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PULMONARY EFFECTS AFTER ACUTE INHALATION OF OIL DISPERSANT (COREXIT EC9500A) IN RATS

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COREXIT EC9500A (COREXIT) was used to disperse crude oil during the 2010 Deepwater Horizon oil spill. While the environmental impact of COREXIT has been examined, the pulmonary effects are unknown. Investigations were undertaken to determine whether inhaled COREXIT elicits airway inflammation, alters pulmonary function or airway reactivity, or exerts pharmacological effects. Male rats were exposed to COREXIT (mean 27 mg/m^3, 5 h). Bronchoalveolar lavage was performed on d 1 and 7 postexposure. Lactate dehydrogenase (LDH) and albumin were measured as indices of lung injury; macrophages, neutrophils, lymphocytes, and eosinophils were quantified to evaluate inflammation; and oxidant production by macrophages and neutrophils was measured. There were no significant effects of COREXIT on LDH, albumin, inflammatory cell levels or oxidant production at either time point. In conscious animals, neither breathing frequency nor specific airway resistance were altered at 1 hr, 1 d and 7 d postexposure. Airway resistance responses to methacholine (MCh) aerosol in anesthetized animals were unaffected at 1 and 7 d postexposure, while dynamic compliance responses were decreased after 1 d but not 7 d. In tracheal strips, in the presence or absence of MCh, low concentrations of COREXIT (0.001% v/v) elicited relaxation; contraction occurred at 0.003–0.1% v/v. In isolated, perfused trachea, intraluminally applied COREXIT produced similar effects but at higher concentrations. COREXIT inhibited neurogenic contractile responses of strips to electrical field stimulation. Our findings suggest that COREXIT inhalation did not initiate lung inflammation, but may transiently increase the difficulty of breathing.

In response to the Deepwater Horizon oil spill, 1.8 million gal of dispersant, primarily COREXIT EC9500A (COREXIT), was used to disperse oil and prevent shoreline contamination, amounting to the greatest volume of dispersant used in an oil spill response. Approximately 1 million gal of the dispersant was delivered to the surface via aerial application, while the remainder was delivered subsea (U.S. Coast Guard 2011). Aerial application and wave action at the sea surface may generate aerosols which may increase the potential for pulmonary exposure to dispersant and respiratory symptoms

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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in cleanup and recovery workers (NIOSH 2010). Although in vitro cytotoxicity (Judson et al. 2010), toxicity in aquatic organisms and ecosystems (NRC 2005; George-Ares and Clark 2000; Coastal Response Research Center 2010), and gastrointestinal effects (George et al. 2001) of dispersants have been studied, little information exists on the potential adverse pulmonary effects of inhaled COREXIT in mammals. On the other hand, various components of COREXIT, such as propylene glycol (PG), organic sulfonic acid salts, and hydro-treated light petroleum distillates (HLPD), are known to alter lung function, produce pulmonary irritation, enhance and induce bronchoconstriction, and produce pulmonary edema and lung injury. In some instances, inhalation of components in COREXIT in an occupational setting might result in neurological, cardiovascular, hematological, and immunological toxicity (ATSDR 1995; 1997; 1999; 2008; NALCO 2010).

PG, a synthetic substance that absorbs water, is frequently used as a solvent and/or deicing component in numerous products (ATSDR 1997), and accounts for approximately 1–5% w/w of COREXIT (NALCO 2010). PG is classified as an irritant and is a known allergen. Few data on adverse pulmonary effects in humans after inhalation of PG are available. Mists of PG are reported to possibly induce respiratory-tract irritation (Dow Chemical 2003; 2006). Animal studies on pulmonary effects associated with PG inhalation are also limited (ATSDR 1997; 2008). Some effects related to reproduction (Carney et al. 1999) and hematology (Robertson et al. 1947; Suber et al. 1989) were noted after inhalation of PG, while effects on the respiratory tract were not considered severe (ATSDR 1997). Acute exposure in rabbits at 10% concentrations was shown to result in degenerative effects on goblet cells in the trachea (Konradova et al. 1978). A 90-d inhalation study in rats demonstrated nasal hemorrhaging at 51 ppm, and goblet-cell hypertrophy and epithelial thickening at concentrations of 321 ppm. Chronic studies (13–18 mo exposure) in rhesus monkeys and rats revealed no adverse effects on pulmonary parameters at a dose of 112 ppm (Robertson et al. 1947).

Dioctyl sodium sulfosuccinate (DSS), the primary organic sulfonic acid salt in COREXIT, accounting for 10–30% w/w (NALCO 2010), is the anionic detergent/surfactant involved in the oil dispersion process and the formation of micelles containing oil, which allow mixing with water (NRC 2010). DSS (1% solution) administered as an aerosol to dogs was found to enhance pulmonary extravascular water volume (an index of pulmonary edema), alter surfactant activity, and increase alveolar surface tension without disrupting membrane permeability (Bredenberg et al. 1986; Nieman and Bredenberg 1985). A 5% solution of DSS administered to rats acutely via aerosol delivery was also shown to produce pulmonary edema (Rao and Das 1994). DSS at concentrations ranging from 0.25 to 5% has been used as a model agent for inducing surfactant dysfunction in rabbits (Dahlbach et al. 2002; Evander et al. 1988; John et al. 1994; Taskar et al. 1997; Wollmer et al. 2000), and acute lung injury/acute respiratory distress syndrome in pigs (Bitzen et al. 2006), to investigate effects on lung function parameters and alveolar transport processes.

Hydro-treated light petroleum distillates (HLPD) account for approximately 10–30% w/w of COREXIT (NALCO 2010). The light petroleum distillates (LPD) were added to the COREXIT mixture in place of the solvent 2-butoxyethanol, a toxic component in the formulation of a related dispersant, COREXIT 9527 (NRC 2005), use of which was discontinued early on in the Deepwater Horizon incident. LPD are derived from crude oil that has been separated into different hydrocarbon fractions based on boiling points. The fractions contain mixtures of aliphatic and aromatic petroleum hydrocarbons, and may contain small amounts of nitrogen, sulfur, and other elements as additives (ATSDR 1999). Hydrotreating LPD removes contaminants (sulfur, nitrogen, metals) and saturates aromatics. NRC (2005) describes the HLPD in COREXIT as mixture of aliphatic hydrocarbons of chain length from 9 to 16.

There are few data on pulmonary effects associated with the aliphatic hydrocarbon mixture used in COREXIT EC9500A. Ritchie et al. (2003) and ATSDR (1999) reviewed the literature available on pulmonary toxicity of fuel mixtures that contain this hydrocarbon chain fraction, including kerosene and kerosene-based jet fuels. It is noteworthy that these fuel oils can also contain up to 24% aromatic hydrocarbons; therefore, responses may not be due solely to the aliphatic portion of the distillate. An epidemiological study of repeated human exposure to kerosene vapor showed an association with bronchoconstriction, asthma, and other airway hyperreactivity conditions (Rodriguez de la Vega et al. 1990). Animal studies of kerosene-based jet fuels also showed that acute (7 d) and intermediate (28–56 d) exposure resulted in increased lung injury and damage to alveolar epithelium in mice (Robledo et al. 2000; Witten 1992; 1993) and rats (Chen et al. 1992), and increased alveolar inflammation and edema in rats (Hays et al. 1995) with doses ranging from approximately 50 to 1,000 mg/m^3. In addition, alterations in tidal volume, dynamic lung compliance, and resistance occurred after acute pulmonary exposure to high doses (approximately 32 g/m^3) of kerosene aerosol in rabbits that lasted 4–9 min (Casaco et al. 1982), and bronchoconstriction was found after high doses in both rabbits (Casaco et al. 1982) and guinea pigs (approximately 20 g/m^3/5 min) (Garcia Mesa et al. 1988). Few studies exist that examined dearomatized distillates that are composed primarily of aliphatic hydrocarbons. A study examining human exposure to dearomatized solvent (white spirit) containing hydrocarbons of chain lengths 9 through 14 (100–300 mg/m^3) reported no adverse effects on lung function, although systemic effects were observed in animal inhalation studies of the same compound (Lam et al. 1994; 1995; Lund et al. 1996).

Related to the pulmonary effects of the individual constituents of COREXIT already discussed, the aim of this study was to evaluate whether inhaled COREXIT may alter lung function, induce pulmonary injury/inflammation, or affect airway resistance and reactivity in response to inhaled methacholine (MCh) aerosol challenge. To address this aim, rats were exposed to 27 mg/m^3 COREXIT for 5 h. At 1 h, 1 d, and 7 d postexposure, breathing rate and specific airway resistance were measured to evaluate lung function. Bronchoalveolar lavage (BAL) was performed on the same rats 1 and 7 d postexposure, and BAL cells and fluid were retained to measure indices of lung injury, inflammation, and oxidant burden. Airway reactivity was evaluated in vivo on d 1 and d 7 postexposure by measuring airway resistance and dynamic compliance and the effects of MCh challenges on these parameters. In addition, in vitro studies were performed using tracheal strips and the isolated, perfused trachea preparation to investigate whether COREXIT elicits contractile and/or relaxant activity or the ability to alter neurogenic responses mediated by endogenous neurotransmitters.

METHODS

Materials

COREXIT EC9500A was provided by Nalco Company (Naperville, IL). In this study the use of the term COREXIT refers to COREXIT 9500A unless stated otherwise. Materials used for analysis of lactate dehydrogenase (LDH) and albumin were purchased from Roche Diagnostic Systems (Montclair, NJ). Kits used for analysis of cytokines and chemokines were purchased from Invitrogen Corporation (Carlsbad, CA). Modified Krebs–Henseleit (MKH) solution (pH 7.4; osmolarity of 281 ± 5 mosM; 37°C) contained 113 m*M* NaCl, 4.8 m*M* KCl, 2.5 m*M* $CaCl_2$, 1.2 m*M* KH_2PO_4, 1.2 m*M* $MgSO_4$, 25 m*M* $NaHCO_3$, and 5.7 m*M* glucose, and was saturated with 95% O_2/5% CO_2. All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Animals

Male Sprague-Dawley (Hla: SD CVF) and Fischer 344 (F344:NHla: CVF) rats weighing approximately 300 g, obtained from Hilltop

Labs (Scottdale, PA), were used for in vivo and in vitro studies, respectively, in accordance with a protocol approved by the Institutional Animal Care and Use Committee. Animals were given a Teklad 2918 diet and tap water ad libitum, housed in a clean air and viral- and antigen-free room with restricted access in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-approved animal facility under a 12 h:12 h light:dark cycle, and allowed to acclimate for 1 wk prior to use.

COREXIT Exposure

An automated whole-body inhalation exposure system was used to expose individually housed rats to COREXIT or air (control). The oil dispersant aerosol was generated with a collision-type atomizer (TSI, 3076) and regulated with software feedback loops to maintain a constant concentration throughout exposures. A detailed description of the exposure system can be found in Goldsmith et al. (2011, this issue). Sprague-Dawley (SD) rats were placed in the inhalation chamber and exposed to air or COREXIT for 5 h. As the effects of COEXIT exposure are largely unknown, a target dose of 15 mg/m^3 was chosen based on exposure limits for "nuisance" dusts, substances considered to be relatively inert. The actual dose delivered was measured in the chamber to be 27 mg/m^3. All animals were returned to the colony room immediately following the exposure.

Lung Function

Breathing rates and specific airway resistance were evaluated in SD rats 1 h following the exposure to COREXIT or air (d 0), and at 1 and 7 d postexposure ($n = 6$ per group per time point). Breathing rates were measured using whole-body plethysmography. Briefly, animals were placed in a closed chamber, and pressure changes due to respiration were measured and digitally recorded at a sampling rate of 500 Hz. Bias flow (2 L/min of 10% CO_2 in air) was continuously supplied through the chamber via high-impedance tubing. Following a 3-min acclimation period, breathing intervals were measured on a breath-by-breath basis. The breathing interval at the peak of the resulting histogram was taken as the measurement for that animal. The breathing rate was then found as the reciprocal of the measured interval.

Specific airway resistance was measured using double-chamber plethysmography. Animals were placed in a double-chamber plethysmograph, and a bias flow of 1 L/min of 10% CO_2 in air was supplied through the head chamber via high-impedance tubing. Flow produced from each chamber was measured and digitally recorded at 2000 Hz. Specific airway resistance was estimated from phase shift between the two flow signals (Pennock et al. 1979).

In Vivo Lung Studies

On d 1 and 7 after exposure to COREXIT or air, BAL was performed by washing the right lungs of the SD rats with aliquots of phosphate-buffered saline (PBS) in order to obtain pulmonary cells for morphological and functional analysis, and the acellular lavage fluid was retained for analysis of indicators of tissue damage and cellular activity ($n = 6$ per group per time point). Rats were euthanized with an overdose of sodium pentobarbital (>100 mg/kg; Sleepaway; Fort Dodge Animal Health, Madison, NJ), the trachea was cannulated, the chest cavity was opened, the left lung was clamped off, and BAL was performed on the right lungs via the tracheal cannula. The acellular fraction of the first lavage was obtained by filling the right lung with 1 ml/100 g body weight PBS, massaging for 30 s, withdrawing, and repeating the process one more time. This concentrated aliquot was withdrawn, retained, kept separately, and was designated as the first fraction of BAL fluid (BALF). The following aliquots were 6 ml in volume, instilled once with light massaging, withdrawn, and combined until a 30-ml volume was obtained. For each animal, both lavage fractions were centrifuged (10 min, 1200 × g), the cell pellets were combined and resuspended in 1 ml PBS,

and the acellular fluid from the first fraction was retained for further analysis.

BALF Analysis: Lactate Dehydrogenase (LDH) Activity, Albumin, and Cytokines The presence of albumin and the LDH activity in the BALF of rats exposed to air or COREXIT were measured to evaluate the loss of integrity of the alveolar–capillary barrier and general cytotoxicity, respectively. Measurements of both albumin and LDH activity in the acellular fluid were obtained using a Cobas Mira analyzer (Roche Diagnostic Systems; Montclair, NJ). Albumin was determined by spectrophotometric measurement of the reaction product of albumin with bromcresol green according to a method by Sigma Diagnostics (St. Louis, MO). LDH activity was quantified by detection of the oxidation of lactate coupled to the reduction of NAD^+ at 340 nm.

Cytokines and chemokines involved in inflammatory and immune responses were measured in the BALF of rats exposed to air or COREXIT using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Invitrogen Corporation; Carlsbad, CA). The following cytokines and chemokines were quantified: tumor necrosis factor-α (TNF-α), interleukin (IL)-2, IL-4, IL-6, IL-10, interferon-γ (IFN-γ), transforming growth factor-β (TGF-β), monocyte chemotactic protein (MCP)-1, and macrophage inflammatory protein (MIP)-2.

Cell Differentials: Alveolar Macrophages (AM), Neutrophils, Lymphocytes, and Eosinophils The totals of BAL cells collected from rats exposed to air or COREXIT were counted using a Coulter Multisizer II (Coulter Electronics; Hialeah, FL). Cell differentials were performed to determine the total number of AM, neutrophils, lymphocytes, and eosinophils. Briefly, 10^5 cells from each rat were spun down onto slides with a Cytospin 3 centrifuge (Shandon Life Sciences International; Cheshire, England) and labeled with Leukostat stain (Fisher Scientific, Pittsburgh, PA) to differentiate cell types. Two hundred cells per slide were counted, and percentages of AM, neutrophils, lymphocytes, and eosinophils were multiplied by the total number of cells to calculate the total number of each cell type.

Chemiluminescence To estimate total lung oxidant production, luminol-dependent chemiluminescence (CL) was performed on BAL cells as a measure of the light generated by the production of reactive oxygen species (ROS) by AM and neutrophils using a Berthold LB953 luminometer (Wallace Inc.; Gaithersburg, MD) as described previously (Antonini et al. 1994). Baseline oxidant production by the cells was measured in the absence of a stimulant. Phorbol 12-myristate 13-acetate (PMA) (10 μ*M*), a soluble stimulant of total BAL phagocytes (AM and neutrophils), or nonopsonized, insoluble zymosan (2 mg/ml), a stimulant of only AM (Castranova et al. 1990), was added to the assay immediately prior to CL measurement to determine the contribution of both AM and neutrophils to the overall production of ROS in the lungs of the rats. Measurement of CL was recorded for 15 min at 37°C, and the integral of counts per minute (cpm) per 10^6 cells versus time was calculated. CL was calculated as the cpm of the stimulated cells minus the cpm of the corresponding resting cells, and the value was normalized to total number of BAL cells for PMA-stimulated CL and total number of AM in the BAL for zymosan-stimulated CL.

In Vivo Airway Reactivity

At 1 and 7 d after COREXIT or air exposure, SD rats were anesthetized with an intraperitoneal (ip) injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). After exposing the trachea through a mid-line incision in the neck, a cannula was inserted into the trachea. The animals were given supplemental ketamine (40 mg/kg suffused onto the muscles of the neck), placed into plethysmographs on top of a warming pad, and ventilated (90 breaths/min, 40 cm H_2O mouth pressure, 5 ml tidal volume). Values were logged at 5-s intervals and averaged (Buxco Research Systems; Wilmington, NC). After basal values of airway resistance (R_L) and dynamic compliance (C_{Dyn}) in air were recorded, the animals were

challenged with saline aerosol (20 μl), the vehicle control for MCh challenge. Following baseline measurements rats were administered aerosols of MCh (20 μl) in stepwise-increasing concentrations (0.3–10 mg/ml). The peak values for MCh-induced R_L and C_{Dyn} responses were quantified.

Effects of COREXIT on Tracheal Strips

Naïve F344 rats were anesthetized with ip sodium pentobarbital (>100 mg/kg) and sacrificed by exanguination. A midline incision was made in the neck and the trachea was removed. After cleaning, trachea strips two cartilage rings wide were prepared. The strips were tied at one end to a holder, placed in an organ chamber (37°C) containing modified MKH solution, and attached to force-displacement transducers for the measurement of isometric tension responses. The preparations were equilibrated for 1 h. At the end of this period, COREXIT was added to the bath in stepwise-increasing concentrations (0.001–0.1% v/v) to evoke responses. Responses are expressed as a change in force (g) from baseline. In another series of experiments the preparations were first contracted with MCh (3×10^{-5} *M*; EC_{50}) before COREXIT was added in stepwise-increasing concentrations (0.1–10% v/v).

Effects of COREXIT in Isolated, Perfused Trachea

This preparation permits the separate application of agents to the intraluminal (IL) or extraluminal (EL) surfaces of the trachea and has been described (Fedan and Frazer 1992). Briefly, after sacrifice, a 25-mm segment of naive F344 rat trachea was removed, cleaned, and mounted on a perfusion holder at its in situ length. The holder, with trachea mounted, was placed into an EL bath (37°C) containing MKH solution and perfused at a constant rate (25 ml/min) with MKH solution from an IL bath (37°C). The perfusion holder contained indwelling side-hole catheters that became inserted into the lumen when the trachea was attached to the holder. These were connected to the positive (inlet) and negative (outlet) sides of a differential pressure transducer. The difference between inlet and outlet pressure (ΔP, cm H_2O), with transmural pressure set to zero, served as an index of tracheal diameter: tracheal contraction increases ΔP and relaxation decreases ΔP. To obtain concentration-response curves, COREXIT was added in stepwise-increasing, cumulative concentrations (0.003–10% v/v) to the IL bath. Responses were obtained from preparations at basal tone and from preparations precontracted with EL MCh (3×10^{-5} *M*) and are expressed as change in ΔP.

Effects of COREXIT on Neurogenic Responses of Tracheal Strips

Tracheal strips prepared as already described were placed between two platinum ring electrodes at either end of the strips. After the 1-h equilibration period, the strips were subjected to electric field stimulation (EFS) by passing 10-s-long trains of square-wave pulses (120 V, 10 ms duration, 10 Hz) between the electrodes at 5-min intervals. Six such stimulation episodes were delivered to obtain control responses. The responses became stable by the fourth through the sixth pulses. These three responses were quantified and averaged to obtain a control value and expressed as percent initial contractile response. The preparations were then washed with fresh MKH solution over a period of 1 h. Five minutes before the end of this period COREXIT was added to the organ bath, in a concentration (0.003% v/v) that was derived from the concentration-response analysis of COREXIT's effects (see Results section). The strips were then stimulated with six additional trains of EFS, responses to which were averaged and compared to the control responses obtained before COREXIT was added to the bath.

Statistical Analysis

Results are expressed as means ± SEM. Statistical analyses for lung function and in

vivo lung toxicity data were carried out with the SigmaStat 11.0 statistical program (Chicago, IL). The significance of the interaction among different treatment groups for the different parameters at each time point was assessed using analysis of variance (ANOVA). The significance of difference between individual groups was analyzed using the Student–Newman–Keuls method. The analysis of measures of basal R_L and C_{Dyn} values were compared using Student's paired and non-paired t-test, as appropriate. The analysis of measures of the effects of MCh on R_L and C_{Dyn} was generated using SAS/STAT software, version 9.1 of the SAS system for Windows (SAS Institute, Inc., Cary, NC). PROC MIXED was utilized to run a two-way factorial analysis of variance with concentration of MCh treated as a repeated measure to account for multiple measures in individual animals. Treatment comparisons were then calculated at each level of MCh utilizing the "slice" option. All differences were considered significant at $p < .05$; n is the number of separate experiments.

RESULTS

Lung Function

After exposure to COREXIT or air, breathing rates (Figure 1A) and specific airway resistance (Figure 1B) were measured immediately following exposure and at 1 and 7 d postexposure. No significant differences were observed in these parameters during the time course.

Pulmonary Toxicity In Vivo

BALF was analyzed for indices of lung injury and inflammation. LDH activity (Figure 2A), an indicator of general cytotoxicity, and albumin content (Figure 2B), a marker for protein present in BALF after injury to the air–blood barrier, did not differ markedly between COREXIT- and air-exposed rats. In addition, there were no significant differences between groups in the inflammatory and immune-related cytokines measured in BALF (data not shown). Total cells recovered from BAL did not differ markedly between COREXIT- and air-exposed rats. Alveolar macrophages (Figure 3A) accounted for greater than 98% of the cell population. There were no significant increases in neutrophils (Figure 3B) in the lungs after exposure to COREXIT, reflecting the absence of an inflammatory response. There was a quantitative rise in lymphocytes at 7 d postexposure (Figure 3C), which accounted for less than 0.5% of the cell population, and there was no eosinophil influx, indicating the absence of an irritant response in either group (data not shown). In addition, oxidant production by recovered BAL cells (Figures 4A and 4B) did not differ markedly among groups at either postexposure time point.

Effects of COREXIT on Basal Pulmonary Function and Reactivity to Inhaled MCh

At 1d after COREXIT exposure, the dispersant exerted no significant effect on basal R_L

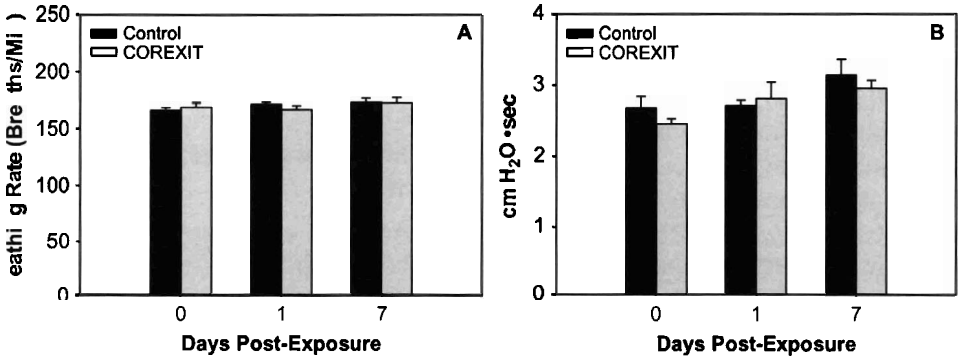

FIGURE 1. (A) Breathing rates and (B) specific airway resistance measured in rats exposed to COREXIT (27 mg/m^3/5 h) or air (control) at 0, 1, and 7 d postexposure; $n = 6$.

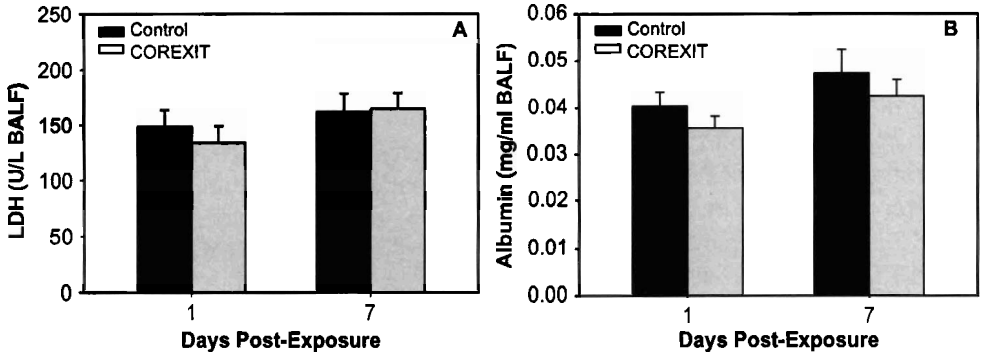

FIGURE 2. (A) Lactate dehydrogenase (LDH) activity and (B) albumin measured in rats exposed to COREXIT (27 mg/m^3/5 h) or air (control) at 1 and 7 d postexposure; $n = 6$.

after breathing air or saline aerosol (MCh vehicle) (Figure 5, upper left panel). However, basal C_{dyn} was reduced nearly significantly ($p = .052$) in COREXIT-exposed animals breathing air, and C_{dyn} was significantly reduced in COREXIT-exposed animals after inhalation of saline aerosol 1 d postexposure (Figure 5, upper right panel). At 7 d postexposure neither basal R_L nor basal C_{dyn} was affected (Figure 5, lower panels). Airway reactivity in responses to MCh challenge is depicted in Figure 6. At 1 d after exposure to COREXIT, R_L responses to MCh were unaffected (upper left panel), whereas C_{dyn} was inhibited (upper right panel). The decrease in C_{dyn} was no longer observed at 7 d after exposure (lower right panel).

Effects of COREXIT on Tracheal Strips

The reduction in C_{dyn} responses to MCh may have involved a direct effect of COREXIT on airway smooth muscle. Therefore, the effects of COREXIT on tracheal strips were investigated. Figure 7 illustrates that addition of the dispersant to the organ chamber led to two types of responses: At a low concentration (0.001 % v/v), COREXIT elicited relaxation. In the same strips, as COREXIT was added cumulatively in higher concentrations, contraction responses were obtained, with the maximum response occurring at approximately 0.03 % v/v. The EC_{50} for the contractile responses was approximately 0.0031% v/v. In MCh-contracted tracheal strips COREXIT (0.01% v/v) elicited contraction in lower concentrations (0.001–0.01% v/v), as it had in non-contracted tracheal strips (data not shown). In both the absence and presence of MCh, it was noted that a white precipitate began to form in the MKH solution at ≥0.1% v/v COREXIT concentrations in association with the development of relaxation responses. Preliminary experiments were performed to identify which salt(s) in the MKH solution became precipitated in the presence of COREXIT. COREXIT, a yellow solution, did not precipitate when added to distilled water; however, it rendered the solution somewhat cloudy. When added to solutions of the individual salts in concentrations present in MKH solution, fine precipitates were, surprisingly, produced after COREXIT was mixed with NaCl (>0.1% v/v) and, to a lesser extent and in order of decreasing intensity, KH_2PO_4, $NaHCO_3$, and $CaCl_2$. $MgSO_4$ and glucose did not yield opacity greater than that of COREXIT in water.

Effects of COREXIT on Isolated, Perfused Trachea

Addition of agents to a bath housing tracheal strips exposes both epithelium and smooth muscle surfaces. An advantage of the isolated, perfused trachea preparation is that it permits separate addition of agents to the luminal or extraluminal baths. Experiments were therefore conducted to investigate COREXIT's

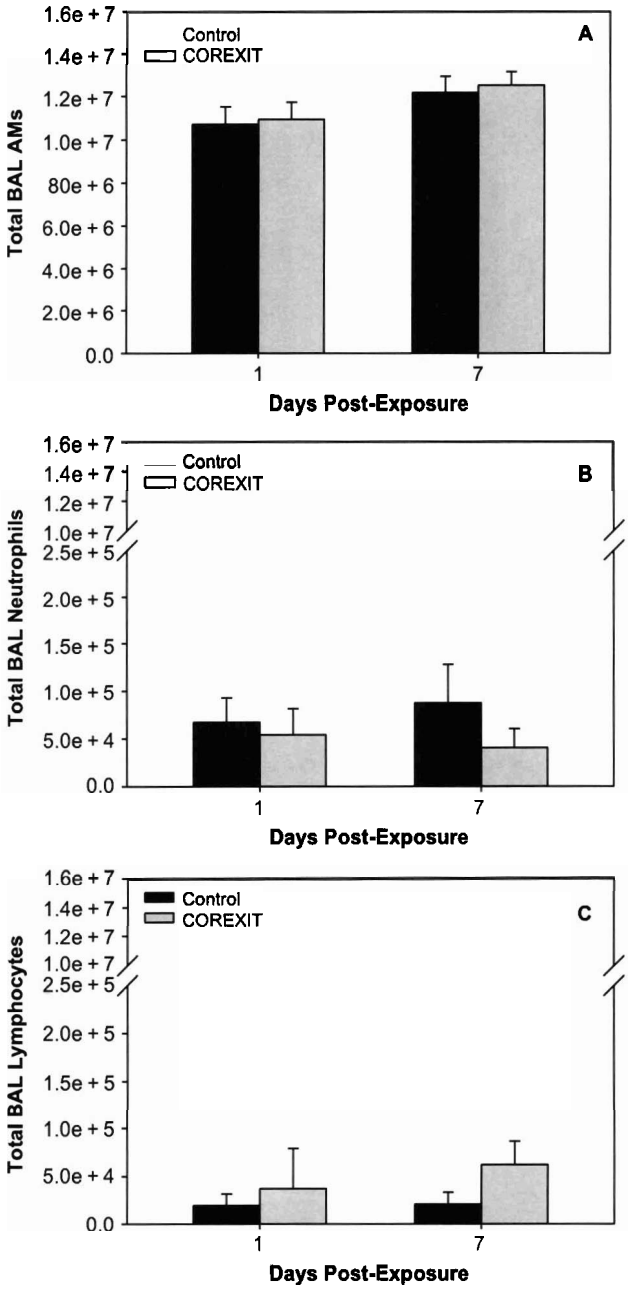

FIGURE 3. (A) Total number of alveolar macrophages (AM), (B) neutrophils, and (C) lymphocytes recovered from BAL in rats exposed to COREXIT (27 mg/m^3/5 h) or air (control) at 1 and 7 d postexposure; $n = 6$.

effects when administered to the lumen of the trachea. The concentrations of intraluminally applied COREXIT that elicited responses of the perfused trachea (Figure 8) were two- to threefold higher compared to those in tracheal strips (Figure 7), owing to the diffusion barrier afforded by the epithelium (Fedan and Frazer 1992). In nonstimulated tracheas low concentrations of COREXIT (0.03–0.1% v/v) produced relaxation, and as the dispersant's concentration was elevated contractile responses were produced (Figure 8A). Similar

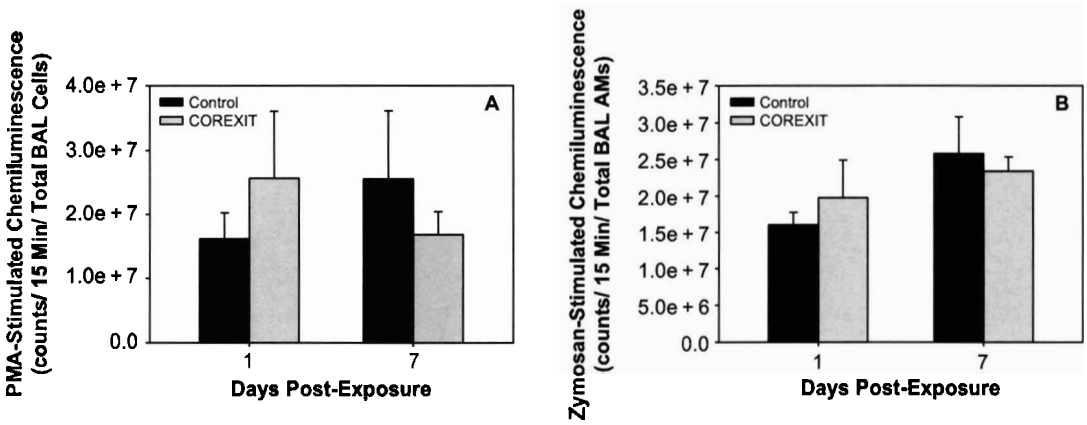

FIGURE 4. Total oxidant production (A) by total BAL cells (AMs and neutrophils) and (B) by total BAL AM only was measured by evaluating chemiluminescence in the presence of phorbol 12-myristate 13-acetate (PMA) or zymosan, respectively, in BAL cells recovered from rats exposed to COREXIT (27 mg/m^3/5 h) or air (control) at 1 and 7 d postexposure; $n = 6$.

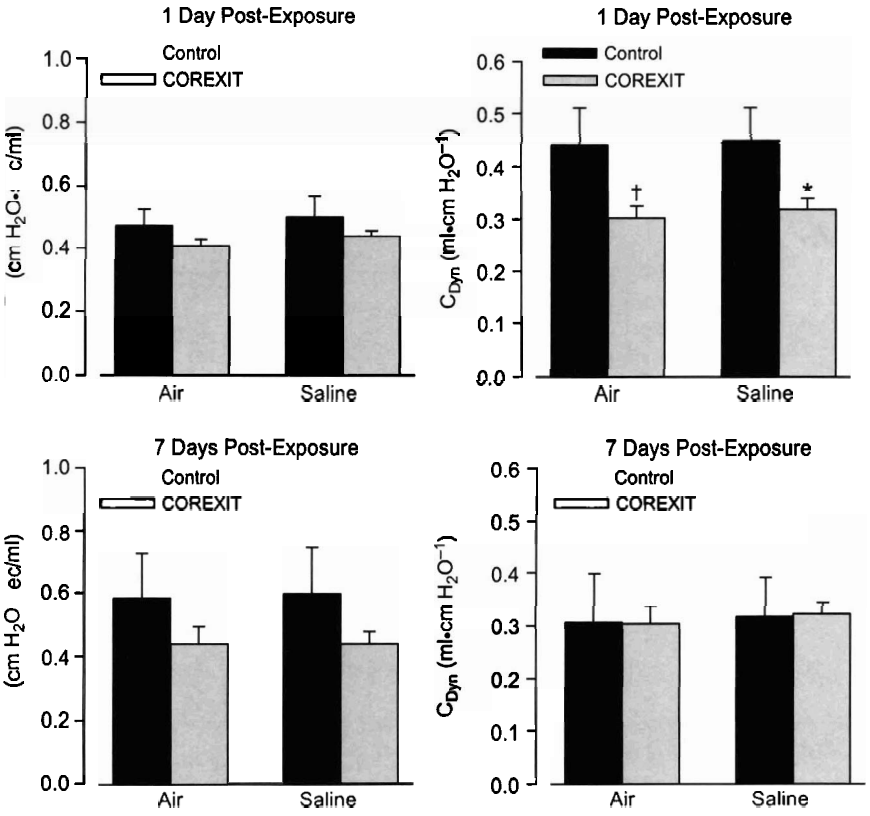

FIGURE 5. Basal values of airway resistance, R_L, and dynamic compliance, C_{Dyn}, 1 and 7 d postexposure to air (Control) or COREXIT (27 mg/m^3/5 h), measured in animals after challenge with air or saline aerosol (vehicle for MCh aerosol). *, Significant difference for control–saline vs. COREXIT–saline, $p < .05$ (nonpaired t-test); $n = 5$–8.

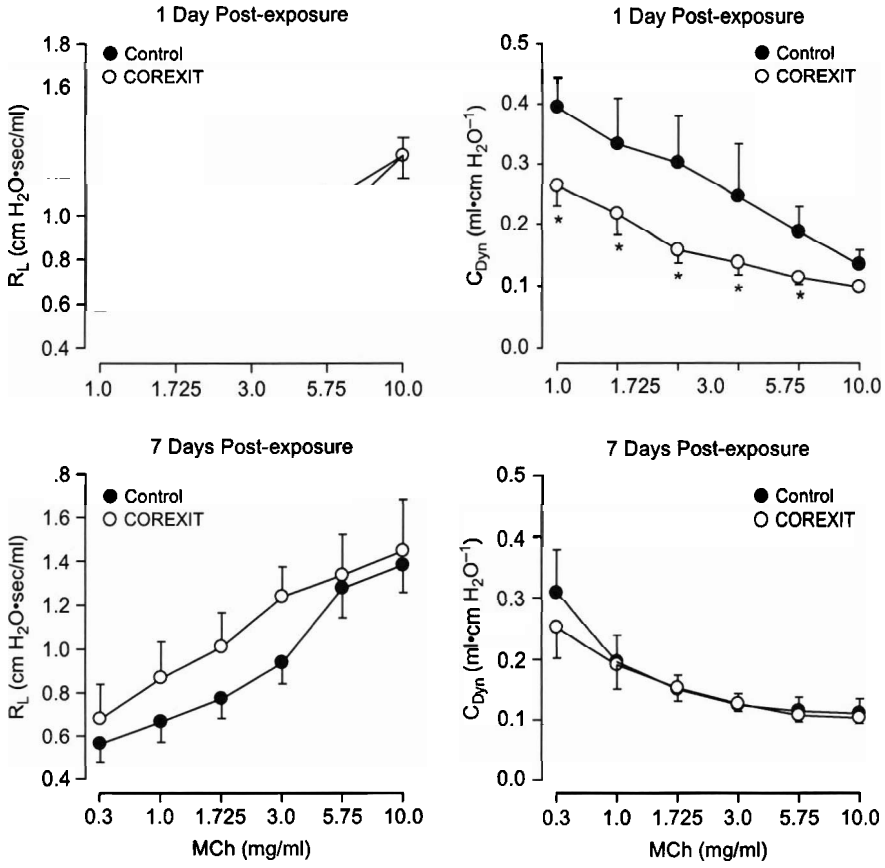

FIGURE 6. Airway reactivity to inhaled MCh aerosol 1 and 7 d after exposure to air (control) or COREXIT (27 mg/m^3/5 h). MCh aerosols were delivered in stepwise-increasing concentrations. Changes in R_L are shown in the left panels; changes in C_{Dyn} are shown in the right panels. Asterisk indicates significantly different from control; $n = 5–8$.

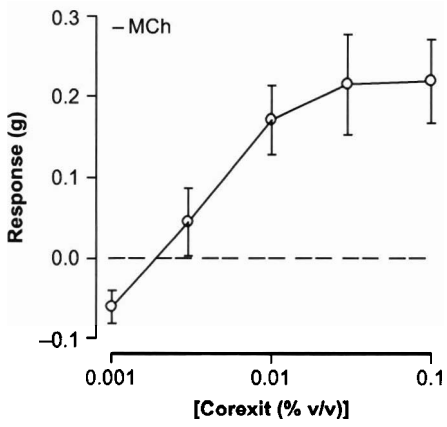

FIGURE 7. Contractile effects of COREXIT on unstimulated rat tracheal strips ($n = 4$).

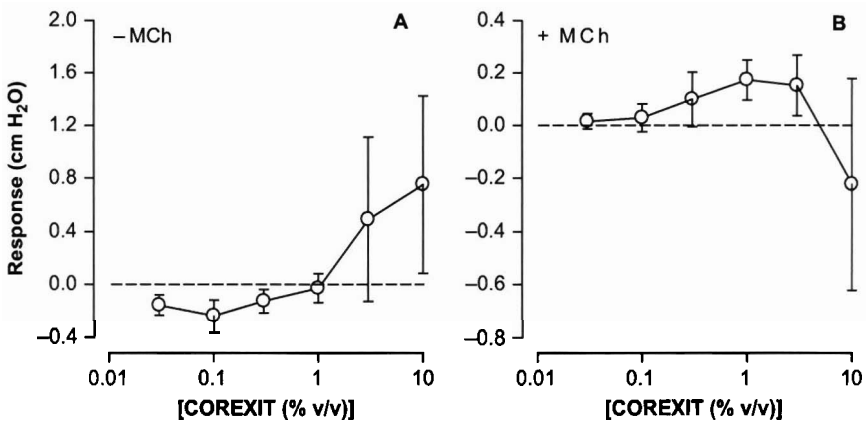

FIGURE 8. Contractile effects of COREXIT on unstimulated (A; $n = 5$) and MCh-contracted (B; $n = 4$) rat isolated, perfused tracheas.

results were observed in MCh-contracted tracheas (Figure 8B). Precipitation occurred in the MKH solution in the intraluminal perfusate, but not in the MKH solution in the extraluminal bath during development of contractions to COREXIT (≥0.1% v/v).

Effect of COREXIT on Neurogenic Responses of Tracheal Strips

It is well known that airway diameter is regulated neurally via parasympathetic postganglionic cholinergic nerves, as well as by sensory and nonadrenergic, noncholinergic nerves. To investigate whether COREXIT interferes with excitatory neurotransmission, tracheal strips were stimulated by endogenous neurotransmitters released by EFS. The results are shown in Figure 9. In control strips, the first three stimulation periods yielded contractions that decreased progressively; by the fourth through sixth stimulation periods the responses became stable and reproducible. Thus, the latter three responses were averaged to serve as the control. After obtaining the control responses and allowing a rest period of 1 h, control strips stimulated again 6 times yielded stable responses that were reduced by approximately 20% compared to those obtained in the initial round of stimulations. These responses served as control for responses obtained in the presence of COREXIT (Figure 9). In the presence of 0.003% v/v COREXIT, added 5 min before EFS, neurogenic contractile responses were inhibited significantly.

DISCUSSION

This study has demonstrated that a 5-h inhalation exposure to COREXIT (27 mg/m^3)

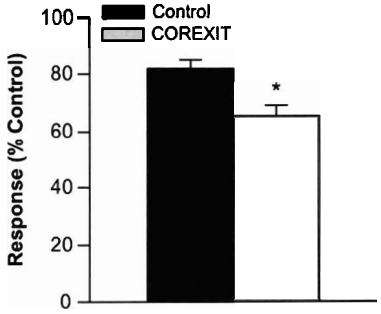

FIGURE 9. Effect of COREXIT (0.003 % v/v) on neurogenic responses of rat tracheal strips. *, Significantly different from control; $n = 4$.

did not elicit inflammation or injury in the lung, but decreased basal C_{Dyn} and C_{Dyn} in the presence of MCh, while not affecting breathing rate, specific airway resistance, or MCh-induced increases in R_L, 1 d after the end of the exposure. The observed changes were not observed 7 d after exposure, indicating responses were reversible. In vitro, the dispersant elicited contraction of airway smooth muscle both in tracheal strips and in the isolated, perfused trachea preparation. COREXIT is, therefore, pharmacologically active in the airways. The observed decreases in C_{Dyn}, indicative of greater lung "stiffness," suggest that the effort of breathing would be increased after inhalation of COREXIT. With no changes in R_L after COREXIT exposure, the dispersant would not appear to have affected the larger resistance airways, e.g., larger bronchi and bronchioles.

COREXIT is a mixture of diverse chemicals, each with a myriad of pharmacological and toxicological effects, designed to disperse oil. Exposure to DSS or LDP was found to increase lung injury and inflammation in rodent models (Bitzen et al. 2006; Chen et al. 1992; Hays et al. 1995; Rao and Das 1994; Robledo et al. 2000; Witten 1992; 1993). In the present study, the acute COREXIT exposure did not induce lung injury or inflammation. Although immune-related cytokines were not altered in the lung, there was a numerical increase in lymphocyte influx into the lungs at d 7; however, this rise was not significant when compared to control values. A likely explanation for the difference between our findings and the earlier pulmonary studies investigating DSS and LPD is that different concentrations of the components in COREXIT and/or greater durations of exposure were employed and resulted in the adverse effects.

Multiple components of COREXIT (PG, DSS, LPDs) were shown to alter lung function parameters, both in humans and in rodent models. It is difficult to ascribe the observed decreases in C_{Dyn} to any one (or more) of the ingredients based on present results. However, one of the main ingredients of COREXIT, DSS, produced surfactant dysfunction and increased lung surface tension (D'Angelo et al. 2007), and may be involved in reducing lung compliance.

In both tracheal strips and isolated, perfused trachea, the first effect on airway smooth muscle produced by the lowest concentrations of COREXIT was relaxation; with increasing concentrations, contraction ensued. The eventual relaxations induced by the highest concentrations of COREXIT were associated with precipitate formation, indicating adulteration of the ionic composition of the MKH solution. Thus, no conclusions can be drawn regarding the mechanisms involved in this relaxation. Nevertheless, the finding that contraction of the perfused trachea did occur when COREXIT was applied to the intraluminal bath, absent any precipitation in the extraluminal MKH solution bathing the smooth muscle, suggests that some active substance(s) in COREXIT did traverse the epithelium to contract the airway smooth muscle.

The reduction in C_{Dyn} after COREXIT exposure, both basal and MCh-induced, might reflect some involvement of airway smooth muscle contraction that was observed in the present investigation. However, specific airway resistance, which is a greater reflection of the diameter of the larger airways, was not affected by treatment with the dispersant. A decrease in neurogenic responses of tracheal strips in the presence of COREXIT is an alteration that would increase airway diameter and compliance.

In addition to evoking responses from airway smooth muscle, COREXIT interfered with excitatory neurotransmission in tracheal strips. This effect may have resulted from an action on intramural nerves to inhibit transmitter, presumably acetylcholine, release, or postjunctionally in the smooth muscle at the level of receptors or signal transduction. Evidence in this study supports that view that that neurotransmitter release was reduced by COREXIT. The concentration of COREXIT (0.003% v/v) that inhibited EFS-induced responses itself had no inhibitory effect on the response to MCh (Figure 7). Inhibition of neurogenic responses by COREXIT is an effect that, if it occurs in vivo, would increase airway diameter and

compliance. However, only a decrease in C_{Dyn} was observed in this investigation.

Lastly, our findings suggest that inhalation of COREXIT in sufficient quantities may lead to the formation of precipitates in the airway surface liquid. Of special note, Figure 5 of Chapman et al. (2007) depicts the formation of a precipitate when dispersant (not specified) was applied at sea.

In summary, the acute 5-h exposure to COREXIT did not induce marked pulmonary lung injury and inflammation, although further investigation of effects on immune parameters in the lung may be warranted due to the trend for an increase in lymphocytes 7 d after exposure. The COREXIT exposure did decrease C_{Dyn} and MCh-induced C_{Dyn} at d 1, but this effect was not evident at d 7. In addition, in vitro studies showed that COREXIT may induce contraction of airway smooth muscle and interfere with excitatory neurotransmission in the airway wall. Longer duration inhalation exposures to COREXIT are underway to investigate how increased lung burden of COREXIT over time may alter pulmonary function and induce lung toxicity.

REFERENCES

Agency for Toxic Substances and Disease Registry. 1995 *Toxicological profile for fuel oils (PB/95/264222/AS)*. Atlanta, GA: U.S. Department of Health and Human Services.

Agency for Toxic Substances and Disease Registry. 1997. *Toxicological profile for propylene glycol (PB2009-103929)*. Atlanta, GA: U.S. Department of Health and Human Services.

Agency for Toxic Substances and Disease Registry. 1999. *Toxicological profile for total petroleum hydrocarbons (PB/99/163370)*. Atlanta, GA: U.S. Department of Health and Human Services.

Agency for Toxic Substances and Disease Registry. 2008. *Addendum to the toxicological profile for propylene glycol (PB2009-103929)*. Atlanta, GA: U.S. Department of Health and Human Services.

Antonini, J. M., Van Dyke, K., Ye, Z., DiMatteo, M., and Reasor, M. J. 1994. Introduction of luminal-dependent chemiluminescence as a method to study silica inflammation in the tissue and phagocytic cells of rat lung. *Environ. Health Perspect.* 102(suppl. 10): 37–42.

Bitzen, U., Enoksson, J., Uttman, L., Niklason, L., Johansson, L., and Jonson, B. 2006. Multiple pressure–volume loops recorded with sinusoidal low flow in a porcine acute respiratory distress syndrome model. *Clin. Physiol. Funct. Imaging*. 26: 113–19.

Bredenberg, C. E., Nieman, G. F., Paskanik, A. M., and Hart, A. K. E. 1986. Microvascular membrane permeability in high surface tension pulmonary edema. *J. Appl. Physiol.* 60: 253–59.

Carney, E. W., Crissman, J. W., Liberacki, A. B., Clements, C. M., and Breslin, W. J. 1999. Assessment of adult and neonatal reproductive parameters in Sprague-Dawley rats exposed to propylene glycol monomethyl ether vapors for two generations. *Toxicol. Sci.* 50: 249–58.

Casaco, A., Gonzalez, R., Arruzazabala, L., Garcia, M., and Rodriguez de la Vega, A. 1982. Studies on the effects of kerosene aerosol on airways of rabbits. *Allergol. Immunopathol. (Madr.)* 10: 361–66.

Castranova, V., Jones, T., Barger, M., Afshari, A., and Frazer, D. J. 1990. Pulmonary responses of guinea pigs to consecutive exposures to cotton dust. In *Proceedings of the 14th Cotton Dust Research Conference*, eds. R. R. Jacobs. P. J. Wakelyn, and L. N. Domelsmith,131–35. Memphis, TN: National Cotton Council.

Chapman, H., Purnell, K., Law, R. J. and Kirby, M. F. 2007. The use of chemical dispersants to combat oil spills at sea: A review of practice and research needs in Europe. *Mar. Pollut. Bull.* 54: 827–38.

Chen, H., Witten, M. L., and Pfaff, J. K. 1992. JP-8 jet fuel exposure increases alveolar epithelial permeability in rats. *FASEB J.* 6: A1064.

Coastal Response Research Center. 2010. *Deepwater Horizon dispersant use*

meeting report. http://www.scribd.com/doc/32904753/Dwh-Dispersants-Use-Meeting-Report

D'Angelo, E., Pecchiari, M., and Gentile, G. 2007. Dependence of lung injury on surface tension during low-volume ventilation in normal open-chest rabbits. *J. Appl. Physiol.* 102: 174–82.

Dahlback, M., Eirefelt, S., Backstrom, K., Larsson, P., Almer, L-O., Wollmer, P., and Jonson, B. 2002. Enhanced insulin absorption in the rabbit airways and lung by sodium dioctyl sulfosuccinate. *J. Aerosol Med.* 15: 27–36.

Dow Chemical, 2006. *Product safety assessment: Propylene glycol*. http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_0074/0901b80380074c91.pdf

Dow Chemical, 2003. *Dow propylene glycol industrial safety data sheet*. http://www.dow.com/PublishedLiterature/dh_0361/09002f1380361176.pdf?filepath=propyleneglycol/pdfs/noreg/117-01515.pdf&fromPage=GetDoc), No. 248, November 17, 2003, p. 1.

Evander, E., Wollmer, P., and Jonson, B. 1988. Pulmonary clearance of inhaled ^{99m}Tc-DPTA: Effect of the detergent dioctyl sodium sulfosuccinate in aerosol. *Clin. Pathol.* 8: 105–11.

Fedan, J. S. Dowdy, J. A., Johnston, R. A., and Van Scott, M. R. 2004. Hyperosmolar solution effects in guinea pig airways. I. Mechanical responses to relative changes in osmolarity. *J. Pharmacol. Exp. Ther.* 308: 10–18.

Fedan, J. S., and Frazer, D. G. 1992. Influence of epithelium on the reactivity of guinea pig isolated, perfused trachea to bronchoactive drugs. *J. Pharmacol. Exp. Ther.* 262: 741–50.

Garcia Mesa, M., Gonzalez Alvarez, R., and Casaco Parada, A. 1988. Biochemical mechanisms in the effects of kerosene on airways of experimental animals. *Allergo. Immmunopathol. (Madr.)* 16: 363–67.

George-Ares, A., and Clark, J. R. 2000. Aquatic toxicity of two Corexit dispersants. *Chemosphere* 40: 897–906.

George, S. E., Nelson, G. M., Kohan, M. J., Warren, S. H., Eischen, B. T., and Brooks, R. L. 2001. Oral treatment of Fischer 344 rats with weathered crude oil and a dispersant influences intestinal metabolism and microbiota. *J. Toxicol. Environ. Health A* 63: 297–316.

Goldsmith, W. T., McKinney, W., Jackson, M., Law, B., Bledsoe, T., Siegel, P., Cumpston, J., and Frazer, D. 2011. A computer-controlled whole-body inhalation exposure system for the oil dispersant COREXIT EC5900A. *J. Toxicol. Environ. Health* 74: 1368–1380.

Hays, A. M., Parliman, G., Pfaff, J. K., Lantz, R. C., Tinajero, J., Hall, J. N., and Witten, M. L. 1995. Changes in lung permeability correlate with lung histology in a chronic exposure model. *Ind. Health* 11: 325–36.

John, J., Wollmer, P., and Jonson, B. 1994. Effect of detergent on alveolar particle clearance due to large tidal ventilation. *Thorax* 49: 147–50.

Judson, R. S., Martin, M. T., Reif, D. M., Houck, K. A., Knudsen, T. B., Rotroff, D. M., Xia, M., Sakamuru, S., Huang, R., Shinn, P., Austin, C. P., Kavlock, R. J., and Dix, D. J. 2010. Analysis of eight oil spill dispersants using rapid, in vitro tests for endocrine and other biological activity. *Environ. Sci. Technol.* 44: 5979–85.

Konradova, V., Vavrova, V., and Janota, J. 1978. Effect of the inhalation of a surface tension-reducing substance (propylene glycol) on the ultrastructure of the epithelium of the respiratory passages in rabbits. *Folia Morphol.* 126: 28–34.

Lam, H. R., Ostergaard, G., and Ladefoged, O. 1995. Three weeks' and six months' exposure to aromatic white spirit affect synaptosomal neurochemistry in rats. *Toxicol. Lett.* 80: 39–48.

Lam, H. R., Ostergaard, G., Guo, S. X., Ladefoged, O., and Bondy, S. C. 1994. Three weeks' exposure of rats to dearomatized white spirit modifies indices of oxidative stress in brain, kidney, and liver. *Biochem. Pharmacol.* 47: 651–57.

Lund, S. P., Simonsen, L., Hass, U., Ladefoged, O., Lam, H. R., and Ostergaard, G.

1996. Dearomatized white spirit inhalation exposure causes long-lasting neurophysiological changes in rats. *Neurotoxicol. Teratol.* 18: 67–76.

NALCO. 2010. *COREXIT ®EC9500A material safety data sheet*. Naperville, IL: Nalco Co. http://www.nalco.com/documents/9500A_MSDS.pdf

National Institute for Occupational Safety and Health. 2010. *Health hazard evaluation of Deepwater Horizon response workers*. HETA 2010-01152010–0129-3138. www.cdc.gov/niosh/hhe/reports/pdfs/2010-0115-0219-3138.

National Research Council. 2005. *Oil spill dispersants: Efficacy and effects*. Washington, DC: National Academic Press.

Nieman, G. F., and Bredenberg, C. E. 1985. High surface tension pulmonary edema induced by detergent aerosol. *J. Appl. Physiol.* 58: 129–36.

Pennock, B. E., Cox, C. P., Rogers, R. M., Cain, W. A., and Wells, J. H. 1979. A noninvasive technique for measurement of changes in specific airway resistance. *J. Appl. Physiol.* 46: 399–406.

Rao, J. G., and Das, P. K. 1994. Pulmonary oedema due to inhalation of detergent aerosol. *Malays. J. Pathol.* 16: 165–66.

Ritchie, G. D., Still, K. R., Rossi, J., Bekkedal, M. Y.-V., Bobb, A. J., and Arfsten, D. P. 2003. Biological and health effects of exposure to kerosene-based jet fuels and performance additives. *J. Toxicol. Environ. Health B Crit. Rev.* 6: 357–451.

Robertson, O. H., Loosli, C. G., Puck, T. T. 1947. Test for chronic toxicity of propylene glycol and triethylene glycol on monkeys and rats by vapor inhalation and oral administration. *J. Pharmacol. Exp. Ther.* 91: 52–76.

Robledo, R. F., Young, R. S., Lantz, R. C., and Witten, M. L. 2000. Short-term pulmonary exposure to inhaled JP-8 jet fuel aerosol in mice. *Toxicol. Pathol.* 28: 656–63.

Rodriguez de la Vega, A., Casaco, A., Garcia, M., Noa, M., Carvajal, D., Arruzazabala, L., and Gonzalez, R. 1990. Kerosene-induced asthma. *Ann. Allergy* 64: 362–63.

Suber, R. L., Deskin, R., Nikiforov, I., Fouillet, X., and Coggins, C. R. 1989. Subchronic nose-only inhalation study of propylene glycol in Sprague-Dawley rats. *Food Chem. Toxicol.* 27: 573–83.

Taskar, V., John, J., Evander, E., Robertson, B., and Jonson, B. 1997. Surfactant dysfunction makes lungs vulnerable to repetitive collapse and reexpansion. *Am. J. Resp. Crit. Care Med.* 155: 313–20.

U.S. Coast Guard. 2011. *BP Deepwater Horizon oil spill incident specific preparedness review final report, January 2011*. www.uscg.mil/foia/docs/DWH/BPDWH.pdf

Witten, M. L. 1992. Chronic effects of JP-8 jet fuel exposure on lungs. *Govt. Rep. Announce. Index (GRA&I)* 17.

Witten, M. L. 1993. Chronic effects of JP-8 jet fuel exposure on lungs. *Govt. Rep. Announce. Index (GRA&I)* 18.

Wollmer, P., Backstrom, K., Zhao, H., Nilsson, P. G., and Jonson, B. 2000. Surface active agents as enhancers of alveolar absorption. *Pharm. Res.* 17: 38–41.