Longitudinal distribution of chlorine absorption in human airways: comparison of nasal and oral quiet breathing

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Nodelman, Vladislav, and James S. Ultman. Longitudinal distribution of chlorine absorption in human airways: comparison of nasal and oral quiet breathing. J. Appl. Physiol. 86(6): 1984–1993, 1999.—The fraction of an inspired chlorine (Cl₂) bolus absorbed during a single breath (A) was measured as a function of bolus penetration (Vₚ) into the respiratory system of five male and five female nonsmokers during both nasal and oral breathing at a quiet respiratory flow of 250 ml/s. The correspondence between Vₚ and specific anatomic landmarks was found for each subject by a combination of acoustic reflection and nitrogen washout measurements. For both nasal and oral breathing, A reached ~0.95 at the distal end of the upper airways and reached 1.00 within the lower conducting airways. The values of a regional mass transfer parameter computed from the A-Vₚ data indicated that the resistance to Cl₂ diffusion in the airway mucosa was negligible compared with the diffusion resistance in the respired gas. Changing the peak inhaled Cl₂ concentration from 0.5 to 3.0 parts/million did not significantly affect the distribution of Cl₂ absorption, suggesting that the underlying mass transport and chemical reaction processes were linear with respect to Cl₂ concentration.

Over their entire lifetime, people might experience long-term cumulative Cl₂ exposure leading to irreversible tissue damage and functional impairment, even at concentrations below 0.5 ppm. To investigate this possibility, female and male B6C3F₁ mice as well as F344 rats were exposed to Cl₂ concentrations of 0.4–2.5 ppm for 6 h/day, 5 days/wk (3 days/wk for female rats) for up to 2 yr (28). Exposure-dependent nonneoplastic lesions were detected at all Cl₂ concentrations, were confined to the nasal cavities of both rodents, and were most severe in the anterior nose. Respiratory and olfactory epithelial degeneration, septal fenestration, and secretory cell metaplasia were some of the Cl₂-induced histological changes that were observed even at an exposure concentration of 0.4 ppm. In a similarly designed study in which rhesus monkeys were exposed to Cl₂ concentrations of 0.1–2.3 ppm for up to 1 yr (11), nonneoplastic nasal lesions were only observed at the highest exposure concentration. As in the rodents, the severity of the lesions decreased with distal distance into nasal cavities. Although lesions in the monkeys were milder than in the rodents, the lesions in the monkeys penetrated beyond the nose into the trachea.

To utilize these observations in the prediction of health effects on people, one must understand the factors that influence the severity and spatial distribution of Cl₂-induced tissue damage. One of these factors is the regional pattern of Cl₂ absorption. Because Cl₂ is a highly soluble gas, its principal site of absorption is most likely the upper airways, the same airways in which the majority of Cl₂-induced lesions have been observed in animal studies. Moreover, a progressive loss of Cl₂ from the inhaled gas stream as it passes over more and more airway surface is a logical explanation for the anterior-to-posterior attenuation of lesion severity observed in monkeys as well as in rodents. The deeper penetration and less severe tissue responses in monkeys may have resulted from slower Cl₂ absorption in their larger-diameter airways and may also have been related to the fact that primates can breathe in an oronasal fashion whereas rodents are obligate nasal breathers.

The purpose of the present study was to observe the longitudinal distribution of Cl₂ absorption in intact human airways during quiet breathing by employing the noninvasive bolus inhalation method that was previously developed for ozone (O₃), another oxidant gas that can pollute inhaled air (8). This first required that the inhalation apparatus be modified so that it could deliver Cl₂ boluses and could continuously monitor Cl₂ concentration. By using the modified apparatus, bolus measurements were compared during nasal and oral breathing to determine whether the site of air pollution; inhalation toxicology; lung dosimetry; regional uptake; mass transfer coefficient; conducting airways.

CHLORINE (Cl₂) is a reactive oxidant gas used in the large-scale production of chlorinated hydrocarbon solvents, polyvinyl chloride plastics, and pharmaceuticals, in the bleaching of paper, and in the disinfection of drinking and swimming pool water. The average Cl₂ level in contemporary occupational settings is mandated to be 0.5 parts/million (ppm) or less over an 8-h workshift (1). This is less than the 1–3 ppm range in which people first perceive irritation of their eyes or respiratory tract (18). This is also below the Cl₂ concentration at which people first perceive irritation of their eyes or respiratory tract (18). This is also below the Cl₂ concentration at which people first perceive irritation of their eyes or respiratory tract (18). This is also below the Cl₂ concentration at which people first perceive irritation of their eyes or respiratory tract (18). This is also below the Cl₂ concentration at which people first perceive irritation of their eyes or respiratory tract (18). This is also below the Cl₂ concentration at which people first perceive irritation of their eyes or respiratory tract (18).
access influenced the penetration of Cl₂ beyond the upper airways. To investigate the possibility of nonlinear chemical reaction effects in the airway mucosa, bolus measurements were also made at different peak inhaled Cl₂ concentrations. The resulting Cl₂ distribution data were referenced to specific anatomic landmarks by using upper airway and total conducting airway volumes measured in each subject with acoustic reflection and nitrogen-washout methods, respectively.

METHODS

Subject population. Five healthy male and five healthy female nonsmokers participated in this investigation. After reading an explanation of the study, each subject completed an informed consent form, a medical questionnaire, and a standard spirometry test to determine his or her forced vital capacity (FVC) and forced expired volume in 1 s (FEV₁). Each subject was selected without regard to body size and was included in the study only if he or she met the following criteria: was between 18 and 40 yr old; had not smoked within the past 3 yr; had no history of hay fever, asthma, allergic rhinitis, chronic respiratory disease, or cardiovascular disease; had not used medication within 1 wk of the experiment; was not exposed to air pollution on a daily basis; did not swim more than once a week in a chlorinated swimming pool; and had an FEV₁-to-FVC ratio >75% of the predicted value (12). Each female participant provided menstrual information, was administered a human chorionic gonadotropin-urine pregnancy test (QuickVue, Quidel) at the beginning of the session, and was excluded from the study if this information suggested that she was pregnant. All procedures were approved by the Institutional Review Board of the Pennsylvania State University.

Instrument development. The design and performance of the bolus inhalation system as configured for O₃ measurements were described in detail in previous publications (8–10). The apparatus originated from a breathing assembly that contained an injection port connected to an O₃ bolus generator, a pneumotachometer to monitor respired flow, and a sampling port connected to a fast-responding O₃ analyzer. For the present study, a new breathing assembly, Cl₂ bolus generator, and fast-responding Cl₂ analyzer were constructed. To minimize absorption of Cl₂, the internal surfaces of these devices were fabricated of Teflon, wherever possible.

The breathing assembly (Fig. 1, 1–3) consisted of a specially machined Teflon tube that incorporated a mild Venturi-type constriction (Fig. 1, 1). The distal end of the breathing assembly could be fitted with one of two breathing fixtures (Fig. 1, 2). One fixture was a flexible plastic mouthpiece, and the other was a nasal cannula. The nasal cannula was composed of rigid plastic manifold holding a pair of flexible rubber connectors that provided a comfortable but tight fit with both nostrils. The internal volume of each breathing fixture was 20 ml. Located near the distal end of the breathing assembly was a 1/8-in. Swagelock fitting connected to the inlet metering valve of the Cl₂ analyzer. Located near the proximal end of the breathing assembly was a 1/4-in. Swagelock fitting that was connected to the Cl₂ bolus generator. The proximal end of the breathing assembly was fitted with a pneumotachometer (Fig. 1, 3) for monitoring respiratory flow.

The bolus generator (Fig. 1, 4–9) utilized a diffusion chamber (Fig. 1, 7) containing a Cl₂ permeation tube (Fig. 1, 8) to produce a chlorinated airstream. The outlet of the diffusion chamber was connected to a pair of three-way solenoid valves (Fig. 1, 4A and 4B) that were separated by a Teflon hold-up tube (Fig. 1, 9). When these valves were in the electrically "off" position, as shown in Fig. 1, chlorinated air continuously flowed through the hold-up tube to an exhaust. To produce a bolus, the valves were simultaneously energized by a data-acquisition system (Fig. 1, 10) such that clean air bypassed the diffusion chamber and all the chlorinated air in the hold-up tube was rapidly propelled into the subject's breathing tube (Fig. 1, 1). To precisely measure the Cl₂ distribution within the airways, boluses with a small volume of ~10 ml and reproducible Cl₂ concentration distributions were necessary. This was achieved by employing a volume of 10 ml for the hold-up tube, a duty cycle of 20 ms for energizing the solenoid valves, and a pressure of 30 psi (Fig. 1, 5B) for propelling the bolus. The peak Cl₂ concentration of the boluses could be conveniently regulated by adjusting the flow rate of clean air (Fig. 1, 6) entering the diffusion chamber.

Because the inner surfaces of the mouthpiece and nasal cannula were constructed of plastic, they might absorb Cl₂ before the inhaled bolus reached the respiratory tract. To check this possibility, the breathing assembly was alternatively fitted with the oral and the nasal fixture, and clean air was supplied to the open end of the pneumotachometer at a flow of 150 ml/s. The concentration of Cl₂ was monitored immediately proximal to the breathing fixture as six boluses were separately injected into the breathing assembly. The Cl₂ concentration was then monitored just distal to the fixture as six additional boluses were injected. By integrating the resulting Cl₂ concentration curves, the mass of Cl₂ entering (proximal sampling) and the mass exiting (distal sampling) the fixture were determined. A two-tailed t-test indicated no significant difference (P > 0.05) between these two sets of measurements for either fixture. Therefore, Cl₂ absorption by
either the mouthpiece or nasal cannula was unlikely, even at the relatively low airflow of 150 ml/s that exaggerated the contact time of Cl2 boluses with the breathing fixtures.

The fast-responding Cl2 analyzer developed for this study (19) was based on the principle of thermionic ionization. The commercial detector used in the analyzer consisted of an electrically heated catalytic cathode that ionized Cl2 to Cl− and a metallic anode that functioned as an electron collector when biased at a positive voltage relative to the cathode. At a sample inlet flow of 600 ml/min, the analyzer had a sensitivity of 3 pA ppm, a minimum detection limit of 0.04 ppm, and a relative humidity range of 3–90% step-response time of 80 ms, and a delay time of 100 ms. The static calibration of the instrument was linear for Cl2 concentrations from 0.03 ppm to 4.0 ppm and was insensitive to variations in temperature, CO2 concentration, and humidity that normally occur in respired air.

In addition to Cl2, the thermionic detector could ionize chlorinated vapors such as chloroform, methyl chloride, and carbon tetrachloride (17). Therefore, chlorinated vapors originating from either endogenous Cl2 metabolism (4, 13, 23) or the reaction of inhaled Cl2 with airway mucosa could be wrongly interpreted as expired Cl2. To check this possibility, the chemical composition of expired breath was measured in three male and two female subjects while they breathed clean air or chlorinated air. In each measurement, 1 liter of slowly expired air was collected in a 9×9-in. Teflon bag (50,090,009, Eagle Picher). The bag was then connected to a gas chromatograph mass spectrometer (589011GC/5792A MSD, Hewlett-Packard) with a Nafion drying tube (MD-070–48F, Perma Pure). The chromatograph utilized a capillary column with a high affinity for halogenated organic compounds (2–4, 154, Supelco). Cryofocusing at the sample inlet of the instrument and again at the head of the chromatographic column concentrated the condensable components of the expired breath sample to achieve a minimum detectable limit of ~0.001 ppm (8533 Cryo-concentrator, Graseby-Nutech).

One expired breath sample was collected immediately after clean air breathing for 2 min, and three replicate breath samples were obtained at the end of a 2-min exposure to 0.5 ppm Cl2. Chlorinated vapors were found in only 2 of the 20 expired breath samples collected from the 5 subjects. In both cases, the concentration of the chlorinated compound in the breath sample was on the order of 0.002 ppm. Because the minimum detection limit of the thermionic ionization analyzer was 10 times larger than this, expired chlorinated compounds were unlikely to affect the Cl2 bolus measurements.

Bolus inhalation measurements. All 10 subjects participated in a 2- to 4-h session in which bolus measurements were made during nasal and oral quiet breathing. The subject was seated comfortably on a stool, wore noseclips during oral breathing, and maintained a closed mouth during nasal breathing. To carry out a bolus test breath, the subject donned the mouthpiece or nasal cannula, activated the inhalation apparatus by depressing a handheld switch, and inhaled beginning at functional residual capacity while viewing a computer monitor on which the integrated pneumotachometer signal (i.e., the expired volume) was displayed in real time. The subject controlled his or her breathing so that the expired volume signal followed a predetermined pattern corresponding to equal inspiratory and expiratory flows of 250 ml/s and a tidal volume of 500 ml. At a predetermined time during inhalation, the data-acquisition system automatically injected a 10-ml Cl2 bolus into the inspired airflow.

Penetration of the bolus into the respiratory system could be systematically varied from breath to breath by changing the bolus injection time relative to the time that the subject was supposed to switch from inhalation to exhalation. The earlier the injection time relative to the end of inhalation, the greater the penetration of the bolus distal to the airway opening. Throughout a test breath, the Cl2 analyzer and pneumotachometer output were continuously recorded on a computerized data-acquisition system at a sampling rate of 200 Hz. The system was also used for triggering a bolus injection valve, integrating the pneumotachometer output to obtain respired volume, and displaying the respired volume on the breathing monitor. The subject took 2–3 test breaths/min, and a collection of 50–70 breaths between bolus penetrations of 0–200 ml constituted a complete experiment.

Three experiments were conducted during the bolus inhalation session: oral breathing with a peak inhaled Cl2 concentration (Cmax) of 3.0 ppm; nasal breathing with a Cmax of 3.0 ppm; and nasal breathing with a Cmax of 0.5 ppm. To avoid possible systematic errors associated with Cl2 preexposure, the sequence in which the experiments were carried within a session was randomized for each subject. In addition, bolus test breaths were arbitrarily carried out at progressively increasing penetration in some experiments but at progressively decreasing penetration in others. In all experiments, a test breath was deemed acceptable if the subject could maintain an average respiratory flow within ±15% of 250 ml/s.

Anatomic measurements. The anatomy of the respiratory system of each subject was characterized by measurements of FVC by using a forced spirometer (model 110 automated spirometer, CDXi), and dead space (Vd) by using a previously described nitrogen-washout apparatus (3). In addition, the nasal volume (VNS), oral volume (VOR), and pharyngeal volume (VPH) of each subject were determined by an acoustic reflection apparatus. The value of FVC represented the average of the highest two of three forced expired volume measurements, Vd was the average of 5–7 washout measurements, and VNS, VOR, and VPH were each determined as the average of six acoustic reflection measurements.

The commercially available acoustic reflection apparatus (Eccovision Acoustic Rhinometry-Pharyngometry System, Hoo Laboratories) consisted of two acoustic wave tubes, one fitted with a rubber mouthpiece and used to obtain oropharyngeal geometry and the other fitted with a rubber nosepiece and used to obtain unilateral nasopharyngeal geometry. During an acoustic measurement, a subject was seated comfortably on a stool and either grasped the mouthpiece or placed the nosepiece adjacent to one nostril. In the case of the nasal measurements, a small amount of petroleum jelly was applied to the tip of the nosepiece to ensure a good seal with the external nares. The subject was instructed to maintain an upright posture and perform a slow expiration while relaxing the glottis. In each measurement, a pulse of white noise was generated in the wave tube, propagated into the subject’s airways, and was partially reflected because of changes in the cross-sectional area of the airway. The incident and reflected sound were monitored with microphones and then converted to a cross-sectional area vs. distance function by using a proprietary computer algorithm.

The length of the nasal cavity and the length of the nasopharynx were estimated from two points of minimum cross section in the nasal area-distance function as previously illustrated by Swift and Proctor (24) (Fig. 2A). Similarly, the length of the oral cavity including the oropharynx and the length of the hypopharynx were estimated from two points of minimum cross section in the oral area-distance function, as previously shown by Fredberg and associates (7) (Fig. 2B).
The volumes of the left and right nasal cavities were determined by integrating each nasal area-distance function over the length of the nasal cavity. The volume of the nasopharynx was calculated as an average of the values obtained by integrating the two nasal area-distance functions over the length of the nasopharynx. The value of VNS was then determined by integrating each nasal area-distance function over its length. The volumes of the left and right nasal cavities were determined by integrating the oral area-distance function over the length of the oral cavity including the oropharynx, and the value of VOR was obtained by integrating the oral area-distance function over the length of the hypopharynx. Because VD measurements were made during oral breathing, the volume of a subject’s lower conducting airways, VLA, was computed as (VD - VOR - VPH).

Analysis of bolus inhalation data. The integrals (M1 and M3), means (VP and VB), and variances (σ2p, σ2b) of the inhaled and the exhaled portion of each test breath were obtained by numerical integration of the Cl2 concentration data with respect to respired volume as illustrated in Fig. 3. These moments were then interpreted as follows: absorbed fraction (A; i.e., 1 - ME/Mi) was the amount of pollutant absorbed during a single respiratory cycle relative to the inhaled amount; penetration volume (VP) represented the mean airway volume that would be reached by inhaled Cl2 molecules relative to the gas-sampling point if no absorption had occurred; breakthrough volume (VBF) symbolized the mean airway volume traversed by unabsorbed Cl2 molecules that reached the gas-sampling point during expiration; and dispersion (σ2; i.e., σ2 - σ2) was a measure of the longitudinal mixing of the unabsorbed Cl2 molecules. A more quantitative definition of these variables can be found elsewhere (8).

By viewing VP as an independent variable that characterized the spatial excursion of an inhaled bolus into the respiratory system, the distributions of A, VP, and σ2 with respect to VP were considered to be the primary dose-distribution information retrieved with the bolus inhalation method. The A-VP distribution for a particular subject during one of the three experimental conditions is illustrated in Fig. 4. To determine the pooled A-VP, VP-VP, and σ2-VP distributions for all subjects at each experimental condition, the A, VP, and σ2 values collected from all test breaths were sorted into 10-ml increments of VP and averaged. The overall SE of each average, including both between-subject and within-subject variations, was computed as previously specified by Kabel and associates (10).

Compartmental analysis and the overall mass transfer coefficient. In modeling Cl2 absorption, the respiratory system was subdivided into nasal-orl (N/O), pharyngeal (PH), lower airway (LA), and respiratory air space (RA) compartments (Fig. 5). Longitudinal position was specified by the penetration VP of a bolus distal to the Cl2-sampling point. The N/O compartment was bounded at its proximal end by the volume of the breathing fixture (VP0 = VPb) consisting of the nasal cannula during nasal breathing and the mouthpiece during oral breathing. The PH compartment was bounded at its proximal end by VP1 = (VBF + VN/O), where VN/O = VNS during nasal breathing and VN/O = VOR during oral breathing. The LA compartment was bounded at its proximal end by VP2 = (VBF + VN/O + VPH + VLA), and the RA compartment was bounded at its proximal end by VP3 = (VBF + VN/O + VPH + VLA).

Fig. 2. Representative acoustic reflection measurements. Area-distance profiles of left nasopharyngeal airways (A) and oropharyngeal airways (B) in 1 subject.

Fig. 3. Representative bolus test breath. Respired volume axis is obtained by integrating respiratory flow relative to end inspiration. The physical interpretation of the integrals (M1, M3), means (VP, VB), and variances (σ2p, σ2b) of the inhaled and exhaled portions of the curve are given in the text.

Fig. 4. Regression of diffusion model to Cl2 distribution data obtained during nasal breathing in 1 subject. Each point represents absorption fraction (A) obtained from a bolus test breath; smooth curve, splined regression of these data according to Eq. 1. Because of extensive absorption in nasal cavity and pharynx, data were insuffcient to determine a mass transfer parameter in lower conducting airways. VP0, VP1, and VP2: boundaries of airway compartments as specified in Fig. 5.
Table 1. Characteristics of the subject population

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 5)</th>
<th></th>
<th>Women (n = 5)</th>
<th></th>
<th>All (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>26.4 ± 6.2</td>
<td>23.0 ± 4.4</td>
<td>24.7 ± 5.4</td>
<td>21–36</td>
<td>18–28</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>81.7 ± 11.7</td>
<td>61.1 ± 5.2</td>
<td>71.4 ± 13.8</td>
<td>64.2–94.8</td>
<td>55.1–68.2</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.81 ± 0.12</td>
<td>1.69 ± 0.07</td>
<td>1.75 ± 0.11</td>
<td>1.68–1.98</td>
<td>1.62–1.98</td>
</tr>
<tr>
<td>FVC, liters</td>
<td>5.7 ± 1.6</td>
<td>3.9 ± 0.9</td>
<td>4.8 ± 1.6</td>
<td>4.0–7.9</td>
<td>2.5–5.0</td>
</tr>
<tr>
<td>Vo, ml</td>
<td>169 ± 30</td>
<td>137 ± 29</td>
<td>153 ± 33</td>
<td>132–208</td>
<td>103–184</td>
</tr>
</tbody>
</table>

n, No. of subjects; FVC, forced vital capacity; Vo, anatomical dead space.

RESULTS

Characteristics of subjects. All the subjects were physically fit individuals; their anthropometric characteristics are summarized in Table 1. Although the men were generally older, taller, heavier, had larger FVC, and had larger Vo than did the women, only the difference in weight was statistically significant (P = 0.007). Compartment volumes of the subjects are summarized in Table 2. Mean values of VNS were similar, VOR was somewhat smaller, and VPH was considerably larger in the men than in the women. The mean value of VNS in the subject population as a whole was somewhat smaller than the corresponding value of VOR. Of these differences in average compartment volumes, only the gender difference in VPH was statistically significant (P < 0.001).
Table 2. Compartment volumes in individual subjects

<table>
<thead>
<tr>
<th>Individual/Average</th>
<th>VNS, ml</th>
<th>VOR, ml</th>
<th>VPH, ml</th>
<th>VLA, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>49.4±1.5</td>
<td>38.2±1.3</td>
<td>26.9±1.4</td>
<td>81.7±10.5</td>
</tr>
<tr>
<td>M</td>
<td>46.6±1.2</td>
<td>21.8±2.2</td>
<td>36.2±3.8</td>
<td>114.0±2.4</td>
</tr>
<tr>
<td>M</td>
<td>42.7±1.5</td>
<td>31.4±1.5</td>
<td>37.4±2.8</td>
<td>63.5±12.1</td>
</tr>
<tr>
<td>M</td>
<td>50.7±0.8</td>
<td>74.6±2.1</td>
<td>27.7±3.0</td>
<td>106.1±16.7</td>
</tr>
<tr>
<td>M</td>
<td>41.9±1.0</td>
<td>76.1±1.5</td>
<td>27.2±3.0</td>
<td>82.5±9.1</td>
</tr>
<tr>
<td>F</td>
<td>38.4±1.5</td>
<td>57.8±1.2</td>
<td>14.7±14.4</td>
<td>111.1±13.5</td>
</tr>
<tr>
<td>F</td>
<td>37.4±0.9</td>
<td>61.3±2.2</td>
<td>11.7±3.0</td>
<td>65.1±6.9</td>
</tr>
<tr>
<td>F</td>
<td>45.1±1.4</td>
<td>79.8±1.9</td>
<td>8.3±2.8</td>
<td>45.2±8.6</td>
</tr>
<tr>
<td>F</td>
<td>43.3±1.0</td>
<td>41.0±1.6</td>
<td>16.8±2.1</td>
<td>70.3±6.6</td>
</tr>
<tr>
<td>F</td>
<td>45.3±0.8</td>
<td>48.3±1.6</td>
<td>14.4±2.2</td>
<td>40.5±13.3</td>
</tr>
<tr>
<td>Average</td>
<td>44±4</td>
<td>54±20</td>
<td>22±10</td>
<td>78±26</td>
</tr>
</tbody>
</table>

Values are means ± SD. VNS, combined volume of the nasal cavity and the nasopharynx; VOR, combined volume of the oral cavity and the oropharynx; VPH, volume of hypopharynx; VLA, volume of the lower conducting airways; M, male; F, female. *6 values measured by acoustic reflection. †Pooled value for all 10 subjects.

There was a large intersubject variability in all the compartment volumes listed in Table 2. As one would expect, Vvo was a good predictor of VLA in the different subjects (coefficient of determination adjusted for number of subjects: r² = 0.71) but was not a predictor of VNS, VOR, and VPH (r² = 0.00, 0.00, and 0.03, respectively). Whereas VD as well as VNS were somewhat correlated with weight (r² = 0.28 and 0.27, respectively), VOR was not correlated with weight (r² = 0.00). The value of Vvo was strongly correlated with height (r² = 0.81), but neither VNS nor VOR was correlated with height (r² = 0.03 and 0.00, respectively). Finally, there was no correlation between VNS and VOR (r² = 0.00).

Cl₂ absorption. The ΔVp distributions pooled for all participants at each of the three experimental conditions (i.e., oral breathing, Cmax = 3.0 ppm; nasal breathing, Cmax = 3.0 ppm; nasal breathing, Cmax = 0.5 ppm) are given in Fig. 6. In general, the inhaled Cl₂ boluses were completely absorbed at a Vp of ~80 ml, which was immediately distal to the upper airways. Considering the magnitude of the SE bars on these graphs, there appears to be little difference between the ΔVp distributions during oral and nasal breathing. On the other hand, decreasing Cmax from 3.0 to 0.5 ppm appears to increase the absorbed fraction of Cl₂ at Vp below 60 ml, which is within the hypopharynx. The Vp-Vp and α²-Vp distributions pooled for all participants during oral and nasal breathing at Cmax = 3.0 ppm are given in Fig. 7. The relationship between Vb and Vp is similar for both modes of breathing, with oral values of Vb being somewhat larger than nasal values at Vp > 30 ml. Values of α² appear relatively insensitive to Vp, with oral values being somewhat larger than nasal values at Vp < 70 ml.

Table 3 summarizes the mass transfer parameters that were calculated from an individual regression of each subject’s ΔVp distribution. The (Ka)NS averaged for all subjects was significantly larger (P = 0.04) than (Ka)OR. On the other hand, there was not a significant difference (P = 0.97) between the average (Ka)NS obtained when Cmax was 0.5 ppm and that when Cmax was 3.0 ppm. Although the data were insufficient to calculate (ΔKa)PH in some of the subjects, there was nevertheless a consistent trend of negative values at all three experimental conditions, indicating that Ka decreases between the nasal or oral cavity and the hypopharynx. The values of (Ka)PH determined by summing the average values of (ΔKa)PH and (Ka)H/O.
Table 3. Compartmental mass transfer parameters in individual subjects during quiet oral and nasal breathing with peak inspired chlorine concentration of 0.5 and 3.0 ppm

<table>
<thead>
<tr>
<th>Individual/Average</th>
<th>Oral, 3 ppm</th>
<th>Nasal, 3 ppm</th>
<th>Nasal, 0.5 ppm</th>
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<tr>
<td></td>
<td>(Ka)</td>
<td>Δ(Ka)</td>
<td>Vp</td>
</tr>
<tr>
<td>M</td>
<td>5.3 ± 0.3</td>
<td>-2.8 ± 1.6</td>
<td>4.8 ± 2.0</td>
</tr>
<tr>
<td>M</td>
<td>6.3 ± 0.7</td>
<td>-3.2 ± 1.1</td>
<td>6.0 ± 3.5</td>
</tr>
<tr>
<td>M</td>
<td>6.5 ± 0.4</td>
<td>-3.8 ± 1.1</td>
<td>8.2 ± 2.2</td>
</tr>
<tr>
<td>M</td>
<td>3.6 ± 0.2</td>
<td>-3.1 ± 1.4</td>
<td>-0.5 ± 2.6</td>
</tr>
<tr>
<td>M</td>
<td>3.7 ± 0.3</td>
<td>-7.4 ± 3.2</td>
<td>7.1 ± 0.4</td>
</tr>
<tr>
<td>F</td>
<td>2.9 ± 0.3</td>
<td>-2.9 ± 4.5</td>
<td>-8.8 ± 4.3</td>
</tr>
<tr>
<td>F</td>
<td>4.2 ± 0.7</td>
<td>-4.2 ± 6.8</td>
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<td>F</td>
<td>4.1 ± 0.2</td>
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<tr>
<td>F</td>
<td>5.8 ± 0.3</td>
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<td>5.6 ± 1.7</td>
</tr>
<tr>
<td>F</td>
<td>5.6 ± 0.3</td>
<td>-3.3 ± 2.2</td>
<td>5.8 ± 0.4</td>
</tr>
<tr>
<td>Average*</td>
<td>4.8 ± 0.4</td>
<td>-3.3 ± 0.2</td>
<td>0.9 ± 1.8</td>
</tr>
</tbody>
</table>

Values are means ± SE (s⁻¹) of mass transfer parameters in oral and nasal compartment ([Ka]orr and [Ka]nnr, respectively); change in Ka between pharyngeal and oral compartments [Δ(Ka)]nn; and breathing assembly volume (Vp, ml) obtained from the splined regression of the individual subject’s data to Eq. 1. ppm, Parts/million. The ordering of individual subject data in this table is the same as in Table 2. *Pooled Ka and Vp value.

were 1.5 s⁻¹ during oral breathing of 3 ppm Cl₂ boluses; 1.1 s⁻¹ during nasal breathing of 3 ppm Cl₂ boluses; and 1.6 s⁻¹ during nasal breathing of 0.5 ppm Cl₂ boluses. This suggests that mass transfer in the hypopharynx was not markedly affected by the mode of breathing or the inspired Cl₂ concentration. Because of extensive absorption of Cl₂ in the NS and OR compartments, there were insufficient A-Vp data in the LA compartment to evaluate (ΔKa)La in any of the subjects.

The ΔA for Cl₂ in the three conducting airway compartments is given in Fig. 8. Nearly all of the inspired Cl₂ was absorbed in the upper airways, and ~90% of the inspired Cl₂ was absorbed in the nose or mouth of all the subjects. This result was independent of the mode of breathing and of Cmax.

DISCUSSION

One purpose of this research was to determine the principal site of Cl₂ uptake in the respiratory system.

From the bolus inhalation measurements, it is clear that nearly all of the Cl₂ inhaled during quiet breathing is absorbed in the upper airways, whether the nose or the mouth is the site of air access. In reaching this conclusion, we relied on measurements of Vg, VNS, and VpH measured by acoustic reflection. The average volume of the nasal cavity, between the nares and the entrance to the nasopharynx, was found to be 23 ml. This is within the range of 20–32 ml reported for the nasal cavity volume by two other research groups who used computerized tomography and magnetic resonance imaging scans in a total of eight subjects (16). The average ± SD volume of the oral cavity between the lips and the entrance to the hypopharynx was found to be 54 ± 20 ml, which is consistent with the values of 41 ± 9 and 50 ± 10 ml previously reported for acoustic reflection and oropharyngeal cast measurements, respectively (10).

Another purpose of this study was to compare Cl₂ uptake by the nose and the mouth. Because (Ka)NN is ~25% larger than (Ka)ON, the absorption rate of Cl₂ per unit volume of airway lumen is somewhat larger for the nose than for the mouth (Table 3). The nose has a smaller volume than the mouth (Table 2), however, so that (Ka)VNN is only ~5% larger than (Ka)VON. It can be concluded that the total absorption rates for the nose and mouth are similar. A third purpose of this research was to examine the influence of inhaled Cl₂ concentration on airway absorption during nasal breathing. The fact that (Ka)VNN retained the same value when Cmax was changed from 0.5 to 3.0 ppm (Table 3) indicates that the dissolution, diffusion, and chemical reactions governing Cl₂ uptake from respired gas to the nasal mucosa are all linear processes.

The continuous inhalation of Cl₂ is the theoretical equivalent of inhaling a train of Cl₂ boluses that is introduced between the beginning and the end of inspiration. The absorption distribution that applies by integrating the ΔVb bolus distribution. To a first approximation, the change in bolus absorption ΔA is an estimate of Cl₂ absorption within a particular airway.
compartment during continuous exposure. This approximation is valid when tidal volume is much larger than \( V_p \) at the proximal end of the compartment of interest. This is always the case for the N/O and PH compartments and is usually true for the LA compartment. Judging from the results of this study, 95% or more of continuously inhaled Cl\(_2\) is absorbed in the upper airways that consist of the N/O and PH compartments (Fig. 8).

Cl\(_2\) undergoes a rapid and reversible hydrolysis in aqueous solutions according to the following chemical reaction

\[
\text{Cl}_2 + \text{H}_2\text{O} \rightarrow \text{Cl}^- + \text{HOCl} + \text{H}^+ \tag{2}
\]

This reaction has an equilibrium constant with the following value at 25°C (27)

\[
10^{-7.6}[\text{Cl}^-][\text{HOCl}]/[\text{Cl}_2] = 5 \times 10^{-4}(\text{mol/l})^2 \tag{3}
\]

where [Cl\(^-\)] and [HOCl] indicate the molar concentrations of the chloride ion and hypochlorous acid in the aqueous phase, respectively, and [Cl\(_2\)] is the concentration of Cl\(_2\) in dissolved form. For mucus that has a [Cl\(^-\)] of ∼0.16 mol/l (14) and a pH of ∼6.6 (2), Eq. 3 indicates that the concentration of Cl\(_2\) in hydrolized form ([HOCl]) is 120,000 times [Cl\(_2\)]. In other words, the effective solubility of Cl\(_2\) between the inspired gas and mucous phase is five orders of magnitude larger than the physical solubility. This large effective solubility explains why >95% of inhaled Cl\(_2\) was absorbed in the upper airways. Moreover, the linearity of the equilibrium relationship between [HOCl] and [Cl\(_2\)] that occurs when pH and [Cl\(^-\)] are fixed is consistent with the observation that (Ka)\(_{\text{NS}}\) did not depend on the peak inhaled Cl\(_2\) concentration.

As Cl\(_2\) absorbs into an airway, it encounters a diffusion resistance created by a respiratory gas boundary layer and a second diffusion resistance imposed by the surrounding mucosa. Because of the large effective solubility of Cl\(_2\), the diffusion resistance in mucus and tissue should be minimal, and gas absorption should be limited primarily by the convection and diffusion processes in the respired gas phase. If this is true, then the overall mass transfer parameter Ka should be similar in value to the gas-phase mass transfer parameter \( k_a \). To make this comparison, the gas-phase mass transfer coefficient \( k_a \) was computed by applying the heat-mass transfer analogy to an empirical correlation developed from heat transfer measurements on casts of the upper airways (20).

\[
k_a = m(d/d)_{n-1}(V/A)^n \tag{4}
\]

where m and n are empirical constants that have respective inspiratory values of 0.028 and 0.854 in the nose and 0.035 and 0.804 in the mouth and respective expiratory values of 0.0045 and 1.08 in the nose and 0.0006 and 1.269 in the mouth; \( D_g \) is the binary diffusivity of Cl\(_2\) in air, which was estimated to be 0.13 cm\(^2\)/s (25); \( d \) is tracheal diameter, which is ∼1.8 cm (26); and \( A \) is the mean tracheal cross-sectional area, which is ∼2.6 cm\(^2\) (26). From previously published anatomic data, the surface-to-volume ratio \( a \) was estimated to be 9.2 cm\(^{-1}\) in the nose (16) and 1.7 cm\(^{-1}\) in the mouth (21).

On the basis of these estimates, the predicted \( k_a \) averaged over inspiration and expiration at a \( V \) of 250 ml/s were 7.7 s\(^{-1}\) in the nose and 1.0 s\(^{-1}\) in the mouth. The \( k_a \) value predicted for the nose is within the intrasubject range of (Ka)\(_{\text{NS}}\) values from the bolus experiments (i.e., 5.0–8.1 s\(^{-1}\)), supporting the conclusion that gas-phase diffusion is the rate-limiting step in the overall absorption process. However, the \( k_a \) predicted for the mouth is much below the range of (Ka)\(_{\text{OR}}\) deduced from the bolus study (i.e., 2.9–6.5 s\(^{-1}\)). This result cannot be explained by the influence of a mucosal resistance that would cause \( k_a \) to be larger, not smaller, than (Ka)\(_{\text{OR}}\). The result is possibly due to a difference in the mouth opening and tongue placement of the cadaver casts used to obtain \( k_a \) and a compared with the mouth and tongue orientations of the subjects who breathed through a mouthpiece during the bolus experiments.

Previous bolus measurements indicated that O\(_3\) had a \( \Lambda \) value of 0.8 in the nasal cavity and 0.5 in the oral cavity (10). The \( \Lambda \) value for Cl\(_2\) in the present investigation was ∼0.9 in both the nasal and oral cavities. The smaller absorbed fractions of O\(_3\) relative to Cl\(_2\) may be due to the fact that O\(_3\) does not hydrolyze in mucus. In other words, the solubility of O\(_3\) may be sufficiently low that the diffusion resistance of O\(_3\) through the mucosa influences the overall absorption process. In addition, because Cl\(_2\) absorption is similar in the mouth and nose but O\(_3\) absorption is less in the mouth than in the nose, it appears that the mucosal diffusion resistance of O\(_3\) is greater in the mouth than in the nose.

The toxicity of Cl\(_2\) is mediated locally by HOCl, which may disrupt the integrity and increase the permeability of the epithelium (6), and by an increase in H\(^+\), which may decrease blood pH, provided that a sufficient dose of Cl\(_2\) is absorbed (29). Cl\(_2\) also reacts with the sulfhydryl groups of the amino acid cysteine, thereby inhibiting various enzymes (6, 15). Because this study revealed that almost all inhaled Cl\(_2\) was absorbed in the nose during nasal breathing and the mouth during oral breathing, it is reasonable to conclude that the upper airways are the most likely site of long-term Cl\(_2\)-induced tissue damage in humans. This is consistent with the findings in laboratory animals that lesions due to the chronic inhalation of Cl\(_2\) are confined primarily to the nasal cavities (11, 28).

One of the general limitations of the bolus inhalation method is a lack of spatial resolution because of the finite width of the bolus. The bolus is most narrow when it enters the respiratory system but becomes progressively more dispersed by longitudinal mixing throughout the test breath. The fact that \( V_{p0} \), the intercept of the \( \Lambda-V_p \) distribution with the abscissa, is less than the 20-ml volume of the nonabsorbing breathing fixture (Table 3) is a symptom of this limitation. In other words, if the width of the bolus had been zero, then \( \Lambda \) would rise above zero only when \( V_{p} \) was greater than \( V_{BF} \). Because the bolus had a finite width, how-
ever, a portion of the bolus penetrated into the respiratory system even when its center of mass was located at values of $V_p < V_{BF}$. This artifact can lead to an incorrect interpretation of the $\Lambda - V_p$ distribution. For example, it appears in Fig. 6A that Cl$_2$ absorption at low $V_p$ is somewhat more efficient during oral than nasal breathing. In fact, because bolus dispersion is greater during oral than nasal breathing (Fig. 7A), $V_{p0}$ is less during oral than during nasal breathing (Table 3), and the $\Lambda - V_p$ distribution for oral breathing is shifted to the left of that for nasal breathing. Judging from the values of $(K_a)_{\text{NS}}$ and $(K_a)_{\text{OR}}$ that are based on the slopes of the $\Lambda - V_p$ data, it appears that the absorption efficiency in the nose is actually greater than in the mouth. The same logic applies to the effect of $C_{\text{max}}$ on Cl$_2$ absorption. From Fig. 6B, it appears that absorption is more efficient at a $C_{\text{max}}$ of 0.5 ppm than at a $C_{\text{max}}$ of 3.0 ppm, whereas the values of $(K_a)_{\text{NS}}$ indicate that there is actually no significant difference.

An important consideration in any dosimetric process is whether the initial uptake of a pollutant causes some disturbance in the chemical or physiological state of the respiratory system that induces a subsequent change in the uptake. For example, exposure to Cl$_2$, whether continuously or by multiple bolus breaths, can result in the accumulation of Cl$_2$ in mucus, with a concomitant decrease in the absorption rate. To test for such an effect, one subject nasally inhaled a series of 3-ppm boluses once every five breaths for 1 h at a $V_p$ target level of 40 ml, corresponding to the posterior nasal cavity. A linear regression of $\Lambda$ against time had a small coefficient of determination ($r^2 = 0.03$) and a slope that was not significantly different from zero. It can thus be concluded that the bolus inhalation method provides a time-invariant measure of Cl$_2$ absorption.

Although no statistically significant gender differences in the values of $K_a$ and the values of $\Delta \Lambda$ were uncovered, this study lacked sufficient power to conclude with high probability that Cl$_2$ absorption is the same for men and women. In particular, the probability of falsely concluding that there was no gender difference was $\approx 0.9$. To reduce this probability of making a type II error to a more reasonable level of 0.2 would have required that 10 female and 10 male subjects be tested. Nevertheless, the apparent lack of a gender difference in Cl$_2$ absorption is consistent with the previous finding for O$_3$ absorption that $K_a$ was related to gender only insofar as the smaller conducting airway volumes of the women produced larger values of $K_a$ than for the men (3). In the case of Cl$_2$, that was primarily absorbed into the N/O compartment, one would expect $(K_a)_{\text{N/O}}$ to be negatively correlated with $V_{\text{N/O}}$, irrespective of gender. This expectation was met for the mouth but not for the nose (Fig. 9). It may be that the shape of the oral cavity was more similar from person to person than was the shape of the nasal cavity and/or that flow patterns in the mouth were more similar among different subjects than in the nose.

The breakthrough volume $V_B$ and dispersion $\sigma^2$ are indirectly affected by the absorption process. Because it represents the average airway volume from which unabsorbed Cl$_2$ molecules originate during expiration, $V_B$ should progressively increase as $V_p$ increases. However, absorption is very efficient in the small airways where $a$ is exceedingly large, so that $V_p$ should level off once $V_p$ is larger than some critical value. This is exactly the type of $V_B - V_p$ behavior that has been observed for Cl$_2$ boluses in the present study (Fig. 7A) as well as for O$_3$ boluses in a previous study (10). As was the case for O$_3$, the $V_B$ for Cl$_2$ tends to be greater during oral breathing than during nasal breathing and is not markedly affected by a change in $C_{\text{max}}$. The value of $\sigma^2$, which characterizes the volume of air in which the test gas becomes distributed by longitudinal mixing, will also tend to level off because boluses are truncated by the highly efficient absorption occurring in the small airways. As was previously observed for O$_3$, $\sigma^2$ for Cl$_2$ is relatively independent of $V_p$ and $C_{\text{max}}$ and is greater during oral breathing than during nasal breathing.

To summarize, measurements of the $\Lambda - V_p$ distribution of Cl$_2$ during nasal as well as oral quiet breathing in five men and five women indicated that >95% of inspired Cl$_2$ was absorbed in the upper airways of all subjects, whereas the dose delivered to the respiratory air spaces was negligible. Although there were no statistically significant gender differences in the results, individual values of $(K_a)_{\text{OR}}$ were inversely correlated with individual values of $V_{\text{OR}}$. Representative overall mass transfer coefficients estimated in the nose were in good agreement with gas-phase mass transfer coefficients calculated from established correlations. This suggested that diffusional resistance in the nasal mucosa was negligible relative to diffusional resistance.
in the respired gas. Both the high absorptivity of Cl₂ in the upper airways and the domination of the gas-phase diffusion resistance were attributable to the rapid hydrolysis of Cl₂ in the mucosa.

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