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Computer-Controlled Ozone Inhalation Exposure System

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Accurate systems designed to expose laboratory animals to carefully controlled concentrations of gases and aerosols are an important tool in inhalation toxicology studies. These systems are necessary for determining the dose-response relationship of toxicants under a variety of exposure conditions. The objective of this project was to develop a system, employing feedback control, to expose small laboratory animals to precise concentrations of ozone. This system needed the capability of maintaining exposures at selected levels between 0.2 to 3.0 ppm over specified periods ranging between 1 and 8 h in order to evaluate health risks associated with ozone. The overall goals of this study were (1) to develop a system capable of automatically controlling the ozone exposure levels so the steady-state error remained less than 1% and (2) to optimize the system's response time. By employing a tuned control algorithm, gas monitors, data acquisition, and a custom computer software program, these two goals were realized.

Ozone gas is the principal air pollutant in photochemical smog found in most large cities. It can be generated in a variety of ways, such as the reaction of sunlight with fossil fuel combustion products, radiation from ultraviolet (UV) sources, and the result of electrical arcs associated with welding and high voltage sources. When ozone is inhaled, it can cause tissue injury in the pulmonary airways (Barry et al., 1985; Fujinaka et al., 1985; Harkema et al., 1993; Wagner et al., 2003) and injure the nasal airway passages of both animals and humans (Graham et al., 1988; Johnson et al., 1990; Harkema et al., 1999). In this study, a computer-controlled ozone inhalation exposure system was developed to enable investigation of the toxicity of inhaled ozone in small laboratory animals. This article focuses on the design and testing of that system.

In the past, several publications detailed the basic requirements and considerations for designing an inhalation exposure system (Raabe et al., 1973; Phalen, 1976; Austin et al., 1978; Wong, 2007). These general considerations were examined and when added to the specific requirements of the National Institute for Occupational Safety and Health (NIOSH) investigators, the following specific requirements for the ozone inhalation system were derived: (1) a leak-free exposure chamber for slight positive and negative internal pressures, (2) the ability to house up to 6 rats in individual cages, (3) a design allowing for quick and easy loading and unloading of the animals, (4) a uniform concentration at all exposure locations to ensure all animals receive the same dose, (5) the ability to expose animals to a wide range of constant ozone levels between 0.2 and 3 ppm, (6) minimization of the exposure concentration response time after the animals have been placed into the chamber, (7) minimization of overshoot in exposure concentration following the initial rise-time transient, (8) automatic control of the exposure concentration at a constant level after the initial rise transient, and (9) ample ventilation between 5 and 20 air changes per hour in order to keep the exposure chamber at room temperature and prevent the build up of CO_2 and ammonia.

SYSTEM DESCRIPTION

System Hardware

A block diagram of the ozone inhalation exposure system is shown in Figure 1. The system employed an Enmet ozone generator (model 04052-11) to produce ozone gas at a nearly steady rate. Charcoal- and HEPA-filtered room air at a flow rate of 1.2 L/min (lpm) was used as the air source for the generator. The generator produced ozone by exposing the air to a low-pressure mercury vapor lamp that provided UV radiation, which was capable of producing up to 150 ppm ozone gas. The output of the ozone generator was mixed with conditioned air before entering the exposure chamber. An ozone analyzer (API

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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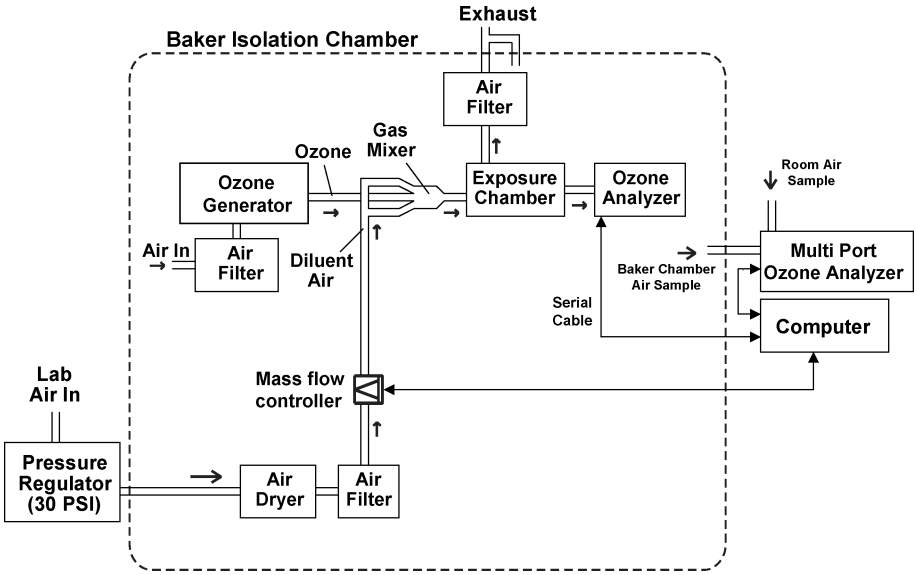

FIG. 1. Ozone exposure system diagram.

model 400A) continuously monitored the ozone level within the exposure chamber. The analyzer detected ozone based on the absorption of 254-nm UV light by ozone molecules. This instrument was equipped with a built-in calibrated ozone source that enabled the user to periodically perform a zero and span check to ensure that it was functioning correctly. The analyzer was capable of detecting ozone concentrations in a range between 0 and 10 ppm at a sample flow rate of 800 ml/min. A computer algorithm compared the ozone concentration in the exposure chamber with the desired concentration and made the appropriate adjustments to the amount of diluent air added at the mixer with a mass flow controller (Aalborg GFC37). The diluent air was supplied by a compressed air source, which passed thorough a regulator set at 30 psi, a drier containing Drierite, and finally a high-efficiency MSA air-line filter. The diluent airflow normally ranged between 7 and 25 lpm, which when added to the constant 1.2 lpm from the ozone generator supplied 8.2 to 26.2 lpm to the exposure chamber. This flow rate resulted in 6.3 to 20 air changes per hour. The custom-built exposure chamber, shown in Figure 2, was constructed of stainless steel and glass, to minimize reactions with the highly reactive ozone gas. Animals were housed in a cage rack that was partitioned into six sections and was easily moved into and out of the exposure chamber on tracks. A pan was placed directly under the cages to simplify the cleaning process following an exposure.

In order to achieve a homogeneous mixture of ozone within the exposure chamber, the ozone and clean air initially passed though a gas mixer and the mix was delivered to four evenly spaced ports on the left side of the chamber and was exhausted from four ports on the right side. The entire system was enclosed in a large isolation chamber (Baker, model AEC), which provided a protective safety barrier for the system operator during exposure periods. During an ozone exposure a second analyzer (API, model 450) was used to sample ozone levels in the isolation chamber and in the exposure room to alert personnel to any potential leaks and to provide safety records.

System Software: User Interface and System Control

The system control software was accessed through a virtual instrument display on the computer monitor as shown in Figure 3. Changes could be made to the exposure control system parameters through the interactive display. The virtual instrument also provided both graphical and numerical measurements of the system's performance. The upper central display showed the instantaneous ozone concentration inside the exposure chamber

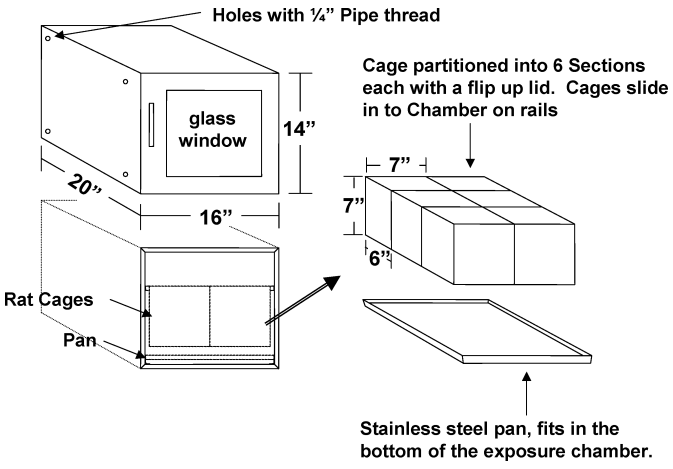

FIG. 2. Custom-built gas inhalation exposure chamber.

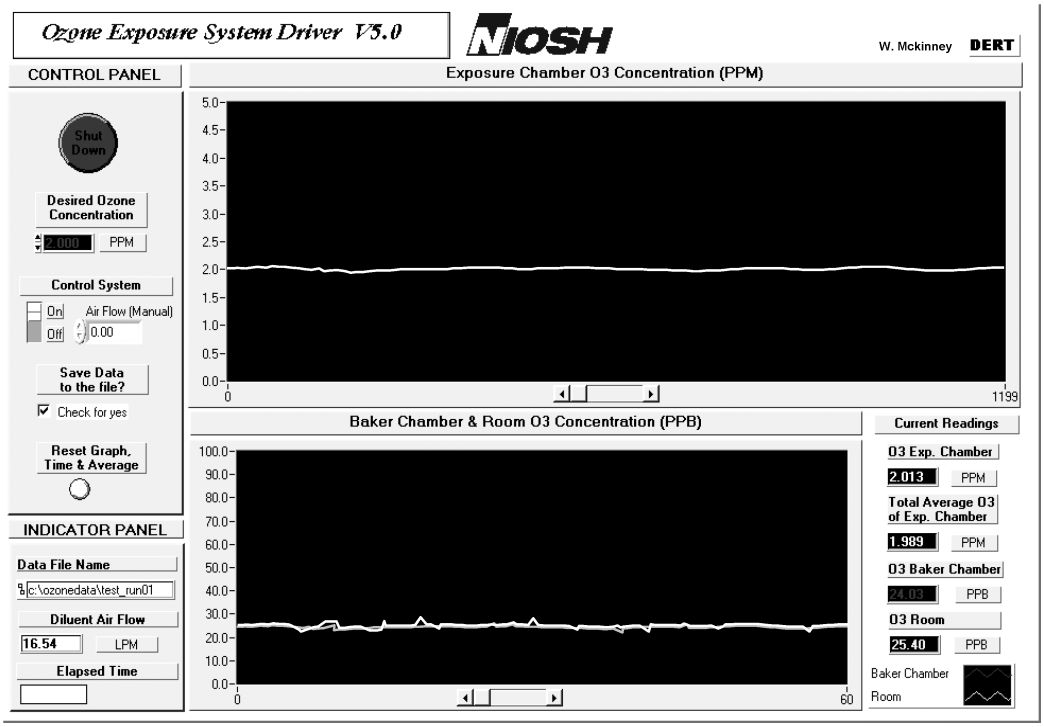

FIG. 3. Ozone exposure system user control screen.

for the previous 30-min period, while the average concentration of ozone from the beginning of the exposure was displayed numerically in the right corner of the screen. In the same area of the display the instantaneous numerical values of the ozone exposure concentration in three locations within the system were given. Those locations included the interior of the exposure chamber, the isolation chamber, and the laboratory room. The second graph in the lower middle of the display showed the instantaneous value of the ozone concentration in both the Baker isolation chamber and the exposure room over the preceding 30 min. All the graphs and numerical displays shown in the virtual instrument were updated once every second and could be stored in memory. The control panel, located in the left-hand region of the user screen, enabled the user to control the exposure system parameters. The user has the capability of stopping the exposure at any time, exporting the exposure data to a file, resetting all graphs and concentration averages, and running the system in either automatic or manual control mode.

During an exposure period, the ozone generator produced a near-constant level of ozone. In order to achieve the desired ozone exposure levels, changes were made to the amount of diluent air added to the generator output. In manual control mode, the user specified the amount of diluent air constantly mixed with the generator output. When the system was in auto control mode, the user needed only to enter the desired ozone concentration and the ozone level would be automatically adjusted by the computer. Under normal operation of the system it would be set to automatic control mode; however, if one of the critical components of the feedback system were to malfunction, the system would be placed in manual control mode. For example, should the serial port on either the computer or ozone monitor fail, the system would need to be operated in manual control mode because the automatic control algorithm would not be reciving the proper ozone levels via the serial port, leading to unpredictable behavior by the system. In this case the user would need to read the values from the ozone monitor's display and manually make the appropriate adjustments to the diluent air. Often the user will set the system to manual mode at the end or beginning of an exposure while the animals are being place or removed from the chamber. This will keep the system from maximizing its ozone output while trying to compensate for the exposure chamber door being open.

Automated Control System

When the system was running in automatic control mode, a proportional-integral-derivative (PID) control algorithm (Nise, 1995) was implemented. The ozone concentration was measured by the ozone analyzer every second. That reading was transmitted from the analyzer through the computer's serial port, where the software employed a PID control algorithm to compute, then transmit, a control signal adjustment to the mass flow controller via a 12-bit digital-to-analog converter. The control parameters for the PID controller—proportional gain, integral gain, and derivative gain—were found by standard techniques until the system responded with a minimum rise time and minimum overshoot that approximated critically damped conditions.

After these values were determined, they were hard coded into the system software.

Animals

All animals used in this project were male Sprague-Dawley [Hla:(SD) CVF] rats weighing 200–300 g (approximately 10 wk old at arrival) obtained from Hilltop Lab Animals (Scottdale, PA). The animals were housed in a AAALAC-accredited, specific-pathogen-free, environmentally controlled facility. The rats were monitored to be free of endogenous viral pathogens, parasites, mycoplasms, *Helicobacter,* and CAR bacillus. Rats were acclimated for at least 5 days before use, and were housed in ventilated cages that were provided HEPA-filtered air, with Alpha-Dri virgin cellulose chips and hardwood Beta-chips used as bedding. The rats were maintained on ProLaB 3500 diet and tap water, both of which were provided ad libitum.

METHODS FOR SYSTEM TESTING AND VALIDATION

Leak tests were performed on the ozone system to ensure the exposure chamber and all air lines were leak free. Without animals in the exposure chamber, the ozone level was set to 3 ppm during a 3-h test run. During those tests, a second ozone analyzer (API model 400) continually sampled room air and air from inside the Baker isolation chamber that enclosed the generator, air mixer, and animal exposure chamber. In the event of an ozone leak, the leak would be quickly detected in the isolation chamber with this second ozone analyzer.

Tests were conducted in order to ensure that all animals would receive the same uniform ozone levels in all regions within the exposure chamber. A multiport ozone analyzer sampled at four locations within the chamber, including the center and three corners of the chamber. A test exposure of 2 ppm concentration lasted for 2 h and the ozone levels were recorded at each location.

Several additional experiments were performed on the system to test the automated control system response. The response of an ideal exposure system is illustrated in Figure 4. The purpose of the first set of experiments, on the control system, was to monitor the rise in ozone levels at the beginning of an exposure period. In this case, the ozone level was held steady at 1 ppm, and the exposure chamber door was opened and the cage rack was placed inside with or without animals. After closing the chamber door, the ozone level was monitored and the time taken to reach 95% of the original value of 1 ppm was noted. This was repeated with and without the control system activated, and with and without animals in the cage rack.

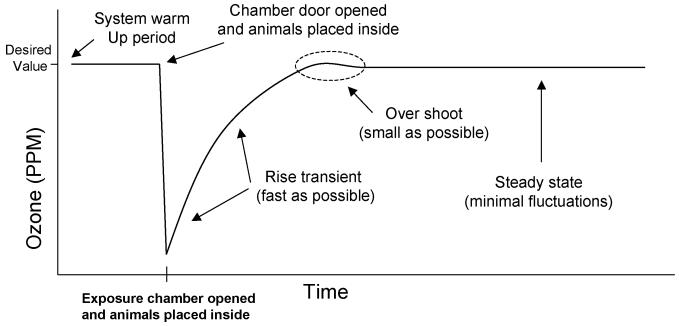

FIG. 4. System response example.

Another set of experiments was performed using the feedback control system to determine how well it could maintain the ozone concentration at a constant value following the initial rise transient. Three animals were placed in the exposure chamber, and the ozone level was allowed to level out at the desired values of either 1.5 or 2 ppm; then the chamber concentration was measured at 1-s intervals. This data was recorded and saved over 3-h periods.

To test that the normal ventilation range between 5 and 20 air changes per hour would be adequate in keeping the exposure chamber at room temperature and prevent the buildup of CO_2 and NO_x, both NO_x (API, model 200a) and CO_2 (INOVA, model 1312) monitors were used to sample air from the ozone exposure chamber exhaust lines during a 3-h test run with animals in the chamber. The temperature in the exposure chamber was also monitored during this test.

RESULTS

During the leak tests the ozone measurements taken outside the exposure chamber never increased and stayed at normal background levels, 0.05 ppm or less. This showed that the ozone gas was not leaking from the system and that the exposure system was virtually air-tight.

The results from the exposure chamber spatial uniform concentration test showed that the ozone concentration varied by less than 1% at all sampled points within the exposure chamber.

Figure 5 shows a comparison of the system transient response when the control system is activated (closed loop) versus not being activated (open loop). There are two plots showing this test with and without animals place inside the exposure chamber. The time it took to reach 95% of the desired ozone concentration, without animals in the chamber, in open loop mode was 35 min, and the time to reach 95% of the desired value in closed loop mode was 18 min. With animals in the exposure chamber the time to reach 95% of the desired ozone level was 30 min with the system in automatic control mode, and when place in open loop mode the ozone level could not reach the desired level even after 1 h of operation. Figure 5 also indicates that the overshoot in ozone concentration during the initial rise transient was virtually zero with the chosen control system parameters.

Figure 6 shows the system's ability to maintain ozone concentrations at constant levels with minimal variations following the initial rise transient. The average and standard deviation of the concentration during these 3-h test runs were 1.492 (0.56% error) $\pm$ 0.077 ppm when the target was 1.5 ppm, and 1.997 (0.13% error) $\pm$ 0.078 ppm when the target was 2.0 ppm.

To test that the system had adequate ventilation, both NO_x (API, model 200a) and CO_2 (INOVA, model 1312) monitors

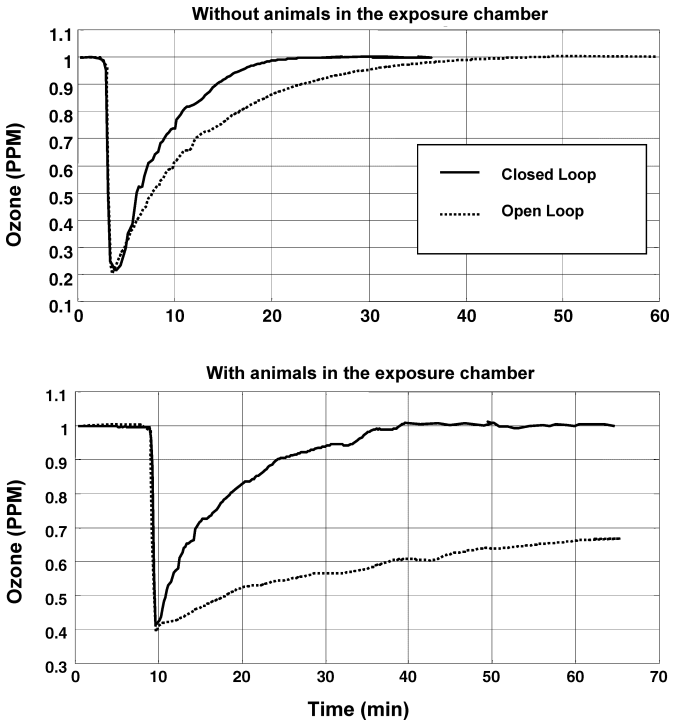

FIG. 5. System transient response, open loop versus closed loop.

were used to sample air from the ozone exposure chamber exhaust lines. They indicated that the gas levels were below normal background levels. The exposure system temperature followed the laboratory room temperature during all tests.

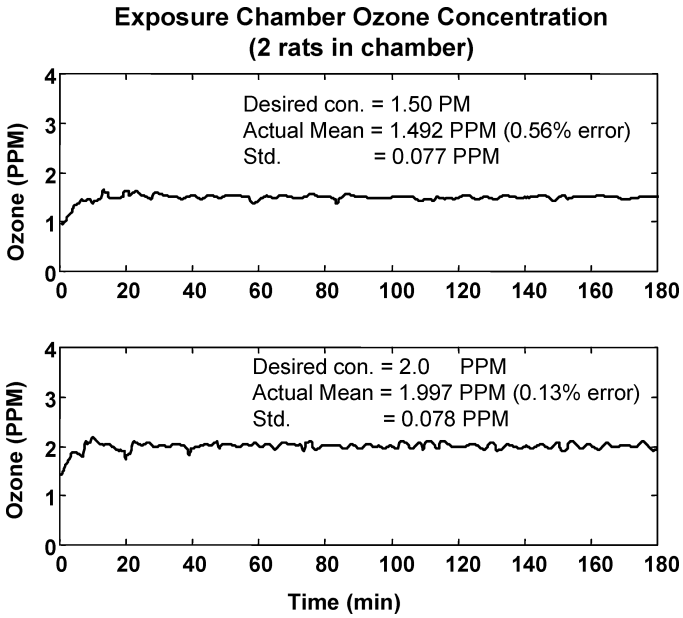

FIG. 6. System steady-state response.

DISCUSSION

A computer-controlled ozone inhalation exposure system was successfully designed, constructed and tested. All the objectives of the project were met: (1) The exposure system was air tight and leak free. As a safety matter, the regions surrounding the exposure chamber were regularly monitored with an ozone analyzer to detect potential leaks. (2) The exposure chamber was capable of housing up to six rats in individual cages. (3) The custom chamber and cage rack designs allowed for quick and easy loading and unloading of the animals. (4) Uniform ozone concentrations existed in all regions of the exposure chamber, which ensured that all animals received the same ozone exposure dose. Test results showed there was less than a 1% variation at multiple sample sites within the exposure chamber. (5) The system had the ability to expose animals to a wide variety of ozone levels ranging between 0.2 and 3 ppm. (6) The exposure system was computer controlled and the ozone levels could be maintained automatically using a PID control algorithm. The system was fine tuned to minimize the rise time of the ozone concentration after the animals were placed into the chamber. A short rise time was desired to insure that the animals were exposed to a constant concentration of ozone rather than a steadily increasing concentration during the initial portion of the exposure period. Figure 5 shows a comparison of the open loop system response and the closed loop PID response during the initial rise time transient. The time it took to reach 95% of the desired ozone concentration, without animals inside the chamber, in open loop mode was 35 min, and the time to reach 95% of the desired value in closed loop mode was 18 min. The closed loop PID algorithm cut the rise time nearly in half. When animals were place in the exposure chamber their fur and skin would react with the ozone gas. This resulted in a sink of ozone concentration. The evidence of this can be seen in Figure 5. This reaction with the ozone is why the concentration never rebounded back to 1 ppm after the animals were placed in the exposure chamber while in open loop mode. The closed loop control system was needed to compensate for this effect by removing a fraction of the diluent air that went into the mixing chamber. The rise time could be reduced even further by making the system underdamped, but then the overshoot would increase. The control system parameter values (P, I, and D) were found though standard techniques until the system had acceptable rise times with little to zero overshoot. (7) Figure 5 indicates that the overshoot in ozone concentration was virtually zero with the chosen system parameters. (8) Figure 6 shows the system could maintain ozone concentrations at constant levels with very slight variations following the initial rise transient. The ozone levels in the exposure chamber during 3-h test runs were within 1% of the desired concentrations, and the standard deviations of the concentrations were less than 5% from the mean during a 3-h test exposure. (9) The exposure chamber was ventilated with 5 to 20 air changes per hour, depending on the amount of diluent air added to the ozone–air mixture. This airflow was effective in keeping the exposure chamber

at room temperature and preventing the buildup of CO_2 and ammonia.

The goals of this project were met and validated through careful design and testing. The computer-controlled ozone exposure system can be used to expose laboratory animals to precisely controlled concentrations of ozone gas and can serve as an important tool in inhalation toxicology studies.

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