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Noninvasive Determination of Respiratory Ozone Absorption: Development of a Fast-Responding Ozone Analyzer

James S. Ultman and Abdellaziz Ben-Jebria
Department of Chemical Engineering, Pennsylvania State University, University Park, PA

Includes the Commentary of the Institute's Health Review Committee

Research Report Number 39

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HEALTH REVIEW COMMITTEE'S COMMENTARY Health Effects Institute

Noninvasive Determination of Respiratory Ozone Absorption: Development of a Fast-Responding Ozone Analyzer

James S. Ultman[1] and Abdellaziz Ben-Jebria

ABSTRACT

We developed a chemiluminescent ozone analyzer and constructed an ozone bolus generator with the eventual goal of using a bolus-response method to measure noninvasively the longitudinal distribution of ozone absorption in human lungs. Because the analyzer will be used to sample gases within a single breath, it must have a sufficiently rapid response to monitor changes in ozone concentration during a four-second breathing period, yet its sampling flow must be small enough that it does not interfere with quiet respiratory flows of 300 mL/sec. Our analyzer, which is based on the chemiluminescent reaction between 2-methyl-2-butene and ozone, has favorable performance characteristics: a 90 percent step-response time of 110 msec; a linear calibration from 0.03 to 10 parts per million (ppm)[2] with a sensitivity of 2.3 nA/ppm; a signal-to-noise ratio of 30 evaluated at 0.5 ppm; and a minimum detection limit of 0.017 ppm. At an airflow corresponding to quiet breathing, the ozone generator is capable of producing single boluses with a peak ozone fraction as high as 4 ppm, but containing only 0.35 μg of ozone dispersed over a small volume of 19 mL.

To test the combination of ozone analyzer and bolus generator, we performed bolus-response experiments at steady airflows of 50 to 200 mL/sec in excised pig and sheep tracheas. In spite of the small surface area available for radial diffusion, we found that 25 to 50 percent of the ozone introduced into the trachea was absorbed. By comparing the mathematical moments of the bolus input and the response curves to the predictions of a diffusion theory, we computed an absorption coefficient (K). The values of K increased with increasing airflow, implying that ozone absorption is limited by diffusion processes in the airway lumen as well as in the surrounding tissue.

INTRODUCTION

Ozone (O_3) resulting from the photochemical reaction of automobile emissions is an urban air pollutant that can have adverse effects on human health, particularly in the lung. In many previous laboratory studies, human subjects have been exposed to controlled levels of 0.1 to 1.0 ppm O_3 for one to four hours, and changes in their lung function have been characterized using routine spirometric tests, such as forced expiratory volume and specific airway resistance (Colucci 1983). From the more recent of these studies, it appears that adverse health effects are possible even during acute exposures of 2.5 hours at concentrations as low as 0.12 ppm (McDonnell et al. 1983). However, there is considerable variation in the response of different subjects to the same ambient O_3 concentration (McDonnell et al. 1985). Undoubtedly, a portion of this variability is due to the use of ambient concentration as a surrogate for the actual O_3 dose delivered to those tissues responsible for changes in pulmonary function.

Mathematical model simulations indicate that O_3 is nonuniformly distributed to lung tissue, and that the proximal alveolar region receives a far greater dose than other lung regions (Miller et al. 1985). Morphometric studies of the lungs of animals exposed subchronically for one to six weeks to O_3 concentrations as low as 0.12 ppm confirm that the proximal alveolar region incurs the most cell injury (Mellick et al. 1977; Barry et al. 1985, 1988). From these results, it is clear that the distribution of O_3 dose among different regions in the lung must be known if unique dose-response relationships are to be established. It is not sufficient to characterize O_3 exposure in terms of ambient O_3 concentration alone.

Respiratory physiologists have developed techniques for assessing the regional distribution of nonreactive indicator gases (Ultman 1985), and it is possible, in theory, to use similar methods for determining the dose distribution of reactive gases such as O_3. For example, in the bolus-response method (Ultman et al. 1978; Ben-Jebria et al. 1981), a small volume of foreign gas is rapidly introduced into the inhaled airstream, and its composition is continually monitored at the lips throughout the remainder of inspiration and the following expiration. If the foreign gas is reactive, then its absorption during this single breath could be computed from the difference between the integrals of inspiration and expiration composition data. In fact, it is possible to map out absorption as a function of cumulative airway volume by using data from a series of test breaths in which bolus injection occurred at a different time during each inhalation.

[1] Correspondence may be addressed to Dr. James S. Ultman, Department of Chemical Engineering, Pennsylvania State University, University Park, PA 16802.

[2] A list of abbreviations appears at the end of this report for your reference.

Application of the single-breath bolus-response technique to O_3 requires the use of a fast-responding analyzer and an ozonator capable of reproducibly generating small, but concentrated, O_3 boluses. Commercially available ozone gas analyzers, intended for monitoring ambient air, have large gas-sampling rates and slow dynamic responses that are entirely unsuitable for respiratory applications. Ozone bolus generators are not commercially available.

SPECIFIC AIMS

The long-range objective of our research is to develop a noninvasive bolus-response method for measuring the longitudinal distribution of O_3 absorption in the lungs of cooperating human subjects. During the current study, we developed and tested the critical instrumentation necessary to carry out such measurements. The three specific aims that guided our work are described below.

1. To construct and test a fast-responding ozone analyzer capable of monitoring the changing O_3 concentration during a single breath. The performance specifications that we wanted to achieve for this instrument were a sample inlet flow of 60 mL/min, a minimum detectable limit of 0.002 ppm, a linear calibration range up to 1 ppm, and a 90 percent step-response time of 100 msec or less.
2. To construct and test a small-scale ozonator capable of producing a bolus suitable for inhalation by a cooperating human subject. To ensure adequate spatial resolution of the bolus-response test, we required that the ozonator generate boluses with volumes of 50 mL or less when injected into inhaled airstreams flowing at 0.25 to 1.0 L/sec. We also aimed for an O_3 content as high as 0.2 μg. This corresponds to a well-mixed O_3 concentration of 0.2 ppm in a 500-mL breath, which should pose little risk to a human subject.
3. To demonstrate the ability of the analyzer-ozonator instrumentation to obtain bolus-response data in a simple in vitro system. We aimed to evaluate the reliability of the instruments by measuring bolus-response during steady airflow through an inert nonabsorbing tube as well as through excised animal tracheas. Whereas data from the nonabsorbing tube must demonstrate mass conservation, data from excised tracheas should provide a precise measure of O_3 absorption.

DESIGN OF APPARATUS

RESPIRATORY OZONE ANALYZER

After considering various measurement methods, including mass spectrometry, ultraviolet photometry, and thermocatalytic detection, we concluded that the chemiluminescence generated by mixing O_3 with an appropriate gas-phase reactant would be the most suitable basis for a respiratory ozone analyzer. Although the feasibility of such an instrument was demonstrated more than 20 years ago (Nederbragt et al. 1965; Fontijn et al. 1970; Stevens and Hodgeson 1973), its commercialization using ethylene as the reacting alkene species resulted in analyzers with dynamic time constants of 1 second or more at sampling rates in excess of 1 L/min. Because the reaction of O_3 with ethylene is relatively slow compared to its reaction with other, more complex alkenes (Japar et al. 1974), we reasoned that a shorter time constant could be achieved at a smaller sampling flow by employing one of these alternative hydrocarbons.

Figure 1 is a schematic diagram of the chemiluminescent analyzer we constructed (numbers in parentheses in the text correspond to parts of Figures). The reaction cell (1) was maintained at a desired vacuum pressure by a pump and bellows valve combination (2–4). A sample stream from the output port of an O_3 calibrator approved by the U.S. Environmental Protection Agency (7) was metered into the reaction cell through a needle valve (5). A three-way solenoid valve (6) between the calibrator and the metering valve allowed rapid changes between calibrator gas and room air for purposes of testing the analyzer response to a step change in O_3 concentration.

Alkene vapor was metered into the reaction cell from a reservoir containing the pure liquid alkene (10). Table 1 lists the physicochemical properties of the alkenes we tested. Because the first six alkenes have vapor pressures below atmospheric pressure, their flow could be controlled with a needle valve (8) alone. The last four alkenes, however, have large vapor pressures, which had to be reduced by using a pressure regulator (not shown in Figure 1) between a compressed gas source and the needle valve.

The alkene and sample streams were rapidly combined in a mixing tee internal to the reaction cell. The light produced by the subsequent O_3-alkene reaction was sensed by a 1-inch-diameter ultraviolet-visible photomultiplier tube (11) in proximity to a fused quartz window mounted at the end of the reaction cell. The photomultiplier cathode was biased at a constant negative voltage (13), and the resulting output current was converted to voltage by a variable-gain electrometer and then electronically filtered (14). The filtered signal was recorded on either a high-speed strip-chart recorder (16) or a computer-based data acquisition system (15).

Figure 2 is a cutaway drawing of the reaction cell, the most critical component of the analyzer. To provide maxi-

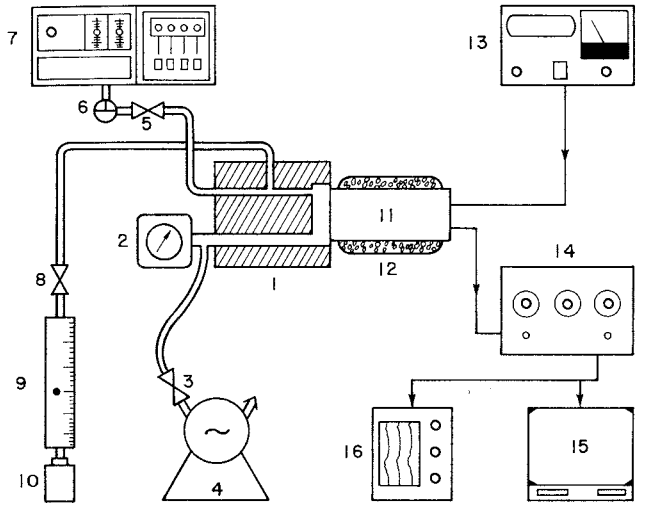

Figure 1. Respiratory ozone analyzer as configured for performance tests. (1) Ozone-alkene chemiluminescent reaction cell (see Figure 2 for details). (2, 3, 4) Vacuum system: Alcatel (Hingham, MA) 1004AC vacuum pump (4); Nupro (Willoughby, OH) 55-8BW bellows valve (3); and Validyne (Northridge, CA) PS309 pressure transducer (2). (5, 6, 7) Sampling and calibration system: Nupro angle-pattern needle metering valve (5); General Valve Corporation (Fairfield, NJ) Series 1 three-way subminiature solenoid valve (6); and Thermoenvironmental Instruments (Franklin, MA) model 49PS photometric ozone calibrator (7). (8, 9, 10) Reactant supply system: 15-mL stainless-steel reservoir containing pure liquid alkene (10); Gilmont (Great Neck, NY) no. 10 rotometer (9); and Nupro double-pattern needle metering valve (8). (11, 12, 13) Photomultiplier system: Hamamatsu (Bridgewater, NJ) R268 photomultiplier tube enclosed in an Oriel (Stratford, CT) no. 77265 shielded housing (11), biased with a Bertan Associates (Hicksville, NY) model 215 adjustable high-voltage power supply (13), and cooled with dry ice (12). (14, 15, 16) Signal conditioning system: Keithley (Cleveland, OH) 600B electrometer and Tektronix (Pittsburgh, PA) AM501 amplifier wired as a low-pass filter (14); Apple (Cupertino, CA) IIe personal computer and Daisi Electronics (Newton Square, PA) AI13 A/D interface (15); and MFE (Salem, NH) strip-chart recorder (16).

mum sensitivity, the volume of the reaction well had to be sufficiently large to allow near completion of the chemiluminescent reaction, but to ensure a rapid dynamic response, the well had to be small enough to limit back mixing of the reacting gases. We selected 10 mL as a reasonable compromise. To obtain a rapid dynamic response, it was also necessary to minimize longitudinal dispersion of O_3 in the sample stream. This was achieved by placing the inlet metering valve (see 5 in Figure 1) at the upstream end of a small bore (that is, $\frac{1}{16}$-inch i.d., $\frac{1}{8}$-inch o.d.) sampling line.

Aerodynamics within the reaction cell were an important consideration. The diameters of the holes constituting the internal mixing tee were chosen so that the line velocities of the two impinging streams were matched when the volumetric flow of the sample stream was 16 times that of the alkene stream. After emerging from this tee, the mixed stream impinged on the rightmost edge of the quartz window and then swept across the entire surface of the window before it was evacuated through a $\frac{3}{16}$-inch-diameter exit hole. The intention of this design was to maximize the light collected by the photomultiplier tube.

The reaction cell, the two metering valves, the vacuum tubing, and all tube fittings were constructed of stainless steel. Sample tubing and alkene inlet tubing were made of Teflon, and seals were fabricated from Teflon, if practical, or Viton. The reaction cell, pressure transducer, and photomultiplier tube housing were mounted on an optical bench with vibration-isolated feet (Oriel no. 11140) to avoid electronic noise associated with movement of the photomultiplier tube.

SMALL-SCALE OZONE BOLUS GENERATOR

Because we planned to measure the bolus-response of O_3 in human subjects, we needed an apparatus that would reproducibly inject boluses with volumes of 50 mL or less into inhaled airflows of 0.25 to 1.0 L/sec. To provide additional versatility, the device also had to be capable of serving as a continuous source of O_3. However, to minimize an uncontrolled release of O_3 in the event of malfunction, the capacity of the generator had to be kept as small as possible.

Figure 3 is a schematic diagram of the bolus generator we constructed. The key component is a small, commercially available ozonator (4), consisting of a heated aluminum block with a 30-mL flow-through cavity, in which O_3 is continuously produced by a Pen-Ray mercury lamp, as described by Hodgeson and associates (1972). The ozonator produced a maximum output concentration of 10 ppm when the inlet airflow was 500 mL/min. Smaller O_3 concentrations could be obtained either by altering the airflow through the cavity (3) or by decreasing the electric current supplied to the Pen-Ray lamp.

Clean dry air was supplied to the inlet of the bolus generator at a pressure of about 40 psig (1). Two regulators internal to the generator provided a low pressure to the ozonator (2b) and a higher pressure to a flow path that bypassed the ozonator (2a). In the "standby" mode, a three-way solenoid valve (5a) provided an open path between the ozonator (4) and a hold-up tube (7), while another three-way solenoid valve (5b) provided an open path between the hold-up tube and an exhaust (10). Therefore, freshly generated O_3 continuously flowed through the hold-up tube, where it reached a steady concentration before it was exhausted to a fume hood. In the standby mode, no O_3 could reach the mouthpiece (8) through which a human subject would normally breathe.

When a bolus was required, an electric pulse generator (6) simultaneously switched the inlet position of valve (5a) and the outlet position of valve (5b) for about 100 msec. Dur-

Table 1. Physicochemical Properties of Alkenes

Substance	Molecular Weight[a]	Normal Boiling Point[a] (°C)	Vapor Pressure at 70°F[a] (torr)	Reaction Rate[b] (10^{-18} cm^3/molecule/sec)	Purity[c] (%)
2,3-Dimethyl-2-butene [$(CH_3)_2C = C(CH_3)_2$]	84.09	73.0	125	1,510	99 +
2-Methyl-2-butene [$CH_3CH = C(CH_3)_2$]	70.08	38.4	466	493	99 +
3-Methyl-2-pentene [$C_2H_5C(CH_3) = CHCH_3$]					
cis	84.09	67.6	152[d]	456	99[e]
trans	84.09	65.7	152[d]	563	
1-Pentene [$CH_3CH_2CH_2CH = CH_2$]	70.08	40.0	638	10.7	99 +
Cyclopentene [C_5H_8]	68.06	45.0	378	813	99
Cyclohexene [C_6H_{10}]	82.08	83.0	88	169	99 +
Ethylene [$CH_2 = CH_2$]	28.03	- 103.9	Gas	1.9	99.5 +
2-Butene [$CH_3CH = CHCH_3$]					
cis	56.06	1.0	1,430	161	99 +[f]
trans	56.06	2.5	1,569	260	
Propylene [$CH_3CH = CH_2$]	42.05	- 47.0	7,755	13	99
Isobutylene [$CH_2 = C(CH_3)_2$]	56.06	- 6.0	2,068	113.6	99

[a] Handbook of Chemistry and Physics (1986).

[b] Ozone-alkene reaction rate constant (Japar et al. 1974).

[c] Reagents obtained from Aldrich Chemical Company (Milwaukee, WI) were used as delivered.

[d] The vapor pressure value of 152 torr is an estimate for both *cis* and *trans* forms based on the vapor pressure of 2-methyl-2-pentene. Accurate vapor pressure values for this compound could not be found.

[e] 3-Methyl-2-pentene was an unspecified mixture of *cis* and *trans* forms.

[f] 2-Butene was a mixture of 55 percent *cis* and 45 percent *trans* forms.

ing this short interval, a flow of clean air bypassed the ozonator and propelled O_3 from the hold-up tube into the proximal end of the mouthpiece assembly (8). The characteristics of the bolus could be controlled by the ejection pressure supplied by regulator (2a), the preejection output concentration from the ozonator, the volume of the hold-up tube, and the duration of the solenoid valve switching pulse.

The mouthpiece assembly was designed for future use with human subjects. Each subject will breathe from the distal end of the mouthpiece assembly. The ozone analyzer (9), with its sampling line attached to the proximal end of the mouthpiece assembly, will monitor the O_3 concentration curve of the inhaled bolus as well as the O_3 concentration curve of the exhaled gas. In some tests designed to evaluate the performance of the ozone generator and the ozone analyzer, a constant airflow was supplied to the proximal end of the mouthpiece assembly to mimic an inhaled airflow (11).

The generator could also be used to supply a constant concentration of O_3. When the generator was used in the standby mode, a constant O_3 concentration was available at the exhaust port (10). Alternatively, if the solenoid valve (5b) was held open to the mouthpiece assembly (8), a constant O_3 concentration would be mixed into the airstream (11).

EVALUATION OF PERFORMANCE

OZONE ANALYZER: SCREENING OF ALKENES

In this series of experiments, analyzer performance was evaluated with 10 alkenes used as reacting species (Table 1). In all cases, the photomultiplier output current was conditioned using electronic components readily available in our laboratory: a Keithley 600B electrometer was used as a current-to-voltage converter; a Tektronix AM501 operational amplifier module was wired as a low-pass filter with a 10-msec time constant; and an Apple IIe personal computer was used with a Daisi Electronics AI13 A/D converter to record 700 digital samples of the electrometer voltage over a 5-sec interval. From these 700 sample points, the computer determined the mean (that is, signal) and the SD of the mean (that is, the root-mean-square [rms] noise) of the electrometer output.

To design an appropriate test protocol, preliminary experiments were carried out with 2,3-dimethyl-2-butene, the

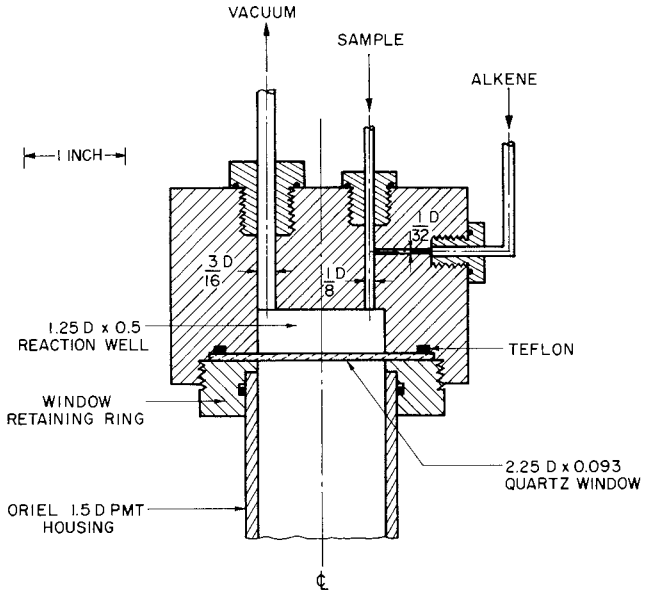

Figure 2. Custom-machined reaction cell. All dimensions are in inches.

most reactive of the 10 alkenes. The photomultiplier tube was biased at alternative voltages from −100 to −1,000, first with the photomultiplier tube at room temperature and then with dry ice to cool the photomultiplier tube housing (see 11 and 12 in Figure 1). A bias voltage of −700 gave the maximum signal-to-noise ratio, and although cooling the photomultiplier tube had virtually no effect on either signal level or noise when O_3 was present, it did reduce the photomultiplier tube dark current by a factor of 4. These experiments also revealed that the analyzer output has a maximum value with respect to reaction cell pressure, increases with alkene inlet flow but eventually reaches a plateau value, and has a reasonable signal-to-noise ratio only if the inlet sampling flow is 200 mL/min or more.

On the basis of these preliminary results, the following standardized test procedures were followed to compare the 10 alkenes. In all cases, the photomultiplier tube was cooled and operated at a bias voltage of −700, and the inlet sampling flow was fixed at 200 mL/min.

- Fixing O_3 mole fraction at 0.5 ppm, the reaction cell pressure and the inlet alkene flow were simultaneously altered to find the nominal values at which the analyzer signal was maximized.
- Fixing O_3 mole fraction at 0.5 ppm and inlet alkene flow at its nominal optimum (that is, plateau) value, the signal and rms noise were recorded over a reaction cell pressure range of 5 to 600 torr. The exact value of optimum pressure was determined from these data.
- Fixing O_3 mole fraction at 0.5 ppm and reaction cell pressure at its optimum value, the signal and rms noise were recorded over an inlet alkene flow of 3 to 100 mL/min. The exact plateau value of alkene flow was determined from these data.

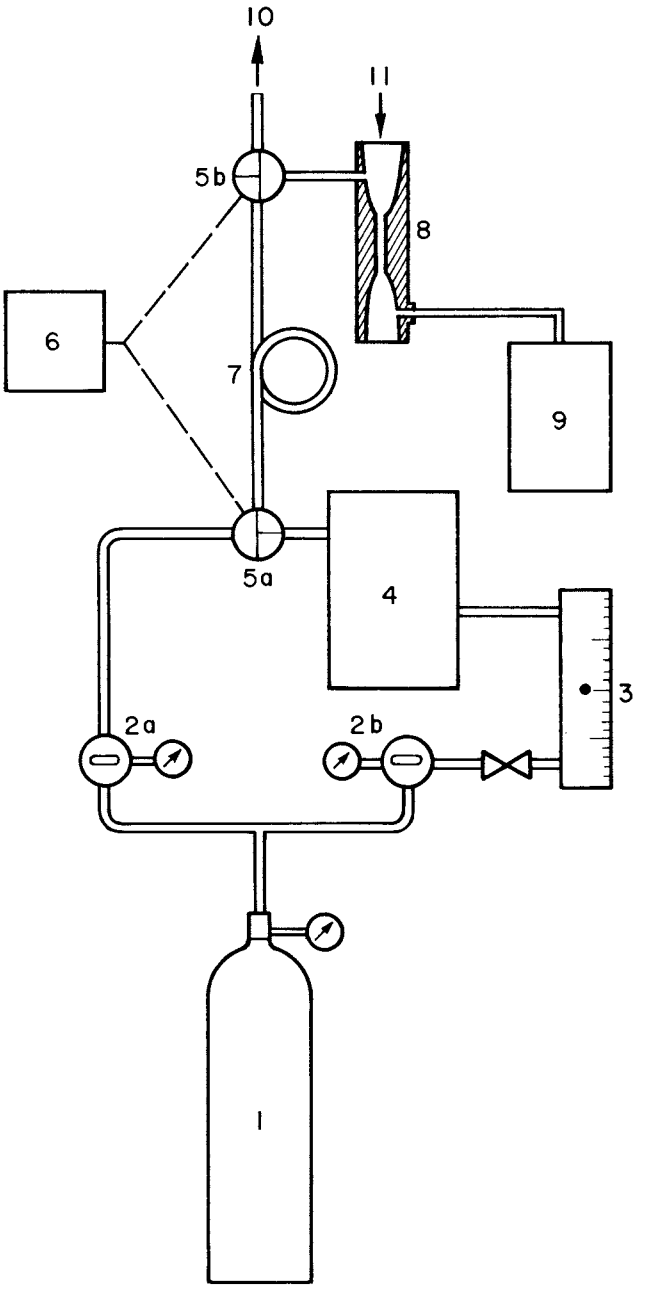

Figure 3. Small-scale ozone bolus generator. (1) High-pressure air supply. (2) Pressure regulators (model R07, Norgren, Littleton, CO) for ozonator (2b) and bypass (2a) flows. (3) Rotometer and needle valve (Series RM, Dwyer, Michigan City, IN). (4) Low-capacity ozonator (Internal Span Source, Thermoenvironmental Instruments, Franklin, MA). (5) Three-way solenoid valves (Series 9, General Valve Corporation, Fairfield, NJ). (6) Pulse generation by data acquisition and control system (model 570, Keithley, Cleveland, OH). (7) Teflon hold-up tube, ¼-inch o.d. (8) Custom-machined mouthpiece assembly. (9) Chemiluminescent ozone analyzer (see Figure 1). (10) Exhaust port. (11) Inspiratory-directed flow.

- Operating at the optimum reaction cell pressure and plateau alkene flow, the signal and rms noise were recorded over an O_3 mole fraction range of 0 to 1 ppm in intervals of 0.1 ppm. The static calibration curve was constructed from these data.
- Strip-chart recordings of the dynamic response were obtained, first to a change in O_3 mole fraction from 0 to 0.5 ppm, and then to a change from 0.5 to 0 ppm. The 90 percent step-response time was measured from these data.

Performance highlights for all 10 alkenes tested are compared in Table 2. As judged from these data, none of the alkenes provided all the performance characteristics required in a respiratory gas analyzer. For example, 1-pentene, ethylene, and propylene were the only compounds that produced linear calibrations, but their response times were relatively long and the signal-to-noise ratio of 1-pentene was unacceptable. On the other hand, 2,3-dimethyl-2-butene, 2-methyl-2-butene, and cyclopentene had suitable response times, but exhibited markedly nonlinear calibration curves. Of these three alkenes, 2-methyl-2-butene had the best signal-to-noise ratio and the highest vapor pressure (Table 1), an important factor in controlling delivery of the reactant vapor.

The complete set of test data for 2-methyl-2-butene in Figures 4 through 7 illustrates the nature of the results that were obtained. The reaction cell pressure test (Figure 4) indicates that the analyzer signal possessed a well-defined maximum value at 51 torr, and the alkene flow test (Figure 5) demonstrates that the signal reaches a plateau value at a 10 mL/min flow of 2-methyl-2-butene. At 51 torr, the calibration curve is nonlinear (Figure 6) but the 90 percent step-response time is sufficiently low at 140 msec (Figure 7).

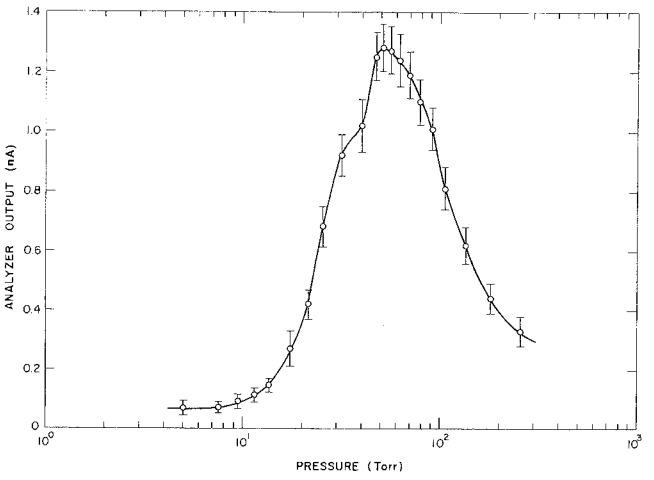

Figure 4. Ozone analyzer output as a function of reaction cell pressure. Sample flow was 200 mL/min, O_3 fraction was 0.5 ppm, and 2-methyl-2-butene flow was 10 mL/min. The analyzer output was sampled at 140 Hz for a 5-sec period. Open circles represent the mean signal and vertical bars represent rms noise.

To determine whether or not the shape of the calibration curve can be modified by reaction cell pressure, additional experiments with 2-methyl-2-butene were carried out both above and below the optimum value of 51 torr. As Figure 6 indicates, an operating pressure of 26 torr led to increased curvature, but a pressure of 107 torr linearized the data taken at 0.1-ppm intervals of O_3 mole fraction. With this improvement, we decided that 2-methyl-2-butene exhibited the best performance of the 10 alkenes. However, linearization of the calibration curve came at some expense to step-response time, which increased from 140 to 200 msec

Table 2. Performance of Ozone Analyzer Employing 10 Alkenes[a]

Substance	Plateau Alkene Flow (mL/min)	Optimum Pressure (torr)	Mean Signal (nA)	Signal/rms Noise Ratio	Percent Nonlinearity[b]	Response Time (msec)[c]
2,3-Dimethyl-2-butene	6.0	40	1.10	13.62	35	140
2-Methyl-2-butene	13.0	51	1.30	15.67	24	140
3-Methyl-2-pentene	16.0	56	0.61	10.10	19	160
1-Pentene	26.0	120	0.14	5.35	0	300
Cyclopentene	15.0	42	0.92	13.94	21	130
Cyclohexene	15.0	51	0.18	7.12	34	180
Ethylene	100.0	400	1.32	14.79	0	540
2-Butene	40.0	62	0.48	9.11	18	160
Propylene	60.0	130	0.37	8.93	0	280
Isobutylene	60.0	120	0.88	12.93	8	260

[a] All data were obtained at a sample flow of 200 mL/min, the plateau alkene flow, the optimum pressure, and with low-pass electronic filtering at a time constant of 10 msec. Data in columns 4 and 5 were obtained at an O_3 fraction of 0.5 ppm.

[b] Computed as $100(i_l - i)/i_l$, where i is the actual signal at 0.5 ppm and i_l is the signal that would have occurred if the analyzer output were linear between 0 and 1.0 ppm.

[c] The 90 percent fall time in response to a step change in O_3 concentration from 0.5 to 0 ppm.

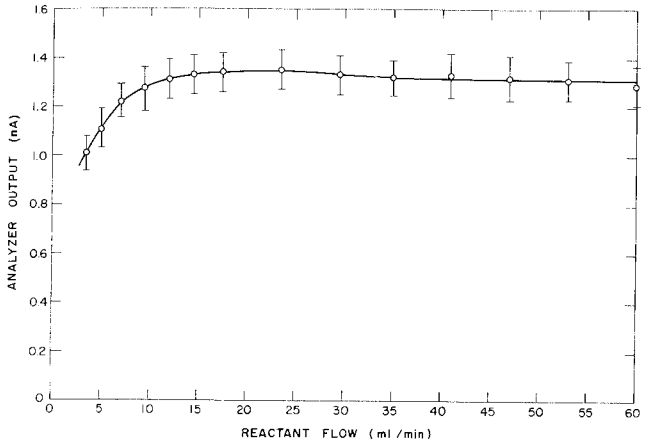

Figure 5. Ozone analyzer output as a function of 2-methyl-2-butene flow. Sample flow was 200 mL/min, O_3 fraction was 0.5 ppm, and reaction cell pressure was 51 torr. The analyzer output was sampled at 140 Hz for a 5-sec period. Open circles represent the mean signal and vertical bars represent rms noise.

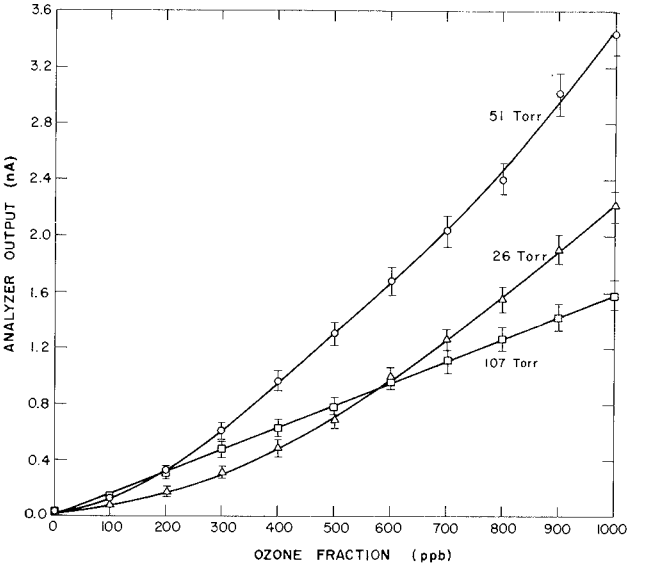

Figure 6. Ozone analyzer output as a function of O_3 concentration and reaction cell pressure. Sample flow was 200 mL/min and 2-methyl-2-butene flow was 10 mL/min. The analyzer output was sampled at 140 Hz for a 5-sec period. Open circles, triangles, and squares represent the mean signal and vertical bars represent rms noise.

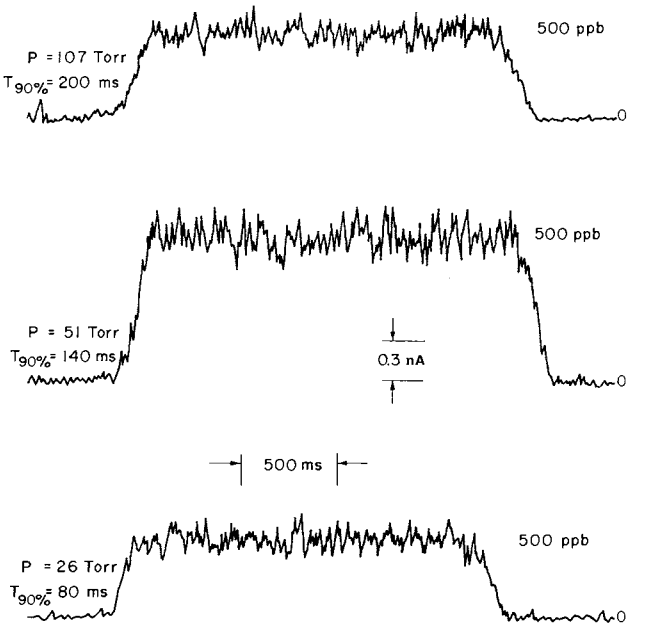

Figure 7. Dynamic response of ozone analyzer for a step change in O_3 fraction from 0.5 to 0 ppm at different reaction cell pressures. Sample flow was 200 mL/min, 2-methyl-2-butene flow was 10 mL/min, and low-pass filtering was performed at a time constant of 10 msec.

Table 3. Effect of Sample Flow on Performance of the Ozone Analyzer[a]

Sample Flow (mL/min)	Pressure (torr)[b]	Sensitivity (nA/ppm)[c]	Signal/rms Noise Ratio[d]	Response Time (msec)[e]
200	140	1.19	5.1	160
300	170	1.69	6.4	120
400	200	2.00	7.1	100
500	240	2.17	7.6	100

[a] All data were obtained at a 2-methyl-2-butene flow of 15 mL/min and with low-pass filtering at a time constant of 10 msec.

[b] Minimum pressure to ensure linear calibration. These values were obtained after replacing the pressure transducer with a more accurate unit. This is why the first entry, 140 torr, differs from the value of 107 torr in Figure 6.

[c] Slope of calibration line.

[d] Signal (that is, mean analyzer output) and rms noise (that is, SD of the mean) were evaluated at an O_3 fraction of 0.5 ppm.

[e] Ninety percent rise time in response to a step change in O_3 inlet concentration from 0 to 0.5 ppm.

when operating pressure was elevated from 51 to 107 torr (Figure 7).

To compensate for the step-response time, we investigated analyzer performance at inlet sample flows greater than 200 mL/min. As shown in Table 3, higher sample flows shortened the response time and simultaneously increased the sensitivity (that is, the slope of the calibration line). Appropriate operating conditions for respiratory applications were a sampling flow of 400 mL/min at a reaction cell pressure of 200 torr.

OZONE ANALYZER: IMPROVING RESOLUTION

At reaction cell pressures sufficiently large to ensure a

linear calibration, the signal-to-noise ratio evaluated at 0.5 ppm O_3 has unacceptable values of 5.1 to 7.6 (Table 3). We attempted to solve this problem by eliminating possible sources of noise. We installed a 20-L surge tank between the vacuum pump and the reaction cell, intending to dampen flow pulsations. We also added a small capillary jet within the reaction well to ensure complete mixing of O_3 with the alkene. Neither of these measures improved the signal-to-noise ratio.

We next tried to reduce noise electronically. We installed a chopper wheel between the quartz window of the reaction cell and the photomultiplier tube, and employed a lock-in amplifier to demodulate the analyzer output. This approach was not successful. We then replaced the Keithley electrometer and Tektronix filter (see 14 in Figure 1) with a Keithley 427 current amplifier. Whereas the Tektronix filter had a fixed time constant of 10 msec and a roll-off of -3 dB/octave, the Keithley current amplifier incorporated a low-pass filter with an adjustable time constant and a much steeper roll-off of -12 dB/octave.

Figure 8 illustrates the effect on step-response recordings of various analog filter constants. The top trace, in which a 10-msec time constant was used, shows results similar to the data obtained with the original electronics (Figure 7). When the filter constant is increased above 10 msec, the noise level drops dramatically. Simultaneously, there is a distortion in the waveform that increases the step-response time. The best trade-off between attenuation of noise and preservation of rapid dynamic response occurs at a filter constant of 30 msec. In that case, the 90 percent step-response is 110 msec, and the signal-to-noise ratio is 18:1.

To reduce noise further, we replaced the Interactive Structures A/D converter and Apple IIe computer (see 15 in Figure 1) with a Keithley 570 data acquisition system and a Zenith Z386 workstation running ASYST signal processing software. The output of the current amplifier was then smoothed using the time domain low-pass filter supplied with ASYST. The effectiveness of this digital filter was evaluated by conducting two series of experiments in which the analog filtering constant was fixed at 30 msec while the digital filtering frequency was varied from 6 to 50 Hz. In the first series the 90 percent response time for a 0 to 0.5 ppm

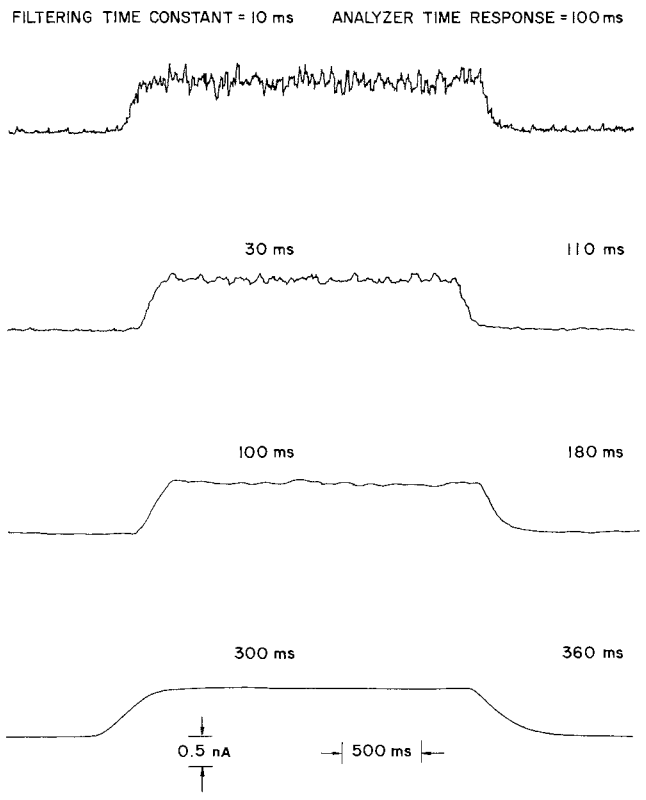

Figure 8. Effect of low-pass analog filtering on a step change in O_3 fraction from 0 to 0.5 ppm. Sample flow was 400 mL/min, 2-methyl-2-butene flow was 4.5 mL/min, and reaction cell pressure was 220 torr.

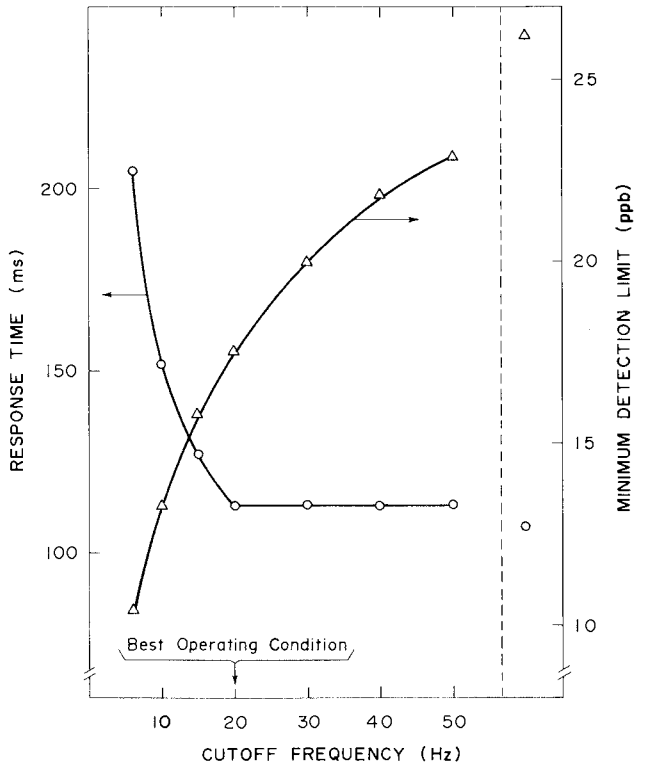

Figure 9. Effect of low-pass digital filtering on dynamic response and on the limit of minimum detection. Sample flow was 400 mL/min, 2-methyl-2-butene flow was 4.5 mL/min, and reaction cell pressure was 220 torr. Analyzer output was analog-filtered at a time constant of 30 msec by the Keithley 427 current amplifier, sampled at 200 Hz for a 10-sec period by the Keithley 570 data acquisition system, and then digitally filtered by ASYST software. Data to the right of the vertical broken line have not been digitally filtered.

step increase in inlet O_3 concentration was determined. In the second series, the mean and rms noise of the filtered output were determined at a series of steady O_3-inlet concentrations from 0.05 to 1.0 ppm, and the minimum detectable limit of the analyzer was defined as that extrapolated O_3 fraction at which the signal-to-noise ratio was unity. As was the case with analog filtering, a trade-off between the resolution of the analyzer and its dynamic response was observed during digital filtering (Figure 9).

A digital cutoff frequency of 20 Hz superimposed on an analog filter time constant of 30 msec gave the best results, a step-response of 110 msec and a minimum detectable limit of 0.017 ppm. With this signal processing scheme, the signal-to-noise ratio was 9:1 at 0.1 ppm, 28:1 at 0.5 ppm, and 40:1 at 1 ppm of O_3. This represents a fourfold improvement from the signal-to-noise ratio of 7:1 at 0.5 ppm observed before the upgrade in electronic components.

OZONE ANALYZER: INTERFERENCES

We evaluated the influence of temperature, humidity, and inlet gas composition on the analyzer signal by employing the best operating conditions defined in previous experiments: 2-methyl-2-butene flow of 4.5 mL/min, sample flow of 400 mL/min, and cell pressure of 220 torr (elevated above 200 torr [see Table 3] to ensure that the static calibration would be linear). In all cases, the ozone generator (Figure 3) introduced a 0.5-L/min flow of dry air with a known O_3 concentration into the injection port of the mouthpiece assembly (8). Within the lumen of this assembly, the ozonated air mixed with a 250-mL/sec flow of test gas that mimicked an inhaled airstream (11).

To evaluate the effect of humidity, the ozonator output was fixed, and 100 percent humidified air and dry air alternately flowed through the mouthpiece assembly during five-minute intervals. No systematic differences were found between the analyzer signal obtained in humid air and that in dry air during the one-hour course of the experiment (Figure 10, upper panel). The influence of temperature was determined by using heated clean air as a test gas. Step changes in test gas temperature had no perceptible effect on the analyzer output (Figure 10, lower panel).

The effect of gas composition on the analyzer signal was evaluated by sampling mixtures of O_3 with different carrier gases from the mouthpiece assembly. In one set of experiments, the carrier gas consisted of binary combinations of oxygen (O_2) with commonly used inert gases (that is, helium, nitrogen, argon, and sulfur hexafluoride). In another set of experiments, the carrier gas was composed of air with a partial substitution of carbon dioxide (CO_2) for O_2, simulating the effect of respiratory gas exchange. In

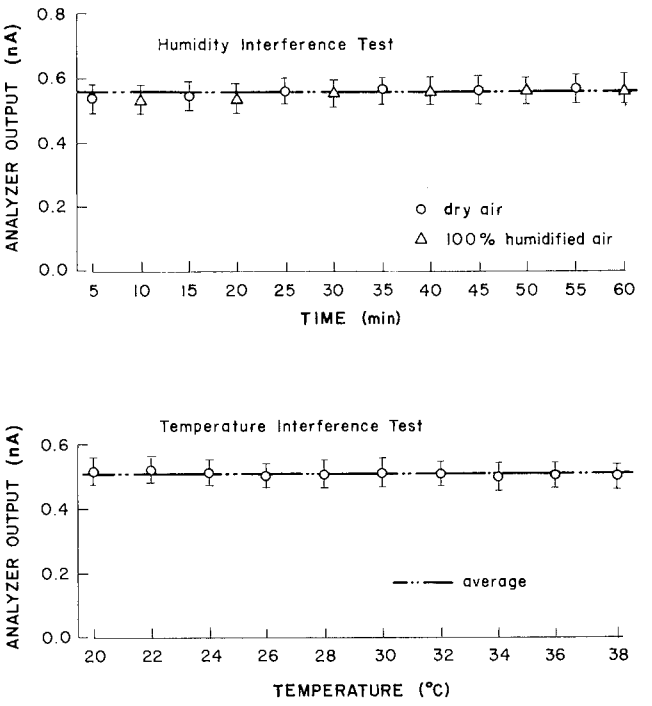

Figure 10. Effect of changes in inlet gas humidity (upper panel) **and temperature** (lower panel) **on the analyzer output.** Sample flow was 400 mL/min, 2-methyl-2-butene flow was 4.5 mL/min, reaction cell pressure was 220 torr, nominal O_3 fraction was 0.25 ppm, and analog filtering time constant was 30 msec (signal was not digitally filtered). The analyzer output was sampled at 0.1 Hz for a 200-sec period. Open circles and triangles represent the mean signal and vertical bars represent rms noise.

each experiment, the ozonator lamp current was adjusted to different levels so that calibration data could be obtained over an O_3 mole fraction range of 0.1 to 1 ppm. The calibrations for all the O_3–carrier gas combinations were linear, but the sensitivity of the analyzer to O_3 was affected by the content and nature of the carrier gas (Table 4). For the O_2–inert carrier gas mixtures, analyzer sensitivity was directly related to the mole fraction and inversely related to the molecular weight of the inert gas. Carrier gas mixtures containing nitrogen and argon, which have similar molecular weights, exhibit virtually the same analyzer sensitivity at any particular mole fraction. We also found that when air was the carrier gas, the substitution of CO_2 for O_2 increased the analyzer sensitivity in proportion to the percentage of CO_2 that was present. A linear regression (r^2 = 0.993) of the data in Table 4 indicates that for every 1 percent increase in the substitution of CO_2 for O_2, there is a 3.8 percent increase in the analyzer sensitivity relative to its value in room air.

OZONE ANALYZER: EXTENDED CALIBRATION

As demonstrated in the next section, the ozone generator

Table 4. Effect of Carrier Gas on Analyzer Output[a]

Gas Species[b] (Molecular Weight)	Inert Gas Concentration (%)	O_3 Sensitivity[c] (nA/ppm)
Nitrogen (28)	52.64	1.14
	66.27	1.44
	75.36	1.91
	80.00	2.23
	84.45	2.71
Argon (40)	52.64	1.10
	66.27	1.46
	75.36	1.84
	80.00	2.23
	84.45	2.61
Helium (4)	52.64	1.44
	66.27	2.03
	75.36	2.86
	80.00	3.78
	84.45	4.18
Sulfur hexafluoride (146)	52.64	0.63
	66.27	0.74
	75.36	1.05
	80.00	1.25
	84.45	1.44
Carbon dioxide (44)	0.00	2.54
	2.00	2.72
	3.00	2.78
	4.00	2.98
	5.00	3.03

[a] Operating conditions for the ozone analyzer were as follows: reaction cell pressure of 220 torr; 2-methyl-2-butene flow of 4.5 mL/min; sample flow of 400 mL/min; and a filter constant of 10 msec.

[b] In the case of nitrogen, argon, helium, and sulfur hexafluoride, the gas mixture was composed of O_2 and the inert gas only. In the case of CO_2, the gas mixture was composed of 79 percent nitrogen and the balance was CO_2 plus O_2.

[c] Slope of O_3 calibration line.

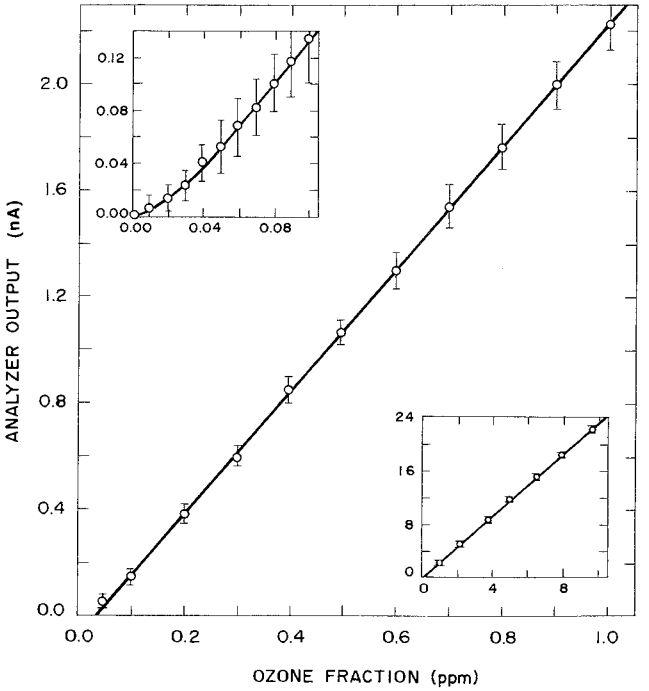

Figure 11. Extended calibration of the ozone analyzer. Sample flow was 400 mL/min, 2-methyl-2-butene flow was 4.5 mL/min, reaction cell pressure was 220 torr, analog filtering time constant was 30 msec (signal was not digitally filtered). Open circles are the mean signal obtained by sampling the analyzer output at 200 Hz for a 10-sec interval, and verticle bars are the associated rms noise. These data were fit to a linear calibration equation [(Analyzer Output) = 2.328 (O_3 Fraction) − 0.108] for O_3 fractions from 0.03 to 10 ppm, and to a nonlinear calibration equation [(Analyzer Output) = 3.000 (O_3 Fraction)$^{1.355}$] for O_3 fractions less than 0.03 ppm. Graph inserts show expanded (top) and extended (bottom) scales of the main graph.

can produce peak bolus concentrations in excess of the 1-ppm limit of our O_3 calibration source. Therefore, we developed an indirect method of extending the calibration above 1 ppm. The bolus generator (Figure 3) was operated in the standby mode, and its exhaust gas (10) was diluted by a factor of 10 with a metered source of clean air. By varying the current supplied to the ozonator lamp, we could then correlate calibrated analyzer measurements in the diluted gas with measurements in the undiluted exhaust gas.

Figure 11 is an extended calibration of the ozone analyzer. Data between 0 and 1 ppm were obtained by using the standard O_3 source, while data above 1 ppm were obtained by the gas dilution technique. The extended calibration is linear over a 30-fold increase in O_3 concentration, from 0.03 to 10 ppm. Below 0.03 ppm, there is some curvature, suggesting that a nonlinear calibration equation may be necessary to process data at low O_3 levels. As illustrated in the caption of Figure 11, we used a two-parameter equation, of the form (Analyzer Output) = A(O_3 Fraction)^B, for this purpose.

OZONE BOLUS GENERATOR

We performed a series of performance tests of the ozone generator (Figure 3) by injecting an O_3 bolus into the proximal injection port of the mouthpiece assembly (8) and monitoring O_3 concentration at the distal sampling port. Simulated inhaled airflows of 250 or 1,000 mL/sec, hold-up tube volumes of 10 to 30 mL, regulator (2a) settings of 5 to 30 psig, regulator (2b) settings of 5 to 10 psig, and solenoid valve pulse durations of 50 to 500 msec were investigated. In all these tests, the ozonator (4) was set at its highest output of 10 ppm in order to determine the maximum amount of O_3 that could be generated in each bolus.

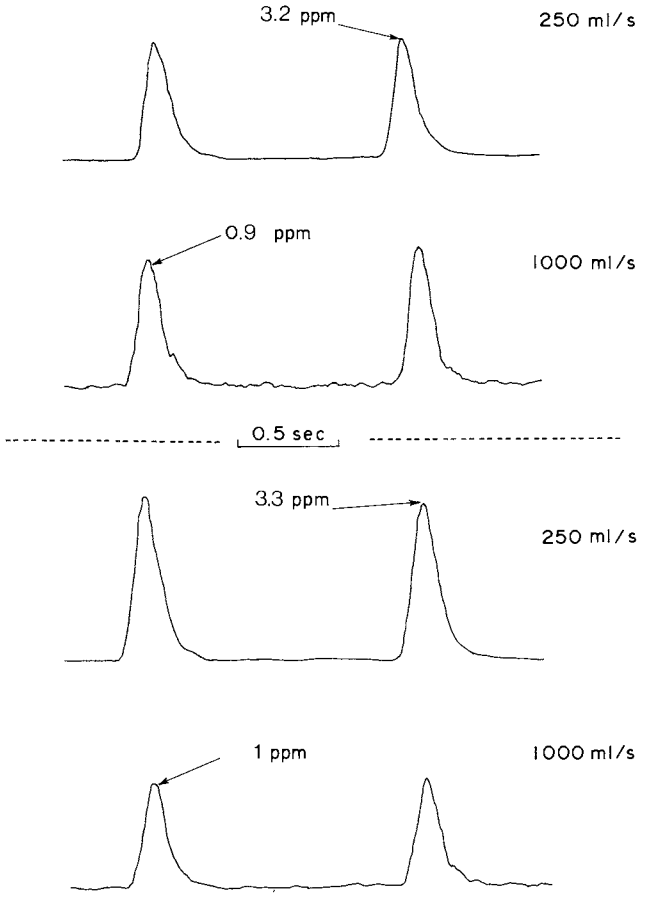

Figure 12. Replicate boluses produced with 10 mL hold-up tubes (upper two curves) **and 20 mL hold-up tubes** (lower two curves) **at simulated inspiratory flows of 250 and 1,000 mL/sec.** Sample flow was 400 mL/min, 2-methyl-2-butene flow was 4.5 mL/min, reaction cell pressure was 220 torr, and analog filtering time constant was 30 msec (signal was not digitally filtered). The solenoid valve pulse duration was 100 msec, and the ozonator was set for its maximum rate of O_3 production. Curves are scaled differently due to use of different gain settings on the strip-chart recorder.

The best test results were obtained with hold-up tube volumes of 10 to 20 mL, valve pulse durations of 100 to 130 msec, and both pressure regulators set at 10 psig. Representative bolus concentration curves (Figure 12) indicate that reproducibility of the bolus shape is good, and that peak O_3 concentrations greater than 1 ppm can be obtained except at the largest inspiratory flow of 1,000 mL/sec. The fact that peak O_3 concentration is inversely proportional to inspiratory airflow (Table 5) is due to dilution of the bolus when it mixes with inhaled air in the mouthpiece assembly.

To quantify further the characteristics of the bolus curves, their mathematical moments were automatically computed by the data acquisition system. Raw moments are defined in terms of the O_3 concentration, C, and time, t, as:

$$I_i = \int_0^\infty Ct^i \, dt \qquad (1)$$

where the index i can assume any nonnegative integer value, but only the first three moments (that is, i = 0, 1, 2) are normally considered. Using these values in conjunction with the airflow, $\dot{V}$, three reduced moments can be computed:

$$M = \dot{V}I_0 \qquad (2)$$

is the total amount of O_3 (μg or ppm•mL [the amount of O_3 contained in a 1-mL volume when the concentration of O_3 is 1 ppm]) within the bolus;

$$\bar{t} = I_1/I_0 \qquad (3)$$

is the mean time (sec) for appearance of the bolus at the sampling site; and

$$S^2 = (I_2/I_0 - (I_1/I_0)^2)\dot{V}^2 \qquad (4)$$

is the volumetric variance or dispersion (mL^2). The square root of the variance (that is, the SD, S) represents the volume of air containing about 50 percent of the O_3 in the bolus. Therefore, S is a measure of the volume of the bolus.

The amount of O_3 in a bolus cannot exceed the O_3 content in the hold-up tube: 100 ppm•mL in the 10-mL tube, and 200 ppm•mL in the 20-mL hold-up tube. Therefore, the values computed for M (see Table 5, column 4) suggest that a solenoid switching pulse with a duration of 100 msec is sufficient to eject all of the O_3 from the 10-mL hold-up tube, but a duration of 130 msec is required for complete ejection from the 20-mL tube. Under all conditions, the amount of O_3 is greater than 100 ppm•mL (that is, 0.2 μg).

Values computed for the bolus volume, ranging from 19 to 76 mL, are independent of tube hold-up volume but are proportional to increases in inhaled airflow (Table 5, column 5). This result reflects the fact that at the higher flows, there is greater axial dispersion of the O_3 between the injection and sampling sites of the mouthpiece assembly.

IN VITRO BOLUS-RESPONSE TESTS

MEASUREMENTS IN EXCISED TRACHEAS

To illustrate and evaluate the application of the ozone analyzer-generator instrumentation, bolus-response studies were carried out with a steady flow of air through excised tracheas. Both pig and sheep tracheas were obtained from the Penn State Meats Laboratory. Each trachea was initially rinsed with physiologic saline to remove extraneous blood and cell debris, and was used within two hours after the animal was butchered.

Table 5. Characteristics of Ozone Boluses[a]

Hold-up Tube Volume (mL)	Simulated Inspiration (mL/sec)	Peak Concentration (ppm)	Amount of O_3, M (ppm•mL)[b]	Bolus Volume, S (mL)[c]
Valve Pulse Duration = 100 msec				
10	250	3.2	115	18
	500	1.8	125	35
	1,000	0.9	128	76
20	250	3.3	123	19
	500	1.9	133	35
	1,000	1.0	133	76
Valve Pulse Duration = 130 msec				
20	250	4.0	163	19
	500	2.5	183	37
	1,000	1.2	190	76

[a] Operating conditions for the ozone analyzer were as follows: reaction cell pressure of 220 torr; 2-methyl-2-butene flow of 4.5 mL/min; sample flow of 400 mL/min; and a filter constant of 10 msec. Ozonator and bypass flow regulators were both set at 10 psig and the ozonator was operated at its full capacity.

[b] Computed as the product of the inspiratory flow and the integral under the bolus concentration curve.

[c] Computed as the product of the inspiratory flow and the SD of the bolus concentration curve.

During an experiment, the proximal end of the trachea was mounted in a cylindrical inlet ring containing an upstream port, into which the O_3 bolus was injected, and a downstream port, from which the bolus input curve was monitored. The distal end of the trachea was mounted in an outlet ring containing a sampling port from which the O_3

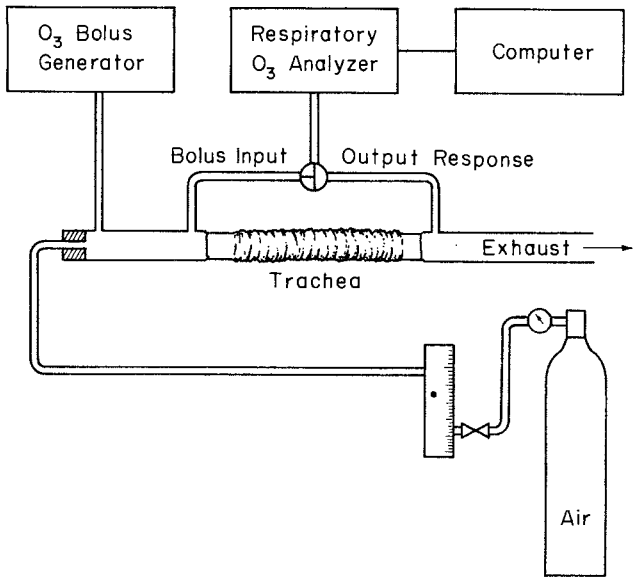

Figure 13. Apparatus employed for bolus-response measurements in excised tracheas. Design details of the ozone bolus generator and the respiratory ozone analyzer are given in Figures 3 and 1, respectively. Note that separate experiments were performed to monitor the bolus input and the output response. The three-way valve is intended to illustrate this, but in reality no valve was used. The analyzer inlet line was moved between the input and output ends of the trachea.

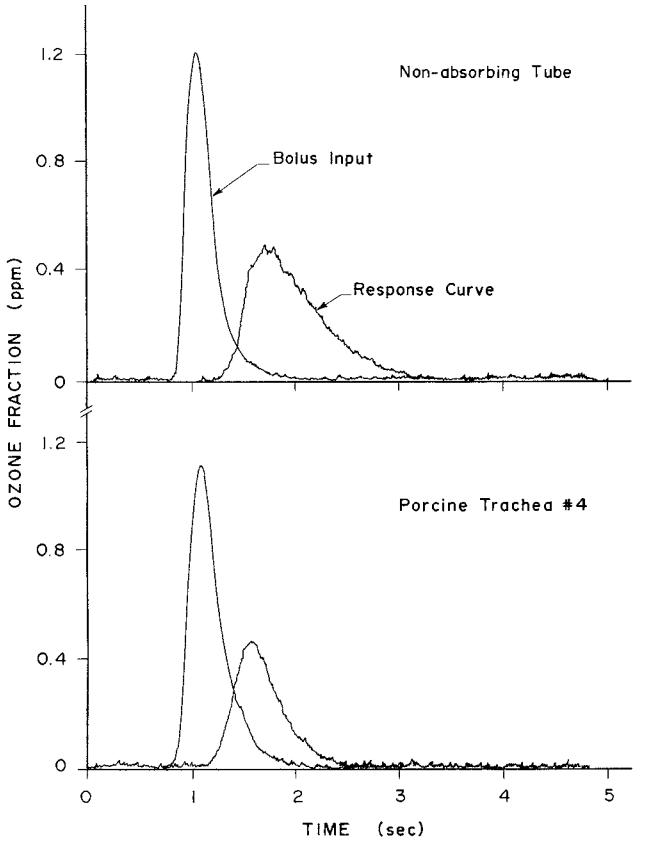

Figure 14. Representative bolus-response data obtained in a nonabsorbing tube and in a pig trachea at a steady flow of 100 mL/sec. Sample flow was 400 mL/min, 2-methyl-2-butene flow was 4.5 mL/min, reaction cell pressure was 220 torr, and analog filtering time constant was 30 msec (signal was not digitally filtered). The changes in moments for the nonabsorbing tube were M_R/M_B = 1.013, Δt = 0.824 sec, and ΔS^2 = 1,010 mL^2. Changes in moments for the trachea were M_R/M_B = 0.595, Δt = 0.478 sec, and ΔS^2 = 209 mL^2.

response curve was monitored (Figure 13). As the analyzer had only one inlet, it was necessary to repeat each experiment at least once in order to measure both the bolus and the response. Separate experiments were carried out at inlet airflows of 50, 100, 150, and 200 mL/sec. Analogous experiments were also carried out in a stainless-steel (nonabsorbing) tube of dimensions similar to those of the tracheas.

Representative tracheal concentration data (Figure 14, lower panel) illustrate three important differences between the bolus and response curves: (1) the area enclosed by the response curve is less than that of the bolus input curve because of absorption into the tracheal wall; (2) the response curve appears at a later time than the bolus input because of the finite time required for O_3 to traverse the tube; and (3) the response curve is broader than the bolus input because of axial mixing. These observations are put on a more quantitative basis in Tables 6, 7, and 8, where the three reduced moments, M, $\bar{t}$, and S^2 of the bolus input (subscript B) and response curve (subscript R) are compared. The ratio M_R/M_B corresponds to the amount of O_3 in the response curve relative to that in the bolus input; and the differences $\Delta t = \bar{t}_R - \bar{t}_B$ and $\Delta S^2 = (S^2)_R - (S^2)_B$ represent the increase in appearance time and in longitudinal dispersion as O_3 traverses the conduit.

For a nonabsorbing tube, there is no loss of O_3 from the airstream so that M_R/M_B should be equal to 1. Moreover, Δt should be equal to the mean residence time of gas in the tube, $V/\dot{V}$ (Himmelblau and Bishoff 1968). In fact, the M_R/M_B data in Table 8 vary from 0.87 to 1.12, and although the Δt data are linearly correlated ($r^2 = 0.986$) with $V/\dot{V}$, the slope of the correlation is equal to 1.16 rather than 1.0. We believe these deviations from ideality are caused by our inability to withdraw radially mixed gas samples into the ozone analyzer. In our apparatus, the sampling needle was placed near the tube wall where gas is convected at lower than the average rate. If radial mixing was not complete, then the portion of the gas stream that we sampled would require more than one mean residence time to traverse the tube.

In an excised trachea, values of M_R/M_B are less than 1 because of O_3 absorption. Table 8 indicates that as flow increases from 50 to 100 mL/sec, such that gas residence time decreases, the fractional absorption ($1 - M_R/M_B$ calculated from Table 8) also decreases, from about 0.5 to 0.25. It is interesting that at any given flow, Δt as well as ΔS^2 values are less for the animal trachea than for the nonabsorbing tube. This observation is supported by the theoretical analysis given below.

DATA ANALYSIS: THE ABSORPTION COEFFICIENT

To interpret more fully the results of the tracheal bolus-response experiments, we developed a mathematical model of absorption in a straight tube of uniform cross-section. We began with the differential equation that ensures conservation of mass in the lumen of the tube:

Table 6. Moments of the Bolus-Response in Excised Tracheas: Bolus Input[a]

	Flow, $\dot{V}$ (mL/sec)	Amount, M_B (ppm•mL)	Appearance Time, $\bar{t}_B$ (sec)	Variance, S_B^2 (mL^2)
Nonabsorbing tube[b]	50	30.35 ± 0.47	1.32 ± 0.009	238.29 ± 21.25
	100	37.50 ± 1.55	1.13 ± 0.003	304.60 ± 22.81
	150	48.00 ± 1.69	1.07 ± 0.003	454.38 ± 42.74
	200	52.80 ± 1.34	1.03 ± 0.004	820.31 ± 78.76
Pig tracheas[c]	50	32.58 ± 1.06	1.33 ± 0.035	233.29 ± 15.07
	100	39.75 ± 1.96	1.14 ± 0.036	297.50 ± 67.30
	150	47.63 ± 1.03	1.06 ± 0.025	392.00 ± 55.24
	200	52.60 ± 1.30	1.03 ± 0.025	635.75 ± 160.86
Sheep tracheas[d]	50	29.78 ± 6.23	1.30 ± 0.101	207.00 ± 70.65
	100	41.68 ± 4.69	1.15 ± 0.034	323.20 ± 57.50
	150	48.93 ± 4.38	1.08 ± 0.033	482.33 ± 82.56
	200	56.33 ± 5.99	1.03 ± 0.032	682.67 ± 188.94

[a] Operating conditions for the ozone analyzer were as follows: reaction cell pressure of 220 torr; 2-methyl-2-butene flow of 4.5 mL/min; sample flow of 400 mL/min; and a filter constant of 10 msec. All values are given as mean ± SD. In footnotes: n = number of replications in nonabsorbing tube or number of tracheas, with one experiment conducted per trachea; V = average luminal volume; l = length between sampling ports; a = luminal surface-to-volume ratio.

[b] $n = 10$, V = 46 mL, l = 14.7 cm, a = 2.0 cm^{-1}.

[c] $n = 4$, V = 30.50 ± 4.2 mL, l = 11.88 ± 0.85 cm, a = 2.22 ± 0.13 cm^{-1}.

[d] $n = 6$, V = 46.83 ± 7.88 mL, l = 16.22 ± 2.73 cm, a = 2.09 ± 0.10 cm^{-1}.

Table 7. Moments of the Bolus-Response in Excised Tracheas: Response[a]

	Flow, $\dot{V}$ (mL/sec)	Amount, M_R (ppm•mL)	Appearance Time, $\bar{t}_R$ (sec)	Variance, S_R^2 (mL^2)
Nonabsorbing tube[b]	50	26.25 ± 1.03	2.74 ± 0.028	911.98 ± 63.89
	100	38.00 ± 2.50	1.95 ± 0.020	1,314.54 ± 94.84
	150	53.70 ± 3.01	1.64 ± 0.018	1,823.95 ± 201.31
	200	56.60 ± 2.78	1.39 ± 0.019	1,560.00 ± 162.12
Pig tracheas[c]	50	13.10 ± 0.92	2.13 ± 0.074	350.47 ± 48.17
	100	22.48 ± 2.19	1.60 ± 0.057	559.50 ± 53.33
	150	33.26 ± 2.91	1.38 ± 0.045	723.50 ± 131.19
	200	41.85 ± 4.02	1.25 ± 0.042	1,015.75 ± 239.58
Sheep tracheas[d]	50	12.93 ± 3.52	2.64 ± 0.261	529.17 ± 262.20
	100	23.92 ± 6.84	1.89 ± 0.173	1,008.20 ± 361.50
	150	33.40 ± 7.16	1.57 ± 0.119	1,257.50 ± 297.20
	200	45.50 ± 11.16	1.38 ± 0.078	1,286.33 ± 270.79

[a] Operating conditions for the ozone analyzer were as follows: reaction cell pressure of 220 torr; 2-methyl-2-butene flow of 4.5 mL/min; sample flow of 400 mL/min; and a filter constant of 10 msec. All values are given as mean ± SD. In footnotes: n = number of replications in nonabsorbing tube or number of tracheas, with one experiment conducted per trachea; V = average luminal volume; l = length between sampling ports; a = luminal surface-to-volume ratio.

[b] n = 10, V = 46 mL, l = 14.7 cm, a = 2.0 cm^{-1}.

[c] n = 4, V = 30.50 ± 4.2 mL, l = 11.88 ± 0.85 cm, a = 2.22 ± 0.13 cm^{-1}.

[d] n = 6, V = 46.83 ± 7.88 mL, l = 16.22 ± 2.73 cm, a = 2.09 ± 0.10 cm^{-1}.

$$\partial C/\partial t = (D)\partial^2 C/\partial z^2 - (\dot{V}/A)\partial C/\partial z - N \qquad (5)$$

where C and N are the concentration and absorptive flux of O_3, which depend on longitudinal position, z, and time, t; $\dot{V}/A$ is the ratio of gas flow to luminal cross-section; and D is the effective longitudinal diffusion coefficient. Equation 5 expresses the fact that in any thin cylindrical control volume, the accumulation rate of O_3 (left-hand side) equals the input rate by axial diffusion (first term, right-hand side) less a loss by bulk convection (second term, right-hand side) and a loss by absorption into the tracheal wall (third term, right-hand side).

To complete the mathematical model, the dependent variables N and C must be related by an appropriate rate expression. In the design of industrial gas-liquid absorption equipment, chemical engineers (for example, Treybal 1980) have traditionally used an expression of the form:

$$N = Ka(C - C') \qquad (6)$$

where K is an overall mass transfer coefficient, a is the surface-to-volume ratio of the tube lumen, C is the gas-phase concentration of the compound being absorbed, and C' is its liquid-phase (or tissue-phase) concentration. Equations 5 and 6 can be integrated after making the reasonable assumption that O_3 is sufficiently reactive in tissue that C' can be neglected.

Using the Laplace transformation with a special moment-generating technique (Himmelblau and Bishoff 1968), we solved equations 5 and 6 for the change in reduced moments occurring over an axial tube length, l. The resulting formulas were normalized by the moments that would be obtained if there were no absorption. For the simplifying case of relatively large convection, the normalized zeroth and second moments are

$$(M_R/M_B)/(M_R/M_B)_{K=0} = \{\exp[-(Ka)\,(\Delta t)_{K=0}]\} \qquad (7)$$

$$(\Delta S^2)/(\Delta S^2)_{K=0} = [1-(Ka)\,(6D/l^2)\,(\Delta t^2)_{K=0}] \qquad (8)$$

The subscript $K=0$ indicates a value obtained in a nonabsorbing tube.

Equation 7 indicates that the fraction of injected O_3 exiting the tube decreases as Ka increases. Because the rate of absorption is proportional to Ka, this result is expected. Equation 8, predicting that dispersion within the tube is reduced as Ka increases, is a surprising result. It does, however, correspond to our experimental observation that ΔS^2 is smaller for animal tracheas than for the nonabsorbing tube. Employing equations 7 and 8 with the measured values of these normalized moments, we have computed Ka and D at each volumetric airflow (Table 9).

DISCUSSION AND CONCLUSIONS

OZONE ANALYZER

The operating characteristics most critical in making an

Table 8. Moments of the Bolus-Response in Excised Tracheas: Comparison of Bolus and Response[a]

	Flow, $\dot{V}$ (mL/sec)	Unabsorbed Fraction, M_R/M_B	Residence Time, $\bar{t}_R - \bar{t}_B$ (sec)	Dispersion, $S_R^2 - S_B^2$ (mL^2)
Nonabsorbing tube[b]	50	0.865 ± 0.040	1.430 ± 0.029	673.69 ± 21.29
	100	1.013 ± 0.048	0.820 ± 0.020	1,009.94 ± 30.84
	150	1.119 ± 0.066	0.570 ± 0.018	1,369.57 ± 65.08
	200	1.072 ± 0.072	0.354 ± 0.019	739.69 ± 56.99
Pig tracheas[c]	50	0.402 ± 0.036	0.794 ± 0.082	117.18 ± 33.78
	100	0.566 ± 0.046	0.464 ± 0.067	261.98 ± 44.71
	150	0.699 ± 0.073	0.321 ± 0.052	331.31 ± 94.91
	200	0.796 ± 0.088	0.226 ± 0.049	380.11 ± 157.42
Sheep tracheas[d]	50	0.452 ± 0.142	1.192 ± 0.351	332.01 ± 221.74
	100	0.572 ± 0.147	0.739 ± 0.151	685.03 ± 334.32
	150	0.685 ± 0.143	0.496 ± 0.096	775.18 ± 251.50
	200	0.806 ± 0.167	0.348 ± 0.058	603.45 ± 159.77

[a] Operating conditions for the ozone analyzer were as follows: reaction cell pressure of 220 torr; 2-methyl-2-butene flow of 4.5 mL/min; sample flow of 400 mL/min; and a filter constant of 10 msec. All values are given as mean ± SD. In footnotes: n = number of replications in nonabsorbing tube or number of tracheas, with one experiment conducted per trachea; V = average luminal volume; l = length between sampling ports; a = luminal surface-to-volume ratio.

[b] n = 10, V = 46 mL, l = 14.7 cm, a = 2.0 cm^{-1}.

[c] n = 4, V = 30.50 ± 4.2 mL, l = 11.88 ± 0.85 cm, a = 2.22 ± 0.13 cm^{-1}.

[d] n = 6, V = 46.83 ± 7.88 mL, l = 16.22 ± 2.73 cm, a = 2.09 ± 0.10 cm^{-1}.

Table 9. Absorption and Dispersion Coefficients Obtained from Reduced Moments of the Bolus Response in Excised Tracheas[a]

Flow, $\dot{V}$ (mL/sec)	Unabsorbed Fraction, $(M_R/M_B)/(M_R/M_B)_{K=0}$	ΔResidence Time Ratio, $(\bar{t}_R - \bar{t}_B)/(\bar{t}_R - \bar{t}_B)_{K=0}$	ΔVariance Ratio, $(S_R^2 - S_B^2)/(S_R^2 - S_B^2)_{K=0}$	Absorption Coefficient, K (cm/sec)	Dispersion Coefficient, D (cm^2/sec)
Pig tracheas					
50	0.465 ± 0.026	0.555 ± 0.043	0.174 ± 0.050	0.242 ± 0.015	19.18 ± 4.65
100	0.559 ± 0.033	0.566 ± 0.035	0.259 ± 0.044	0.311 ± 0.035	40.52 ± 9.73
150	0.625 ± 0.049	0.563 ± 0.028	0.242 ± 0.069	0.364 ± 0.062	58.20 ± 13.39
200	0.743 ± 0.057	0.638 ± 0.026	0.514 ± 0.128	0.428 ± 0.114	76.22 ± 23.91
Sheep tracheas					
50	0.523 ± 0.060	0.834 ± 0.144	0.493 ± 0.229	0.262 ± 0.136	26.07 ± 11.48
100	0.565 ± 0.063	0.901 ± 0.062	0.678 ± 0.231	0.353 ± 0.169	32.99 ± 10.63
150	0.612 ± 0.064	0.870 ± 0.040	0.566 ± 0.183	0.391 ± 0.191	46.67 ± 13.58
200	0.752 ± 0.074	0.983 ± 0.025	0.816 ± 0.215	0.426 ± 0.218	56.66 ± 14.04

[a] Mean values ± SD based on single experiments in four pig tracheas and six sheep tracheas.

analyzer useful in respiratory measurements are a sufficiently fast dynamic response so that changes in O_3 concentration can be reliably monitored during the four-second period of a normal breath, and a sampling flow that is small enough to not interfere with quiet respiratory flows of 300 mL/sec. The analyzer we have developed meets these criteria.

A few accounts of fast-responding ozone analyzers have appeared in the literature. Ray and colleagues (1986) constructed an analyzer based on the chemiluminescence of O_3 with a solution of eosin Y dye in ethylene glycol. They achieved a frequency response of 7 Hz (that is, a 90 percent step-response time of about 52 msec), but this required a large sample inlet flow of 9 L/min. Pearson and Stedman

(1980) constructed a chemiluminescent analyzer employing the reaction of O_3 with nitric oxide. This instrument had a frequency response of 10 Hz at an inlet sampling flow of about 2 L/min. Not only is this sampling flow still too large for respiratory applications, but the use of a toxic reagent such as nitric oxide poses a significant risk when human subjects are involved. Gerrity and coworkers (1988) modified the reaction cell of a commercially available chemiluminescent analyzer that utilized the reaction of O_3 and ethylene. Although their sampling flow of 300 mL/min is appropriate for respiratory applications, their modifications only improved the step-response from 2,300 to 700 msec.

In the current study, we constructed our own chemiluminescent analyzer in which we tested nine alkene reactants as possible alternatives to ethylene. We also designed the sample inlet system and reaction cell geometry to minimize sluggish response due to back mixing. We achieved the best analyzer performance using 2-methyl-2-butene, a nontoxic simple asphyxiant (Budavari et al. 1989) that has an intrinsic reaction rate with O_3 that is 500 times faster than that of ethylene. With this reactant gas, we encountered little difficulty in achieving a rapid dynamic response. The optimum analyzer performance we observed included a 90 percent step-response time of 110 msec; a linear calibration from 30 parts per billion (ppb) to 10 ppm with a sensitivity of 2.3 nA/ppm; a signal-to-noise ratio of 30 evaluated at 0.5 ppm; and a minimum detection limit of 0.017 ppm.

Some of these performance specifications do not strictly meet our original aims, but we believe that the analyzer is well suited for many respiratory applications including the bolus-response method. The sample flow of 400 mL/min, although larger than the 60 mL/min we originally anticipated, represents only about 3 percent of a resting minute volume. Thus, a disturbance in airflow patterns due to gas sampling at the mouth is unlikely during a normal breathing maneuver. The minimum detectable limit of 0.017 ppm, although greater than the 0.002 ppm value we initially strived for, is still acceptable provided that peak O_3 fractions of at least 0.1 ppm are to be measured. The presence of a curvilinear calibration below 0.03 ppm is an unexpected result, probably due to the complexity of the chemiluminescent reaction between 2-methyl-2-butene and O_3. In some applications, this behavior will require that calibration data be fit piecewise to a straight line above 0.03 ppm and a curved line at or below 0.03 ppm (Figure 11).

The results of interference tests indicate that the temperature and humidity of the sampled gas have no influence on the analyzer output (Figure 10). However, the analyzer is affected by the nature and composition of the carrier gas in which O_3 is mixed (Table 4). This may occur because of differences in the chemiluminescent quenching rates of various carrier gases (Aimedieu and Barat 1981). An important observation is that a 1 percent replacement of O_2 with CO_2 causes a 3.8 percent increase in analyzer sensitivity when air is the carrier gas. Therefore, if O_3 is monitored in exhaled breaths in which respiratory O_2–CO_2 exchange occurs, CO_2 concentration will also have to be measured so that an appropriate correction can be made.

OZONE BOLUS GENERATOR

Although commercial ozone generators are available, these devices are usually designed for large rates of O_3 production. In a laboratory setting where human subjects are to be tested, it is safer to limit the production of O_3. Therefore, we constructed our own small-scale device, which has provisions for either continuous O_3 generation or delivery of O_3 boluses. Except at the largest flow tested, this device meets our original specification of bolus volumes no larger than 50 mL with O_3 contents as large as 0.2 μg.

We characterized the boluses produced by the generator when it was operated at its maximum capacity (Table 5). At an inhaled airflow of 250 mL/sec, the ozone generator was capable of producing a bolus with a peak concentration of 4 ppm and an O_3 content of 163 ppm•mL (that is, 0.35 μg). In future bolus-response studies in human subjects, we expect a 10:1 attenuation in O_3 concentration between inspired and expired breaths. Therefore, a 4-ppm peak inspired concentration may be desirable in order to maximize resolution of expired O_3 measurements. With respect to safety, inspiring a bolus containing 163 ppm•mL of O_3 is equivalent to taking a 540-mL breath at a constant O_3 fraction of 0.3 ppm, and this maneuver poses an acceptably low risk to human subjects (see, for example, McDonnell et al. 1983; Gerrity et al. 1988).

An important concern in future O_3 bolus-response experiments is the spatial resolution of the absorption distribution, which we plan to compute. Spatial resolution is ultimately limited by the volume of the bolus, which we have characterized by its SD (Table 5, column 5). As the bolus volume is only 19 mL at an inhaled airflow of 250 mL/sec, we expect very good resolution during quiet breathing. At higher flows, however, resolution will diminish because of the larger bolus volume.

IN VITRO BOLUS-RESPONSE EXPERIMENTS

The bolus-response experiments performed in excised pig and sheep tracheas demonstrate that the method is capable of detecting and quantifying O_3 absorption, even

in a conduit of limited surface area. In making these measurements, we intentionally employed small airflows below the physiologic range to exaggerate absorption. As flow increased from 50 to 200 mL/sec, the percentage of O_3 absorbed from the bolus input decreased from about 50 to 25 percent. This inverse relationship is to be expected because, at larger flows, there is less residence time available for absorption to occur.

To quantify O_3 absorption, we computed the change in mathematical moments between the bolus input and the response curve. Moreover, we derived theoretical expressions allowing us to relate the experimentally measured change in moments to fundamental system parameters, namely, an absorption coefficient, K, and an effective longitudinal diffusion coefficient, D. In addition to providing a means of interpreting the excised trachea experiments, this theory provides a starting point for the interpretation of bolus-response data in human subjects.

From the change in the zeroth moment (that is, the integral), K was computed (Table 9). Values of K more than doubled when the flow rate quadrupled, indicating that O_3 absorption is limited by diffusion processes in the airway lumen as well as in the surrounding tissue. We also observed that changes in the first moment (that is, the appearance time) and in the second moment (that is, the dispersion) were both reduced by O_3 absorption when compared to corresponding data from the nonabsorbing tube. This unexpected result, which was predicted, however, by the theoretical analysis, illustrates the importance of applying an appropriate diffusion model to the interpretation of bolus-response data.

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ABOUT THE AUTHORS

James S. Ultman is Professor of Chemical Engineering at the Pennsylvania State University, where he has been a faculty member since 1970. He received his Ph.D. in Chemical Engineering at the University of Delaware in 1969, and then served as a National Institutes of Health Postdoctoral Fellow at the University of Minnesota. In 1978, Professor Ultman was a Fulbright Lecturer at the Technion (Israel Institute of Technology), and a visiting researcher at the Silverberg Medical School (Haifa, Israel). In 1989, he was a Visiting Research Professor in the Department of Medicine of Duke University Medical School. Dr. Ultman is interested in the application of the physical principles of fluid flow, diffusion, and chemical reaction to problems in pulmonary physiology, pathology, and toxicology.

Abdellaziz Ben-Jebria is currently a Visiting Associate Professor in the Department of Chemical Engineering of the Pennsylvania State University. He earned his Ph.D. and State Doctorate degrees in biophysics and in natural science, respectively, at the University of P. and M. Curie in Paris. Since 1980, he has been a permanent researcher at the Institut National de la Santé et de la Recherche Médicale, working in the Respiratory Pathophysiology Research Unit in Paris until 1984, and then in the Physiology Laboratory at the University of Bordeaux-II. His major research interest is pulmonary gas transport and uptake processes.

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ABBREVIATIONS

CO_2	carbon dioxide
O_2	oxygen
O_3	ozone
ppm	parts per million
rms	root-mean-square
SD	standard deviation

INTRODUCTION

A Request for Preliminary Applications (RFPA 87-3), which solicited proposals for "New Methods for Assessing Health Risks from Automotive Emissions," was issued by the Health Effects Institute (HEI) in the summer of 1987. In response to the RFPA, Dr. J.S. Ultman, from Pennsylvania State University, submitted a preliminary application entitled "Noninvasive Measurement of Ozone Uptake Distribution in Cooperating Human Subjects Using a Bolus-Response Method." The HEI Research Committee requested that a limited proposal be prepared that would concentrate only on the development of the instrumentation. In response, Dr. Ultman submitted a proposal entitled "Noninvasive Determination of Respiratory Ozone Absorption: Development of a Fast-Responding Ozone Analyzer." The eighteen-month project began in June 1988, and total expenditures were $166,899. The Investigators' Report was received at the HEI in December 1989, and was accepted by the Health Review Committee in April 1990. During the review of the Investigators' Report, the Review Committee and the principal investigator had the opportunity to exchange comments and to clarify issues in the Investigators' Report and in the Review Committee's Commentary. The Health Review Committee's Commentary is intended to place the Investigators' Report in perspective as an aid to the sponsors of the HEI and to the public.

REGULATORY BACKGROUND

The U.S. Environmental Protection Agency (EPA) sets standards for oxidants (and other pollutants) under Section 202 of the Clean Air Act, as amended in 1990. Section 202(a)(1) directs the Administrator to "prescribe (and from time to time revise) . . . standards applicable to the emission of any air pollutant from any class or classes of new motor vehicles or new motor vehicle engines, which in his judgment cause, or contribute to, air pollution which may reasonably be anticipated to endanger public health or welfare." Sections 202(a), (b)(1), (g), and (h) and Sections 207(c)(4) through (6) impose specific requirements for reductions in motor vehicle emissions of certain oxidants (and other pollutants) and, in some cases, provide the EPA with limited discretion to modify those requirements.

In addition, Section 109 of the Clean Air Act provides for the establishment of National Ambient Air Quality Standards (NAAQS) to protect the public health. The current primary and secondary NAAQS standard for ozone is 0.12 parts per million (ppm). This standard is met when the number of days per year with maximum hourly average concentrations above 0.12 ppm is equal to or less than one. Section 181 of the Act classifies the 1989 nonattainment areas according to the degree to which they exceed the NAAQS standards and assigns a primary standard attainment date for each classification.

SCIENTIFIC BACKGROUND

Ozone is a highly reactive gas and a major constituent of photochemical smog. Although not directly emitted by automobiles and other combustion sources, it is formed in the atmosphere by a complex series of photocatalyzed reactions with nitrogen oxides and volatile organic compounds, such as olefinic hydrocarbons and formaldehyde, that are directly released (reviewed by Lippmann 1989). In the presence of sunlight and these pollutants, atmospheric ozone levels often exceed the current NAAQS that specifies that the average ozone concentration over a one-hour period shall not exceed 0.12 ppm for more than one day per year. Incidents of noncompliance occur most frequently during the summer months in heavily populated areas such as southern California, the Northeast corridor, and other metropolitan areas. Substantial uncertainty remains about whether or not the current standard is sufficient to protect healthy humans exposed to ambient levels of ozone for extended periods of time. There is additional concern over whether or not individuals who may be even more sensitive to ambient ozone, such as people with asthma and elderly persons, are also adequately protected (Van Bree et al. 1990).

Experimental studies with animals have important implications for human health risks from ozone exposure. Because studies of biological effects of ozone indicate a marked similarity in interspecies response, it is possible that results obtained with animals may be directly related to human response, particularly because some of these data were obtained with nonhuman primates. It is clear from inhalation studies that the primary site of damage is the centriacinar region of the lung where the small mucus-coated conducting passages merge with the surfactant-coated gas exchange airways. Inflammation and a shift in cell populations resulting from damage to and loss of a set of cells (ciliated bronchiolar cells and type I alveolar epithelial cells) and replacement by proliferation of their respective progenitor

cells (nonciliated bronchiolar [Clara] cells and type II epithelial cells) are characteristic effects. These responses have been noted in rhesus monkeys (Mellick et al. 1977; Castleman et al. 1980) and in rats (Stephens et al. 1973; Schwartz et al. 1976; Plopper et al. 1978; Barry et al. 1985). The inflammatory and proliferative responses were diminished in subchronic and chronic studies with rats (Boorman et al. 1980; Moore and Schwartz 1981; Gross and White 1987) and monkeys (Eustis et al. 1981; Fujinaka et al. 1985) when ozone concentrations were reduced and exposure times were increased.

It has not been established whether or not the centriacinar region of the lung in humans is damaged by ozone exposure. However, Miller and colleagues (1985) developed a mathematical model of ozone dosimetry for the human lung, taking into account chemical and physical parameters related to the diffusion and reactivity of ozone, as well as morphological and physical properties of the lung tissues. Although the model excluded ozone absorption by the nasopharyngeal tissues, it predicted that maximal absorption of ozone would occur in the centriacinar region, where damage has been demonstrated in ozone-exposed animals.

Ozone-induced changes in measurements of pulmonary function in humans have been consistently demonstrated, although structural damage of the small airways has not been related to ozone exposure. There are a number of acute studies in which healthy young human adults were experimentally exposed to ozone concentrations ranging from 0.08 to 0.5 ppm for periods of one to seven hours (Gliner et al. 1983; McDonnell et al. 1983, 1985; Kehrl et al. 1987; Folinsbee et al. 1988; Horstman et al. 1989). Various parameters of pulmonary function, including forced vital capacity, forced expiratory volume in one second, and specific airways resistance, were measured. In general, statistically significant decrements in pulmonary function were consistently observed at ozone concentrations equal to or greater than the NAAQS of 0.12 ppm. Each of these studies confirmed the existence of marked differences among tested individuals with respect to their response to ozone. Typical individual decreases in forced expiratory volume in one second were calculated or reported to range from no effect to approximately 50 percent.

Two salient facts were established in these studies with humans exposed to environmentally relevant levels of ozone. First, the decrease in human pulmonary function was related to ozone concentration and duration of exposure (McDonnell et al. 1983; Folinsbee et al. 1988; Horstman et al. 1989). Second, an individual's response to ozone, however deviant from the norm, appeared to be reproducible. McDonnell and coauthors (1985) exposed subjects for 2.5 hours to ozone concentrations of 0.12 to 0.4 ppm, then repeated the exposures at least once at an average interval of nearly three months. Changes in several measures of pulmonary function in individuals were highly reproducible for ozone concentrations equal to or greater than 0.18 ppm and for intervals between exposures of up to 10 months.

The dose-response–related decrements in pulmonary function and the interindividual variations observed among humans exposed to ozone, as well as the implication of the centriacinar region as the site of ozone-induced lung damage in animals, should be related in some manner to the actual dose of ozone delivered to the target tissues and, ultimately, to the specific molecular receptors or sites within the target tissues. The amount of ozone available to these critical sites is dependent on a host of factors, including ambient ozone concentration, the total amount of ozone inspired into the respiratory tract during the exposure period, the portion of the inspired ozone dose reaching the presumed target cells in the centriacinar region (which, in turn, is dependent upon nonspecific absorption by other respiratory tract tissues), and the amount of ozone selectively absorbed by the target cells. Quantitative information on tissue absorption of ozone throughout the length of the respiratory tract, and particularly in the centriacinar region, is, therefore, essential to identifying target tissues and to understanding the mechanisms of action underlying individual sensitivity. This information is also a prerequisite for evaluating the consequences to public health and making informed regulatory decisions about ozone exposure limits.

It is of significant interest to explore approaches by which the actual dose of ozone to target tissues can be measured. The current state of knowledge in this area is rudimentary because, apart from some theoretical modeling studies (Miller et al. 1985), measurements of ozone absorption in animals and humans have been limited to the total respiratory tract or to the extrathoracic (nose, mouth, larynx, and pharynx) or intrathoracic (trachea, bronchi, and conducting and respiratory tissues of the lung) subdivisions.

Gerrity and coworkers (1988) measured ozone absorption by the extrathoracic or intrathoracic airways by removing samples of inspired and expired air via a tube placed in the posterior pharynx. They estimated that approximately 95 percent of the inhaled ozone was removed by the entire respiratory tract, with 40 percent removed by the extrathoracic airways and 55 percent by the intrathoracic airways. It was noted that ozone absorption, expressed as a percentage, was independent of initial concentration over a range of 0.1 to 0.4 ppm. Significantly, interindividual variation in ozone absorption was observed, complementing the variability in physical parameters of pulmonary function discussed previously. Total ozone removal by the respiratory tract in 18 healthy young males varied from 69 to 100 percent, reflecting a difference in extrathoracic removal of

ozone of between 14 and 75 percent, and in intrathoracic removal of between 42 and 100 percent of the ozone remaining after extrathoracic absorption. Though helpful, as a first step, in providing basic information on total ozone absorption, this approach is inadequate for resolving unanswered questions of ozone dose delivered to specific areas of the respiratory tract.

A potentially useful methodology that could be adapted for ozone absorption studies is the noninvasive bolus-response technique previously described for pulmonary airway mixing of the inert gases helium and sulfur hexafluoride (Ultman et al. 1978). A bolus input curve (a plot of gas concentration versus cumulative respired gas volume) is produced by rapidly injecting an appropriate short pulse (or bolus) of tracer gas into the airflow that is inhaled by a human subject. Upon exhalation, a response curve is obtained that differs from the input curve by having a reduced height, a broader base, and an altered time of appearance. These features contain information that reflects the absorption and mixing characteristics of the tracer gas in the respiratory tract. The reduced area under the response curve is related to the total absorption of gas by the tissues through which the bolus passed during inspiration and expiration. The time of appearance of the response curve reflects the depth into the respiratory tract that the bolus was inspired, and can be controlled by varying the point in the breathing cycle at which the bolus is introduced into the airflow. This, in effect, permits mapping the absorption of gas along the length of the respiratory tree by introducing boluses at different points in individual inspirations and measuring loss in response peak areas. Finally, the degree of broadening of the response peak is a function of the amount of axial mixing occurring during passage of the bolus.

Three major questions could be addressed if the bolus-response technique was applied to ozone absorption studies in humans. First, can the ozone-induced response observed in laboratory animals be extrapolated to humans? This query would be supported if it could be demonstrated that the centriacinar region of the human lung receives a proportionately larger dose of ozone than other areas of the respiratory tract; this demonstration would be in agreement with the theoretical absorption model developed by Miller and colleagues (1985). Second, where and to what extent is ozone absorbed in the respiratory tract? Third, can individual variability in pulmonary function measurements be explained by potential differences in respiratory tract absorption of ozone?

The applicability of the bolus-response technique depends upon the development of suitable instrumentation that fulfills two requirements: (1) it can rapidly make a series of sequential ozone analyses over the approximately four-second interval during the inspiration and expiration of the bolus, and (2) it is sufficiently sensitive to measure ozone in low-level exposure studies or at the low level expected after substantial absorption of ozone when a bolus is inspired deep into the respiratory tract. Furthermore, these analyses need to be performed in low sample volumes and at flow rates consistent with a single 500-mL breath taken at a quiet breathing rate of approximately 300 mL/sec. These conditions impose design specifications not found in commercially available ozone analyzers that were originally designed for ozone measurements in either the atmosphere or in environmental exposure chambers, where successive rapid analyses and small sample volumes and flow rates are not required for adequate instrument performance.

Commercial ozone analyzers depend either upon optical absorption or chemiluminescence detection principles. Commercial photometric analyzers, depending upon absorption of ultra-violet light, are the current standard for monitoring ambient ozone levels (Stevens and Hodgeson 1973). However, measurements of the low ozone concentrations would require an instrument with an absorption path length that is so long that the dynamic response of the instrument is unsuitable for human bolus-response measurements.

Chemiluminescent reactions of ozone with suitable reactant substances represent the most promising technology for rapid sensitive ozone analysis (Fontijn et al. 1970; Stevens and Hodgeson 1973). The level of light emitted is proportional to the amount of ozone and reactive substances present in the reaction zone at the surface of a photomultiplier tube, which is employed as the detector (Nederbragt et al. 1965; Bowman and Alexander 1966).

The earliest use of chemiluminescence for ozone measurements employed the solid-state reaction of ozone with a luminescent powder adsorbed to a bed of silica gel (Regener 1960). Interference from moisture was eliminated by coating the surface of the silica gel with a moisture-resistant silicone resin (Hodgeson et al. 1970). The reaction was specific for ozone, with a detection limit of less than 0.001 ppm and with a linear response up to 0.4 ppm. More recent modifications compared a variety of chemiluminescent dyes dissolved in selected organic solvents (Ray et al. 1986). Optimal ozone analyzer response was obtained with a solution of eosin Y dissolved in ethylene glycol. Solid-state ozone analyzers require frequent calibration because of sensitivity changes in the chemiluminescent surface (Stevens and Hodgeson 1973).

Gas-phase ozone analysis, based on the chemiluminescent reaction of ozone with a reactant gas, such as nitric oxide (Pearson and Stedman 1980), ethylene (Nederbragt et al. 1965), or 2-methyl-2-butene (Aimedieu and Barat 1981), under homogeneous conditions represents an improved meth-

odology. Several commercial ozone analyzers are available that use ethylene as the reactant (Stevens and Hodgeson 1973). As was the case with previous solid-state ozone analyzers, these instruments are primarily designed to measure ozone in ambient air, and, therefore, do not have the appropriate combination of short instrument response time, low sample volume, and low inlet flow rate necessary for noninvasive respiratory measurements in humans. As an example, the ozone analyzer developed by Pearson and Stedman (1980), although having an acceptably short response time of 42 msec, had a high inlet sample flow rate. Likewise, a gas-phase chemiluminescent analyzer developed for measuring stratospheric ozone concentrations had an unacceptable instrument response time of 1 second and an inlet sample flow rate of 30 L/min (Aimedieu and Barat 1981).

Gerrity and coauthors (1988) modified a commercial ethylene-based analyzer to continuously monitor ozone in airways of humans exposed to a constant ozone level. The volume of the sampling system was reduced and the reaction chamber altered to permit a continuous flow of ethylene and ozone. Although an appropriate sample flow of 300 mL/min was obtained, the instrument response time was reduced from 2.3 seconds to only 700 msec. This response time, though sufficient to permit breath-by-breath ozone measurements, was still considered by these investigators to be sufficiently long to introduce possible overestimations of extrathoracic ozone absorption and underestimations of intrathoracic ozone absorption.

An additional requirement for the applicability of the bolus response method to the study of ozone absorption in human subjects is the design and development of an ozone bolus generator, an instrument that is not commercially available. A suitable design would include the ability to produce ozone boluses of variable volumes and concentrations and to inject these boluses at appropriate times into inhaled airflows, all under conditions that assured the safety of personnel conducting and participating in the experiment.

In summary, there is a critical lack of experimental measurement of ozone absorption along the respiratory tract of human subjects. This information is needed to identify lung tissues that receive the highest amounts of ozone, and that may, therefore, be designated as areas potentially the most susceptible to ozone-induced damage. Maximal damage from ozone occurs in the centriacinar region of the lung in rats and monkeys, and absorption modeling studies predict that maximal absorption will also occur in this region in humans. Experimental verification of this prediction will provide, for the first time, evidence to justify the extrapolation of data obtained with animal studies to humans. Furthermore, individual differences in ozone absorption patterns in humans could provide a quantitative basis for explaining interindividual variation in susceptibility to ozone, as consistently manifested by decrements in pulmonary function following exposure. The bolus-response technique was demonstrated to be a novel methodology for the noninvasive measurement of absorption and mixing of inert gases in the human respiratory tract, and promises to be suitable for the mapping of ozone absorption along the course of the tract as well. The design and development of instrumentation for producing an ozone bolus, suitable for inhalation by human subjects, and an ozone analyzer capable of making rapid, successive measurements of ozone in inspired and expired air from a single breath in human subjects, will be a major step forward in addressing these objectives.

JUSTIFICATION FOR THE STUDY

The HEI sought proposals for the development of better methods to provide quantitative data on human exposure to automotive emissions and their constituents, with particular emphasis placed on exposure by inhalation. Priority was given to new methods and procedures that would permit quantification of dose and dose-response relationships to target tissues.

Dr. Ultman proposed to develop a fast-responding ozone analyzer that would be capable of continuously monitoring ozone concentrations in inspired and expired air by human subjects exposed to a bolus of air containing variable amounts of ozone. Commercially available ozone analyzers need relatively large samples of gas for analysis and are not able to make the measurements in the short time period necessary for the measurement of rapidly changing ozone concentrations in a single breath. Additionally, he proposed to develop an ozonator to produce and deliver the small ozone boluses required for these studies, and for eventual studies with human subjects. Commercial ozone bolus generators are not available. Concerns for human safety require that only limited amounts of ozone be produced in a laboratory setting.

The investigator was considered well qualified for this study because he had pioneered the development and description of the bolus-response method (Ultman et al. 1978). This noninvasive technique was previously used to measure mixing and penetration of inert gases into the tracheobronchial tree by mathematical treatment of gas concentration curves produced during inspiration and expiration of a single breath containing a bolus of the gas under study. When applied to ozone, the bolus-response method can provide information on the internal distribution and actual

dose levels of gas absorbed by different tissues along the length of the respiratory tract.

These studies are an important step in the documentation of respiratory tract uptake of ozone in humans. Experiments performed with ambient levels of ozone will be of central importance to the assessment of risk of exposure to ozone, and will provide a scientific basis for the extrapolation of results obtained with animals. In addition, this approach should provide information on the large degree of variability in the observed response of humans to ozone, which may reflect, in part, the delivery and uptake of ozone at various levels of the respiratory tract. Understanding such differences is central to understanding the variability in human response.

OBJECTIVES AND STUDY DESIGN

Dr. Ultman proposed to develop and validate instrumentation that produced and analyzed a bolus of ozone for inhalation by cooperating human subjects. This instrumentation would be used in future studies to obtain quantitative information on ozone absorption, mixing, and penetration into the respiratory tract. Specific aims of the proposal were:

1. To construct a fast-responding ozone analyzer capable of monitoring the changing ozone concentration during the inspiration and expiration of a single breath of air containing a bolus of ozone.
2. To construct a small-scale ozone bolus generator capable of producing a bolus suitable for inhalation in eventual noninvasive studies of human respiratory tract absorption of ozone.
3. To test the ability of the analyzer and bolus generator to obtain bolus-response data with in vitro systems, using a nonabsorbing steel tube and excised pig and sheep tracheas.

In specific aim 1, the performance specifications set for the ozone analyzer were linked to the capability of on-line monitoring of the rapidly changing ozone concentrations expected during the inhalation and expiration of a breath of air containing a fixed amount of ozone. This led to design specifications of a sample inlet flow rate of 60 mL/min, a minimum detectable limit of 0.002 ppm ozone, a linear calibration range of up to 1 ppm ozone, and a 90 percent step-response time of 100 msec or less. The reaction parameters of ozone with a variety of complex alkenes were to be examined to select an alkene with the optimum balance of performance characteristics.

The design of the small-scale ozone bolus generator in specific aim 2 focused upon the capability of generating boluses with volumes of 50 mL or less when injected into airstreams flowing at 0.25 to 1.0 L/sec, corresponding to a well-mixed ozone concentration of 0.2 ppm. These specifications also served to avoid risk to future human subjects.

In specific aim 3, the fast-responding ozone analyzer and the small-scale ozone bolus generator were to be evaluated in bolus-response studies using a nonabsorbing stainless-steel tube and excised pig and sheep tracheas. The ozone bolus was injected into the proximal end of the tube or trachea and ozone concentration was monitored through a port at the distal end. The characteristics of the measured ozone bolus curves were further quantified by computing their mathematical moments.

TECHNICAL EVALUATION

ATTAINMENT OF STUDY OBJECTIVES

This was a carefully designed and conducted study. The investigators were able to confirm their expectation that complex alkenes would have a faster (approximately 500 times) intrinsic reaction rate with ozone than does ethylene, which is presently employed in commercial ozone analyzers. The investigators appear to have fully met their overall objective of developing a markedly improved ozone analyzer that can be applied to experimental studies of ozone absorption in single respirations in human subjects, despite the fact that not all of the initial instrument design specifications were met.

The 90 percent step-response time of 110 msec was very close to the design specification of 100 msec or less, but the goal of a sample flow of 60 mL/min was not obtained. The minimum detectable limit for ozone was 0.017 ppm, not the original goal of 0.002 ppm. This means that bolus levels of at least 0.1 ppm ozone will be required to ensure that sufficient ozone can be detected upon expiration after accounting for absorption by tissues of the respiratory tract. The goal of a linear calibration curve from 0 to 1 ppm ozone was only partially met, as linearity was observed only over the range of 0.3 to 10 ppm.

The investigators also successfully developed an ozone bolus generator for laboratory studies with human subjects. The original specifications for ozone bolus generation were met or exceeded except at the very highest flow rates. At airflows corresponding to quiet breathing (250 mL/sec), single bolus peaks containing 0.35 μg of ozone in a volume of 19 mL were generated.

The in vitro bolus-response experiments with excised

tracheas and stainless-steel tubes and the mathematical analysis of bolus input and response curves are important accomplishments preparatory to performing ozone absorption studies in human subjects.

METHODS AND STUDY DESIGN

The investigators did an excellent job of attaining their goals and describing the data that allowed them to draw their conclusions.

STATISTICAL METHODS

Because this study was primarily directed toward the development of instrumentation, there was relatively little need for data analysis apart from replicative measurements of moments derived from ozone bolus-response curves. Statistical analyses were uncomplicated computations of means, standard errors, and levels of significance.

RESULTS AND INTERPRETATION

Development of Instrumentation

The investigators decided that the chemiluminescent gas-phase reaction of ozone with an alkene was the only feasible methodology for continuously monitoring ozone levels in inspired and expired air in humans at quiet respiratory flows of 300 mL/sec. Alternative technologies, such as mass spectrometry, ultraviolet photometry, or thermocatalytic detection, were considered inappropriate for respiratory measurements because of their requirement for high gas sampling flow rates and their limited ability to respond rapidly to changes in ozone concentration. Although commercial ozone analyzers that employ a chemiluminescent reaction between ozone and ethylene are available, they also have very high sample inlet flow rates that render them unsuitable for respiratory applications.

An ozone analyzer was constructed featuring an aerodynamically redesigned reaction cell that permitted the chemiluminescent reaction of ozone with the selected alkene to go to near completion. Ten alkenes (including ethylene) were selected for testing in the ozone analyzer on the basis of their published reactivities with ozone (Japar et al. 1974).

A number of variables, including alkene inlet flow rate, reaction cell pressure, and ozone concentration, were systematically altered for each of the ten alkenes to provide data on signal-to-noise ratio, step-response time, and linearity of the analyzer response to actual ozone concentrations. None of the tested alkenes had all of the desired performance characteristics. However, on the basis of these studies, 2-methyl-2-butene was selected for future use because of its low step-response time and high signal-to-noise ratio, although its calibration curve was markedly nonlinear at ozone levels below 0.03 ppm.

The curvilinear calibration curve in the critical region below 0.03 ppm ozone, where important measurements in the bolus response curve will be made, will require attention. The investigators suggested that this nonlinearity was due to complexities in the reaction of ozone with 2-methyl-2-butene. However, the degree of this nonlinearity does not appear severe, and the investigators have derived a nonlinear calibration equation to correct for this circumstance.

The investigators also obtained a sample flow rate of 400 mL/min, rather than the target flow rate of 60 mL/min. Because this sample flow rate represented only 3 percent of a resting minute volume, they concluded that this would not result in airflow disturbances in the mouthpiece assembly during normal breathing. The implication is that some problems might be anticipated with lower minute volumes or with breath holding.

In addition, a small-scale commercially available ozone generator was modified to provide a suitable bolus of ozone for optimization and evaluation of the operating conditions of the ozone analyzer. The characteristics of the ozone bolus generator were evaluated with different simulated inhaled airflows, hold-up tube volumes, pressure regulator settings, and pulse durations for the solenoid valves. Optimum settings were chosen based on the reproducibility of the bolus shape and the ability of the generator to produce peak ozone concentrations greater than 1 ppm at airflows consistent with those measured during quiet breathing in human subjects.

The investigators stated that ozone bolus volume would increase with higher inhaled airflows. At simulated quiet airflows of 250 mL/sec, the bolus volume was 19 mL/sec, and the spatial resolution of inferred ozone distributions should not pose a problem. However, at an airflow of 1,000 mL/sec characteristic of moderate exercise, the bolus volume was 76 mL and this could limit resolution. The relative impact of these factors on the ability to generate usable bolus-response curves can be assessed only by actual experiments with human subjects.

Measurement of Interference

Factors that might influence ozone measurements in future studies with human subjects were examined. Variables evaluated were temperature, humidity, and gas composition using mixtures of selected inert gases with oxygen, or carbon dioxide with air.

No effects of humidity were observed on the ozone analyzer signal when 100 percent humidified air and dry air were alternately passed through the apparatus. Temperature also did not interfere with ozone measurement over a range of 20° to 38°C. The calibration curves for combina-

tions of inert gases with oxygen were linear, but sensitivity of the ozone analyzer was increased in direct proportion to the mole fraction of the carrier gas, as well as to its molecular weight.

Interference was also noted when carbon dioxide was combined with air at concentrations simulating respiratory gas exchange, with analyzer sensitivity increased in direct proportion to carbon dioxide concentration. This effect will require correction, as there may be different carbon dioxide levels in the expired air at different points along a given bolus response curve. Carbon dioxide levels will also be expected to vary as a function of breathing rate and exercise level in the same subject. Also, interindividual differences in expired carbon dioxide can be predicted because of variations in body mass, age, and metabolic level, to list some of the obvious confounding factors. At the very least, preliminary carbon dioxide measurements will be required to ascertain what the range of error in ozone measurements will be, and to what extent this will affect the usability of the data obtained from the mathematical moments of the bolus input and response curves. If precise measurements of ozone are required, then simultaneous measurements of carbon dioxide may also have to be obtained and appropriate corrections applied.

In Vitro Bolus-Response Tests

The applicability of the ozone analyzer and ozone bolus generator was demonstrated in a series of in vitro bolus response experiments with excised tracheas and nonabsorbing stainless-steel tubes. These studies were a highly useful preliminary step to performing ozone bolus-response studies in human subjects.

The resultant bolus input and response curves exhibited three important differences that reflected the absorption characteristics of ozone by the trachea. First, the integrated area enclosed by the response curve was less than that of the bolus input curve. This difference was due to the absorption of ozone by the trachea. When a nonabsorbing stainless-steel tube of similar dimensions was substituted for a trachea, the response curves produced had the same area as the input curve. Second, the peak of the response curve appeared at a later time than the peak of the input curve because of the additional time it took for the bolus to traverse the steel tube or trachea. Third, the response curve was broadened by passage through the tube or trachea. The degree of broadening reflected the amount of axial mixing during passage.

Each of these three properties of the bolus curves was expressed quantitatively by calculating reduced moments, which represented the amount of ozone in the peak, the appearance time of the peak, and a measure of longitudinal dispersion. The ratios of the amount of ozone in the input and response curves and the differences in the appearance time and variance were determined and used for the calculation of absorption (K) and dispersion (D) coefficients. The investigators' mathematical treatment of these data should prove valuable in interpreting bolus-response experiments.

IMPLICATIONS FOR FUTURE RESEARCH

Although measurement of ozone absorption in humans was not an objective of this study, there are a number of implications relating to the applicability of this instrumentation to future investigations with humans. These issues relate to sensitivity of the ozone analyzer, factors interfering with the ozone analysis, and configuration of the mouthpiece assembly.

There is a question of whether or not limits in the sensitivity of the ozone analyzer will compromise its ability to measure ozone in a bolus-response curve when the bolus is inspired deep into the lung airways and ozone absorption is correspondingly greater. The investigators state that the minimum detectable limit of the analyzer is 0.017 ppm ozone and that they estimate that there will be a 10:1 attenuation in ozone concentration between inspired and expired air due to absorption and mixing. This estimate is probably low, compared to the results of Gerrity and colleagues (1988). They reported that approximately 95 percent of inhaled ozone was removed by the respiratory tract of humans in exposure chamber studies, of which 40 percent was absorbed by nasal and pharyngeal (extrathoracic) tissues. Moreover, there was substantial variation among the subjects, with total ozone removal values ranging from 69 to 100 percent. This means that ozone analysis would require peak fractions of approximately 0.2 ppm ozone in the inspired bolus as the minimum condition necessary for measurement of a bolus-response curve. Without further improvements in analyzer sensitivity, human studies would be limited to relatively high exposure levels of ozone. Experiments using lower ozone doses would, therefore, be limited to studies of ozone absorption in the upper airways of the lung. It will be important, however, in future experiments to measure ozone absorption in the centriacinar region of the lung, where most of the focal damage from ozone exposure occurred in laboratory animals (Lippmann 1989).

The development and characterization of an improved fast-responding ozone analyzer, suitable for respiratory measurements of ozone in inspired and expired air in humans, have several important implications. In conjunction with the design and construction of a novel ozone bolus generator, the amount of the ozone bolus absorbed by the human respiratory tract during a single breath can be quan-

tified. Thus, the actual dose delivered to different portions of the respiratory tract can be measured on an individual basis, rather than relying on measurements of ambient concentrations of ozone in exposure chambers as a crude estimate of dose. This information can be expected to prove particularly valuable in helping to resolve questions pertaining to interindividual sensitivity to ozone exposure. There is also an opportunity to determine the actual distribution of the ozone bolus to tissues along the respiratory tract by varying the point during inspiration at which an ozone bolus is delivered. Absorption of ozone as a function of penetration depth may then be calculated. This is an important step toward the interpretation of the anatomical and physiological sequelae observed as consequences of ozone exposure. Finally, analysis of data generated by appropriate bolus inhalation studies would serve to validate and refine mathematical models presented for ozone absorption in the human respiratory tract (Miller et al. 1985).

Dr. Ultman is currently applying this instrumentation and methodology to human subjects with an HEI-funded two-year study entitled "Noninvasive Determination of Respiratory Ozone Absorption: The Bolus-Response Method." The specific aims of this study are to measure ozone absorption and distribution in the respiratory tract of healthy male subjects with the bolus-response method as a function of different expiratory and inspiratory flow rates, inspired ozone concentration, and nasal versus oral breathing patterns.

CONCLUSIONS

The investigators designed and constructed two instruments essential for the noninvasive measurement of respiratory absorption of ozone in human subjects: (1) a fast-responding ozone analyzer capable of continuous monitoring of ozone concentrations during the four-second period of a normal breath, and (2) a small-scale ozone bolus generator suitable for producing boluses of specified volume and concentration, The investigators met their major objective of developing instrumentation suitable for use in applying the bolus-response technique in humans.

The investigators substantiated their hypothesis that by using an alkene more reactive than the ethylene employed in commercially available ozone analyzers, instrument response time would be significantly reduced. This modification, coupled with aerodynamic optimization of reaction cell design and introduction of electronic filtering to maximize the signal-to-noise ratio, resulted in a markedly improved instrument design. Although analyzer response time was similar to the goal set in the study objectives, some limitations in instrument application can be expected because of higher-than-optimal sample flows and reduced sensitivity. The nonlinearity of the calibration curve below 0.03 ppm ozone will require application of appropriate correction factors.

Experiments on the effects of possible interference from variables associated with the measurement of respiratory ozone absorption indicated that temperature and humidity did not alter ozone analyzer response. However, carbon dioxide, at concentrations in the range expected in exhaled breath, increased analyzer sensitivity by 3.8 percent for every 1 percent increase in carbon dioxide concentration. Simultaneous continuous measurements of carbon dioxide may be necessary so that appropriate corrections can be applied.

The development of a small-scale ozone bolus generator, applicable to ozone absorption studies in humans, was also a unique contribution of this study.

Although measurement of respiratory absorption of ozone in humans was not an objective of this study, sufficient data were presented on instrument specifications, potential interfering factors, and mathematical treatment of ozone absorption in excised tracheas, to indicate that valuable bolus-response data also should be successfully obtained in humans.

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LIST OF HEI PUBLICATIONS

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Special Reports

Title	Publication Date
Gasoline Vapor Exposure and Human Cancer: Evaluation of Existing Scientific Information and Recommendations for Future Research	September 1985
Automotive Methanol Vapors and Human Health: An Evaluation of Existing Scientific Information and Issues for Future Research	May 1987
Gasoline Vapor Exposure and Human Cancer: Evaluation of Existing Scientific Information and Recommendations for Future Research (Supplement)	January 1988

Research Reports

Report No.	Title	Principal Investigator	Publication Date
1	Estimation of Risk of Glucose 6-Phosphate Dehydrogenase-Deficient Red Cells to Ozone and Nitrogen Dioxide	M. Amoruso	August 1985
2	Disposition and Metabolism of Free and Particle-Associated Nitropyrenes After Inhalation	J. Bond	February 1986
3	Transport of Macromolecules and Particles at Target Sites for Deposition of Air Pollutants	T. Crocker	February 1986
4	The Metabolic Activation and DNA Adducts of Dinitropyrenes	F.A. Beland	August 1986
5	An Investigation into the Effect of a Ceramic Particle Trap on the Chemical Mutagens in Diesel Exhaust	S.T. Bagley	January 1987
6	Effect of Nitrogen Dioxide, Ozone, and Peroxyacetyl Nitrate on Metabolic and Pulmonary Function	D.M. Drechsler-Parks	April 1987
7	DNA Adducts of Nitropyrene Detected by Specific Antibodies	J.D. Groopman	April 1987
8	Effects of Inhaled Nitrogen Dioxide and Diesel Exhaust on Developing Lung	J.L. Mauderly	May 1987
9	Biochemical and Metabolic Response to Nitrogen Dioxide-Induced Endothelial Injury	J.M. Patel	June 1987
10	Predictive Models for Deposition of Inhaled Diesel Exhaust Particles in Humans and Laboratory Species	C.P. Yu	July 1987
11	Effects of Ozone and Nitrogen Dioxide on Human Lung Proteinase Inhibitors	D.A. Johnson	August 1987
12	Neurotoxicity of Prenatal Carbon Monoxide Exposure	L.D. Fechter	September 1987
13	Effects of Nitrogen Dioxide on Alveolar Epithelial Barrier Properties	E.D. Crandall	October 1987
14	The Effects of Ozone and Nitrogen Dioxide on Lung Function in Healthy and Asthmatic Adolescents	J.Q. Koenig	January 1988
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16	Metabolism and Biological Effects of Nitropyrene and Related Compounds	C.M. King	February 1988

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Report No.	Title	Principal Investigator	Publication Date
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18	Respiratory Infections in Coal Miners Exposed to Nitrogen Oxides	M. Jacobsen	July 1988
19	Factors Affecting Possible Carcinogenecity of Inhaled Nitropyrene Aerosols	R.K. Wolff	August 1988
20	Modulation of Pulmonary Defense Mechanisms Against Viral and Bacterial Infections by Acute Exposures to Nitrogen Dioxide	G.J. Jakab	October 1988
21	Maximal Aerobic Capacity at Several Ambient Concentrations of Carbon Monoxide at Several Altitudes	S.M. Horvath	December 1988
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25	Acute Effects of Carbon Monoxide Exposure on Individuals with Coronary Artery Disease	HEI Multicenter CO Study Team	November 1989
26	Investigation of a Potential Cotumorigenic Effect of the Dioxides of Nitrogen and Sulfur, and of Diesel-Engine Exhaust, on the Respiratory Tract of Syrian Golden Hamsters	U. Mohr (U. Heinrich)	May 1989
27	Cardiovascular Effects of Chronic Carbon Monoxide and High-Altitude Exposure	J.J. McGrath	July 1989
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29	Early Markers of Lung Injury	J.N. Evans	September 1989
30	Influence of Experimental Pulmonary Emphysema on Toxicological Effects from Inhaled Nitrogen Dioxide and Diesel Exhaust	J.L. Mauderly	October 1989
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The Health Effects Institute (HEI) is an independent nonprofit corporation that is "organized and operated . . . to conduct, or support the conduct of, and to evaluate research and testing relating to the health effects of emissions from motor vehicles." It is organized in the following ways to pursue this purpose.

INDEPENDENCE IN GOVERNANCE

The Institute is governed by a four-member Board of Directors whose members are Archibald Cox (Chairman of the Board), Carl M. Loeb University Professor (Emeritus) at Harvard University; William O. Baker, Chairman (Emeritus) of Bell Laboratories and Chairman of the Board of Rockefeller University; Donald Kennedy, President of Stanford University; and Walter A. Rosenblith, Institute Professor (Emeritus), Massachusetts Institute of Technology.

TWO-SECTOR FINANCIAL SUPPORT

The Institute receives half of its funds from the United States government through the Environmental Protection Agency, and half from the automotive industry. Twenty-eight domestic and foreign manufacturers of vehicles or engines contribute to the Institute's budget in shares proportionate to the number of vehicles or engines that they sell in the United States.

THE HEI RESEARCH PROCESS

The Institute is structured to define, select, support, and review research that is aimed at investigating the possible health effects of mobile source emissions. Its research program is developed by the Health Research Committee, a multidisciplinary group of scientists knowledgeable about the complex problems involved in determining the health effects of mobile source emissions. The Committee seeks advice from HEI's sponsors and from other sources prior to independently determining the research priorities of the Institute.

After the Health Research Committee has defined an area of inquiry, the Institute announces to the scientific community that research proposals are being solicited on a specific topic. Applications are reviewed first for scientific quality by an appropriate expert panel. Then they are reviewed by the Health Research Committee both for quality and for relevance to HEI's mission-oriented research program. Studies recommended by the Committee undergo final evaluation by the Board of Directors, who review the merits of the study as well as the procedures, independence, and quality of the selection process.

THE HEI REVIEW PROCESS

When a study is completed, a final report authored by the investigator(s) is reviewed by the Health Review Committee. The Health Review Committee has no role either in the review of applications or in the selection of projects and investigators for funding. Members are also expert scientists representing a broad range of experience in environmental health sciences. The Committee assesses the scientific quality of each study and evaluates its contribution to unresolved scientific questions.

Each Investigator's Report is peer-reviewed, generally by a biostatistician and three outside technical reviewers chosen by the Review Committee. At one of its regularly scheduled meetings, the Review Committee discusses the Investigator's Report. The comments of the Committee and the peer reviewers are sent to the investigator, and he or she is asked to respond to those comments and, if necessary, revise the report. The Review Committee then prepares its Commentary, which includes a general background on the study, a technical evaluation of the work, a discussion of the remaining uncertainties and areas for future research, and implications of the findings for public health. After evaluation by the HEI Board of Directors, the HEI Research Report, which includes the Investigator's Report and the Review Committee's Commentary, is published in monograph form. The Research Reports are made available to the sponsors, the public, and many scientific and medical libraries, and are registered with NTIS, MEDLINE, and Chemical Abstracts.

All HEI investigators are urged to publish the results of their work in the peer-reviewed literature. The timing of the release of an HEI Research Report is tailored to ensure that it does not interfere with the journal publication process.

HEI HEALTH EFFECTS INSTITUTE 141 Portland Street, Cambridge, MA 02139 (617) 621-0266

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