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Near-Lifetime Exposure of the Rat to a Simulated Urban Profile of Nitrogen Dioxide: Pulmonary Function Evaluation[1]

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To investigate the potential for up to a near-lifetime exposure to high-ambient levels of nitrogen dioxide (NO_2) to induce functional lung damage, groups of rats were exposed to air or a simulated urban profile of NO_2 (0.5 ppm background, 1.5 ppm peak) for 1, 3, 13, 52, or 78 weeks. The dynamic, static, and diffusional characteristics of the lung were evaluated postexposure in anesthetized rats. Furthermore, for the 13-, 52-, and 78-week groups, additional animals were tested after a 6-, 26-, or 17-week period in filtered air, respectively. No significant NO_2 differences between exposed and control animals were found for the nitrogen washout, compliance, lung volume, or diffusion capacity of carbon monoxide measurements. At 78 weeks, however, a reduction in $\Delta FEF_{25\%}$, an estimate of convexity in the later portion of the forced expiratory flow volume curve, was observed. Breathing patterns and mechanics were also assessed postexposure in a parallel group of similarly exposed unanesthetized rats. These rats were examined during a filtered air, 4 and 8% carbon dioxide (CO_2) challenge. In the unanesthetized rat, frequency of breathing was significantly decreased and tidal volume, expiratory resistance, and inspiratory and expiratory times tended to increase. For several of these variables, the largest response also occurred at 78 weeks and seemed to be exacerbated by CO_2 challenge. For both unanesthetized and anesthetized test groups, the magnitude of the changes in pulmonary function were small and their significance was borderline, thus indicating that near-lifetime exposure to the rat of a high ambient urban profile of NO_2 does not lead to dysfunction suggestive of degenerative lung disease. © 1993 Society of Toxicology.

Nitrogen dioxide (NO_2) is an oxidant gas common to the urban environment that primarily arises directly (as NO_2) or indirectly (from photochemical transformation of nitric oxide) from stationary and vehicular emissions. Due to its reactivity with sunlight and various organic chemicals in the atmosphere, a continuous low-level baseline is maintained, upon which peaks that cycle with automobile traffic patterns are superimposed (U.S. EPA, 1982). In some cities, the 24-hr NO_2 concentration can range from 0.05 to 0.2 ppm, with occasional 1-hr spikes as high as 0.5 ppm (California Air Resources Board, 1983).

Serious adverse functional or morphological injury has not been associated with ambient concentrations of NO_2 exposure in laboratory animals. However, chronic NO_2 exposures at high concentrations can induce an emphysematous lesion in the lung (12 ppm, rabbits, Haydon *et al.*, 1967; 15–20 ppm, rats, Freeman *et al.*, 1972). Similar findings have been reported in beagle dogs exposed for 16 hr daily for 68 months to 0.6 ppm NO_2 with 0.16 ppm nitric oxide (Hyde *et al.*, 1978). The lesions described in these studies meet the established criteria for emphysema in that the lungs were fixed in an inflated state and both alveolar airspace enlargement and septal destruction occurred (Snider *et al.*, 1985). Other studies of NO_2 exposures in different animal species, conducted at lower concentrations or for shorter periods, also have reported enlarged airspaces, but without alveolar destruction; such findings have been considered consistent with an emphysema-like lesion (U.S. EPA, 1982).

Although morphologically defined emphysema-like lesions can occur with exposure to NO_2, sparse and often contradictory functional evidence of emphysema has been reported. Several studies have examined pulmonary function after subchronic (<90 days) NO_2 exposure. Six-week exposures to 2 ppm NO_2, with 1-hr, 6-ppm spikes occurring twice daily, yielded a slight decrease in lung compliance in adult rats,

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uncharacteristic of emphysema (Stevens *et al.*, 1988). Similarly, a continuous 9-month exposure to 2.9 ppm NO_2 resulted in decreased compliance and lung volume, a finding more consistent with a restrictive lung lesion, although the authors attributed the functional effects to reduced surfactant lipid content (Arner and Rhoades, 1973). In contrast, alterations in lung function more characteristic of emphysema, such as enlarged lung volumes and increased compliance, have also been observed. Lafuma *et al.* (1987) reported no alterations in vital capacity or compliance after 8 weeks of exposure to 2 ppm NO_2, but found an increase in lung volume as determined by morphometric techniques. A similar, but nonsignificant tendency for lung volume to be elevated at low inflation pressures was reported in rats after a 30-day exposure to 5.4 ppm NO_2 (Yokoyama *et al.*, 1980). Most recently, Mauderly *et al.* (1990) reported that exposure to 9.5 ppm NO_2 for 7 hr/day, 5 days/week for 24 months resulted in larger, heavier, and more compliant lungs. However, in the latter study, total lung collagen was increased in the exposed animals and no enlargement of alveolar terminal airspaces or destruction of septal tissues was observed.

To date, the epidemiological data also have not shown a clear relationship between exposure to NO_2 and chronic lung disease (Samet and Utell, 1990). However, NO_2 has been implicated in the increase in respiratory infections of children who live in homes with gas stoves (Samet *et al.*, 1987). Peak hourly concentrations of 0.1 to 0.4 ppm have been recorded in kitchens where gas stoves are used for cooking (Spengler and Sexton, 1983). Acute controlled human exposures to NO_2 have revealed significant alterations in airway resistance and reactivity with exposure to 1.5 ppm or greater (Frampton *et al.*, 1990; Beil and Ulmer, 1976; Mohsenin, 1987). In some studies, exercising asthmatics and patients with chronic obstructive pulmonary disease show changes in airway reactivity and spirometry after acute exposures to 0.5 ppm or less (Bauer *et al.*, 1986, 1987). Whether such transient effects reflect a potential for long-term pathological consequences remains uncertain.

Because of the uncertainty of outcome associated with prolonged ambient exposures, a large multidisciplinary study was conducted to evaluate if a near-lifetime NO_2 exposure simulating an outdoor diurnal urban profile would produce lung dysfunction suggestive of degenerative lung disease in the rat. The exposure scenario used for this study was constructed from time and concentration profiles of a typical Los Angeles basin summer pollution episode (Davies *et al.*, 1987) as recorded in the National Aerometric Data Bank of the Office of Air Quality Planning and Standards (Environmental Protection Agency) and the South Coast Air Quality Management District (provided by Southern California Edison). Concentrations were approximately 2 to 5 times the worse-case air-shed scenario. Evaluation intervals of 1, 3, 13, 52, and 78 weeks were selected to represent acute, subchronic, and near-life long exposures. The major objectives were to determine (1) if a simulated urban pattern of NO_2 produced lung disease, (2) whether the effects changed with continued exposure, and (3) if there was reversibility of the lesions during a clean-air postexposure period. Only the results from pulmonary function studies will be presented herein. Pulmonary histomorphometric and lung biochemistry results are to be reported elsewhere.

METHODS

Animals

Male, 60-day-old, Fischer 344 rats (CDF [F-344]CrlBR, VAF+ animals, Charles River Breeding Laboratories, Inc., Kingston, NY) were ear-tagged and housed in individual wire cages with food (Purina Rodent Lab Chow, St. Louis, MO) and water available *ad libitum*. Animals were randomly assigned to the air or NO_2 exposure groups and were quarantined in a barrier-maintained exposure facility for 1 week prior to exposure. Separate groups of rats were purchased and handled identically for the 1-, 3-, 13-, 52-, and 78-week exposure studies. Serum was obtained upon arrival from 5% of rats in each shipment and from sentinel rats exposed along with test rats. Sera were found to be free of antibodies to the following viruses: reovirus Type 3, pneumonia virus of mice, encephalomyelitis, Sendai, mouse adenovirus, sialodacryo adenitis, Toolan H-1, Kilham rat virus, lymphocytic choriomeningitis, and rat coronavirus. In addition, lung washes were found to be free of *Mycoplasma pulmonis*, and nothing unusual appeared in bacterial cultures from the nasopharynx, trachea, and gut. Test rats were also found to be free of ectoparasites and endoparasites.

Exposure Regimen and Facility

All rats (except those in the 52-week group) were exposed in an automated exposure facility containing 14.2-m^3 walk-in environmental rooms as previously described (Davies *et al.*, 1987). Control and NO_2 exposures of the 52-week group were conducted in a separate facility utilizing identical procedures, except for the use of Hazelton-2000 exposure chambers. This deviation from the protocol was instituted to minimize the total length of the study and to maximize the number of animals per group. Exposure concentration, temperature (74 ± 3°F), humidity (60 ± 10%), room ventilation, and lighting (6:00 AM to 6:00 PM) were controlled and monitored using a computer-interfaced mechanical system. Nitrogen dioxide was cylinder-supplied to the exposure chamber (National Specialty Gas Co.) and was monitored using continuous chemiluminescent analyzers (Bendix Model 8101 C NO_x) that were calibrated biweekly (RFNA-0777-022). The NO_2-exposure profile consisted of a 0.50 ppm background level for 16 hr, a 6-hr exposure spike, and a 2-

hr downtime (8:30 AM to 10:30 AM) for animal and equipment service (Fig. 1). During the 6-hr exposure spike, the concentration gradually rose from 0.5 to 1.5 ppm, remained at 1.5 ppm for 2 hr, then returned to 0.5 ppm. This profile was run each weekday, but on Saturdays and Sundays, the baseline concentration was run for 22 hr per day.

Separate groups of rats were exposed to this simulated urban profile for 1, 3, 13, 52, or 78 weeks. At each exposure-specific assessment, the rats slated for analysis were maintained at the background NO_2 concentration (0.5 ppm) for at least 2 days, but not more than 5 days, without the superimposed spike of NO_2. This procedure was instituted to avoid acute response-related artifacts in the biological end points. Upon completion of the 13-, 52-, and 78-week exposures, a portion of the group was removed to alternate housing and received filtered air for 6, 26, or 17 weeks, respectively. A 17-week, rather than 26-week, postexposure evaluation was chosen to reduce the likelihood of encountering senescence-related deaths after the 78-week exposure. Due to limitations in chamber space, not all end points could be accommodated for the postexposure evaluation.

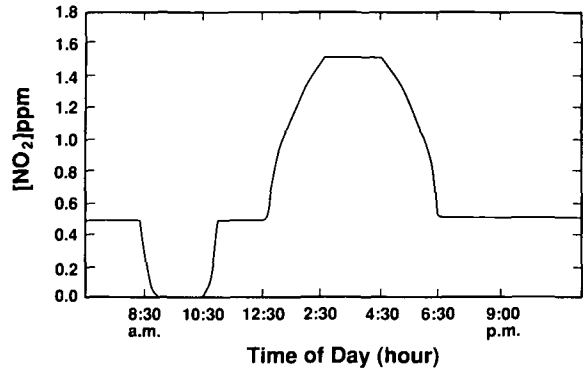

FIG. 1. Schematic showing the timing and concentration of the daily NO_2 profile.

Anesthetized Pulmonary Function Testing

Animal preparation. Immediately prior to testing, each rat was anesthetized with 50 mg/kg sodium pentobarbital (ip) and an appropriately sized Luer stub adapter was surgically inserted into the trachea. For the 52- and 78-week study animals, a transoral tracheal adapter was initially inserted for measurement of the forced expiratory parameters, after which the transoral tube was removed and the animals were tracheotomized as described above. Prior to the measurement of lung compliance and nitrogen (N_2) washout, the animal was paralyzed with succinylcholine (4 mg) and ventilated with a Harvard rodent respiratory at 80 breaths/min and a tidal volume equal to their tidal volume during spontaneous breathing.

Pulmonary function tests. The rats were placed in a whole-body pressure plethysmograph for *in vivo* lung function measurements. Detailed methods for the measurements of static lung volume in rodents using this system have been described previously (Raub *et al.*, 1982). All measurements were conducted in duplicate or triplicate. Briefly, vital capacity (VC) was measured between airway pressures (Pao) of −20 and +30 cm water (H_2O), total lung capacity (TLC) was obtained by gas dilution methods, and residual volume (RV) was computed as the difference between TLC and VC. End-expiratory volume (EEV) was measured using an application of Boyle's Law. Single-breath diffusing capacity of carbon monoxide (DL_{CO}) was obtained as described by Takezawa *et al.* (1980).

The quasistatic volume–pressure curve was used to calculate the measurement of respiratory system compliance (C_{rs}). The curve was obtained by slowly inflating the lungs (3 ml/sec) to TLC (+30 cm H_2O, Pao) and then slowly deflating them to RV (−20 cm H_2O). The data are reported as the slope from a nonlinear least-squares regression of the deflation wing of the quasistatic pressure–volume curve. A shape constant determined by an exponential fit ($V = 1 - V_0e^{-kp}$) of the deflation wing was used as a volume-independent description of lung compliance above EEV (Pengelly, 1977). The terms V and p are corresponding volumes and pressure points along the curve, V_0 is the infinite asymptote of V at infinite p, and k is the volume-independent slope constant. The distribution of ventilation (multibreath N_2 washout) was assessed by computing the slope of the log end-tidal N_2 concentration vs the breath number from the multibreath N_2–washout curve. This measurement was performed at fixed tidal volume (Vt) and frequency of breathing (FOB) during ventilation with 100% oxygen.

During the 52- and 78-week studies only, small-airway function was evaluated using a forced expiration technique that had recently been added to our testing system (Tepper *et al.*, 1987). These measurements were obtained prior to the previously described pulmonary function measurements using a transoral tracheal adaptor and a separate plethysmograph. The maneuver was performed by slowly inflating the rats to +30 cm H_2O (Pao) and, under computer control, quickly opening a wide-bore solenoid exposing the rat's airway to −40 cm H_2O pressure. Previous studies have demonstrated that the plethysmograph system is linear to 50 Hz (Raub *et al.*, 1982) and peak flow is achieved in less than 50 msec (unpublished data). From the resultant maximum expiratory flow–volume curve, forced vital capacity (FVC); peak flow; and flow at 50% ($FEF_{50\%}$), 25% ($FEF_{25\%}$), and 10% ($FEF_{10\%}$) of FVC were analyzed for treatment differences. Additionally, the delta flow at 25% ($\Delta FEF_{25\%}$) of FVC was calculated by subtracting the chord slope value of $FEF_{25\%}$ from the actual $FEF_{25\%}$ (Tepper *et al.*, 1989). This value indicates the deviation from the normal convexity of the effort-independent portion of the curve.

Data collection and statistical analysis. Analog outputs from the transducers and Nitralyzer (Med Science, St. Louis, MO) were recorded simultaneously on an eight-channel recorder and were digitized by an on-line microcomputer. The computer also was used to calculate the various measurements of pulmonary function.

A multivariate analysis of variance (ANOVA) (SAS 516, SAS Institute, Cary, NC) was used four times to evaluate overall main and interactive effects to help avoid Type I errors (false positives) associated with making multiple univariate comparisons. Each analysis modeled exposure at two levels (air vs NO_2) as one factor, duration of exposure (2–5 levels, see below) as the second factor, and the interaction between exposure and duration as a third factor. The N_2 slope, C_{rs}, VC, EEV, TLC, and DL_{CO} were considered primary variables at five levels (1, 3, 13, 52, and 78 weeks) for the initial multivariate analysis. A second multivariate analysis, using the same primary variables, was used to examine effects related to the 6-, 26- and 17-week postexposure filtered-air periods. Another set of multivariate analyses had to be performed for the flow volume measurements because they were examined only at two levels (52 and 78 weeks). The FVC and peak flow, representing large airway function, and the $FEF_{25\%}$, representing small airway function, were designated as primary variables. Finally, a similar analysis was performed using these variables for the 26- and 17-week postexposure periods.

According to the study protocol, if significant ($p \leq 0.1$) multivariate exposure effects or significant exposure by time interactions were found, further univariate analyses were to be performed. When univariate comparisons at different exposure and time levels were made, the p values were corrected to control the overall Type I error rate. The Type I error rate was set at 0.05 and the Type II error rate was set at 0.20. Ancillary measurements (i.e., measurements derived from other measures) were examined using a univariate ANOVAs. Type I error rate was maintained for individual measurements, but not for the collective group of ancillary measurements. The ancillary measures examined were RV, k, $FEF_{50\%}$, $\Delta FEF_{25\%}$ and $FEF_{10\%}$. Data presented in tabular form represent the mean ± the standard error of the mean.

Unanesthetized Pulmonary Function Testing

Surgery. Rats were weighed and anesthetized with sodium pentobarbital using 40 mg/kg body weight q.s. to 1 ml injection volume with saline. As described previously (Wiester *et al.*, 1985), silastic catheters (0.030 in. i.d. × 0.065 in. o.d.) were inserted aseptically into the intrapleural space at the level of the seventh rib to allow measurement of intrapleural pressure (Ppl). Recovery from surgery (18–24 hr) was in filtered room air prior to evaluation. For the 78-week group, the dose of pentobarbital was reduced from 40 to 25 mg/kg because several animals from both the air and NO_2 group died prior to surgery at the higher anesthetic dose level. Due to limitations in chamber space for the 13-, 52-, and 78-week exposures, additional animals were not available for unanesthetized pulmonary function measurements after the filtered air postexposure holding period.

Testing apparatus. On the day of evaluation, four rats were fitted with cotton jackets, placed into acrylic restrainers, and sealed in stainless steel plethysmographs with their heads facing into a 0.3-m^3 Rochester exposure chamber (Tepper *et al.*, 1988). The Ppl catheter was filled with saline and attached to a pressure transducer (Narco Biosystems, Houston, TX). Platinum needle electrodes were attached subcutaneously on the ventral surface at the junctures of the extremities to monitor the electrocardiogram (ECG). Tidal volume was measured by a differential pressure transducer (Validyne Model MP45, Northridge, CA) as air pressure changed inside the plethysmograph due to the expansion and contraction of the rat's chest wall. Previous studies indicated that the plethysmograph and the pleural pressure transducer systems were linear and in phase to 10 Hz (Tepper *et al.*, 1988). The analog outputs from these transducers were recorded simultaneously on an eight-channel recorder and were digitized by an on-line microcomputer. The computer automated the experiment, controlling timing, sampling, analysis, and regulation of the concentration of carbon dioxide (CO_2) used for postexposure challenges (see below).

Testing protocol. All testing in the four-chamber pulmonary function system was conducted blind to treatment and such that both control and NO_2 rats were present in every run. The overall sequence of pulmonary measurement periods in relation to CO_2 challenges is described in Fig. 2. Seventeen minutes after the rats were placed in the plethysmograph, control readings in filtered air were obtained (C1 in Fig. 2). Challenges with CO_2 then were initiated to test the rats' abilities to compensate to a hypercapnic stress. First, a 7-min challenge to 4% CO_2 was obtained by metering 100% CO_2 into the chamber dilution air via a mass flow controller. Subsequent to this challenge, there was a 15-min rest period

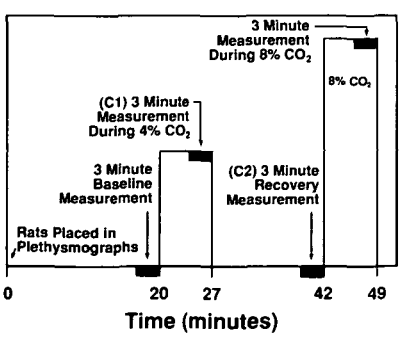

FIG. 2. Schematic showing the timing and concentration of the postexposure CO_2 challenge testing protocol.

in filtered air, at the end of which, recovery measurements were obtained (C2 in Fig. 2). Next, the pulmonary response to a 7-min 8% CO_2 challenge was ascertained. Carbon dioxide concentration was monitored continuously using a CO_2 analyzer (Beckman Instrument Co., Schiller Park, IL).

Data collection and statistical analysis. During the last 3 min of each of the four measurement periods (control [C1], 4% CO_2, air recovery [C2], and 8% CO_2), ventilatory function measurements were obtained (Fig. 2). The measurements computed were Vt, FOB, minute volume, Ppl, inspiratory resistance, expiratory resistance (Re), dynamic compliance (Cdyn), and inspiratory (Ti) and expiratory time (Te). The ECG was evaluated only in the 52- and 78-week exposed rats. The following parameters were obtained from the ECG during each of the four postexposure measurement periods: heart rate, R-R interval, P-R interval, P-R segment, and P-αT interval (Watkinson *et al.*, 1985). Data presented in tabular form represent the mean ± the standard error of the mean.

A two-way repeated measures ANOVA was used to evaluate significant main and interactive effects in the functional responses (SAS 516, SAS Institute, Cary, NC). The analysis modeled exposure (air vs NO_2) as one independent factor and time (1, 3, 13, 52, and 78 weeks) as the second. The dependent variables were the responses at the various concentrations of CO_2 (0, 4, and 8%). For each end point yielding significant, interactive effects in the repeated measures analysis, a two-way ANOVA examining the differences (contrasts) in the responses among the levels of exposure and time was performed. Contrasts were corrected for multiple comparisons and Type 1 and Type 2 errors were fixed at 0.05 and 0.20, respectively.

RESULTS

Anesthetized Pulmonary Function Testing

The six primary variables that were analyzed statistically were the slope from the N_2–washout curve, C_{rs}, EEV, TLC, DL_{CO}, and VC. Primary variable data and the ancillary measurements, k and RV, from air- and NO_2-exposed rats after 13, 52, and 78 weeks are presented in Table 1. The multivariate analysis indicated that there were no significant exposure by time interactions ($p = 0.89$) or any exposure-only effects ($p = 0.41$), even when pooled over all evaluation times for these six primary variables. Univariate tests were used to examine exposure-only or exposure by time interactions for the ancillary measurements and, again, no significant effects were detected. Furthermore, no significant multivariate exposure ($p = 0.26$) or exposure by time interactions ($p = 0.29$) were observed during the filtered air postexposure period for the 13-, 52-, or 78-week exposure groups for either primary or ancillary variables (Table 2).

Because the forced-expiratory maneuver was performed only for the 52- and 78-week exposure studies, a separate multivariate ANOVA had to be performed. The analysis indicated that for the primary variables (FVC, peak flow, and $FEF_{25\%}$) there was no significant exposure by time ($p = 0.98$) interaction; however, a significant multivariate exposure-only effect was observed ($p = 0.09$). Although the a priori criterion was met, further univariate subtesting indicated that none of these variables were significantly affected by NO_2 exposure. Similarly, after the filtered-air postexposure period, a significant multivariate exposure effect was observed ($p = 0.05$). Again, univariate analysis of the primary variables indicated that there were no significant NO_2-related effects.

For the flow-derived ancillary parameters, the univariate contrast analysis (52 weeks air minus 52 weeks NO_2 or 78 weeks air minus 78 weeks NO_2) revealed that $\Delta FEF_{25\%}$ was significantly smaller ($p = 0.004$) after 78 weeks of NO_2 exposure. This indicates that the effort-independent portion of the forced expiratory curve was less outwardly convex (Table 3). After a 17-week holding period in filtered air the contrast analysis indicated that the $\Delta FEF_{25\%}$ of the NO_2-exposed rats was back to within normal limits. None of the other ancillary measures ($FEF_{50\%}$, and $FEF_{10\%}$) evaluated at 52- and 78-weeks or at 26- and 17-weeks postexposure were significantly different from control (Table 3).

Unanesthetized Pulmonary Function Testing

A two-way repeated measures ANOVA indicated that significant exposure effects were observed for FOB and a significant exposure by CO_2 challenge interaction was seen for Cdyn. Additionally, Vt, Ti, Te, and Re showed noteworthy trends. The magnitude and direction of these results are described in more detail below. Repeated measures analysis of the ECG indicated that there were no significant exposure-related consequences at either the 52- or 78-week exposure evaluation (Table 4).

Overall, the Vt, Re, Ti, and Te were generally greater and FOB was significantly slower in NO_2-exposed rats compared to control-exposed rats at all of the time points evaluated (Fig. 3). Three of the variables (Re, FOB, and Te) showed the greatest difference between the control and exposed rats' responses at the 78-week testing point. These interesting, however, nonsignificant, time-related trends also showed a CO_2 challenge concentration–response relationship at the 78-week evaluation (Fig. 4). The normal increase in FOB induced by 8% CO_2 challenge was reduced by 13% in the NO_2-exposed rats. Most of the reduction in FOB could be explained by a lengthening of expiration rather than an equal change in Ti and Te.

A significant ($p < 0.01$) exposure/CO_2-challenge interaction was found for the measurement of Cdyn. This resulted because of a difference in response between the two control measurements (C1 and C2, Fig. 2) and was considered un-

TABLE 1
NO_2 Chronic Exposure

Exposure duration:	13 weeks		52 weeks		78 weeks	
	Air	NO_2	Air	NO_2	Air	NO_2
Number	16	16	12	12	11	12
Body weight (g)	338 ± 5	340 ± 5	460 ± 7	470 ± 5	402 ± 8	420 ± 4
TLC (ml)	12.43 ± 0.16	12.48 ± 0.18	14.13 ± 0.17	14.16 ± 0.20	13.40 ± 0.14	13.28 ± 0.22
VC (ml)	11.33 ± 0.11	11.29 ± 0.12	12.81 ± 0.15	13.22 ± 0.20	11.81 ± 0.19	11.88 ± 0.18
EEV (ml)	2.58 ± 0.07	2.64 ± 0.12	2.56 ± 0.06	2.65 ± 0.09	2.36 ± 0.06	2.46 ± 0.09
RV (ml)	1.33 ± 0.08	1.40 ± 0.07	1.56 ± 0.06	1.46 ± 0.05	1.67 ± 0.07	1.43 ± 0.06
C_{rs} (ml/cm H_2O)	0.86 ± 0.01	0.87 ± 0.02	1.01 ± 0.02	1.03 ± 0.02	0.99 ± 0.02	0.99 ± 0.02
k	0.126 ± 0.002	0.130 ± 0.001	0.125 ± 0.002	0.128 ± 0.002	0.126 ± 0.004	0.131 ± 0.002
N_2 Slope[a]	−0.28 ± 0.01	−0.27 ± 0.01	−0.31 ± 0.01	−0.32 ± 0.01	−0.34 ± 0.01	−0.33 ± 0.01
DL_{CO} (ml/min * Torr)	0.211 ± 0.008	0.206 ± 0.006	0.242 ± 0.005	0.239 ± 0.007	0.225 ± 0.008	0.245 ± 0.012

[a] Units for N_2 Slope: slope log % N_2/number of breaths.

important. Specifically, the air-exposed group had a slightly lower Cdyn (C2-C1 = −0.021 cm H_2O) during the second control period (C2) compared to the first control period (C1, see Fig. 2) whereas in NO_2-exposed rats, the opposite occurred, with Cdyn being slightly larger (C2-C1 = 0.037 cm H_2O) in C2 than during C1. No significant differences in Cdyn were found comparing air versus NO_2 for any single control period (C1 or C2), indicating that only the differences (C1-C2) were significant.

DISCUSSION

In laboratory animals, exposures to NO_2 at concentrations well above those typically encountered in the environment have been associated with changes in lung structure that are generally interpreted as "emphysema-like" (Freeman *et al.*, 1972; Haydon *et al.*, 1967, Hyde *et al.*, 1978). Several studies of rats and other laboratory animals chronically exposed to NO_2 have observed enlarged alveoli without destruction of the septal region (U.S. EPA, 1982). Thus, the appropriateness of calling these lesions emphysema would be considered questionable according to the National Institutes of Health morphological definition (Snider *et al.*, 1985).

More recent studies involving prolonged exposures have not been consistent with these early findings. Exposures to 2 ppm for 6 weeks (with daily morning and afternoon 1-hr spikes to 6 ppm) resulted in a functionally stiffer lung (Stevens *et al.*, 1988) with thickened alveolar septum and interstitium (Chang *et al.*, 1986). After a 24-month exposure to 9.5 ppm, rats exhibited enlarged lung volumes, but had a total lung

TABLE 2
NO_2 Chronic Exposure Recovery

Exposure duration:	13 weeks		52 weeks		78 weeks	
Recovery:	+6 weeks		+27 weeks		+17 weeks	
	Air	NO_2	Air	NO_2	Air	NO_2
Number	16	17	9	9	7	9
Body weight (g)	352 ± 6	365 ± 4	510 ± 13	497 ± 5	401 ± 14	404 ± 9
TLC (ml)	12.69 ± 0.17	12.87 ± 0.15	14.81 ± 0.16	14.64 ± 0.28	13.23 ± 0.19	14.08 ± 0.32
VC (ml)	11.24 ± 0.15	11.39 ± 0.12	13.06 ± 0.12	12.08 ± 0.23	11.69 ± 0.19	12.41 ± 0.15
EEV (ml)	3.08 ± 0.08	3.26 ± 0.16	2.59 ± 0.13	2.55 ± 0.09	2.60 ± 0.10	2.80 ± 0.10
RV (ml)	1.44 ± 0.06	1.49 ± 0.06	1.76 ± 0.08	1.84 ± 0.11	1.55 ± 0.06	1.67 ± 0.19
C_{rs} (ml/cm H_2O)	0.88 ± 0.02	0.87 ± 0.02	1.03 ± 0.04	0.96 ± 0.09	0.86 ± 0.03	0.82 ± 0.05
k	0.135 ± 0.002	0.134 ± 0.003	—	—	0.134 ± 0.007	0.142 ± 0.001
N_2 Slope[a]	−0 29 ± 0.01	−0.292 ± 0.00	−0.36 ± 0.01	−0.34 ± 0.01	−0.32 ± 0.02	−0.32 ± 0.02
DL_{CO} (ml/min * Torr)	0.220 ± 0.007	0.223 ± 0.007	0.219 ± 0.009	0.219 ± 0.009	0.183 ± 0.012	0.231 ± 0.019

[a] Units for N_2 Slope: slope log % N_2/number of breaths.

TABLE 3
NO_2 Chronic Exposure and Recovery Flow Volume Parameters

Exposure duration:	52 weeks				78 weeks			
Recovery:	0 weeks		+27 weeks		0 weeks		+17 weeks	
	Air	NO_2	Air	NO_2	Air	NO_2	Air	NO_2
Number	12	12	9	9	11	12	7	9
FVC (ml)	13.0 ± 0.2	13.5 ± 0.2	12.6 ± 0.3	12.2 ± 0.3	11.1 ± 0.2	11.4 ± 0.2	10.8 ± 0.2	11.5 ± 0.2
Peak flow (ml/sec)	136.5 ± 3.0	138.2 ± 3.1	131.2 ± 2.0	136.8 ± 4.1	119.9 ± 4.5	122.2 ± 2.4	125.4 ± 3.2	129.5 ± 3.1
$FEF_{50\%}$ (ml/sec)	108.4 ± 4.8	101.4 ± 4.1	94.8 ± 2.4	97.3 ± 6.9	90.2 ± 4.7	99.6 ± 2.3	96.6 ± 8.5	95.6 ± 4.1
$FEF_{25\%}$ (ml/sec)	61.3 ± 2.6	59.3 ± 2.8	57.5 ± 2.0	51.6 ± 2.9	55.4 ± 1.7	52.7 ± 2.5	52.6 ± 4.6	50.4 ± 2.5
$\Delta FEF_{25\%}$ (ml/sec)	7.1 ± 1.4	8.6 ± 2.2	10.0 ± 2.1	2.9 ± 2.5	10.3 ± 1.0	3.0 ± 1.9[a]	4.3 ± 2.9	2.6 ± 2.0
$FEF_{10\%}$ (ml/sec)	28.8 ± 1.3	27.7 ± 1.7	21.2 ± 1.3	21.5 ± 1.2	21.2 ± 1.4	21.3 ± 0.9	18.6 ± 1.3	21.9 ± 1.2

[a] Significantly different from air ($p = 0.004$).

collagen content that was increased and airspaces that were neither enlarged nor destroyed (Mauderly *et al.*, 1990. It is possible that the lesions described in rats by Freeman *et al.* (1972) were the result of a greater exposure concentration (15 ppm) or longer daily (23 hr/day) exposure periods. Perhaps, as has been suggested, older rat exposure studies were commonly confounded by various lung infections that might contribute in some unknown fashion to alter the development of the morphological lesions (Mauderly *et al.*, 1990; Castleman, 1983).

The evidence of corroborative morphological injury in the lung resulting from this NO_2 exposure is unclear. No histopathological changes in the lungs of these rats were found using standard light microscopy (data not presented). Similarly, there appears to have been little, if any, alteration in the epithelial cell make-up and characteristics in the proximal alveolar region (PAR) of the lung, as might have been predicted based on earlier reports and modeled dosimetry patterns (L. Y. Chang, personal communication). A recent abstract has described changes in the interstitial matrix of the PAR, indicating quantitative losses of the elastic network in this region in a small sample of rats exposed to this NO_2 profile (Vincent *et al.*, 1990), but these findings remained to be confirmed in the entire sample population (R. Vincent, personal communication). Should the loss of elastin matrix at this distal airway site be substantiated, the subtle functional defects would be corroborated because it is generally acknowledged that elastin is critical to the infrastructure of the distal lung and the maintenance of airflow integrity during forced expiration.

Taken together, our findings do not support the contention that high-ambient levels of NO_2 are emphysemagenic; however, we cannot totally dismiss that significant, albeit small, functional alterations were observed and that these effects occurred primarily after 78 weeks of exposure. It is possible that these significant effects are statistical artifacts related to the large number of comparisons examined. However, the timing (effects occurred only at 78 weeks), independence between variables (tidal breathing and mechanics versus the forced expiration maneuver), and the direction of the aggregate effects (FOB decreased, Re increased, and $\Delta FEF_{25\%}$ decreased) suggest a random coincidence to be unlikely. Ad-

TABLE 4
NO_2 Chronic Exposure Electrocardiogram Parameters

Exposure duration:	52 weeks		78 weeks	
	Air	NO_2	Air	NO_2
Number[a]	6–7	6–9	4–5	9–10
Heart rate (beats/min)	474 ± 9	491 ± 7	475 ± 11	466 ± 6
R-R interval (sec)	1.27 ± 0.02	1.24 ± 0.02	1.25 ± 0.03	1.30 ± 0.02
P-R interval (sec)	0.44 ± 0.02	0.43 ± 0.01	0.47 ± 0.01	0.50 ± 0.02
P-R segment	0.35 ± 0.01	0.34 ± 0.01	0.36 ± 0.01	0.36 ± 0.01
P-αT interval (sec)	0.87 ± 0.02	0.81 ± 0.02	0.90 ± 0.03	0.86 ± 0.01

[a] Range of number of animals used for all five parameters.

ditionally, the data were obtained from both the anesthetized and the unanesthetized assessments of airway function, tests performed in separate groups of animals. For example, the $\Delta FEF_{25\%}$, which is typically a positive number indicating the normal convexity of the latter portion of the flow–volume curve (Tepper *et al.*, 1989), decreased. Although this measurement shows considerable variability, there was a statistically significant decrease in $\Delta FEF_{25\%}$ in the 78-week NO_2-exposed rats relative to the air-exposed control group. With a subsequent 17-week period in filtered air, the NO_2-exposed animals apparently lost this abnormality in the flow-volume curve, suggesting that the functional lesion and perhaps the associated tissue abnormalities had resolved or otherwise undergone repair.

The trends in the unanesthetized pulmonary function data at the 78-week evaluation point could be viewed as being consistent with the noted mild alteration of the flow–volume curve. Breathing patterns were altered at this time point and some of these effects were exacerbated by imposing increasing concentrations of CO_2 (Fig. 4). The FOB was significantly decreased largely by a lengthening of the normal expiratory phase rather than by an equal change in Ti and Te. This unequal distribution of the breath suggests that increased resistance might also be observed at this time point. Although not significantly increased, Re was indeed elevated compared to control and the CO_2 challenge exacerbated this effect (Fig. 4). Interestingly, ozone also caused an elevation in Re after 78 weeks of exposure (Tepper *et al.*, 1991). Similar to the speculation in the ozone study, the increased resistance may reflect an oxidant-induced premature aging phenomenon. Although the measurement of Re is theoretically thought to reflect mostly large airway function, recent evidence affirming the correlation between Re and Cdyn in laboratory animals and the apparent role of tissue viscous resistance of the lung suggest that Re might also be a measurement of changes in the smaller airways and parenchyma (Mitzner *et al.*, 1990). While the apparent consistency of the trends from several seemingly unrelated measurements, after the same length of exposure, might lead to the speculation that these data indicate an obstructive lesion, the collective data, including the reversibility of those trends during clean-air postexposure, more strongly support the conclusion that this near-lifetime exposure to an urban pattern of NO_2 did not result in degenerative lung disease.

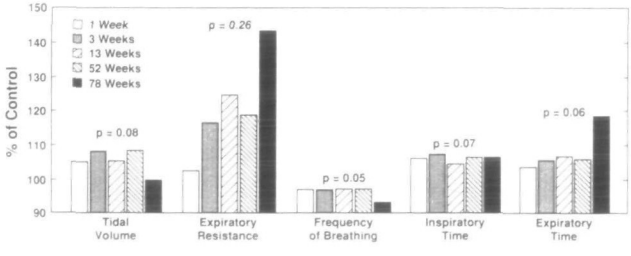

FIG. 3. Breathing patterns and mechanics (tidal volume, expiratory resistance, frequency of breathing, and inspiratory and expiratory times) are depicted as a percentage of the time-equivalent filtered-air exposure group. The *p* values are associated with a difference between air and NO_2 only and do not reflect interactions with time or postexposure CO_2 challenge.

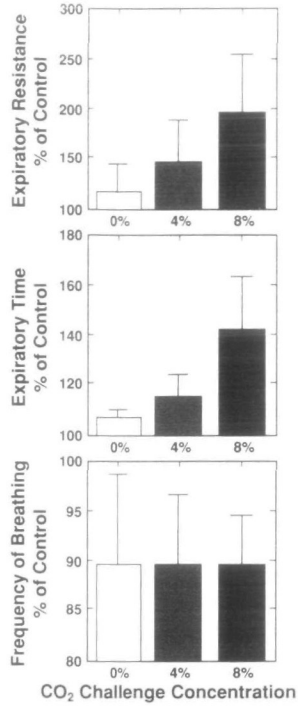

FIG. 4. The effect of CO_2 postexposure challenge on expiratory resistance, expiratory time, and frequency of breathing expressed as a percentage of the 78-week filtered-air exposure group's CO_2-challenge response

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