Inhalation Toxicology, 18:127–136, 2006

ISSN: 0895-8378 print / 1091-7691 online
DOI: 10.1080/08958370500306107

Taylor & Francis
Taylor & Francis Group

Comparison of Chamber 6.6-h Exposures to 0.04–0.08 PPM Ozone via Square-wave and Triangular Profiles on Pulmonary Responses

William C. Adams
Human Performance Laboratory, Exercise Biology Program, University of California, Davis, California, USA

It has become increasingly well realized that laboratory simulations of air pollution risk assessment need to employ O_3 concentration profiles that more accurately mimic those encountered during summer daylight ambient air pollution episodes. The present study was designed to compare the pulmonary function and symptoms of breathing discomfort responses to a 6.6-h square-wave 0.08-ppm O_3 chamber exposure to those observed in a triangular O_3 exposure profile (mean of 0.08 ppm), as well as to both a 0.06-ppm square-wave and triangular mean 0.06-ppm exposure, and to those observed during a triangular mean 0.04-ppm exposure and to a filtered air (FA) square-wave exposure. Thirty young adults (15 of each gender) served as subjects, each completing all exposures. While the 6.6-h postexposure responses to the acute triangular exposure to a mean O_3 concentration of 0.08 ppm did not differ significantly from those observed in the square-wave exposure, forced expiratory volume in 1 s $(FEV)_{1.0}$ and total symptoms severity (TSS) were significantly different from preexposure at 4.6 h (when O_3 concentration was 0.15 ppm) in the triangular exposure, but not until 6.6 h in the square-wave exposure. Thus, significant pulmonary function and symptoms responses were observed over a longer period in the triangular exposure protocol at a mean O_3 concentration of 0.08 ppm. These results support previous evidence that O_3 concentration has a greater singular effect in the total inhaled O_3 dose than do V_E and exposure duration. Subtracting pulmonary function effects consequent to O_3 exposure to existent 8-h average background levels (e.g., ∼0.04 ppm, rather than those observed in FA exposures) from those observed at higher concentrations (e.g., ∼0.08 ppm) represents a means of focusing the regulatory effort on effects that can be controlled. The greatest pulmonary function and symptoms responses observed for a 0.04-ppm triangular exposure were nearly the same as those for the FA square-wave exposure. Thus, results of the present study show that calculating the net pulmonary function effect of exposure to 0.08 ppm with "correction" for FA response, or for that incurred for 0.04 ppm O_3, does not result in any statistically significant difference.

Received 4 June 2005; accepted 28 July 2005.

The author thanks Glen R. Mangseth, Associate Development Engineer and Manager, Human Performance Laboratory, who directed the development of pulmonary and respiratory metabolic measurement systems and the computer data acquisition system for the chamber exposure facility. He also provided technical assistance in the calibration of all equipment utilized in this study, as well as keeping it operational. James D. Shaffrath, MD, performed human subject medical screening and supervision throughout the project. Kerry Proctor assisted with pilot work, conducted experimental protocols, and assisted with data reduction. Jon Eastman, Jessie Holly, Kaela Holly, Matt Laye, Amy Sekhon, and Heather Werth assisted in the conduct of experimental protocols. Sincere appreciation is extended to the subjects for their considerable contribution of time and effort. The author acknowledges Allen S. Lefohn, A.S.L. and Associates, Helena, MT, Steven Ziman, Chevron/Texaco, Richmond, CA, and Will Ollison, American Petroleum Institute, Washinton, DC, for developing the hour-by-hour diurnal triangular O_3 concentration profiles used in this study. This research was supported, in part, by a gift from the American Petroleum Institute (no. 01-0000-4913). The statements and conclusions in this article are those of the author and not necessarily those of the American Petroleum Institute.

Address correspondence to Prof. William C. Adams, 1110 Ridgecrest Dr., SE, Albuquerque, NM 87108, USA. E-mail: wcadams@ucdavis.edu

In the initial prolonged ozone (O_3) inhalation effects study, Folinsbee et al. (1988) exposed 10 young adult males for 6.6 h to 0.12 ppm throughout, with 5 h of quasi-continuous exercise at an average expired ventilation volume (V_E) of $\sim$ 20 L/min/m^2 body surface area (BSA). Forced vital capacity (FVC) and forced expiratory volume in 1 s ($FEV_{1.0}$) decreased in a near linear manner during this square-wave 0.12 ppm exposure, falling by 8.3% and 13%, respectively, after 6.6 h. Symptoms of cough and chest discomfort were significantly increased following exposure. Utilizing the same 6.6-h quasi-continuous exercise protocol, Horstman et al. (1990) demonstrated the importance of exposure to lower concentrations, that is, 0.08 to 0.12 ppm. These investigators found a significantly reduced $FEV_{1.0}$ after 3 h at 0.12 ppm, 4.6 h at 0.10 ppm, and 5.6 h at 0.08 ppm, indicating an interrelationship between duration of exposure and O_3 concentration.

Several investigators (Adams & Ollison, 1997; Lefohn, 1997; Lefohn & Foley, 1993) have advanced the notion that laboratory simulations of air pollution risk assessment need to employ O_3 concentration profiles that more accurately mimic those encountered during summer daylight ambient air pollution episodes. Neither square-wave O_3 exposures nor the one study (Hazucha et al., 1992) that utilized an 8-h triangular shaped varied O_3 exposure (i.e., 0 ppm increased to 0.24 ppm at 4 h, and back to 0 ppm at 8 h), adequately mimics the variable diurnal daylight O_3 concentration pattern observed in many urban areas experiencing air pollution episodes. Accordingly, in a recent study (Adams, 2003), two types of 6.6 h exposure patterns were used: (1) the usual square-wave profile with O_3 concentration maintained constant at 0.08 ppm, and (2) an acute triangular profile, in which O_3 concentration was increased stepwise each hour from 0.03 ppm to 0.15 ppm in the fourth hour and then decreased each hour to 0.05 ppm in the last hour (mean = 0.08 ppm). Thirty young adults (15 of each gender) served as subjects. Near identical total inhaled (O_3) doses in both the square-wave and triangular exposure profiles were observed in both face-mask and chamber exposures, and very similar postexposure pulmonary function, symptoms, and exercise ventilatory pattern responses were observed. However, pulmonary function and symptoms changes from preexposure became statistically significant at 4.6 h (when $[O_3] = 0.15$ ppm) in the triangular chamber and face-mask exposures, but not until 6.6 h in the square-wave 0.08 ppm exposures. These results support previously cited evidence that O_3 concentration has a greater singular effect in the total inhaled O_3 dose than do V_E and exposure duration (Adams et al., 1981; Folinsbee et al., 1978; Hazucha, 1987; Silverman et al., 1976).

Horstman et al. (1995) contended that a more accurate assessment of O_3 effects could be obtained by subtracting responses observed at a particular concentration from those observed in an identical filtered air (FA) exposure. Lefohn (1997), however, has pointed out that if the actual 8-h background O_3 levels at the cleanest sites in the United States are 0.04 ppm or somewhat higher, then health effects may well be overestimated if FA is used as the background control. Further, background levels of O_3 concentration do not necessarily follow a square-wave pattern but instead vary diurnally (Lefohn, 1997; Rombout et al., 1986). In our laboratory, prolonged exposures to O_3 concentrations less than 0.08 ppm (and greater than FA) have only been done in a square-wave protocol with the face mask system (at 0.04 ppm [Adams, 2002,] and at 0.06 ppm [Adams, 1998]). Hence, a direct examination of responses to diurnally observed triangular O_3 concentration exposures at mean concentrations of 0.04 and 0.06 ppm seems advisable.

The present study was designed to compare the pulmonary function and symptoms of breathing discomfort responses to a 6.6-h square-wave 0.08-ppm O_3 chamber exposure to those observed in a triangular O_3 exposure profile (mean of 0.08 ppm), as well as to both a 0.06-ppm square-wave and a triangular mean 0.06-ppm exposure, to those observed during a triangular mean 0.04-ppm exposure, and to those observed consequent to the usual square-wave FA exposure.

METHODS

Subjects and Subject Orientation

Thirty young adults, 15 of each gender, who were nonsmokers and had not lived in an area for the prior 6 months where the hourly State of California air quality standard for O_3 (viz. 0.09 ppm) was exceeded, served as subjects. Subjects were solicited volunteers from the University of California, Davis, or surrounding community. They were screened for absence of asthma or significant allergies, and had normal baseline pulmonary function. Prospective participants read and signed an Institutional Review Board-approved informed consent form. They were shown the equipment to be used in the study and any questions were answered before they signed the consent form.

Subjects participated in a $1^1/_4$-h orientation session, during which they first had their height and body weight measured. The subject then walked to another section of the laboratory in which a free-standing stainless steel environmental chamber (model 1328-M, Vista Scientific, Ivyland, PA) was located. Following performance of at least 3 maximum forced expiratory maneuvers, each subject then pedaled an electronically braked cycle ergometer (Quinton, model 829E, Varberg, Sweden) at 3 or 4 work rates for at least 3 min each until they reached a steady-state V_E of $\sim$20 L/min/m^2 BSA. Following 5 min rest, the subject walked on a motor driven treadmill (Odyssey LSD, Bodyguard Fitness, Hackensack, NJ) at 3.4 mph, at 2 or 3 grades between 1 and 8% for at least 3 min each until a steady-state V_E value of $\sim$20 L/min/m^2 BSA was reached. During the performance of these activities, V_E determinations were made via the subject breathing FA through a Teflon-coated respiratory valve (Hans Rudolph, Kansas City, MO) with obligatory mouthpiece inhalation. Heart rate (HR) was monitored via three leads connected to an electrocardiograph R-wave detector. The orientation session was completed with the performance of at least two maximum forced expiratory maneuvers.

Experimental Design and Protocols

The six 6.6-h chamber exposures completed by each subject included: (1) a repeat of the Folinsbee et al. (1988) protocol entailing a square-wave chamber exposure to FA, with six 50-min exercise bouts at a mean equivalent ventilation rate (EVR) of ~20 L/min/m^2 BSA: (2) the same protocol as number 1, but with a square-wave exposure to 0.08 ppm O_3; (3) the same protocol as number 1 while exposed to a different constant O_3 concentration level each hour (0.03, 0.07, 0.10, 0.15, 0.08, and 0.05, averaging 0.08 ppm); (4) the same protocol as number 1, but with a square-wave exposure to 0.06 ppm O_3; (5) the same protocol as number 1 while exposed to a different constant O_3 concentration level each hour (0.04, 0.07, 0.09, 0.07, 0.05, and 0.04, averaging 0.06 ppm); and (6) the same protocol as number 1 while exposed to a different constant O_3 concentration level each hour (0.03, 0.04, 0.05, 0.05, 0.04, and 0.03, averaging 0.04 ppm). The protocols were conducted in single-blind fashion and completed by each subject in near random order, with a minimum of 4 days intervening between protocols (Schonfeld et al., 1989).

During each protocol, subjects breathed the air mixture provided continuously during exercise and rest according to the 6.6-h exposure protocol used by Folinsbee et al. (1988). Subject exposure was continuous for 6.6 h except for an average of 3.74 min (range = 0–9 min) when the subject exited the chamber to use a nearby rest room. A 35-min lunch break after completion of h 3 was taken at rest in the chamber with the O_3 concentration maintained at that used in h 3. The 50-min exercise bouts were done alternately on the cycle ergometer and treadmill. Temperature and relative humidity were maintained between 21 and 25°C and between 40 and 60%. Airflow between 1 and 2 m/s was directed at the subject's frontal aspect during exercise by a small industrial-grade fan (model 9318, Air King Ltd., Brampton, Ontario, Canada) mounted on a wall.

Pulmonary Function Measurements

Subjects performed 2 to 4 forced expiratory maneuvers immediately before and after each experimental exposure, during the last 3 min of the 30 min lunch break, and during the last 3 min of each hour. Measurements of FVC and $FEV_{1.0}$ were made with a UCI-500 spirometry system (Vacumetrics, Ventura, CA). If the sums of the FVC and $FEV_{1.0}$ values for the first 2 maneuvers were within 200 ml of each other, the mean for each was used in statistical analyses. If not, then additional maneuvers were performed until this criterion was met for any two maneuvers.

Exercise and Resting Measurements

Data acquisition instruments interfaced to a Lab View software package, supported by a Macintosh-compatible Power Center 120 computer, included a VMM-400 ventilation measurement module (Interface Associates, Aliso Viejo, CA), a CD3A CO_2 analyzer (Applied Electrochemistry, Pasadena, CA), an OM-11 O_2 analyzer (Beckman Instruments, Fullerton, CA), an electrocardiograph R-wave detector, and a temperature thermistor located in the expired gas line. Minute-by-minute HR values were obtained every 15 s throughout all exposures. When the subject breathed through a Teflon-coated respiratory valve with mouthpiece and noseclip, minute-by-minute values for V_E, tidal volume (V_T), breathing frequency (f), percent O_2 and CO_2 in expired gas, expired gas temperature, and oxygen uptake (VO_2) were displayed each 15 s on an Apple Multiple Scan 15 monitor interfaced to the Power Center 120 computer. During the first 2 h of each protocol, this occurred between 8 and 12 min and between 45 and 49 min. If V_E was more than 2 L/min above or below the subject's target value, the cycle ergometer work rate or treadmill grade was adjusted. Thereafter, exercise data acquisition occurred only between 45 and 49 min of each hour. If the total mean V_E at the end of each measurement period was more than 1 L/min above or below the subject's target value, the cycle ergometer work rate or treadmill grade was adjusted at the beginning of exercise during the following hour.

Symptoms were monitored initially after 8 min of the first exercise bout in each exposure; thereafter, they were evaluated during the next to the last minute of each exercise bout. Subjects were asked to rate the severity of each of four symptoms—throat tickle, cough, shortness of breath (SOB), and pain on deep inspiration (PDI)—by pointing to a visual display. Each symptom was rated according to a severity scale (ranging from 0, not present, to 40, incapacitating) previously described (Adams et al., 1987). Total symptoms severity (TSS) was calculated as the sum of the severity ratings for the four individual symptoms.

O_3 Administration and Monitoring

The stainless-steel chamber is a closed system with provision for some damper-controlled fresh air intake. The damper controls the cross-sectional area of an 8-in-diameter duct. The damper is adjusted so that approximately 20% of the cross-sectional area of the duct is open to room air. After entering, the room air is mixed with air returning from the chamber. This air passes through two chemisorbent filters (Purafil Chemisorbent Media, Doraville, GA), through a particle filter, through the blower, and over the humidifier, dehumidifier, and cooling and heating coils in succession. This FA is returned to the chamber through an 8-in × 12-in sheet metal duct. O_3 is introduced into this duct ~2 m prior to its outlet into the chamber. During the O_3 exposures, a known concentration of O_3 was generated by passing dry purified cylinder O_2 through an ozonizer (Sander model 200, Sander Aquarientechnik, Am Ostenberg, Germany). The O_3 was drawn through Teflon tubing into the chamber. During the FA exposure, the O_2 tank and ozonizer were off. The filter system of the chamber ensured that even with low ambient O_3 concentrations in the laboratory, measured O_3 in the chamber was <0.003 ppm throughout the FA exposure.

Sampling of O_3 occurred through 0.64-cm inner diameter Teflon tubing connected to a Dasibi O_3 monitor; the sample tubing outlet was located on a wall adjacent to the treadmill at a height of ~1 m from the floor. Continuous measurement of

O_3 was accomplished by an online data acquisition system with minute-by-minute averages obtained from the voltage output generated by the Dasibi monitor. The Dasibi monitor was calibrated before and after the study (change <0.003 ppm O_3 within the range used), using the ultraviolet (UV) absorption photometric method, traceable to a National Institute of Standards and Technology standard photometer, at the Primate Research Center, University of California, Davis.

Subject Characterization

Following completion of all experimental exposures, each subject had his or her body composition and maximal aerobic capacity (VO_{2max}) measured. Body composition was determined by hydrostatic weighing utilizing procedures described previously (Madsen et al., 1998). Maximal oxygen uptake was determined by a progressively incremented cycle ergometer (model 800S, Sensormedics, Yorba Linda, CA) test to volitional exhaustion (Adams & Schelegle, 1983). Pedal frequency was set at 70 full revolutions/min (rpm), with progressive increments in resistance of 20 to 30 W effected every 2 min, starting with 120–130 W for females and 150–170 W for males. A plateau in VO_2 (i.e., less than 0.10-L/min increase with the last work rate increment equivalent to between 0.25 and 0.30 L/min) was the criterion used to ensure that VO_{2max} had been achieved (McArdle et al., 1996).

Statistical Procedures

Minute-by-minute V_E values were added separately for exercise and rest periods of each exposure, with separate averages for total ventilation (V_{tot}) calculated for each subject, and then for the whole group. The latter, together with exposure duration and mean O_3 concentration, was used to determine the group mean total inhaled O_3 dose for each protocol. Duplicate (occasionally, triplicate) spirometric volumes and flows for pre and postexposure and at hourly intervals during the 6.6-h protocols were obtained. The treatment effect was determined as percent change from the preexposure value. Similarly, values taken at 8 and 10 min of the first exercise bout (i.e., "initial" value) and the final 3 min of each exercise bout for VO_2, V_E, f, V_T, and HR were utilized to calculate percent change from the initial value. The PDI and TSS ratings for all reported symptoms were analyzed as absolute changes from zero. The data were analyzed for statistical significance ($p < .05$) using a two-way analysis of variance (ANOVA) with repeated measures, which tested for gas concentration (including square-wave or triangular exposure mode) effects and exposure time effects. Upon obtaining a significant F value, the Scheffé post hoc test (Kleinbaum et al., 1988) was applied to determine which particular mean values were significantly different from each other.

RESULTS

A summary of the female and male subjects' anthropometry, VO_{2max}, and pulmonary function is given in Table 1. The 30 subjects, with 4 exceptions (2 females), were not competitive athletes, although all of the nonathletes were engaged in some form of regular recreational aerobic activity. All subjects had normal pulmonary function, with the ratio of $FEV_{1.0}/FVC$ ranging from 71.0 to 92.6%.

The group mean total exercise V_E and total V_E (including estimated total rest V_E) values for the six 6.6-h protocols, together with mean O_3 concentrations and total inhaled O_3 dose, are given in Table 2. There were no significant differences between total V_E values for the six protocols (protocols 1 through 6). The total inhaled O_3 dose for protocol 1 (FA) was significantly less than those for the other five protocols. The total inhaled O_3 doses for the two 0.08-ppm exposures (protocols 2 and 3) were not significantly different from each other. The total O_3 inhaled doses for the two 0.06-ppm exposures (protocols 4 and 5) were not significantly different from each other, but were significantly less than for the values for the 0.08-ppm exposures and significantly greater than for the 0.04 ppm exposure (protocol 6).

Preexposure group mean pulmonary function and postexposure percent change values for the six protocols, together with the protocol postexposure statistically significant specific mean differences, are given in Table 3. None of the preexposure FVC values for the protocols differed significantly from each other, which was also true for $FEV_{1.0}$ and percent $FEV_{1.0}/FVC$. Postexposure percent change in $FEV_{1.0}$ for the FA protocol (protocol 1) was not significantly different from those for the two 0.06-ppm exposures (protocols 4 and 5) or for protocol 6 (0.04-ppm triangular, exposure). The postexposure percent change in $FEV_{1.0}$ for the two 0.08-ppm protocols (square-wave and triangular) were significantly greater than for all other protocols, but did not differ significantly from each other. Postexposure percent change

TABLE 1
Summary of subjects' anthropometric and functional characteristics

Gender	Age (yr)	Height (cm)	Weight (kg)	Body fat (%)	BSA (m^2)	VO_{2max} (L/min)	FVC (L)	$FEV_{1.0}$ (L/s)	$FEV_{1.0}/FVC$ (%)
Female	22.8	166.8	59.3	21.0	1.65	2.40	4.26	3.65	85.8
	(1.2)	(5.7)	(5.1)	(4.3)	(0.09)	(0.35)	(0.48)	(0.40)	(3.7)
Male	23.5	179.9	80.8	17.3	1.98	3.56	5.92	4.68	79.5
	(3.0)	(7.3)	(10.7)	(7.0)	(0.14)	(0.39)	(0.65)	(0.40)	(5.8)

Note. Numerical values are group means (standard deviations in parentheses).

TABLE 2
Group mean O_3 concentration, mean total exercise V_E, total rest V_E, total lunch V_E, and total inhaled O_3 dose for the six protocols

Protocol	Mean [O_3] (ppm)	Total exercise V_E (L)	Total V_E (L)[a]	Total inhaled dose (ppm · L)[b]
1	0.0005	10,886 ± 1282	11,998 ± 1404	5.8
2	0.0799	10,903 ± 1240	12,015 ± 1362	960
3	0.0799	11,018 ± 1288	12,130 ± 1410	969
4	0.0600	10,877 ± 1322	11,989 ± 1446	719
5	0.0601	10,898 ± 1339	12,010 ± 1461	722
6	0.0401	10,937 ± 1319	12,049 ± 1441	483

Note. Plus and minus values are one standard deviation.
[a]Total V_E equals total exercise plus resting values for each hour and the 0.6 h lunch break (in liters) for 6.6 h of exposure.
[b]Total inhaled dose equals the product of total V_E (L) and mean O_3 concentration (ppm).

for FVC for all protocols closely paralleled those for $FEV_{1.0}$. Those for percent change in $FEV_{1.0}$/FVC were more variable than those for $FEV_{1.0}$ and FVC.

Hourly percent changes in $FEV_{1.0}$ for the six protocols are shown in Figure 1. None of the hourly $FEV_{1.0}$ percent changes from preexposure for protocol 1 (FA) were significantly increased. Those observed for the triangular exposure to a mean concentration of 0.04 ppm (i.e., protocol 6) did not differ significantly from those for FA. $FEV_{1.0}$ percent change values observed for both 0.06-ppm protocols (i.e., square-wave and triangular) did not differ significantly from each other, and were not significantly greater than those for FA at 6.6 h. The percent change in $FEV_{1.0}$ for the triangular O_3 exposure averaging 0.08 ppm (i.e., protocol 3) was significantly decreased from preexposure at 4.6 h, with no significant differences in values between 4.6 h and 6.6 h. Values for the 0.08 ppm square-wave exposure (protocol 2) did not become statistically significant until 6.6 h.

Group mean final PDI and total symptoms responses to the six exposures are given in Table 4. There was no significant difference in PDI or TSS values at 6.6 h of exposure to FA (protocol 1) which were significantly less than those for the two 0.08-ppm protocols (protocols 2 and 3). Both PDI and TSS values observed at the end of protocols 2 and 3 were significantly greater than for the O_3 exposures to a mean concentration of 0.06 ppm (protocols 4 and 5) and for the exposure to a mean concentration of 0.04 ppm (protocol 6). End exposure values for the two 0.06-ppm exposures were not significantly greater than those for the FA or the 0.04-ppm exposures. Hour-by-hour TSS ratings for the six protocols are depicted in Figure 2. TSS did not change significantly during the FA exposure (protocol 1) or for the 0.04-ppm exposure (protocol 6). Hour-by-hour TSS values for the square-wave 0.08-ppm O_3 exposure (protocol 2) reached statistical significance at 5.6 h, while that for the triangular 0.08-ppm exposure (protocol 3) was significant at 4.6 h. Further, the TSS values for the latter were significantly greater than for the square-wave exposure (protocol 2) at 4.6 h and 5.6 h. Hour-by-hour TSS values for the square-wave 0.06-ppm O_3 exposure (protocol 4) did not reach statistical significance at 6.6 h. However, TSS values for the triangular 0.06-ppm exposure (protocol 5) reached statistical significance at 5.6 and 6.6 h, but were not significantly greater than those for the square-wave exposure (protocol 4) at 5.6 h and 6.6 h.

TABLE 3
Group mean pulmonary function postexposure responses for the six protocols

Protocol number and description	FVC (L)[a]		$FEV_{1.0}$ (L)[a]		$FEV_{1.0}$/FVC[b] (%)	
	Pre	Change (%)	Pre	Change (%)	Pre	Change (%)
1, FA, sq.-w.	5.033 ± 1.044	−0.44 ± 2.15	4.113 ± 0.674	+1.35 ± 2.98	82.5 ± 6.3	+0.92 ± 2.61
2, 0.08 ppm, sq.-w.	5.112 ± 1.015	−4.46 ± 7.26	4.194 ± 0.684	−4.72 ± 8.65	82.7 ± 5.7	−0.36 ± 3.31
3, 0.08 ppm, triang.	5.077 ± 1.046	−4.78 ± 6.23	4.145 ± 0.694	−5.65 ± 8.08	82.4 ± 6.3	−1.00 ± 3.90
4, 0.06 ppm, sq.-w.	5.066 ± 0.988	−0.89 ± 3.12	4.125 ± 0.694	−1.51 ± 4.24	82.1 ± 6.6	−0.62 ± 3.04
5, 0.06 ppm, triang.	5.047 ± 0.991	−1.72 ± 5.15	4.137 ± 0.648	−1.43 ± 5.95	82.8 ± 6.6	+0.31 ± 3.36
6, 0.04 ppm, triang.	5.038 ± 1.105	−0.74 ± 2.05	4.112 ± 0.691	+1.17 ± 2.97	82.3 ± 5.7	+0.43 ± 2.31

Note. Plus and minus values are one standard deviation; sq.-w., square-wave; triang., triangular.
[a]Specific significant mean differences for protocol pairs 1–2, 1–3, 2–4, 2–5, 2–6, 3–4, 3–5, and 3–6.
[b]Specific significant mean differences for protocol pairs 1–2, 1–3, and 1–4.

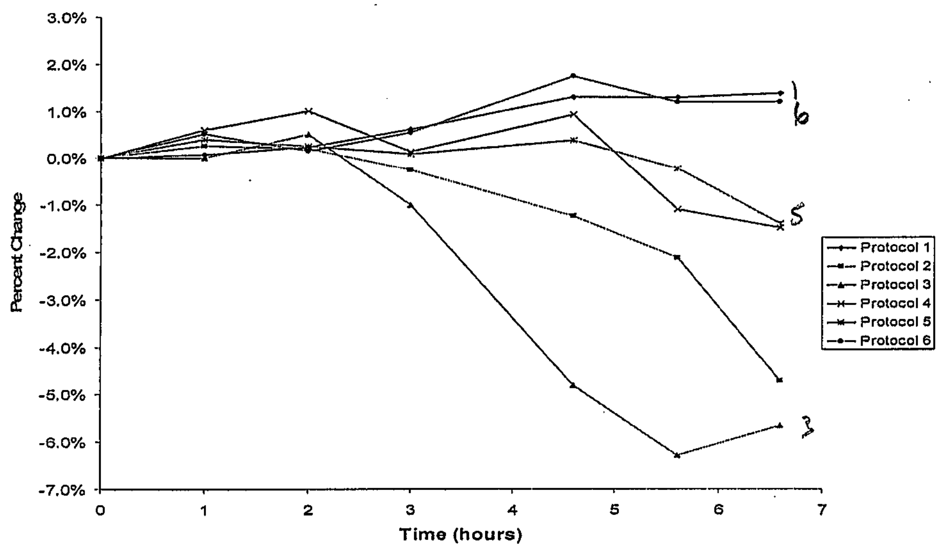

FIG. 1. Hour by hour percent change in $FEV_{1.0}$.

Hour-by-hour PDI scores followed a closely similar pattern as those for TSS.

Group mean values for cardiorespiratory and ventilatory responses for the first exercise period (between 8 and 10 min) and the last minute of exercise (i.e., ~6.6 h) for the six protocols are given in Table 5. As expected, the initial values for each of these protocols were not significantly different for any of the five variables. At ~6.6 h, HR VO_2. and V_E values were not consistently greater than the "initial" value for the six protocols. The prolonged quasi-continuous exercise (5.0 h of 6.6 h total) induced a significant rapid shallow breathing pattern (i.e., increased f and decreased V_T) at the end of the FA exposure (protocol 1). The increased f and decreased V_T observed for the 6.6-h O_3 exposures (protocols 2–6) were numerically greater than those observed for the FA exposure, but only the final values for the two 0.08-ppm exposures (protocols 2 and 3) were statistically significant.

TABLE 4
Group mean final subjective symptoms responses for the six protocols

Protocol number and description	Pain on deep inspiration[a]	Total subjective symptoms[a]
1, FA, sq.-w.	0.2 ± 0.9	0.6 ± 2.2
2, 0.08 ppm, sq.-w.	6.0 ± 7.1	9.6 ± 10.6
3, 0.08 ppm, triang.	5.6 ± 5.2	8.1 ± 9.0
4, 0.06 ppm, sq.-w.	1.4 ± 2.9	2.5 ± 4.9
5, 0.06 ppm, triang.	2.0 ± 4.4	3.9 ± 7.4
6, 0.04 ppm, triang.	0.7 ± 1.8	2.0 ± 4.5

Note. Plus and minus values are one standard deviation; sq.-w., square-wave; triang., triangular.

[a]Specific significant mean differences for protocol pairs 1–2, 1–3, 2–4, 2–5, 2–6, 3–4, 3–5, and 3–6.

DISCUSSION

Comparison of Pulmonary Function and Symptoms Responses to Square-Wave and Triangular Profile Exposures with Equivalent Mean O_3 Concentration of 0.08 ppm

In a recent study (Adams, 2003), the net postexposure mean $FEV_{1.0}$ responses for the 2 square wave 0.08-ppm exposures were −6.14% for the face-mask system and −6.16% for the chamber. In the present study, the net postexposure mean $FEV_{1.0}$ response was −6.06% for the square-wave exposure and −6.99% for the triangular exposure. Thus, not only were the postexposure $FEV_{1.0}$ responses in the present study very similar, but their close agreement with those observed in our previous study (Adams, 2003) indicates that sensitivity to O_3 was similar in both groups of subjects. Further, these responses were only slightly less than the −7.4% net response (including +0.8% response to FA) observed in a chamber exposure to 0.08 ppm O_3 with exercise V_E of 20 L/min/m^2 BSA by Horstman et al. (1990) and the −7.7% net response (including −0.6% response to FA) observed by McDonnell et al. (1991).

It has become increasingly well realized that laboratory simulations of air pollution risk assessment need to employ O_3 concentration profiles that more accurately mimic those encountered during summer daylight ambient air pollution episodes (Adams & Ollison, 1997; Lefohn & Foley, 1993). Accordingly, in a recently published study (Adams, 2003) two types of exposure patterns were used: (1) the usual square-wave profile with O_3 concentration maintained constant at 0.08 ppm, and (2) an acute triangular profile, in which O_3 concentration was

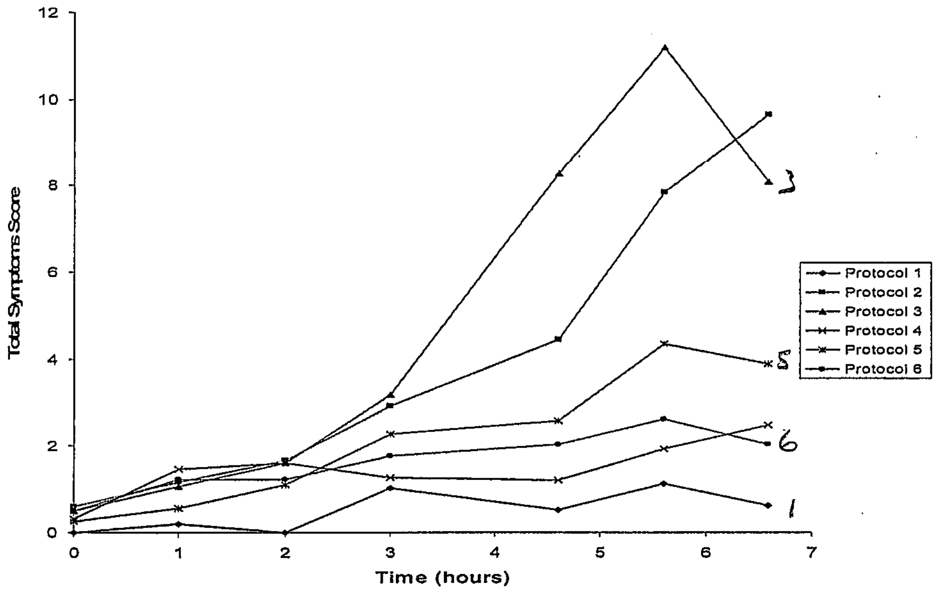

FIG. 2. Hour-by-hour change in total symptoms score.

increased stepwise each hour from 0.03 ppm to 0.15 ppm in 4 h and then decreased stepwise each hour to 0.05 ppm (mean = 0.08 ppm). Near-identical total inhaled O_3 doses in both the square-wave and triangular exposure profiles for both the face-mask and chamber inhalation protocols were observed. Further, very similar postexposure pulmonary function, symptoms, and exercise ventilatory pattern responses were observed. However, earlier significant $FEV_{1.0}$ response for the triangular protocol was paralleled by significant differences in TSS and PDI at 4.6 h (when O_3 concenration was 0.15 ppm) that continued on through 5.6 h and 6.6 h when the mean O_3 concentration was 0.065 ppm. The total O_3 dose in the chamber triangular exposure at the end of 4.6 h was 74.5% of the total square-wave exposure dose (including 35 min of rest during the lunch break while exposed to 0.10 ppm).

In the present study, as shown in Table 3, there was no significant difference in postexposure $FEV_{1.0}$ decrement for the two 0.08-ppm exposures. However, the square-wave value was not significantly reduced until 6.6 h. $FEV_{1.0}$ response was significant in the triangular exposure beginning at 4.6 h (when O_3 concentration was 0.15 ppm), peaked at 5.6 h, and then decreased slightly at 6.6 h. The earlier significant $FEV_{1.0}$ responses for the triangular exposure were paralleled by significant differences in TSS and PDI beginning at 4.6 h, peaking at 5.6 h, and then decreasing somewhat at 6.6 h. Collectively, these results are consistent with previous evidence that O_3 concentration has a greater singular effect in the total inhaled O_3 dose than do V_E and exposure duration (Adams et al., 1981; Folinsbee et al., 1978; Hazucha, 1987; Silverman et al., 1976). They also indicate that subjects spend more time with reduced lung function and symptoms of breathing discomfort during an environmentally realistic variable O_3 pattern averaging 0.08 ppm than when exposed to the constant, square-wave 0.08-ppm O_3 profile. It would be very interesting to determine whether later occurring inflammatory indicators are different in the longer occurring significant pulmonary effects observed in the triangular exposures than in the square-wave exposures, especially since they were not measured by Hazucha et al. (1992), in our earlier study (Adams, 2003), or in the present study.

Comparison of Pulmonary Function and Symptoms Responses to Square-Wave and Triangular Profile Exposures with Equivalent Mean O_3 Concentrations of 0.06 and 0.04 ppm

No previous 6.6-h chamber exposure has been done at 0.06 ppm O_3, although the results of a face-mask square-wave exposure (with exercise $V_E = 23$ L/min/m^2 BSA) was reported in a final report to the American Petroleum Institute (Adams, 1998). In that study, $FEV_{1.0}$ change was reduced to -1.51% at end exposure, with TSS of 5.2, but with neither being statistically different from values observed for a FA exposure (+1.97% and 2.2, respectively). In the present study, final $FEV_{1.0}$ response was also -1.51% for the 0.06 ppm square-wave exposure (protocol 4), while the final TSS value was 2.5. Neither of these values differed significantly from those observed for the FA exposure (protocol 1; +1.35% and 0.6, respectively) or for the $FEV_{1.0}$ (+1.17%) and TSS (2.0) values observed for the 0.04-ppm triangular exposure (protocol 6). Maximum response values for the 0.06-ppm triangular exposure in the present study (protocol 5) were -1.43% for $FEV_{1.0}$ (at 6.6 h) and 4.3 for TSS (at 5.6 h). Again, neither of these values differed significantly from those observed for the FA exposure or for the 0.04-ppm triangular

TABLE 5
Group mean first exercise cardiorespiratory and ventilatory responses and final percent change for the six protocols

	HR (beats/min)		VO_2 (L/min)		V_E (L/min)		f (breaths/min)[a]		V_T (L)[a]	
Protocol number and description	Exer. 1 (8–10 min)	Final change (%)	Exer. 1 (8–10 min)	Final change (%)	Exer. 1 (8–10 min)	Final change (%)	Exer. 1 (8–10 min)	Final change (%)	Exer. 1 (8–10 min)	Final change (%)
1, FA, sq.-w.	125.2 ± 16.3	+0.9 ± 8.6	1.42 ± 0.24	−0.7 ± 8.4	36.1 ± 4.7	−0.1 ± 7.0	26.5 ± 4.2	+11.0 ± 11.6	1.40 ± 0.28	−9.4 ± 10.9
2, 0.08 ppm, sq.-w.	123.9 ± 15.1	+0.3 ± 7.1	1.43 ± 0.27	−1.7 ± 8.2	36.8 ± 4.2	−2.8 ± 6.6	26.3 ± 4.2	+21.8 ± 17.6	1.44 ± 0.29	−18.9 ± 10.9
3, 0.08 ppm, triang.	125.2 ± 12.6	−1.2 ± 8.3	1.41 ± 0.25	−3.0 ± 7.2	36.7 ± 4.8	−1.8 ± 6.6	26.0 ± 4.1	+22.9 ± 17.6	1.44 ± 0.27	−18.6 ± 10.6
4, 0.06 ppm, sq.-w.	122.6 ± 15.5	+1.2 ± 8.1	1.40 ± 0.26	−1.6 ± 7.9	36.3 ± 4.5	−1.1 ± 7.9	27.0 ± 4.5	+17.9 ± 17.3	1.38 ± 0.26	−14.8 ± 9.7
5, 0.06 ppm, triang.	123.8 ± 12.6	+1.4 ± 9.3	1.40 ± 0.25	−0.4 ± 6.8	36.3 ± 4.8	−0.3 ± 6.9	26.6 ± 4.4	+17.9 ± 14.6	1.41 ± 0.30	−14.6 ± 8.6
6, 0.04 ppm, triang.	124.5 ± 15.4	−0.8 ± 6.3	1.49 ± 0.25	−1.6 ± 7.7	36.7 ± 4.5	−2.0 ± 7.2	26.8 ± 4.2	+16.4 ± 13.4	1.40 ± 0.29	−15.1 ± 8.9

Note. Plus and minus values are one standard deviation; sq.-w., square-wave; triang., triangular.
[a]Specific significant percent change mean differences between protocols 1 and 2, and 1 and 3.

exposure. TSS for the 0.06-ppm triangular exposure (protocol 5) were significantly greater at 5.6 h and 6.6 h than the initial value obtained at 8 min of exposure, but were not significantly greater than TSS observed at those times in the 0.06-ppm square-wave exposure. Final exercise ventilatory pattern measurements (f and V_T) for both 0.06-ppm exposures were not significantly different from those observed for the FA and 0.04 ppm triangular exposures, but were significantly less than those observed for both 0.08 ppm exposures (Table 5).

No previous 6.6-h chamber exposure has been done at 0.04 ppm O_3, but in an earlier study (Adams, 2002), a 0.04-ppm square-wave face-mask exposure was completed. The postexposure $FEV_{1.0}$ response value (+1.15%) was not significantly different from that for a FA exposure (+2.39%). The greatest $FEV_{1.0}$ response for the 0.04-ppm triangular exposure in the present study (protocol 6) was +1.17% at both 5.6 h and 6.6 h, which, as shown in Figure 1, was nearly the same as those for the FA exposure (protocol 1). Further, no significant difference between the final TSS response for the 0.04-ppm triangular exposure and that observed for the FA exposure (protocol 1) was observed. Mean TSS responses for the 0.04-ppm O_3 triangular exposure in the present study (protocol 6) were numerically quite similar to those observed previously in the 0.04-ppm O_3 square-wave face mask exposure, which did not differ significantly from those observed for the FA exposure (Adams, 2002).

Subtraction of Pulmonary Responses to Background O_3 Concentrations, Instead of Those Consequent to FA Exposure, in Calculating the Net Pulmonary Effects of Exposure to 0.08 ppm

In a comparison of pulmonary responses of asthmatic and nonasthmatic subjects exposed to O_3, Horstman et al. (1995) contend that a more accurate assessment of O_3 effects could be obtained by subtracting responses observed at a particular concentration from those observed in an identical FA exposure. Lefohn (1997), however, has pointed out that if the actual 8-h background O_3 levels at the cleanest sites in the United States are 0.04 ppm (or perhaps somewhat higher), then health effects well may be overestimated in the U.S. Environmental Protection Agency (EPA) risk assessment if FA is used as the background control. Thus, perhaps subtracting pulmonary function effects consequent to O_3 exposure to existent 8-h background levels (i.e., ~0.04–0.06 ppm) from those observed at higher concentrations represents a more commendable means of focusing the regulatory effort on effects that can be controlled. In addition, background levels of O_3 concentration do not necessarily follow a square-wave pattern but instead vary diurnally (Lefohn, 1997; Rombout et al., 1986). Results of the present study, together with those of Adams (2002), show that calculating the net pulmonary effect of exposure to 0.08 ppm with "correction" for FA response, or for that incurred for 0.04 ppm O_3 (whether square-wave or triangular), does not result in any statistically significant difference between net values for $FEV_{1.0}$ or TSS. Further, in the present study, subtracting the final $FEV_{1.0}$ response for the 0.06-ppm exposures (protocols 4 and 5) from those observed for the two 0.08-ppm exposures (protocols 2 and 3) gave results not statistically different from the values obtained upon subtracting the final FA (protocol 1) value from those for the two final values observed for the two 0.08-ppm exposures ($p < .15$).

REFERENCES

Adams, W. C. 1998. *Dose-response effects of varied equivalent minute ventilation rates on pulmonary function responses during exposure to ozone.* Final Report to the American Petroleum Institute, Washington, DC. September 29.

Adams, W. C. 2002. Comparison of chamber and face mask 6.6-hour exposures to ozone on pulmonary function and symptoms responses. *Inhal. Toxicol.* 14:745–764.

Adams, W. C. 2003. Comparison of chamber and face mask 6.6-hour exposures to 0.08 ppm ozone via square-wave and triangular profiles on pulmonary responses. *Inhalat. Toxicol.* 15:265–281.

Adams, W. C., and Ollison, W. M. 1997. Effects of prolonged simulated ambient ozone dosing patterns on human pulmonary function and symptomatology. *Proc. Air Waste Manage. Assoc.* June 8–13, 97-MP9.02, pp. 1–16.

Adams, W. C., and Schelegle, E. S. 1983. Ozone and high ventilation effects on pulmonary function and endurance performance. *J. Appl. Physiol.* 55:805–812.

Adams, W. C., Savin, W. M., and Christo, A. E. 1981. Detection of ozone toxicity during continuous exercise via the effective dose concept. *J. Appl. Physiol.* 51:415–422.

Adams, W. C., Brookes, K. A., and Schelegle, E. S. 1987. Effects of NO_2 alone and in combination with O_3 on young men and women. *J. Appl. Physiol.* 62:1698–1704.

Folinsbee, L. J., Drinkwater, B. L., Bedi, J. F., and Horvath, S. M. 1978. The influence of exercise on the pulmonary function changes due to low concentrations of ozone. In *Environmental stress,* eds. L. J. Folinsbee, J. A. Wagner, J. F. Borgia, B. L. Drinkwater, J. A. Gliner, and J. F. Bedi, pp. 125–145. New York: Academic Press.

Folinsbee, L. J., McDonnell, W. F., and Horstman, D. H. 1988. Pulmonary function and symptom responses after 6.6-hour exposure to 0.12 ppm ozone with moderate exercise. *J. Air Pollut. Control Assoc.* 38:28–35.

Hazucha, M. J. 1987. Relationship between ozone exposure and pulmonary function changes. *J. Appl. Physiol.* 62:1671–1680.

Hazucha, M. J., Folinsbee, L.J., and Seal, E., Jr. 1992. Effects of steady-state and variable ozone concentration profiles on pulmonary function. *Am. J. Respir. Crit. Care Med.* 146:1487–1493.

Horstman, D. H., Folinsbee, L. J., Ives, P. J., Abdul-Salaam, S., and McDonnell, W. F. 1990. Ozone concentration and pulmonary response relationships for 6.6-hour exposures with five hours of moderate exercise to 0.08, 0.10, and 0.12 ppm. *Am. Rev. Respir. Dis.* 142:1158–1163.

Horstman, D.H., Ball, B.A., Brown, J., Gerrity, T., and Folinsbee, L.J.1995. Comparison of pulmonary responses of asthmatic and nonasthmatic subjects performing light exercise while exposed to a low level of ozone. *Toxicol Ind. Health* 11:369–385.

Kleinbaum, D. G., Kupper, L. L., and Muller, K. W. 1988. *Applied regression analysis and other multivariable methods*, pp. 368–372. Boston, PWS-Kent.

Lefohn, A. S. 1997. Science, uncertainty, and EPA's new ozone standards. *Environ. Sci. Technol.* 31(6):280A–284A.

Lefohn, A. S., and Foley, J.K. 1993. Establishing relevant ozone standards to protect vegetation and human health: Exposure/dose-response considerations. *J. Air Waste Manage. Assoc.* 43:106–112.

Madsen, K. L., Adams, W. C., and Van Loan, M. D. 1998. Effects of physical activity, body weight and composition, and muscular strength on bone density in young women. *Med. Sci. Sports Exercise.* 30:114–120.

McArdle, W. D., Katch, F. I., and Katch, V. L. 1996. *Exercise physiology: Energy, nutrition, and human performance*, 4th ed., pp. 198–200. Malvern, PA: Lea & Febiger.

McDonnell, W. F., Kehrl, H. R., Abdul-Salaam, S., Ives, P. J., Folinsbee, L. J., Devlin, R. B., O'Neil, J. J., and Horstman, D. H. 1991. Respiratory response of humans exposed to low levels of ozone for 6.6 hours. *Arch. Environ. Health.* 46:145–150.

Rombout, P. J. A., Lioy, P. J., and Goldstein, B. D. 1986. Rationale for an eight-hour ozone standard. *J. Air Pollut. Control Assoc.* 36:913–917.

Schonfeld, B. R., Adams, W. C., and Schelegle, E. S. 1989. Duration of enhanced responsiveness upon re-exposure to ozone. *Arch. Environ. Health* 44:229–236.

Silverman, F., Folinsbee, L. J., Barnard, J., and Shephard, R. J. 1976. Pulmonary function changes in ozone—Interaction of concentration and ventilation. *J. Appl. Physiol.* 41:859–864.

APPENDIX

In designing the triangular diurnal patterns, it was important to select a plausible but acutely peaked 6.6-h pattern to test the influence of nonconstant hourly values averaging the same multihour O_3 ppm level as a square-wave exposure. In a previous study (Adams, 2003), this was done by scanning the Air Quality System (AQS) database for sites just meeting both the 8-h and 1-h National Ambient Air Quality Standards—that is, site-years with annual fourth highest daily maximum 8-h values under 0.085 ppm O_3 and second highest daily maximum 1-h values below 0.125 ppm O_3. A peak hourly value of 0.15 ppm O_3 was picked to approximate the highest daily maximum 1-h values (that ranged from 0.142 to 0.147 ppm) for the top 4 daily maximum 8-h days at each of four sites (550790041, 1997; 060850004, 1998; 360610010, 1997; 220190008, 1997) found to meet these attainment criteria. The adjacent hourly diurnal values were adjusted to average 0.08 ppm O_3 over a 6.6-h exposure in a pattern approximating the shape of the 8-h AQS sequences. This 0.08-ppm 6.6-h diurnal triangular profile was used again in the present study. Further, a similar procedure was used to select a site (060010005, 6/21/1996) in which an 8-h diurnal profile with a maximum of 0.088 ppm and a mean of 0.059 ppm was observed. A site (060670012, 7/12/2000), in which an 8-h diurnal profile with a maximum of 0.047 ppm and a mean of 0.041 ppm was observed. In both cases, diurnal values were adjusted to average 0.06 ppm and 0.04 ppm, respectively, over a 6.6-h exposure in a pattern approximating the shape of the 8-h AQS sequences.