Uptake of Acetaldehyde Vapor and Aldehyde Dehydrogenase Levels in the Upper Respiratory Tracts of the Mouse, Rat, Hamster, and Guinea Pig

JOHN B. MORRIS

Toxicology Program, Department of Pharmaceutical Sciences, University of Connecticut, Storrs, Connecticut 06269

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Acetaldehyde is a ubiquitous indoor and outdoor air pollutant. This vapor is a respiratory tract irritant and a rodent inhalation carcinogen. The current study was aimed at examining the upper respiratory tract (URT) deposition efficiency for this vapor at inspired concentrations of 1, 10, 100, or 1000 ppm in four rodent species: B6C3F1 mouse, Sprague-Dawley rat, Syrian hamster, and Hartley guinea pig. For measurement of vapor uptake, the URT was isolated in urethane-anesthetized animals via insertion of a polyethylene cannula in the trachea such that its tip lay at the larynx. Uptake was measured under constant velocity unidirectional inspiratory flow at flow rates of approximately 50, 100, 200, and 300% of the predicted minute ventilation of each species and also under pseudo-cyclic flow (sinusoidal flow at 100% of the predicted minute ventilation with a constant 7 ml/min bleed for analysis). In addition, aldehyde dehydrogenase (AldDH) activities were measured in whole nasal tissue homogenates from each species for comparative purposes. In all species a high-affinity ($K_m < 0.2$ mM), low-capacity AldDH isozyme was observed. In the mouse, hamster, and rat, a low-affinity ($K_m > 10$ mM), high-capacity isozyme was observed; this isozyme was not observed in nasal tissue homogenates of the guinea pig. In all species, URT deposition efficiency was strongly dependent on the inspired concentration, with uptake being two- to three-fold more efficient at inspired concentrations of 1 or 10 ppm than at 1000 ppm. For example, at flows approximating the twice-minute ventilation rate URT uptake efficiency averaged 43, 49, 28, and 43% in the mouse, hamster, rat, and guinea pig, respectively, at an inspired concentration of 10 ppm, compared to 27, 14, 16, and 6% at an inspired concentration of 1000 ppm. Species differences were observed in uptake efficiency. At an inspired concentration of 1000 ppm a two-factor analysis of variance followed by a Newman–Keuls test revealed that uptake was significantly higher in the mouse, rat, and hamster than in the guinea pig. In contrast, at 10 ppm uptake was significantly lower in the rat than in any other species. Thus, the rank order of these species on the basis of ability to scrub acetaldehyde from the airstream differed at high compared to low inspired concentrations. The documentation of greatly differing deposition efficiencies as well as differing relative dosimetric relationships among species at high compared to low exposure concentrations highlights the potential complexities of quantitative extrapolation of high-concentration rodent inhalation toxicity data. © 1997 Society of Toxicology.

Acetaldehyde is a ubiquitous pollutant present in both indoor and outdoor air (Puxbaum et al., 1988; Lofroth et al., 1989). On a concentration basis, it is one of the major aldehydes present in mainstream and environmental tobacco smoke. Concentrations of acetaldehyde in smoky atmospheres may reach 0.1 ppm (Badre et al., 1978). Acetaldehyde is also an important industrial chemical. Industrial emissions of acetaldehyde in 1993 were estimated to exceed 9,000,000 pounds (TRI, 1996). Although not highly potent, acetaldehyde is a respiratory tract irritant and carcinogen in the rodent. The OSHA PEL for acetaldehyde is 100 ppm and is based on irritation (OSHA, 1996). In the rat, the RD50 for sensory irritation is approximately 3000 ppm (Babiuk et al., 1985). Inhalation exposure produces respiratory tract damage in the trachea and nasal cavity of rodents. Within the nose, the olfactory epithelium is more sensitive to acetaldehyde than the respiratory epithelium. Subchronic exposure (4 weeks 6 hr/day, 5 days/week) of the rat to 400 ppm results in nasal olfactory mucosal degeneration without producing damage in the respiratory mucosa (Appelman et al., 1982). In contrast, in the hamster subchronic exposure (90 day, 6 hr/d, 5 d/week) to 1340 ppm did not produce nasal lesions but did result in tracheal metaplasia (Kruysse et al., 1975). In this study both nasal and tracheal lesions were observed in the hamster at exposure concentrations of over 4000 ppm. Thus, the hamster nose appears more resistant than the rat nose to the irritating effects of acetaldehyde.

The EPA has classified acetaldehyde as a class B2 probable carcinogen based on increased incidence of nasal tumors in rats and laryngeal tumors in hamsters after inhalation exposure (IRIS, 1996). Chronic exposure of the rat to concentrations of 750 ppm or more results in a significant increase in nasal adenocarcinomas. An increased incidence of
both nasal adenocarcinomas and squamous cell carcinomas was observed at exposure concentrations of 1500 ppm or more (Woutersen et al., 1986). No other tumor types were observed. A significant increase in laryngeal tumors was observed in hamsters exposed to 2000 ppm acetaldehyde (32-week exposure, 29-week recovery period, Feron et al., 1982). Thus, acetaldehyde exposure produces lesions in the large airways with the site of injury being more proximal (nasal cavity) in the rat and more distal (larynx, trachea) in the hamster. The reasons for this species difference are not known.

Previous studies in this laboratory have focused on the upper respiratory tract deposition of acetaldehyde in the F344 rat (Morris and Blanchard, 1992). (The term upper respiratory tract, URT, is used to describe all regions of the respiratory tract anterior to and including the larynx. The terms deposition and uptake are used synonymously to describe the net transfer of vapor from the airstream to nasal tissues.) These studies revealed that URT dosimetric relationships differed markedly at high compared to low exposure concentrations. For example, when measured under pseudo-cyclic flow conditions (100 ml/min sinusoidal flow with 7 ml/min drawn off at constant flow for analysis), average URT deposition efficiencies of 76, 48, 41, and 26% were observed, respectively, at inspired concentrations of 1, 10, 100, and 1000 ppm, suggesting that a saturable process was involved in uptake. Other studies in this laboratory have shown that nasal metabolism via carboxylesterase (Morris, 1990; Morris and Frederick, 1995), alcohol dehydrogenase, or mixed function oxidase (Morris, 1993) served to enhance vapor uptake in that site. Aldehyde dehydrogenase (AldDH) is known to be present in the nose of the rat and is thought to represent a detoxification pathway for aldehydes (Casanova-Schmitz et al., 1984; Bogdanffy et al., 1985; Lindahl, 1992). Thus, it was hypothesized that inspired acetaldehyde was significantly metabolized in the nose via this enzyme and that capacity limitation of nasal metabolism might play a role in the inspired concentration dependence in uptake that was observed (Morris and Blanchard, 1992). The term capacity limitation is used to describe the situation in which the rate at which acetaldehyde is delivered to nasal tissues (µmol/min, measured as V<sub>max</sub>) of that site.

The current study was designed to confirm and extend our previous results in the F344 rat. Specifically, it is not known if altered dosimetric relationships at high versus low exposure concentrations represent phenomena specific to the F344 rat or if they are more generalized in nature. Information on the presence of AldDH in the nose of additional species is also unavailable, as is information on the quantitative relationships between acetaldehyde uptake and detoxification capacity via this enzyme. To provide such information, the current study included measurement of URT deposition efficiencies at inspired concentrations of 1, 10, 100, and 1000 ppm. In addition, previously used methods (Casanova-Schmitz et al., 1984) were used to provide initial characterization of AldDH kinetics in nasal tissues of the B6C3F1 mouse, Sprague-Dawley (SD) rat, Syrian hamster, and Hartley guinea pig. The overall aim was to determine if any species-specific relationships exist in nasal uptake, and, if so, to provide an initial examination of possible correlations between uptake and aldehyde dehydrogenase levels.

**METHODS**

**Animals and reagents.** Specific pathogen-free male B6C3F1 mice (B6C3F1/CrlBR, 19–21 g), Syrian golden hamsters (HarV/GsYBR, 91–100 g), Sprague–Dawley (SD) rats (VAF/Plus Crl:CDBR, 170–230 g), and Hartley guinea pigs (Crl:HA BR, 250–300 g) were obtained from Charles River Laboratories. Animals were housed over hard wood bedding in animal rooms maintained at 22–25°C with a 12-hr light–dark cycle (lights on 6:30 AM). Animals were acclimated for at least 1 week prior to use and were used within 4 weeks of arrival. Acetaldehyde (99% pure) was obtained from Aldrich Chemical (Milwaukee, WI). All other reagents were obtained from local suppliers and were of the highest purity available.

**Animal 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 into the trachea such that its tip lay at the larynx. The animal was then placed in a nose-only inhalation chamber (CH Technologies, Westwood, NJ) and chamber air was drawn through the isolated URT for 60 min under the flow conditions described below. Respiration rates were counted at 10-min intervals during the exposure. Immediately after the end of exposure each animal was killed by exsanguination. The nasal cavity was then lavaged once with 3 ml saline via the endotracheal tube, with the saline being collected at the external nares. Average recovery of fluid exceeded 2.5 ml.

To collect nasal tissues for AldDH determination, the skull of unexposed, urethane-anesthetized animals were split sagittally and tissues from the entire nasal cavity were removed and homogenized in cold Krebs–Ringer buffer. Final homogenate volumes were 5, 6, and 8 ml, respectively, for the hamster, SD rat, and guinea pig. For the mouse studies, tissues from three mice were pooled; the final homogenate volume was 9 ml. Homogenates were spun (10,000g) and the supernatant was stored on ice for subsequent AldDH activity determination as described below. Protein concentrations were less than 3 mg/ml in the supernatants.

**Deposition measurement.** To measure URT deposition, vapor-laden air was drawn through the isolated URT under the airflow conditions described below and vapor concentrations were measured in chamber air (i.e., air entering the URT, C<sub>I</sub>) and air exiting the URT (C<sub>O</sub>). Deposition efficiency was calculated from the difference between these two concentrations (Morris, 1990). C<sub>O</sub> was measured throughout the 60-min exposure period. For each animal, chamber air concentration (C<sub>I</sub>) was measured immediately before and again immediately after C<sub>O</sub> determination using the same flow conditions as used for C<sub>O</sub> determination. The average value of these chamber concentrations was used to calculate deposition efficiency.

An indirect index of the steadiness of the chamber vapor concentrations during exposure is provided by comparison of the "before" and "after" chamber concentrations. For the mouse, hamster, rat, and guinea pig studies. The after samples averaged 99 ± 5, 97 ± 5, 96 ± 7, and 97 ± 5% (mean ± SD), respectively, of the before samples. Since the before and after samples were separated by 60 min these ratios suggest that chamber air levels changed by 0.1% or less per minute during the exposure (assuming a linear change during the 60-min exposure period).
NASAL UPTAKE AND METABOLISM OF ACETALDEHYDE

TABLE 1  
Inspiratory Flow Rates and Predicted Minute Ventilation

<table>
<thead>
<tr>
<th>Species</th>
<th>$V_m$ (ml/min)</th>
<th>Inspiratory flow rates (ml/min)</th>
<th>Inspiratory flow rates (% predicted $V_m$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse*</td>
<td>23</td>
<td>25 35 70</td>
<td>52 40 80</td>
</tr>
<tr>
<td>(24 g)</td>
<td></td>
<td></td>
<td>109 152 304</td>
</tr>
<tr>
<td>Hamster</td>
<td>88</td>
<td>70 100 200</td>
<td>52 48 80</td>
</tr>
<tr>
<td>(140 g)</td>
<td></td>
<td></td>
<td>109 152 304</td>
</tr>
<tr>
<td>SD rat</td>
<td>150</td>
<td>200 400 750</td>
<td>52 48 80</td>
</tr>
<tr>
<td>(275 g)</td>
<td></td>
<td></td>
<td>109 152 304</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>210</td>
<td>400 750</td>
<td>52 48 80</td>
</tr>
<tr>
<td>(440 g)</td>
<td></td>
<td></td>
<td>109 152 304</td>
</tr>
</tbody>
</table>

*Minute ventilation for each species was predicted from the equation of Guyton (1947) as described by Phalen (1984). The body weights are the actual average body weights of the animals used in the studies.

Air sampling. The sampling system used for drawing air samples consisted of a glass trap (6 ml) connected to two vacuum sources. Air was drawn off the trap (approx 7 ml/min) and into a gas chromatograph (see below) via heated tubing. Air samples were injected into the chromatograph every 3.0 min to provide continuous monitoring. Polyethylene tubing was used to connect the trap to the chamber sampling port ($C_m$ determination) or the animal endotracheal tube ($C_e$ determination). Total air flow rates were maintained at the desired flow by a rotameter which had been previously calibrated in the sample line.

Flow conditions. Selection of unidirectional inspiratory flow rates was based on three criteria. First, flows were selected to be consistent with our previous studies (Morris 1990, 1993, 1994; Morris and Blanchard, 1992). Second, it was desired that the flow rates cover a wide, but physiologically reasonable range and that they reflect approximately equal fractions of the predicted minute ventilation of each species. Finally, to facilitate possible future comparisons among species it was desired that whenever possible identical flow rates also be used in each species. The mouse studies also included flows of 18 and 100 ml/min, corresponding to 78 and 435% of the predicted minute ventilation rate, respectively.

Airborne water content (wet/dry bulb) averaged about 40 mg/liter corresponding to roughly 90% 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 endotrachéal tubing.

Chamber atmospheres were generated with a syringe pump system. Aqueous acetaldehyde solutions (prepared daily) were fed into a 500-ml flask maintained at 100°C. Air (0.6 liters/min) was passed through the flask and into the chamber diluting air line. Chamber concentrations were controlled by changing the solvent concentration in the aqueous generation fluid. The chamber was operated for at least 45 min prior to measurement of deposition to allow for equilibration.

Nominal acetaldehyde concentrations for these studies were 1, 10, 100, and 1000 ppm. The actual concentrations averaged 1.8, 18, 160, and 1600 µg/liter corresponding to 1.1, 11, 94, and 940 ppm. The coefficient of variation for day-to-day variability in chamber concentration was approximately 13%; within each day, chamber concentration variability was approximately 5% (see above).

Analytical techniques. Acetaldehyde concentrations were measured in a Varian Model 3600 gas chromatograph (GC) 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 33°C and a carrier gas (N$_2$) flow rate of 30 ml/min. For analysis, air was continuously passed through one of two sample loops in an eight-port gas sampling valve. Samples were injected on the column every 3.0 min to provide continuous monitoring. Peak areas (Varian Model 4290 integrator) were converted to concentrations on the basis of a standard curve by injecting 4-µl aliquots of aqueous acetaldehyde standards into teflon gas sampling bags (Cole-Parmer, Niles, IL) which were then filled with 0.8 liter of clean air. After at least 1 hr to allow for evaporation, air was drawn from the bags through the sample line used for deposition measurement and into the GC gas sampling valve for analysis.

Partition coefficient determination. Since acetaldehyde is directly reactive with sulfhydryl and amino groups it was not possible to determine a blood:air or tissue:air partition coefficient. As a surrogate, the water:air partition coefficient was determined as described previously (Morris, 1990; Gargas et al., 1989). Briefly, known amounts of acetaldehyde were added to 40-ml gas sampling vials containing 0.2 ml of fluid. After incubation at 37°C for 1 hr or more in a 1-ml headspace air sample was withdrawn with a warm syringe and injected to the gas sampling valve of the GC for analysis of acetaldehyde concentration as described above. Headspace air concentrations ranged between 10 and 1800 ppm. The water:air partition coefficient was determined based on the decreased headspace air concentration in the fluid-containing vials assuming mass balance.

Biochemical measures. Lavage fluid was analyzed for protein by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Nasal homogenate 10,000g supernatant AldDH activity was measured spectrophotometrically by quantitating acetaldehyde-dependent formation of NADH at 340 nm during the first 1–5 min after addition of acetaldehyde as described by Casanova-Schmitz et al. (1984). (Initial studies revealed that the activity in the 10,000g pellet was quite low, averaging sevenfold or more lower than in the supernatant.) The total incubation volume was 1.0 ml, including the 0.2- to 0.4-ml aliquot of homogenate that was added. Final NAD concentration was 4 mM (Morris and Blanchard, 1992). The
alcohol dehydrogenase inhibitor, 4-methylpyrazole, was included at a concentration of 0.75 mM. To establish enzyme kinetics acetaldehyde as added to achieve final concentrations of 0.05–60 mM. This is approximately the same span of concentrations utilized by Casanova-Schmitz et al. (1984) in their studies of AldDH kinetics in the F344 rat nose. At substrate concentrations less than 0.05 mM enzyme velocities were not easily detectable (less than 0.001 absorbance unit per minute). Limits of detection could not be increased by using more concentrated homogenates due to the absorbance of the homogenate itself. The NADH formation in the absence of acetaldehyde was used as a control and subtracted from the NADH formation rate measured in the presence of acetaldehyde. The NADH formation rate was linear with protein concentration, and was calculated for the period in which it was linear with time.

**Mathematical analysis.** All data are presented as means ± SD unless otherwise indicated. All statistical calculations were performed with Statistica software (Statsoft, Inc., Tulsa, OK), with $p < 0.05$ being required for significance.

**RESULTS**

*Partition coefficient.* The water:air partition coefficient for acetaldehyde averaged $140 ± 20$ $(n = 6)$. The value was similar over the entire range of acetaldehyde concentrations. Similar values were obtained regardless of the amount of fluid present in the vials.

*Aldehyde dehydrogenase kinetics.* Shown in Fig. 1 are representative Eadie–Hofstee plots for metabolism of acetaldehyde in nasal homogenates. In the mouse, rat, and hamster apparent high-affinity and low-affinity isozymes were observed (Fig. 1A). In contrast, only a single (high-affinity) form was detected in guinea pig nose homogenates (Fig. 1B). Nonlinear least squares regression analysis of enzyme velocity versus substrate concentration (assuming two isozymes for all but the guinea pig) was used to estimate the kinetic parameters for each species. These parameters were then used to generate the fit lines that are shown.

Shown in Table 2 are the kinetic parameters for each species. The body weights and inspiratory flow rates used in each species differed dramatically. Therefore, to facilitate direct cross-species comparisons, the estimated $V_{\text{max}}$ values for each species were normalized to the predicted minute ventilation of that species (based on body weight). This is the most straightforward comparison because the flow rates used in these studies were also normalized to predicted minute ventilation. It should be noted that nasal tissue volumes are also related to body weight, thus a body weight-based flow rate normalization intrinsically incorporates some degree of tissue volume normalization. In absolute units, total metabolic capacity, defined as the sum of $V_{\text{max}}$ for both high- and low-affinity isozymes, averaged $0.04, 0.16, 0.56$, and $0.14 \mu\text{mol/min/whole nose}$ for the mouse, hamster, SD rat, and guinea pig, respectively.

Significant differences were observed among species for the high-affinity–low-capacity isozyme (analysis of variance, $p < 0.05$). Specifically, for the guinea pig the $V_{\text{max}}$ was significantly greater than for the other species; the $K_m$ was significantly higher than for the hamster or rat, but not for the mouse. No significant differences were observed among the mouse, rat, and hamster. The kinetic data for the high-affinity–low-capacity isozyme should be interpreted with caution as the lowest substrate concentration used was $0.05 \text{mm}$, a concentration twofold greater than the estimated $K_m$ for most species. $V_{\text{max}}/K_m$ ratios were not calculated due to the uncertainty in the estimated $K_m$ value.

The low-affinity–high-capacity isozyme was not detected in the guinea pig nose, but was observed for the mouse, hamster, and rat. It is, of course, possible that its presence went undetected due either to a very low $V_{\text{max}}$ or a high $K_m$. The $K_m$ values for the low-affinity isozyme...
were similar for the rat and hamster, but significantly higher in the mouse (Newman–Keuls test). In contrast, the \( V_{\text{max}} \) value was significantly greater in the rat than in either the mouse or the hamster (Newman–Keuls test). At low substrate concentrations, the metabolism rate may be best approximated by the \( V_{\text{max}}/K_m \) ratio. This ratio (normalized for ventilation rate) was significantly different in all species with the least activity in the mouse followed by the hamster and then the rat (Table 2).

**Deposition—individual species analysis.** In all four species, URT uptake of acetaldehyde quickly attained and maintained an apparent steady state during the exposure. In the mouse, hamster, rat, and guinea pig steady-state deposition efficiency (calculated from the \( C_{\text{ex}} \) values obtained between 20 and 60 min of exposure) changed on the average by 0.05 \( \pm 0.02 \) for the unidirectional flows for each species at inspired concentrations of 10, 100, and 1000 ppm averaged two- to threefold less that at 1 or 10 ppm. In all species similar relationships were seen in the unidirectional and cyclic flow regimes.

It is possible to calculate the steady-state delivered dosage rate (\( \mu \text{mol/min} \cdot \text{nose} \)) for the unidirectional flows for each species by multiplying the inspired concentration by the flow rate and the deposition efficiency. At each concentration, the dosage rate depends on the flow rate. The range of dosage rates observed at each concentration is shown in Table 3. The total AldDH metabolic capacity as measured in vitro for that species is shown for comparative purposes. In all species the dosage rate increased with increasing exposure concentration. The increase was not linear, however, because deposition efficiencies decreased with increasing concentration. In all species the delivered dosage rate at an inspired concentration of 1000 ppm exceeded the total metabolic capacity as determined in vitro. In the mouse, hamster, and guinea pig, but not in the rat, the delivered dosage rates at 100 ppm may have exceeded the metabolic capacity.

**Deposition—species comparison.** Deposition among the species at inspired concentrations of 10, 100, and 1000 ppm were compared by three-factor ANOVA with the factors being species, exposure concentration, and flow rate. Significant effects of species \((p < 0.001)\), exposure concentration \((p < 0.001)\), and flow rate \((p < 0.001)\) were observed. In addition several statistically significant interactions were detected: between species and concentration \((p < 0.001)\), concentration and dosage rate \((p < 0.001)\), and dosage rate and exposure concentration \((p < 0.001)\).
concentration and flow \((p < 0.001)\), and among species, concentration, and flow \((p < 0.01)\). No interaction between species and flow \((p > 0.50)\) was observed, perhaps because the flow rates were normalized to minute ventilation. Therefore, separate two-factor analyses of variance were done for the 1000-, 100-, and 10-ppm data, as well as for the mouse, hamster, and rat 1-ppm data. The two factors were species and flow rate.

At no exposure concentration was a significant interaction between species and flow observed by two-factor ANOVA \((p > 0.05\) for each case). However, significant differences among species were detected at each exposure concentration. These relationships are depicted graphically in Fig. 3. Shown for each exposure concentration is the average deposition efficiency (at unidirectional flows of approximately 50, 100, 200, and 275% of the minute ventilation) of all animals at that concentration. Since no interaction between species and flow rate were observed, statistically similar effects occurred at all flows. The average deposition efficiency among all flows was selected for this plot as the best representation of the overall effects in each species.

Surprisingly, the differences among species were dependent on the exposure concentration. For example, at 1000 ppm, uptake efficiency was significantly greater in the mouse than in the hamster, whereas at 10 and 1 ppm the opposite was observed. At 1000 ppm uptake efficiency in the guinea pig was significantly lower than that in any other species. In contrast at 10 ppm uptake in the guinea pig was significantly higher than that in the rat and was similar to that in the mouse.

**Biologic parameters.** Respiration were counted every 10 min during the exposure and were not altered from control levels by exposure to acetaldehyde (ANOVA, \(p > 0.05\)) in any species. Respiration frequency averaged approximately 150 breaths/min in the mouse, hamster, and SD rat and 100 breaths per minute in the guinea pig. Nasal lavage protein levels averaged approximately 140, 300, 400, and 600 \(\mu\)g/ml in mice, hamsters, rats, and guinea pigs, respectively, and were not significantly different between any groups, suggesting the absence of marked toxicity at 1000 ppm versus 1 ppm.

**DISCUSSION**

The documentation of diminished nasal acetaldehyde uptake efficiencies at high versus low inspired concentrations in the mouse, hamster, SD rat, and guinea pig, and under either unidirectional or pseudo-cyclic flow conditions, provides strong evidence that the concentration dependence on nasal uptake efficiency is a generalized phenomenon among rodent species. The magnitude of this effect is very similar in all animal models examined to date, including the F344 rat (Morris and Blanchard, 1992). In all species, the concentration dependence was quite large, with deposition efficiency being two- to threefold more efficient at inspired concentrations of 1 or 10 ppm compared to 1000 ppm. The mechanisms of this effect are not known, but the diminished uptake at high concentrations suggests that some saturable process is involved.

Irrespective of the mechanism(s) involved, these data indicate that exposure concentration-delivered dose relationships for the nose are very different at high exposure concentrations such as those used in toxicity testing, compared to low, more environmentally relevant concentrations. Whether

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**TABLE 3**

Delivered Dosage Rates (nmol/min/nose) and AldDH Metabolic Capacities*  

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Delivered dosage rate (nmol/min/nose)</th>
<th>Metabolic capacity (nmol/min/nose)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mouse</td>
<td>Hamster</td>
</tr>
<tr>
<td>1</td>
<td>0.4-1</td>
<td>2-5</td>
</tr>
<tr>
<td>10</td>
<td>3-8</td>
<td>14-32</td>
</tr>
<tr>
<td>100</td>
<td>30-60</td>
<td>100-200</td>
</tr>
<tr>
<td>1000</td>
<td>180-500</td>
<td>400-100</td>
</tr>
</tbody>
</table>

* Delivered dosage rates are calculated as the product of the inspired concentration (nmol/liter), the flow rate (liter/min), and the deposition efficiency. A range is given because the delivered dosage rate at each inspired concentration differed for each of the inspiratory flow rates. Metabolic capacity is defined as the sum of the \(V_{\text{max}}\) values for the high-affinity-low-capacity and the low-affinity-high-capacity forms of AldDH.
this phenomenon occurs in the human is unknown; however, the current results suggest the need for caution in directly extrapolating high inhalation concentration rodent data to predict effects at lower exposure concentrations. This phenomenon may be important for interpretation of both nasal and pulmonary toxicity data, because diminished nasal uptake could lead to greater dose delivery to the lungs.

The potential for an inspired concentration dependence on deposition efficiency has been studied for a few vapors. Uptake of the nonreactive nonmetabolized vapor, acetone, in the nose of the B6C3F1 mouse remained constant at exposure concentrations ranging from 1500 to 20,000 μg/liter (Morris, 1991), indicating that a concentration dependence on uptake is not universally present among vapors. This result is consistent with the hypothesis that acetone uptake is dependent on diffusion to and removal from nasal tissues via the bloodstream, which is a first-order nonsaturable process (Morris et al., 1993). For the reactive vapor acrolein, uptake efficiency in the F344 rat nose was found to be roughly twofold less at an inspired concentration of 10 compared to 1 ppm (Morris, 1996). Uptake of sulfur dioxide, another reactive vapor, was found to be much more efficient at high compared to low concentrations in the rabbit nose (Strandberg, 1964); however, other investigators did not observe an effect of similar magnitude in the dog (Frank et al., 1969).

Mechanisms of URT vapor uptake have been discussed previously (Morris, 1994; Morris et al., 1993; Gerde and Dahl, 1991). Acetaldehyde is both reactive and metabolized in nasal tissues. Both reaction-based uptake, via depletion of tissue substrates, and metabolism-based uptake, via saturation or capacity limitation, would be expected to be saturable processes. From the current results it is not known with certainty if metabolism via AldDH serves to enhance uptake of acetaldehyde. This seems likely as the current studies indicate that acetaldehyde is extensively metabolized by nasal tissues in vitro and our previous work has demonstrated that nasal metabolism via carboxylesterase, mixed function oxidases, or alcohol dehydrogenase (Morris, 1990, 1993) serves to enhance uptake efficiencies for vapors which are substrates for those enzymes. Moreover, initial studies indicated that AldDH inhibition diminished uptake of acetaldehyde vapor in the F344 rat nose (Morris and Blanchard, 1992).

The current results provide strong evidence that in all four species of rodents, nasal AldDH metabolism is overwhelmed at inspired concentrations of 1000 and, perhaps, 100 ppm. This provides a likely explanation for the diminished uptake at 100 or 1000 ppm compared to that at lower concentrations. In all species, the delivered dosage rates at 1000 ppm (nmol/min) exceeded by severalfold the AldDH V_{\text{max}} values (nmol/min) as determined under optimized in vitro conditions (Table 3). Thus, even if acetaldehyde were metabolized in vivo at optimal rates and even if tissue concentrations were well above the \( K_m \), only a small fraction of the deposited burden could be metabolized via this enzyme. At low inspired concentration, the AldDH V_{\text{max}} values are not exceeded and it is possible that a large fraction of the deposited burden could be metabolized via this detoxifying enzyme.

The current study utilized nonphysiological flow conditions in an anesthetized animal model; thus the actual deposition efficiencies in awake, normally breathing animals are not known. It is noted, however, that the dose intervals in the current study were spaced by orders of magnitude, and the estimated metabolism and deposition rates at 1000 ppm differ by as much as 10-fold, suggesting that similar relationships would occur in normally breathing animals even if the measured deposition efficiencies were not strictly reflective of those in normal animals. In general, the toxic and carcinogenic effects of acetaldehyde in nasal tissues are only observed at concentra-

FIG. 3. Shown is the composite URT deposition efficiency for each species at each inspired concentration. The composite deposition efficiency is the overall average deposition efficiency at the four unidirectional inspiratory flow rates corresponding to approximately 50, 100, 200, and 375% of the predicted minute ventilation for each species (see text for details). At each concentration data were analyzed by two-factor analysis of variance. A significant effect of species and flow rate was observed at each concentration, but no significant interactions indicating statistically similar relationships were observed among all species regardless of the flow rate. Labels above the bars refer to the comparisons of deposition among species: bars with differing superscripts are significantly different from each other (\( p < 0.05 \), Newman–Keuls test).
tions exceeding 400 ppm (Appelman et al., 1982; Kruysse, 1975; Morris, 1996b). This may reflect that nasal detoxification capacities become overwhelmed at concentrations of 400 ppm or more in animals breathing normally.

As evidenced by acetaldehyde-stimulated reduction of NAD to NADH, AldDH activity was demonstrated in tissues of all four species utilized in this study, suggesting that this enzyme is ubiquitously expressed in rodents. Unfortunately, the methodology for measuring AldDH activity was not ideal, as activities could not be measured at low substrate concentrations. Precise quantitation of nasal AldDH kinetics may require development of enhanced methodologies. Species differences were detected in AldDH activity. The most dramatic difference is, perhaps, the apparent lack of a high-capacity, low-affinity isozyme in the guinea pig nose. This may be compensated for, in part, by the higher $V_{\text{max}}$ and $K_m$ values of the low-capacity, high-affinity isozyme in this species. Also apparent were the relatively high levels of the high-capacity-low-affinity isozyme in the rat compared to the other species. The F344 rat also appears to possess high levels of this isozyme. From the data of Casanova-Schmitz et al. (1984), total nasal cavity AldDH activities of 13–16 $\mu$mol/min per liter/min ventilation are estimated (assuming 4–5 mg protein in respiratory and olfactory mucosa and 100 ml/min ventilation rate, Morris and Blanchard, 1992). These values are even higher than that observed in the SD rat in the current study. Interestingly, species differences in sensitivity of acetaldehyde do not appear to correlate with tissue levels of AldDH. Specifically, the rat nose appears to be more sensitive to acetaldehyde than the hamster nose (see Introduction), yet the rat nasal mucosa appears to express higher levels of AldDH. Perhaps the metabolic product of AldDH, acetic acid, plays a role in the olfactory toxicity of acetaldehyde in the rat. The olfactory mucosa of the rat is known to be sensitive to acids (Morris, 1996b; Stott and McKenna, 1985; Trela and Bogdanffy, 1991). Whether AldDH is expressed in human nasal tissue is not known; however, its widespread presence in nasal tissue among at least four other species and its presence in many human tissues (Lindahl, 1992) may suggest that it may also be expressed in the human nose.

Statistically significant species differences in URT acetaldehyde uptake were observed in the current study. Based on the limited AldDH metabolic capacity compared to the delivered dosage rates at 1000 ppm in the current study (see above) it is unlikely that AldDH plays a role in the species differences that were observed at high concentrations. Interestingly, the rank order of species on the basis of ability to scrub acetone from the airstream, as measured in urethane-anesthetized animal in our previous studies, is mouse. SD rat, hamster, and guinea pig (Morris, 1994). Exactly the same rank order was observed for acetaldehyde uptake at 1000 ppm (Fig. 2), suggesting that at 1000 ppm the species differences in acetaldehyde uptake were reflective of generalized differences among species in ability to scrub vapors in the nasal cavity.

Species differences observed at low inspired concentrations were different from those observed at 1000 ppm. The most dramatic differences were observed among the guinea pig versus rat and the mouse versus the hamster. Uptake was more efficient in the rat than the guinea pig and the mouse than the hamster at 1000 ppm, but the reverse was observed at 1 or 10 ppm (Fig. 3). The relationships at low concentrations may or may not reflect species differences in AldDH. Comparison of metabolism kinetics (Table 2) with species differences (Fig. 3) reveals no clear-cut correlation between uptake efficiency and metabolism except, perhaps, for the guinea pig. At 10 ppm, uptake in the guinea pig was similar to that in the mouse and greater than that in the rat, yet at 100 ppm uptake in the guinea pig was the least of all species and significantly less than in the rat. The diminished uptake at 100 ppm in the guinea pig may correlate with the absence of a high-capacity-low-affinity isozyme. Further studies are needed, however, to assess the potential role of AldDH in the species differences in acetaldehyde uptake. Irrespective of the mechanism involved, it is important to recognize that the current study indicates that dosimetric relationships among rodent species may differ significantly at high compared to low exposure concentrations.

In summary, the current results indicate that aldehyde dehydrogenase is present in nasal tissue of four rodent species, the mouse, hamster, rat, and guinea pig. Species differences were detected in both the amount of enzyme activity present and the presence of specific isoforms. URT uptake of acetaldehyde was strongly dependent on the inspired concentration, with uptake efficiency at an exposure concentration of 10 or 1 ppm exceeding that at 1000 ppm by two- to threefold in all four species. These differing dosimetric relationships indicate the need for caution in direct linear extrapolation of high-exposure concentration data to predict toxic effects at lower concentrations. Species differences were observed in acetaldehyde uptake efficiency, with quantitatively differing dosimetric relationships among the species being observed at high versus low concentrations. Indeed, even the rank order of species on the basis of deposition efficiency was found to be markedly different at inspired concentrations of 1 or 10 versus 1000 ppm. This, again, indicates that inhalation dosimetric relationships observed at high exposure concentrations may not be reflective of those occurring at lower, more environmentally or occupationally relevant levels. In toto, these results highlight the potential dosimetric complexities involved in the extrapolation of high concentration inhalation toxicity data.

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