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Nasal Uptake of Inhaled Acrolein in Rats

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An improved understanding of the relationship between inspired concentration of the potent nasal toxicant acrolein and delivered dose is needed to support quantitative risk assessments. The uptake efficiency (UE) of 0.6, 1.8, or 3.6 ppm acrolein was measured in the isolated upper respiratory tract (URT) of anesthetized naive rats under constant-velocity unidirectional inspiratory flow rates of 100 or 300 ml/min for up to 80 min. An additional group of animals was exposed to 0.6 or 1.8 ppm acrolein, 6 h/day, 5 days/wk, for 14 days prior to performing nasal uptake studies (with 1.8 or 3.6 ppm acrolein) at a 100 ml/min airflow rate. Olfactory and respiratory glutathione (GSH) concentrations were also evaluated in naive and acrolein-preexposed rats. Acrolein UE in naive animals was dependent on the concentration of inspired acrolein, airflow rate, and duration of exposure, with increased UE occurring with lower acrolein exposure concentrations. A statistically significant decline in UE occurred during the exposures. Exposure to acrolein vapor resulted in reduced respiratory epithelial GSH concentrations. In acrolein-preexposed animals, URT acrolein UE was also dependent on the acrolein concentration used prior to the uptake exposure, with preexposed rats having higher UE than their naive counterparts. Despite having increased acrolein UE, GSH concentrations in the respiratory epithelium of acrolein preexposed rats were higher at the end of the 80 min acrolein uptake experiment than their in naive rat counterparts, suggesting that an adaptive response in GSH metabolism occurred following acrolein preexposure.

Acrolein, an α,β-unsaturated aldehyde, is used as a water system biocide and by the chemical industry as a synthetic intermediate. Acrolein is also found in tobacco smoke, gasoline and diesel engine exhausts, partially combusted animal fats and vegetable oils, and certain foods (IARC, 1995; U.S. EPA, 2003). Acrolein concentrations in mainstream cigarette smoke can exceed 50 ppm (Brunnemann et al., 1990). Acrolein is also produced in structural fires; smoke concentrations associated with fires can exceed 3 ppm (Slaughter et al., 2004). Ambient air measurements in the United States have detected acrolein at concentrations ranging from 2 to 7 ppb. Acrolein is metabolized by aldehyde dehydrogenase to acrylic acid (Patel et al., 1980), although other metabolites are also produced (Beauchamp et al., 1985).

Acrolein is chemically reactive, highly water soluble, and produces cytotoxicity at the site of initial contact. Inhaled acrolein is a potent lacrimator and respiratory tract irritant (WHO, 1992). The atmospheric acrolein concentration required to reduce the respiratory rate of F344 rats by 50% (RD_{50}) is 6 ppm (Babiuk et al., 1985). Inspired acrolein reacts rapidly with glutathione (GSH) and other cellular nucleophiles (Kehrer & Biswal, 2000), depletes rat nasal and lung GSH (Arumugam et al., 1999; Lam et al., 1985, McNulty et al., 1984), stimulates cell proliferation in the rat respiratory tract (Roemer et al., 1993), and induces nasal pathology in rodents (Buckley et al., 1984; Cassee et al. 1996; Dorman et al., 2008; Feron et al., 1978; Lyon et al., 1970). Short-term (6 h/day for 3 days) exposure of rats to 0.7 ppm acrolein results in respiratory epithelial metaplasia and olfactory mucosal damage (Cassee et al., 1996). Nasal lesions following acrolein exposure have been replicated in multiple species. Acrolein also impairs normal tracheal cilia function (Dalhamn & Rosengren, 1971).

Nasal lesions induced by acrolein in rodents have been used by the U.S. Environmental Protection Agency (U.S. EPA) to establish an inhalation reference concentration (RfC) for this chemical. The RfC is an estimate of a continuous inhalation exposure to humans that is likely to be without appreciable risk

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of adverse effects over a person's lifetime (U.S. EPA, 1994). The current RfC for acrolein is 2×10^{-5} mg/m^3 (Integrated Risk Information System, http://www.epa.gov/iris, accessed May 2007), based on nasal pathology seen in rats following subchronic exposure (Feron et al., 1978). Derivation of a scientifically based RfC and other quantitative risk assessments for inhaled acrolein requires knowledge of the relationship between inspired concentration and delivered dose. The U.S. EPA RfC calculation relied on dosimetric adjustment factors to account for species differences in delivered dose. This interspecies dosimetric extrapolation considered relative minute volume to upper respiratory tract (URT) surface area ratios between the rat and the human. An alternative to this approach relies on the use of anatomically accurate three-dimensional computational fluid dynamics (CFD) models of the URT to simulate steady-state inspiratory airflow and vapor uptake in the rat nasal passages (Kimbell & Subramaniam, 2001). Human nasal CFD models can then be used to support interspecies extrapolation for a noncancer risk assessment for human exposure to inhaled acrolein. This approach has been recently used with hydrogen sulfide, yielding a refined prediction of the risk posed by that nasal toxicant (Schroeter et al., 2006).

This experiment was designed to provide additional data needed to support these quantitative risk assessment approaches. Previous investigators have examined the nasal uptake of acrolein in the surgically isolated rat nasal cavity (Morris, 1996; Morris et al., 1999). This study was designed in part to replicate these previous studies. Studies evaluating the effect of prior chemical exposure on nasal uptake have been rarely conducted. Thus, one novel aspect of our study involved measurement of nasal tissue glutathione (GSH) concentrations following a short-term acrolein inhalation challenge in naive and acrolein preexposed rats.

MATERIALS AND METHODS

Study Design

This study consisted of three phases. The first phase evaluated the nasal uptake of inhaled acrolein (0.6, 1.8, or 3.6 ppm) at constant unidirectional flow rates of 100 or 300 ml/min for up to 80 min ($n = 5$–6 rats/acrolein concentration/flow rate). Nasal epithelial (respiratory and olfactory) GSH concentrations were also determined at the end of the acrolein exposure. The acrolein exposure concentrations used in this experiment ($\geq$0.6 ppm) have been associated with nasal pathology following short-term (e.g., 4 days at 6 h/day) and subchronic inhalation (Dorman et al., 2008; Feron et al., 1978) and allowed suitable detection with the gas chromatography system used in this experiment. Acrolein-induced depletion of nasal respiratory GSH has been reported by others (Lam et al. 1985; McNulty et al. 1984); however, these studies largely focused on the respiratory epithelium. Thus, phase II evaluated nasal epithelial (respiratory and olfactory) GSH concentrations following 0 (naive rats) 20, 40, or 60 min of acrolein exposure ($n = 5$–6 rats/flow rate/time). Phase II also examined the nasal uptake of inhaled acrolein (3.6 ppm acrolein) at constant unidirectional flow rates of 100 or 300 ml/min for up to 60 min.

Acrolein induces morphological changes in the nasal epithelium that may affect the nasal uptake of this chemical. Phase III evaluated the nasal uptake of inhaled acrolein (1.8 or 3.6 ppm acrolein at a constant unidirectional flow rate of 100 ml/min for up to 80 min) in animals that were preexposed to acrolein (0.6 or 1.8 ppm acrolein, 6 h/day, 5 days/wk, for 14 exposure days) ($n = 5$–6 rats/nasal uptake concentration/preexposure concentration). Nasal uptake studies were performed the day after the animal's last preexposure to acrolein. Nasal epithelial (respiratory and olfactory) GSH concentrations were determined at the end of the acrolein nasal uptake exposure. Maximal exposure durations (80 min) were chosen to minimize anesthesia-induced changes in core body temperature and other body functions.

Chemicals

High-purity (>99%) acrolein (CAS number 107-02-08) containing 200 ppm hydroquinone was purchased from Aldrich Chemical Company (Milwaukee, WI). Cylinders containing acrolein were purchased from Linde Gas, (Cary, NC) and manufactured by Quality Standards and Research Gases (Pasadena, TX).

Animals and Their Husbandry

This study was conducted under federal guidelines for the care and use of laboratory animals (National Research Council, 1996) and was approved by the CIIT Centers for Health Research Institutional Animal Care and Use Committee. In total, 104 male F344 rats, 6 wk of age at purchase, were obtained from Charles River Laboratory (Kingston, NY). Animals were housed on direct bedding (Alpha-DriTM, Shepard Specialty Papers, Kalamazoo, MI) in filter-capped cages and were given certified NIH-07 feed (Zeigler Brothers, Gardners, PA) and reverse-osmosis water (HydroService and Supplies, Research Triangle Park, NC) ad libitum. Animal rooms were ventilated with HEPA-filtered air and maintained at 18–26°C with relative humidity of 30–70% on a 12-h dark–light cycle. The general condition of the animals was checked daily. Body weights were recorded at least once weekly. Animals were acclimated for at least 2 wk prior to exposure. At the time of use, the average body weight ($\pm$ SEM) of naive animals was 190 $\pm$ 2.1 g.

Acrolein Nasal Uptake

Animals were anesthetized with urethane (Acros Organics, MorrisPlains, NJ) at approximately 1.3 g/kg (ip). Anesthetized rats were placed on a heated pad (SnuggleSafe, South Holland, IL) in a supine position, and the trachea was surgically exposed by blunt dissection using methods described by Morris (1999). A 2.5-cm polyethylene endotracheal tube (PE 205, Clay Adams, Parsippany, NJ) was positioned toward the lungs while a 14-G catheter (4058-20 Jelco, Cincinnati, OH) was inserted into a

second tracheal incision so that the catheter tip was positioned near the larynx. The animal's head and nose were placed in an anesthesia nose-only cone (Euthanex Corp., Palmer, PA) that was used to deliver acrolein. Nasal uptake was measured under constant velocity unidirectional inspiratory flow at rates approximating 67 and 200% of the predicted minute ventilation (150 ml/min) of the adult male rat. Air was drawn through the isolated URT for up to 80 min under the desired flow conditions. Anesthesia was monitored and administered as needed throughout the exposure period. Animals were killed via exsanguination immediately after the end of exposure and tissues collected within 5–10 min.

Acrolein Vapor Generation and Characterization

A schematic of the exposure system is provided in Figure 1. Test atmospheres of acrolein were generated with certified gas cylinders containing either 40 or 80 ppm acrolein in nitrogen. Acrolein exposure concentrations were generated by metering acrolein in nitrogen from the gas cylinder through a mass flow controller (MKS Instruments, Andover, MA) into a "T" in the dilution airflow, where the acrolein was mixed with dilution air to achieve the target concentrations. The dilution air was drawn from an instrument grade compressed air supply, a portion of which was humidified. The acrolein exposure concentration was controlled by changing the acrolein delivery rate and the dry and humidified airflow rates to achieve the desired concentration. Total flow rates through the system were maintained at 300 to 800 ml/min for URT flow rates of 100 or 300 ml/min, respectively. Acrolein-laden, humidified air (to 45–55% relative humidity) was drawn continuously through the isolated URT with a rotary-vane vacuum pump (GAST Manufacturing, Benton Harbor, MI). The test atmosphere was presented to the breathing zone of the animal at an excess flow rate at least 100 ml/min above the projected URT flow and sampling flow rate. The average air temperature and relative humidity maintained during the exposures ranged from 21.7 to 23.0°C and from 45 to 55%, respectively. The exposure portion of the system was contained within a vented portable hood.

Air samples were analyzed approximately every 3 min. The sampling system used to draw samples consisted of Teflon tubing and fittings. Two tees were used to connect the sampling system to either the test atmosphere near the breathing area in the rat nose cone (pre-nose) or the endotracheal tube (post-nose). Samples were drawn through a multiport gas sampling valve connected to a gas chromatograph (GC) at a flow rate of 30 ml/min

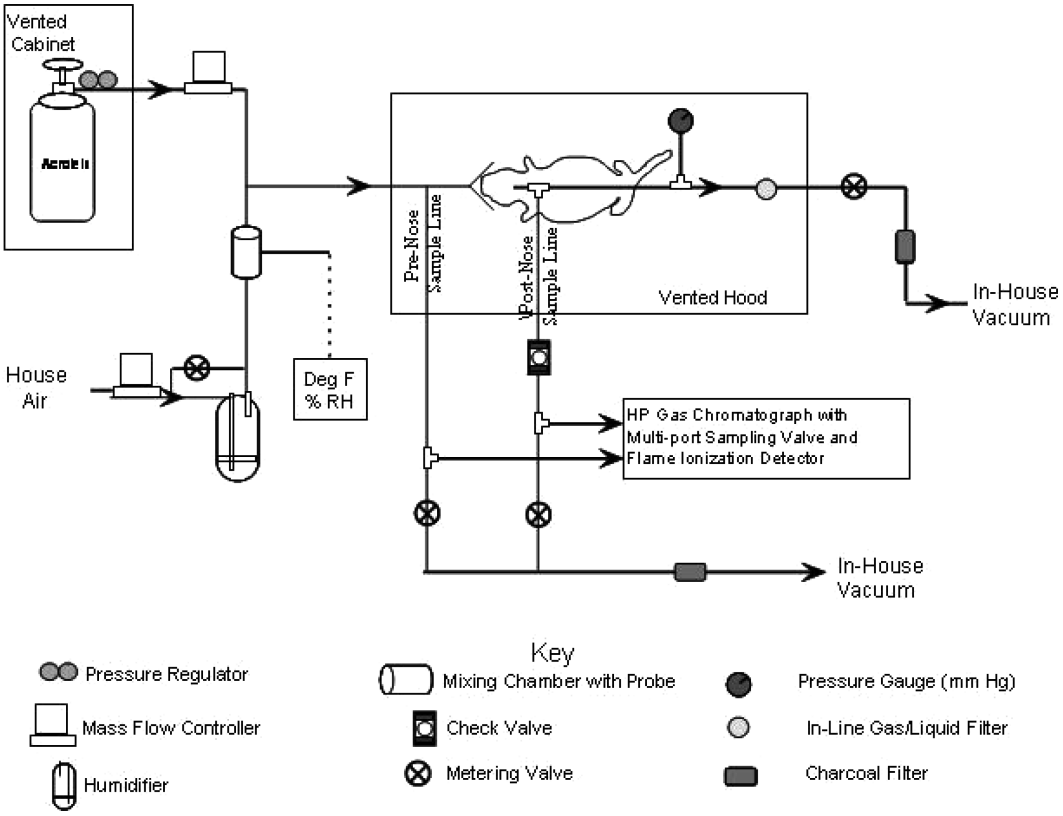

FIG. 1. Diagram of exposure system showing major components. Humidified air (to 45–55%) containing acrolein was drawn through the isolated rat nose by house vaccum under a constant unidirectional airflow (see text for details). Two gas chromatograph sampling ports positioned at the air inlet ("pre-nose") and near the pharynx of the rat ("post-nose") were used to measure acrolein concentrations and determine nasal uptake.

from either location. Sampling airflow rates were controlled with rotameters and needle valves, which were verified using a BIOS DC-1 flow calibrator (Bios International Corporation, Pompton Plains, NJ). Acrolein concentrations were measured with a Hewlett Packard 5890 Series II GC (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector (FID) and an oven temperature of 90–100°C. For the 1.8-ppm and 3.6-ppm exposures, a 5% phenyl-/95% methylpolysiloxane column (EC-5 , Alltech Chromatography, Deerfield, IL) was used (elution time ≈ 0.64 min), while a packed column (10% FFAP on CW-AW 80/100, Alltech Chromatography, Deerfield, IL) was used for the 0.6-ppm acrolein exposures (elution time ≈ 1.36 min). An eight-position gas sampling valve was used by the GC to sample at a programmed sequence of locations from both pre- and post-nose locations from both exposure systems. The GC was calibrated with standards made by injecting certified high-purity acrolein (3000 ppm) into Tedlar gas sampling bags (SKC, Inc., Eighty-Four, PA), which were further filled with a measured volume of instrument-grade air to achieve the desired range of concentrations. Area counts were converted to concentrations in parts per million of acrolein.

Glutathione Measurement

Immediately following the end of the acrolein exposure, anesthetized rats were killed by decapitation, and the head was sectioned sagitally on the bridge of the nose. The nasal respiratory and olfactory mucosa with the supporting turbinates were visually identified and dissected from the nasal cavity. Freshly harvested tissue (40–100 mg of mucosa and turbinate) was placed directly into 400 μl cold 5% meta-phosphoric acid (MPA) and homogenized. Separate 100-μl aliquots of the homogenate were used for measurement of total protein, total glutathione, and GSSG. The GSSG aliquot also included 5 μl of the glutathione scavenger 1-methyl-2-vinylpyridinium trifluoromethanesulfonate (M2VP). All aliquots were frozen at −80°C until analysis.

Samples were subsequently thawed and centrifuged at 1000 × g for 10 min. Total GSH and oxidized GSH (GSSG) were determined with the Bioxytech GSH/GSSG-412 kit (OxisResearch, Portland, OR) and methods developed by Griffith (1980). A 5-μl aliquot of triethanolamine (TEAM) was added to 80 μl of the GSSG supernatant to ensure proper pH for the scavenging of reduced GSH to proceed. Samples used for total protein were thawed, weighed, and centrifuged at 1000 × g for 1 min. Pellets were dried in a fume hood for at least 1 h, weighed, and returned to their original wet weight by adding TRIZMA pH 8.0. Resuspended pellets were analyzed for protein with the Pierce Coomassie Plus protein assay (Pierce, Rockland, IL).

Data Analysis

The concentration of acrolein entering the URT (pre-nose) and exiting the URT (post-nose) was measured approximately every 13 min after initiation of flow through the URT through the end of the 80-min acrolein exposure. More frequent sampling (e.g., every 3–6 min) were performed during shorter uptake experiments. Inhalation uptake efficiency (UE) was calculated as [(pre-nose sample – post-nose sample)/pre-nose sample] × 100, and expressed as a percentage. The grand mean UE was calculated as the overall average of the six samples for each animal. To test for the effects of acrolein concentration, airflow rate, and exposure time, and also their interactions, experimental data were analyzed by a three-factor multivariate analysis of variance for a design with repeated measures. If the concentration or flow rate effect was significant, and the effect was not involved in a significant interaction, Tukey's multiple comparison procedure for the overall mean UE data was used to determine which concentrations and/or flow rates were different. Statistical analyses were performed with JMP Statistical Software (SAS Institute, Inc., Cary, NC). A probability value of .05 was used as the level of significance for all statistical tests. Unless otherwise indicated, all data presented represents mean ± standard error of the mean (SEM).

RESULTS

Nasal Uptake of Acrolein in Naive Animals

URT uptake efficiency of acrolein was dependent on the concentration of inspired acrolein (Figure 2), duration of exposure (Figure 3), and airflow rate (Figure 2). Overall average UE was significantly greater at the lowest concentration than at either of the higher concentrations (0.6 > 1.8 ≈ 3.6 ppm) ($p < .0001$, Tukey's test) (Figure 2). At a 100 ml/min airflow rate, mean UE of inspired concentrations of 0.6 ppm acrolein (97.8 ± 1.5%) was nearly double the efficiency of uptake of 3.6 ppm (49.5 ± 4.8%). A general decline in UE during the 80-min exposure was observed and a repeated-measures analysis of variance (ANOVA) revealed time as a significant effect on

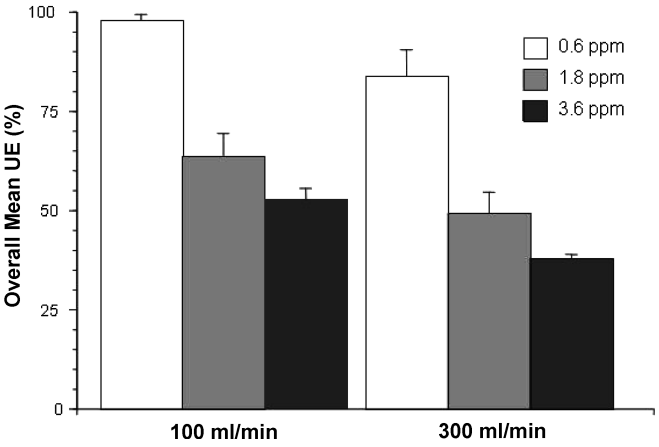

FIG. 2. Averaged URT uptake efficiencies (UE) of naive F344 rats (mean + SEM, $n = 6$ rats/concentration/airflow rate) over the course of a single 80-min inhalation exposure to 0.6, 1.8, or 3.6 ppm acrolein at 100 or 300 ml/min. Acrolein concentrations and airflow significantly affected UE (0.6 > 1.8 ≈ 3.6 ppm; $p <$ .0001, 100 > 300 ml/min, $p < .0061$; Tukey's test).

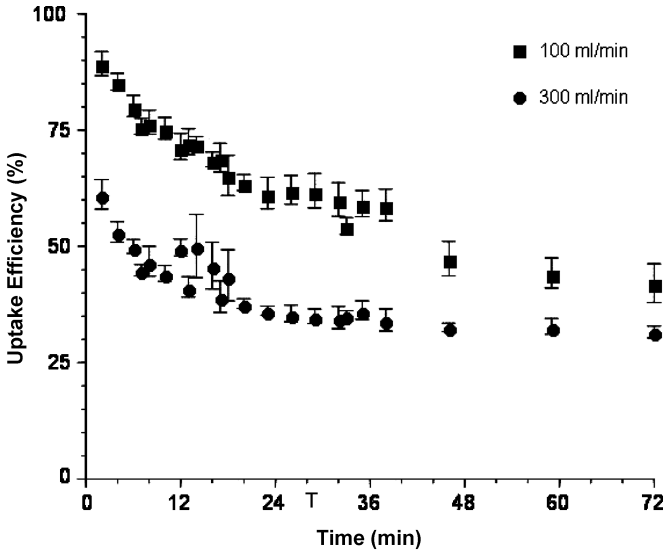

FIG. 3. Mean URT acrolein uptake efficiency (UE) in naive F344 rats (± SEM, $n = 6$ rats/airflow rate) during the course of a single 80-min exposure to 3.6 ppm acrolein. Airflow rate and time significantly affected UE.

uptake efficiency ($p < .001$). For example, animals exposed to an inspired concentration of 3.6 ppm acrolein had an approximate 50% decrease in UE during the 80-min exposure (Figure 3). Significant interactions between acrolein concentration and airflow ($p = .95$) and time and flow rate ($p = .12$) were not observed. A significant interaction between acrolein concentration and time ($p = .01$) was, however, observed.

Nasal GSH Levels Following Short-Term Acrolein Inhalation by Naive Animals

Nasal epithelial GSH concentrations (μmol GSH/μg protein) following short-term (up to 80 min) acrolein exposure of naive animals are presented in Figure 4. Nasal epithelial GSH concentrations were dependent on the concentration of inspired acrolein ($p = .0002$), airflow rate ($p = .0007$), and epithelial subtype (olfactory vs. respiratory; $p = .0577$). The duration of exposure did not have an overall significant effect on end-of-exposure epithelial GSH concentrations ($p = .226$). The duration of exposure had a marginally significant effect on end-of-exposure epithelial GSH concentrations ($p = .0705$). Exposure to acrolein vapor also reduced GSH concentrations in the respiratory epithelium (Figure 4). In contrast, we did not observe acrolein-induced GSH depletion in the olfactory epithelium of naive rats (Figure 4).

The relative amounts of GSH and GSSG (expressed as the GSH/GSSG ratio) were dependent on the concentration of inspired acrolein ($p = .0007$), airflow rate ($p = .053$), and epithelial subtype (olfactory vs. respiratory; $p < .0001$) (Data not shown).

Nasal Uptake of Acrolein in Acrolein Preexposed Animals

Some animals were exposed to 0.6 or 1.8 ppm acrolein, 6 h/day, 5 days/wk, for 14 exposure days prior to performing acrolein nasal uptake studies at a single airflow rate (100 ml/min). As with our naive animals, URT uptake efficiency of acrolein was time dependent ($p < .0001$). URT uptake efficiency of acrolein in pre-exposed animals was also dependent on the acrolein concentration used prior to ($p = 0.01$) and during the uptake exposure ($p = .06$) (Figure 5). Animals that were pre-exposed to acrolein had higher UE than did naive animals exposed to air. Overall mean UE of acrolein in preexposed animals was approximately 13 to 38% higher than in air-exposed controls. A significant interaction between these two acrolein concentrations was not observed ($p = .42$).

Nasal GSH Levels Following Combined Short-Term Acrolein Inhalation and Acrolein Preexposure

Nasal epithelial GSH concentrations (μmol GSH/μg protein) in rats that were preexposed to acrolein for 14 exposure days and then underwent an 80-min acrolein exposure are presented in Figure 6. Nasal epithelial GSH concentration was dependent on the concentration of acrolein used during the preexposure ($p = .006$) and epithelial subtype (olfactory vs. respiratory; $p < .0001$). The acrolein concentration used for the uptake experiment did not have an overall significant effect on end-of-exposure epithelial GSH concentrations ($p = .97$). The relative amounts of GSH and GSSG (expressed as the GSH/GSSG ratio) were dependent on the epithelial subtype (olfactory vs. respiratory; $p < .0001$) but were only marginally dependent on either the concentration of inspired acrolein ($p = .097$) or the acrolein concentration used prior to the uptake experiment ($p = .095$) (data not shown).

DISCUSSION

The U.S. EPA RfC calculation for acrolein relied on dosimetric adjustment factors to account for species differences in delivered dose. Dosimetric species extrapolation for Category I gases depends upon a calculation of the relative minute volume to URT surface area ratios between the rat and the human, and assumes complete extraction of the gas (i.e., UE = 100%) with a uniform dose throughout the URT. Our experiments show the assumption of complete extraction is nearly met when exposures occur at resting inspiratory airflow, relatively low acrolein exposure concentrations (0.6 ppm), and short-term exposure duration. We found that the average URT uptake efficiency of acrolein is dependent on the concentration of inspired acrolein, the exposure duration, and airflow rate. At an airflow rate roughly equivalent to the predicted minute volume, the average UE of an inspired concentration of 0.6 ppm acrolein was nearly double the uptake efficiency of 3.6 ppm. Further, as ventilation rate for acrolein increased we saw a negative tendency on the UE, due to the decreased residence time of the gas in the URT and tissue acrolein concentration. Similar results have been reported

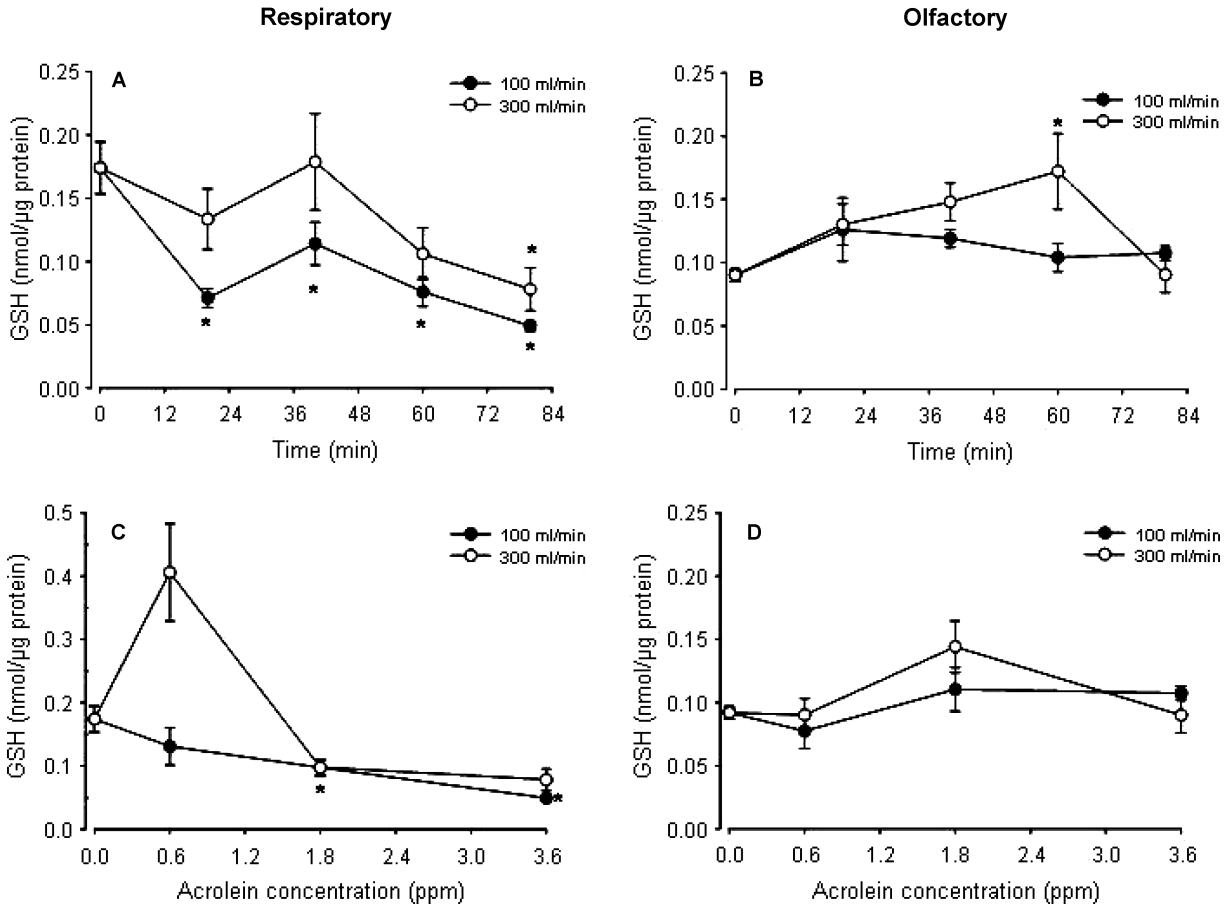

FIG. 4. Mean GSH concentrations in the respiratory (Left) and olfactory (Right) epithelium of naive F344 rats (± SEM, n = 5–6 rats/airflow rate/concentration/time point) during the course of a single exposure to acrolein. (A and B) Results following a single 3.6 ppm acrolein of 20 to 80 min duration; (C and D) dose-comparison results following the end of a single 80-min acrolein exposure. End-of-exposure GSH concentrations were dependent on the concentration of inspired acrolein, airflow rate, epithelial subtype, and duration of exposure. Asterisk indicates decreased end-of-exposure GSH concentrations vs. naive control ($p < .05$).

by Morris (1996) in his study of nasal uptake of 0.9, 4.5, or 9.1 ppm acrolein in urethane-anesthetized F344 rats under unidirectional inspiratory flow rates of 50 to 300 ml/min over a 40 minute period. Morris reported that the nasal uptake of acrolein was dependent on inspired concentration and airflow conditions with deposition efficiency decreasing as inspired acrolein concentration increased. Concentration-dependent nasal extraction of inhaled acrolein in rats is suggestive of saturable metabolic or reactive processes in nasal tissue.

We also observed a time-dependent reduction in net uptake during the exposure. For example, at a flow rate of 300 ml/min, URT acrolein UE decreased approximately 30% in animals exposed to 1.8 or 3.6 ppm acrolein for 80 min. Morris (1996) also reported time dependency for acrolein uptake in rats. He observed that during the last 20 min of the exposure an approximate 0.5% per minute decrease in extraction efficiency occurred with a 40 min exposure to 0.9 ppm acrolein. The mechanisms of these responses are not known; however, acrolein exposure has been shown to rapidly induce nasal vasodilation and progressive thickening of the air–blood barrier, due perhaps to excess mucus secretion and/or tissue swelling (Morris, 1996; Morris et al., 1999).

Although we observed trends similar to those reported by Morris, important differences also occurred. The uptake data from the current study appear consistently higher than those reported by Morris (1996). For example, Morris (1996) reported an overall uptake efficiency of approximately 50% in male F344 rats exposed to 0.9 ppm acrolein for 40 min under a unidirectional flow rate of 300 ml/min. In contrast, we observed an overall uptake efficiency of approximately 85% in male F344 rats exposed to 0.6 ppm acrolein for 80 min under a unidirectional flow rate of 300 ml/min. Humidification of the inspired air is an important difference between the Morris studies (which involved prehumidification of the air to 90% relative humidity) and ours, which used air with a relative humidity of 45 to 55%. Decreased acrolein concentrations would occur as the air becomes

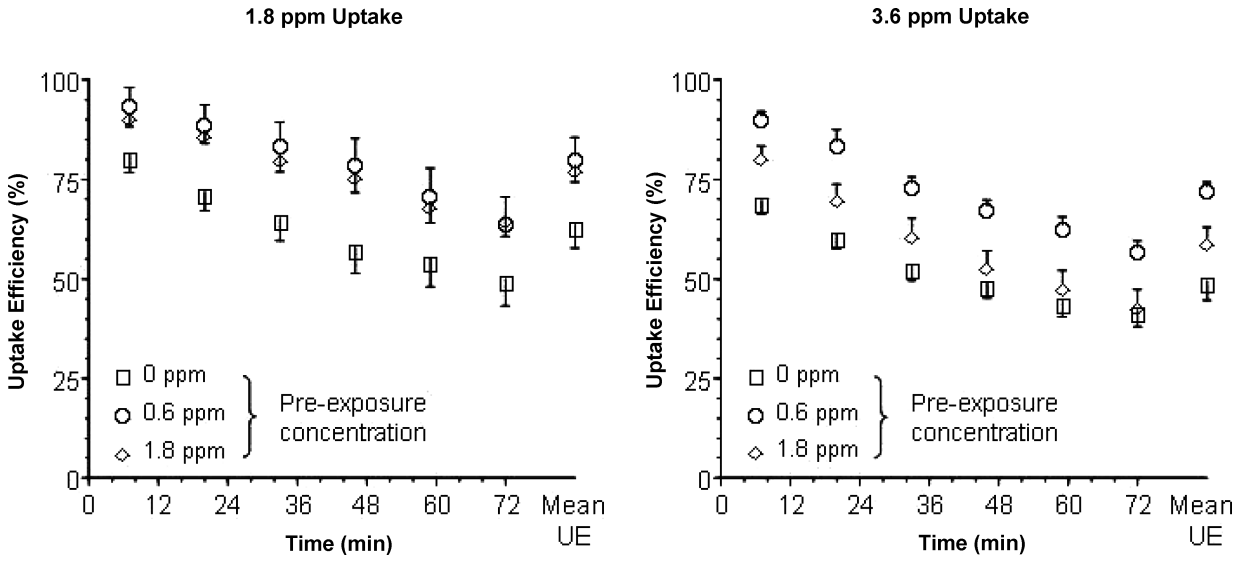

FIG. 5. Mean uptake efficiency (UE) of acrolein in the URT of preexposed F344 rats during the course of a single 80-min exposure to either 1.8 or 3.6 ppm acrolein at an airflow rate of 100 ml/min (± SEM, $n = 6$ rats/exposure concentration). Rats were exposed to 0, 0.6, or 1.8 ppm acrolein, 6 h/day, 5 days/wk, for 14 exposure days prior to performing acrolein nasal uptake studies. Acrolein preexposure and time significantly affected UE ($p < .05$). Overall mean UE for the 80 min exposure is also shown.

humidified within the nasal cavity; thus our results may overstate the actual extent of nasal extraction (e.g., by approximately 10%) and may also demonstrate a flow-rate dependency. Moreover, the degree of desiccation of nasal tissues that might have occurred during the present study and its effect, if any, on uptake efficiency are not known. Husbandry conditions for our animals also differed from those used by Morris. In particular, rats in the Morris study were housed on hardwood bedding and the

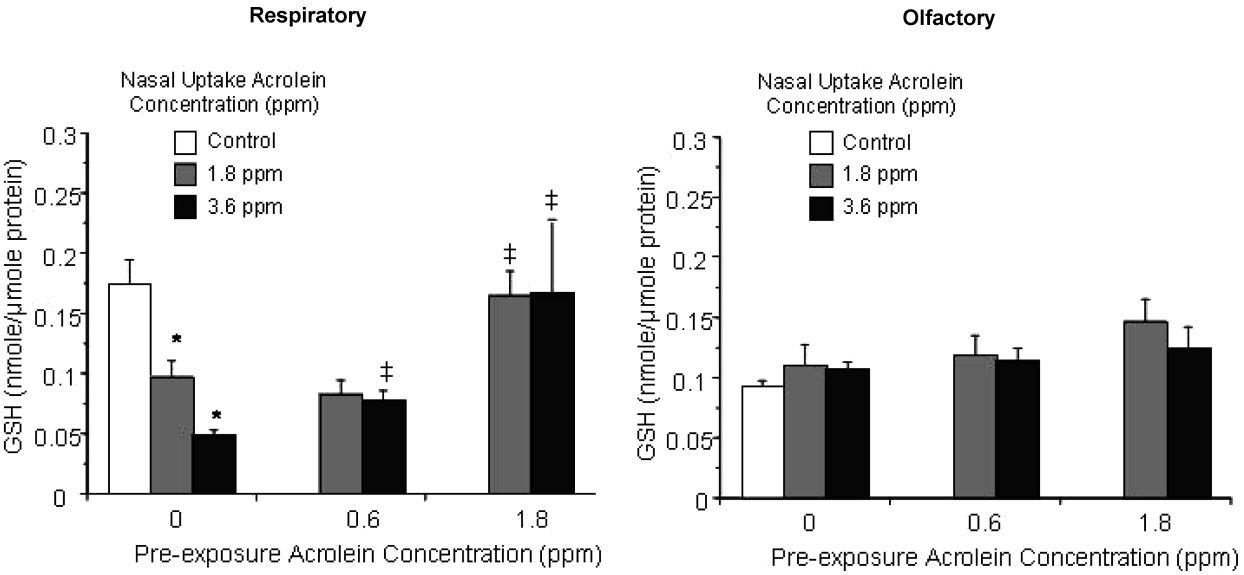

FIG. 6. Mean GSH concentration in the F344 rat at the end of an URT uptake experiment with acrolein (flow rate = 100 ml/min) in preexposed animals (± SEM, $n = 5$–6 rats/preexposure concentration/uptake concentration). Nasal epithelial GSH concentration was dependent on the concentration of acrolein used during the preexposure ($p = .006$) and epithelial subtype (olfactory vs. respiratory; $p < .0001$). The acrolein concentration used for the uptake experiment did not have an overall significant effect on end-of-exposure epithelial GSH concentrations ($p = .97$). Asterisk indicates decreased end-of-exposure GSH concentrations vs. air exposed control ($p < .05$); ‡, increased end-of-exposure GSH concentrations vs. control animals with an air preexposure ($p < .05$).

frequency of bedding changes was not reported. Ammonia and other nasal irritants present in urine can induce nasal pathology (Bolon et al., 1991) and can result in subtle nasal pathology that alters tissue responses to methyl bromide and presumably other nasal toxicants. Exposure to acrolein vapor also reduced GSH concentrations in the respiratory epithelium. Naive rats exposed to either 1.8 or 3.6 ppm acrolein for 80 min had an approximate 34 and 72% reduction in respiratory GSH concentrations, respectively. Acrolein-induced depletion of nasal respiratory GSH has been reported by others (Lam et al., 1985; McNulty et al., 1984). Lam and coworkers (1985) reported a greater than 33% reduction in rat nasal GSH concentrations following a 3-h exposure to 0.5 to 2.5 ppm acrolein. An increase in respiratory GSH concentrations, albeit not statistically significant, was seen in rats exposed to 0.6 ppm acrolein with a flow rate of 300 ml/min. Responses seen in this group were highly variable and uptake efficiency in this group also varied. The source of this variability could not be identified during the course of the experiment.

In contrast, we did not observe acrolein-induced GSH depletion in the olfactory epithelium of naive rats. Basal GSH levels in the olfactory epithelium were significantly lower than those found in the respiratory epithelium. Potter et al. (1995) likewise reported that GSH levels in the rat olfactory epithelium were approximately 15% lower than those seen in the respiratory epithelium. Potter and colleagues (1995) also reported that the apparent first-order rapid-phase (nonmitochondrial) rate constant for GSH turnover and synthesis in the rat respiratory epithelium was markedly shorter ($t_{1/2} = 4.4$ h) when compared to the olfactory epithelium ($t_{1/2} = 42.4$ h). This rapid turnover suggests that the effects of acrolein-induced depletion of GSH may be magnified in the respiratory epithelium.

Reduced antioxidant capacity in the olfactory epithelium is also reflected by the presence of lower catalase, GSH peroxidase, and GSH reductase activities in this tissue (Reed et al., 2003). Our prior studies showed that rats exposed to 0.6 or 1.8 ppm acrolein for $\geq$4 days develop inflammation, hyperplasia, and squamous metaplasia of the respiratory epithelium, while olfactory epithelial inflammation and olfactory neuronal loss occurred in acrolein-exposed animals following exposure to 1.8 ppm acrolein (Dorman et al., 2008). Epithelial antioxidant status does not solely account for these observed responses. Acrolein and other highly water-soluble and reactive chemicals are largely removed by the more anterior portions of the nasal cavity, thus sparing the olfactory epithelium that lines the more caudal part of the nose. Acrolein-induced GSH depletion in the olfactory epithelium, and subsequent cytotoxicity, would therefore be favored when UE of this chemical is reduced (i.e., high ambient acrolein concentrations, high airflow rates, prolonged duration).

One novel aspect of the present study was our investigation of the role of acrolein preexposure on the nasal uptake of acrolein. As noted earlier, animals exposed to acrolein quickly develop respiratory epithelial lesions. Ongoing acrolein exposure altered the UE of this chemical. Acrolein UE was dependent on the acrolein concentration used both prior to and during the uptake exposure. Animals that were preexposed to acrolein had higher UE than did naive animals exposed to air. Overall mean UE of acrolein in preexposed animals was approximately 13 to 38% higher than air-exposed controls. Rats exposed to acrolein for several weeks were also more resistant to GSH depletion following an additional 80-min challenge to this chemical. Indeed, despite increased acrolein UE, GSH concentrations in the respiratory epithelium of acrolein-preexposed rats were higher at the end of the 80-min acrolein uptake experiment than their naive rat counterparts suggesting that an adaptive response in GSH metabolism occurred following acrolein preexposure. Tissue GSH concentrations in response to acrolein exposure can be quite dynamic, with increased concentrations reflecting enhanced de novo synthesis of this cysteine-containing peptide (Horton et al., 1997). Importantly, pathological responses seen in the respiratory epithelium showed little progression with ongoing (subchronic) acrolein exposure, suggesting that adaptive responses emerged to restrict the extent of epithelial injury at this site (Dorman et al., 2008).

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