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14. ABSTRACT

Exposures to diesel exhaust (DE) and other petrochemical combustion products were the exposures reported by the greatest percentage of all Gulf War veterans (GWV). Along with diesel exhaust and other chemical exposures, psychological stress has been implicated in the onset of unexplained symptoms such as chemical sensitivity among GWV. The purpose of the proposed study is to test a model for chemical sensitivity in GWV, in which simultaneous acute exposures to DE and psychological stress cause increased symptoms via the acute phase response (APR), in susceptible individuals. Individuals who are low or high in the susceptibility factor of chemical intolerance (CI) will be exposed to DE either with or without a public speaking task, an acute psychological stressor. To date, 72 subjects have completed the protocol. The mean concentration in any single diesel exposure remained within ± 10% of the target PM mass concentration of 300µg/m3. Preliminary data indicates that relative to clean air, subjects report a small increase in symptoms following the onset of diesel exposure. Relative to pre-stress values, CD4/CD8 ratios are reduced and NK cells increased following the stressor. Analysis of induced sputum cell differential counts show a high proportion of macrophages, verifying that the sputum originated in the airways. The percentages of neutrophils and macrophages are similar to reference values reported in other studies (Spanevello, et al. 2000). No hypothesis tests will be performed until the sample is complete.

15. SUBJECT TERMS
Diesel exhaust, chemical sensitivity, stress, acute phase response, symptoms, inflammation, chemical intolerance

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INTRODUCTION

Exposure to DE, kerosene, and/or other petrochemical vapors and incomplete combustion products was the exposure reported by the greatest percentage of all GWV (Kang et al., 2000) and was, along with numerous other self-reported exposures, associated with increased risk of various medically unexplained symptoms, including chemical sensitivity symptoms (Spencer, 2001; Fiedler et al. , 2004, Wolfe et al., 2002). Many airborne chemical exposures may cause irritation of the eyes and respiratory tract, and recent controlled exposure studies have shown that DE (DE) can cause an acute inflammatory response in the respiratory tract. Such chemical exposure alone, however does not satisfactorily account for the multiple unexplained symptoms of veterans, and the stress of deployment and war has been cited as a contributory factor (Presidential Advisory Committee on GWV' Illnesses, 1996). We are proposing to test a model for low-level chemical sensitivity in GWV (GWV's), in which simultaneous, acute exposure to DE and psychological stress cause symptoms through a common pathway, the acute phase response (APR). As demonstrated in experimental studies, the APR is activated independently by both psychological stress and local airway inflammation caused by acute inhalation of DE and other air contaminants. A main objective of the proposed study is to test the effects of an interaction between acute psychological stress and airway inflammation due to DE, an interaction that has not been studied previously. We will test several hypotheses suggested by this model, namely that: 1) Acute inhalation exposure to DE will cause a measurable local inflammatory response in the upper and lower respiratory tract, 2) Acute DE exposure alone will cause an APR, 3) An acute psychological stressor alone will cause an APR , 4) Simultaneous exposure to DE and an acute psychological stressor will interact additively or synergistically to enhance the APR, and 5) An enhanced APR will be associated with increased symptoms.

While exogenous exposures, such as DE and acute psychological stress, may well contribute to the symptoms of Gulf war illness, many veterans had no apparent health effects, suggesting that some individual psychological or physiological differences may have contributed to the response, and implicating some form of increased susceptibility. Studies of symptomatic GWV, however, cannot adequately test the interaction of susceptibility and exposure due to the potentially confounding effects of illness. Therefore, we also propose to test the effects of exposure to DE and stress among healthy subjects who are low or high in the susceptibility factor of self-reported chemical intolerance, a phenomenon of undefined mechanism.

BODY

Goals and Objectives for Year 3:

August, 2005 – July, 2006

A. Complete exposure sessions, data coding, and entry for remaining 40 subjects

Final DOD and UMDNJ IRB approval was received in January of 2005. Since that time, 72 subjects have completed the study. We expect to complete running all subjects by December, 2006 when data analysis will commence. Recent efforts to increase subject recruitment include mail drop advertising to 7500 homes in Piscataway, New Jersey, and continual postings on list serves, bulletin boards, and student mailboxes at Rutgers University. We have performed preliminary analyses on selected data, the results of which we report below.

Summary of Subject Participation to Date:

DID NOT PASS TELEPHONE SCREENING:		Total
Ineligible age	1	
Asthma	5	
Smoker	6	
Known blood draw difficulties	1	
Medical reason (i.e., Hepatitis B, Medication, year-round allergies)	12	25

DROPPED OUT BEFORE PHYSICAL EXAM (Passed Telephone Screening):		
Parents Concerned	2	
Exposure/cancer concerns	3	
Schedule conflicts	19	
No show for physical exam/ could no longer contact	16	40

MEDICAL FAILURES:		
Problems with blood draw	6	
Medical or lab abnormalities	16	
Other:		
Could not perform nasal lavage	1	23

DROPPED OUT AFTER PHYSICAL EXAM:

Not able to contact	4	
Parents concerned	1	
Schedule conflicts	4	8

REMOVED FROM PARTICIPATION:

Dropped-not able to contact	1	
Vasovagal	1	
Blood draw problems	3	
Illness	3	8

COMPLETE STUDY BY END OF AUGUST 2006: 72

B. & C. Conduct data analysis, prepare manuscripts and final report

Summary data for 49 subjects who completed the exposure protocol prior to July 31, 2006 are presented below. Table 1 gives the demographics for these subjects.

Table 1

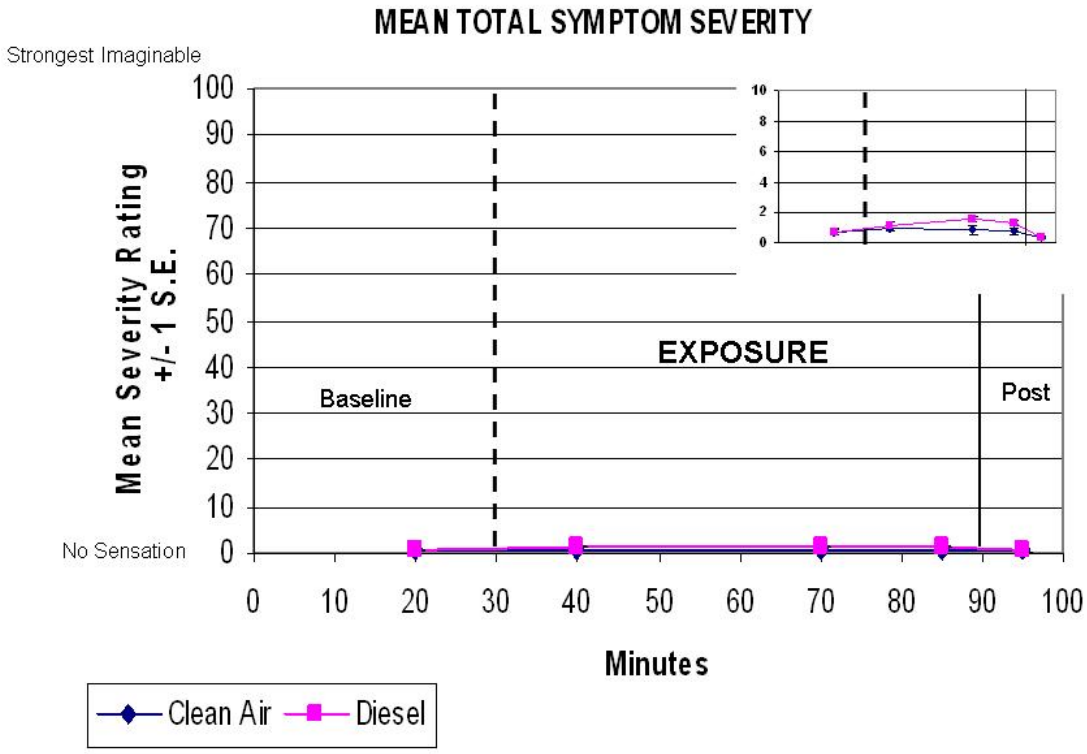
DEMOGRAPHICS OF STUDY VOLUNTEERS (N=49)

	Females/Males
Gender	14/35
	Mean (S.D.)
Age	24.5 (5.5)
Years Education	16.4 (2.5)
Chemical Odor Intolerance Index	6.5 (2.0)
	Percent (N)
Race:	
Asian	43 (21)
White	33 (16)
Hispanic	20 (10)
Black	4 (2)

Symptom data for 49 subjects at time points before, during, and after exposure are reported in Figure 1. "Total symptom severity" represents aggregate mean symptom severity on a 100-point scale for 37 symptoms elicited by questionnaire. Additional symptom severity

ratings at 6 hours and 24 hours post exposure have not been summarized yet. Results reported here show a modest increase in acute symptoms from the diesel exposure compared to the clean air control. Individual symptom means have not been examined to date, but are likely to show clearer effects for specific symptoms that have been associated with diesel exhaust exposure in previous studies (eg. eye irritation, respiratory tract irritation).

Figure 1



Blood cell counts and differentials (Flow cytometry)

Pilot analyses have been performed to affirm that our quality control procedures are working adequately for the laboratory analyses. Table 2 presents data for peripheral white blood cell counts, lymphocyte percentages, CD4 and CD8 subsets, and CD4/CD8 ratio from baseline (prior to exposure) blood collections. These results are within normal reference ranges reported. Statistically significant decreases in CD4/CD8 ratio and increases in natural killer cell counts immediately after the stressor are consistent with results reported in the literature after similar stress exposures.

Table 2

Mean Blood Leukocyte Counts at Baseline and 45 min for Each Exposure Condition

	DE/Stress			CA/Stress		
Leukocyte Type (cells/mm^3)	**Baseline Mean(SE)**	**45 min. Mean(SE)**	**% Change Mean(SE)**	**Baseline Mean(SE)**	**45 min. Mean(SE)**	**% Change Mean(SE)**
Neutrophils	3427(255)	3808(298)	12(6)	3434 (164)	3745 (240)	9(5)
Total Lymphocyte	1741(108)	1971 (106)	15(4)	1691 (59)	2009 (61)	19(3)
CD_8	458 (47)	482 (48)	8(4)	428 (25)	492 (30)	14(2)
CD_4	709 (41)	673 (32)	-5(2)	714 (33)	700 (31)	0(2)
CD_4/CD_8	1.77 (0.12)	1.63 (0.13)	-11(2)*	1.81 (0.12)	1.57(0.12)	-12(2)*
NK	158 (22)	366 (49)	144(20)*	154 (15)	348 (35)	126(13)*
	DE/No Stress			**CA/No Stress**		
Leukocyte Type (cells/mm^3)	**Baseline Mean(SE)**	**45 min. Mean(SE)**	**% Change Mean(SE)**	**Baseline Mean(SE)**	**45 min. Mean(SE)**	**% Change Mean(SE)**
Neutrophils	3498(285)	3580(305)	7(3)	3531(293)	3730(287)	8(5)
Total Lymphocyte	1775(97)	1870(103)	9(4)	1648(93)	1800(90)	11(3)
CD_8	449(39)	457(41)	7(4)	414(37)	442(39)	8(4)
CD_4	749(53)	731(51)	1(3)	693(50)	702(44)	3(3)
CD_4/CD_8	1.80(0.13)	1.75(0.12)	-5(1)*	1.81(0.11)	1.75(0.12)	-4(1)*
NK	155(17)	222(22)	54(13)*	155(15)	221(20)	50(9)*

SE – Standard Error
% Change = Post-stress-Baseline/Baseline x100
* significant for main effect of stress at $p<0.01$

Plasma Fibrinogen

Table 3 shows baseline and 24-hr post exposure fibrinogen concentrations in plasma for a subset of subjects. These results are within normal reference ranges for plasma fibrinogen in young, healthy adults. Preliminary statistical analysis of this data with a linear mixed model showed no significant interaction between stress and DE exposures, and no significant main effects of the exposures. However, the number of subjects analyzed is too few to draw conclusions from this preliminary analysis.

Table 3

Mean Fibrinogen (mg/dl) & Standard Deviations at 24 Hours Post and Baseline for Each Exposure Condition

DE/Stress (N=21)			**CA/Stress (N=21)**		
Baseline	24 Hrs. Post		Baseline	24 Hrs. Post	
Mean(SD)	Mean(SD)	Effect*	Mean(SD)	Mean(SD)	Effect*
265.6(76.4)	267.1(76.4)	1.52	241.9(75.7)	235.8(77.4)	-6.09
DE/No Stress (N=16)			**CA/No Stress (N=16)**		
Baseline	24 Hrs. Post		Baseline	24 Hrs. Post	
225.0(56.1)	249.9(77.2)	25.00	257.7(71.4)	254.9(85.8)	-2.84
*Mean of individual differences					

Plasma Cortisol

Plasma cortisol was measured at baseline after acclimatization to the CEF (baseline), immediately prior to (pre-stress) and 20 minutes after (post-stress) the stressor or control condition. Mean values for a subset of subjects are presented in Table 4. The values are within normal reported ranges for plasma cortisol in young healthy adults. Results indicate a modest effect of the stressor on plasma cortisol comparing pre-stress to post-stress values. Conclusions await further analysis including separate analyses of a subset of individuals identified as “stress responders.”

Table 4

Mean Plasma Cortisol Levels and Standard Errors at Baseline, Pre-stress, Post-stress for Each Exposure Condition

	DE/Stress (N=26)			CA/Stress (N=26)		
Cortisol (µg/dl)	**Baseline**	**Pre-stress**	**Post-stress**	**Baseline**	**Pre-stress**	**Post-stress**
	Mean(SE)	**Mean(SE)**	**Mean(SE)**	**Mean(SE)**	**Mean(SE)**	**Mean(SE)**
	14.7(1.10)	12.8(1.08)	13.7(0.84)	13.1(1.19)	12.4(1.19)	14.6(1.09)
	DE/No Stress (N=20)			**CA/No Stress (N=20)**		
Cortisol (µg/dl)	**Baseline**	**Pre-stress**	**Post-stress**	**Baseline**	**Pre-stress**	**Post-stress**
	Mean(SE)	**Mean(SE)**	**Mean(SE)**	**Mean(SE)**	**Mean(SE)**	**Mean(SE)**
	12.9(1.30)	10.7(1.16)	10.9(1.07)	12.1(1.21)	10.8(1.23)	12.1(1.26)

SE – Standard Error

Induced sputum

Sputum induction and collection continued as described previously, with 72 subjects now completed. Differential cell counts are ongoing and results await further analysis. A subset of 20 sputum differential slides were reviewed by a hematopathologist at UMDNJ-Robert Wood Johnson Medical School, and her white blood cell differential counts were found to be in agreement with counts done in our laboratories.

KEY RESEARCH ACCOMPLISHMENTS

"Non-invasive measures of oxidative stress and inflammatory responses to diesel exhaust particles in human respiratory epithelium. Abstract presentation at the American Thoracic Society, 2005.

"The effects of diesel exhaust and stress on systemic inflammation and the acute phase response." Abstract presentation at the "Mechanisms of Action of Inhaled Fibers, Particles, and Nanoparticles in Lung and Cardiovascular Disease" conference, sponsored by NIEHS and NIOSH, on October 25-28, 2005.

"The effects of diesel exhaust and stress on the acute phase response and symptoms in the chemically intolerant" Symposium: environmental modulation of neurotoxicants in military-relevant environments". International Neurotoxicology Conference, September, 2006.

"Effects of diesel exhaust and stress on systemic inflammation and the acute phase response in humans: Acute peripheral leukocyte counts." Presented at EOHSI Research Day, May, 2006.

"Diesel exhaust and psychological stress: Redistribution of human peripheral blood leukocytes." Presented at the American Thoracic Society (ATS) 2006 International Conference, San Diego, CA, May 22, 2006.

REPORTABLE OUTCOMES

Tests of study hypotheses will be completed following testing of 100 subjects. However, based on the development of our diesel exposure capabilities, the following projects have been initiated to augment outcome measures in the current study as well as additional studies using similar exposure paradigms and outcome measures.

Kipen, H (principal investigator). Responses to Fresh Aerosol in Susceptible Subjects, funded by EPA, R832144, $1,521,398. The purpose of this study is to evaluate cardiovascular effects of diesel exhaust. Subjects will be exposed to 200 $\mu g/m^3$ $PM_{2.5}$ for 2 hours and platelet activation, endothelial dysfunction, and pulmonary inflammation through induced sputum will be measured.

"Noninvasive Measures of Oxidative Stress and Inflammatory Responses to Diesel Exhaust in Human Respiratory Epithelium." Laumbach RJ (PI). 12/2004-6/2005 Funded by NIOSH ERC Pilot project. $10,000. This study examines molecular markers of oxidative stress and inflammation in nasal respiratory epithelium samples from human subjects after controlled exposure to diesel exhaust. The goal is to develop and validate a new noninvasive techniques for studying responses to diesel exhaust in controlled exposure and epidemiology studies.

"Mechanisms of Responses to Diesel Exhaust and Stress." Laumbach RJ (PI). K08 Career Development, NIEHS. $125,000 x 5 years, This career award will provide support for development of the PI's capability to perform complementary studies of responses to diesel exhaust and stress in relevant animal models and human subjects.

"Validation of Diesel Exhaust Biomarkers". Zhang J (PI); Fiedler N (Co-I); Ohman-Strickland P (Co-I); Zhang L (Co-I); Lioy P (Co-I); Kipen H (Co-I); Laumbach R (Co-I); and Stern A (Co-I). 5/1/05 – 4/30/08. Funded by Environmental Protection Agency (EPA), Star Grant #RD832097. $572,497. The major goal of this project is to develop and validate urinary markers for exposure to diesel exhaust in human subjects utilizing controlled human exposures.

"Diesel Exposure in Mild and Moderate Asthmatics: Gene-envrionment Interactions." Hussain S (PI); Kipen HM (Co-I); Laumbach RJ (Co-I); Zhang J (Co-I). 4/1/06 – 3/31/07. Funded by NIEHS Center Pilot Project ES005022-19, $30,394. The major goal of this project is to characterize the effect of diesel inhalation on acute inflammation in mild to moderate stable asthmatics, using non-invasive measures in a controlled exposure setting.

"Diabetic Susceptibility to Procoagulant Effects of Traffic-generated Particulate Pollution." Rich D (PI); Kipen HM (Co-I), Zhang J (Co-I). 4/1/06-3/31/07. Funded by NIEHS Center Pilot Project. ES005022-19, $29,617. The major goal of this study is to examine the change in coagulation/cardiovascular indices in healthy Type II diabetics following in-car exposure to traffic pollution.

"Effects of Diesel Exhaust Particles on Human Antimycobacterial Immunity." Schwander SK (PI); Kipen HM (Co-I), Zhang J (Co-I). 4/1/06-3/31/07. Funded by NIEHS Center Pilot Project. ES005022-19, $29,822. The purpose of this study is to assess in human blood cells, how exposure to diesel exhaust particulates (DEP) affects immune responses that are required to control the growth of Mycobacterium turberculosis (M.tb).

CONCLUSIONS

Recruitment of subjects was significantly delayed due to deferral of review by the DOD IRB. Since IRB approval was received in January 2005, 13 pilot and 72 full protocol subjects have completed the study. During the pilot phase, additional technical improvements were developed and implemented. We are presently recruiting at a rate of 8 subjects per week and anticipate that the study will be completed by December 2006. Additional exposure characterization has demonstrated the reliability of our diesel exposure system. Based on preliminary review of data, techniques for measuring outcomes in induced sputum have been improved.

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