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A COMPUTER-CONTROLLED WHOLE-BODY INHALATION EXPOSURE SYSTEM FOR THE OIL DISPERSANT COREXIT EC9500A

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An automated whole-body inhalation exposure system capable of exposing 12 individually housed rats was designed to examine the potential adverse health effects of the oil dispersant COREXIT EC9500A, used extensively during the Deepwater Horizon oil spill. A computer–controlled syringe pump injected the COREXIT EC9500A into an atomizer where droplets and vapor were formed and mixed with diluent air. The aerosolized COREXIT EC9500A was passed into a customized exposure chamber where a calibrated light-scattering instrument estimated the real-time particle mass concentration of the aerosol in the chamber. Software feedback loops controlled the chamber aerosol concentration and pressure throughout each exposure. The particle size distribution of the dispersant aerosol was measured and shown to have a count median aerodynamic diameter of 285 nm with a geometric standard deviation of 1.7. The total chamber concentration (particulate + vapor) was determined using a modification of the acidified methylene blue spectrophotometric assay for anionic surfactants. Tests were conducted to show the effectiveness of closed loop control of chamber concentration and to verify chamber concentration homogeneity. Five automated 5-h animal exposures were performed that produced controlled and consistent COREXIT EC9500A concentrations (27.1 ± 2.9 mg/m^3, mean ± SD).

On April 20, 2010, the Deepwater Horizon (DH) drilling rig exploded in the Gulf of Mexico about 40 miles southeast of the Louisiana coast. Eleven workers were killed due to the explosion, and damage to the rig caused an estimated 206 million gallons of crude oil to be spilled into the Gulf (U.S. Coast Guard 2011).

Oil spills in the past have been shown to produce adverse health effects in cleanup workers (Aguilera et al. 2010). Remediation processes such as "oil burning" and the use of chemical dispersants were employed in response to oil spills. Oil dispersants were typically applied to oil slicks by spraying from boats and airplanes. Dispersants are a mixture of solvents and surfactants that reduce the interfacial tension between the water and oil, thus, facilitating the breakup of the oil into tiny droplets, which are more easily dispersed by wind and wave action (Chapman et al. 2007). During the DH response, the dispersant most widely utilized was COREXIT EC9500A (CE). It is estimated that more than 1.8 million gal of dispersant was applied to the spill, of which 1 million gal was sprayed via aerial application (U.S. Coast Guard 2011). The aerosolized CE may have been inhaled by cleanup workers and inhabitants in proximity to the sprayers

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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(National Institute for Occupational Safety and Health 2010).

The adverse health effects of inhaled CE on cleanup workers were unknown at the time of the DH oil spill and response. Small-animal inhalation exposures can be an effective method for collecting animal model data to ascertain the possible health effects of inhaled particulates and vapors on humans. There are various types of exposure systems that focus on different methods of toxicant generation and delivery (Wong 2007; Phalen 2009). In the past, our laboratory has utilized small-animal, whole-body inhalation exposure systems to determine the health effects of liquid aerosols and vapors present in various workplace environments (Hubbs et al. 2002; 2008; Shvedova et al. 2002). Recently, a proportional-derivative (PD) software concentration controller was developed and used in exposures to ozone (McKinney and Frazer 2008) and carbon nanotubes (McKinney et al. 2009). The controller has resulted in more tightly regulated concentration control and an automation level that has minimized technician interaction with the system.

The overall objective of this study was to develop a rat model that would examine the adverse health effects of inhaled CE. This study deals with the design, testing, and implementation of an inhalation exposure system for this purpose. The specific goals of this study were: (1) Develop a method to generate CE aerosol and vapor that could be delivered to individually housed animals in a whole body exposure system, (2) verify that the aerosol was of a respirable size for the animals used in the study, (3) design an exposure chamber that could achieve a homogenous concentration of CE throughout the chamber, (4) develop a method to measure the total (particulate + vapor) concentration of CE in the animal's breathing zone, (5) estimate and control the exposure concentration in real time through the use of feedback control technologies, and (6) develop software that would monitor exposure conditions, record environmental conditions, and control the system parameters as needed in an automated fashion.

METHODS

Exposure System Overview

A whole-body inhalation exposure system was designed to expose 12 rats simultaneously to the oil dispersant COREXIT EC9500A (CE; Nalco Energy Services, L.P., Sugar Land, TX), widely utilized in response to the Gulf oil spill. A diagram of the system is shown in Figure 1 and a picture of the system is shown in Figure 2.

At the beginning of each exposure a 20-ml glass syringe was filled with CE and positioned within a syringe pump (210, KD Scientific, Inc., Holliston, MA). Since CE settled in the syringe over time, a Teflon-coated stir bead was placed within the barrel of the syringe and was activated with a magnetic stirrer placed in close proximity to the syringe. Droplets of CE were aerosolized with an air-pressure-type atomizer (3076, TSI, Inc., Shoreview, MN). The pressurized supply air was dried and HEPA filtered. The syringe pump injected the CE liquid into the atomizer, which used a small orifice held at an air pressure of 35 psi to generate the particles. The airflow through the generator was 3.5 L/min. Diluent air (25 L/min) was regulated with a mass flow controller (MFC) (GFC37, Aalborg, Orangeburg, NY) and was mixed with the CE aerosol. The diluted aerosol was then introduced into a custom exposure chamber. CE aerosol leaving the exposure chamber passed through a HEPA filter and exited through an exhaust MFC attached to the house vacuum. The flow through the exhaust MFC was adjusted by the computer software to maintain a negative pressure within the exposure chamber to prevent CE from escaping into the surrounding areas. The pressure inside the chamber was measured with a Setra type 264 pressure transducer (Boxborough, MA). A personal DataRAM (pDR-1000AN, Thermo Electron Co., Franklin, MA) was used to estimate the total mass particulate concentration of aerosol in the exposure chamber based on the light-scattering characteristics of the aerosol. Values were updated through an RS-232 connection to the computer every second. The real-time values of the DataRAM were

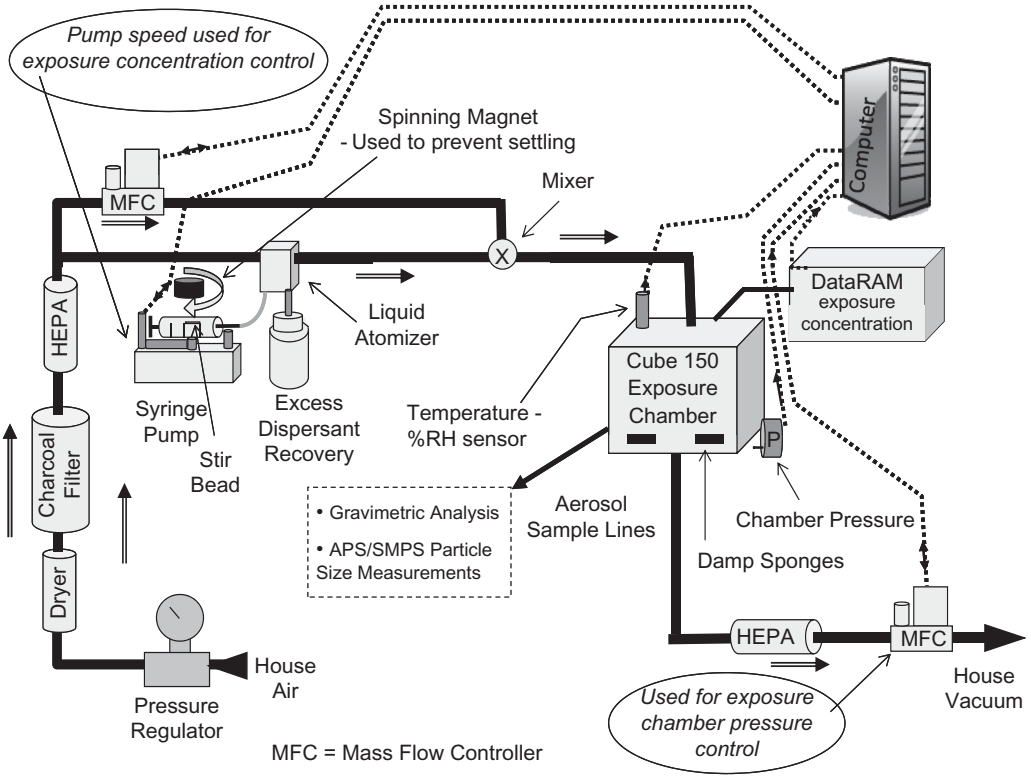

FIGURE 1. Block diagram of the inhalation exposure system used to expose rats to COREXIT EC9500A.

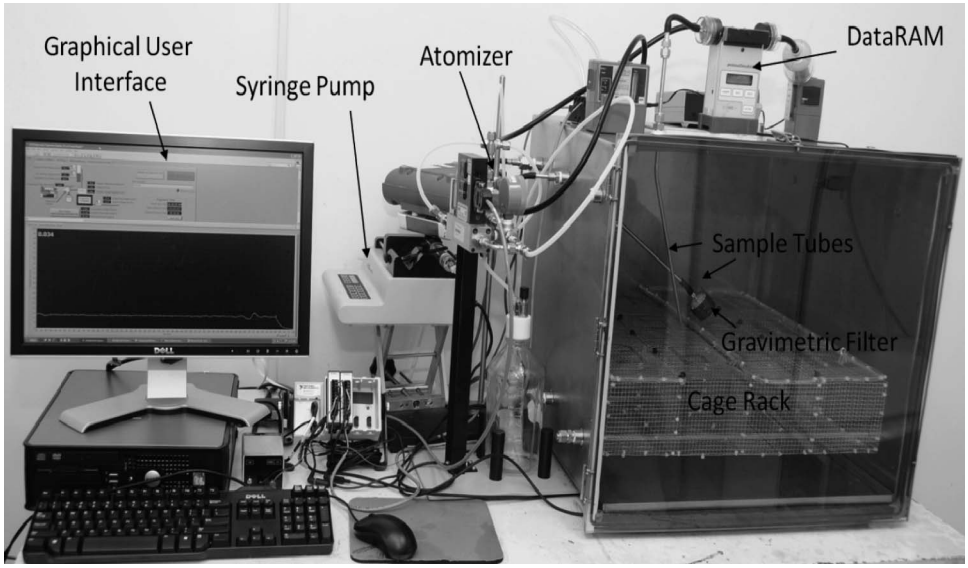

FIGURE 2. Picture of the inhalation exposure system used to expose rats to COREXIT EC9500A.

calibrated by making simultaneous gravimetric measurements during exposure tests and runs. The PD controllers were tuned to optimally adjust the exhaust MFC and the syringe speed to control the chamber pressure and chamber concentration, respectively. Damp sponges were placed in the bottom of the chamber to provide humidity throughout the

exposure. Temperature and relative humidity were continually recorded during an exposure period with a Vaisala HMP60 humidity and temperature probe (Helsinki, Finland). Analog input and output signals were processed with a National Instruments multifunction data acquisition board (PCI-6229, National Instruments, Austin, TX). Software was developed using LabVIEW (National Instruments, Austin, TX), which displayed exposure information, saved pertinent data, and controlled system parameters.

Exposure Chamber Design

A 150-L whole-body exposure system (Cube 150) was designed and constructed out of stainless steel and Plexiglas for the oil dispersant exposures. A schematic of the system is shown in Figure 3. The chamber measured 22 × 22 × 20 inches. The hinged front door of the chamber was constructed of ½-inch-thick Plexiglas. The remainder of the chamber was constructed with 0.0625-inch-thick type 304 stainless steel. Three stainless-steel 3/8-inch tubes functioned as both cage supports and exhaust ventilators. A stainless-steel cage rack, consisting of 12 individual wire mesh cages, each 5 × 7 × 3 inches, sat on the supports and a stainless-steel pan was placed in the bottom of the chamber to collect animal waste. Exhaust holes were drilled in the bottom of the support tubes under the center of each cage to direct the flow of CE to each of the animals to be exposed. CE aerosol and vapor entered the chamber from the top center of the chamber and exited from the 12 exhaust holes. Sampling ports in the chamber permitted the measurement of temperature, relative humidity, and chamber pressure in addition to gravimetric and DataRAM measurements of CE concentration.

System Software

In order to automate the exposure process for lab technicians, a software graphical user interface (GUI) was developed. The GUI appeared as a "virtual instrument" that allowed users to view environmental conditions, change system variables, and record pertinent information.

The GUI contained multiple "tabs" that could be accessed to display additional system

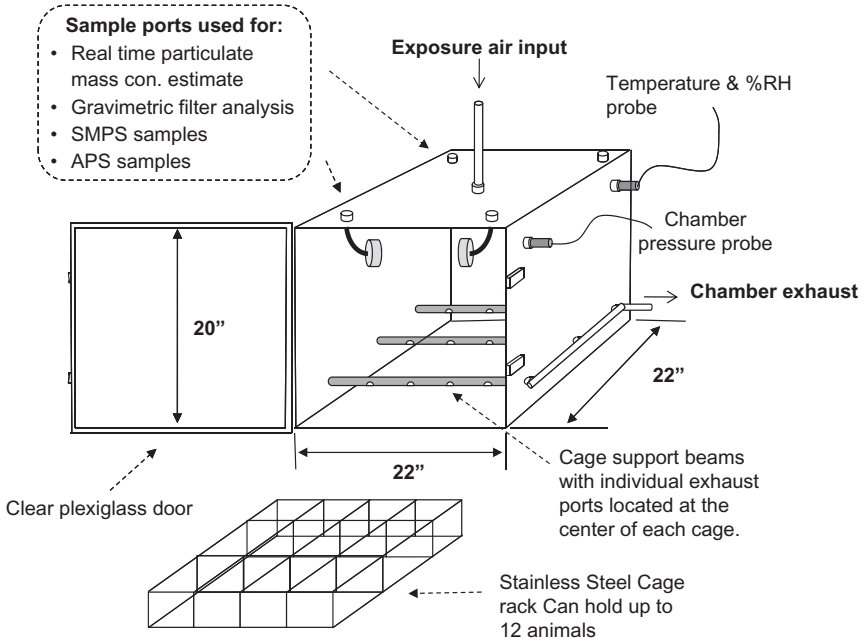

FIGURE 3. Illustration of the custom exposure chamber (Cube 150) that housed the rats during inhalation exposures to COREXIT EC9500A.

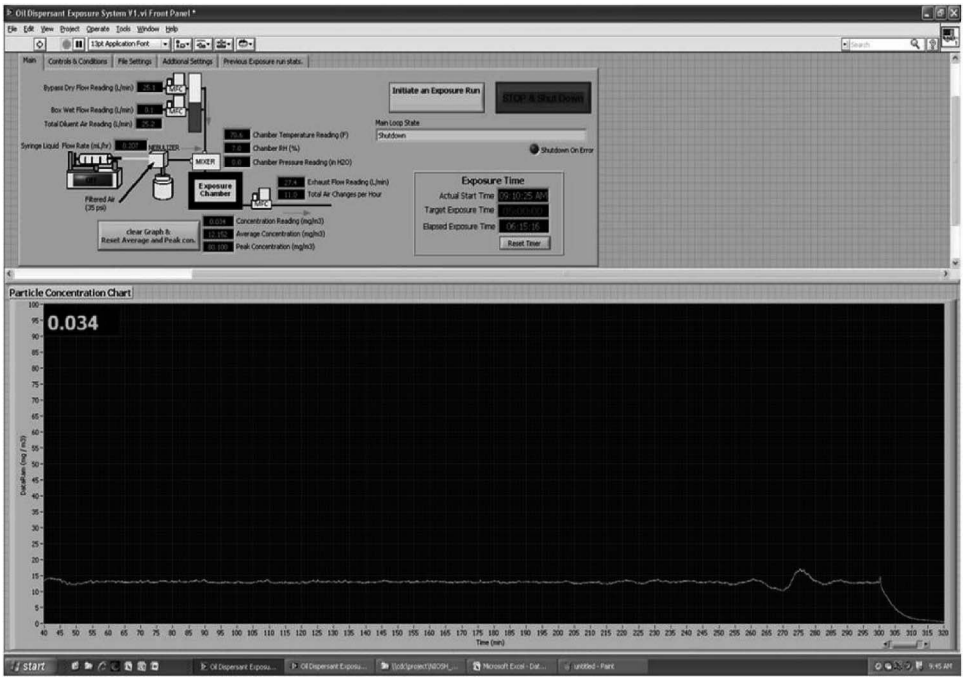

FIGURE 4. "Main" tab of the software graphical user interface used to acquire, record, and control system parameters during an inhalation exposure.

performance information. The "Main" tab (shown in Figure 4) displayed a schematic of the exposure system with the real-time readings from various instruments and also displayed the average particulate concentration and elapsed exposure time. Control buttons on this tab permitted the user to begin or prematurely end an exposure. The "Controls and Conditions" tab displayed real-time instrument readings, and controls were accessible to manually change the set points for the variables these instruments were monitoring. Variables displayed included particle concentration, chamber pressure, relative humidity, chamber temperature, diluent airflow, exhaust flow, and syringe pump flow. The ability to plot select variables versus time was also implemented on this tab. The "File Settings" tab permitted the user to change the data file name and structure. The "Additional Settings" tab allowed the user to change system settings such as syringe diameter, DataRAM gain, and pressure transducer zero offset. The "Previous Exposure Run Stats" recorded prior exposure concentration data that was used to calibrate the DataRAM for future studies.

A PD control algorithm, similar to that described in McKinney et al. (2009), was included in the software. The first feedback loop regulated the CE particulate concentration within the exposure chamber. The PD controller adjusted the speed of the syringe pump based on the real-time concentration estimations provided by the DataRAM. In this study, a target particulate concentration of 15 mg/m^3 was used for all exposures and the system was tuned to optimally achieve this value. A second feedback loop altered the exhaust flow based on the readings from the pressure transducer in order to hold the chamber at a slightly negative pressure (–0.05 inches H_2O). This was undertaken to ensure that any leaks in the exposure chamber would pull surrounding air into the chamber instead of releasing CE into the room environment around the chamber where technicians could potentially be exposed to CE.

To maximize the automation of the exposure system for technician use, a virtual button labeled "Initiate an Exposure Run" could be

accessed on the "Main" tab. This button served many functions. Initially, the syringe pump was activated and set to a high rate (5 ml/h) to confirm that the fluid quickly reached the atomizer and accelerate the concentration rise time. After the DataRAM detected a mass concentration of 0.5 mg/m^3, the PD control algorithm was activated. The controller regulated the syringe rate throughout the rest of the exposure to achieve an aerosol concentration of 15 mg/m^3. Actuation of the "Initiate" button also: (1) queried the user to specify a data file name, the target CE particulate concentration and exposure time length, (2) reset the graph and exposure averages, (3) activated the PD controller to regulate pressure, and (4) initiated recording of data. After the exposure time period had completed, the software disabled the syringe pump and continued to monitor the CE particulate concentration within the chamber. Once the concentration had dropped to a safe level for animal removal from the chamber, an alert was issued to signal the technicians. At this point, the total elapsed time and average concentration were noted for calibration purposes, data recording was ended, and the animals were removed from the chamber.

Aerosol Measurements

Two ¼-inch stainless-steel tubes were passed through individual sample ports in the top of the chamber. The distal ends of the tubes were positioned directly above the center of the cage rack in order to sample the CE concentration within the chamber. The opposite ends of the tubes could be attached to the DataRAM input, sample pumps, or particle sizers, depending on the tests being conducted. All gravimetric tests were conducted by pulling air through 37-mm Telfon filters in closed-faced cassettes. The cassettes were attached to the distal end of one of the tubes directly over the animals' breathing zone.

During test runs of the system, the DataRAM was calibrated by taking its average reading over an exposure period and comparing it to gravimetric filter measurements made over the same time period. Test runs were conducted with the target CE particulate concentration (15 mg/m^3) used during exposures. Multiple samples were used to establish a DataRAM calibration factor of 0.50 for the CE aerosol.

In order to verify that each animal would be exposed to a comparable concentration of CE in their breathing zones, tests were conducted to examine the spatial homogeneity of the aerosol distribution within the exposure chamber. Gravimetric filter measurements were made at various locations within the chamber. One filter was placed on the sampling tube directly above the center of the cage rack and was referred to as the "reference sample." Eight additional sampling pumps, set with flow rates of 0.2 L/min, were used for each experiment. The pumps and filters were distributed throughout the chamber in separate cages. Pump placement was altered for each test run to gain an understanding of the CE concentration within each cage as it related to the others. Average normalized values were calculated and compared for each of the cages and the reference sample.

Particle size measurements were collected from the "reference" sample tube. Test runs were conducted for the exposure system, and the calibrated DataRAM was used to ensure the CE particle concentration was at the steady-state exposure level of 15 mg/m^3. An aerodynamic particle sizer (APS; TSI, Inc., Shoreview, MN) and a scanning mobility particle sizer (SMPS; TSI, Inc., Shoreview, MN) were used to determine the size distribution of the CE aerosol within the exposure chamber. The SMPS measured aerosols between 15 and 660 nm, while the APS measured those between 660 nm and 20 μm. Number distributions were converted to mass distributions assuming spherical particles with a density of 0.91 (obtained at room temperature, gravimetrically).

Measurement of Chamber COREXIT EC9500A Concentration During Exposures

Due to the potential volatility of the CE aerosol, an analysis was performed to examine

the amount of CE that would appear on filters before and after evaporation. Four 37-mm Teflon filters were spiked with approximately 1 mg CE. The weight change of the filter was measured directly after spiking. After weighing, the filters were placed in a desiccant chamber overnight to facilitate evaporation. The filters were then reweighed to determine the percent CE remaining on the filters. Analysis of these filters indicated significant evaporation had occurred. Since gravimetric analysis would only provide the nonevaporated portion of the CE concentration, it was determined that an additional assay would be required to establish the total (particulate + vapor) CE concentration present during exposures.

During each exposure, gravimetric samples were collected during the entire exposure period of approximately 5 h with a filter flow rate of 0.2 L/min. The particulate concentration for each exposure was based on the gravimetric analysis. After weighing, the filters were then placed back in the cassette and resealed. Those filters and subsequent filter extracts were then stored at 4°C. In subsequent analyses, filters were removed from the cassettes and extracted in 5 ml distilled/deionized (DI) water/filter by sonication at room temperature for 30 min. CE mass was assessed by analysis of dioctyl sulfosuccinate sodium salt (DOS; Sigma Chemical, St. Louis, MO) using a modification of the acidified methylene blue spectrophotometric method for anionic surfactants (Koga et al. 1999). Filter extracts were diluted 1:10 with DI water, and 1 ml diluted extract was transferred to a 15-ml silanated glass centrifuge tube. One hundred microliters of 1 m*M* methylene blue (Sigma Chemical, St. Louis, MO) acidified in 117 m*M* H_2SO_4 was added, followed by 2 ml chloroform. The tube was capped, shaken vigorously for 1 min, and centrifuged at 120 × g for 1 min at room temperature. One milliliter of the chloroform layer containing the methylene blue–DOS complex was transferred to a cuvette, capped to prevent evaporation, and read immediately at 654 nm on the spectrophotometer. CE standards (130 nl to 4 nl/ml DI water) and a DI water blank were run concurrently. The volume of CE/filter was extrapolated from the standard plot and converted to total concentration (mg/m^3) by multiplying by the density of 0.91 g/ml and the volume of air sampled.

Animals

Male Sprague-Dawley rats (Hla: SD CVF, 8–10 weeks old) were obtained from Hilltop Labs (Scottdale, PA). The animals were housed in the AAALAC-approved National Institute for Occupational Safety and Health (NIOSH) Animal Facility (12-h light/dark cycle; 20–25°C), with food and water available ad libitum. Rats were acclimated to the facilities for 1 wk prior to exposures. The NIOSH Animal Care and Use Committee approved all experimental procedures followed in this study.

COREXIT EC9500A Inhalation Exposures

In order to determine the health effects of CE, five acute exposures were conducted with the CE inhalation exposure system. Up to 12 rats were exposed to a target concentration of 15 mg/m^3 particulate (27 mg/m^3 total) for 5 h. Each rat was exposed only once and was sacrificed at either 1 or 7 d post-exposure. Collaborating investigators examined the health effects on the circulatory, respiratory, and central nervous systems of the animals. Exposure goals included: (1) a rapid rise and fall time of the CE concentration, (2) a constant value of 15 mg/m^3 particulate concentration after the steady-state value had been reached, and (3) temperature (20–23°C) and humidity (30–70% RH) readings within comfortable (Institute for Laboratory Animal Research 2011) limits throughout each exposure. Rise time (time to reach 90% of the steady-state target particulate concentration value from the beginning of the exposure), fall time (time to reach 10% of steady-state target particulate concentration value after the syringe pump was turned off), and average concentrations were determined for each exposure. An open loop (no concentration feedback) test exposure was recorded and compared to the closed-loop exposures to illustrate the benefit

of concentration feedback in exposure systems. Five matched control exposures were also conducted with rats under similar environmental conditions with no CE present.

RESULTS

Aerosol Measurements

Analysis of the gravimetric filters used during chamber homogeneity tests verified that the aerosol concentration was in fact uniform in each animal cage and at the reference position (Figure 5). Averaged normalized cage concentration values varied between 2 and 4% from the mean for all cages and the reference. The concentration of the aerosol at the reference position was within 1% of the mean. No significant differences were observed for any of the cages or the reference sample.

Particle size measurements of the CE aerosol were determined with APS and SMPS aerosol analyzers. The particles were assumed to be described by lognormal particle size distributions. When data for the two devices were merged and the size distribution was calculated by a standard graphical method (Baron and Willeke 2001), the CE aerosols had a count median aerodynamic diameter of 285 nm with a geometric standard deviation (GSD) of 1.7 (Figure 6). The mass median aerodynamic diameter estimation for this merged distribution was 655 nm with a GSD of 1.7.

Measurement of Chamber COREXIT EC9500A Concentration During Exposures

Gravimetric filters were analyzed to determine the CE particulate exposure concentration. A modification of the acidified methylene blue spectrophotometric assay for anionic surfactants was applied to the filters to establish the total CE concentration during the exposures. The results of the filter measurements are shown in Table 1. The CE particulate concentration ranged from 14.62 to 15.33 mg/m^3 with a mean of 14.95 mg/m^3. The total CE concentration

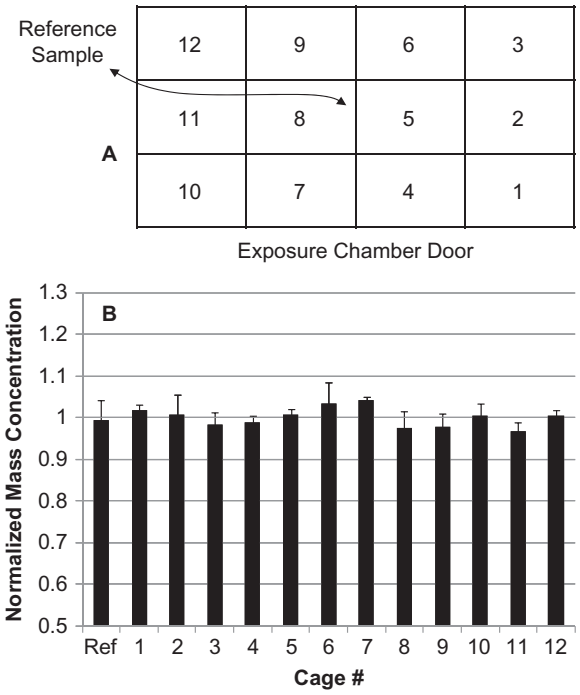

FIGURE 5. Results of chamber aerosol concentration homogeneity: (A) cage number positions within the exposure chamber and (B) the normalized average concentrations from gravimetric filter samples from four test runs. Data are shown for each cage and the reference sample that pulled COREXIT EC9500A aerosol from directly above the center of the cage rack. Error bars represent standard errors.

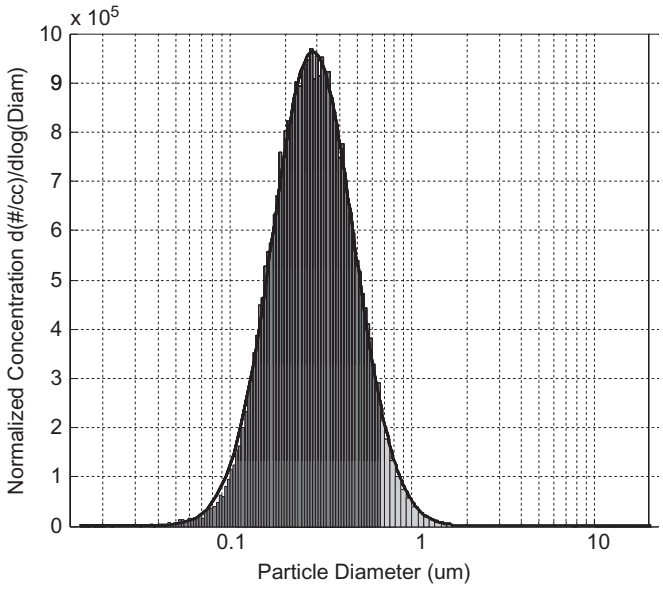

FIGURE 6. Particle size distribution of the aerosol (in terms of counts) in the breathing zone of the rats during the COREXIT EC9500A inhalation exposures as measured with an aerodynamic particle sizer (APS) and a scanning mobility particle sizer (SMPS). The dark line on the graph represents a lognormal distribution with a count median aerodynamic diameter of 285 nm and geometric standard deviation of 1.7.

TABLE 1. Results From Gravimetric Filters Used to Sample COREXIT EC9500A (CE) in the Breathing Zone of the Animals During Inhalation Exposures

Exposure Filter #	Pre-Weight (mg)	Post-Weight (mg)	Air Volume (L)	CE Particulate Conc. (mg/m^3)	Total CE Conc. (mg/m^3)	Gravimetric/ Total Corexit
1	49.873	50.806	63.2	14.76	28.08	0.53
2	50.107	51.074	63.2	15.30	30.06	0.51
3	49.231	50.212	64.0	15.33	22.43	0.68
4	48.590	49.511	63.0	14.62	26.34	0.56
5	54.790	55.719	63.0	14.75	28.66	0.51
Average				**14.95**	**27.11**	**0.56**
SE				**0.15**	**1.31**	**0.03**

Note. A calibrated sample pump (0.2 L/min) pulled air through 37-mm Teflon filters in closed-face cassettes to collect the samples. Particulate concentration was determined based on the weight change of the filters, and total CE concentration was evaluated with a modification of the acidified methylene blue spectrophotometric assay for anionic surfactants.

ranged from 22.43 to 30.06 mg/m^3 with a mean of 27.11 mg/m^3. On average, the particulate concentration was 56% of the total concentration. This matched the results seen with the spiked filter experiments. Each of the 4 spiked filters retained 56% of their initial weight after overnight desiccation.

COREXIT EC9500A Inhalation Exposures

Five inhalation exposures to CE were conducted. Each exposure lasted for approximately 5 h, and up to 12 rats were exposed at one time. An example of the real-time particulate concentration measured during an exposure is shown in Figure 7. Average steady-state DataRAM concentrations for each exposure ranged from 15 to 15.1 mg/m^3. The rise times varied from 4.7 to 9.8 min with a mean of 6.7 min. The fall times varied from 8.2 to 10.3 min with a mean of 9.5 min. Average temperatures ranged from 20.9 to 23.0°C and average relative humidities ranged from 52.3 to 63.6%. The aerosol

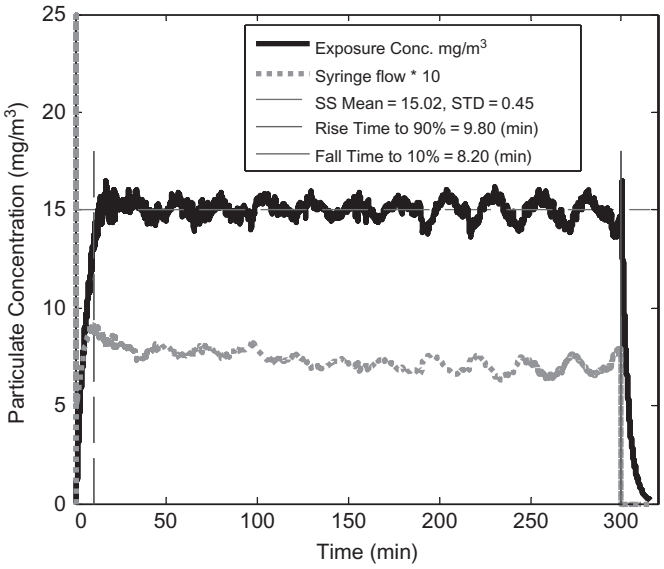

FIGURE 7. Example of the real-time particulate concentration during a COREXIT EC9500A inhalation exposure. The syringe flow, which is regulated by the feedback loop that controls the concentration, is also shown and multiplied by 10 for display purposes. SS Mean and STD refer to the steady state mean and standard deviation of the concentration. The SS is defined as the concentration values between when the concentration reaches 90% of its target value and when the syringe pump is turned off at the 5-h point in the exposure.

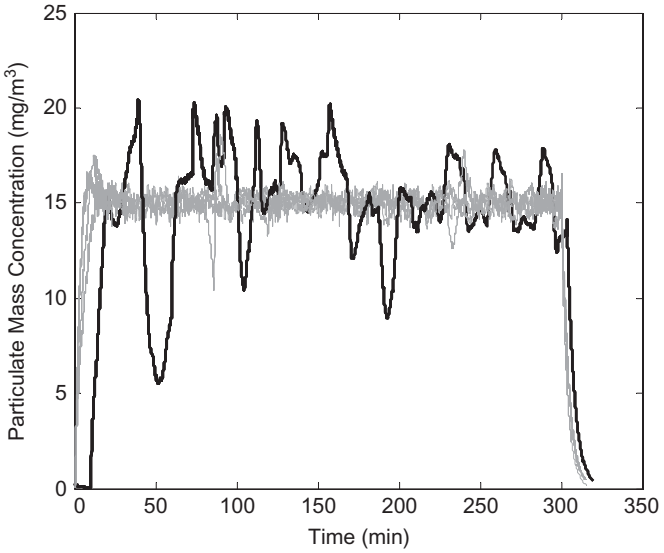

FIGURE 8. Real-time particulate concentrations during the 5-h COREXIT EC9500A inhalation exposures (where feedback was utilized, illustrated with the lighter lines), along with a test run initiated without feedback control (indicated with the dark line). Notice that a much more consistent concentration throughout each exposure is achieved with the addition of feedback control.

concentration versus time plots for the closed-loop exposures, along with a test run with no feedback, are shown in Figure 8. Faster rise times and a more consistent steady-state concentration are visually apparent when feedback is utilized.

DISCUSSION

The results of this study provided an automated inhalation exposure system for the oil dispersant COREXIT EC9500A. The system was constructed, tested, and implemented. Five

animal exposures were conducted to examine the acute adverse health effects of inhaled CE.

Initial evaporation tests with gravimetric filters demonstrated the volatility of the CE liquid. These tests showed that 56% of the initial weight of spiked filters remained after desiccation. This could have been due to either part of the CE being volatilized after impacting on the sampling filter material or elements of the CE liquid volatilizing as aerosols were being created by the atomizer. In order to make sure the vapor phase of CE was not ignored, it was determined that an additional assay would be required to measure the "total" amount of CE. The modified acidified methylene blue assay estimated the amount found on the gravimetric filter to be 56% of the total amount of CE within the chamber during exposures. This value was identical to the spiked filter experiments, which further verified that 44% of the CE volatilized, but additional study is required to answer the question of how much of the CE vaporized before impacting on the filter.

The uniformity of distribution in the concentration of aerosols within a whole-body exposure chamber needs to be verified prior to the start of inhalation exposures (Wong 2007). Environmental conditions, such as air currents, particle momentum, and poor mixing, may produce regions of higher aerosol concentration within a chamber. Disparities of the aerosol concentrations in the breathing zones of animals may produce different doses to be delivered to animals within the same exposure. The design of the Cube 150 exposure chamber included placement of separate exhaust ports under each cage to ensure that aerosols were distributed uniformly to each animal. Chamber homogeneity tests for the CE exposure system established uniform aerosol concentration throughout the Cube 150 (normalized concentration values for each cage and the reference measurement were between 0.98 and 1.04). Though these uniformity results were satisfactory for the CE aerosol, new tests needs to be conducted for each new aerosol generated. The relatively small size of the CE aerosols (mass peak at 655 nm) compared to aerosols typically used in inhalation studies (1–4 μm) (Wong 2007) may have contributed to the homogeneity of the aerosols throughout the exposure chamber.

Particle size distribution measurements indicated a lognormal distribution of CE aerosols with a count peak centered at approximately 0.29 μm and a mass peak centered around 0.66 μm. Particle deposition experiments carried out by Raabe et al. (1988) in rats showed that particles with aerodynamic diameters of 0.29 and 1.02 μm were deposited in both the tracheobronchial and alveolar regions. Though the deposition efficiency between these two regions is not well understood in rats, a straight-line interpolation of the Raabe et al. (1988) deposition efficiency measurements indicates that the CE aerosols in this study were respirable and would likely be deposited in both the tracheobronchial and alveolar regions. It is noteworthy that the size distribution of aerosols that resulted from spraying during the DH response may have been considerably different. In addition, the aerosol deposition relationship for humans is different from that for rats, and this needs to be taken into account when estimating and comparing deposited doses.

An exposure chamber environment that provides adequate fresh air, delivers a consistent user-defined dose of potential toxin, removes waste product gases, and keeps the animal comfortable in terms of temperature and relative humidity is essential when conducting laboratory inhalation exposures. The airflow through the chamber during exposures in this study was 28.5 L/min. This corresponded to air change rate of 11.4 changes/h. When coupled with the fact that average temperature and relative humidity values during the exposures were within the comfort levels for rats (Institute for Laboratory Animal Research 2011), a healthy environment for the rats, minus the toxin, was achieved.

Due to generator instabilities and transport losses, consistent exposure chamber concentrations with aerosols can be notoriously difficult to attain (Wong 2007). The addition of feedback controllers to maintain stable exposure levels in these systems is critical to achieving

a constant steady-state dose. Feedback control may also reduce the rise and fall time periods during an exposure. A modified controller was designed that was based on a system described by McKinney et al. (2009). As can be seen from Figure 8, the steady-state value of the particulate concentration was much more stable when feedback control was implemented. The total CE concentration, which included a combination of a particulate and vapor phase, ranged from 22.5 to 30.1 mg/m^3. Steady-state DataRAM averages for each of the five exposures varied over an extremely tight range (15–15.1 mg/m^3). Rise times were generally reduced to less than 10 min. The "five-hour" exposure began when the syringe pump began to inject aerosol into the atomizer. After 5 h the syringe pump stopped, the diluent air was increased, and the animals were exposed to lesser concentrations of CE until the level was safe to remove the animals from the exposure chamber. The total exposure lasted approximately 315 min. Because the concentration was going up at the beginning and down at the end of the exposure, concentration levels during these periods were lower than target values. The extra time (approximately 25 min) at these "lower" concentrations were adjusted so that the exposures were equivalent to 5-h exposures at the steady-state level.

The rats from the 5 inhalation exposures (along with controls) were sacrificed 1 or 7 d postexposure, and different organ systems in the rats were examined. The acute health effects observed for rats exposed to CE are examined in the companion articles described in this issue. These results showed a transient increase in heart rate and blood pressure, reduction in vascular responsiveness to vasodilating factors (Krajnak et al. 2011), upregulation of the expression of the voltage-gated calcium channel Cav1.3, altered synaptic and cytoskeletal protein content in discrete brain areas (Sriram et al. 2011), and negligible lung inflammation or injury (Roberts et al. 2011).

In summary, the objective of this study was to produce an inhalation exposure system to study the adverse health effects of inhaled CE. The system was capable of delivering a consistent respirable CE aerosol that was uniform throughout the exposure chamber. An assay was developed to measure the total CE concentration within the chamber with the use of typical gravimetric filter samples. Feedback controllers ensured a tightly controlled steady-state concentration with quick rise and fall times. Software was developed to provide an interface between the exposure and technicians to automate all aspects of the exposures. The system has been used successfully to examine the acute health effects of inhaled CE in small laboratory animals.

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