

# Ozone Uptake in Healthy Adult Males during Quiet Breathing<sup>1</sup>

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Received February 17, 1995; accepted July 20, 1995

Ozone Uptake in Healthy Adult Males during Quiet Breathing. WIESTER, M. J., STEVENS, M. A., MENACHE, M. G., MCKEE, J. L., JR., AND GERRITY, T. R. (1996). *Fundam. Appl. Toxicol.* 29, 102-109.

Experimental measurements of ozone (O<sub>3</sub>) uptake are needed for validation of dosimetry model parameters and in predictions as well as for determining factors affecting uptake and for making comparisons between subpopulations or across species. In this study, 10 healthy adult male subjects were exposed to 0.3 ppm O<sub>3</sub> while seated and breathing naturally through the nose or mouth. Total respiratory tract O<sub>3</sub> uptake, spontaneous breathing parameters, and respiratory gas exchange were measured for 10 min under steady-state conditions. The exposure protocol was replicated in each subject approximately 2 weeks after the first visit. On each visit, health exams were performed and spirometric lung measurements were obtained. The experimental design provided comparisons of total O<sub>3</sub> uptake during nasal and oral breathing, differences in uptake in an individual at two time points, and an examination of between-subject variability in O<sub>3</sub> uptake. Exposure to O<sub>3</sub> had no effect on the breathing parameters or gas exchange. Oral and nasal breathing frequency averaged  $16.2 \pm 1.1$  (SE) and  $16.0 \pm 1.2$  breaths per minute with tidal volumes averaging  $651 \pm 46$  and  $669 \pm 67$  ml, respectively. A significant correlation ( $p < 0.01$ ) was found for the minute volume during resting breathing with the percentage of uptake. The percentage of O<sub>3</sub> uptake was consistently higher ( $p = 0.02$ ) during oral breathing ( $76.5\% \pm 3.3$ ) than during nasal breathing ( $73.1\% \pm 3.0$ ) although this difference may not be biologically significant. The variability in percentage of uptake between subjects was substantial with calculated uptakes ranging from 51 to 96%, a difference of about 45%. Variability in percentage of uptake for an individual was less with the maximal difference between the first and second visits being about 20%; the average difference, however, was only about 3%. We conclude that total percentage of O<sub>3</sub> uptake is approximately 75% in adult males

during resting breathing. It is slightly greater during oral than during nasal breathing, will vary considerably among subjects, and is moderately reproducible within a subject. © 1996 Society of Toxicology

Ozone (O<sub>3</sub>) is often found in ambient air at levels exceeding the National Ambient Air Quality Standard of 0.12 ppm averaged over a 1 hr period. Many detailed reviews of studies regarding O<sub>3</sub> health effects on humans and animals have been published (Lippmann, 1989; Dungworth, 1989; Warheit *et al.*, 1989; Mauderly, 1988).

Similar types of O<sub>3</sub> effects on lung tissue have been reported in rodents and nonhuman primates. Qualitatively similar effects have been observed in humans and animals, particularly for endpoints relating to pulmonary function and inflammation. Although the relationships between animal and human effects are qualitatively similar, the quantitative relationships needed for health risk assessment require information on dose delivered to the target site and sensitivity of the target.

Dosimetry models provide estimates of the amount of inhaled O<sub>3</sub> that is delivered to the target tissue using equations for gas transport and uptake that are applied to the complex branching system of the respiratory tract. Such dose estimates may be used not only for animal to human extrapolation but also for intraspecies dose comparisons, reconciliation and interpretation of data from different experiments, prediction of doses in situations that cannot be reproduced experimentally, and assessment of potential mechanisms of action associated with observed target tissue response.

Experimental measurements in different species are needed for determining factors affecting O<sub>3</sub> uptake, making comparisons between subpopulations and across species, as well as for validating both parameters used in and predictions generated by dosimetry models. Much of the animal toxicology for O<sub>3</sub> effects has been done in rats. More limited toxicological data are available for other rodents and for nonhuman primates. Although several dosimetry studies have been performed in dogs (Yokoyama and Frank, 1972; Vaughan *et al.*, 1969; Moorman *et al.*, 1973), rabbits (Miller *et al.*, 1979), and guinea pigs (Miller *et al.*, 1979), the animals in

<sup>1</sup> This work was supported in part by EPA cooperative agreement CR813113 and a contract from Southern California Edison Co. This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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these studies were anesthetized, ventilated, and/or cannulated. Wiester and colleagues (1987, 1988) measured total O<sub>3</sub> uptake and breathing parameters in awake spontaneously breathing rats and guinea pigs during nose-only exposures and reported uptakes between 45 and 47%. Several O<sub>3</sub> uptake studies have been performed in humans (Gerrity *et al.*, 1988, 1995; Hu *et al.*, 1992). These studies have reported total O<sub>3</sub> uptakes of 80% and greater. The human exposure systems varied between studies and were quite different from the nose-only exposure apparatus used in the animal studies of Wiester *et al.* (1987, 1988). Because there is a need to compare human O<sub>3</sub> uptake in the respiratory tract with that in the rat, we have measured total respiratory tract uptake in humans using an up-scaled system similar to that used for rats and guinea pigs (Wiester *et al.*, 1987, 1988). Uptake was determined for 10 healthy males over 10-min periods, during steady-state exposure and breathing conditions. The subjects were seated and quietly breathing 0.3 ppm O<sub>3</sub> by nose or by mouth. Measurements were repeated for each subject after approximately 2 weeks.

## METHODS

Ten male subjects, 19–32 years of age, were recruited from the community in and around Chapel Hill, North Carolina. Excluded from participation in the study was anyone who smoked, had a history of asthma, allergic rhinitis, cardiac disease, acute respiratory disease within the previous 4 weeks, or had facial hair (beard or mustache). Screening procedures included a medical history, a physical examination, and a complete blood count plus differential white cell count. Accepted subjects were informed of the purpose of the study, the experimental methods, and the potential risks of participation before signing a statement of informed consent. This study was approved by the Committee on the Protection of the Rights of Human Subjects of the University of North Carolina School of Medicine.

**Experimental apparatus.** A diagram of the O<sub>3</sub> uptake exposure apparatus is shown in Fig. 1. The exposure apparatus was located in a 4 × 6 × 3.2-m stainless-steel walk-in environmental chamber. The chamber was maintained at 22°C and 40% relative humidity. Ozone was produced for the chamber by passing incoming air through a series of ultraviolet light O<sub>3</sub> generator tubes. Ozone concentration was monitored by chemiluminescence detection using Bendix Model 8002 analyzers which were calibrated against an ultraviolet O<sub>3</sub> photometer (Dasibi Model 1003AH). A detailed description of the environmental chamber operation has been published (Glover *et al.*, 1981).

The subject exposure apparatus consisted of 4-in. stainless-steel pipe which opened to the environmental chamber at one end and attached to a fan/damper assembly at the other end. Chamber air was drawn down through the apparatus by the fan/damper assembly. Ports were cut into the apparatus for sampling of O<sub>3</sub>, O<sub>2</sub>, and CO<sub>2</sub>. A face port was also cut into the pipe for the subject to insert his nose and mouth and breathe from the airstream flowing through the apparatus. The exposure apparatus flow rate was approximately 40 liters/min. At this rate, the downstream (relative to the face port) dilution of exhaled air with the airstream was similar to that used in the rat and guinea pig studies (Wiester *et al.*, 1987, 1988). Testing indicated that this rate was sufficient to prevent the rebreathing of downstream air by the subject and to supply an adequate volume of upstream air during maximum inspiratory flow. Flow measurements were determined using a Roots meter (Model 5M). Additionally, flows were checked for accuracy with a Gilibrator Primary Flow Calibrator (Gilian Instrument Corp., West

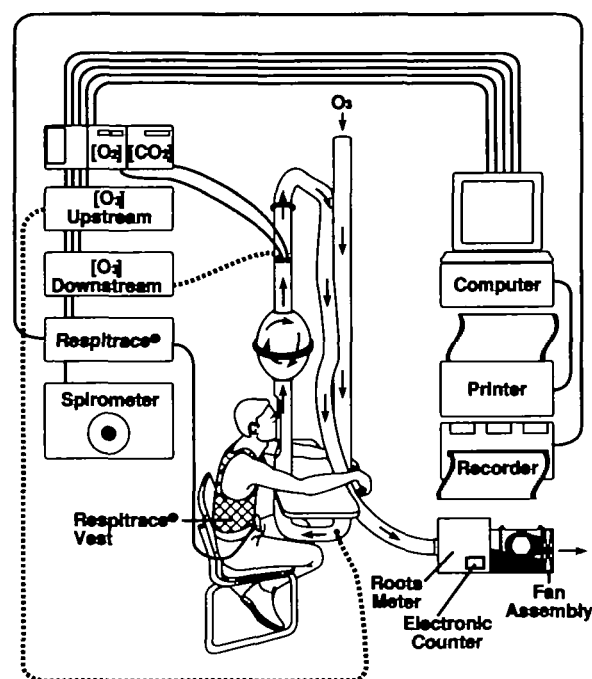


FIG. 1. Exposure apparatus and data acquisition system. The stainless-steel exposure apparatus was attached to an adjustable tray table so that the face port could be placed at the correct height for each subject. The apparatus was constructed of 4-in. pipe with a 30-liter mixing chamber that contained three perforated baffles. The pipe was indented 1½ in. to accommodate the face. The indented portion was flat with a triangular cutout face port. An air-filled cushion, cut from a disposable plastic face mask (Intertech Resources Inc., OH) was glued around the opening to ensure a tight seal at the face. Chamber room exposure air entered into the system through 4-in. flexible stainless-steel tubing, traveled through the stainless-steel pipe, and exited via 4-in. flexible plastic tubing. A Roots flow meter (Model 5M) was located upstream of the fan/damper assembly to monitor gas flow. An optical-electrical counter device was installed on the Roots meter's magnetically coupled counter to assist in the counting of impeller revolutions. Flow time was measured using a digital stopwatch. Ozone monitors were linked to the apparatus with Teflon tubing and the O<sub>2</sub> and CO<sub>2</sub> probes were placed at the side ports.

Caldwell, NJ). Flow values checked by these two methods were in agreement with each other to within 1%. A mixing chamber was placed downstream of the face port. The mixing chamber served two purposes: (1) to dampen the downstream pressure pulses caused by subject breathing and (2) to mix the exhaled air with the uninhaled air in the airstream before it reached the downstream analyzer probes. Thus, by the time the exhaled air arrived at the level of the analyzer probes, it was free from fluctuations in pressure, temperature, and humidity and it was well mixed with the uninhaled air. The transit time required for an exhaled breath to reach the downstream monitors was determined by airstream dilution. A bolus of nitrogen was injected into the airstream at the face port and dilutions of O<sub>2</sub> and CO<sub>2</sub> were recorded downstream. This time-delay constant was used to synchronize monitor readings with corresponding subject breathing parameters. The upstream and downstream inlets used for monitoring O<sub>3</sub>, were placed approximately 1 m before and after the face port, respectively. This distance resulted in a delay in the time needed for the airstream to travel from the upstream monitor to the face port, and from the face port through the mixing chamber and to the downstream monitor. These temporal delays were measured using a point-source UV light ozone generator and were

used to synchronize O<sub>3</sub> sampling with the corresponding subject breathing parameters. Downstream O<sub>2</sub> and CO<sub>2</sub> were sampled for determination of respiratory quotient (RQ) using Radiometer TCM<sub>2</sub> and TCM<sub>20</sub> monitors, respectively

**Breathing parameters.** Subject chest wall movements caused by breathing efforts were measured using Respirace inductance plethysmography chest bands (Respirace, Ambulatory Monitoring, Inc., Ardsley, NY). The fitted Respirace was calibrated by having the subject quietly breathe into a rolling seal spirometer that had been previously calibrated with a 1-liter syringe. The ensuing Respirace excursions were matched with the calibrated spirometer excursions to determine a volume conversion factor which was used for the subsequent experiment. This calibration was repeated at the end of the experiment to account for sliding or other repositioning of the bands during the experiment. The pre- and postconversion factors were averaged together and final breathing parameters were calculated at the end of the experiment. Tidal breathing measurements were obtained during the experiment by sampling the Respirace signal at 12 msec intervals for 20 sec each minute. Breathing parameters reported represent averaged values from all breaths completed during this 20-sec sampling period (usually four to six breaths). These include tidal volume ( $V_T$ ); breathing frequency ( $f$ ); expiratory minute volume ( $\dot{V}_E$ ); maximum inspiratory and expiratory flows ( $\dot{V}_{i_{max}}$ ,  $\dot{V}_{e_{max}}$ ); inspiratory, expiratory, and apnea times ( $T_{ins}$ ,  $T_{exp}$ ,  $T_{apnea}$ ); oxygen consumed per minute ( $\dot{V}_{O_2}$ ); carbon dioxide produced per minute ( $\dot{V}_{CO_2}$ ); and RQ.

**Data acquisition.** All monitor output voltages as well as Respirace voltage outputs were digitized, collected, and analyzed using software custom-written in FORTRAN-77 and MACRO-11 ASSEMBLY languages and executed on a PDP-11/23 microcomputer (Digital Equipment Co., Maynard, MA). Additionally, hard copies of Respirace traces and O<sub>3</sub> concentration traces from the monitors were obtained using chart recorders.

**Experimental protocol.** Subjects were measured, using the same experimental protocol, on two visits. These visits were separated by at least 14 days.

On the day of an exposure, the subject completed a symptom questionnaire and performed forced expiratory maneuvers. These consisted of three forced vital capacity (FVC) maneuvers performed on a 12-1 dry seal spirometer (CPI model 220). Peak expiratory flow rate (PEF) and forced expiratory volume at 1 sec (FEV<sub>1.0</sub>) were calculated for each maneuver. The subject was then fitted with a telemetered ECG monitor and the Respirace bands.

With filtered air flowing into the environmental chamber, the subject was seated in front of the exposure apparatus and the volume conversion factor was determined. The subject then placed his mouth and nose into the face port of the apparatus, and a 15-min period of quiet nasal or oral breathing ensued (subjects were either instructed to "nose-breathe" or were forced to "mouth-breathe" by placing a swimmer's clip on the nose). The first 5 min were to allow for system equilibration. This was followed by a 10-min sampling period to collect air exposure values. System airflow was measured during the 10-min sampling period. After completing similar runs of both nose and mouth breathing, the subject was led out of the environmental chamber. The subject remained out of the chamber while it filled with 0.3 ppm O<sub>3</sub>. Once the O<sub>3</sub> in the chamber stabilized and the O<sub>3</sub> monitors were calibrated, the subject returned, was seated, and then the Respirace was recalibrated. Prior to placing the mouth and nose back into the face port of the apparatus, the port was closed and the background level of O<sub>3</sub> loss to the interior walls of the apparatus was measured by comparing the upstream and downstream O<sub>3</sub> concentrations. The background loss was measured both before and after subject exposure and was found to vary less than 1 ppb. This background loss value was used in the calculation of the percentage of O<sub>3</sub> uptake at the end of the experiment. The subject then placed the nose and mouth into the face port of the exposure apparatus, using the same procedure that was followed for the air exposures (i.e., 5 min of quiet breathing to allow for system equilibration followed by 10 min of sampling). The percentage of O<sub>3</sub> uptake was calculated using

% O<sub>3</sub> Uptake

$$= \frac{[O_3 \text{ upstream} - O_3 \text{ downstream} + O_3 \text{ background loss}] \times \text{SAF}}{O_3 \text{ upstream} \times \dot{V}_E} \times 100,$$

O<sub>3</sub> upstream ( $\mu\text{g/liter}$ ); O<sub>3</sub> downstream ( $\mu\text{g/liter}$ ); O<sub>3</sub> background loss ( $\mu\text{g/liter}$ ); SAF is the system airflow (liters/min); and  $\dot{V}_E$  (liters/min).

Figure 2 shows an example of data and monitor tracings for a subject during one 10-min session while breathing O<sub>3</sub> orally. The monitor tracings of ozone concentrations recorded during nasal breathing are shown for visual comparison with those obtained during oral breathing.

**Statistical analysis.** All breathing parameters and the percentage of O<sub>3</sub> uptake were examined for time-related trends over the 10-min exposure period using linear regressions. These were calculated for each subject and breathing mode during each exposure. Because no trends were found, the individual minute by minute data were averaged for each subject during each exposure for each breathing mode. These means were used as the unit of observation for the subsequent analyses.

A two-way multivariate analysis of variance (MANOVA) was used to examine the effects of "route" of exposure (nasal or oral), "visit" (first or second), and the interaction between these two factors on the percentage of O<sub>3</sub> uptake. Significance of an effect was determined using the Hotelling-Lawley trace. Because there were only two categories for each main effect comparison (i.e., nasal versus oral or visit 1 versus visit 2), no further subtesting was required for significant main effects. Interactions between route and visit were tested using paired *t* tests. Three-way MANOVAs were used to examine the effects of route, visit, and exposure (air versus O<sub>3</sub>) and all possible interactions in the breathing data.

Pearson correlation coefficients were calculated to test for linear relationships between the percentage of O<sub>3</sub> uptake and the breathing parameters, spirometric measurements of lung function, and subject weight and height.

## RESULTS

Subject characteristics and preexposure pulmonary function results are shown in Table 1. The 10 volunteers selected for this study ranged in age from 19 to 32 years and, generally, had similar anthropomorphic characteristics. No statistically significant differences were found between spirometric measurements made on the first and second visits (Table 1).

Table 2 contains breathing parameters measured or derived from parameters measured during the 10-min exposures and averaged over that period. The absence of statistically significant changes in breathing parameters among the exposure periods indicates that the subjects were breathing at steady state and that the O<sub>3</sub> exposures were low enough not to affect ventilation. Because the multivariate analyses indicated that there were generally no differences between the first and second visits, the means of the continuous measurements from both visits (for a given exposure-breathing combination) were then averaged to provide a single value for each subject for each exposure-breathing combination.

Figure 3 shows individual subject values for the percentage of O<sub>3</sub> uptake in six columns of numbers, paired into three panels. A single number identifies the same subject in all six columns and lines join the two values in each panel. Panel 1 corresponds to visit 1 and visit 2 for oral breathing

Min	V <sub>T</sub>	f	V̇ <sub>E</sub>	T <sub>ins</sub>	T <sub>exp</sub>	T <sub>apnea</sub>	V̇ <sub>I</sub> max	V̇ <sub>E</sub> max	V̇O <sub>2</sub>	V̇CO <sub>2</sub>	RQ	O <sub>3</sub> Dose (μg/min)		O <sub>3</sub> UP
												Inhaled	Retained	
* 1	488	19.0	9285	1.17	1.68	0.31	575	672	314	224	0.71	5.27	4.04	78.8
2	516	19.4	10026	1.11	1.47	0.52	590	825	314	224	0.71	5.66	3.74	66.1
3	576	18.0	10356	1.18	1.55	0.61	606	896	314	224	0.71	5.91	4.45	75.3
4	674	21.8	14682	1.01	1.54	0.21	1142	918	314	224	0.71	8.35	3.81	45.6
5	571	17.2	9835	1.19	1.82	0.49	557	781	314	224	0.71	5.58	3.73	66.8
6	570	17.9	10191	1.18	1.62	0.56	634	863	314	224	0.71	5.74	3.86	67.2
7	530	18.5	9821	1.16	1.61	0.47	628	710	269	179	0.87	5.56	4.00	72.0
8	552	17.7	9781	1.14	1.80	0.45	574	809	314	224	0.71	5.49	3.82	69.6
9	504	18.4	9243	1.14	1.67	0.46	541	721	314	224	0.71	5.20	3.72	71.5
10	529	18.2	9631	1.13	1.81	0.38	617	781	314	224	0.71	5.45	4.04	74.1

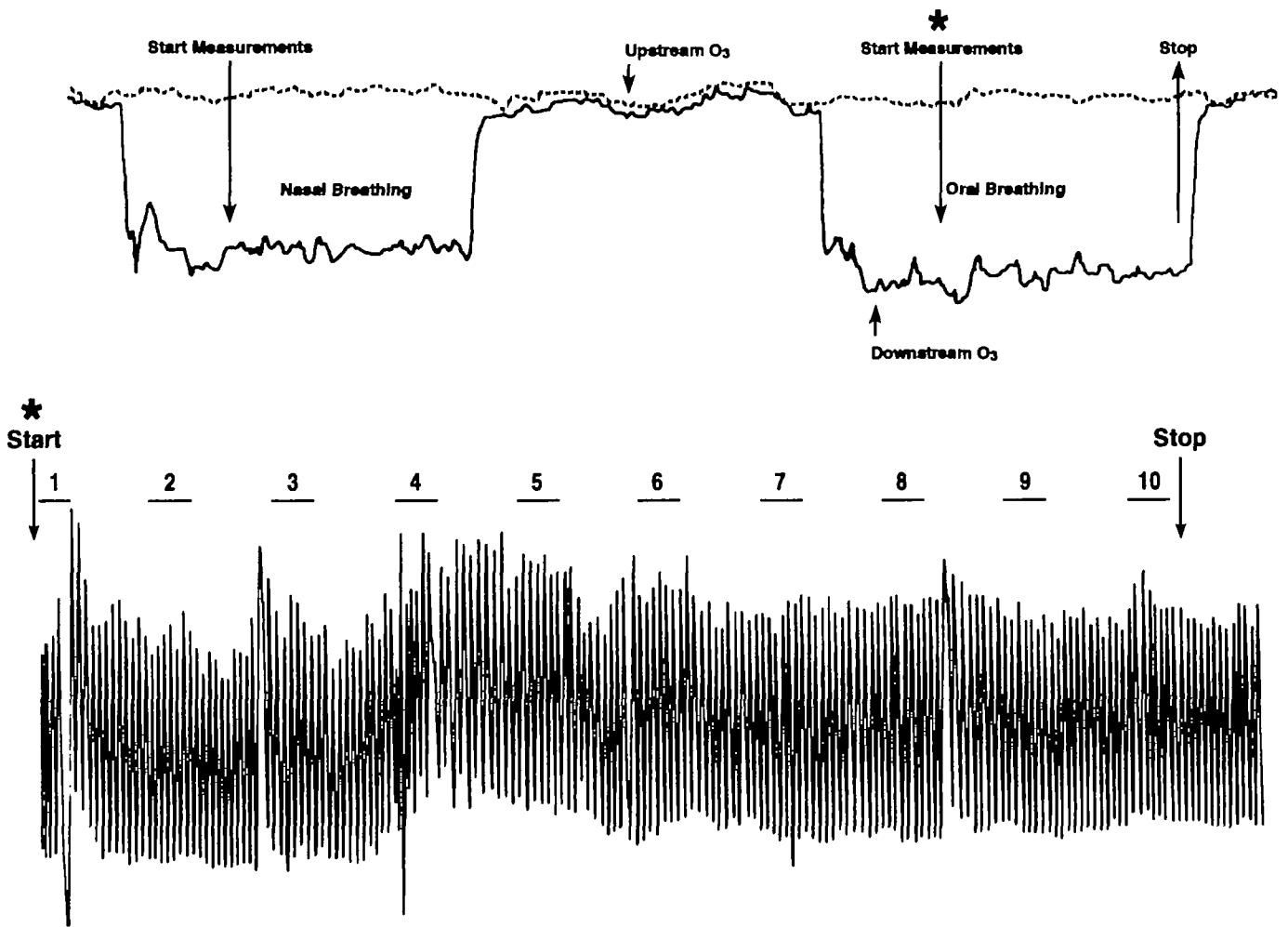


FIG. 2. (Top) Minute-by-minute respiratory measurements, obtained for one subject breathing 0.3 ppm O<sub>3</sub> by mouth for 10 min. (Middle) Tracings for two O<sub>3</sub> monitors showing the effects of breathing on the downstream O<sub>3</sub> concentration. (Bottom) Respirator tracings showing breathing excursions during the 10-min oral breathing exposure. An asterisk designates the beginning of the measuring sequence. V<sub>T</sub>, tidal volume (ml/min); f, breathing frequency (breaths/min); V̇<sub>E</sub>, expiratory minute volume (ml/min); T<sub>ins</sub>, T<sub>exp</sub>, and T<sub>apnea</sub>, inspiratory, expiratory, and apnea times (sec); V̇<sub>I</sub>max and V̇<sub>E</sub>max, maximum inspiratory and expiratory flow (ml/sec); V̇O<sub>2</sub> and V̇CO<sub>2</sub>, O<sub>2</sub> consumption and CO<sub>2</sub> production (ml/min STPD). Percentage of oral uptake for this subject, 68.5% ± 2.8 (SE); n = 10.

TABLE 1

Subject characteristics		
Age (years)	27 ± 1	
Body weight (kg)	74 ± 3	
Body height (cm)	178 ± 2	
Subject Spirometry	Visit 1	Visit 2
FVC (liters)	4.92 ± 0.18	4.94 ± 0.18
PEF (liters/sec)	9.91 ± 0.46	10.42 ± 0.41
FEV <sub>10</sub> (liters)	3.98 ± 0.17	4.04 ± 0.16

Note: The sample size is 10. Data displayed as means ± SE. FVC, forced vital capacity; PEF, peak expiratory flow; FEV<sub>10</sub>, forced expiratory volume in 1 sec.

and panel 3 shows the two replicates for nasal breathing. In panel 2, visits 1 and 2 are averaged for oral and nasal breathing respectively. Means and standard errors are listed at the bottom of each column.

There was no significant effect of visit on the percentage of O<sub>3</sub> uptake detected in the MANOVA ( $p = 0.63$ ), demonstrating the reproducibility of the measurement. There was a slight but significant route effect ( $p = 0.02$ ), that can be seen in Fig. 3, panel 2. The mean percentage of O<sub>3</sub> uptake when breathing orally was 76.5% ± 3.3 SE compared to 73.1% ± 3.0 when breathing nasally. There was no interaction between visit and route ( $p = 0.43$ ).

The relative extent of intra- and intersubject variability is illustrated in Fig. 3. The variability between subjects was substantial. The percentage of O<sub>3</sub> uptake was lowest in sub-

ject 0, ranging from about 50 to 60% whether breathing orally or nasally. Among the nine other subjects, the percentage of O<sub>3</sub> uptake ranged between about 65 and 95%, a difference of about 30%. In general, the variability in percentage of O<sub>3</sub> uptake for an individual subject was less than this although in some cases (e.g., oral breathing for subject 4), there was a difference of not quite 25% between the first and second visits. The average difference in percentage of uptake between the first and second visits for oral breathing was  $-3.1\% \pm 3.7$  SE and for nasal breathing was  $-0.2\% \pm 3.9$ . Because neither of these values are statistically significantly different from zero, this suggests that the percentage of uptake was equally likely to increase or decrease between visits as indicated the lack of significance in the MANOVA effect for visit. Although the difference between the first and second visits for an individual subject might vary by as much as 25%, the average difference was close to zero.

The variance components for the proportions of the variability attributable to the subjects, visit, and route were also examined. Over 48% of the variability was due to differences between subjects. Of the remaining within subject variability, 30.4% was attributable to variability between visits and 21.2% to variability associated with the two exposure routes. These numbers suggest that the intersubject variability exceeds the differences in percentage of O<sub>3</sub> uptake within subjects that is associated with different visits.

Other studies have observed a negative relationship between the percentage of O<sub>3</sub> uptake and flow rates. The relationship between  $\dot{V}_E$  and the percentage of O<sub>3</sub> uptake is graphed in Fig. 4. From this figure it can be noted that the

TABLE 2  
Respiratory Parameters for Adult Males during Oral and Nasal Tidal Breathing

Breathing route:	Oral		Nasal	
	Air	Ozone	Air	Ozone
$V_t$ (ml)	607 ± 50	651 ± 46	619 ± 48	669 ± 67
$f$ (breaths/min)	16.1 ± 1.2	16.2 ± 1.1	16.7 ± 1.1	16.0 ± 1.2
$\dot{V}_E$ (liters/min)	9.4 ± 0.7	10.0 ± 0.5	9.9 ± 0.6	9.9 ± 0.4
$\dot{V}_{i_{max}}$ (ml/sec)	714 ± 59	743 ± 38	728 ± 49	751 ± 42
$\dot{V}_{e_{max}}$ (ml/sec)	789 ± 57	814 ± 33	802 ± 41	835 ± 43
$T_{ins}$ (sec)	1.21 ± 0.12	1.16 ± 0.12	1.16 ± 0.11	1.24 ± 0.14
$T_{exp}$ (sec)	1.56 ± 0.11	1.60 ± 0.13	1.63 ± 0.14	1.69 ± 0.17
$T_{apnea}$ (sec)	1.23 ± 0.12	1.27 ± 0.18	1.06 ± 0.15	1.14 ± 0.19
$\dot{V}_{O_2}$ (ml/min)	324 ± 16	332 ± 14	330 ± 14	332 ± 16
$\dot{V}_{CO_2}$ (ml/min)	225 ± 16	237 ± 11	228 ± 12	246 ± 13
RQ	0.70 ± 0.03	0.72 ± 0.03	0.69 ± 0.02	0.75 ± 0.04

Note: Data displayed as means ± SE for 10 subjects and averaged over two visits.  $V_t$ , tidal volume;  $f$ , frequency of breathing;  $\dot{V}_E$ , expiratory minute volume;  $\dot{V}_{i_{max}}$  and  $\dot{V}_{e_{max}}$ , maximum inspiratory and expiratory flows;  $T_{ins}$ ,  $T_{exp}$ , and  $T_{apnea}$ , inspiratory, expiratory, and apnea times;  $\dot{V}_{O_2}$ , oxygen consumption at STPD;  $\dot{V}_{CO_2}$ , carbon dioxide production at STPD; RQ, respiratory quotient.

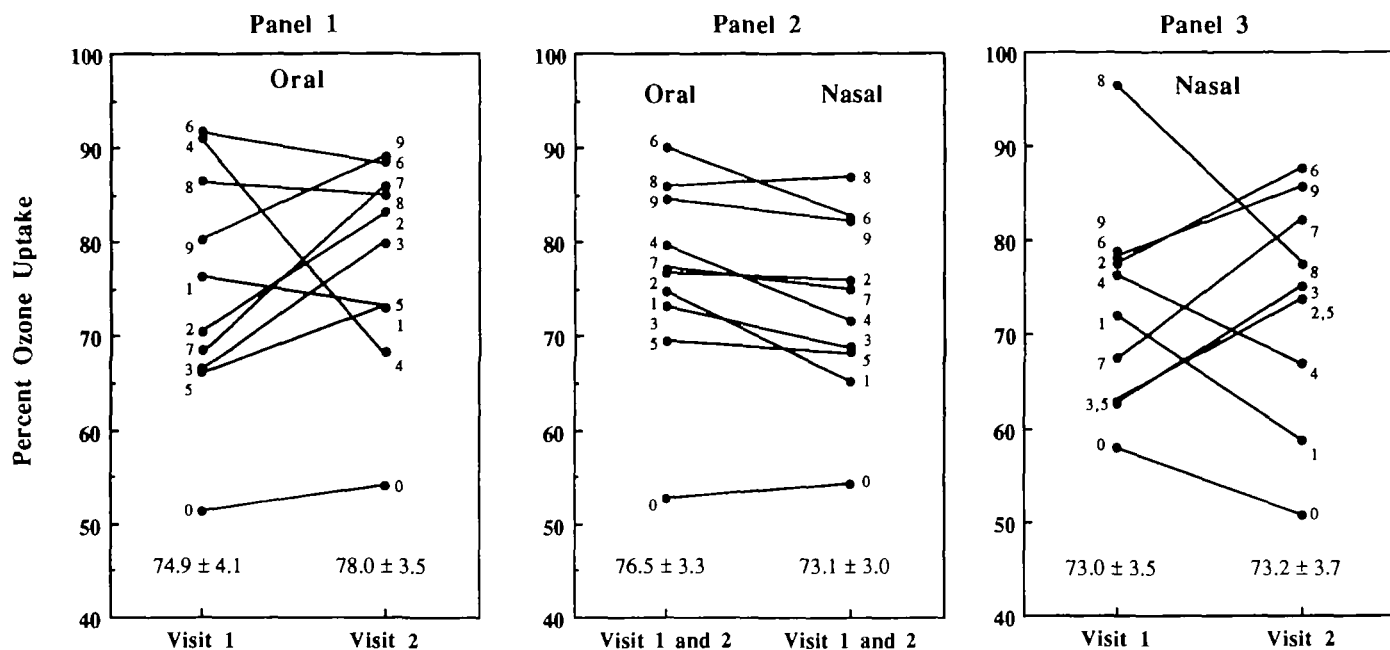


FIG. 3. Individual graphs of the percentage of O<sub>3</sub> uptake for 10 subjects. Panel 1, data obtained with oral breathing on two visits; panel 3, data for nasal breathing on two visits; and panel 2, oral and nasal data averaged over both visits. MANOVA analysis showed that route had a significant effect on the percentage of O<sub>3</sub> uptake ( $p < 0.02$ ). However, neither visit nor route  $\times$  visit had significant effects ( $p < 0.63$  and  $0.43$ , respectively).

resting  $\dot{V}_E$  tended to be high for subject 0; thus, it would be expected that his percentage of O<sub>3</sub> uptake would be somewhat low. The Pearson correlation coefficient for  $\dot{V}_E$  and the percentage of O<sub>3</sub> uptake was  $-0.74$  for all 10 subjects and  $-0.61$  excluding subject 0. Both correlation coefficients were significantly different from zero ( $p < 0.01$ ).

#### DISCUSSION

Total respiratory uptake of inhaled O<sub>3</sub> was measured in healthy adult male subjects during normal quiet breathing

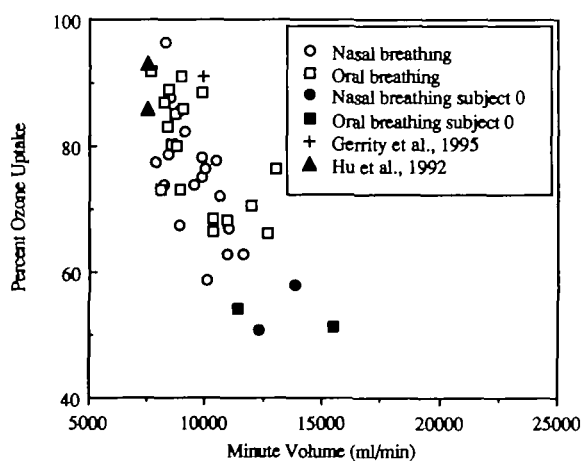


FIG. 4. The graph shows the relationship between  $\dot{V}_E$  and the percentage O<sub>3</sub> uptake for individual subjects measured in this paper. Also shown are averaged data for healthy young adult subjects that have been reported in two other publications. The Pearson correlation coefficient for the 10 subjects in this paper was  $-0.74$  ( $p < 0.01$ ).

by mouth and by nose under steady-state exposure and breathing conditions. The subjects were exposed to O<sub>3</sub> at a dose well below that expected to affect spontaneous breathing patterns. In the present study, this was confirmed by the absence of an O<sub>3</sub> effect on breathing parameters. During the 10-min exposure, the percentage of uptake was uniform and time independent. A similar uniformity in percentage of O<sub>3</sub> uptake was seen in rats exposed to 0.3, 0.6, or 1.0 ppm O<sub>3</sub> over 1 hr, and in guinea pigs exposed to 0.3 ppm O<sub>3</sub> for 1 hr (Wiester *et al.*, 1987, 1988).

Among the 10 subjects, the average percentage of O<sub>3</sub> uptake was slightly, but statistically significantly greater, with oral breathing (76%) than with nasal breathing (73%). Gerrity *et al.* (1988) also found a slight but significant increase in percentage of O<sub>3</sub> uptake by the extrathoracic airways (all airways superior and anterior to the posterior pharynx) for healthy adult males during oral breathing compared to nasal breathing (40.0 and 36.4%, respectively). Their data were obtained using a technique that sampled O<sub>3</sub> from a small polyethylene tube with the tip positioned in the posterior pharynx. They suggested that greater uptake during oral breathing may occur because (1) higher linear velocities through nasal passages relative to the oral cavity may lead to shorter residence times for O<sub>3</sub> in the nasal airways, (2) endogenous sulfated compounds that are strongly reactive with O<sub>3</sub> are present in the mouth but not in the nose, and (3) the presence of the sampling tube in the nose may have affected the nasal measurement. It is possible that the first two reasons are more likely explanations since we did not

use a sampling tube and our results were in the same direction. Consistency between the two studies enhances the possibility that there is a slight but real increase in uptake during oral breathing.

In our study, intersubject variability in percentage of O<sub>3</sub> uptake was as great as 50% for all route–visit combinations, and intrasubject variability did not exceed 25%. A similar degree of intersubject variability was found by Gerrity *et al.* (1988). This pattern of inter- and intrasubject variability in uptake may not be surprising, given that pulmonary responses to O<sub>3</sub> can differ widely among healthy subjects of similar age and gender (Gliner *et al.*, 1983; McDonnell *et al.*, 1983) but, over time, will show a high degree of reproducibility within a subject (McDonnell *et al.*, 1985). The latter study (McDonnell *et al.*, 1985), reporting reproducibility in O<sub>3</sub> responses in individuals over 3 weeks to 14 months, concluded that the previously observed intersubject variability was due to large differences in intrinsic responsiveness to O<sub>3</sub>. We suggest that the variabilities in percentage of O<sub>3</sub> uptake also contribute to the variability in O<sub>3</sub> responsiveness. Experiments in rats have indicated that the accumulated dose during an exposure is a factor in the expression of response, as are intrinsic factors like those associated with O<sub>3</sub> adaptation (Wiester *et al.*, 1995).

In our subjects, young adult males with larger  $\dot{V}_E$  retained a smaller percentage of inhaled O<sub>3</sub> than males with lower  $\dot{V}_E$  (Fig. 4). Two other studies have been performed in subjects at resting or tidal breathing (Gerrity *et al.*, 1995; Hu *et al.*, 1992). Their data are also plotted in Fig. 4. Despite differences in methodology, the percentage of O<sub>3</sub> uptake in all three studies fall within the same range with respect to  $\dot{V}_E$ . These results are also consistent with intrasubject data reported by Gerrity *et al.* (1988) in which the percentage of O<sub>3</sub> uptake was statistically significantly less in subjects breathing at 24 breaths per minute than when breathing at 12 breaths per minute with equal tidal volumes. Their data suggest that O<sub>3</sub> residence time in the respiratory tract may modify the percentage of O<sub>3</sub> uptake.

The studies of Wiester *et al.* (1987, 1988) have shown that the percentage of O<sub>3</sub> uptake in rodents is lower than that in humans. The much greater frequencies in the rats (~120 breaths per minute) compared to humans (~16 breaths per minute) result in much shorter residence times. It is possible that the association of decreased percentage of O<sub>3</sub> uptake with decreased residence time (Gerrity *et al.*, 1988) observed in humans may provide a partial explanation for the substantially lower percentage of O<sub>3</sub> uptake measured in rats compared to humans.

The usefulness of toxicological animal data depends on whether a certain animal is an appropriate surrogate for humans with respect to its dose–response characteristics for a particular xenobiotic substance. There is good reason to believe that rats are appropriate animal models to use for pre-

TABLE 3  
Ozone Uptake in Humans and Rodents Evaluated  
under Similar Measurement Conditions

Species (No./group)	Route	Exposure		Percentage of O <sub>3</sub> uptake (mean ± SE)
		(ppm O <sub>3</sub> )	Time (min)	
Human (10)	Oral	0.3	10	76 ± 3
Human (10)	Nasal	0.3	10	73 ± 3
Guinea pig (6) <sup>a</sup>	Nasal	0.3	60	53 ± 5
Fischer 344 rat (6) <sup>a</sup>	Nasal	0.3	60	44 ± 5
Sprague–Dawley rat (6) <sup>a</sup>	Nasal	0.3	60	46 ± 9
Long Evans rat (6) <sup>a</sup>	Nasal	0.3	60	47 ± 4

<sup>a</sup>Wiester *et al.* (1988).

dicting toxic effects of O<sub>3</sub> in humans for both acute (Tepper *et al.*, 1989) and long-term environmental-type exposures (Wiester *et al.*, 1995).

This is the only study we know in which uptake measurements were obtained for humans using a technique similar to the one used for small laboratory rodents (Wiester *et al.*, 1988). In both human and rat studies, the subjects were awake, quietly inhaling 0.3 ppm O<sub>3</sub> that flowed upward past the face. In addition, the flow rates were proportional to  $\dot{V}_E$  (flow/ $\dot{V}_E$  = ~4.2) and adequate to prevent rebreathing of downstream air. The percentage of O<sub>3</sub> uptake was determined under steady-state conditions using similar equations for computing the amount of O<sub>3</sub> removed from downstream air. Comparative data (Table 3) show that during nasal breathing, humans retain approximately 75% of inhaled O<sub>3</sub> compared to 45–47% for rats. The consistency of the human data from our study, along with the other human data, suggests that the lower uptake in rats is not a function of methodology employed.

In conclusion, these data provide further insights into the variability in health effects observed between humans; in this study the percentage of O<sub>3</sub> uptake ranged from approximately 50 to over 95% in 10 subjects. This study also illustrated that the percentage of O<sub>3</sub> uptake is reproducible within subjects, varying by about 25% or less. In evaluating O<sub>3</sub> effects for quantitative health risk assessment, it is important to be aware of the variability in uptake for a population to estimate the range of predicted health-related responses. In determining mechanisms of effects using animal models, these data provide information on the predicted percentage of O<sub>3</sub> uptake needed to extrapolate from animals to humans.

#### ACKNOWLEDGMENTS

The authors thank Drs. W. F. McDonnell and E. Seal for assisting with the spirometry measurements, J. A. Raub and Drs. J. H. Overton, Jr., and J. A. Dye for their thoughtful reviews of the manuscript, and Joanne Cook for secretarial services.

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