UE-versus-time relationship for each animal, and the resulting slope values for the animals in each group were averaged to statistically evaluate steady-state UE behavior. Both positive and negative averages of slope values were observed in the naïve and metyrapone-pretreated rat groups. In no case, were the average slopes different from zero, indicating that steady state UE was maintained in these groups. Slope data among groups were compared by 2-factor ANOVA with the factors being concentration and metyrapone pretreatment. This analysis detected no significant effect of metyrapone ($p = 0.70$), no effect of styrene ($p = 0.93$), and no interaction between styrene and metyrapone ($p = 0.96$). In toto, these results indicate that, unlike the mice, steady state UE of styrene was maintained from 15 to 45 min of exposure in the rat.

The average UE between 15 and 45 min of exposure are

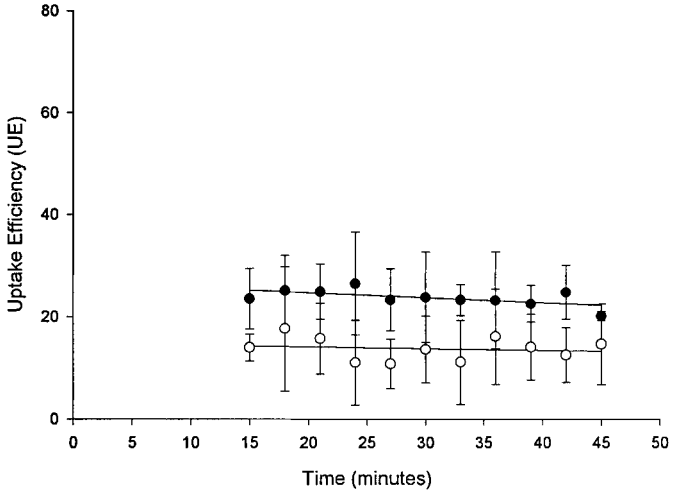

FIG. 2. Shown is the average uptake efficiency (expressed as a percent) obtained at each time point of the naïve (closed circles) and metyrapone-pretreated (open circles) rats. The exposure concentration averaged 5.4 ± 0.9 (mean ± SD) among the 7 naïve and 6 metyrapone-pretreated rats. Data are presented as mean ± SD. The linear regression lines (uptake efficiency versus time) are shown.

TABLE 3
Upper Respiratory Tract Styrene Uptake Efficiency in the Rat

	Inspired styrene concentration (ppm)					
	5	10	25	50	100	200
Naïve	23.7 ± 2.9[a] (7)	22.4 ± 4.2[a] (6)	15.3 ± 3.4[b] (6)	13.1 ± 4.7[b] (9)	8.7 ± 4.6[b] (7)	10.1 ± 3.8[b] (7)
Metyrapone	13.6 ± 5.0[b] (6)	10.5 ± 4.1[b] (6)	9.9 ± 5.0[b] (6)	7.6 ± 3.9[b] (6)	7.7 ± 3.9[b] (6)	8.5 ± 3.1[b] (6)

Note. Average uptake efficiency between 15 and 45 min, expressed as percent. Numbers in parentheses refer to numbers of animals (*n*). Data are reported as mean ± SD; naïve animals received no pretreatment, metyrapone animals received 150-mg/kg metyrapone, sc, 30–60 min prior to exposure. Data were analyzed by 2-factor ANOVA which detected a significant effect of metyrapone ($p = 0.000001$), of styrene concentration ($p = 0.00001$) and an interaction between metyrapone and styrene concentration ($p = 0.007$). Each group was then compared by Newman-Keuls test, groups with differing superscripts differ significantly ($p < 0.05$) from each other. Measured exposure concentrations for the rat studies were 5.4 ± 0.8, 11.2 ± 0.8, 27.1 ± 1.0, 52.1 ± 6.5, 103 ± 10, and 198 ± 19 ppm (mean ± SD).

shown in Table 3. These values were compared among the groups by 2-factor ANOVA with the factors being styrene concentration and metyrapone pretreatment. A significant effect of exposure concentration, metyrapone pretreatment and a significant interaction were detected. The interaction demonstrates that metyrapone had statistically differing effects at the different exposure concentrations. Groups were then compared by Newman-Keuls test. In the naïve animals, UE at exposure concentrations of 5 or 10 ppm were significantly higher than at higher concentrations, an effect similar to that observed in the mice (see Table 2). In contrast, UE were similar at all exposure concentrations in the metyrapone-pretreated animals Among the 36 metyrapone pretreated rats, UE averaged 9.7 ± 4.4%. Thus, metyrapone pretreatment abolished the inspired concentration dependence of UE in rats as it did in mice (see Table 2). The UE in all metyrapone-pretreated animal groups were similar to those observed at the high concentrations in the naïve animals. At the two lowest exposure concentrations (5 or 10 ppm), UE averaged significantly higher in the naïve than in the metyrapone-pretreated animals.

Uptake rates (μg/min) averaged approximately 0.35 to 6 μg/min at exposure concentrations of 5 to 200 ppm, respectively.

DISCUSSION

Under constant velocity inspiratory flow conditions styrene was removed from the air stream in the nasal passages of the rat and mouse with moderate efficiency. UE ranged between 4 and 42% depending on the inspired concentration and inspiratory flow rate. The styrene UE observed in the study at exposure concentrations of 25 ppm or higher were similar to those observed in the rat and hamster for xylene and bromobenzene at similar inspired concentrations (Morris, 1993). The blood:air partition coefficients for xylene and bromobenzene (25 and 40, respectively) are also similar to that of styrene (40, Andersen *et al*., 1984). As for xylene and bromobenzene (Morris, 1993), styrene UE was strongly dependent on the inspiratory flow rate with diminished UE being observed at high flow rates in both the rat and mouse. This behavior has been observed for numerous vapors in many laboratory species (Morris, 1994).

Prolonged uptake of inspired metabolized vapors is dependent upon the ability of the nose to remove vapors from nasal tissues via both the bloodstream and metabolism. Uptake due to bloodstream clearance is a first-order process and, in the absence of toxicity, increases linearly with increasing exposure concentration (Morris, 1994, 1999). In contrast, uptake via metabolic clearance is first order at low concentrations but zero order (saturable) at high-inspired concentrations (Morris *et al*., 1993; Morris, 1994, 1999). At sufficiently high concentration, uptake will be dominated by the blood-clearance process. Although metabolism may be occurring at Vmax, it may account for only a small fraction of the total amount of vapor being removed via the bloodstream.

Styrene is a known CYP450 substrate (Sumner and Fennell, 1994) and nasal tissues express CYP 450 (Thornton-Manning, 1997). That styrene UE was diminished by the CYP450 inhibitor metyrapone (Tables 2 and 3) provides strong evidence that styrene is metabolized *in situ* by CYP450, and that this process serves to enhance uptake. For comparative purposes, it is noted that metyrapone exerts similar effects on nasal UE of xylene and bromobenzene (Morris, 1993). Similar results have also been obtained with carboxylesterase inhibition and inspired ester vapors (Morris, 1990; Morris and Frederick, 1995), alcohol dehydrogenase inhibition and isoamyl alcohol vapor (Morris, 1993), aldehdye dehdyrogenase inhibition, and acetaldehyde vapor (Stanek and Morris, 1999).

In both the rat and mouse, styrene UE was concentration-dependent. Enhanced UE at low- compared to high-exposure concentrations has been observed for other metabolized vapors including propanol (Morris and Cavanagh, 1987) and acetaldehyde (Morris and Blanchard, 1992). For acetaldehyde vapor, comparison of the nasal metabolic potential (as measured by the *in vitro* Vmax for nasal aldehyde dehydrogenase) with the uptake rates suggested the diminished UE was due to metabolic capacity limitation. Specifically, diminished UE was observed

at inspired concentrations that were sufficiently high that uptake rates (μg/min) greatly exceeded nasal Vmax. Subsequent studies utilizing the aldehyde dehydrogenase inhibitor cyanamide, demonstrated that the concentration dependence on acetaldehyde UE was, indeed, attributable to local metabolism (Stanek and Morris, 1999).

In both the rat and mouse, nasal UE of styrene was significantly lower at inspired concentrations of 50, 100, or 200 ppm than at 5 or 10 ppm. The concentration dependence of styrene UE was abolished by metyrapone. In fact, metyrapone diminished UE at 5 or 10 ppm to the level observed at 100 or 200 ppm in the naïve animals. These results provide strong evidence that the concentration dependence is attributable to local metabolism. These results suggest that at inspired concentrations of 50 ppm or more, nasal tissue concentrations are sufficiently great that metabolism is no longer first order. Data on nasal styrene metabolism are needed to comprehensively evaluate the precise kinetics of the concentrations dependence.

It is not likely that the inhibitory effects of metyrapone on styrene UE were due to some unanticipated side effects. First, were metyrapone to induce non-specific changes in UE, then diminished UE would be anticipated at all exposure concentrations. This was not observed; metyrapone diminished UE only at low inspired concentrations. It is at low concentrations that the enhancing effects of local metabolism should be fully manifested. Second, and more importantly, metyrapone is without effect on nasal UE of acetone, a vapor which is not significantly metabolized by nasal tissue CYP450 (Morris, 1993).

When measured by the technique used in the current study, nasal UE of most vapors rapidly attains a steady state, which is maintained for prolonged periods (Morris, 1994, 1996, 1999; Medinsky *et al.*, 1999). This behavior was observed for styrene in naïve and metyrapone-pretreated rats. Steady state UE was not observed in naïve (non-pretreated) mice but was observed in metyrapone-pretreated mice, suggesting a CYP450 metabolite is responsible for the non-steady state behavior in this species.

The mechanism(s) responsible for the non-steady state behavior are not known. Under steady state conditions, the rate at which vapor is removed from the air stream is exactly balanced by the rate at which it is removed from the air:tissue interface by diffusion and clearance by metabolism and/or the bloodstream (Morris, 1994, 1999). The continual decline in UE may be due to several factors including a continual decline in metabolism rate, a continual decrease in the perfusion rate, and/or a continual thickening of the air blood barrier. Perhaps a mouse-specific CYP450 metabolic product inhibits metabolism thus diminishing UE as the exposure progressed. Hynes *et al*. (1999) has noted differences between the rat and mouse in pulmonary metabolism of styrene. However, the non-steady state behavior, as quantitated by the change in UE (%/min), was similar at all exposure concentrations of styrene (5–200 ppm), and UE is not dependent on metabolism at the high-exposure concentrations (Table 2), making this possibility seem unlikely. Acrolein vapor also demonstrates non-steady state UE and co-exposure to acrolein induces non-steady state UE behavior for other vapors including acetone and acetaldehyde (Morris, 1996, 1997). Recent studies have suggested that acrolein induces this response via stimulation of nasal sensory nerves and release of substance P, and induction of a neurogenic edema (Morris *et al*., 1999). Perhaps a styrene metabolite initiates a similar sensory neuronal response in the mouse although the lowest reported RD50 in mice (157 ppm; Alarie, 1973) is considerably higher than the lowest concentration that exhibited non-steady state behavior (5 ppm) in the current study.

The non-steady state UE behavior that was observed in the mouse but not the rat may be reflective of physiological/toxicological response differences between species, or it may reflect a proportionately greater metabolism rate and/or the formation of a different metabolite in the mouse. The mouse is more sensitive than the rat to styrene-induced olfactory injury (Cruzan *et al*., 1997). Because the kinetics of styrene UE differed in the rat and mouse (steady state vs. non-steady state) it is not possible to directly compare the UE data between these species to assess species differences in metabolism. However, it does appear that metyrapone has a much greater effect on UE in the mouse than the rat, reducing UE by ~20% at 5 ppm in the mouse (Table 2) compared to only ~10% at 5 ppm in the rat (Table 3). This greater effect in mice, if real, may be due to a greater metabolic capacity in the mouse than in the rat.

The distribution of enzymatic activity throughout nasal tissues is important in influencing inspired vapor metabolism (Morris *et al*., 1993). In the rat, only ~10% of the inspired air stream passes through the olfactory-line ethmoturbinates (Kimbell *et al*., 1993). Thus, regardless of the amount of enzyme expressed in this area of the nose, metabolic clearance in the olfactory mucosa alone cannot exceed ~10% of the inspired burden. Presumably, regional airflow patterns are similar in the mouse; however, we are not aware of any direct information in this regard. A proportionately greater styrene metabolic capacity in the mouse than the rat may, therefore, reflect a proportionately greater expression of total enzymatic activity and/or a differing anatomical distribution of that activity. Further studies are needed to clarify these possibilities.

In summary, the current study revealed styrene vapor was scrubbed from the air stream in the nasal passages of the rat and mouse with moderate efficiency. In both species, UE was inhibited by treatment with the CYP450 inhibitor metyrapone, providing strong evidence that styrene is metabolized in nasal tissues *in situ* and that this process serves to enhance UE. In both species UE was concentration-dependent, with diminished UE efficiencies being observed at inspired concentrations exceeding 50 ppm. The concentration dependence was abolished by pretreatment with metyrapone, suggesting it is due to metabolic saturation and/or capacity limitation at high inspired concentrations.

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