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# POTENTIAL IMMUNOTOXICOLOGICAL HEALTH EFFECTS FOLLOWING EXPOSURE TO COREXIT 9500A DURING CLEANUP OF THE DEEPWATER HORIZON OIL SPILL

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**Workers involved in the Deepwater Horizon oil spill cleanup efforts reported acute pulmonary and dermatological adverse health effects. These studies were undertaken to assess the immunotoxicity of COREXIT 9500A, the primary dispersant used in cleanup efforts, as a potential causative agent. COREXIT 9500A and one of its active ingredients, dioctyl sodium sulfosuccinate (DSS), were evaluated using murine models for hypersensitivity and immune suppression, including the local lymph node assay (LLNA), phenotypic analysis of draining lymph node cells (DLN), mouse ear swelling test (MEST), total serum immunoglobulin E (IgE), and the plaque-forming cell (PFC) assay. Dermal exposure to COREXIT 9500A and DSS induced dose-responsive increases in dermal irritation and lymphocyte proliferation. The EC3 values for COREXIT 9500A and DSS were 0.4% and 3.9%, respectively, resulting in a classification of COREXIT 9500A as a potent sensitizer and DSS as a moderate sensitizer. A T-cell-mediated mechanism underlying the LLNA was supported by positive responses in the MEST assay for COREXIT and DSS, indicated by a significant increase in ear swelling 48 h post challenge. There were no marked alterations in total serum IgE or B220+/IgE+ lymph-node cell populations following exposure to COREXIT 9500A. Significant elevations in interferon (IFN)-γ but not interleukin (IL)-4 protein were also observed in stimulated lymph node cells. The absence of increases in IgE and IL-4 in the presence of enhanced lymphocyte proliferation, positive MEST responses, and elevations in IFN-γ suggest a T-cell-mediated mechanism. COREXIT 9500A did not induce immunosuppression in the murine model.**



The Deepwater Horizon oil spill in the Gulf of Mexico prompted many concerns over adverse effects on human health due to inhalation or dermal contact with the oil and dispersant chemicals used in the cleanup. COREXIT 9580 and 9500A were originally approved by the U.S. Environmental Protection Agency (EPA) for use in emergencies such as the Gulf oil spill, but due to increased concerns over public safety, the U.S. EPA recommended the use of less toxic and equally effective alternatives to COREXIT (U.S. EPA 2011). However, due to issues such as limited availability, effectiveness, and toxicity of the other approved dispersants, the use of COREXIT 9500A was continued and it was ultimately selected as the primary dispersant to be used for cleanup efforts in response to the Deepwater Horizon oil spill. Approximately 1.84 million gal of dispersant was applied during cleanup efforts in the Gulf of Mexico, with 1.07 million gal on the surface and 771,000 gal sub-sea since the start of the oil spill (U.S. Coast Guard 2011). COREXIT 9500A primary ingredients include sorbitan, butanedioic acid, hydro-treated light petroleum distillates (HLPD), propylene glycol (PG), and dioctyl sodium sulfosuccinate (DSS) (Nalco 2010).



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, Centers for Disease Control and Prevention.

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Increasing numbers of U.S. Gulf Coast residents attribute ongoing sicknesses to BP's oil disaster and use of cleanup dispersants. Cleanup workers reported symptoms such as headaches, dizziness, nausea, vomiting, cough, respiratory distress, chest pains, dermatitis, and secondary skin infections (NIOSH 2010; Solomon and Janssen 2010). According to the Alaska Community Action on Toxics, the use of COREXIT 9527A during the *Exxon Valdez* oil spill produced human "respiratory, nervous system, liver, kidney, and blood disorders." Laboratory studies supported this association (George et al. 2001; Scarlett et al. 2005). COREXIT 9527A is stated by its manufacturer (MSDS sheet) to be potentially harmful to red blood cells, kidneys, and liver, and may irritate eyes and skin. The chemical 2-butoxyethanol, which is found in COREXIT 9527A but not in 9500A, was identified as potentially having produced chronic health problems in workers involved in the cleanup of the *Exxon Valdez* oil spill. The relative contributions to adverse health effects of COREXIT 9500A and similar types of dispersants are difficult to determine due to insufficient scientific data. Testing requested by the U.S. EPA on eight dispersants found similar toxicities based on standard toxicity tests conducted on sensitive aquatic organisms found in the Gulf. These results suggested Corexit 9500A is generally no more or less toxic than the other available alternatives (Hemmer 2010a; 2010b; Judson et l 2010). A May 2010 report by the Centers for Disease Control (CDC) concluded that "because of the strict guidelines that must be followed to utilize dispersants, it is unlikely that the general public will be exposed [directly] to [the] product." The report further stated that "ingredients are not considered to cause chemical sensitization; the dispersants contain proven, biodegradable and low toxicity surfactants" (CDC 2011).

However, due to increasing concerns of the general public, speculative reported adverse health effects, large application volume, potential for worker exposure, and lack of definitive toxicity data, these studies were undertaken to evaluate the irritancy and immunotoxicity, including hypersensitivity and immune suppression, following dermal exposure to COREXIT 9500A and one of its main ingredients (DSS).

## MATERIALS AND METHODS

### Test Articles

Dioctyl sodium sulfosuccinate (99+%) (DSS; CAS number 577-11-7), alpha-hexylcinnamaldehyde (HCA; CAS number 101-86-0), propylene glycol (PG; CAS number 57-55-6), toluene-2,4-diisocyanate (TDI; CAS 584-84-9), and 2,4-dinitrofluorobenzene (DNFB; CAS number 70-34-8) were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). COREXIT 9500A was obtained from Nalco (Naperville, IL).

### Species Selection

Female BALB/*c* and B6C3F1 mice were used in these studies. BALB/*c* mice possess a Th2 bias and are commonly used to evaluate potential immunoglobulin (Ig) E-mediated sensitization, and thus are used in the hypersensitivity studies (Klink and Meade 2003; Woolhiser et al. 2000). B6C3F1 mice are the strain of choice for immunosuppression studies and are used to evaluate the IgM response to SRBC (Luster et al. 1992). Mice were purchased from Taconic (Germantown, NY) at 6–8 wk of age, weighing approximately 20 g. Upon arrival, the animals were allowed to acclimate for a minimum of 5 d. Each shipment of animals was randomly assigned to a treatment group, weighed, and individually identified via tail marking using a permanent marker. A preliminary analysis of variance on body weights was performed to ensure a homogeneous distribution of animals across treatment groups. The animals were housed at a maximum of five per cage in ventilated plastic shoebox cages with hardwood chip bedding. NIH-31 modified 6% irradiated rodent diet (Harlan Teklad) and bottled tap water were provided ad libitum. The temperature in the animal facility was maintained between 20 and 22°C and relative humidity between 36 and 57%. The

light/dark cycle was maintained on 12-h intervals. All animal experiments were performed in the AAALAC-accredited National Institute for Occupational Safety and Health (NIOSH) animal facility in accordance with an animal protocol approved by the Institutional Animal Care and Use Committee.

### Concentration Range-Finding Studies

Concentration range-finding studies were performed to select the concentrations of COREXIT 9500 and DSS to be used for dermal exposures. BALB/c mice were exposed topically to PG vehicle or increasing concentrations of test article (12–100%) in PG on the dorsal surface of each ear (25 μl per ear) for 3 consecutive days. Animals were allowed to rest for 2 d following the last exposure and then weighed and examined for signs of toxicity, such as loss of body weight, fatigue/lack of activity, and ungroomed fur. The maximum concentration selected for the subsequent studies was based on limits of toxicity.

### Combined Local Lymph Node and Irritancy Assay

To determine the irritancy and sensitization potential of COREXIT 9500A and DSS, a combined LLNA was conducted. COREXIT (0.25–25%) and DSS (0.05–15%) dosing concentrations were based on the results of a concentration range-finding study. The LLNA was performed according to the method described in the ICCVAM Peer Review Panel report (1999) with minor modifications. Briefly, mice (5 per group) were topically treated with PG vehicle, increasing concentrations of test article, or positive control (30% alpha-hexylcinnamaldehyde; HCA) on the dorsal surface of each ear (25 μl per ear) for 3 consecutive days. 2,4-Dinitrofluorobenzene (DNFB) was used as a positive control for the irritancy portion of the experiment. Irritancy measurements were performed as previously described (Woolhiser et al. 1999). The thickness of the right and left ear pinnae of each mouse was measured using a modified engineer's micrometer (Mitutoyo Co.) before the first chemical administration and 24 h following the final exposure. The mean percent ear swelling was calculated based on the following equation: [(Mean postchallenge ear thickness – mean prechallenge ear thickness)/mean prechallenge thickness] × 100. Animals were allowed to rest for 2 d following the last exposure. On d 6, mice were injected, intravenously (iv), via the lateral tail vein with 20 μCi [$^3$H]thymidine (Dupont NEN; specific activity 2 μCi/mmol). Five hours after [$^3$H]thymidine injection, animals were euthanized via $CO_2$ inhalation, and the left and right superficial parotid draining lymph nodes (DLN) located at the bifurcation of the jugular vein were excised and pooled for each animal. Single-cell suspensions were made and incubated overnight in 5% trichloroacetic acid (TCA), and samples were counted using a Packard Tri-Carb 2500TR liquid scintillation analyzer (Perkin Elmer). Stimulation indices (SI) were calculated by dividing the mean disintegrations per minute (DPM) per test group by the mean DPM for the vehicle control group. EC3 values (concentration of chemical required to induce a threefold increase over the vehicle control) were calculated based on the equation from Basketter et al. (1999).

### Phenotypic Analysis of Lymphocytes

To further evaluate the mechanisms of the hypersensitivity response, phenotypic analysis of lymphoid tissue was conducted following dermal exposure to COREXIT 9500A using flow cytometry. For the phenotypic analysis, COREXIT 9500A was tested at concentrations up to 25%. Toluene-2,4-diisocyanate (TDI) (1.5%) was included as a positive control for the total IgE, cytokine, and phenotyping portions of the experiment. Lymph-node cell phenotypes were analyzed using flow cytometry as described by Manetz and Meade (1999). Mice were topically exposed to PG or increasing concentrations of COREXIT 9500A on the dorsal surface of each ear (25 μl per ear) for 4 consecutive days. Animals were allowed to rest for 6 d after the final treatment and then

euthanized on d 10 by $CO_2$ inhalation. Animals were weighed and examined for gross pathology at the end of the experiment. The following organs were removed, cleaned of connective tissue, and weighed: liver, spleen, kidneys, and thymus. DLN (two nodes/animal/tube) and spleens were collected separately in 3 ml phosphate-buffered saline (PBS) and dissociated using the frosted ends of two microscope slides. Cell counts were performed using a Coulter Counter (Z2 model, Beckman Coulter), and $1 \times 10^6$ cells per sample were added to the wells of a 96-well plate. Cells were washed using staining buffer (1% bovine serum albumin/0.1% sodium azide in PBS) and then incubated with Fc block (clone 2.4G2). For IgE+/B220+ analysis, the cells were incubated with anti-CD45RA/B220 (PE, clone RA3-6B2) and anti-IgE antibodies (FITC, clone R-35-72) or appropriate isotype control diluted in staining buffer. For analysis of T-cell subsets, cells were incubated with anti-mouse CD3e antibody (APC, clone 145-2C11), anti-mouse CD4 antibody (FITC, clone RM4-5), and anti-mouse CD8a antibody (PE, clone 53-6.7) or the appropriate isotype controls diluted 1:100 in staining buffer. All antibodies and isotype controls were purchased from BD Pharmingen. Cells were then washed and incubated with propidium iodide (PI). After a final wash, cells were resuspended in staining buffer and analyzed with a Becton Dickinson FACSCalibur flow cytometer using a PI viability gate.

### Total Serum IgE

Following euthanasia of animals used in the phenotypic/IgE/cytokine analysis assays, blood samples were collected via cardiac puncture. Sera were separated by centrifugation and frozen at –20°C for subsequent analysis of IgE by enzyme-linked immunosorbent assay (ELISA). The standard colorimetric sandwich ELISA was performed as previously described (Butler 2000). All antibodies and isotype controls were purchased from BD Pharmingen. Briefly, 96-well flat bottom plates (Dynatec Immulon-2) were coated with (2 μg/ml in PBS) purified monoclonal rat anti-mouse IgE antibody (clone R35-72), sealed with plate sealers, and incubated overnight at 4°C. The plates were washed thrice with PBS/Tween 20 and then blocked for 1 h with 2% newborn calf serum (NCS) and 0.05% sodium azide at room temperature. Initial dilutions (1:10) were made from the serum samples, and IgE control standards were prepared at 500 ng/ml. All dilutions were made in 2% NCS and 0.05% sodium azide. Serum samples and IgE control standard (mouse IgE anti-TNP, clone C38-2) were serially diluted (1:2), added to the coated plates in a 100 μl volume, and incubated at room temperature for 1 h. The plates were washed thrice with PBS/Tween 20. Biotin-conjugated rat anti-mouse IgE (clone R35-92) was added in a 100-μl volume and plates were incubated at room temperature for 1 h. The plates were washed thrice with PBS/0.05%Tween 20. Streptavidin–alkaline phosphatase was added (100 μl of a 1:400 dilution) and plates were incubated for 1 h at room temperature. *p*-Nitrophenyl phosphate (Sigma) was used as the alkaline phosphatase substrate and added to the plates in a 100-μl volume. The plates were allowed to develop at room temperature for up to 30 min or until the optical density (OD) reading of the highest standard reached 3. Absorbance was determined using a Spectramax Vmax plate reader (Molecular Devices) at 405–605 nm. Data analysis was performed using the IBM Softmax Pro 3.1 (Molecular Devices), and the IgE concentrations for each sample were interpolated from a standard curve using multipoint analysis.

### Cytokine Analysis

An aliquot of DLN cells from the mice used for phenotyping/IgE studies was evaluated to determine cytokine protein production by lymphocytes. Half a million cells (adjusted by dilution in sterile RPMI media containing 10% FCS) were added to a 48-well plate in a 500-μl volume, stimulated with α-CD3 and α-CD28 (2 μg/ml of each; BD Pharmingen), and incubated for 24 h at 37°C and 5% $CO_2$. Supernatants were analyzed for IL-4 and IFN-γ production using an OptEIA

ELISA kit purchased from BD Biosciences according to the manufacturer's instructions. Supernatants collected from each culture (two stimulated and two unstimulated for each mouse) were added to the plates in triplicate along with serial dilutions of the standards. Plates were read at 450 nm (OD values for standards ranging from 0.77 to 1.93) using a SpectraMax M2 spectrophotometer (Molecular Devices). Cytokine concentration was extrapolated from the standard curve. The final data are expressed as the mean value generated when the concentration identified for the nonstimulated cultures was subtracted from the value generated from the stimulated cultures for each mouse.

### Mouse Ear Swelling Test (MEST)

The mouse ear swelling test (MEST) followed the original procedure outlined by Gad et al. (1986) with minor modifications. Mice (5 mice per group) were exposed to 50 μl of the acetone vehicle, increasing concentrations of COREXIT 9500A (5, 10, or 20%) or DSS (3, 7, or 15%), or positive control (0.5% DNFB) on the dorsal clipped thorax once daily for 4 consecutive days (d 1–4). Concentrations evaluated were nonirritating based on the results from the irritancy assay (see Figures 1A and 3A). The animals were rested on d 5–10. On d 11, the ears of the animals were premeasured in duplicate using a modified engineer's micrometer and then the animals were challenged with 25 μl acetone, 7% COREXIT, 7% DSS, or 0.15% DNFB on the dorsal surface of the right ear pinna. Post treatment ear measurements were taken at 24, 48, and 72 h time points. Mice were sacrificed after the 72-h ear measurement. A percent ear swelling for each animal was calculated as described for the irritancy assay. Mean percent ear swelling for each dose group was compared to the mean percent ear swelling for the background control group, to control for irritation of the challenge dose. The background control group (Student's *t*-test) was compared to unexposed vehicle control to assess swelling caused by nonspecific irritation.

### In Vivo IgM Response to the T-Cell-Dependent Antigen SRBC

The primary IgM response to sheep red blood cells (SRBC) was enumerated using a modified hemolytic plaque-forming cell (PFC) assay of Jerne and Nordin (1963). B6C3F1 mice were dermally exposed to COREXIT 9500A (0.25–5%) for 14 d (25 μl/ear). Four days prior to euthanasia (day 11), the mice were immunized with 7.5 × 107 SRBC by iv injection in a 200-μl volume. All SRBC for these studies were drawn from a single donor animal (Lampire Laboratories, Pipersville, PA). On d 14, mice were euthanized by $CO_2$ asphyxiation, body and organ weights were recorded, and spleens were collected in 3 ml Hanks balanced salt solution (HBSS). Single-cell suspensions of the spleens from individual animals were prepared in HBSS by disrupting the spleen between the frosted ends of microscopic slides. To identify the total number of spleen cells, 20 μl of cells was added to 10 ml isoton buffer (1:500) and 2 drops of Zap-o-globin were added to lyse red blood cells. Cells were then counted using a Coulter Counter. Dilutions at 1:30 and 1:120 were made of spleen cells. One hundred microliters of a dilution was added to a test tube containing 0.5 ml warm agar/dextran mixture (0.5% Bacto-Agar, DIFCO; and 0.05% DEAE dextran, Sigma), 25 μl of 1:1 ratio of SRBC suspension, and 25 μl of 1:4 dilution (1 ml lyophilized) guinea pig complement (Cedarlane Labs). Each sample was vortexed, poured onto a petri dish, covered with a microscope coverslip, and incubated for 3 h at 37°C. The plaques (representing antibody-forming B-lymphocytes) were viewed and quantified after this incubation. Results were expressed as specific activity (IgM PFC per $10^6$ spleen cells) and total activity (IgM PFC per spleen).

### Statistical Analysis

Data were first tested for homogeneity using Bartlett's chi-squared test. If homogeneous, a one-way analysis of variance (ANOVA) was conducted. If the ANOVA showed significance at $p < .05$, Dunnett's multiple-range

*t* test was used to compare treatment groups with the control group. Linear trend analysis was performed to determine whether the test article had exposure concentration-related effects for the specified endpoints. Results were considered significant at $p < .05$.

## RESULTS

### In Vivo Treatment with COREXIT Induced Systemic Toxicity

There were no COREXIT 9500A- or DSS-related animal deaths in these studies. However, animals exposed to concentrations of COREXIT 9500A greater than 25% exhibited severe irritation and a loss (>10%) in body weight. Mice exposed to concentrations of COREXIT 9500A (25% or less) or DSS (15% or less) appeared clinically normal with no overt clinical toxicity (based on visual monitoring for appearance of ruffled fur, and discharge from eyes, nose, or anus) and no significant loss of body weight (data not shown). Due to the toxicity described earlier, 25% COREXIT 9500 was the highest concentration used for the subsequent studies.

### In Vivo Studies Identified COREXIT to Be an Irritant and Allergic Sensitizer

To evaluate irritancy potential, ear swelling was measured in mice following dermal exposure to COREXIT 9500A. A dose-dependent significant increase in ear swelling was observed after COREXIT 9500A treatment at 15% dose (72% ear swelling) 24 h post final chemical exposure (Figure 1A). DNFB at 0.3% was used as a positive control for irritancy studies and resulted in an average increase of 122% ear swelling post application. In the LLNA, dose-dependent significant elevation in DLN proliferation was noted after treatment with COREXIT 9500A, with counts from the

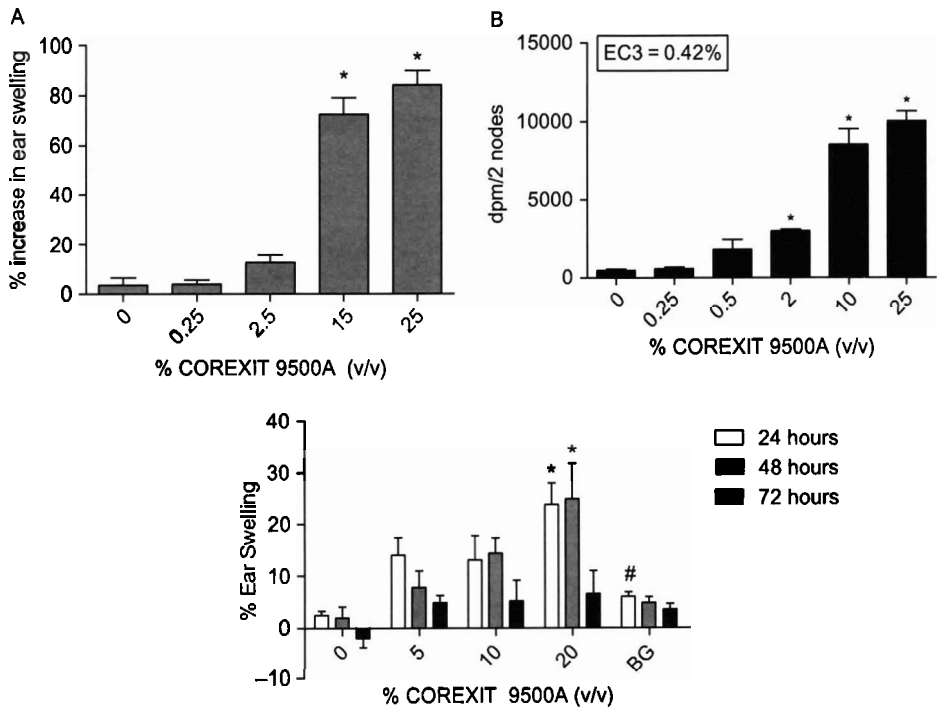


**FIGURE 1.** Irritancy and allergic sensitization potential following dermal exposure to COREXIT 9500A. Analysis of irritancy (A) and allergic sensitization potential [LLNA (B), MEST (C)] following dermal exposure to COREXIT 9500A. For the MEST, measurements were taken on the right ear while the left ear was included as a negative control. Bars represent means ± SE of five mice per group. Levels of statistical significance are denoted by asterisk for $p \leq .05$ compared to background (BG) control or vehicle control (irritancy and LLNA); # indicates statistically significant compare to the vehicle control (Student's *t*-test).

2, 10, and 25% exposed animals (Figure 1B). An EC3 value of 0.42% was calculated. HCA (30%) was used as a positive control for these experiments and resulted in an average SI value of 13.7.

### Evaluation of Th1 and Th2 Responses Following Exposure to COREXIT

**Lack of IL-4 Cytokine Production and Local/Systemic IgE Do Not Support a Th2 Response** Exposure to COREXIT 9500A (at any concentration) did not markedly elevate IL-4 production by stimulated DLN (Figure 2A). In addition, no significant changes were observed in total serum IgE levels following exposure in any treatment group (Table 1). Consistent with the total IgE data, no marked alterations were noted in the IgE+B220+ DLN cell population (percentage or absolute number). No significant changes were observed in the spleen for any of the above mentioned cell populations (data not shown). There were no marked alterations in organ or body weights of these animals (data not shown). Dermal exposure to the positive control respiratory sensitizer TDI (1.5%) significantly elevated the B220+ (38.65 ± 3.6%) and IgE+B220+ (13.35 ± 3.1%) cell populations in the DLN, along with total serum IgE (1756 ± 201ng/ml) and IL-4 production (258 ± 68 pg/ml).

**A Positive MEST Response Along with Increases in IFN-γ Support a Th1 Response** Levels of IFN-γ cytokine production by stimulated draining lymphoid cells were analyzed to further evaluate the effect of COREXIT 9500A exposure on Th1/Th2 balance. A dose-responsive significant rise in IFN-γ protein production by DLN was observed after dermal exposure to COREXIT 9500A. Significant elevations in cytokine production were found following exposure to 25% COREXIT (Figure 2B).

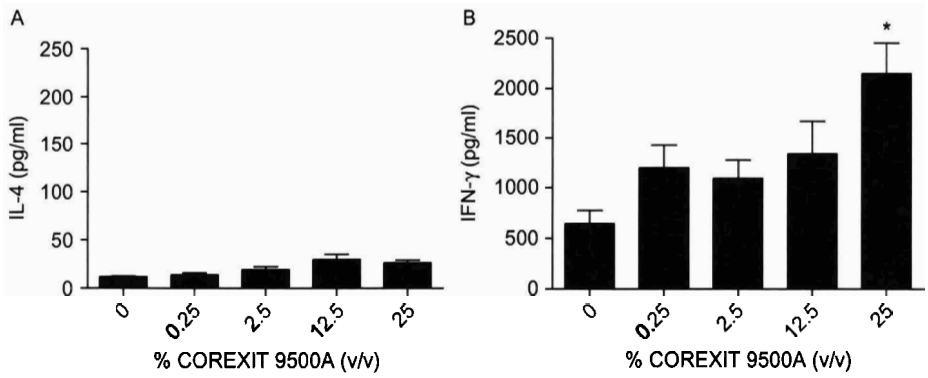


**FIGURE 2.** COREXIT 9500A sensitization results in increased IFN-γ protein expression in the draining lymph nodes. Analysis of IL-4 (A) and IFN-γ (B) and protein expression generated by stimulated DLN after dermal exposure to COREXIT 9500A. Bars represent mean ± SE of five mice per group. Levels of statistical significance are denoted by asterisk for $p \leq .05$ as compared to PG control.

**TABLE 1.** Phenotypic and IgE Analysis After in Vivo COREXIT 9500A Treatment

| Dose group | T cells (% lymphocyte population) | Cells × $10^6$ | IgE+B220+ (% lymphocyte population) | | B220+(% lymphocyte population) | | Total IgE (ng/ml) |
|---|---|---|---|---|---|---|---|
| | | | % | Cells × $10^6$ | % | Cells × $10^6$ | |
| PG COREXIT 9500A | 90.68 ± 1.13 | 3.40 ± 0.23 | 1.51 ± 0.04 | 0.06 ± 0.003 | 13.31 ± 0.76 | 0.49 ± 0.04 | 342 ± 132 |
| 0.25% | 90.07 ± 0.79 | 3.70 ± 0.54 | 0.08 ± 0.12 | 0.03 ± 0.005 | 13.00 ± 0.82 | 0.54 ± 0.09 | 192 ± 40 |
| 2.5% | 88.47 ± 2.36 | 5.09 ± 0.84 | 1.11 ± 0.34 | 0.06 ± 0.030 | 10.47 ± 2.8 | 0.69 ± 0.24 | 376 ± 109 |
| 12.5% | 87.04 ± 0.76 | 11.25 ± 0.90* | 0.58 ± 0.05 | 0.07 ± 0.012 | 13.49 ± 0.55 | 1.76 ± 0.21* | 255 ± 128 |
| 25% | 82.30 ± 0.96 | 12.61 ± 0.57* | 0.06 ± 0.04 | 0.16 ± 0.011 | 16.35 ± 0.67 | 2.50 ± 0.14* | 524 ± 262 |

*Note.* Values present group mean (n = 5) ± SE. Levels of statistical significance are denoted by asterisk for $p \leq .05$ compared to vehicle (PG).

The maximal rise in IFN-γ protein expression was calculated to be 2140 ± 307 pg/ml. Phenotypic analysis of the DLN cells also identified significant dose responsive increases in the absolute number of B cells and T cells at 12.5% treatment (Table 1). Similar to the trend observed with total T cells, the absolute numbers of CD4+ and CD8+ cells were significantly increased following exposure to COREXIT 9500A (12.5% and higher; data not shown). After prior sensitization to COREXIT 9500A (20%), significant elevation in ear swelling were observed at 24 and 48 h post challenge (7%) with the peak increase occurring at 48 h (25% rise) (Figure 1C). A quantitative increase in ear swelling was observed for the background control animals (irritancy control) at the 24-h time point following COREXIT exposure. DNFB induced significant elevation in ear swelling at 24, 48, and 72 h post challenge (108 ± 14.4%, 152 ± 13.7%, and 134 ± 17.1%).

### Dermal Exposure to COREXIT 9500 Did Not Alter the IgM Response to SRBC

To evaluate immunosuppressive potential, the murine splenic IgM response to SRBC was examined following a 14-d exposure to COREXIT 9500A. No biologically significant changes in total (PFC/spleen) or specific (PFC/$10^6$ cells) IgM antibody activity to SRBC were observed following exposure to any concentration of COREXIT 9500A (Figure 3). Animals exposed to the positive control, cyclophosphamide, displayed a significantly reduced specific spleen IgM response (87%) and total IgM response (59%) compared to vehicle control. No marked changes in body or organ weights were observed for these animals (data not shown).

**In Vivo Studies Identify DSS to be an Irritant and Allergic Sensitizer** In an attempt to identify the contribution of DSS to the hypersensitivity response elicited by COREXIT, DSS was evaluated for irritancy and allergic potential. A dose-dependent increase in ear swelling was observed after DSS exposure, reaching statistical significance at 7.5% (18%) and 15% (44%) concentrations 24 h post final chemical exposure (Figure 4A). DNFB at 0.3% was used as a positive control for irritancy studies and resulted in an average significant elevation of 167% ear swelling post application. In the LLNA, a dose-dependent significant increase in DLN proliferation was identified after treatment with DSS, with counts from the 7.5% and 15% exposed animals (Figure 4B). An EC3 value of 3.9% was calculated. HCA (30%) was used as a positive control for these experiments and resulted in an average SI value of 13.7. After prior sensitization to DSS, significant elevation in ear swelling was noted in all dose groups (3–15%) at 24 and 48 h post challenge (Figure 4C), with peak rise for each group occurring at 48 h (72% for 3%, 86% for 7%,

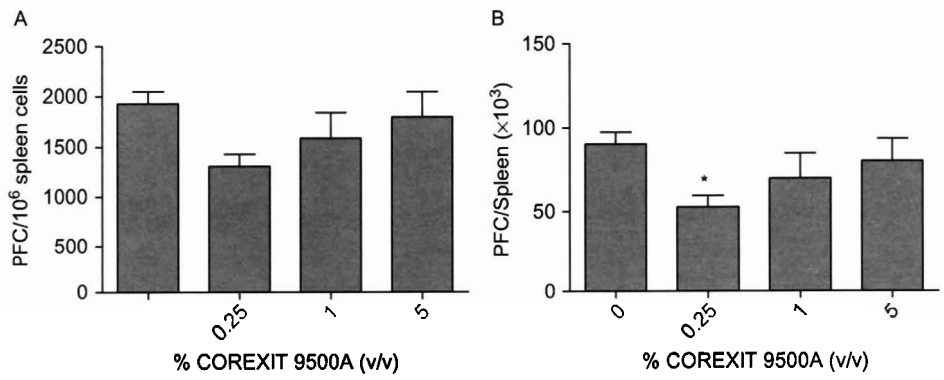


**FIGURE 3.** COREXIT 9500A exposure does not alter the IgM response to SRBC. Analysis of antibody producing spleen cells after a 14-d dermal exposure to COREXIT 9500A did not produce alterations in the total (A) and specific activity (B) IgM response to SRBC. Bars represent mean ± SE of five mice per group.

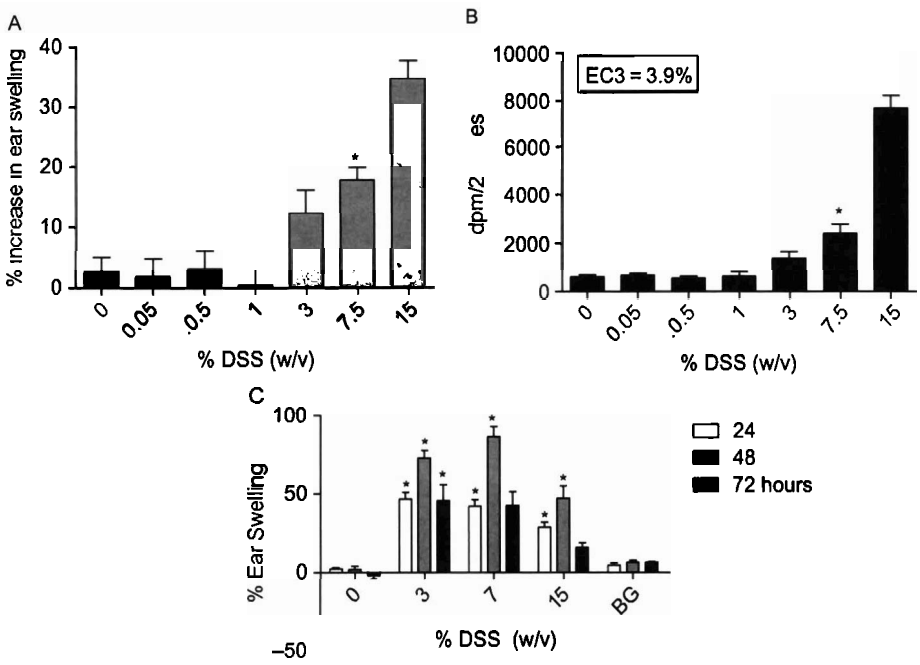


**FIGURE 4.** Irritancy and allergic sensitization potential following dermal exposure to DSS. Analysis of irritancy (A) and allergic sensitization potential (B, C) of DSS. For the MEST, measurements were taken on the right ear while the left ear was included as a negative control. Bars represent means ± SE of five mice per group. Levels of statistical significance are denoted by asterisk for $p \leq .05$ as compared to background (BG) control or vehicle control (irritancy and LLNA); # indicates statistically significant compare to the vehicle control (Student's *t*-test).

and 47% for 15%). No swelling was observed in the DSS background control animals. The DNFB-exposed positive control group experienced significant elevation in ear swelling at 24, 48, and 72 h post challenge (108 ± 14.4%, 152 ± 13.7%, and 134 ± 17.1%).

## DISCUSSION

More than 13 million workers in the United States are potentially exposed to chemicals, such as the ones described here that are absorbed through the skin. Chemical exposure might lead to contact dermatitis, the most common occupational skin disorder, which is responsible for up to 30% of all cases of occupational disease in industrialized nations. Epidemiologic data suggest that contact dermatitis accounts for 90–95% of all cases of occupational skin disease that potentially reduce quality of life and impose considerable social and economic implications (Ingber and Merims 2004; Lushniak 2004). Time off from work, loss of workplace productivity, and medical and worker's compensation costs are several factors accounting for the loss of billions of dollars.

The standard LLNA was not originally evaluated for the testing of formulations. However, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICVAAM) recently recommended, due to a nomination by the U.S. Consumer Product Safety Commission, reevaluation of the LLNA applicability domain allowing the LLNA to be used to test any chemical or product, including pesticide formulations, metals, substances in aqueous solutions, and other products such as natural complex substances and dyes, unless the chemical or product to be tested has properties that may interfere with the ability of the LLNA to detect skin-sensitizing substances (ICCVAM 2010). This assay was selected for

use in these studies to evaluate a complex mixture and its active ingredient for hypersensitivity. Using a similar dosing regimen, DLN were excised and analyzed to the identify mechanism of the allergic response.

In addition to those involved in the Deepwater Horizon cleanup, the results from these studies are also relevant to the general public. In addition to its use as a dispersant, examples of everyday products with specific ingredients in common with COREXIT 9500A include use as a wetting agent in dry gelatin, beverage mixtures and fruit juice drinks, skin creams and body shampoos, baby bath liquids, cosmetics, and surface-active agents, and as emulsifiers for agents used in food contact, household cleaning products, hand creams and lotions, odorless paints, and stain blockers. Exposure to these products may therefore present a potential adverse health risk, dependent upon which chemicals are present and at what concentration (Nalco 2010). The MSDS sheet for COREXIT 9500A stated that it is not expected to be a sensitizer; however, sufficient data supporting this statement were not identified. While the studies described here did not find COREXIT 9500A exposure to be immunosuppressive, the results suggest that COREXIT 9500A and chemicals contained within this complex mixture may be capable of inducing an allergic hypersensitivity reaction following dermal exposure. The potential for occupational exposure to COREXIT 9500A and other dispersants is high, especially during disasters such as the Gulf oil spill. It is recommended by the manufacturer that COREXIT 9500A be applied in an undiluted form to generate a dispersant-to-oil ratio of 1:50 to 1:10. The maximum concentration (25%) tested in these studies induced significant increases in lymphocyte proliferation (EC3 = 0.42%), suggesting that COREXIT 9500A may induce allergic sensitization at working concentrations.

DSS, an organic sulfonate, is a synthetic chemical detergent used as a surfactant to emulsify oil and allow its dispersion into water. DSS is considered to be the active ingredient and, based on data presented here, most likely the causative agent of sensitization by COREXIT 9500A. The manufacturer's MSDS for COREXIT 9500A states that there is approximately 10–30% of DSS present in the product. The concentrations tested here (EC3 = 3.9%) coincide with working concentrations of COREXIT 9500A and raise concern for potential worker sensitization. The lower EC3 value for COREXIT 9500A (0.42%) compared to DSS may be a result of the complexity of the mixture. Factors such as the interaction of irritants with sensitizing chemicals, such as DSS, may produce alterations in chemical absorption and/or additional increases in lymphocyte proliferation. There is also the potential for other sensitizing chemicals to be present in COREXIT 9500A, which may result in a lower EC3 value via a synergistic or additive effect. These data also have implications beyond those involved in the Gulf oil spill and cleanup. DSS is a high-volume chemical with production exceeding 1 million lb annually in the United States (U.S. EPA 1990). In addition to its use as a component of the dispersant COREXIT 9500A, DSS is also as an emulsifying and wetting agent, as a pesticide, and as a common ingredient in numerous consumer products, including laxatives of the stool softener type, acrylic adhesives, water-thinned interior paints and tinting bases, glass window cleaning preparations, laundry starch preparations, cosmetic creams (except eye and manicuring creams), other interior water-thinned coatings, specialty performance sealants, and water-thinned interior undercoaters and primers.

The MSDS indicates that DSS is a potent irritant for eyes and lungs but is not known to be carcinogenic, mutagenic, or teratogenic. There are limited sensitization studies describing results for DSS. A repeated-insult patch test (RIPT) of a product containing DSS (concentration of 0.42%) was conducted using 100 volunteers. Following a 3-wk treatment (9 exposures), none of the volunteers displayed a reaction to the challenge (CTFA 1991). These results are not contradictory to the findings of this study, given that the concentration of DSS was well below the identified EC3 value of 3.9%. Dermatitis coinciding with the application of a plaster-of-Paris cast lined with an

orthopedic wool was observed in six patients (Staniforth and Lovell 1981). Staniforth and Lovell (1981) patch tested these patients with 4 chemicals used to process the wool at 1, 10, and 100% concentrations along with gypsona (100%), benzalkonium chloride (0.1%), and cetrimide (0.1%). Only DSS yielded a positive reaction in all patients. In addition, a case report was identified that describes allergic contact dermatitis in a 28-year old woman resulting from DSS present in a topical corticosteroid (Lee and Lee 1998).

This study describes the use of the LLNA to identify a sensitizing component of COREXIT 9500A, the dispersant used in the cleanup of the Deepwater Horizon Gulf oil spill. COREXIT 9500A was identified as an irritant and tested positive for allergic contact sensitization in the LLNA and MEST when tested at working concentrations, supporting a Th1 hypersensitivity response. The active ingredient in COREXIT 9500A, DSS, also tested positive for irritancy and sensitization potential, suggesting implications for workers and the general public beyond those involved in the Gulf oil spill.

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