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14. ABSTRACT

Jet propulsion fuel 8 (JP8) has been recognized by the DoD as the single largest chemical exposure for its personnel. The primary aim of the project (OJENES, Occupational JP8 Exposure Neuroepidemiology Study) was to conduct an epidemiological field study to examine the relationship between JP8 fuel exposure and adverse neurological health in military (US Air Force) personnel. The research objectives include 1) determination of the individual service member's level of exposure to JP8 components measured by specified biomarkers of exposure, and 2) examination of whether exposure to JP8 over a work week is significantly associated with hypothesized neurobehavioral and neurophysiologic performance outcomes. The project was conducted in two phases: 1) onsite exposure assessment to fully characterize JP8 exposure parameters in the military occupational field setting (Tier I), 2) neuroepidemiology field study to examine predicted dose-response relationships (Tier II). Data analyses of core research hypotheses are complete. Findings describe JP8 exposure levels in a range of AF job activities. Three core manuscripts are in press or published, with six more manuscripts either submitted or in the final stages of preparation.

15. SUBJECT TERMS

Jet fuel, JP-8, neurobehavior, neurological health, exposure assessment

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Introduction

Jet propulsion fuel 8 (JP8) has been recognized by the Department of Defense (DoD) as the single largest chemical exposure for its personnel. The primary aim of the project is to conduct an epidemiological field study to examine the relationship between JP-8 fuel exposure and neurological functioning in military personnel. The research objectives include 1) determination of the individual service member's level of exposure to JP-8 components while carrying out his/her job tasks, as measured by specified biomarkers of exposure, and 2) examination of whether acute, or cumulative exposure to JP-8 over a work week is significantly associated with hypothesized neurobehavioral and neurophysiologic performance outcomes. The project has two phases: Tier I is to conduct onsite exposure assessment techniques to fully characterize JP-8 exposure parameters in the military occupational field setting required for the planned field study; Tier II is the conduct of the full-scale neuroepidemiology field study to examine predicted dose-response relationships. The field study is being carried out with military (Air Force) personnel.

Research Report Body

Statement of Work

Due to administrative delays that occurred at the beginning of this project, a request to modify the timeline of the study Statement of Work (SOW) was submitted in March 2007. Thus, the current, approved SOW (Table 1) incorporates those modifications and reflects the timeline of required tasks.

Table 1. Modified SOW, approved June, 2007

Year 1	**Months 1-12**	***Task 1***	-Obtain all required administrative approvals.
		Task 2	-Conduct planning steps, which include field site exposure measurements and samples analyzed.
		Task 3	-Convene Working Groups.
Year 2	**Months 13-24**	***Task 4***	-Conduct Tier I phase.
		Task 5	-Carry out analyses of environmental/biological samples from Tier I phase.
		Task 6	-Perform data management tasks to integrate multiple data sources for data analyses.
		Task 7	-Convene Workshop.
		Task 8	-Initiate Tier II phase.
Year 3	**Months 25-32**	***Task 9***	-Complete analyses of environmental and biological samples (Tier II).
		Task 10	-Complete data analyses of exposure-outcome hypothesis relationships (Tier II).
		Task 11	-Prepare Final Report and manuscript(s).

The project was funded November 1, 2005 and has been working under a no-cost extension since June 30, 2008. The progress made during the first 8 months of the project was reported in the 2006 Annual Report. Specifically, ***Task 1***, obtaining the required Army and Air Force administrative approvals, was completed, and progress on ***Tasks 2 & 3*** described. Progress made during months 9-20 of the project was reported in the 2007 Annual Report. Specifically, ***Tasks 2-5*** were completed; ***Task 6*** was in progress. Progress made on tasks outlined in the modified SOW for months 21 through 32 was reported in the 2008 Annual Report. Specifically, ***Tasks 6-8*** were completed; ***Tasks 9-11*** were in progress. Progress made on ***Tasks 9-11*** was reported in the 2009 Annual Report. Specifically, ***Task 9*** was completed; ***Tasks 10-11*** were in progress. Progress made on **Tasks 10-11** was reported in the 2010 Annual Report.

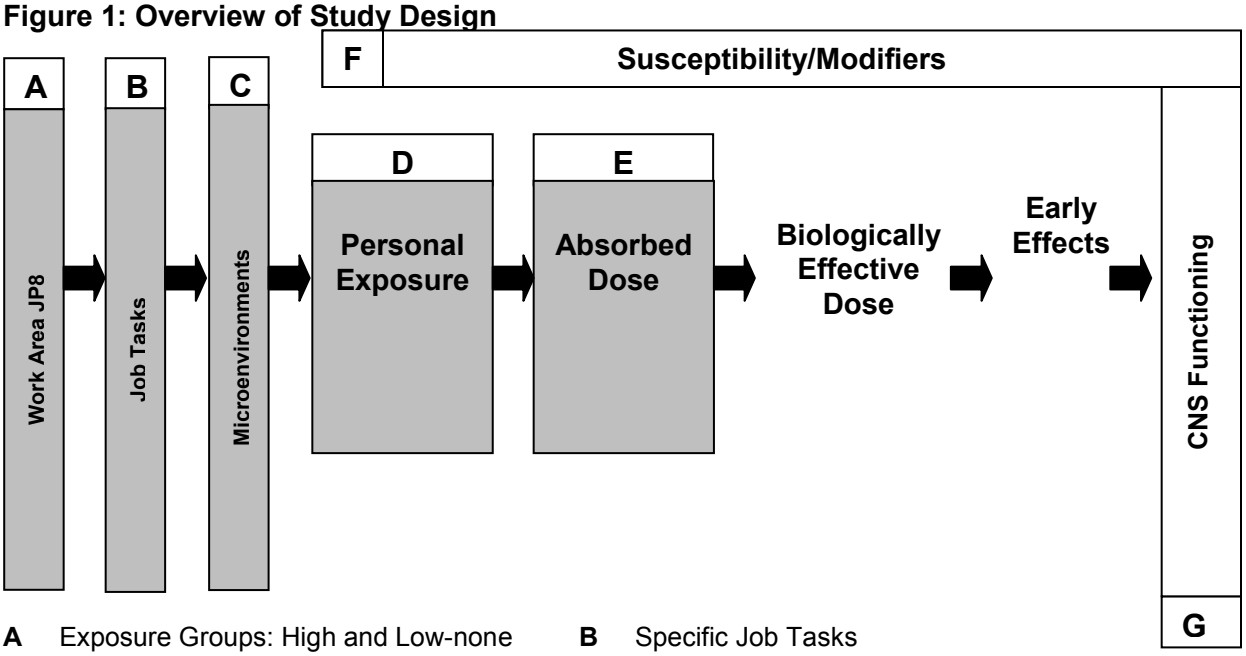
Research Progress- Final Report Summary

Summary of the work conducted over the period of performance, 1 Nov 2005 through 30 June 2011 is described below (excerpted from prior Annual Reports). Several manuscripts describing findings are published or in press; other than the completion of writing and publication of several remaining Core manuscripts, all SOW Tasks are complete.

One of the most prevalent workplace chemical exposures historically and currently confronting both the global military and civilian workforce is jet propellant (JP) fuel (e.g., JP4, JP5, JP8, jet A1). To date, numerous protective and preventive strategies (e.g., federal exposure limits, workplace procedure protocols, protective gear such as goggles, respirator use, gloves, and coveralls) have been put in place to minimize acutely toxic exposure levels. However, questions remain regarding the effect of repeated exposures at lower (than regulated) levels of JP fuel. The **Occupational JP8 Exposure Neuroepidemiology Study (OJENES)** was designed to examine the relationships between occupational JP8 exposure over multiple, repeated workdays and specific aspects of central nervous system (CNS) functioning among Air Force (AF) personnel (Proctor et al., in press).

The primary research objectives of the OJENES were 1) to characterize JP8 exposure and biological dose in an occupational setting over a typical workweek 2) to evaluate the impact of JP8 exposure on CNS functioning, specifically by examining neuropsychological and neurophysiologic (postural sway) performances, and 3) to identify potential modifiers of occupational JP8 exposure including those that may alter exposure levels (i.e., use of personal protective equipment), and those that may modify the relationship between JP8 exposure and CNS functioning (i.e. lifestyle behaviors (smoking status) and genetic polymorphisms). Two studies (tiers) were carried out as part of the OJENES project (**Figure 1**).

Figure 1: Overview of Study Design



A Exposure Groups: High and Low-none **B** Specific Job Tasks
C Job Task work area microenvironments, in terms of exposure, temperature and humidity
D Personal Exposure measures: Breathing space air and dermal samples
E Absorbed Dose measure: Exhaled breath, urine, blood
F Lifestyle factors (smoking), use of protective equipment (gloves, respirator, etc.)
G Neuropsychological and postural sway (balance) performances

In Tier I, we completed a comprehensive exposure assessment to characterize JP8 exposure and biological dose over three consecutive days across a number of AF occupational job categories and work environments (Smith et al. 2010; Smith et al. in press). Tier II was designed to understand the relationships between repeated exposure to JP8 and both personal exposure and absorbed dose levels. Using the information garnered about high and low exposure job-type categories characterized in Tier I, a second study (Tier II) was conducted using a neuroepidemiology field study design to prospectively (using repeated measures) assess JP8 exposure and CNS functioning over a 6-day work schedule.

Tier I Summary

As summarized in previous years' Annual Reports, Tier I phase data collection conducted in Jan 2007 involved a total of 24 participants at one Air Force base (AFB) (**Table 1**).

Table 1. Descriptive Characteristics of Participants in Tier I (n=24)

		Count	%	Mean	Standard Deviation	Min.	Max.
Age (yrs)				27.7	6.8	19.1	42.6
Body Mass Index (BMI = weight in lbs. X 703 / height in inches^2)				26.5	2.7	21.7	33.1
Education (yrs)				13.0	1.4	12.0	17.0
Time in Active Air Force (AF, yrs)				7.0	6.6	.5	23.0
Time in AF Current Job (yrs)				5.7	6.6	.3	22.0
Race, Ethnicity:	White, Caucasian	21/24	(87.5%)				
Sex:	Male	21/24	(87.5%)				
Smoking Status:	Yes, current smoker	7/24	(29.2%)				
Drink Alcohol:	Yes, currently	17/24	(70.8%)				
Chew Tobacco:	Yes, currently	5/24	(20.8%)				
Voluntary Exercise:	Yes	15/24	(62.5%)				
Currently Live on Base:	Yes	17/24	(70.8%)				
Ave. Hrs Worked Day:	8-10 hours	20/24	(83.3%)				

In the Tier I phase, we measured total hydrocarbon levels of personal air (area and in-tank) samples because regulated exposure levels to JP8 (by NIOSH, Army, Air Force) are based on total hydrocarbons. Naphthalene and benzene were measured primarily in order to provide historical benchmarks of exposure compared to other occupational epidemiological studies of jet fuel, some of which have been focused on carcinogenic aspects. Toluene, ethylbenzene, xylene(s), and to some degree, benzene, levels were determined as they represent the neurotoxicant components of interest with JP8 exposure. 1- and 2- naphthol levels in urine were measured as they are considered the more specific indicators of exposure to JP8 as absorbed dose markers for the naphthalene exposure resulting from JP8. (Benzene exposure may be confounded by smoking status and exposure to gasoline.)

Personal air sampling from each participant (over each of the 3 workdays; 72 samples) was conducted and the following primary analytes of interest were measured: total hydrocarbons, benzene, toluene, ethylbenzene, xylene, and naphthalene. Dermal samples were collected post-shift on each of the 3 workdays from all 24 participants (72 samples) and the following primary analytes of interest were measured: total hydrocarbons, benzene, toluene, ethylbenzene, xylene, and naphthalene. To assess personal absorbed dose levels to JP8 components, exhaled breath and urine samples were collected. Pre- and post- shift exhaled breath samples were collected on each of the 3 workdays from all 24 participants (144 samples)

and the following primary analytes of interest were measured: benzene, toluene, ethylbenzene, xylene, and naphthalene. Pre- and post- shift urine samples were collected on each of the 3 workdays from all 24 participants (72 samples) and 1- and 2- naphthols were measured as biomarkers of exposure to naphthalene.

Table 2 summarizes the results for each analyte collapsed across all 24 participants and across the study period (pre-shift, 3 work days and post-shift). The results are presented in this manner to provide the range of JP8 exposure and absorbed dose levels detected during the Tier I phase.

Table 2. Summary of Analyte Sample Results from Tier I Phase

		Analyte	Mean	Std Dev	Median	Minimum	Maximum
Urine		1-Naphthol (ng/ml)	8.97	16.30	2.57	0.15	102.60
		2-Naphthol (ng/ml)	9.68	12.02	5.80	0.38	83.97
Dermal		Total Hydrocarbons (ug/cm^2)	3.62	1.42	3.13	0.00	8.13
		Benzene (ng/cm^2)	21.54	8.17	19.38	8.75	58.13
		Toluene (ng/cm^2)	5.78	2.14	5.63	1.25	11.88
		Ethylbenzene (ng/cm^2)	1.81	1.74	1.25	0.63	12.50
		m/p-Xylene (ng/cm^2)	7.05	8.58	4.38	1.25	61.25
		o-Xylene (ng/cm^2)	2.13	2.22	1.25	0.63	15.63
		Naphthalene (ng/cm^2)	0.84	0.61	0.63	0.00	4.38
Air **		Total Hydrocarbons (mg/m^3)	5.68	13.55	1.53	0.00	101.46
		Benzene (ug/m^3)	5.85	17.22	1.48	-0.38	135.94
		Toluene (ug/m^3)	19.13	56.42	4.39	0.40	448.84
		Ethylbenzene (ug/m^3)	11.10	33.82	1.50	0.00	265.43
		m/p-Xylene (ug/m^3)	34.13	102.14	4.58	0.18	797.76
		o-Xylene (ug/m^3)	17.24	52.49	2.25	0.00	410.50
		Naphthalene (ug/m^3)	0.79	1.62	0.21	0.00	10.71
Breath		Benzene (ug/m^3)	20.10	11.50	17.74	2.91	75.26
		Toluene (ug/m^3)	26.69	29.61	17.60	-1.52	195.84
		Ethylbenzene (ug/m^3)	11.47	28.79	3.60	0.00	284.13
		m/p-xylene (ug/m^3)	39.12	123.98	9.84	0.00	1292.17
		o-xylene (ug/m^3)	19.28	66.19	2.22	0.00	654.05
		Naphthalene (ug/m^3)	0.06	0.37	0.00	0.00	3.60

** Results presented are from personal air sampling. Work area air samples (n=19) and fuel cell tank area samples (n=4) were also collected and analyzed.

Findings from Tier I indicate:

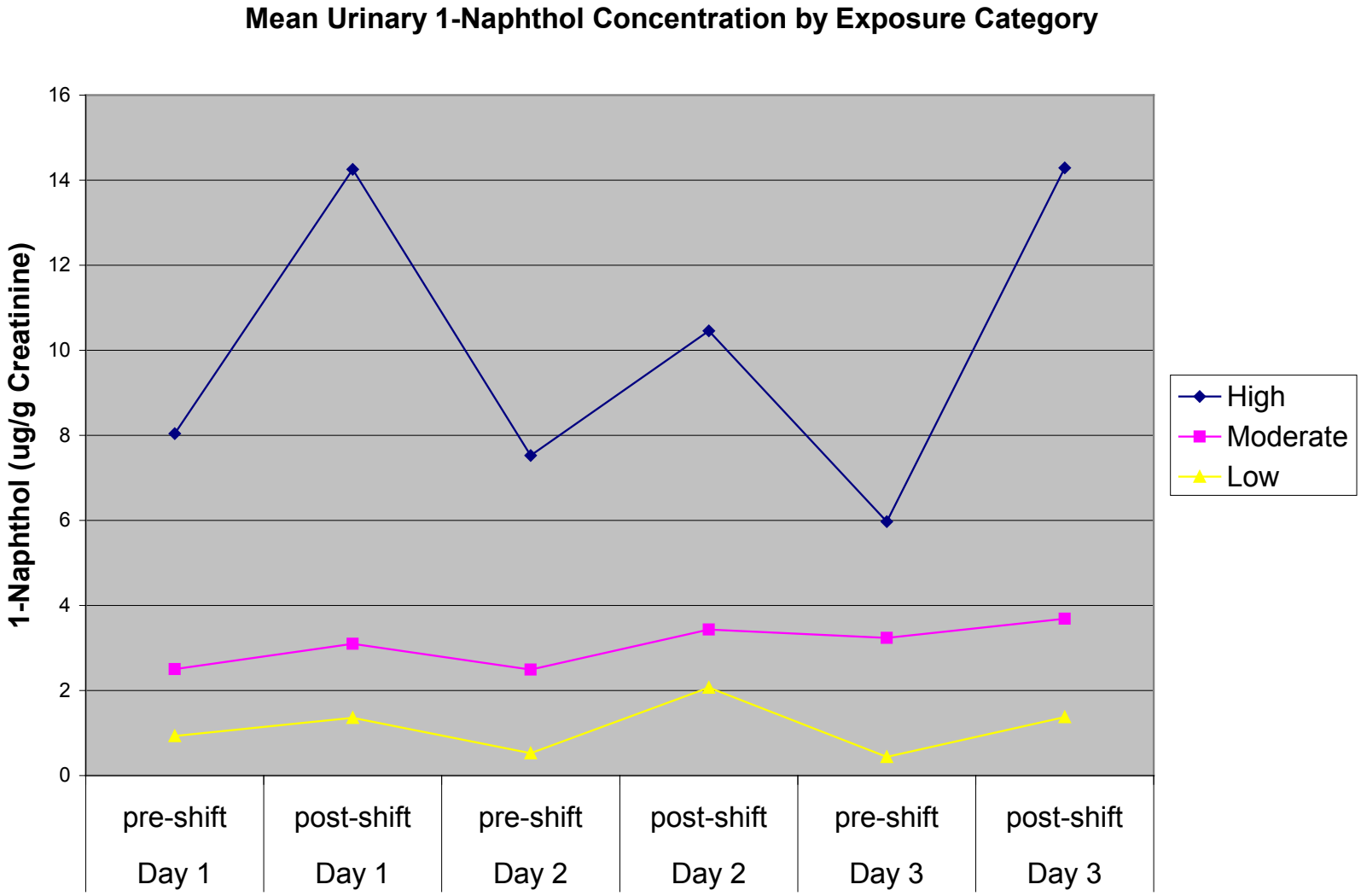
- *A priori* designated high, moderate, and low exposure groupings do distinguish personal degree of exposure to JP8. **Table 3** presents results for total hydrocarbons, benzene, and naphthalene air levels for each work day; results for toluene, ethylbenzene, and xylenes indicate similar group differences (Smith et al; 2010)
- Exposure across work days is variable within the *a priori* exposure groups related to degree of direct work task exposure to JP8, particularly within the high exposure group
- Differences are observed between absorbed doses pre- to post- shift across workdays (**Figure 2** and Smith et al; *in press*).

Table 3. Air Sampling Summary (mean values) for Exposure Groups over 3 Workdays.

	Day 1	Day 2	Day 3
Total Hydrocarbons (mg/m3)			
High exposure group	15.46	3.44	8.81
Moderate exposure group	1.86	2.84*	4.18
Low-to-no exposure group	0.03	0.00	0.05
Benzene (ug/m3)			
High exposure group	12.28	1.48	6.09
Moderate exposure group	3.56	6.11*	3.10
Low-to-no exposure group	0.92	0.57	0.32
Naphthalene (ug/m3)			
High exposure group	2.40	0.68	1.22
Moderate exposure group	0.22	0.35*	0.60
Low-to-no exposure group	0.07	0.02	0.04

* One member of the moderate group on Day 2 had outlier levels of exposure that are not included in the presented group mean summaries.

Figure 2. Tier I results



Tier II Summary

Tier II phase data collection was carried out with a total of 74 participants between January-April 2008 at three different US Air Force bases (AFB1: 25 Jan- 1 Feb 2008; AFB2: 28 Mar-4 Apr 2008; AFB3: 18-25 April 2008). In brief, the study design involved recruiting participants from higher and lower exposure group categories (based on review of their job titles and activities involving the degree of direct and routine exposure to JP8 they encountered) (**Table 4**).

Each participant was asked to participate in the study over a period of 6 work days, with his/her study participation starting on a Friday afternoon (Day 1) and continuing Monday morning through Friday morning (Days 2-6) the following week. Biological and/or environmental samples of JP8 exposure were collected from each participant every day, along with daily questionnaires and scheduled neurobehavioral task and postural sway testing. (Also see Proctor et al. *in press* for a summary of Study 2/Tier II design and methods.)

Table 4. JP-8 Tier II Baseline Survey Descriptive Statistics (n=74)

	Overall Group (n=74)	High Exposure Group + (n=38)	Low Exposure Group (n=36)	Test statistic*	p-value
Age, mean years (SD) [range]	25.8 (6.25) [18.6-43.0]	25.4 (6.23)	26.18 (6.33)	0.51	0.61
Education, mean years (SD) [range]	12.5 (1.36) [12.0-20.0]	12.3 (0.88)	12.69 (1.72)	1.12	0.27
Years of AF service, mean (SD) [range]	5.8 (5.35) [0.5-20.0]	5.6 (5.07)	6.09 (5.68)	0.51	0.61
Male, n (%)	62 (83.8)	37 (97.4)	25 (69.4)	10.61	0.001
White, Caucasian, n (%)	53 (71.6)	27 (71.1)	26 (71.2)	0.012	0.91
Current smoker, n (%)	41 (55.4)	18 (47.4)	23 (63.9)	2.04	0.15

+ High and Low exposure groups from *a priori* categorizations based on job-type activities.
* Comparison between High and Low exposure groups; Student's t-test statistic for comparison of continuous variables or Chi-square test statistic for comparison of categorical variables.

In Tier II, we collected area air, personal breathing zone air, urine, dermal, exhaled breath, and blood from each participant on multiple work days.

- Area air samples were collected on each of 4 consecutive work days (Mon-Thurs) during the 6-day study from each of the workplace areas were study participants worked and sent to the Organic Chemistry Analytical Laboratory at the Harvard School of Public Health (HSPH) and analyzed[1] for total hydrocarbon (THC), benzene, toluene, ethylbenzene, m,p-xylene, o-xylene, and naphthalene.
- Personal breathing zone air samples were collected via active sampling methods from each participant on each of 4 consecutive work days during the 6-day study and sent to the Organic Chemistry Analytical Laboratory at HSPH and analyzed[1] for THC, benzene, toluene, ethylbenzene, m,p-xylene, o-xylene, and naphthalene.

[1] Air samples were analyzed for THC with gas chromatography with flame ionization detection (GC/FID) (NIOSH method 1550). Air samples were analyzed for benzene, toluene, ethylbenzene, m,p-xylene, o-xylene (BTEX) via GC/MS in SIM (Mattorano, Kupper et al., 2004), as was for naphthalene (modified version of OSHA35 methodology). (Measurement of THC and BTEX concentrations was made from extraction from charcoal sorbent tube; for naphthalene Chromosorb 106 sorbent tube was used.)

- Pre- and post- shift exhaled breath samples were collected from each participant on Day 5 of the study and sent to the Organic Chemistry Analytical Laboratory at HSPH, where they were analyzed[2] for benzene, toluene, m,p-xylene, o-xylene, and naphthalene.
- Dermal samples were collected post-shift from each participant on Day 5 of the study and sent to the Organic Chemistry Analytical Laboratory at HSPH, where they were analyzed[3] for THC, benzene, toluene, ethylbenzene, m,p-xylene, o-xylene, and naphthalene.
- Pre- and post-shift urine samples were collected over the 6 days of the study from each participant and sent to several CDC Laboratories, where they were analyzed for:
    - 1-, & 2-naphthol; 2-,3-, & 9- hydroxyfluorene; 1-,2-,3-, & 4-hydroxyphenanthrene; and 1-hydroxypyrene [4] (CDC Combustion Products and Persistent Pollutants Biomonitoring Lab)
    - volatile organic compounds (VOC) mercapurates including N-acetyl-S-(benzyl)-L-cysteine (parent compound: toluene) and N-acetyl-S-(phenyl)-L-cysteine (parent compound: benzene)[5] (CDC VOC and Perchlorate Laboratory)
    - creatinine, and
    - cotinine and other nicotine analytes[6] (CDC Tobacco Exposure Biomarkers Laboratory), to complement self-reported smoking histories
- Blood was collected in 2 tubes post-shift on Day 5 of the study and analyzed for:
    - trace level amount (ppt) quantification of VOC fuel components including benzene, ethyl benzene, m-/p-/o- xylenes, and toluene (CDC VOC and Perchlorate Laboratory) using the solid-phase microextraction (SPME) [7]
    - presence of GST enzyme polymorphisms[8] (Brown University).

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[2] Breath samples were analyzed for BTEX and naphthalene via GC/MS in SIM (using carbopack b tubes for passive transfer from the glass bulbs for analyses.)

[3] Dermal samples were collected using a tape-stripping method and analyzed via methods described above for air samples.

[4] Method references:

-Li Z, Romanoff LC, Trinidad DA, Hussain N, Jones RS, Porter EN, Patterson DG Jr., Sjodin A. Measurement of Urinary Mono-Hydroxy Polycyclic Aromatic Hydrocarbons Using Automated Liquid-Liquid Extraction and Gas Chromatography/Isotope Dilution High Resolution Mass Spectrometry. Anal Chem 2006; 78 (16): 5744-575.

-Li Z, Sandau CD, Romanoff LCS, Caudill SP, Sjodin A, Patterson Jr. DG. Concentrations and Profiles of Urinary Polycyclic Aromatic Hydrocarbon Metabolites in the General U.S. Population, 2001-2002. Environ Res 2008*;* 107(3): 320-331.

[5] These determinations were performed using a modified version of our established HPLC-tandem mass spectrometry (MS/MS) method: Ding YS, Blount BC, Valentin-Blasini L, Applewhite HS, Xia Y, Watson CH, Ashley DL. Simultaneous determination of six mercapturic acid metabolites of volatile organic compounds in human urine. Chemical ResToxicol 2009; 22(6):1018-1025.

[6] Method reference: Bernert JT, Harmon TL, Sosnoff CS, McGuffey JE. Use of cotinine immunoassay test strips for preclassifying urine samples from smokers and nonsmokers prior to analysis by LC-MA-MS. J Analyt Toxicol 2005; 29:814-818.

[7] Method reference: Blount BC, Kobelski RJ, McElprang D, Ashley DL, Morrow JC, Chambers DM, Cardinali FL. Quantification of 31 volatile organic compounds in whole blood using solid-phase microextraction and gas chromatography-mass spectrometry. J. Chromatography B. 832(2):292-301 (2006).

[8] Method reference: Schwartz J, Park SK, O'Neill MA, Vokonas PS, Sparrow D, Weiss S, Kelsey K (2005). Glutathione-S-transferase M1, obesity, statins and autonomic effects of particles. Am J Resp Crit Care Med 172:1529-1533.

Table 5 presents a summary of the average levels of total hydrocarbons measured in personal breathing air samples.

TABLE 5. Concentrations (mcg/m3)* of JP8 constituents in personal breathing-zones averaged over study period (4 days, Day 2-5), reported by *a priori* Exposure Group.

	Low					High				
Analyte§	**N**	**N-days**	**% <LOD**	**GM (GSD)**	**Range**	**N**	**N-days**	**% <LOD**	**GM (GSD)**	**Range**
Total Hydrocarbons*	35	138	61	0.52 (2.9)	0.2-22	38	149	12	2.64 (4.3)	0.2-74
Benzene	35	138	46	0.88 (3.6)	0.2-250	38	149	15	1.98 (4.0)	0.2-99
Toluene	35	138	0	6.16 (5.6)	0.3-3754	38	149	1	9.78 (4.6)	0.1-713
Ethylbenzene	35	138	39	0.72 (5.8)	0.1-224	38	149	3	5.57 (5.3)	0.2-390
m,p-Xylenes	35	138	12	1.93 (7.5)	0.2-900	38	149	2	16.71 (5.6)	0.2-1038
o-Xylenes	35	138	44	0.91 (5.8)	0.2-265	38	149	3	8.34 (5.4)	0.2-501
Naphthalene	35	138	79	0.37 (2.2)	0.2-11	38	149	17	2.25 (4.5)	0.2-55

* Total hydrocarbons concentrations are reported in mg/m^3
§ Analytes are representative of 8-hour time-weighted averages.
N= number of participants
N-days=total number of samples collected and analyzed (reflective of the number of participants and multiple days sampled)
LOD=limit of detection
GM (GSD)=geometric mean (geometric standard deviation), for any sample analyte value reported as less than the laboratory-determined LOD (<LOD), half (½) the LOD value was used to compute GM.

Summary of results focused on exposure:

- Air levels of JP8 components were significantly higher among the hypothesized higher exposure group
- Total hydrocarbon(THC) air levels were significantly different among job task categories
- THC levels were significantly correlated with naphthalene levels in air
- Post-shift urinary 1-naphthol levels were significantly higher than pre-shift levels among the high exposure group
- THC air levels were significantly associated with urinary 1-, and 2-naphthol levels
- Naphthalene air levels were slightly stronger predictors of urinary 1-,2-naphthol levels than THC air levels

To examine study hypotheses regarding occupational exposure to JP8 and neuropsychological functioning, neuropsychological testing was conducted at the end of shift on the first day of the study (Day 1 Battery) and subsequently on at the start of shift on Day 2, Day 4, and Day 6 (Repeated Day Battery). The neurobehavioral task batteries **(Table 6A & B)** were designed to be feasible in a field study environment, given time and environmental constraints, and to provide appropriate and reliable measurements of performance in a repeat testing scenario.

Table 6A. Neuropsychological Day 1 Battery: Task Descriptions

Test	Domain Assessed	Outcomes Measured	Possible Score Range	Reference
Shipley Vocabulary	General academic ability	# of correct responses	0-40	Shipley, 1946
Hooper Visual Organization Test	Visuospatial ability	# of correct responses	0-30	Hooper , 2004
Hopkins Verbal Learning Test: Total Recall	Verbal learning	# correct, sum of trials 1-3	0-36	Brandt, 1991
Delayed Recall	Verbal memory	Number correct, trial 4	0-12	
Retention (%)	Verbal memory	Delayed Recall/(Higher of recall score 2 or 3)*100	0-100	
Recognition Discrimination Index	Verbal memory	Total True Positives – Total False Positives	0-12	

To increase experimenter reliability and facilitate administration and data management efficiency, several tasks are administered in a computer-assisted format (tasks from the Automated Neuropsychological Assessment Metrics (version 4, ANAM4) test battery, C-SHOP-ANAM4 2007). Other traditional paper-pencil neuropsychological tasks that focus on particular functional domains of importance, but not tapped via the computer-assisted tasks, were included. Also, at the time of each neuropsychological test session, participants were administered the Positive and Negative Affect Scale (PANAS) to assess current mood state, and completed the ANAM4 Sleepiness Scale. On Day 1, all participants were administered trial 1 of the Test of Memory Malingering (TOMM), which is a simple 50-item visual memory test assessing cognitive engagement. It was administered for the purpose of excluding persons

from the analyses who exhibit low levels of engagement in the objective cognitive tests. Previous research examining the sensitivity and specificity of the TOMM indicates that a score below 38 on trial 1 of the TOMM suggests insufficient task engagement; in this study, no participant scored below 38.

Table 6B. Repeated Day Battery*:Task Descriptions

Test	Domain Assessed	Outcomes for Analyses	Possible Score Range	Reference
ANAM4 Match to Sample	Visuospatial ability, visual memory	Throughput	-	Vincent et al., 2008 others
ANAM4 Simple Reaction Time	Attention, psychomotor ability	Throughput	-	
ANAM4 Standard CPT	Sustained attention	Response time # NR (Omission) errors # FP (Commission) errors	-	
ANAM4 Finger Tapping Dominant hand Non-dominant hand	Psychomotor speed	Mean # of taps, from 2 trials	-	
Auditory Consonant Trigrams – 9 s delay – 18 s delay – 36 s delay	Executive function, memory	 # correct # correct # correct Total correct	 0-15 0-15 0-15 0-45	Stuss et al, 1987; Strauss et al, 2006
WAIS3 Digit Span Forward Backward	Attention	 # correct spans	 0-16 0-14	Wechsler, 1981; Strauss et al, 2006
Grooved Pegboard Dominant hand Non-dominant hand	Fine motor abilities	 Time to complete	 0-300 0-300	Matthews and Klove, 1964; Strauss et al, 2006

***administered on Study Days 2, 4, 6 (Mon, Wed, Fri mornings)**

Summary of findings focused on exposure-outcome associations:

- Only subtle differences in neurocognitive functioning are noted in those persons with greater than 10 years of Air Force service and those currently working in jobs with higher JP8 jobs (analyses of performances on the Day 1 battery)
- Overall, performance on most all cognitive task performances was observed to improve over the workweek
- Significant patterns of association between JP8 exposure and cognitive performance over the workweek were observed on tasks involving sustained attention (analyses of performances on the Repeated Day battery), but not observed on other tasks
- Minimal to no significant changes in balance parameters are observed over a work shift

Currently, we are in the final stages of completing and submitting the remaining Core OJENES manuscripts for publication (**Table 7**).

Table 7. List of CORE OJENES manuscripts and current status.

	Title or [Topic]	Manuscript Status
Overview	The Occupational JP8 Exposure Neuroepidemiology Study (OJENES): Repeated workday exposure and central nervous system functioning among Air Force personnel.	*In press*, NeuroToxicology
Tier I		
	Inhalation exposure to jet fuel (JP8) among US Air Force personnel	Published: Smith KW, Proctor SP, Ozonoff A, McClean MD. J. Occupational and Environmental Hygiene, 2010; 7:563-572.
	Urinary biomarkers of occupational jet fuel (JP8) exposure among Air Force personnel	*In press*, JESEE
	Biomarkers of exposure to jet fuel (JP8) in exhaled breath among Air Force personnel	Under final internal review
Tier II		
	Characteristics of jet fuel inhalation exposure among US Air Force personnel	Submitted for publication
	JP8 exposure history and neuropsychological performance	Manuscript in preparation
	Repeated workday exposure to JP8 and changes in neurocognitive performances over a workweek	Manuscript in preparation
	Workday exposure to JP8 and balance	Under final internal review
	Repeated measures of urinary biomarkers of occupational jet fuel (JP8) exposure	Manuscript in preparation

Key Research Accomplishments

2005- 2006:

- Sites visits to the AFB of interest were made to brief the pertinent Command Group and plan logistics for upcoming study.

2006- 2007:

- Tier I data collection conducted/completed in January 2007
- Tier I sample analyses by the Harvard University Organics Lab (under approved contractual arrangements) completed.

2007- 2008:

- A Workshop 'Research Issues related to JP8 Exposure Assessment' was organized by the PI and HM Jackson Fdn staff and convened 3 Oct 2007. The goal of the Workshop was to bring together a group of experts and in a Working Group-type structure, to share and discuss findings from recent epidemiologic research efforts involving JP8 exposure assessment. The areas of discussion focused around three research issues raised by the results from the Tier I field study (conducted in January 2007). The research issues were those that had an impact on certain study design and protocol specifics for the planned Tier II phase (to be conducted in early 2008). The issues included i) discussion of sampling structure of exposure groups by job titles/tasks, ii) discussion of the repeated JP8 exposures over consecutive workdays, and iii) discussion of the best and most appropriate markers of JP8 exposure and absorbed dose, particularly for the examination of neurological effects. Attendees included: M Butler, P Egeghy, Col R Gibson, KJ Heaton, J Hinz, D Kim, B. LaBrecque, MD McClean, L Nylander-French, T Risby, SP Proctor, KW Smith, Maj D Widing, Maj J Vietas.
- Sites visits to the three AFBs of interest were made to brief the pertinent Command Group and plan logistics for upcoming study.
- Tier II phase data collection was conducted/completed during field site trips to three AFBs between January-April 2008

2008-2009:

- Analyses of the biological and environmental samples collected during Tier II and sent to the Harvard University Organics Lab and Brown University (under approved contractual arrangements) was completed.

2009- 2010:

- The PI and several members of the study team visited and/or communicated with the AFBs involved in Tier II to provide them with briefings of the preliminary results (Fall 2009).
- Final analyses of all biological samples collected during Tier II and sent to CDC (under approved contractual arrangements) were completed.

Over the total study period-2005-2011:

- Initial and subsequent Annual Continuing Review Approvals granted by the Air Force Research Laboratory (AFRL) IRB, USARIEM HURC, and USAMRMC HRPO over the course of the study.
- The Exposure Assessment Methodology Working Group, with Core members from the Boston area, met regularly throughout the study period.
- The Data Management and Logistics Working Group met on an *ad hoc, as needed* basis.

Reportable Outcomes

1. Reports, manuscripts, abstracts

- Three peer-reviewed publications published or in press (Appendix 1)
- 6 additional Core study manuscripts have been submitted or are in final preparation stages for submission (see **Table 7**)
- 5 presentations made at national/international scientific meeting, with Abstracts published (Appendix 2)

2. Degree and student work supported by this award

- Over the course of the study, three graduate students and a recent doctoral graduate received funding support, each of them also serving as integral members of the Tier I and/or II field study teams.
- In addition, a group of 16 graduate students or undergraduate seniors from several US colleges/universities (University of North Dakota, Gonzaga University, Lyon College, University of Arkansas Medical School, and BUSPH) assisted in the Tier II data collection phase.

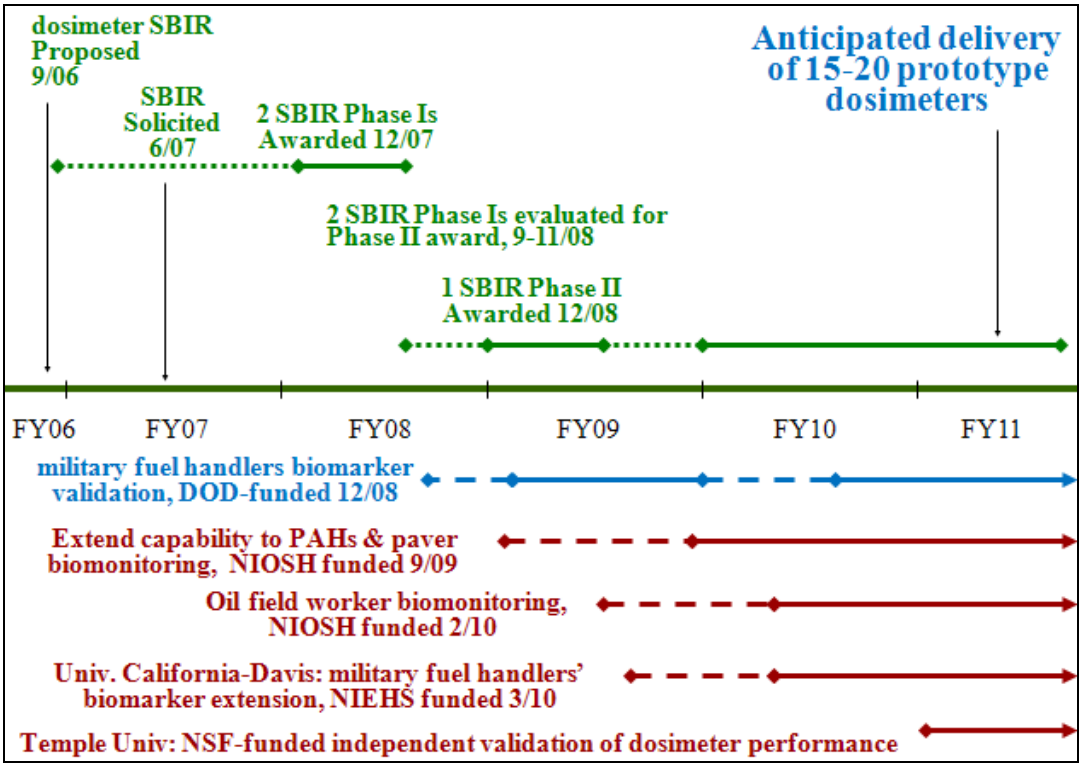
3. Collaborative funding applications related to work supported by this award

- Henk C. Trap, BSc, from TNO Defense Security and Safety (The Netherlands) completed a USAMRMC-funded project titled "Profiling Jet Fuel on Neurotoxic Components with 'Comprehensive Two-Dimensional GC'" (#W81XWH-07-1-0002). The PI served as a collaborator for this project, helping to advise on aspects related to neurotoxicity. Samples of JP8 fuel were provided to TNO for analyses. In this project, samples of jet fuel were screened for the presence and quantitative mixture composition of suspected neurotoxic compounds using a relatively new and effective instrumental technology, 'comprehensive two-dimensional GC', in combination with a Time of Flight Mass spectrometer (ToF-MS). Experiments were performed to monitor the vapor concentration time profile of a maximum of 20 compounds of interest specifically in JP-8.

- The PI is involved (at co-PI level) in a new project 'Measuring Naphthalene and Biological Markers of Exposure among Military Fuel-Worker Personnel: The Naphthalene Dosimeter Field Validation Study' (USARIEM protocol #H10-10).
    - The goals of this study are to test and validate the ability of the Army Research Office- Small Business Innovative Research (SBIR) Program- developed naphthalene (wearable) dosimeter prototype instruments to efficiently and accurately measure workplace levels of naphthalene in (near) real time and to examine the extent to which the naphthalene exposure levels measured with the newly developed instrument correlate with environmental and biological indices of exposure to naphthalene. The project managers on the naphthalene dosimeter SBIR project are Dr. Janis Hulla, US Army Corps of Engineers and Dr. Micheline Strand, Army Research Office. This research validation study has been funded initially by the Office of the Secretary of Defense (OSD) Chemical and Materials Risk Management Directorate to the Army Research Office (Dr. Strand, Contract Officer's Representative), with implementation through the CDC Foundation. The other 2 PIs include: Janis Hulla, PhD, DABT (Army Corps of Engineers) and John Snawder, PhD, DABT (CDC/NIOSH).

- In addition to the Army-funded SBIR project (described above), there are currently five ancillary projects funded by the DOD, National Science Foundation, NIOSH (two) and National Institute of Environmental Health Sciences (**Figure 2).** The human subject validation research project described above is depicted in blue.

Figure 3. Timeline for the Naphthalene Dosimeter SBIR Project and the five ancillary projects.



- An overview of DoD Naphthalene Dosimeter research has been presented in several Defense Knowledge Online website locations. A poster summarizing this work was presented at the 2010 Society of Toxicology meeting in Salt Lake City, March 2010.
    - Hulla, JE, Snawder JE, Proctor SP, Chapman GD. DoD impact assessment and management of naphthalene-related risks. Abstract published in The Toxicologist 2010; 114: 400.
- In 2011, the PI has initiated and submitted a program project-type proposal to DMRDP/RAD3 for funding consideration to include research related to deployment pulmonary health and OCONUS dosimeter validation, 'Validation of Exposure Assessment Tools for High Risk Military Personnel in Operational Environments' (PI: S Proctor); total budget ~$2.7 mill.

4. Related projects and collaborations initiated

- On 15 September, 2006, the PI participated and presented (via teleconference) in a 1-Day Workshop, "Naphthalene: Exposure, Epidemiology, Human Effects & Cancer"; Brooks City Base, San Antonio, Texas. During the meeting, she briefed COL Gibson and other attendees on the study design of this jet fuel study.
- On June 18, 2007, Daan Noort and Henk Trap from TNO Defence, Security, and Safety (The Netherlands) visited USARIEM for a collaborative meeting. An update on the jet

fuel study was presented by Dr. Proctor, and Dr. Trap updated us on the status of his research on jet fuel analysis by 2-dimentional GC (see above). Future collaborations were also discussed.

- Since 2008, the PI has been serving as a member of the Naphthalene Dosimeter Advisory Group, chartered from the Office of the Secretary of Defense, Emerging Contaminants Directorate (now *Chemical and Materials Risk Management Directorate*).
- The PI has collaborated with Drs. Langenberg and Trap at TNO on the USAMRMC-funded project titled "Profiling Jet Fuel on Neurotoxic Components with 'Comprehensive Two-Dimensional GC'" (#W81XWH-07-1-0002). The PI and Dr. Heaton visited visited TNO March 2009 to discuss findings and update TNO colleagues on the jet fuel study.
- The PI and Boston University Exposure Assessment team have provided collaborative assistance to the NIOSH Biomonitoring Research Team (Dr. John Snawder) in preparation of a new initiative to study and characterize workplace exposures via direct read monitors.
- The PI was invited to attend the MOMRP Pulmonary Health Task Working Group Meeting in June 2010 in Frederick, MD. This meeting and other DoD initiatives have focused increased attention on exposure assessment and biomarker efforts under operational conditions.
- The PI was invited to attend the scientific symposium entitled "Assessing Potentially Hazardous Environmental Exposures among Military Populations" at USUHS in May 2010. The meeting was sponsored by the Armed Forces Health Surveillance Center (AFHSC) and the Uniformed Services University of the Health Sciences (USUHS). A summary of that meeting has been published in as a Supplement to Military Medicine vol. 176, July 2011.

Conclusions

The study has provided important occupational health and exposure assessment information concerning JP8 in repeated workday settings supported with objective measures of multiple aspects of JP8 exposure combined with objective measurement of neuropsychological and postural sway (balance) outcomes.

In our foundation report (Proctor et al., in press), we focused attention on descriptive analyses to rule out major sources of bias that could contribute key sources of errors when addressing the OJENES research objectives. Results indicated minimal differences between participants in the high and lower exposure groups in terms of descriptive characteristics, other than daily JP8 exposure levels ($p<.001$). In addition, neuropsychological task performances for most task measures were not found to be significantly different from reported reference ranges. These findings demonstrated that confounding and misclassification of exposure and outcome status are not major concerns for the study. Furthermore, the more focused research questions regarding associations between JP8 exposure and CNS functioning are likely to provide valid conclusions, as they will be less influenced by these research biases.

In summary, we quantify levels of JP8 components found while performing US AF work tasks in- and around JP8 and find that levels are generally low, when appropriate protective gear is utilized. However, certain job activities have the potential for higher exposure levels. We find subtle but significant patterns of association between JP8 exposure and cognitive performance over the workweek on tasks involving sustained attention (analyses of performances on the Repeated Day battery), but not for other tasks. And, we find minimal changes in balance parameters are observed over a work shift.

The study's strength is its focus of better understanding the effect of consecutive, acute workday exposures to JP8 and specific CNS functioning endpoints. That said, there are limits to the study's ability to measure task-specific, short-lived spikes in exposures, as the OJENES utilizes day-specific, 8- hour TWAs and not real-time exposure level (minutes or hours) changes.

OJENES builds on existing knowledge concerning exposure assessment concerning JP8 exposure and extends the state of the science to evaluate the influence of repeated, consecutive workday exposure to JP8 on CNS functioning measured via standardized objective measures. The OJENES design directly addresses the NRC (2003) report's recommendation for the conduct of field research studies that combine the in-depth assessment of on-the-job exposure levels with concurrent assessment of adverse health effects are needed and contribute significantly to the knowledge of the subclinical effects of both acute and chronic exposure to occupational solvent exposures.

References

Proctor SP, Heaton KJ, Smith KW , Rodrigues EG, Widing DE, Herrick R, Vasterling JJ , McClean MD. The Occupational JP8 Exposure Neuroepidemiology Study (OJENES): Repeated workday exposure and central nervous system functioning among US Air Force personnel. Neurotoxicology, *in press.*

Smith KW, Proctor SP, Ozonoff A, McClean MD. Inhalation exposure to jet fuel (JP8) among U.S. Air Force personnel. Journal of Occupational and Environmental Hygiene; 2010;7: 563-572.

Smith KW, Proctor SP, Ozonoff A, McClean MD, Urinary biomarkers of occupational jet fuel exposure among Air Force personnel. Journal of Exposure Science and Environmental Epidemiology, *in press*.

Subcommittee on Jet-Propulsion Fuel 8, Committee on Toxicology, National Research Council. (2003). *Toxicologic Assessment of Jet-Propulsion Fuel 8*. Washington, D.C.: The National Academies Press.

List of Personnel Receiving Pay from this Award

Joseph Allen
Elisabeth Gentry
Robert Herrick
Karl Kelsey
Gabriela Kernan
Elisabeth Kryskow
Nicole Longcore
Alexis Maule
Michael McClean
Ema Rodrigues
Deborah Watkins

Appendix

ABSTRACTS

1. Smith KW, Allen JG, **Proctor SP**, McClean MD. Repeated measures of urinary 1- and 2-naphthol among jet fuel exposed Air Force personnel. Published in Occupational and Environmental Medicine 2007; 64:21. Presented at the 19th International Conference on Epidemiology in Occupational Health (EPICOH) in Banff, Alberta, Canada in October, 2007

2. Smith KW, Proctor SP, McClean MD. Repeated measures of inhalation and dermal exposure to jet fuel among Air Force personnel. Published in Epidemiology 2008; 19:S179. (Tier I results)

- The abstract presented both as a presentation and a poster at the International Society of Exposure Assessment (ISEA) meeting in October 2008 in Pasadena, CA was Kristen Smith MPH was awarded second place in the Student Poster Award 2008 competition at the ISEA Conference. The award is given in recognition of outstanding research conducted by a student in the area of Human Exposure Science.

3. Smith KW, Proctor SP, McClean MD. Relationships between inhalation exposure, urinary and end exhaled-breath biomarkers among jet fuel exposed Air Force personnel. Epidemiology 2009; 20:S167. (Tier I results) Presented at the International Society for Environmental Epidemiology Conference (Dublin, Ireland), August 2009.

4. Rodrigues EG, Merchant-Borna K, Smith KW, Proctor SP, McClean M. Characterization of jet fuel inhalation exposure and urinary metabolites in US Air Force personnel. Epidemiology 2009; 20:S60. (from Tier II results) Presented at the International Society for Environmental Epidemiology Conference (Dublin, Ireland), August 2009.

5. Alwis KU, Blount BC, Sheppard A, Proctor SP, Ashley DL. Simultaneous analysis of eleven VOC metabolites in human urine. Abstract published in The Toxicologist 2010; 114: 277-278. Colleagues at CDC presented on the volatile organic compound (VOC) analysis method used in Tier II. Presented at the 2010 Society of Toxicology meeting in Salt Lake City, March 2010.

6. Supplementary Abstract-Description of DoD Naphthalene Projects
Hulla, JE, Snawder JE, Proctor SP, Chapman GD. DoD impact assessment and management of naphthalene-related risks, The Toxicologist 2010; 114: 400. Presented at the 2010 Society of Toxicology meeting in Salt Lake City, March 2010.

PUBLICATIONS

1. Smith KW, Proctor SP, Ozonoff A, McClean MD. Inhalation exposure to jet fuel (JP8) among U.S. Air Force personnel. Journal of Occupational and Environmental Hygiene; 2010;7: 563-572.

2. Smith KW, Proctor SP, Ozonoff A, McClean MD, Urinary biomarkers of occupational jet fuel exposure among Air Force personnel. Journal of Exposure Science and Environmental Epidemiology, *in press*.

3. Proctor SP, Heaton KJ, Smith KW , Rodrigues EG, Widing DE, Herrick R, Vasterling JJ , McClean MD. The Occupational JP8 Exposure Neuroepidemiology Study (OJENES): Repeated workday exposure and central nervous system functioning among US Air Force personnel. Neurotoxicology, *in press.*

Biomarkers 1

C. G. Parks, E. C. McCanlies, D. B. Miller, R. M. Cawthon, L. A. DeRoo, D. B. Sandler, S. Peters, G. Talaska, B. A. G. Jönsson, H. Kromhout, R. Vermeulen, K. J. Aronson, M. Sanchez, A. Grundy, H. Richardson, J. Tranmer, M. Borugian, C. Graham, K. W. Smith, J. G. Allen, S. P. Proctor, M. D. McClean, D. J. McLean, A. Eng, C. Walls, E. Dryson, J. Harawira, A. 't Mannetje, M. Gray, P. Shoemack, N. Pearce and C. Brooks

Occup. Environ. Med. 2007;64;21-

Abstracts

Biomarkers 1

098 TELOMERE LENGTH AND WORK SCHEDULE CHARACTERISTICS IN THE NIEHS SISTER STUDY

C. G. Parks[1], E. C. McCanlies[1], D. B. Miller[1], R. M. Cawthon[2], L. A. DeRoo[3], D. B. Sandler[3]. *[1]CDC/NIOSH/HELD; [2]University of Utah; [3]NIH/NIEHS*

Objectives: Telomeres are protective DNA sequences on the ends of chromosomes, which can shorten with repeated cell replication and contribute to senescence. Shorter telomere length has been associated with chronic stress, age and obesity in women, and with metabolic and cardiovascular disease outcomes. In combination with lifestyle and socioeconomic factors, work schedule may be a source of occupational stress in women. We hypothesised that cumulative lifetime years of full-time and over-time work, rotating shift-work or irregular hours, may be related to shorter telomere length in women.
Methods: Average leukocyte telomere length was estimated by quantitative PCR on a sample of 677 women selected for a study of biomarkers and perceived stress in the NIEHS Sister Study cohort (median age 55, range 35–75). Questionnaire data included lifetime job history and work schedule for each job reported. Age-adjusted regression models estimated associations with telomere length. We also examined whether associations were mediated or modified by education, age, and risk factors such as inadequate sleep, elevated stress and body mass index.
Results: Currently holding a full-time job and years of full-time work were significantly associated with shorter telomere length ($\beta = -0.003$ per year, $p = 0.002$) independent of the effects of age ($\beta = -0.006$ per year, $p < 0.0001$). These findings persisted in women currently working at enrolment and were not confounded by education, current sleep, BMI, perceived stress, smoking and health status. The odds of being in the shortest quartile of telomere length increased with increasing years of full-time work among women over age 55 (OR 3.4; 95% CI 1.4 to 8.2; $\geq$24.5 years vs <5.2 years), those with higher than average perceived stress (OR 3.7; 95% CI 1.5 to 9.3) and those with some college or a bachelors degree (OR 5.7; 95% CI 1.9 to 16.7) but not higher levels of education. Years in jobs characterised as over-time, shift-work and irregular hours were not consistently related to telomere length.
Conclusion: Telomere length may be associated with lifetime years of full-time work in some women. Further investigation is needed to understand the contribution of job strain, work–life balance and socioeconomic factors. Telomere length may provide a novel biomarker for studies of chronic occupational stress.
Key words: telomere length; stress; occupational

099 PAH EXPOSURE, URINARY MUTAGENICITY AND DNA ADDUCTS IN RUBBER WORKERS

S. Peters[1], G. Talaska[2], B. A. G. Jönsson[3], H. Kromhout[1], R. Vermeulen[1]. *[1]Institute for Risk Assessment Sciences, Environmental Epidemiology Division, Utrecht University, Utrecht, The Netherlands; [2]Department of Environmental Health, University of Cincinnati, Cincinnati, OH; [3]Department of Occupational and Environmental Medicine, University Hospital, Lund, Sweden*

Objectives: Several studies have suggested that genotoxic risks might still be present in the contemporary rubber industry. Previously we observed elevated levels of urinary DNA adducts in rubber workers. In this study we investigated whether DNA adducts in lymphocytes and/or urothelial cells may be caused by PAHs or by other bioactivated genotoxic compounds.
Methods: Spot urine samples from 102 rubber workers were collected on Sunday and during the workweek on Tuesday, Wednesday, and Thursday at ~4 pm to determine 1-hydroxypyrene (1-HP) and mutagenicity levels. In addition, 24 h urine samples were collected from 52 non-smoking workers to measure the presence of urothelial cell DNA adducts. Lymphocyte bulky DNA adducts were measured in 65 workers.
Results: For all workers, urinary 1-HP levels were significantly higher in urine samples during the workweek compared to Sunday ($p < 0.0001$). The increase in 1-HP levels was, however, not uniform across tasks and factories and only reached statistical significance for the production functions mixing, moulding (both $p < 0.005$), and curing ($p < 0.0001$). The overall higher weekday urinary 1-HP levels might be mostly due to rubber fumes measured as cyclohexane soluble matter (CSM; $p < 0.005$), while among moulding workers dermal exposure to CSM seemed to be the main cause. Weekday urinary mutagenicity (corrected for cotinine) was significantly increased in the mixing (+5%) and curing (+6%) workers when compared to the Sunday urine sample. Mixing and curing workers also showed higher amounts of four identified urothelial cell DNA adducts compared to the other rubber workers. No pattern in lymphocyte DNA adducts was observed for the several production functions. Total urothelial cell DNA adducts were significantly related to urinary 1-HP ($p = 0.021$) and mutagenicity ($p = 0.027$). No significant relationships were found between the identified lymphocyte and urothelial cell DNA adducts or urinary 1-HP and mutagenicity.
Conclusion: The results indicate that mixing and curing workers are at the highest genotoxic risk among rubber workers. Increased levels of 1-HP, urinary mutagenicity and urothelial cell DNA adducts were found in these workers. Urothelial cell DNA adducts were not related to lymphocyte DNA adducts, hinting possibly at the presence of specific bladder carcinogens present in the rubber industry.
Key words: rubber industry; 1-hydroxypyrene; DNA adducts

100 LIGHT INTENSITY AND URINARY MELATONIN LEVELS AMONG NURSES

K. J. Aronson[1], M. Sanchez[1], A. Grundy[1], H. Richardson[1], J. Tranmer[1], M. Borugian[2], C. Graham[1]. *[1]Queen's University; [2]British Columbia Cancer Agency*

Objectives: To describe differences in light exposure and biomarkers of melatonin production among nurses, and to determine if light intensity during sleep and other variables are associated with peak melatonin levels.
Methods: 60 female clinical nurses at an acute care hospital who worked rotating day/night shifts consented to participate. During a 72 h period, nurses working either 2 days or 2 nights (age frequency matched) wore light intensity data loggers and completed a diary. The principal metabolite of melatonin, 6-sulfatoxymelatonin, was measured in a single urine void taken upon arising after sleep following their last shift.
Results: Nurses who worked the day shift experienced lower intensity of light during sleep than night workers, and night workers were four times more likely to have low melatonin levels than day workers (OR 4.35, 95% CI 1.43 to 13.20). Multivariable linear regression indicated that light intensity during sleep was inversely associated with urinary melatonin level ($p = 0.001$). Of the other variables included, only age was independently associated with the outcome, and no variable confounded this association.
Conclusion: Recent epidemiological studies suggest that higher frequency of night shift work and increased light at night exposure could increase cancer risk. One hypothesised pathway is through the hormone melatonin: the presence of light inhibits its production, and decreased melatonin may increase reproductive hormone levels that may in turn increase the proliferation of hormone sensitive cells, potentially enhancing tumour development. In this study, there was a statistically significant inverse association between light intensity during sleep and metabolites of melatonin, as hypothesised. If light at night is associated with increased cancer risk, the mechanism may be through melatonin; however, longitudinal studies are needed. Since it is necessary that some nurses work at night, occupational policies must give more consideration to the implications of exposure to light at night.
Support: CIHR Transdisciplinary Cancer Training Program; Breast Cancer Action Kingston; Programme of Research in Environmental Etiology of Cancer, NCIC.
Key words: nurses; biomarkers; shift work

101 REPEATED MEASURES OF URINARY 1- AND 2-NAPHTHOL AMONG JET FUEL EXPOSED AIR FORCE PERSONNEL

K. W. Smith[1], J. G. Allen[1], S. P. Proctor[2], M. D. McClean[1]. *[1]Boston University School of Public Health; [2]US Army Research Institute of Environmental Medicine*

Objectives: The primary objectives of this study were to evaluate jet propulsion fuel 8 (JP8) exposure by examining potential differences in urinary 1- and 2-naphthols (absorbed dose) between a priori designated exposure groups and assess the relationship between absorbed dose and concurrent measurements of inhalation and dermal exposure levels.
Methods: The study population included 24 Air Force (AF) personnel from six to eight different job types from an active USAF base. Based on a review of job activities, the participants were recruited from three a priori designated exposure groups (low: six workers with administrative or office roles;

moderate: nine workers with fuel distribution jobs, and high: nine workers from fuel systems maintenance). In January 2007, urine samples (n=144) were collected pre- and post-shift over three consecutive workshifts and analysed for 1- and 2-naphthol via gas chromatography mass spectrometry (GC/MS). Personal air (n=72) and dermal tape-strip (n=72) samples were collected concurrently from each worker and analysed for benzene, toluene, ethylbenzene, xylene (BTEX) and naphthalene via GC/MS. Linear mixed effects models were used to evaluate the exposure data.
Results: In post-shift urine samples, the mean urinary 1-naphthol measurements in the high exposure group were sevenfold higher than in the moderate group ($p=0.0005$) and ninefold higher than in the low group ($p=0.0004$). Similarly, the mean urinary 2-naphthol measurements in the high exposure group were fourfold higher than both the moderate ($p=0.0007$) and low groups ($p=0.002$). However, the 1- and 2-naphthol measurements in the moderate group were not significantly higher than in the low group. Exposure group and smoking status explain 62% and 63% of the between-worker variability for 1- and 2-naphthol, respectively. Analyses of personal air and dermal samples are forthcoming and will be used to evaluate the effect of inhalation and dermal exposure on absorbed dose.
Conclusion: The a priori exposure categories and smoking status are significant determinants of urinary naphthols. Based on absorbed dose levels, the fuel systems maintenance workers experience higher JP8 exposures than the fuel distribution and office workers, while levels among fuel distribution workers are not significantly higher than the office workers.
Key words: jet fuel; biomarkers; inhalation and dermal exposure

102 SERUM DIOXIN LEVELS IN FORMER SAWMILL WORKERS 20 YEARS AFTER EXPOSURE TO PENTACHLOROPHENOL (PCP) CEASED

D. J. McLean[1], A. Eng[2], C. Walls[3], E. Dryson[3], J. Harawira[4], A 't. Mannetje[2], M. Gray[2], P. Shoemack[5], N. Pearce[2], C. Brooks[2]. *[1]Massey University; [2]Centre for Public Health Research, Massey University; [3]Occupational Medicine Specialists; [4]Ngatiawa Social and Health Services; [5]Bay of Plenty District Health Board*

Objectives: From the 1950s to the late 1980s fungicides containing pentachlorophenol (PCP) were widely used in the New Zealand sawmill industry to prevent the proliferation of sapstain fungi. Workers involved in the treatment process or handling treated timber are known to have experienced significant PCP exposure. Commercial grade PCP contained contaminants including the 2,3,7,8-substituted polychlorinated dibenzo-p-dioxin (PCDD) and dibenzofuran (PCDF) congeners. The objectives of this study were to test serum dioxin levels in former sawmill workers 20 years after PCP use had ceased and to compare these with levels in the general population and also to establish whether elevated dioxin levels were the result of occupational PCP exposure.
Methods: Serum dioxin levels were analysed in two groups of former sawmill workers, 22 volunteers who had lodged claims for compensation (known as Sawmill Workers Against Poisons or SWAP) and 58 individuals randomly selected from surviving members of a cohort enumerated for a study of mortality and cancer incidence in former sawmill workers. This latter group was divided into 34 exposed and 24 non-exposed individuals based on work history. Age-adjusted serum dioxin levels in the general New Zealand population determined in a 1991 survey were compared with levels found in former sawmill workers with a correction based on a 7-year half-life. To establish the link with occupational exposure we compared dioxin congener profiles with those found in the general population and also in commercial grade PCP. We also tested the correlation between dioxin levels and known PCP in urine levels associated with different job titles.
Results: For SWAP members, both the WHO-TEQ and levels of specific hexa-, hepta- and octa-chlorinated congeners were at least 10 times those in the general population. Preliminary analyses of the randomly selected group suggest similar elevations in WHO-TEQ and the same specific congeners. Additional results of tests of the second group, and of the association with specific jobs, will be presented.
Conclusion: Serum dioxin levels in former sawmill workers in New Zealand are significantly elevated 20 years after the use of PCP ceased, and the congener profiles indicate that the source is past occupational exposure to PCP.
Key words: sawmill workers; dioxins; chlorophenols

humidity and temperature. The region was divided in 9 areas with homogeneous average air pollution levels. Each woman was assigned to one area according to her residence at the time of delivery. Time variables were dealt with to represent the exposure time-window considered relevant for the different health outcome of concern. Effects were evaluated using multiple regression models controlling for the mentioned confounding factors.

Results: Pregnant women were exposed to considerable levels of pollutant (mean PM_{10} = 49 $\mu g/m^3$, IQR = 26–63), with important seasonal variability (PM_{10} = 84 $\mu g/m^3$ in January and 23 $\mu g/m^3$ in August). We observed an inverse relationship between birth-weight and PM_{10} and NO_2 exposure levels. The magnitude of the effect seems to vary according to windows of exposure during pregnancy: we observed a decrease of 12 and 8 grams in birthweight for 10 $\mu g/m^3$ increase in PM_{10} during the fourth and the fifth month of pregnancy respectively (P = 0.001 and P = 0.03). We also observed an increased risk of LBW (<2500 g) related to higher levels of exposure at fifth months of gestation (AdjOR = 1.006, P = 0.04 for PM_{10} and AdjOR = 1.012, P = 0.05 for NO_2). PM_{10} and NO_2 exposure during the two months before delivery resulted also associated with a decreased placental weight (β for PM_{10} = −3, P = 0.03; β for NO_2 = −7, P = 0.02). CO seemed not to influence any of the examined outcomes.

Conclusions: Our preliminary results show that current air pollution levels in urbanised areas might affect fetal growth giving further support to rapid and efficient policy to decrease atmospheric pollutant levels.

ISEE-981

Repeated Measures of Inhalation and Dermal Exposure to Jet Fuel Among Air Force Personnel

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Objectives: The primary objectives of this study were to characterize inhalation and dermal exposure to jet propulsion fuel 8 (JP-8) based on measured levels of total hydrocarbons, naphthalene, benzene, toluene, ethylbenzene, and xylene (BTEX) in personal air and dermal tape-strip samples. We evaluated potential differences in exposure between *a priori* designated exposure groups, identified significant determinants of inhalation and dermal exposure to JP-8 constituents, and evaluated the relationships between total hydrocarbons, naphthalene, and BTEX.

Methods: The study population included 24 Air Force (AF) personnel recruited from an active USAF base. Based on job title and a review of job activities, participants were recruited from three *a priori* designated exposure groups (low: 6 office workers with no regular exposure to JP-8; moderate: 9 workers with fuel distribution jobs with intermittent exposure to JP-8, and high: 9 workers from fuel systems maintenance with regular exposure to JP-8). In January 2007, personal air samples were collected from the breathing zone of each worker over three consecutive work-shifts (n = 72) and analyzed for total hydrocarbons via GC/FID, as well as BTEX and naphthalene via GC/MS. Dermal tape-strip samples were collected post-shift over three consecutive work-shifts (n = 72) and analyzed for the same analytes. Linear mixed effects models were used to evaluate the exposure data.

Results: The geometric mean air concentrations for participants in the low, moderate, and high exposure groups were 0.3, 1.7, 5.1 mg/m^3 for total hydrocarbons and 0.3, 0.4, 0.9 $\mu g/m^3$ for naphthalene, while results for BTEX were similarly ordered. The correlations between THC and the other analytes were strong as indicated by correlation coefficients ranging from 0.83 to 0.95. Significant predictors of inhalation exposure to JP-8 included the *a priori* assigned exposure categories (low, moderate, high) and task (working in the hangar, hangar office, refueling maintenance, fuel handling, other). Among highly exposed workers, time spent in the hangar was a significant predictor of inhalation exposure to JP-8. In this group, inhalation exposure appeared to be affected by job (entrant, attendant/runner, other) and purpose of work (inspect, find leak, repair leak, other), though the results for these variables were not statistically significant. For all analytes, dermal tape-strip concentrations were below the limit of detection in >75% of the samples.

Conclusions: Total hydrocarbons exposure was strongly correlated with naphthalene and BTEX, suggesting that exposures came from the same source. Significant determinants of inhalation exposure levels were the *a priori* exposure groups and the worker's task.

ISEE-984

The Relationship Between Blood Serum Dioxin Levels and Breast Feeding

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Background: Studies of blood and breast milk from mothers have demonstrated that dioxins are eliminated from the body during breast feeding. The purpose of this paper is to explore the relationship between dioxin levels in blood and breast feeding for participants in a large population-based exposure study. The focus is on exploring 1) the effects of breast feeding a first child compared to additional children and 2) a time period interaction related to the changing levels of dioxins in the environment over time.

Methods: The data come from the University of Michigan Dioxin Exposure Study (UMDES) which was conducted to identify exposure pathways by which dioxin contamination in the environment contribute to dioxin concentrations in blood. Blood samples were collected from 946 study participants in Midland, Saginaw, Bay, Jackson, and Calhoun counties in Michigan in 2004–2005. Information on pregnancy and breast feeding were obtained during an hour-long interview of each study participant. Linear regression models were run for the log10 of the blood TEQ (calculated based on the WHO 2005 TEFs), adjusting for all other covariates in the UMDES model including age, body mass index, smoking status, food consumption, and recreational activities.

Results: 532 of the study participants were women, 442 of the women had at least one child, and 240 of the women with at least one child breast fed for at least one month. After adjusting for covariates, breast feeding a first child and additional children were both significantly associated with a lower TEQ in blood. The effect of breast feeding a first child was larger than the effect of breast feeding additional children. The parameter estimates indicate that each six month increase in breast feeding a first child was associated with a 7.6% decrease in blood TEQ and each six month increase in breast feeding additional children was associated with a 2.3% decrease in blood TEQ.

Conclusion: In general, breast feeding before 1959 was significantly associated with a higher TEQ in blood while breast feeding from 1960–1979 and after 1980 were significantly associated with a lower TEQ in blood. This suggests that breast feeding during the historic period when the dioxin content in the food supply was high was not associated with reductions in blood TEQ, whereas breast feeding more recently when the women were eating less contaminated foods was associated with reductions in blood TEQ.

ISEE-985

Inflammation of Airways Occurs Soon After Inception of Exposure to Flour Dust and Airborne Irritants in Bakery, Pastry Cooking and Hairdressing Apprentices: A Follow-Up Study of the Risk of Occupational Asthma

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ISEE-0485

Association Between Cotinine and Metals in Maternal and Cord Blood in Non-Smoking Mothers

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Background and Objective: Environmental tobacco smoke (ETS) contains over 4000 compounds, including numerous heavy and trace metals such as arsenic, lead, cadmium, and selenium. Smokers have been reported to have higher blood lead and cadmium levels than do nonsmokers. The objective of this study was to explore the association between cotinine and metals in maternal and umbilical cord blood in non-smoking mothers.

Methods: The study population consisted of 328 postpartum women collected from four hospitals and clinics in northern Taiwan. We interviewed them by a structured questionnaire after delivery and collected maternal and umbilical cord blood at birth. Cotinine in blood as an indicator of ETS was analyzed by using HPLC-MS/MS and the metals were analyzed by Agilent 7500 C inductively coupled plasma mass spectrometry (ICP-MS). We examined the association between cotinine and $\log_{10}$ transformed metal levels by linear regression models.

Result: After adjusting for maternal age and education, there were negative association between cadmium ($\beta \pm SE = -0.00005 \pm 0.0011$, P-value = 0.961), antimony ($\beta \pm SE = -0.00026 \pm 0.0007$, P-value = 0.689) and barium ($\beta \pm SE = -0.00414 \pm 0.0025$, P-value = 0.095) and cotinine in maternal blood. In umbilical cord blood, a negative association was found for antimony ($\beta \pm SE = -0.00113 \pm 0.0005$, P-value = 0.020) while positive associations were shown for thorium ($\beta \pm SE = 0.00237 \pm 0.0011$, P-value = 0.028) and uranium ($\beta \pm SE = 0.00307 \pm 0.0015$, P-value = 0.046).

Conclusions: Although cotinine were associated with some metals in blood, environmental tobacco smoke may not be the major source of metals in the non-smoking population.

ISEE-0488

Relationships Between Inhalation Exposure, Urinary and End Exhaled-Breath Biomarkers Among Jet Fuel Exposed Air Force Personnel

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Background and Objective: Jet propulsion fuel 8 (JP8) and similar jet fuels are widely used by the US military and commercial airline industry, resulting in widespread occupational exposures that could potentially cause adverse neurological health effects. The objectives of this study were to characterize JP8 exposure by examining exhaled-breath biomarkers between a priori designated exposure groups and assessing relationships with both inhalation exposure and urinary biomarkers.

Methods: Air Force (AF) personnel (n = 24) were recruited from an active USAF base into low, moderate, and high a priori designated exposure groups. Exhaled-breath samples were collected over three consecutive work-days and analyzed for benzene, toluene, ethylbenzene, xylene (BTEX), hexane, and naphthalene. Urine samples were collected concurrently and analyzed for 1- and 2-naphthol. Breathing-zone air samples were collected over the work-shift and analyzed for total hydrocarbons (THC), BTEX, and naphthalene. Linear mixed effects models were used to evaluate the exposure data.

Results: The geometric mean post-shift exhaled-breath concentrations for participants in the low, moderate, and high exposure groups were <6.5 ug/m^3, 9.0 ug/m^3, and 10.4 ug/m^3 for hexane; results for BTEX were similarly ordered. Exhaled-breath naphthalene concentrations were excluded from the analyses due to a low limit of detection. In post-shift exhaled breath samples, exposure group was a significant predictor of hexane ($P = 0.01$), ethylbenzene ($P < 0.0001$), m-/p-xylene ($P < 0.0001$), and o-xylene ($P < 0.0001$) with levels increasing across the low to high exposure groups. In pre-shift exhaled breath samples, exposure group was also a significant predictor of ethylbenzene ($P = 0.01$), m-/p-xylene ($P = 0.005$), and o-xylene ($P = 0.01$). Post-shift exhaled-breath hexane and BTEX measurements were weakly to moderately correlated with THC measured in personal air (r = 0.1–0.5) and moderately correlated with post-shift urinary 1- and 2-naphthol (r = 0.4–0.6).

Conclusion: Exhaled-breath concentrations increased across the low to high a priori designated exposure groups and were correlated with urinary biomarkers.

ISEE-0489

Effects of Household Use of Cleaning Products on Birth Weight

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Background and Objective: Associations between frequent use of household chemicals during pregnancy and wheezing and diarrhoea in offspring have been reported, but there are no studies on the effects of such exposures on birth weight (BW). The aim of this study is to assess the association between the use of domestic cleaning products during pregnancy and BW.

Methods: In the Spanish longitudinal INMA-Sabadell birth cohort study, 619 pregnant women were followed from the first trimester of pregnancy until delivery. Birth outcomes were obtained from clinical records of 617 newborns (≥34 weeks of gestation). The use of cleaning products in the home was obtained from an interviewer-led questionnaire administered at the third trimester of pregnancy. Associations between the use of cleaning products and BW were evaluated using multivariable linear regression models adjusting for sex, gestational age, mother's height, weight and number of previous pregnancies.

Results: The median BW was 3288g (interquartile range 2970 to 3520g). The most commonly used cleaning products were glass cleaners (77%), bleach (74%), furniture polishes (42%) and ammonia (25%). Women who used bleach had newborns with a higher BW (mean difference 87g; 95%CI 17 to 152). Similar results were found for ammonia (mean difference 60g; 95% CI −11 to 130). The association between bleach and BW remained apparent after additional adjustment for tobacco smoking during pregnancy, maternal education and employment in cleaning work (mean difference 71g; 95%CI: −3 to 146) and showed a dose-related trend (mean difference 62 and 96g for frequency of cleaning ≤1 and >1 time/week, respectively). Other products were not associated with BW.

Conclusions: Household use of bleach during pregnancy was associated with a higher BW. We hypothesise that a higher degree of disinfection of the living environment could be beneficial for foetal development.

ISEE-0492

Initial Enrollment of Asthmatic Children in a Woodstove Intervention Study

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Background: This study utilizes in-home woodstove interventions to assess the impact of indoor biomass smoke on asthmatic children. Initial enrollment efforts, methodologies, and the descriptive characteristics of the first cohort of participants are described here.

Methods: Asthma screening surveys were administered to school children (n = 1,185) to identify subjects. Baseline indoor air sampling and health measures were conducted during the winter of 2008/09. Air sampling

ISEE-0487

Quantification of Short Term Effects of Pollen Counts and Sentinel Botanic Garden Observations on Pollinosis Symptoms: A French Panel Study

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Background and Objective: In recent decades, numerous epidemiological studies investigating the change of prevalence of pollinosis showed an increase in the occurrence of this disease all over the world. In order to explain this phenomenon and to build a specific prevention tool, a sentinel botanic garden was realized in Nantes city (France). The objective of this study was to explore short term relationships between pollinosis symptoms and either grass pollen counts or pollen observations in this garden.

Methods: 81 volunteers suffering from pollinosis and living in Nantes metropolis area were recruited by allergy physicians. The panel took place while the grass pollen season (March to July 2007). Daily number of plants in flower was collected by botanists and daily pollen counts were measured by a central stationary sampler. Daily symptoms of rhinoconjunctivitis (eye, nose, throat, respiration) were reported in a diary by volunteers as well as pollinosis treatment intake.

Marginal (GEE) and mixed models were realized to explore short term effects of pollen covariates on pollinosis symptoms and treatment intake. Models were adjusted for confounding variables (time trend, meteorology, pollution levels) and took lags and autocorrelation into account.

Results: The response rate was excellent: 97%. Most of the volunteers were treated by antihistaminic and immunotherapy. A positive and significant association between prevalent pollinosis symptoms and grass pollen counts was shown for nose (OR = 1.043; 95%CI [1.026–1.060]) and eyes (OR = 1.035; 95%CI [1.018–1.052]) symptoms. Short term effects of pollen observations were also observed in the period before the pollen grass peak for total symptoms (OR = 1.02; 95%CI [1.00–1.05]).

Conclusion: Short term effects of grass pollen on pollinosis symptoms were clearly showed and quantified. Those results provide information for better prevention and care of pollinosis and have contributed to the establishment of a new panel study realized in 2009.

ISEE-0491

Characterization of Jet Fuel Inhalation Exposure and Urinary Metabolites in U.S. Air Force Personnel

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Background and Objective: Jet propulsion fuel-8 (JP-8) is the primary jet fuel used by the U.S. military, collectively consuming about 2.53 billion gallons annually. Previous reports suggest that JP-8 is potentially toxic to the immune, respiratory, and nervous systems. The objectives of this study were to evaluate inhalation exposure to JP-8 as well as absorption of JP-8 constituents among U.S. Air Force (USAF) personnel while performing job-related tasks.

Methods: Seventy-three full-time USAF personnel from three active bases were categorized a priori as having low (n = 35) or high (n = 38) exposure to JP-8 based on job title and tasks. Personal air samples were collected from each participant during four consecutive workdays using air pumps and sorbent tubes.

Using gas chromatography/mass spectrometry, charcoal sorbent tubes were analyzed for benzene, ethylbenzene, toluene, xylenes, and total hydrocarbons (THC) while Chromosorb® tubes were analyzed for naphthalene. Pre- and post-shift urine samples were also collected from each worker each day and analyzed for 1- and 2- naphthols, 2-, 3-, and 9-hydroxyflourene, 1-, 2-, 3-, and 4-hydroxyphenanthrene, and 1-hydroxypyrene. Linear mixed-effects models were used to explore the association between inhalation exposure and post-shift urinary metabolites, adjusting for creatinine and pre-shift urinary concentrations.

Results: THC air concentrations were significantly different between the exposure groups (2.6 vs. 0.5 mg/m^3, $P < 0.0001$). Similar differences were observed for the other analytes measured in air. Among the high exposure group, post-shift urinary 1- and 2-naphthol levels were significantly higher than pre-shift levels (both $P < 0.05$). Inhalation exposure to THC was significantly associated with post-shift urinary 1-naphthol ($\beta = 0.21$, $P < 0.0001$), 2-naphthol ($\beta = 0.11$, $P = 0.0006$) and 2-hydroxyflourene levels ($\beta = 0.08$, $P = 0.005$). Naphthalene air concentrations displayed similar significant associations with post-shift urinary 1-naphthol ($\beta = 0.26$, $P < 0.0001$) and 2-naphthol levels ($\beta = 0.13$, $P < 0.0001$).

Conclusion: USAF personnel experience inhalation exposure to JP-8 which is associated with absorption of JP-8 constituents while performing normal job-related tasks.

ISEE-0498

A Panel Study on Epigenetics, Markers of Oxidative Stress, and Lung Function Among Children with Respiratory Disease Exposed to Industrial Air Pollution

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Objectives: To study DNA methylation, exhaled nitric oxide (FeNO), lung function (FEV1) in children with respiratory symptoms exposed to industrial air pollution.

Methods: A panel study of 39 children aged 8–11 years followed on 2007/12–2008/4 was conducted in Valle-del-Mela (Sicily-Italy), a High Risk Area (55504 inhabitants) with oil refineries and energy plants. Symptomatic children were screened by modified ISAAC questionnaire (2506, 89.5% responders). The 39 selected children were divided into 9 groups matched by school, monitored for 7 consecutive days. DNA Methylation was measured on nasal mucosa cells collected by swab, twice per subject on day fourth and seventh of the same week. Personal $PM_{2.5}$ active, NO_2, SO_2 passive sampling were done on one child witness of a 4-child group. Ambient $PM_{2.5}$ monitor, meteo station, passive NO_2 SO_2 samplers in 21 schoolyards were used. Diaries filled in by parents recorded symptoms, therapy, indoor sources. Data were analyzed with mixed models controlling for confounders.

Results: Average daily ambient $PM_{2.5}$ was 23.0 μg/m^3, weekly ambient SO_2 over 20 μg/m^3 in three locations. Average daily (90 percentile) personal $PM_{2.5}$ was 44.5 (86.6), SO_2 17.7 (32.8). Effect measures were expressed for 10 μg/m^3 increase of pollutant concentration.

We found FEV_1 reduction −4.3% (90% Confidence Interval −6.1; −2.6%) for SO_2 lag2 ($P < 0.01$), FeNO increment 10.8% (3.2–18.4%) for SO_2 lag01 ($P = 0.022$); a decrease −1.0% of global DNA Methylation (Alu elements 90% CI −2.0; −0.6%) and −4.1% of iNOS (−7.8; −0.4%) for SO_2 lag12; −1.8% (−3.0; −0.6%) of iNOS for $PM_{2.5}$ lag12. DNA Methylation of interleukin 6 position was reduced when FEV_1 was

PS 1297 INVESTIGATION OF THE NEUROTOXIC MECHANISMS INVOLVED IN BETA-AMYLOID DEPOSITION IN PSAPP MICE.

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Introduction: Amyloid-beta is endogenously formed neuronal peptide which has been proved to have a causal relationship with neurodegeneration in Alzheimer's disease (AD). MAP kinase, CREB, ERK and pCREB have played a vital role in memory regulation. The present study employed PSAPP mice expressing the "Swedish" amyloid precursor protein and M146L presenilin-1 (PSAPP) mutations to study the cellular mechanisms and biomarkers involved in Aβ toxicity in relation to the loss of memory. Experimental Procedures: PSAPP mice and non-transgenic controls (eight months old) were subjected to behavioral and biochemical studies. Brains were dissected, hippocampus and cortex were removed. Behavioral experiments such as Y-maze and open field were performed along with Aβ deposition (1-40 and 1-42). Enzymatic activity of beta secretase as well as the alteration in the cellular signaling pathways (ERK MAP kinase, STAT and CREB pathways were analyzed by multiplex microbeads method. ANOVA and Dunnett's test were used to compare the results with non-transgenic mice. Results and Conclusion: This study reveals the alterations in behavioral and cellular processes that occur due to Aβ deposition in PSAPP transgenic mice. PSAPP mice exhibited significant Aβ deposition (1-40 and 1-42) and behavioral deficits (y-maze and Open field) compared to the control non-transgenic mice. Beta-secretase activity was significantly increased in the PSAPP mice in the cortex and hippocampus. The kinases such as ERK MAP kinase, JNK, p70S6 kinase exhibited down regulation in the transgenic animals. Other cellular biomarkers such as STAT5, STAT and CREB also showed the same trend. Administration of exogenous Aβ peptide has also shown to induce characteristic neurodegeneration in the hippocampus. However, the cellular mechanisms differ as compared to the endogenous deposition of Aβ. The study of these cellular processes and their changes can divulge important targets that could be utilized for newer drug discovery. Acknowledgement: Alzheimer's Association grant (NIRG-08-91816)

PS 1298 NEONATAL EXPOSURE OF MALE RATS TO BISPHENOL A IMPAIRS EXPRESSION OF SERTOLI CELL JUNCTIONAL PROTEINS IN THE TESTIS.

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Sertoli cell junctional proteins (SCJP) (viz. adhesion, gap and tight junctions) are important for spermatogenesis and perturbations in expression of these proteins are associated with impairments in process of sperm production. Bisphenol A (BPA) is an endocrine disrupter that has been associated with impaired spermatogenesis. However the mechanistic basis of impaired spermatogenesis is unknown, whether BPA is a Sertoli cell toxicant has not yet been fully investigated. The present study was undertaken to decipher the effects of neonatal exposure of male rats to BPA on the testicular expression of SCJP during development. Neonatal male rats were s.c injected with 2.4 µg/day (300 µg/kg bw) of BPA in sesame oil from postnatal day 1-5 and controls received vehicle. Immunohistochemical localization for Connexin 43 (Cx-43, gap junctional), Zona Occludin-1 (ZO-1, tight junctions) and N-cadherin (adherens junction) was carried out on testicular tissue sections obtained from PNDs 15, 30, 45 and 90 of rats exposed to the lowest dose of BPA(2.4 µg/day) that impaired fertility. A significant reduction in the expression of Cx-43 (PND 45 and 90) and increases in the expression of N-cadherin (PND 45 and 90) and ZO-1(PND 90) were observed in the testes of rats exposed neonatally to BPA. Interestingly, there was an altered expression pattern of Cx43 amongst the sloughed cells in the testes of the experimental rats as compared to controls. Neonatal exposure of BPA to rats has the potential to induce perturbations in SCJP. These perturbations may be one of the contributing factors that lead to impairments in spermatogenesis in the exposed animals and can be used as potential biomarkers to study BPA- induced effects on testes.

PS 1299 DETECTING BIOMARKERS OF CHRONIC ARSENIC EXPOSURE BY USING SELDI-TOF-MS PROTEIN CHIP TECHNOLOGY.

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Background: Chronic exposure to high levels of inorganic arsenic that is naturally present in drinking water in certain geographic regions has become a major public health concern in China. In this study we used surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) to determine if arsenic exposure in drinking water could induce serum protein changes and to identify new protein biomarkers for chronic arsenic exposure. Methods: A total of 178 subjects were selected in three groups based on arsenic exposure levels in drinking water (3.2±2.5 µg/L, 22.1±4.7 µg/L, and 177.6±23.8 µg/L, respectively). Serum proteomic profiles were analyzed by SELDI-TOF-MS with a CM10 Protein Chip. Diagnostic model was constructed by decision tree algorithm in a training set with 120 subjects and validated in a testing set with other 58 subjects. Results: Relative intensities of 41 protein peaks were found differently among three groups. A panel of five proteins with mass-to-charge ratio (m/z) of 2872.48, 6121.42, 7580.58, 9432.56 and 5552.66 was selected to build the diagnostic model. Among these markers, the 2872.48 Da and the 7580.58 Da were significantly up-regulated or down-regulated only in the group of subjects exposed to 177.6±23.8 µg/L of arsenic. The 6121.42 Da and the 5552.66 Da were significantly down-regulated in the groups with arsenic exposure levels of 22.1±4.7 µg/L and 177.6±23.8 µg/L. The 9432.56 Da content was the lowest in the group exposed to 22.1±4.7 µg/L of arsenic. The power to detect differences among three groups in the testing set was evaluated with the sensitivity of 80.00%-86.67%, and the specificity of 86.67%-95.35%. Conclusion: Exposure to 22.1±4.7 µg/L of arsenic in drinking water is enough to cause changes in serum protein profiles. This proteomic technology showed very promising in detecting levels of arsenic exposure and discovering new biomarkers.

PS 1300 EFFECTS OF ORAL ADMINISTRATION OF PIOGLITAZONE, SODIUM SACCHARIN OR SODIUM O-PHENYLPHENATE ON THE EXPRESSION OF ONCOMODULIN IN THE BLADDER EPITHELIUM OF MALE F344 RATS.

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Currently there are no reliable markers for early detection of urinary bladder cancer. Recent studies have shown that expression of the oncomodulin gene is increased in urothelium from rats treated with various bladder carcinogens. Pioglitazone is a PPARγ agonist which induces rat bladder tumors. We administered pioglitazone in 0.5% methylcellulose (MC) intragastrically (i.g.) to evaluate the level of oncomodulin expression in F344 rat urinary bladder epithelium. Sixty male F344 rats were randomized into 4 groups of 15 rats each and treated for 4 weeks with: 1) control diet and daily MC i.g.; 2) control diet and 16 mg/kg pioglitazone in MC i.g.; 3) diet containing 7.5% sodium saccharin (NaSac) and MC i.g.; or 4) diet containing 2.0% sodium o-phenylphenate (NaOPP) and MC i.g. RT-PCR was employed to detect expression of oncomodulin in the urothelium. Light microscopy, SEM, and immunohistochemical detection of BrdU were used to examine cytotoxic and proliferative urothelial effects. Expression of oncomodulin was significantly increased in NaSac or NaOPP-treated groups compared to controls, but not in the pioglitazone group. All test chemicals induced superficial necrosis by SEM and increased BrdU labeling index indicative of increased cell proliferation. In vitro, PPARγ agonists induced differentiation in rat urothelial cells (MYP3), decreased proliferation, and decreased oncomodulin expression. Unlike NaSac and NaOPP, pioglitazone did not induce an increase in oncomodulin expression, possibly related to its competing effects of, 1) indirectly increasing urothelial proliferation by inducing production of urinary solids, and 2) decreasing proliferation due to direct effects on urothelial PPARγ.

PS 1301 SIMULTANEOUS ANALYSIS OF ELEVEN VOC METABOLITES IN HUMAN URINE.

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Volatile organic compounds (VOCs) are ubiquitous in the environment, originating from many different natural and anthropogenic resources, including tobacco smoke. Long-term exposure to certain VOCs may increase the risk for cancer, birth defects, and neurocognitive impairment. Therefore, VOC exposure is an area of significant public health concern. We developed a reversed-phase high performance liquid chromatography coupled with electro-spray ionization tandem mass spectrometry (LC-ESI/MSMS) method to quantify urinary VOC metabolites as biomarkers of exposure. In the current method we monitor N-acetyl-S-(2-hydroxyethyl)-L-cysteine (HEMA), N-acetyl-S-(3-hydroxypropyl)-L-cysteine (HPMA),

S-(1-hydroxy-3-buten-2-yl)-N-Acetyl-L-cysteine (MHBMA), N-acetyl-S-(3,4-dihydroxybutyl)-L-cysteine (DHBMA), N-acetyl-S-(2-carboxyethyl)-L-cysteine (CEMA), and N-acetyl-S-(phenyl)-L-cysteine (PMA), N-Acetyl-S-(benzyl)-L-cysteine (BMA), 2-thioxothiazolidine-4-carboxylic acid (TTCA), N-Acetyl-S-(N-methylcarbamoyl)-L-cysteine (AMCC), N-Acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA) and N-Acetyl-S-(trichlorovinyl)-L-cysteine (TCVMA) in human urine. These analytes are metabolites of 1,3-butadiene (MHBMA, DHBMA), benzene (PMA), toluene (BMA), acrylamide (AAMA), carbon disulfide (TTCA), N,N-dimethylformamide (AMCC), acrolein (CEMA, HPMA), tetrachloroethylene (TCVMA), acrylonitrile, vinyl chloride, and ethylene oxide (HEMA). For matrix spike experiments the mean accuracy ranges from 98-107% and the mean percent difference ranges from 0.43-9.54%. The limit of detection ranges from 0.01-0.21 µg/L. By spiking urine with pure isomers and retention time interpretation, we could identify the correct diastereoisomer of MHBMA in human urine as S-(1-hydroxy-3-buten-2-yl)-N-Acetyl-L-cysteine. We applied this method to 690 urine samples collected (10 samples each) from 25 smokers and 44 non-smokers (categorized based on blood 2,5-dimethylfuran levels) to find that smokers have significantly elevated levels of AAMA, CEMA, DHBMA, HEMA, HPMA and PMA.

PS 1302 TOXICOGENOMIC IDENTIFICATION OF BIOMARKERS OF ACUTE RESPIRATORY EXPOSURE TO SENSITIZING AGENTS.

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Allergy induction requires multiple exposures to an agent. Therefore the development of high-throughput or in vitro assays for effective screening of potential sensitizers will require the identification of biomarkers. The goal of this preliminary study was to identify potential biomarkers that differentiate the response to allergen vs non-allergen agents following an acute exposure in naïve individuals. Female BALB/c mice received a single intratracheal aspiration exposure to Metarhizium anisopliae crude antigen (MACA) or bovine serum albumin (BSA) in Hank's Balanced Salt Solution (HBSS) or HBSS alone. Mice were sacrificed after 1, 3, 6, 12, 18 and 24h. Bronchoalveolar lavage fluid (BALF) was evaluated to determine total and differential cellularity, total protein concentration and LDH activity. RNA was isolated from lung tissue for microarray analysis and RT-PCR. MACA administration induced a rapid increase in BALF neutrophils, lymphocytes, eosinophils and total protein levels as compared to BSA or HBSS. Microarray analysis demonstrated differential expression of genes involved in cytokine production, signaling, inflammatory cell recruitment, adhesion and activation in 3h and 12h MACA-treated samples as compared to BSA or HBSS. Further statistical and pathway analyses allowed identification of ~100 candidate biomarker genes. Eleven genes were selected for further assessment by qRT-PCR. Of these, 6 demonstrated persistently increased expression (Ccl17, Ccl22, Ccl7, Cxcl10, Cxcl2, Saa1), while C3ar1 increased from 6-24h. In conclusion, a single respiratory exposure of mice to an allergenic mold extract induces an inflammatory response which is distinct in phenotype and gene expression from the response to a control protein. Validation of these biomarker genes with additional allergens and irritants is in progress. Biomarkers identified in these analyses will facilitate improvements in screening methods. (Supported by UNC/EPA Cooperative Training Agreement CR83323701. This abstract does not reflect EPA policy).

PS 1303 DEPLETION OF KUPFFER CELLS AS A MECHANISM FOR INCREASED SERUM ENZYMES.

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In clinical studies, evaluation of certain serum enzymes is routinely performed in order to assess the possibility of tissue injury. For example, an increase in the serum level of the enzyme alanine aminotransferase (ALT) is a sensitive indicator of hepatitis. However, changes in steady state levels of certain serum enzymes may reflect a change in either their rate of release to the bloodstream or in their clearance from the bloodstream. Since the turnover of many serum enzymes occurs via receptor mediated endocytosis by Kupffer cells (KCs) in the liver, it is possible that inhibition or depletion of KCs may also contribute to serum enzyme elevation. In order to better understand the role of KCs in serum enzyme clearance, KCs were depleted from rat liver using intra-venous injection of clodronate (CLO) liposomes. KC depletion was monitored using immunohistochemistry with antibodies to ED1 and ED2, which detect immature and mature (ED1) or just mature (ED2) KCs. ED2-positive cells were undetectable at 24 hours, with repopulation evident at 72 hours, and near to base-line levels by 8 days post CLO administration. The serum levels of ALT, aspartate aminotransferase (AST), creatine kinase (CK), glutamate dehydrogenase (GLDH), and lactate dehydrogenase (LDH) ALT were measured at 4, 8, 24, 48, 72, 96 hours, and 8 days post administration of CLO liposomes. The maximal increase was 8x at 8 hours for CK, and 4x, 10x, and 25x at 24 hours for AST, GLDH, and LDH, respectively with minimal changes in ALT. The increases in serum enzymes were inversely correlated to decreases in levels of KCs and returned to baseline by day 8. Histopathology of liver, heart, and skeletal muscle was normal and no changes to troponin 1 were noted, suggesting that CLO administration did not cause direct injury to these tissues. These data further demonstrate the role of KCs in serum enzyme clearance and support another mechanism for serum enzyme elevation that is not related to liver or muscle injury.

PS 1304 ANALYSIS OF LYMPHOCYTE SUBSETS IN PERIPHERAL BLOOD AMONG EXPOSED WORKERS AND PATIENTS WITH HYPERSENSITIVITY DERMATITIS INDUCED BY TRICHLOROETHYLENE.

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Trichloroethylene (TCE) is an important industrial chemical and is widely used. TCE is considered to have immune toxicity, hypersensitivity dermatitis have been described among workers exposed to TCE. The study consists of 16 patients with hypersensitivity dermatitis, 30 healthy TCE-exposed workers and 28 healthy workers unexposed to TCE. The lymphocyte subsets including CD4+ T cell, CD8+ T cell, B cell, NK cell, CD8+CD28+(-) T cell were measured in addition to the standard blood count analyses. All of the subjects in 3 groups were frequency matched by age and sex. The results showed that the absolute counts of lymphocyte, T cell, CD4+ T cell, CD8+ T cell, CD8+CD28- T cell were significantly increased in patients with hypersensitivity dermatitis compared with TCE exposed workers and unexposed workers, meanwhile, no significant differences in counts of lymphocyte, T cell, CD4+ T cell were demonstrated between exposed and unexposed groups. CD4+/CD8+ ratio and CD8+CD28+/ CD8+CD28- ratio were significantly decreased in both groups of hypersensitivity dermatitis and TCE exposed workers compared with unexposed group, both ratios were similarly in hypersensitivity dermatitis case and TCE exposed groups. The count of NK cell among 3 groups was in the increased tendency of unexposed control group >exposed group> case group, and the difference was significantly. No significant difference in count of B cell between 3 groups was found. These data provide evidence that occupational exposure of TCE causes change of lymphocyte subsets, especially T lymphocyte subsets. The counts of lymphocyte, T cell and CD4+ T cell might be biomarkers for screening cases with hypersensitivity dermatitis from TCE-exposed workers.

PS 1305 GROUP SPECIFIC COMPONENT: URINARY BIOMARKER OF SUBCLINICAL RENAL INJURY IN NEPHROTOXIN MODELS.

C. Mauzy, J. Frey, V. Chan, R. Pitsch and P. Shiyanov. *Applied Biotechnology Branch-Human Effectiveness Directorate, Air Force Research Laboratory, Wright-Patterson AFB, OH.* Sponsor: J. Schlager.

Urine proteomics analysis by in-house efforts have identified Group Specific Component (GSC), also called Vitamin D binding protein, as a potential biomarker to early subclinical kidney degradation. To model subclinical renal toxin injury, low levels of D-serine were used to induce kidney damage at the proximal straight tubules. Male Fisher 344 rats were dosed intraperitoneally with control (0), 200, or 500 mg/kg D-serine in 0.9% saline and urine collected pre-dose and at timed increments post-dose. Renal damage was verified by histopathological examination of kidney tissue. Peptides based on rat GSC protein sequence were synthesized and used as antigens for polyclonal antibody development. Western blots using polyclonal GSC 1242 tested against urine from D-serine dosed rats demonstrated a strong signal as early as 12 hours postdose to a 52 kDal protein as well as a ~70 kDal protein. An examination of control urine demonstrated very low levels of the 52 and 70 kDal protein with background signal from secondary antibody alone negligible. GSC 1242 immunostaining of kidney tissue from animals dosed at 0 and 500 mg/kg D-serine confirmed that GSC protein is strongly induced in the kidney after dosing. To examine the GSC response to renal glomerular damage rather than the proximal tubule injury, urine samples from a rat study using puromycin were examined. The response of GSC in this nephrotoxin model significantly increases at 96 hours post-dose, and is seen at high levels up to 168 hours post-dose. Interesting, an examination of differential RNA expression of dosed versus control kidney demonstrated that GSC expression decreases upon nephrotoxin exposures. These pre-validation studies on rat group specific component indicate that it is present in the urine at low levels which dramatically increase upon exposure to both puromycin and D-serine, an indication that GSC response is not localized to proximal tubule damage.

was collected prior to dosing and at 12 hour intervals for a total of 168 hours. Urine samples were assayed by ELISA for clusterin, retinol binding protein (RBP) 4, heme oxygenase (HO)-1, osteopontin (OPN), Yb1 (mu) glutathione S-transferase (GST), α glutathione S-transferase (αGST), metalloproteinase tissue inhibitor (TIMP)-1 and β_2-microglobulin. Neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (Kim-1) were assayed by Meso Scale Discovery (MSD) Multi-Spot® Assay. Biomarker levels remained constant for control animals throughout the time course. However, for animals dosed with 200 and 500 mg/kg D-serine, significant increases were observed with peaks at 12 hours post-dose (HO-1, Yb1 GST and αGST), 24 hours post-dose (clusterin, RBP4, TIMP-1 and β2-microglobulin), 96 hours post-dose (Kim-1 and NGAL) or 120 hours post-dose (OPN). Biomarkers returned to baseline levels at 36 hours (Yb1 GST and αGST), ≥ 48 hours (HO-1, β_2-microglobulin, TIMP-1, clusterin and RBP4) or ≥ 168 hours (NGAL, Kim-1 and OPN). Gene expression studies were also conducted in control and dosed kidney tissue, and significant increases in transcription were seen in most biomarkers examined. RBP4, however, demonstrated significantly lower expression upon nephrotoxin exposure. Expression profiles indicate that this protein set differed in maximal response times. Their collective detection in urine is a potential noninvasive strategy to determine early onset of low level subclinical kidney damage in response to toxin exposures, ultimately leading to development of rapid field monitoring for the prediction of health hazards associated with chemical exposure.

PS 1879 TRANSPLACENTAL DISTRIBUTION OF METALS AND THEIR INTERACTIONS ASSESSED BY BIOMONITORING IN MOTHER/CHILD PAIRS.

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Exposure of the fetus to (heavy) metals has been associated with adverse health outcomes including developmental toxicity. However, few data exist on the transplacental passage of metals and their interaction with each other in the maternal-fetal unit. In our study, venous and umbilical cord blood samples from 50 mother/child pairs were studied for exposure to multiple heavy metals, essential minerals and trace elements. Smoking status was assessed by cotinine in urine. Lead (Pb) showed the highest median concentration of heavy metals in maternal samples (11.5 µg/L) followed by nearly equal concentrations of mercury (Hg, 0.44 µg/L) and cadmium (Cd, 0.34 µg/L). Smokers showed higher Cd levels than non-smokes (0.73 vs. 0.29 µg/L, P<0.001). Slightly but significantly lower levels of Pb were observed in fetal blood (10.3 µg/L, P<0.004), whereas Cd was strongly reduced (0.05 µg/L). In contrast, higher concentrations of Hg were detected in fetal samples (1.48 µg/L, P<0.0001). Selenium (Se) and iron (Fe) showed a similar distribution in the maternal/fetal unit as observed for Pb, whereas the distribution of manganese (Mn) was similar to Hg. Copper (Cu) and Zn were strongly reduced in the fetus and distribution was more similar to Cd. Linear regression analysis revealed positive associations between maternal and fetal concentrations for Pb, Mn and Hg (P≤0.014). No associations between maternal and fetal blood were found for Cd, Cu, Fe and Zn. Exposure to heavy metals (single or in combination) did not influence the levels of essential minerals such as Zn. In conclusion, the placenta provides a barrier for Cd, Cu and Zn, whereas Fe, Pb and Se enter the fetal environment unaffected. Mn and Hg are unequivocally transported to the fetus resulting in increased exposures compared to the mother. However, homeostasis of essential elements remains unaffected by exposure to heavy metals at low exposures. Overall, our results contribute to the risk assessment of heavy metals and adverse health outcome in the most vulnerable population, the fetus.

PS 1880 SURVEILLANCE FOR SYSTEMIC EFFECTS OF METALS AND OTHER MATERIALS RELEASED FROM RETAINED EMBEDDED FRAGMENTS IN U.S. SOLDIERS.

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Concern has heightened regarding long term health effects associated with embedded fragments in soldiers. In the past, fragments embedded in muscle tissue were thought to be relatively inert, however recent work has shown that veterans with embedded depleted uranium (DU) fragments have elevated blood and urine uranium levels more than 18 years after injury involving DU munitions during the first Gulf War. This finding is supported by studies showing release of metals from certain types of medical implants. To better understand and prevent health problems resulting from retained metal and non-metal fragments in soldiers, the Department of Veterans Affairs has established a program charged with developing clinical management guidelines for embedded fragments. These will be based on results from analysis of fragment content, health surveillance and biomonitoring of veterans with prolonged systemic exposure to chemicals released from fragment material over time. Chemical characterization of over 400 removed fragments has shown that most are metal alloys (83%) while others are different types of organic material, plastics, wood and stones. Based on this information and knowledge of the toxicity of metals, a biomonitoring protocol utilizing primarily urine has been developed to characterize systemic exposure to the following carcinogenic and cytotoxic metals: Al, As, Cd, Cr, Co, Cu, Fe, Mn, Ni, Pb, U, W and Zn. Customized health surveillance and management guidelines will be developed for veterans with chronically elevated excretion of specific metals using biomarkers of potential effects of the metal(s) of concern. Biomonitoring protocols for compounds released from non-metallic fragment materials, such as isocyanate, phthalates and acrylics, will continue to be developed as our knowledge of the breakdown of fragments embedded in muscle tissue increases. *Supported by Department of Veterans Affairs and the Armed Forces Institute of Pathology*

PS 1881 DOD IMPACT ASSESSMENT AND MANAGEMENT OF NAPHTHALENE-RELATED RISKS.

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The Department of Defense Chemical and Materials Risk Management Directorate is using a scan-watch-action process to identify, rank and manage risks associated with emerging contaminants. Naphthalene is characterized as a likely human carcinogen by the NTP and in the EPA's most recent draft health risk assessment. Thus, naphthalene-related environmental health regulations are evolving. The potential impacts have been assessed, using multi-criteria decision analysis, for five of the Department's functional areas. One of the areas of concern is exposure to naphthalene among fuel handlers. To determine whether these exposures present unacceptable risk, the Army Research Office awarded a Small Business Innovative Research Project for the development of a miniature real-time naphthalene sensor. NIOSH's Biomoniting Team and Investigators from the Army Research Institute for Environmental Medicine, UC-Davis and the Army Corps of Engineers are collaborating on a second DOD-funded project. This project will validate the prototype sensor as a dosimeter by defining correlations between measured exposures and biomarkers of exposures to-be-collected from military fuel handlers. To date, naphthalene specificity with sensitivity of 0.5 mg/m3 has been demonstrated and definition of the firmware chemometrics is underway. Implementation of the human subjects research protocol is pending institutional review boards' approval.

PS 1882 EFFECTS OF STYRENE CO-EXPOSURE ON FORMATION OF 1, 3-BUTADIENE DERIVED N7-GUANINE ADDUCTS.

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Protein and DNA adducts have been widely applied for monitoring the internal dose of reactive compounds and metabolites after environmental or occupational exposures. Formation of DNA and protein adducts correlate well with external exposures in rodents and human studies. A recent study in butadiene (BD) exposed workers demonstrated that BD-specific protein adducts correlate with external BD exposure (R2 = 0.6) in BD monomer workers and not in BD-styrene polymer workers (R2= 0.08), despite the fact that the BD exposures were 3-fold higher in the polymer workers. Styrene co-exposure was 14-fold higher in the polymer workers than in the monomer workers. It is suggested that styrene co-exposure effects BD metabolism, since both are metabolized by P450 2e1 to DNA reactive epoxides. Subsequent in vitro studies showed inhibition of P450 2e1 activity by styrene oxide. We report herein the effects of styrene co-exposures on the formation of N7-guanine adducts in vivo. Female B6C3F1 mice were exposed to filtered air, 20 ppm BD, 250 ppm styrene or 20 ppm BD plus 250 ppm styrene for 6 h /day, 5 days/week for 2 weeks. A method was developed for simultaneous quantitation of the isomeric N7-hydroxybuten-guanine (N7-HB-Gua), N7-trihydroxybutan-gua-

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Inhalation Exposure to Jet Fuel (JP8) Among U.S. Air Force Personnel

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Inhalation Exposure to Jet Fuel (JP8) Among U.S. Air Force Personnel

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As jet fuel is a common occupational exposure among military and civilian populations, this study was conducted to characterize jet fuel (JP8) exposure among active duty U.S. Air Force personnel. Personnel (n = 24) were divided a priori *into high, moderate, and low exposure groups. Questionnaires and personal air samples (breathing zone) were collected from each worker over 3 consecutive days (72 worker-days) and analyzed for total hydrocarbons (THC), benzene, toluene, ethylbenzene, xylenes, and naphthalene. Air samples were collected from inside the fuel tank and analyzed for the same analytes. Linear mixed-effects models were used to evaluate the exposure data. Our results show that the correlation of THC (a measure of overall JP8 inhalation exposure) with all other analytes was moderate to strong in the* a priori *high and moderate exposure groups combined. Inhalation exposure to all analytes varied significantly by self-reported JP8 exposure (THC levels higher among workers reporting JP8 exposure),* a priori *exposure group (THC levels in high group > moderate group > low group), and more specific job task groupings (THC levels among workers in fuel systems hangar group > refueling maintenance group > fuel systems office group > fuel handling group > clinic group), with task groupings explaining the most between-worker variability. Among highly exposed workers, statistically significant job task-related predictors of inhalation exposure to THC indicated that increased time in the hangar, working close to the fuel tank (inside > less than 25 ft > greater than 25 ft), primary job (entrant > attendant/runner/fireguard > outside hangar), and performing various tasks near the fuel tank, such as searching for a leak, resulted in higher JP8 exposure. This study shows that while* a priori *exposure groups were useful in distinguishing JP8 exposure levels, job task-based categories should be considered in epidemiologic study designs to improve exposure classification. Finally, the strong correlation of THC with naphthalene suggests that naphthalene may be an appropriate surrogate of JP8 exposure.*

[Supplementary materials are available for this article. Go to the publisher's online edition of the *Journal of Occupational and Environmental Hygiene* for the following free supplemental resource: a pdf file containing a table detailing concentrations of JP8 components.]

Keywords exposure assessment, inhalation exposure, jet fuel, JP8

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INTRODUCTION

Jet propulsion fuel 8 (JP8) is the primary military fuel used by the United States and North Atlantic Treaty Organization (NATO) member countries, with over 5 billion gallons used per year.[1] Due to the widespread use of JP8 and similar jet fuels in the military and commercial airline industry, over 2 million people per year are occupationally exposed.[1]

Information on the health consequences of human exposure to JP8 is limited,[1,2] though there is some evidence that JP8 may be toxic to the immune system, respiratory tract, and nervous system at exposure concentrations near 350 mg/m^3.[3] The current ACGIH® threshold limit value (TLV®) for kerosene and jet fuels is 200 mg/m^3 (total hydrocarbon vapor),[4] which is also the current occupational exposure limit (OEL) recommended by the U.S. Air Force for 8-hour exposure (though there is no enforced Air Force-wide standard for JP8 exposure). Although occupational standards are set for inhalation exposure to JP8, there are no such standards for dermal contact, which is another route of occupational

exposure that has been shown to contribute to total absorbed dose.[5–8]

The composition of JP8 is similar to kerosene and varies by batch, containing many aliphatic and aromatic hydrocarbon compounds (C9-C17+), including varying concentrations of toxic components, such as benzene and naphthalene, plus nonhydrocarbon performance additives.[1–3,9]

The primary objectives of this study were to (1) quantify personal exposure to JP8 using total hydrocarbons (THC) as well as constituents of JP8, including benzene, toluene, ethylbenzene, *m*-/*p*-xylene, *o*-xylene (BTEX), and naphthalene; (2) determine if JP8 exposure differs between our *a priori* assigned (high, moderate, low) exposure groups and evaluate multiple JP8 exposure metrics to assess their utility; and (3) identify potential job-related predictors of JP8 exposure within the high exposure group.

While previous studies have characterized occupational exposure to JP8,[5–7,10–17] this study adds to our limited understanding of JP8 exposure in a number of ways. First, the repeated measures study design allows for a characterization of JP8 exposures that can vary considerably over a workweek while performing multiple tasks. Second, in addition to THC, we quantified JP8 constituents that are potentially neurotoxic and/or carcinogenic (BTEX and naphthalene). Third, JP8 exposures are likely to vary by base and time due to different job characteristics (type of aircraft maintained and ventilation inside of the hangar) and variations in fuel composition. Fourth, personal air exposure was measured throughout the entire work shift but excluding the time while the worker was wearing a respirator and while smoking, thus focusing more specifically on personal exposure to JP8.

MATERIALS AND METHODS

Study Population

Three groups of active duty personnel (n = 24) were recruited from an active U.S. Air Force base and assigned to *a priori* low (n = 6), moderate (n = 9), and high (n = 9) exposure groups based on the likelihood of JP8 exposure in their jobs (determined by a review of historical exposure records and information collected during preliminary base visits). This categorization scheme was chosen to facilitate comparison of our results with previous JP8 studies (e.g., Egeghy et al.[7]) and to reflect a scheme that may be used in epidemiologic studies assessing exposure and health outcomes.

The high exposure group included aircraft fuel systems maintenance workers with routine direct contact with JP8. These participants worked primarily either in the hangar performing maintenance activities on KC-135 Stratotanker refueler aircraft or in an office attached to the hangar performing administrative duties. KC-135 Stratotanker refueler aircraft carry fuel stores for in-air refueling and do not routinely contain fire suppressant foam (the aircraft worked on in this study did not contain fire suppressant foam). The moderate exposure group included workers with regular contact with JP8 via fuel handling (fuels storage, distribution, laboratory testing) or refueling maintenance (performed maintenance activities on fuel distribution trucks). The low exposure group worked in office jobs (health clinic) and did not have regular contact with JP8. This group was categorized as "low" (rather than "no") exposure because there is the potential for everyone on an Air Force base to have some exposure to JP8.[11]

Exposure measurements were collected from the 24 participants during 3 consecutive days (72 worker-days) while performing their normal duties. Each worker-day included collection of questionnaires and personal air and dermal tape-strip samples. Fuel tank air samples were also collected each day. Liquid JP8 samples were collected to determine the concentrations of various components of the fuel (see supplemental material in online edition). The protocol was approved by Army (U.S. Army Research Institute of Environmental Medicine) and Air Force (Wright-Patterson Air Force Base) institutional review boards, and written informed consent was obtained from all participants.

Study Design

A baseline questionnaire was collected from each participant, prior to the work shift on the first sampling day, to obtain information about demographic factors, work history, and tobacco use. Daily post-shift questionnaires were also collected to obtain information about tobacco use, chemical exposures, and protective equipment during each work shift. The high exposure group was asked to provide additional information about exposure scenarios specific to their work environment and duties (e.g., entering fuel tanks, approximate distance from the tank). An observation log detailing work tasks and personal protective equipment was recorded daily by study personnel.

Personal air samples were collected from the breathing zone of each worker during the entire duration of each work shift. The air samples were collected using an active sampling method in accordance with National Institute for Occupational Safety and Health (NIOSH) methods 1501[18] and 1550,[19] a method that has been used in previous assessments of JP8 exposure.[10,12,15,17] Battery-operated sampling pumps were used to collect vapor samples on coconut shell charcoal in two-section (100 mg/50 mg) glass sorbent tubes (Anasorb; SKC Inc., Eighty Four, Pa.) at a flow rate of 0.2 L/min (0.195–0.205 L/min). Personal pumps were paused if the worker left the work area (e.g., for lunch, an errand, or a cigarette break) or entered the fuel tank (when wearing a respirator). A minimum of one sample was collected each day for approximately 30 min from within the fuel tank while an entrant (high exposure group member) was working inside of the tank. Field blanks (n = 12) were collected on each day of sampling. The sorbent tubes were wrapped in foil and shipped in coolers to the Organic Chemistry Analytical Laboratory at the Harvard School of Public Health (HSPH) in Boston, Massachusetts, where the samples were stored at approximately −1°C until analyzed.

In addition to air samples, dermal samples were collected at the end of the work shift using a tape stripping method that has

been previously described.[5,20,21] Adhesive tape (Cover-Roll stretch; BSN medical GmbH, Hamburg, Germany) was precut to 2 × 4 cm, and two successive samples were collected from the same location on the back of the dominant hand. The hand has been shown to be among the two body regions (the arm is the other) most frequently exposed to JP8[5] and thus was chosen for this study. Although a previous dermal JP8 exposure study[5] assessed three body surfaces, additional body regions were not assessed in this study to minimize the burden on study participants as extensive exposure sampling (in addition to that presented here) was conducted.

Each tape strip was applied with constant pressure, left in place for 2 min, removed using clean forceps, and placed in a clean scintillation vial (20 mL; Wheaton, Millville, N.J.) containing 5 mL of acetone. Field blank tape strips were collected each day (n = 12), while duplicate samples were not collected to minimize the burden on study participants. The vials were wrapped in foil and shipped in coolers to the Organic Chemistry Analytical Laboratory at the HSPH (Boston, Mass.) where the samples were stored at approximately −1°C until analyzed.

Air and Dermal Sample Analyses

Air and dermal samples were analyzed for BTEX and naphthalene using gas chromatography mass spectrometry (GC/MS) in selective ion monitoring (SIM) mode,[20,21] and for THC using gas chromatography with flame ionization detection (GC/FID) (NIOSH 1550).[19] Air samples were extracted using NIOSH method 1550.[19] Briefly the charcoal from the sorbent tube was placed in a vial with a Teflon-lined cap, 1 mL of CS_2 was added, and stood for 30 min. An aliquot of the extract was transferred to a GC vial for analysis. Dermal samples were extracted using a previously described method.[21] Briefly the vials containing 5 mL of acetone and the tape strip were placed on a shaker table for 30 min, and the acetone extracts were concentrated from 5 to 0.5 mL. For BTEX and naphthalene, 10 μL of internal standard Napthalene-d8 was added to each sample. A 100 μL aliquot of the extract was transferred to a GC vial for analysis. Following procedures of Chao et al.,[5] we made the *a priori* decision not to analyze the second tape strips if the first tape strips were below the limit of detection.

BTEX and naphthalene were analyzed by GC/MS in SIM using a Hewlett-Packard 6890 GC with temperature and pressure programming capabilities and a split/splitless injector. A capillary column (HP-5MS, 30 m, 250 μm diameter, 0.25 μm film thickness; J&W Scientific, Folsom, Calif.), was used along with the following instrument conditions: injector at 250°C, MS source at 230°C, initial oven temperature at 45°C, held for 2 min, heated to 72°C at 2°/min, then to 280°C at 50°/min, and held for 2 min. The column flow was ramped from 1.5 mL/min (held for 22.0 min) to 1.8 mL/min at a rate of 10 mL/min and then held for 3 min.

THC was analyzed by GC/FID using a Hewlett-Packard 6890 GC. A capillary column (DB-1, 60 m, 250 μm diameter, 1.0 μm film thickness; J&W Scientific) and the following instrument conditions were used: injector at 300°C, detector at 250°C, initial oven temperature at 100°C, held for 5 min, heated to 230°C at 8°/min, and held for 10 min. The column flow was constant at 1 mL/min. FID hydrogen flow was 40 mL/min, airflow was 450 mL/min, and the make-up gas was helium at a flow rate of approximately 45 mL/min.

Statistical Analyses

Air data were analyzed using descriptive statistics, scatter plots, correlation coefficients, and linear mixed-effects models. Units for THC are presented as mg/m^3, whereas units for BTEX and naphthalene are presented as μg/m^3. Values were blank corrected as appropriate using the mean of the field blanks, and all values less than the LOD were replaced with LOD/2. Personal air values exhibited a log-normal distribution and were natural log-transformed prior to analysis. All statistical analyses were conducted using SAS statistical software version 9.1.3 (SAS Institute Inc, Cary, N.C.), and statistical significance is reported at the 0.05 level. The dermal data were not included in statistical analyses due to the low percent of detected measurements (0–24% detect for all of the analytes).

Three air samples were excluded from the analysis. Two sorbent tubes (collected from the high exposure group) broke during the laboratory processing. A third sample (collected from the moderate exposure group) was excluded because there was evidence that the sample was an outlier value and not representative of the worker's actual exposure. The participant may have removed the air pump and placed it near an exposure source, or the sorbent tube may have become contaminated with liquid JP8. Thus, there were 69 air samples included in the final analysis. To address the potential influence of the outlier sample value on results, *post hoc* regression models were run with the sample.

Linear mixed-effects models were used to estimate correlation coefficients and analyze predictors of the exposure levels.[22,23] Models were constructed to assess three JP8 exposure metrics: (1) self-reported JP8 exposure (yes, no); (2) the *a priori* exposure group (high, moderate, low); and (3) job task group (fuel systems hangar, fuel systems office, refueling maintenance, fuel handling, and clinic) for all participants. The fuel handling task group includes those workers from fuels storage, distribution, and testing in the *a priori* moderate exposure group.

Among participants in the *a priori* high exposure group, a second set of models examined job-related predictors of JP8 exposure: time spent in the hangar (hours); distance from the fuel tank during tank work (inside the tank, <25 ft, >25 ft); primary job (entrant, attendant, runner or fireguard, or jobs outside the hangar); searched for a leak (inside or outside the fuel tank); repaired a leak (inside or outside the fuel tank); removed bolts from the tank door; removed the tank door; depuddled; held ventilation in place;, and handed tools to the entrant.

Additional covariates such as smoking status, seniority (based on Air Force specialty codes), and co-exposures to other

TABLE I. Personal Air Summary Statistics by Exposure Group

		Percent			
	N	Detect (%)	GM[A]	(GSD)[B]	Range
THC (LOD[C] = 0.7 mg/m^3)					
Overall	69	64	1.6	(4.3)	<0.7–45.7
High[D]	25	92	5.1	(3.1)	<0.7–45.7
Moderate	26	81	1.7	(3.1)	<0.7–16.5
Low	18	0	<0.7	(NA)	NA–NA
Benzene (LOD[C] = 0.9 μg/m^3)					
Overall	69	64	1.6	(3.5)	<0.9–36.4
High[D]	25	80	2.9	(3.4)	<0.9–36.4
Moderate	26	81	2.1	(3.2)	<0.9–31.7
Low	18	17	<0.9	(NA)	<0.9–3.4
Toluene (LOD[C] = 0.2 μg/m^3)					
Overall	69	100	5.4	(3.6)	0.4–134
High[D]	25	100	11.2	(3.6)	1.3–134
Moderate	26	100	5.5	(3.2)	0.5–58.6
Low	18	100	1.8	(1.7)	0.4–6.6
Ethylbenzene (LOD[C] = 0.4 μg/m^3)					
Overall	69	75	1.8	(6.0)	<0.4–92.1
High[D]	25	96	6.8	(4.1)	0.7–92.1
Moderate	26	96	2.2	(3.7)	<0.4–34.4
Low	18	17	<0.4	(NA)	<0.4–1.0
m-/*p*-Xylene (LOD[C] = 0.2 μg/m^3)					
Overall	69	99	5.3	(6.8)	<0.2–290
High[D]	25	100	21.1	(4.0)	2.3–290
Moderate	26	100	7.1	(3.8)	0.3–107
Low	18	94	0.5	(2.0)	<0.2–3.3
o−Xylene (LOD[C] = 0.6 μg/m^3)					
Overall	69	74	2.6	(6.5)	<0.6–148
High[D]	25	96	10.6	(4.1)	1.0–148
Moderate	26	96	3.3	(3.8)	<0.6–54.7
Low	18	11	<0.6	(NA)	<0.6–1.0
Naphthalene (LOD[C] = 0.7 μg/m^3)					
Overall	69	29	<0.7	(NA)	<0.7–6.6
High[D]	25	52	0.9	(2.6)	<0.7–6.6
Moderate	26	27	<0.7	(NA)	<0.7–2.7
Low	18	0	<0.7	(NA)	NA–NA

Note: NA = not applicable.
[A]Geometric mean (GM).
[B]Geometric standard deviation (GSD).
[C]Average limit of detection (LOD) calculated using flow rate and total time pump was running from personal air samples.
[D]Values were not adjusted to take into account estimated exposure while working in the tank and therefore may be underestimated for some of the high exposure group workers.

chemicals (i.e., gasoline vapors, degreasers or other cleaners) were considered and excluded from final models if the variables were not significant predictors or were determined to be surrogates for other reported variables. Smoking status was not a significant predictor of analytes in air and was excluded from the final models, a result that was expected given that the air pump was removed whenever participants smoked a cigarette. An example of the model used can be described as follows:

$$Y_{ijk} = \ln(X_{ijk}) = \beta_0 + \beta_{1k}\text{EXPGRP}_{ik} + b_i + \varepsilon_{ijk}$$

where X_{ijk} represents the inhalation exposure level of the ith participant on the jth day, and Y_{ijk} is the natural logarithm of measurement X_{ijk}. The β is the fixed effect for the covariate, such that for the *a priori* exposure group variable (EXPGRP) k = (high, moderate, low). The b_i represents the random effect

for each subject, and ε represents the error. Models for the mean were compared using the percent of between-worker variability explained by the fixed-effects model as well as Akaike's Information Criteria (AIC) values (AIC values were obtained using maximum likelihood estimation). A compound symmetric covariance structure was used to fit the models, and the final models were fit using restricted maximum likelihood estimation.

For workers who entered the fuel tank (entrants), the in-tank air samples and NIOSH assigned protection factor (APF) of 50[24] for a full-face, continuous flow supplied-air respirator equipped with a tight-fitting face piece were used to adjust personal air levels, taking into account estimated exposure while working in the tank. The APF 50 adjusted personal air data were used for the scatter plots, correlation coefficients, and regression models. The personal air levels were also adjusted assuming that the participant did not wear the respirator while inside the tank.

RESULTS

The study population included 21 (87.5%) males, 21 (87.5%) participants who described themselves as white, and 7 (29.2%) current smokers. The group averaged 27.7 ± 6.8 years of age and had spent on average 7.0 ± 6.6 years in the Air Force.

Table I presents the summary statistics for THC, BTEX, and naphthalene in personal air samples by exposure group. The geometric mean concentrations for all analytes decreased from the high to low exposure groups. In univariate regression models assessing study day (1–3) as a categorical predictor of the air levels, THC, BTEX, and naphthalene varied significantly by day in the high exposure group ($p < 0.0001$–0.01), whereas ethylbenzene, *m*-/*p*-xylene, *o*−xylene, and naphthalene varied significantly in the moderate exposure group ($p = 0.004$–0.01). The levels did not vary by day in the low exposure group.

The overall within- and between-worker variability (with standard error) for each analyte are as follows: THC: 0.65 (0.14), 1.53 (0.52); benzene: 0.90 (0.19), 0.66 (0.30); toluene: 0.92 (0.20), 0.71 (0.32); ethylbenzene: 0.86 (0.18), 2.49 (0.83); *m*-/*p*-xylene: 0.91 (0.19), 2.89 (0.95); *o*-xylene: 0.84 (0.18), 2.81 (0.92); and naphthalene: 0.24 (0.05), 0.43 (0.15). The ratio of within- to between-worker variability is generally less than one (except for benzene and toluene), indicating that there is more between-worker variability than within-worker variability overall. However, there is generally more within-worker variability than between-worker variability within each *a priori* exposure group. For example, the within- and between-worker variability (with standard error) for THC in the high exposure group are 0.70 (0.14) and 0.45 (0.36), and in the moderate exposure group are 1.05 (0.37) and 0.29 (0.38).

THC was moderately to strongly correlated with all analytes (Table II). Correlations among all other analytes were generally strong, although naphthalene and benzene were moderately correlated. Correlations were generally stronger in

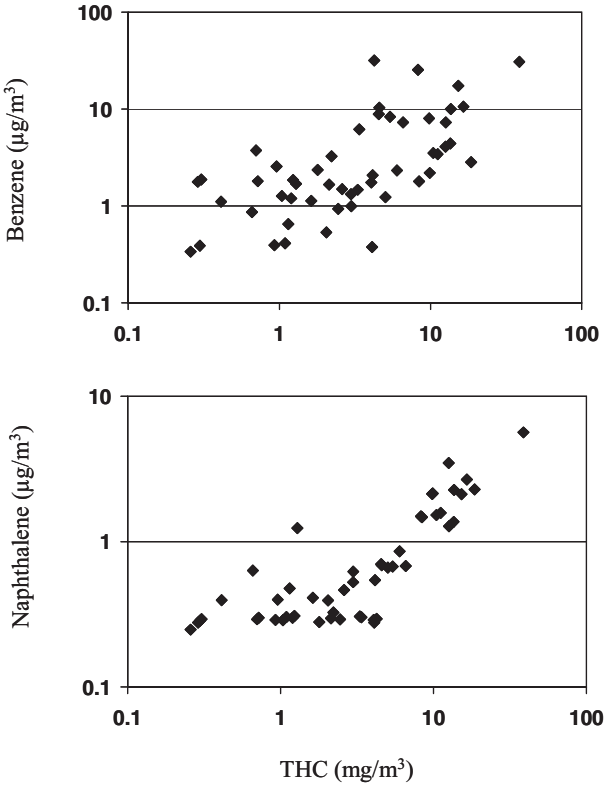

FIGURE 1. Scatterplots of THC with benzene and naphthalene for the high and moderate exposure groups combined (presented on the log scale)

TABLE II. Correlation Coefficients for All Analytes for the High and Moderate Exposure Groups Combined (thc: Mg/M^3, BTEX and Naphthalene: μg/m^3)

	Benzene	Toluene	Ethylbenzene	*m*-/*p*-Xylene	*o*-Xylene	Naphthalene
THC	0.66	0.86	0.91	0.91	0.92	0.81
Benzene		0.84	0.75	0.75	0.72	0.59
Toluene			0.97	0.97	0.95	0.73
Ethylbenzene				1.00	1.00	0.80
m-/*p*-Xylene					1.00	0.79
o-Xylene						0.80

TABLE III. Results of Final Models Evaluating Inhalation Exposure for All Participants (24 workers, n = 69 worker-days)

	THC		Benzene		Toluene		Ethylbenzene		*m*-/*p*-Xylene		*o*-Xylene		Naphthalene	
Parameters	**β(SE)**	**P-values**	**β(SE)**	**P-values**	**β(SE)**	**P-values**	**β(SE)**	**P-values**	**β(SE)**	**P-values**	**β(SE)**	**P-values**	**β(SE)**	**P-values**
Model I														
Intercept	−0.95 (0.34)		−0.46 (0.28)		0.75 (0.30)		−1.19 (0.42)		−0.31 (0.44)		−0.94 (0.44)		−1.10 (0.25)	
Reported JP8		<0.0001		<0.0001		0.0001		<0.0001		<0.0001		<0.0001		0.02
Exposure														
Yes	2.11 (0.41)		1.38 (0.34)		1.39 (0.36)		2.69 (0.50)		2.95 (0.52)		2.88 (0.52)		0.67 (0.29)	
No	0 (Ref)		0 (Ref)		0 (Ref)		0 (Ref)		0 (Ref)		0 (Ref)		0 (Ref)	
Model II														
Intercept	−1.16 (0.29)		−0.66 (0.28)		0.61 (0.29)		−1.51 (0.36)		−0.68 (0.38)		−1.29 (0.37)		−1.20 (0.20)	
Exposure Group		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001
High	2.84 (0.38)		1.71 (0.37)		1.86 (0.38)		3.58 (0.47)		3.87 (0.49)		3.81 (0.48)		1.22 (0.26)	
Moderate	1.72 (0.38)		1.44 (0.37)		1.11 (0.37)		2.37 (0.47)		2.68 (0.49)		2.55 (0.48)		0.32 (0.26)	
Low	0 (Ref)		0 (Ref)		0 (Ref)		0 (Ref)		0 (Ref)		0 (Ref)		0 (Ref)	
Model III														
Intercept	−1.16 (0.16)		−0.66 (0.22)		0.61 (0.17)		−1.51 (0.18)		−0.68 (0.21)		−1.29 (0.18)		−1.20 (0.13)	
Task Group		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001
Fuel Systems Hangar	3.21 (0.23)		1.97 (0.30)		2.18 (0.24)		3.97 (0.25)		4.27 (0.29)		4.22 (0.25)		1.43 (0.18)	
Refueling Maintenance	3.01 (0.35)		2.63 (0.46)		2.53 (0.37)		4.23 (0.40)		4.56 (0.45)		4.46 (0.39)		1.15 (0.28)	
Fuel Systems Office	1.63 (0.32)		0.84 (0.43)		0.86 (0.34)		2.27 (0.36)		2.56 (0.41)		2.45 (0.36)		0.48 (0.26)	
Fuel Handling	1.37 (0.22)		1.13 (0.29)		0.74 (0.23)		1.86 (0.25)		2.17 (0.28)		2.03 (0.24)		0.09 (0.18)	
Clinic	0 (Ref)		0 (Ref)		0 (Ref)		0 (Ref)		0 (Ref)		0 (Ref)		0 (Ref)	

Notes: Units: THC (LN(mg/m^3), BTEX, and naphthalene (LN(μg/m^3)).

TABLE IV. Results of Univariate Analyses Evaluating Inhalation Exposure Among Fuel Systems Maintenance Workers (9 workers, n = 25 worker-days)

	THC (LN(mg/m^3))	
Parameters	**β(SE)**	**P-values**
Time in hangar		0.0002
Hours	0.30 (0.08)	
Distance from tank (during tank work)[A]		<0.0001
Inside	2.00 (0.38)	
<25 ft	0.58 (0.41)	
>25 ft	0 (Ref)	
Job		0.008
Entrant	1.24 (0.49)	
Attendant/runner/fireguard	0.17 (0.54)	
Other (outside hangar)	0 (Ref)	
Searched for leak		0.02
Yes	1.09 (0.46)	
No	0 (Ref)	
Repaired leak		0.7
Yes	0.16 (0.38)	
No	0 (Ref)	
Removed bolts from tank door		0.2
Yes	0.62 (0.48)	
No	0 (Ref)	
Removed tank door		0.1
Yes	0.65 (0.43)	
No	0 (Ref)	
Depuddled		0.1
Yes	1.06 (0.69)	
No	0 (Ref)	
Held ventilation in place		0.09
Yes	0.86 (0.50)	
No	0 (Ref)	
Handed tools to entrant		1.0
Yes	0.01 (0.39)	
No	0 (Ref)	

[A] n = 24 due to missing value of independent variable.

the high exposure group compared to the moderate exposure group (results not presented). Scatterplots of THC with benzene and naphthalene are presented in Figure 1.

The mean air levels measured inside the fuel tank were 402 ± 288 mg/m^3 for THC, 78.8 ± 71.9 μg/m^3 for benzene, 755 ± 484 μg/m^3 for toluene, 764 ± 514 μg/m^3 for ethylbenzene, 2400 ± 1604 μg/m^3 for *m*-/*p*-xylene, 1260 ± 831 μg/m^3 for *o*-xylene, and 77.5 ± 52.7 μg/m^3 for naphthalene.

Exposure Metrics — All Exposure Groups

Table III presents parameter estimates and p-values for three regression models evaluating exposure metrics as predictors of inhalation exposure for all study participants. The results of Model 1 indicate that self-reported JP8 exposure was a significant predictor of THC exposure such that levels were approximately eight times higher (exponentiated β from the model) among workers who reported JP8 exposure. The fixed-effects model explained 61% of the between-worker variability (AIC value of 203.1) but none of the within-worker variability, given that self-reported JP8 exposure did not change over time. Self-reported JP8 exposure was a significant predictor of all other analytes as well.

The results of Model 2 indicate that *a priori* assigned exposure group was a significant predictor of THC exposure such that levels in the high group were 17 times higher than the low group, while levels in the moderate group were six times higher than the low group, reflective of the results presented in Table I. The fixed-effects model explained 81% of the between-worker variability (AIC value of 193.3). *A priori* assigned exposure group was a significant predictor of all other analytes as well.

The results of Model 3 indicate that job task group was a significant predictor of THC exposure such that levels were ranked as follows: fuel systems hangar (25-fold higher than the clinic) > refueling maintenance (20-fold higher than the clinic) > fuel systems office (5-fold higher than the clinic) > fuel handling (4-fold higher than the clinic). The fixed-effects model explained 100% of the between-worker variability (AIC value of 166.7) but none of the within-worker variability, given that task groups did not change over time. Task group was a significant predictor for all other analytes and generally followed the THC task ranking, with a few slight differences.

In the *post hoc* sensitivity analyses, including the one outlier sample, all models remained statistically significant. However, the order of the task groups was impacted in Model 3 such that THC and naphthalene exposure was higher in refueling maintenance than the fuel systems hangar task group.

Job-Related Predictors of Exposure — High Exposure Group

Table IV presents parameter estimates and p-values for univariate regression models evaluating predictors of inhalation exposure to THC for fuel systems maintenance workers (high exposure group, n = 9) over the 3-day study period. Inhalation exposure to THC, as well as BTEX and naphthalene (results not presented), was found to significantly increase with increasing time spent in the hangar during the work shift. Distance from the fuel tank was also a significant predictor of inhalation exposure to THC, as well as all other analytes except benzene, with exposure generally increasing the closer the participant was to the fuel tank.

The participant's job activity was a significant predictor of inhalation exposure to THC, as well as all other analytes except benzene, and generally was ordered as follows: entrants > attendant/runner/fireguard > jobs outside the hangar. The job task of searching for fuel tank leaks was a significant predictor of inhalation exposure to THC, as well as all other analytes, such that exposures were consistently higher among workers whose job tasks involved searching for leaks compared with those that did not.

Removing bolts from the tank door, removing the tank door, depuddling, and holding ventilation in place were not significant predictors of inhalation exposure to THC but were significant for some other analytes. Repairing a leak and handing tools to the entrant were not significant predictors of inhalation exposure. While statistical significance varied, the results of these models consistently indicated that performing these various job tasks led to higher inhalation exposure.

Respirator Protection Adjustments

The geometric mean for the APF 50 adjusted THC data for the tank entrants (7 workers, 11 worker-days) was 8.7 ± 2.3 mg/m^3(range: 1.6–38.8 mg/m^3), while the geometric mean when assuming that the entrant did not wear a respirator inside of the fuel tank was almost 10 times higher (82.6 ± 2.1 mg/m^3, range: 29.5–262 mg/m^3). The relationship between the APF 50 adjusted levels and those assuming that the entrant did not wear a respirator are similar for the other analytes assessed. The mean time spent in the fuel tank was 86 ± 48 min, ranging from 30 to 165 min.

DISCUSSION

Overall, we found that personal exposure levels generally varied over the study days, supporting the statement JP8 exposure varies over time. The utility of the surrogate JP8 exposure metrics increased from self-reported JP8 exposure, to *a priori* assigned exposure group, to job task group being the most informative, suggesting that task-based information provides the most useful surrogate for JP8 exposure. Several job-related predictors of JP8 exposure among fuel systems maintenance workers (*a priori* high exposure group) were also found, indicating that increased time in the hangar, working close to the fuel tank, and performing various job tasks near the fuel tank resulted in higher JP8 exposure. Personal exposure levels for the entrants were higher when assuming the worker did not wear a respirator while working inside the fuel tank, thus highlighting the importance of wearing a respirator while working inside the fuel tank, as exposure levels may exceed 200 mg/m^3(the Air Force-recommended OEL) if the respirator is not worn.

Personal Air Concentrations

All personal exposure levels for THC were below the Air Force-recommended OEL. Similarly, exposures to other analytes were below NIOSH recommended exposure limits (REL). The QA/QC data for naphthalene showed that recovery was low (15%) and likely due to the use of a sorbent that was too strong for naphthalene's higher molecular weight. However, the extraction efficiency for naphthalene was likely reduced in a fairly consistent manner, since naphthalene was highly correlated with THC (87% recovery) in the high and moderate exposure groups combined, and naphthalene was still found to differ significantly by exposure group.

The THC exposure levels in our high and moderate exposure groups were generally lower than those reported previously. Carlton and Smith[10] reported full-shift mean JP8 (THC) levels of 14.2 mg/m^3 during fuel tank entry and repair, activities that should be comparable to our high exposure group. Puhala et al.[12] reported full-shift mean naphtha levels of 1.33 ppm (10 mg/m^3) for aircraft maintenance workers (a category consistent with our high exposure group) and levels of 0.607 ppm (4.5 mg/m^3) for fuel-handling workers (a category consistent with our moderate exposure group).

The benzene exposure levels in our high, moderate, and low exposure groups were also lower than those reported previously. Egeghy et al.[7] reported median benzene levels of 252 μg/m^3, 7.4 μg/m^3, and 3.1 μg/m^3 in similar exposure groups (collected over approximately 4 hr). Puhala et al.[12] reported full-shift mean benzene levels 0.00690 ppm (22 μg/m^3) for aircraft maintenance workers and levels of 0.00573 ppm (18 μg/m^3) for fuel-handling workers.

Within the high exposure group, we expected that personal air exposure levels would be lower than in previous studies, since participants in other studies wore air monitors during tank entry while wearing their respirators,[7] whereas we removed the air monitoring pumps. Our adjusted personal air exposure levels showed much higher levels when assuming that the entrants did not wear a respirator. As mentioned by Puhala et al.,[12] exposure levels would also be expected to vary by base, which may depend on variations in fuel composition, job tasks, work practices, level of work activity, and if the aircraft being worked on contains fire suppressant foam,[7] which would likely result in higher exposure levels.[10]

The adjustment of personal air exposure levels, assuming the entrants did not wear a respirator while working inside the fuel tank, was instructive because, although the measured personal air exposure levels were below the OELs for all analytes, THC exposure levels would have exceeded the Air Force-recommended OEL of 200 mg/m^{3}for one worker-day if this participant had not worn respiratory protection. Similarly, THC exposure levels would have exceeded 100 mg/m^3 on 5 worker-days if the proper respiratory protection had not been worn.

Tank Air Concentrations

As with personal air levels, fuel tank air levels in this study were generally lower than those reported previously. Pleil et al.[11] reported air levels collected inside the fuel tanks (comparable to our interior fuel tank area), with a mean air level for benzene of 2987 ppbv (9543 μg/m^3). The over 100-fold difference between the interior fuel tank area levels measured in this study compared with those reported by Pleil et al.[11] may be due to the lack of fire-suppressant foam used on the aircraft in the present study, differences in the length of time the fuel tank was ventilated prior to sample collection, and differences in the formulation of the JP8 used.

Predictors of Inhalation Exposure

Participants reporting JP8 exposure had significantly increased exposure levels, implying that workers' self-reported JP8 exposure may be a useful surrogate for inhalation

exposure. A more informative predictor of exposure (based on the between-worker variability explained and AIC values) was the *a priori* assigned exposure group based on general job level categorization. However, additional analyses examining exposure levels according to job task group revealed that refueling maintenance workers (part of the *a priori* moderate exposure group and performed maintenance activities on fuel distribution trucks) had higher exposure than fuel systems office workers (part of the *a priori* high exposure group and worked primarily in an office attached to the hangar). The explained between-worker variability of 100% for this model is likely due to the small sample size, and though the use of job task-based categories may reduce the potential for exposure misclassification, it would not eliminate this possibility. The existing potential for exposure misclassification is important to consider given that surrogate categorization schema are often employed in epidemiologic studies to examine relationships between exposure and health outcomes.

The examination of task groups revealed that THC and naphthalene levels were highest among those who worked primarily in the fuel systems hangar, followed by those who worked in refueling maintenance. However, for BTEX, the order of these task groups was reversed, suggesting that exposure to BTEX, at least in the moderate exposure group, may have come from other sources in addition to JP8 (e.g., degreasers or gasoline). Benzene, which had the weakest correlation with THC in the high and moderate group, may have come from other sources (e.g., degreasers or gasoline) in both groups.

The examination of the fuel systems maintenance workers (*a priori* high exposure group) revealed that several job-related factors resulted in increased exposure. Time spent in the hangar during the work shift, distance from the fuel tank, job activity, and searching for fuel tank leaks were all generally significant predictors of the analytes. Although participants wore respirators when entering the fuel tank, entrants likely had higher inhalation exposure compared with the other job activities due to additional time spent outside the tank without a respirator. Searching for fuel tank leaks could have occurred inside (while wearing a respirator) or outside the fuel tank and could be associated with higher inhalation exposure for similar reasons. Our results are generally consistent with those of Egeghy et al.[7] who also found that job (entrant, attendant, other); purpose of maintenance activity (inspect, find leak, repair, other); and distance of the worker from the fuel tank (>3 m, <3 m, inside) were significant predictors of exposure to naphthalene levels in personal air.

Strengths and Limitations

We used a repeated measures study design, collecting samples over 3 consecutive workdays, allowing for a comprehensive characterization of JP8 exposure. This design was important because personal air exposure varied over the study days. Inhalation exposure was measured throughout the work shift, excluding while the worker was wearing a respirator or smoking, thus reducing confounding by these factors. This study also adds to the previous jet fuel literature because JP8 exposure varies by base and time due to variations in job tasks, characteristics, and fuel composition.

Although measured, dermal exposure could not be quantified due to the low percentage of samples with concentrations above detection limits. In spite of this, these findings are important to document because this information could be useful in informing the design of future JP8 exposure and health effects studies. The QA/QC data for THC, ethylbenzene, *m*-/*p*-xylene, *o*-xylene, and naphthalene showed acceptable recovery, although the recovery of the lower molecular weight compounds (benzene and toluene) was low (<30%), which may have been due to volatilization during sample preparation. Therefore, laboratory methodology was sufficient for all of the analytes except benzene and toluene

One explanation for the low detection is that dermal exposures were simply lower in this study than in previous studies that have used this method, as other studies involved examination of fuel systems maintenance workers who had to remove fire suppressant foam from the fuel tanks as part of their work tasks.[5] Another explanation is that the time period between exposure and tape stripping was too long in our study (increased penetration or volatilization time), which may reduce the analyte levels in the upper layers of the skin.[5] A previous study that measured dermal exposure to JP8 did so after a 4-hr work shift, as compared to our full shift, and also measured three exposed body regions with potential for JP8 exposure.[5,6]

Dermal absorption has been shown to be a major route of exposure to JP8,[5–8] and it is important to note that these findings do not reflect a lack of potential for dermal exposure but an inability to capture this exposure at the end of a full work shift using this tape stripping method in this worker population.

The modest sample size (24 workers, 69 worker-days) limited our ability to model personal air exposure levels with multiple parameters. Data for this study came from a single Air Force base, and since exposure scenarios are likely to vary across bases, it is important for future studies to collect data from more than one base to improve generalizability. While adjusting personal air exposure levels among the entrants using the assigned protection factor of 50 is more realistic than assuming 100% respirator protection while inside the fuel tank, in future jet fuel exposure studies it would be more useful to measure the actual exposure levels inside the respirator. We also likely underestimated the naphthalene levels in personal air. For future studies we recommend sample collection procedures using a weaker sorbent that is better suited for determining lower level exposures to a chemical with the molecular weight of naphthalene.

CONCLUSIONS

Exposure levels varied throughout the workweek and were lower than those reported in previous studies, which further supports the idea that exposure levels vary considerably over time and by Air Force base. While self-reported JP8

exposure and the *a priori* assigned exposure groups were useful in significantly distinguishing JP8 exposure levels among our participants, task-based categories may provide further reduction in potential exposure misclassification when used in epidemiologic studies.

Naphthalene was strongly correlated with THC in the high and moderate exposure groups combined, suggesting that naphthalene may be an appropriate surrogate of exposure to JP8. Finally, our results underscore the importance of wearing respirators at all times while working inside the fuel tank, as the potential exists for exposure levels to exceed the Air Force-recommended OEL if the respirator is not worn.

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REFERENCES

1. **Ritchie, G., K. Still, J. Rossi 3rd, et al.:** Biological and health effects of exposure to kerosene-based jet fuels and performance additives. *J. Toxicol. Environ. Health Part B: Crit. Rev. 6(4)*:357–451 (2003).
2. **Agency for Toxic Substances and Disease Registry (ATSDR):** *Toxicological Profile for JP-5 and JP-8*. Atlanta: U.S. Department of Health and Human Services, Public Health Service, 1998.
3. **National Research Council (NRC):** *Toxicologic Assessment of Jet-Propulsion Fuel 8*. Washington, D.C.: NRC, 2003.
4. **ACGIH:** *Kerosene/Jet Fuels: TLV Chemical Substances 7th Edition Documentation*. Cincinnati, Ohio: ACGIH, 2003.
5. **Chao, Y.C., R.L. Gibson, and L.A. Nylander-French:** Dermal exposure to jet fuel (JP-8) in U.S. Air Force personnel. *Ann. Occup. Hyg. 49(7)*:639–645 (2005).
6. **Chao, Y.C., L.L. Kupper, B. Serdar, et al.:** Dermal exposure to jet fuel JP-8 significantly contributes to the production of urinary naphthols in fuel-cell maintenance workers. *Environ. Health Perspect. 114(2)*:182–185 (2006).
7. **Egeghy, P.P., L. Hauf-Cabalo, R. Gibson, et al.:** Benzene and naphthalene in air and breath as indicators of exposure to jet fuel. *Occup. Environ. Med. 60(12)*:969–976 (2003).
8. **Kim, D., M.E. Andersen, and L.A. Nylander-French:** Dermal absorption and penetration of jet fuel components in humans. *Toxicol. Lett. 165(1)*:11–21 (2006).
9. **Zeiger, E., and L. Smith:** The First International Conference on the Environmental Health and Safety of Jet Fuel. *Environ. Health Perspect. 106(11)*:763–764 (1998).
10. **Carlton, G.N., and L.B. Smith:** Exposures to jet fuel and benzene during aircraft fuel tank repair in the U.S. Air Force. *Appl. Occup. Environ. Hyg. 15(6)*:485–491 (2000).
11. **Pleil, J.D., L.B. Smith, and S.D. Zelnick:** Personal exposure to JP-8 jet fuel vapors and exhaust at air force bases. *Environ. Health Perspect. 108(3)*:183–192 (2000).
12. **Puhala, E., G. Lemasters, L. Smith, et al.:** Jet fuel exposure in the United States Air Force. *Appl. Occup. Environ. Hyg. 12(9)*:606–610 (1997).
13. **Serdar, B., P.P. Egeghy, R. Gibson, et al.:** Dose-dependent production of urinary naphthols among workers exposed to jet fuel (JP-8). *Am. J. Ind. Med. 46(3)*:234–244 (2004).
14. **Serdar, B., P.P. Egeghy, S. Waidyanatha, et al.:** Urinary biomarkers of exposure to jet fuel (JP-8). *Environ. Health Perspect. 111(14)*:1760–1764 (2003).
15. **Smith, L.B., A. Bhattacharya, G. Lemasters, et al.:** Effect of chronic low-level exposure to jet fuel on postural balance of U.S. Air Force personnel. *J. Occup. Environ. Med. 39(7)*:623–632 (1997).
16. **Tu, R.H., C.S. Mitchell, G.G. Kay, et al.:** Human exposure to the jet fuel, JP-8. *Aviat. Space Environ. Med. 75(1)*:49–59 (2004).
17. "A Pilot Study of Occupational Assessment of Air Force Personnel Exposure to Jet Fuel before and after Conversion to JP-8." [Online] Available at http://www.dtic.mil/cgi-bin/GetTRDoc?AD=ADA367582&Location=U2&doc=GetTRDoc.pdf (Accessed January 4, 2010).
18. **National Institute for Occupational Safety and Health (NIOSH):** Hydrocarbons, aromatic: Method 1501. In *NIOSH Manual of Analytical Methods (NMAM),* Fourth Edition, P.C. Schlecht and P.F. O'Connor (eds.). Cincinnati, Ohio: Centers for Disease Control and Prevention, NIOSH, 2003.
19. **National Institute for Occupational Safety and Health (NIOSH):** Naphthas: Method 1550. In *NIOSH Manual of Analytical Methods (NMAM),* Fourth Edition, P.C. Schlecht and P.F. O'Connor (eds.). Cincinnati, Ohio: Centers for Disease Control and Prevention, NIOSH, 1994.
20. **Chao, Y.C., and L.A. Nylander-French:** Determination of keratin protein in a tape-stripped skin sample from jet fuel exposed skin. *Ann. Occup. Hyg. 48(1)*:65–73 (2004).
21. **Mattorano, D.A., L.L. Kupper, and L.A. Nylander-French:** Estimating dermal exposure to jet fuel (naphthalene) using adhesive tape strip samples. *Ann. Occup. Hyg. 48(2)*:139–146 (2004).
22. **Hamlett, A., L. Ryan, P. Serrano-Trespalacios, et al.:** Mixed models for assessing correlation in the presence of replication. *J. Air Waste Manag. Assoc. 53(4)*:442–450 (2003).
23. **McClean, M.D., R.D. Rinehart, L. Ngo, et al.:** Inhalation and dermal exposure among asphalt paving workers. *Ann. Occup. Hyg. 48(8)*:663–671 (2004).
24. "NIOSH Respirator Selection Logic 2004." [Online] Available at http://www.cdc.gov/niosh/docs/2005-100/ (Accessed September 8, 2008).

Inhalation Exposure to Jet Fuel (JP8) Among U.S. Air Force Personnel

Supplemental Material

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Keywords exposure assessment, inhalation exposure, jet fuel, JP8

LIQUID JP8 SAMPLES

Two samples of liquid JP8 were collected from the Air Force base in order to characterize components of the fuel: BTEX and naphthalene. The samples were obtained from a KC-135 Stratotanker refueler aircraft and a fuel storage tank. The samples were collected in glass containers and stored at room temperature until shipped to TNO Defence, Safety and Security in The Netherlands, where they were stored at –18ºC prior to analysis by comprehensive gas chromatography with time-of-flight mass spectrometry (GC*GC-TOF-MS).[(1)] Concentrations of BTEX and naphthalene are reported in Table S1.

TABLE S1. Concentrations of JP8 Components

Component	**Concentration (w/v%)**[A]
Benzene	0.004% – 0.007%
Toluene	0.066% – 0.078%
Ethylbenzene	0.104% – 0.104%
m-/*p*-Xylene	0.311% – 0.322%
o-Xylene	0.042% – 0.046%
Naphthalene	0.168% – 0.177%

[A]w/v% = weight/volume % = g/100mL; two liquid fuel samples collected at Air Force base.

REFERENCE

1. Trap H.C.: "Profiling Jet Fuel on Neurotoxic Components with Comprehensive Two-Dimensional GC." Rijswijk, The Netherlands: TNO Defence, Safety and Security, 2007. [Online] Available at http://www.dtic.mil/cgi-bin/GetTRDoc?AD=ADA475160&Location=U2&doc=GetTRDoc.pdf (Accessed December 18, 2009).

Urinary Biomarkers of Occupational Jet Fuel Exposure among Air Force Personnel

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Abstract

There is a potential for widespread occupational exposure to jet fuel among military and civilian personnel. Urinary metabolites of naphthalene have been suggested for use as short-term biomarkers of exposure to jet fuel (JP8). In this study, urinary biomarkers of JP8 were evaluated among US Air Force personnel. Personnel (n=24) were divided *a priori* into high, moderate, and low exposure groups. Pre- and post-shift urine samples were collected from each worker over three workdays and analyzed for metabolites of naphthalene (1- and 2-naphthol). Questionnaires and breathing-zone naphthalene samples were collected from each worker during the same workdays. Linear mixed-effects models were used to evaluate the exposure data. Post-shift 1- and 2-naphthol varied significantly by *a priori* exposure group (levels in high group > moderate group > low group), and breathing-zone naphthalene was a significant predictor of post-shift levels of 1- and 2-naphthol indicating that for every unit increase in breathing-zone naphthalene there was an increase in naphthol levels. These results indicate that post-shift levels of urinary 1- and 2-naphthol reflect JP8 exposure during the work-shift and may be useful surrogates of JP8 exposure. Among the high exposed workers, significant job-related predictors of post-shift 1- and 2-naphthol included entering the fuel tank, repairing leaks, direct skin contact with JP8, and not wearing gloves during the work-shift. The job-related predictors of 1- and 2-naphthol emphasize the importance of reducing inhalation and dermal exposure through the use of personal protective equipment while working in an environment with JP8.

Key words: jet fuel; JP8; urinary biomarkers; 1-naphthol; 2-naphthol; naphthalene

Introduction

Jet propulsion fuel 8 (JP8) is widely used by the militaries of the United States (US) and North Atlantic Treaty Organization member countries, while similar jet fuels are used by the commercial airline industry (Ritchie et al., 2003). There is the potential for widespread occupational exposure to jet fuels, primarily through inhalation and dermal absorption, which is potentially toxic to the immune system, respiratory tract, and nervous system (NRC, 2003).

Naphthalene, a polycyclic aromatic hydrocarbon, is found in JP8 mixtures in low levels (McDougal et al., 2000; Smith et al., 2010), and is classified as Group 2B: "possibly carcinogenic to humans" (IARC, 2002). Naphthalene, which has been used as a surrogate marker for JP8 exposure in previous studies (Chao et al., 2005; Chao et al., 2006; Egeghy et al., 2003; Serdar et al., 2003a; Serdar et al., 2004), has been shown to strongly correlate with overall JP8 inhalation exposure as measured by total hydrocarbons (Smith et al., 2010) and reflects the potential for JP8 exposure through inhalation and dermal absorption (Chao et al., 2005; Egeghy et al., 2003; Kim et al., 2006).

Once absorbed, naphthalene is metabolized through cytrochrome P-450 oxygenases producing 1,2-naphthalene oxide, which can spontaneously rearrange to form 1- and 2-naphthol (1N and 2N) (ATSDR, 2005). Urinary 1N and 2N have been used as biomarkers of total absorbed dose of JP8, reflecting both inhalation and dermal exposure (Chao et al., 2006; Serdar et al., 2004) and have been suggested as short-term biomarkers of JP8 exposure (Serdar et al., 2003a).

The objectives of this repeated measures study were to characterize the absorbed dose of JP8 using 1N and 2N. Specifically we aimed to: 1) describe pre- and post-shift levels of 1N and 2N during several consecutive workdays; 2) determine if 1N and 2N levels differ among our *a*

priori assigned exposure groups; 3) assess the relationship between 1N and 2N with breathing zone naphthalene levels and a dermal exposure surrogate; 4) identify other potential job-related predictors of 1N and 2N levels (e.g. job task, personal protective equipment, etc.); and 5) calculate the half-lives of 1N and 2N.

Materials and Methods

Study population. A total of 24 active duty personnel were recruited from a US Air Force Base, as previously described (Smith et al., 2010). Briefly, three groups of workers were *a priori* assigned to low (n=6), moderate (n=9), and high (n=9) exposure groups. The high exposure group included aircraft fuel systems maintenance workers, who worked primarily in the hangar performing maintenance activities on KC-135 Stratotanker refueler aircraft or in an office attached to the hangar (with some work in the hangar), and had routine direct contact with JP8. The moderate exposure group included personnel who worked in fuels storage, distribution, testing, and refueling maintenance and had regular contact with JP8. The low exposure group included workers with office jobs (health clinic) who did not have regular contact with JP8.

Study design. Exposure measurements were collected from the participants during three consecutive workdays (72 worker-days) while performing their normal duties. Each worker-day of sampling included collection of questionnaires, a pre- and post-shift urine sample, and a personal air sample. The protocol was approved by Army (US Army Research Institute of Environmental Medicine) and Air Force (Wright-Patterson Air Force Base) institutional review boards and written informed consent was obtained from all participants.

A baseline questionnaire and daily pre- and post-shift questionnaires were collected from all participants. The baseline questionnaire, collected prior to the work-shift on the first

sampling day, was used to obtain information on demographic factors, work history, and tobacco use. Daily pre- and post-shift questionnaires were used to obtain information about tobacco use and chemical exposures before and during the work-shift, as well as personal protective equipment use during each work-shift. The post-shift questionnaire also included a subset of questions that were specific to workers in the high exposure group (e.g. entering the aircraft's fuel tanks, approximate distance from the fuel tank, etc.). Information about work tasks and personal protective equipment was recorded in a daily observation log.

During each of the three workdays, two spot urine samples were collected from each worker, one immediately prior to the work-shift and a second immediately following the work-shift. The pre- and post-shift urine samples were collected in 90 mL polyethylene specimen containers, wrapped in foil, and shipped in coolers to the Organic Chemistry Analytical Laboratory at the Harvard School of Public Health (Boston, MA) where the samples were stored at approximately -18˚C until analyzed.

The urine samples were extracted and analyzed for 1N and 2N by gas chromatography mass spectrometry (GC/MS) in selective ion monitoring (SIM) mode using a modification of a previously described method (Serdar et al., 2003b). Briefly, urine samples were enzymatically deconjugated using β-glucuronidase (10 mg of Type H-1 β-glucuronidase per sample) and pH was adjusted to 5.5 using a 1 N (normal) sodium acetate buffer and 3 N hydrochloric acid when needed. The samples were incubated overnight at 37˚C, then extracted twice with pentane (5 mL x 2). The combined extracts were evaporated to dryness, and reconstituted with 190 µL of hexane and 10 µL of Tri-Sil TBT to convert hydroxyls to trimethylsilylated derivatives for analysis using GC/MS. Analysis using GC/MS in SIM mode was done using a Hewlett-Packard 6890 GC with temperature and pressure programming capabilities and a split/splitless injector.

A capillary column was used (HP-5MS, 30 m, 250 µm diameter, 0.25 µm film thickness) (J&W Scientific, Folsom, CA) under the following instrument conditions: injector at 250°C, MS source at 230°C, initial oven temperature at 75°C, hold for 0.4 min, heat to 200°C at 20.5°/min, hold for 3.00 min, then to 230°C at 18°/min, hold for 5.5 min, then to 240°C at 20.5°/min, hold for 11.7 min. The column flow was constant at 1.0 ml/min. Additionally, urinary creatinine levels were analyzed by Quest Diagnostics Inc.

Personal air samples were collected and analyzed for naphthalene via GC/MS in SIM mode as described previously (Smith et al., 2010). Briefly, air samples were collected from the breathing zone of each worker using an active sampling method during the entire duration of each work-shift in accordance with National Institute for Occupational Safety and Health (NIOSH) method 1501 (NIOSH, 2003). The air pumps were paused if the worker left the work area (e.g. cigarette break) or entered the fuel tank (since they wore respirators during entry).

Statistical analyses. Urinary biomarker data were analyzed using descriptive statistics, scatter plots, correlation coefficients, and linear mixed-effects models. Reported values less than the limit of detection (LOD) were used in all analyses. Rather than adjusting urinary 1N and 2N levels for creatinine (i.e. converting to microgram 1N per gram creatinine), urinary 1N and 2N levels were analyzed as microgram per liter urine while controlling for creatinine in the regression models (Barr et al., 2005). Participants with outlying creatinine levels (<0.3 g/L or >3.0 g/L) were considered for exclusion from the regression models (ACGIH, 2009). However, the results were similar with and without exclusion and thus all samples were included in the final models. One urine sample was not analyzed for creatinine (low exposure group, post-shift)

due to insufficient sample volume. Urinary 1N and 2N exhibited a log-normal distribution and were natural log-transformed prior to analysis.

Breathing-zone naphthalene levels (unit = microgram per meter cubed) were adjusted using an 8-hour time weighted average (TWA). For tank entrants, the 8-hour TWA accounted for in-tank exposures using a combination of in-tank naphthalene measurements and an assigned protection factor of 50 (NIOSH, 2004) for a full-face continuous flow supplied-air respirator (Smith et al., 2010). Three breathing-zone naphthalene samples were excluded from the analyses, two (from the high exposure group) that broke during laboratory processing and a third (from the moderate exposure group) that was excluded as an outlier (Smith et al., 2010). All statistical analyses were conducted using SAS statistical software version 9.1.3 (SAS Institute Inc, Cary, NC) and statistical significance is reported at the 0.05 level.

Linear mixed-effects models were used to examine whether biomarker levels (1) increased from pre- to post-shift, (2) accumulated over the three study days (using pre-shift biomarker levels), (3) differed by *a priori* exposure group, or (4) were associated with breathing-zone naphthalene levels. *A priori* assigned exposure group was used to evaluate both pre- and post-shift 1N and 2N levels; however, in the analyses where breathing-zone naphthalene was used as the independent variable, the same-day shift breathing-zone levels were evaluated as predictors of post-shift 1N and 2N levels whereas the preceding day's shift breathing-zone levels were evaluated as predictors of pre-shift 1N and 2N levels. Linear mixed-effects models were also used to estimate correlation coefficients (Hamlett et al., 2003; McClean et al., 2004) as well as the half-lives of 1N and 2N (Sobus et al., 2009).

In addition to the pre-shift biomarker level (when appropriate) and creatinine level, all models controlled for current smoking status (yes/no) since naphthalene is present in tobacco

smoke (IARC, 2002). Alternative smoking variables were considered (i.e. number of cigarettes smoked during shift, number of cigarettes smoked prior to shift) and current smoking status generally provided the best fit. Interactions of current smoking status with exposure group and breathing-zone naphthalene were assessed.

A separate set of analyses was restricted to the high exposure group (fuel system maintenance workers) since some variables were only relevant to this group . Linear mixed-effects models were used to examine whether post-shift biomarker levels were associated with (1) breathing-zone naphthalene levels and time in the fuel tank (a potential surrogate of dermal exposure), and (2) job-related factors (e.g. entering the fuel tank or repairing a leak). The pre-shift biomarker level, post-shift creatinine level, and smoking status (yes/no) were controlled for in all models.

Other potential independent variables derived from questionnaires that were related to 1N and 2N in univariate models ($p \leq 0.1$) were considered for inclusion. Variables considered among all participants included: self-reported JP8 exposure, personal characteristics (e.g. age, race, sex, body mass index (BMI), time in the active Air Force (years), seniority (high, moderate, low based on Air Force Specialty Codes), and exercise), diet-related variables (e.g. consumption of grilled foods or alcohol), health-related variables (e.g. illness in the past week), co-exposures to other chemicals (e.g. gasoline vapors), and personal protective equipment use (e.g. gloves). Among participants in the high exposure group additional variables considered included: time spent in the hangar (hours), primary job (entrant, attendant, runner or fireguard), job tasks performed (e.g. removed bolts from fuel tank door, repaired a leak), distance from the fuel tank during tank work (inside, <25 feet, >25 feet), and reported direct skin contact with JP8. Variables were excluded from the final models when not statistically significant or when

determined to be surrogates for other included variables. Correlation coefficients and scatter plots were used to evaluate continuous variables as surrogates for one another, while the degree of overlap among categorical variables was evaluated. For instance, all participants that reported removing bolts from the fuel tank door also reported removing the fuel tank door.

An example of the final model used to examine *a priori* exposure group as a predictor of post-shift biomarker levels can be described as follows:

$$Y_{ijkl} = \ln(X_{ijkl}) = \beta_0 + \beta_{1k}EXPGRP_{ik} + \beta_{2l}SMK_{il} + \beta_3PREX_{ij} + \beta_4CREATININE_{ij} + b_i + \varepsilon_{ijkl}$$

where X_{ijkl} represents the post-shift biomarker level (1N or 2N) of the i^{th} participant on the j^{th} day, and Y_{ijkl} is the natural logarithm of measurement X_{ijkl}. The βs are the fixed effects for the covariates, such that for the *a priori* exposure group variable (EXPGRP) k = (high, moderate, low), for current smoking status (SMK) l = (yes or no), the pre-shift biomarker level (PREX) = microgram per liter (1N or 2N), and the post-shift creatinine level (CREATININE) = gram per liter. A compound symmetric covariance structure was used to fit the models and generally yielded the lowest Akaike Information Criterion (AIC) value. Models for the mean were compared using AIC values obtained through maximum likelihood estimation and the final models were fit using restricted maximum likelihood estimation. Estimates of the explained variation for the regression models were obtained using the between- and within-worker variance estimates (σ^2_{BW} and σ^2_{WW}, respectively). The explained between-worker variability was calculated as follows: $(\sigma^2_{BW\ (intercept\text{-}only\ model)} - \sigma^2_{BW\ (full\ model)}) / \sigma^2_{BW\ (intercept\text{-}only\ model)} * 100\%$. The explained within-worker variability was calculated as follows: $(\sigma^2_{WW\ (intercept\text{-}only\ model)} - \sigma^2_{WW\ (full\ model)}) / \sigma^2_{WW\ (intercept\text{-}only\ model)} * 100\%$. The total variance was calculated by summing the between- and within-worker variance estimates ($\sigma^2_{BW} + \sigma^2_{WW} = \sigma^2_{Total}$). The explained total

variability was calculated as follows: $(\sigma^2_{\text{Total (intercept-only model)}} - \sigma^2_{\text{Total (full model)}}) / \sigma^2_{\text{Total (intercept-only model)}} * 100\%$.

Results

Table 1 presents population characteristics by exposure group. The study population consists primarily of non-smoking white males. Table 2 presents summary statistics for pre- and post-shift 1N and 2N by exposure group. The geometric mean concentrations of both biomarkers in both pre- and post-shift samples decreased from the high to low exposure groups. The percent of detected measurements in pre- and post-shift samples was high, except for 1N among the low exposure group. Using mixed models the differences in 1N and 2N levels from pre- to post-shift were estimated separately for each exposure group while controlling for smoking status and creatinine level. Post-shift 1N levels were significantly higher than pre-shift levels in the high (β (SE) = 0.75 (0.24), p=0.002) (exponentiated parameter estimate of 0.75 = 2.1 times higher post-shift), moderate (β (SE) = 0.47 (0.14), p=0.0009), and low (β (SE) = 0.74 (0.31), p=0.02) exposure groups. Post-shift 2N levels were significantly higher than pre-shift levels in the high (β (SE) = 0.65 (0.18), p=0.0002) and moderate (β (SE) = 0.21 (0.11), p=0.05) exposure groups, but not the low exposure group (β (SE) = 0.22 (0.27), p=0.4).

Figure 1 presents a scatter plot of post-shift 1N and 2N by exposure group. The correlations were strong for the high (r=0.9) and moderate (r=0.9) exposure groups, and weak for the low exposure group (r=0.3). The correlation between pre-shift 1N and 2N overall was moderate (r=0.6), and was weak to moderate in the high (r=0.5), moderate (r=0.8), and low (r=0.4) exposure groups.

Predictors of urinary biomarkers (all participants). Table 3 (model 1) presents parameter estimates and p-values for regression models assessing *a priori* assigned exposure group as a predictor of post-shift 1N and 2N while controlling for smoking status, pre-shift 1N or 2N level, and post-shift creatinine level. The results indicate that *a priori* exposure group was a significant predictor of post-shift 1N and 2N, such that levels in the high exposure group were 5.2-times and 2.9-times higher than the low group for 1N and 2N, respectively, while levels in the moderate group were 1.4-times and 1.2-times higher than the low group for 1N and 2N, respectively. The model explained 69% and 71% of the total variation for 1N and 2N, respectively, with 83% and 92% of the between-worker variability explained for 1N and 2N, respectively, and 5% and 18% of the within-worker variability explained for 1N and 2N, respectively.

Table 3 (model 2) presents parameter estimates and p-values for regression models assessing breathing-zone naphthalene as a predictor of post-shift 1N and 2N while controlling for the same covariates. The results indicate that breathing-zone naphthalene was a significant predictor of post-shift 1N and 2N, such that levels increased 2.0-times and 1.6-times for 1N and 2N, respectively, with every 1 $\mu g/m^3$ increase in breathing-zone naphthalene level (approximately the median breathing-zone naphthalene level in the high exposure group). The model explained 62% and 64% of the total variation for 1N and 2N, respectively, with 76% and 88% of the between-worker variability explained for 1N and 2N, respectively, and 2% and 6% of the within-worker variability explained for 1N and 2N, respectively.

Table 4 (model 1) presents parameter estimates and p-values for regression models assessing *a priori* assigned exposure group as a predictor of pre-shift 1N and 2N while controlling for smoking status and pre-shift creatinine level. The results indicate that *a priori* exposure group was a significant predictor of pre-shift 1N, but not 2N, such that levels in the

high exposure group were 5.9-times and 1.6-times higher than the low group for 1N and 2N, respectively, while levels in the moderate group were 2.2-times and 1.3-times higher than the low group for 1N and 2N, respectively. The model explained 56% and 53% of the total variation for 1N and 2N, respectively, with 69% and 49% of the between-worker variability explained for 1N and 2N, respectively, and 35% and 55% of the within-worker variability explained for 1N and 2N, respectively.

. Table 4 (model 2) presents parameter estimates and p-values for regression models assessing breathing-zone naphthalene from the previous day as a predictor of pre-shift 1N and 2N while controlling for the same covariates. Only pre-shift 1N and 2N samples from days 2 and 3 were included in these models as breathing-zone naphthalene levels were not collected prior to day 1. The results indicate that breathing-zone naphthalene was a significant predictor of pre-shift 1N, but not 2N, such that levels increased 1.8-times and 1.2-times for 1N and 2N, respectively, with every 1 $\mu g/m^3$ increase in breathing-zone naphthalene level. The model explained 36% and 45% of the total variation for 1N and 2N, respectively, with 46% and 47% of the between-worker variability explained for 1N and 2N, respectively, and 24% and 44% of the within-worker variability explained for 1N and 2N, respectively.

When controlling for current smoking status and pre-shift creatinine, we found a significant interaction between day of sample collection (days 1-3 as a continuous variable) and exposure group (high, moderate, low as a categorical variable) for pre-shift 1N (p=0.05). Further stratifying the model by exposure group, pre-shift 1N was found to increase by 1.2-times over each of the three study days in the high exposure group (parameter estimate (β) (SE) = 0.17 (0.22)); p=0.4), and by 1.3-times in the moderate exposure group (β (SE) = 0.27 (0.10); p=0.008). Pre-shift 1N decreased by 0.7-times over each of the three study days in the low

exposure group (β (SE) = -0.38 (0.20); p=0.06). Controlling for the same covariates there was not a significant interaction between day of sample collection and exposure group for pre-shift 2N (p=1.0). Upon removing the interaction term and exposure group from the model there was no evidence of accumulation of pre-shift 2N over the study days (β (SE) = -0.009 (0.08); p=0.9).

Predictors of urinary biomarkers (high exposure group). Breathing-zone naphthalene and time in the fuel tank (minutes) were assessed as predictors of post-shift 1N and 2N while controlling for smoking status, pre-shift 1N or 2N level, and post-shift creatinine level. In this analysis, restricted to the high exposure group, breathing-zone naphthalene was a significant predictor of post-shift 1N (β (SE) = 0.30 (0.16); p=0.05) (but not 2N: β (SE) = 0.19 (0.22); p=0.4) while time in the fuel tank was a significant predictor of both 1N (β (SE) = 0.01 (0.003); p=0.0001) and 2N (β (SE) = 0.01 (0.003); p=0.001).

Table 5 presents parameter estimates and p-values for regression models assessing job-related factors (entrant (yes, no), repaired leak (yes, no), direct skin contact (any, none), wore gloves during shift (yes, no)) as predictors of post-shift 1N and 2N in the high exposure group while controlling for smoking status, pre-shift 1N or 2N level, and post-shift creatinine level. Job activities including entering the fuel tank (entrant) and self-reported repairing a leak, self-reported direct skin contact with JP8, and self-reported wearing gloves during the shift were all significant or borderline significant predictors of post-shift 1N and 2N. Entering the fuel tank (entrant) resulted in a 3.0-times and 2.7-times increase in 1N and 2N levels, respectively. Repairing a leak resulted in a 1.8-times and 1.9-times increase in 1N and 2N levels, respectively. Direct skin contact resulted in a 1.7-times and 3.0-times increase in 1N and 2N levels, respectively. Wearing gloves during the shift resulted in a 49% decrease in 1N levels and an

84% decrease in 2N levels compared to those who did not report wearing gloves during the shift. The model explained 80% of the total variation for 1N and 67% for 2N.

Half-lives of urinary biomarkers. The estimated half-lives of 1N and 2N were calculated using post-shift 1N and 2N levels along with pre-shift levels from the following day, excluding the pre-shift samples on the first collection day and the post-shift samples on the third collection day. The covariates in the model included: time (hours: post-shift assigned value of 0 hours and pre-shift the next day assigned hours since hour 0), *a priori* exposure group, current smoking status, and creatinine level. Half-lives of elimination were estimated as in Sobus et al. (2009) using the parameter estimate for time as the elimination rate constant (k) and calculating the half-life as ($T_{1/2} = -0.693/k$). Using the elimination rate constants (1N: β (SE) = -0.02817 (0.009398); 2N: β (SE) = -0.01621 (0.007660)), the estimated half-life was 24.6 hr (95% confidence interval (CI): 14.9 hr, 71.1 hr) for 1N and 42.8 hr (95% CI: 22.2 hr, 579.2 hr) for 2N. Further restricting the regression model to 1N and 2N measurements that decreased with time from post-shift to pre-shift the next day (as in Egeghy et al., 2003) and using the elimination rate constants (1N: β (SE) = -0.05103 (0.01130); 2N: β (SE) = -0.04352 (0.007343)), the estimated half-life was 13.6 hr (95% CI: 9.5 hr, 24.0 hr) for 1N and 15.9 hr (95% CI: 12.0 hr, 23.8 hr) for 2N.

Discussion

Overall, we found that the *a priori* assigned exposure group and breathing-zone naphthalene were significant predictors of post-shift levels of both urinary biomarkers and that there may be accumulation of 1N over the work week. Job-related activities such as entering the fuel tank and wearing gloves during the work-shift were found to influence post-shift urinary biomarker levels among participants in the high exposure group.

Urinary biomarker concentrations. The 1N and 2N levels in our study were generally similar to or lower than those in previous studies. Chao et al. (2006) reported geometric mean pre- and post-shift 1N levels of 4,200 ng/L (4.2 µg/L) and 28,000 ng/L (28 µg/L), respectively (and pre- and post-shift 2N levels of 4,350 ng/L (4.4 µg/L) and 38,400 ng/L (38.4 µg/L), respectively) among fuel-cell maintenance workers, which should be equivalent to our high exposure group. Post-shift levels of the urinary biomarkers were lower in our study, while the pre-shift levels were similar. In another study, Serdar et al. (2003a) reported geometric mean pre- and post-shift 1N and 2N levels in similar high, moderate, and low exposure groups. Our pre- and post-shift levels in each exposure group were generally lower than or similar to levels reported by Serdar et al. (2003a).

The post-shift urinary biomarker levels in our high exposure group were expected to be lower than in previous studies (Chao et al., 2006; Serdar et al., 2003a) since measured air levels in our study were also lower (Smith et al., 2010) and relatedly due to a lack of exposure from handling fire-suppressant foam among our participants. Fire-suppressant foam can become saturated with JP8 and would likely result in higher exposure levels compared to working on aircraft without foam (Carlton and Smith, 2000). Jet fuel exposure is also expected to vary from base to base (Puhala et al., 1997) resulting from variations in work activity and practices, as well as the fuel composition.

Pre- and post-shift levels in our moderate and low exposure groups were generally similar to the geometric mean levels of 1N and 2N measured in the US population as part of the National Health and Nutrition Examination Survey (NHANES, 2003-2004) (CDC, 2009). However, pre- and post-shift levels in the high exposure group in our study were higher. Geometric mean 1N levels in NHANES 2003-2004 were reported as 2680 ng/L (2.68 µg/L)

overall, 3020 ng/L (3.02 µg/L) for adults 20 years or older, and 3170 ng/L (3.17 µg/L) for males. Geometric mean 2N levels in NHANES 2003-2004 were reported as 3180 ng/L (3.18 µg/L) overall, 3360 ng/L (3.36 µg/L) for adults 20 years or older, and 3520 ng/L (3.52 µg/L) for males. These results indicate that urinary levels of 1N and 2N are higher in our high exposure group than the general population (maximum levels exceeding the 95^{th} percentiles from NHANES 2003-2004). However, levels in our moderate and low exposure groups were similar to the general population indicating that working on an Air Force base in jobs without high levels of exposure to JP8 does not necessarily lead to increased 1N and 2N levels compared to the general population.

Post-shift urinary biomarkers. After controlling for smoking status and creatinine level, post-shift 1N and 2N levels were generally higher at the end of the work-shift compared to prior to the start of the work-shift, suggesting that exposures during the work day in all exposure groups led to increased urinary biomarker levels (not a significant increase in the low exposure group for 2N). Although participants in the low exposure group were not expected to have job-related exposure to jet fuel, the modest increases in this group could be due to low level jet fuel exposure associated with working on an Air Force base or due to residual confounding by smoking. Post-shift 1N and 2N were strongly correlated in the high and moderate exposure groups suggesting that the likely source of 1N and 2N was naphthalene and not another source such as the pesticide carbaryl (which does not metabolize to 2N) (Meeker et al., 2007; Maroni et al., 2000).

In separate models, *a priori* assigned exposure group and breathing-zone naphthalene exposure were significant predictors of post-shift 1N and 2N, with the exposure group model explaining more of the total variability (although both models explained a high percentage of the

between worker variability). This difference in explained variability may be due to the inability to account for dermal exposure (Smith et al., 2010). Quantitative measures of dermal exposure were not available because of the low percent of detected samples.

Among participants in the high exposure group, breathing-zone naphthalene exposure was a significant predictor of post-shift 1N, but not 2N. However, time spent in the fuel tank (a potential dermal exposure surrogate) was a significant predictor of both 1N and 2N, providing anecdotal evidence that dermal exposure may contribute to the urinary biomarker levels. Since time spent in the fuel tank (while wearing respirators) and not breathing-zone naphthalene was a significant predictor of 2N, dermal absorption may be a more influential route of exposure for 2N compared to inhalation. Although it is not clear that time in the fuel tank is a suitable surrogate for dermal exposure this may be the case if the entrants had good compliance wearing their respirators and the respirators provided adequate protection against inhalation exposure (since during this time period the majority of their exposure would be dermal).

Job-related predictors of 1N and 2N among participants in the high exposure group included entering the fuel tank (status of entrant), repairing a leak, reported direct skin contact with JP8, and wearing gloves during the shift (found to be protective). These factors are likely surrogates for a combination of inhalation and dermal JP8 exposure. Those that entered the fuel tank had higher 1N and 2N levels compared to those that did not. The primary exposure to participants while they were inside of the fuel tank was likely dermal as they wore respiratory protection, which points to the importance of dermal exposure and thus the use of personal protective equipment. However, entrants likely spent time close to the entry hatch of the fuel tank prior to attaching their respirator, thus had the potential for higher inhalation exposure as well. Participants that reported repairing a leak also had higher 1N and 2N levels compared to

those that did not. Repairing a leak likely reflects both inhalation and dermal exposure as leaks were repaired both inside (with respirator) and outside (without respirator) of the fuel tank. Reported direct skin contact with JP8 resulted in higher 1N and 2N levels. Participants reporting direct skin contact likely also had a higher potential for inhalation exposure. Those participants that wore gloves during the shift had lower 1N and 2N levels, suggesting that wearing gloves reduces dermal exposure to JP8. While reported direct skin contact and wearing gloves during shift were significant for 2N, they were only borderline significant for 1N, again suggesting that 2N levels may be more highly influenced by dermal exposure.

While we found that both 1N and 2N are likely influenced by dermal JP8 exposure, our results suggest that 2N may be more highly influenced by exposure through dermal absorption. A previous study (Chao et al., 2006) found that dermal exposure was a significant predictor of urinary 2N levels, but not 1N levels, among fuel-cell maintenance workers (comparable to our high exposure group). Urinary 2N may be more highly influenced by dermal exposure due to differences in the metabolism of naphthalene in the skin (Chao et al., 2006).

Pre-shift urinary biomarkers. Pre-shift 1N and 2N increased across the low to high exposure groups (significant for 1N) suggesting that exposure from the previous day may influence biomarker levels the following day. Breathing-zone naphthalene exposure from the previous day was also associated with pre-shift 1N and 2N (significant for 1N). However, more of the total variability in 1N and 2N levels was explained by the exposure group model, compared to the previous day naphthalene exposure model, which may be due to the inability to account for dermal exposure.

The increase in pre-shift 1N and 2N levels from the low to high exposure groups as well as the influence of breathing-zone naphthalene exposure from the previous day suggests that

previous day exposure may contribute to the biomarker levels. Previous studies have reported the estimated half-life of 1N to be 4 – 14 hr (Bieniek, 1994), and of 1N and 2N combined to be 25.7 hr (95% CI: 14.1 hr, 116 hr) (Sobus et al., 2009). We estimated half-lives of 24.6 hr for 1N and 42.8 hr for 2N, which may be overestimated due to unaccounted for cigarette smoke or other exposures such as continued JP8 exposure after the end of the work-shift (possibly from their clothing). These half-life estimates decreased when the regression model was restricted to 1N and 2N measurements that decreased over time (13.6 hr for 1N and 15.9 hr for 2N). These lower estimates may be more accurate since we were not able to control for all sources of exposure once the worker leaves the work area, and those subjects whose 1N or 2N levels increase over time (from post-shift to pre-shift the next day) may have a higher chance for residual confounding.

The influence of *a priori* exposure group and breathing-zone naphthalene exposure from the previous day also suggests that there may have been an accumulation of the urinary biomarkers over the work week. An increase in pre-shift 1N levels over the three study days was found in the high and moderate exposure groups (significant for the moderate exposure group). There was no evidence of an accumulation of pre-shift 2N levels. The lack of a significant increase in pre-shift 1N in the high exposure group may be due to the fact that the study was conducted Wednesday through Friday (following a Monday holiday) for this group, whereas the study was conducted Monday through Wednesday in the other groups. The urinary biomarker levels within the high exposure group may have been lowest after having been away from exposure for the weekend, subsequently increased after the first day back at work, but by mid-week the accumulation may have tapered somewhat.

Strengths and limitations. The collection of pre- and post-shift biomarker samples over three consecutive work-days allowed us to assess the accumulation of JP8 over several days' time, estimate the half-lives of 1N and 2N, and provide a comprehensive characterization of JP8 exposure that accounted for variability between and within workers, which to our knowledge has not been done in previous JP8 studies. The collection of personal air in addition to biomarker data allowed us to assess the relationship between the two measures. Our measurements of breathing-zone naphthalene exposure improve upon those in previous JP8 studies (e.g. Chao et al., 2006), which were likely overestimated, since air monitors in our study were removed while tank entrants were wearing respirators (Smith et al., 2010) and therefore the estimated association between breathing-zone napthalene and 1N and 2N is likely improved.

Genetic data were not collected and thus we were not able to consider differences in the metabolism of naphthalene based on genetic polymorphisms of enzymes, such as glutathione S-transferase M1 (GSTM1) deficiency or the c1/c2 or c2/c2 type of cytochrome P450 2E1 (CYP2E1) (Nan et al., 2001; Yang et al., 1999). Additional females and racial/ethnic minorities, which were not widely represented in this study, should be included in future studies due to potential variations in the metabolism of naphthalene.

As previously reported (Smith et al., 2010), few dermal samples were above detection limits, thus we were not able to utilize quantitative measures of dermal exposure as a predictor of the biomarker levels. Instead, we incorporated time in the fuel tank as a potential surrogate measure of dermal exposure. The estimated half-lives of 1N and 2N may be overestimated due to unreported or underreported cigarette use prior to and during the work-shift. Finally, urine and breathing-zone air samples were not collected on the same work days in all three exposure

groups and were collected for three days instead of the full work-week, which may have limited our ability to assess accumulation of the urinary biomarkers over time.

In conclusion, urinary 1N and 2N levels increased over the work-shift and *a priori* assigned exposure group as well as measured breathing-zone naphthalene level were significant predictors of post-shift 1N and 2N. These findings, suggesting that post-shift levels of urinary 1N and 2N reflect JP8 exposure during the work-shift, in combination with the ease of sample collection, make urinary 1N and 2N desirable biomarkers for future JP8 studies. The job-related predictors of post-shift 1N and 2N in the high exposure group emphasize the importance of reducing dermal exposure (in addition to inhalation exposure) through the use of personal protective equipment such as wearing gloves while working in an environment with JP8.

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References

ACGIH. 2009. *2009 TLVs and BEIs Based on the Documentation of the Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices.* Cincinnati, OH:American Conference of Governmental Industrial Hygienists.

ATSDR. 2005. *Toxicological Profile For Napthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene.* Atlanta, GA:Agency for Toxic Substances and Disease Registry.

Barr D.B., Wilder L.C., Caudill S.P., Gonzalez A.J., Needham L.L., Pirkle J.L. 2005. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. *Environ Health Perspect* 113(2): 192-200.

Bieniek G. 1994. The presence of 1-naphthol in the urine of industrial workers exposed to naphthalene. *Occup Environ Med* 51(5): 357-359.

Carlton G.N., Smith L.B. 2000. Exposures to jet fuel and benzene during aircraft fuel tank repair in the U.S. Air Force. *Appl Occup Environ Hyg* 15(6): 485-491.

CDC (Centers for Disease Control and Prevention). December 2009. *Fourth National Report on Human Exposure to Environmental Chemicals*. http://www.cdc.gov/exposurereport/. Accessed on January 7, 2011)

Chao Y.C., Gibson R.L., Nylander-French L.A. 2005. Dermal exposure to jet fuel (JP-8) in US Air Force personnel. *Ann Occup Hyg* 49(7): 639-645.

Chao Y.C., Kupper L.L., Serdar B., Egeghy P.P., Rappaport S.M., Nylander-French L.A. 2006. Dermal exposure to jet fuel JP-8 significantly contributes to the production of urinary naphthols in fuel-cell maintenance workers. *Environ Health Perspect* 114(2): 182-185.

Egeghy P.P., Hauf-Cabalo L., Gibson R., Rappaport S.M.. 2003. Benzene and naphthalene in air and breath as indicators of exposure to jet fuel. *Occup Environ Med* 60(12): 969-976.

Hamlett A., Ryan L., Serrano-Trespalacios P., Wolfinger R. 2003. Mixed models for assessing correlation in the presence of replication. *J Air Waste Manag Assoc* 53(4): 442-450.

IARC (International Agency for Research on Cancer). 2002. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Naphthalene.* 82: 367-435.

Kim D., Andersen M.E., Nylander-French L.A. 2006. Dermal absorption and penetration of jet fuel components in humans. *Toxicol Lett* 165(1): 11-21.

Maroni M., Colosio C., Ferioli A., Fait A. 2000. Biological Monitoring of Pesticide Exposure: a review. Introduction. *Toxicology* 143(1): 1-118.

McClean M.D., Rinehart R.D., Ngo L., Eisen E.A., Kelsey K.T., Herrick R.F. 2004. Inhalation and dermal exposure among asphalt paving workers. *Ann Occup Hyg* 48(8): 663-671.

McDougal J.N., Pollard D.L., Weisman W., Garrett C.M., Miller T.E. 2000. Assessment of skin absorption and penetration of JP-8 jet fuel and its components. *Toxicol Sci* 55(2): 247-255.

Meeker J.D., Barr D.B., Serdar B., Rappaport S.M., Hauser R. 2007. Utility of urinary 1-naphthol and 2-naphthol levels to assess environmental carbaryl and naphthalene exposure in an epidemiology study. *J Expo Sci Environ Epidemiol* 17(4): 314-320.

Nan H.M., Kim H., Lim H.S., Choi J.K., Kawamoto T., Kang J.W. *et al*. 2001. Effects of occupation, lifestyle and genetic polymorphisms of CYP1A1, CYP2E1, GSTM1 and GSTT1 on urinary 1-hydroxypyrene and 2-naphthol concentrations. *Carcinogenesis* 22(5): 787-793.

NIOSH. 2003. *Hydrocarbons, Aromatic: Method 1501*. NIOSH Manual of Analytical Methods (NMAM), Fourth Edition. NIOSH Publication No. 2003-154. Cincinnati, OH: National Institute for Occupational Safety and Health.

NIOSH. 2004. *NIOSH Respirator Selection Logic.* NIOSH Publication No. 2005-100. Cincinnati, OH: National Institute for Occupational Safety and Health.

NRC. 2003. *Toxicologic Assessment of Jet-Propulsion Fuel 8.* Washington, DC: National Research Council, National Academy of Sciences.

Puhala E., Lemasters G., Smith L., Talaska G., Simpson S., Joyce J. *et al*. 1997. Jet fuel exposure in the United States Air Force. *Appl Occup Environ Hyg* 12(9): 606-610.

Ritchie G., Still K., Rossi J. 3rd, Bekkedal M., Bobb A., Arfsten D. 2003. Biological and health effects of exposure to kerosene-based jet fuels and performance additives. *J Toxicol Environ Health B Crit Rev* 6(4): 357-451.

Serdar B., Egeghy P.P., Gibson R., Rappaport S.M. 2004. Dose-dependent production of urinary naphthols among workers exposed to jet fuel (JP-8). *Am J Ind Med* 46(3): 234-244.

Serdar B., Egeghy P.P., Waidyanatha S., Gibson R., Rappaport S.M. 2003a. Urinary biomarkers of exposure to jet fuel (JP-8). *Environ Health Perspect* 111(14): 1760-1764.

Serdar B., Waidyanatha S., Zheng Y., Rappaport S.M. 2003b. Simultaneous determination of urinary 1- and 2-naphthols, 3- and 9-phenanthrols, and 1-pyrenol in coke oven workers. *Biomarkers* 8(2): 93-109.

Smith K.W., Proctor S.P., Ozonoff A., McClean M.D. 2010. Inhalation exposure to jet fuel (JP8) among U.S. Air Force personnel. *J Occup Environ Hyg* 7(10): 563-572.

Sobus J.R., McClean M.D., Herrick R.F., Waidyanatha S., Onyemauwa F., Kupper L.L. *et al*. 2009. Investigation of PAH biomarkers in the urine of workers exposed to hot asphalt. *Ann Occup Hyg* 53(6): 551-560.

Yang M., Koga M., Katoh T., Kawamoto T. 1999. A study for the proper application of urinary naphthols, new biomarkers for airborne polycyclic aromatic hydrocarbons. *Arch Environ Contam Toxicol* 36(1): 99-108.

Figure Legend

Figure 1. Scatter plot of post-shift 1N and 2N by exposure group (log scale)[a]
[a]Solid diamond=High exposure group (r=0.9); X=Moderate exposure group (r=0.9); Open circle=Low exposure group (r=0.3); Overall (r=0.8)

Table 1. Population characteristics by exposure group

		Overall (n=24)	High (n=9)	Moderate (n=9)	Low (n=6)
Sex					
(Male)	Count (%)	21 (87.5%)	9 (100%)	8 (88.9%)	4 (66.7%)
Age					
(years)	Mean (SD)	27.7 (6.8)	29.4 (8.5)	25.9 (6.5)	28.0 (4.2)
	Range	(19.1 - 42.6)			
Race, Ethnicity					
(White)	Count (%)	21 (87.5%)	9 (100%)	8 (88.9%)	4 (66.7%)
Body Mass Index					
(BMI)[a]	Mean (SD)	26.5 (2.7)	26.5 (2.0)	26.7 (3.1)	26.4 (3.3)
	Range	(21.7 - 33.1)			
Education					
(years)	Mean (SD)	13.0 (1.4)	13.0 (1.1)	12.2 (0.7)	14.2 (2.0)
	Range	(12.0 – 17.0)			
Time in Active Air Force					
(years)	Mean (SD)	7.0 (6.6)	8.6 (7.9)	6.1 (6.3)	6.1 (5.4)
	Range	(0.5 - 23.0)			
Smoking Status					
(Smoker)	Count (%)	7 (29.2%)	3 (33.3%)	3 (33.3%)	1 (16.7%)

[a]BMI: (weight in lbs. x 703) / height in inches^2

Table 2. Pre- and post-shift 1N and 2N levels by exposure group

		Pre-shift			Post-shift	
	N	% Detect[a]	GM (GSD)	Range	% Detect[a]	GM (GSD)	Range
1-Naphthol (µg/L)							
Overall	72	68%	2.8 (4.2)	0.2 - 85.2	72%	3.5 (4.7)	0.2 - 102.6
High	27	81%	6.2 (4.5)	0.3 - 85.2	100%	12.0 (3.2)	1.6 - 102.6
Moderate	27	67%	2.2 (3.1)	0.4 - 21.7	67%	2.0 (3.6)	0.2 - 18.5
Low	18	50%	1.1 (3.1)	0.2 - 9.9	39%	1.2 (3.1)	0.2 - 13.5
2-Naphthol (µg/L)							
Overall	72	82%	5.5 (2.9)	0.7 - 49.0	81%	4.9 (3.5)	0.4 - 84.0
High	27	81%	6.8 (3.1)	0.7 - 25.3	93%	12.0 (3.2)	0.4 - 84.0
Moderate	27	81%	5.1 (2.6)	0.7 - 41.2	70%	3.1 (2.8)	0.5 - 19.8
Low	18	83%	4.7 (3.2)	0.7 - 49.0	78%	2.6 (2.4)	0.6 - 10.9

[a]Limit of detection (LOD): 1.22 µg/L (1N), 1.69 µg/L (2N) (values <LOD not replaced)

Table 3. Final models evaluating the effect of exposure group and breathing-zone naphthalene on post-shift 1N and 2N levels (all participants)

	1-Naphthol (ln(µg/L))		2-Naphthol (ln(µg/L))	
Parameters	β (SE)	P-values	β (SE)	P-values
Model 1[a]				
Intercept	-0.73 (0.35)		-0.32 (0.25)	
Exposure Group		<0.0001		<0.0001
High	1.64 (0.39)		1.06 (0.26)	
Moderate	0.35 (0.38)		0.16 (0.25)	
Low	0 (Ref)		0 (Ref)	
Smoking status		0.0002		<0.0001
Yes	1.22 (0.33)		1.07 (0.22)	
No	0 (Ref)		0 (Ref)	
1N or 2N (µg/L) (pre-shift)	0.02 (0.009)	0.06	0.02 (0.009)	0.009
Creatinine (g/L) (post-shift)	0.52 (0.15)	0.0005	0.62 (0.12)	<0.0001
Variance estimates				
σ^2_{BW} (Full model)[b]	0.35 (0.18)		0.09 (0.08)	
σ^2_{WW} (Full model) [b]	0.42 (0.09)		0.38 (0.08)	
σ^2_{BW} (Intercept only)[c]	2.00 (0.63)		1.13 (0.38)	
σ^2_{WW} (Intercept only)[c]	0.44 (0.09)		0.47 (0.10)	
Model 2[d]				
Intercept	-0.54 (0.31)		-0.24 (0.23)	
Naphthalene (µg/m^3)	0.70 (0.19)	0.0003	0.46 (0.16)	0.005
Smoking status		0.0003		<0.0001
Yes	1.35 (0.37)		1.22 (0.25)	
No	0 (Ref)		0 (Ref)	
1N or 2N (µg/L) (pre-shift)	0.02 (0.01)	0.007	0.02 (0.01)	0.03
Creatinine (g/L) (post-shift)	0.53 (0.16)	0.0008	0.67 (0.14)	<0.0001
Variance estimates				
σ^2_{BW} (Full model)[b]	0.49 (0.22)		0.13 (0.11)	
σ^2_{WW} (Full model) [b]	0.43 (0.10)		0.44 (0.10)	
σ^2_{BW} (Intercept only)[c]	2.00 (0.64)		1.11 (0.37)	
σ^2_{WW} (Intercept only)[c]	0.44 (0.09)		0.47 (0.10)	

[a]n=71; 1 missing post-shift creatinine sample (low exposure group)
[b]between-worker (σ^2_{BW}) and within-worker (σ^2_{WW}) variance estimates from full model
[c]between-worker (σ^2_{BW}) and within-worker (σ^2_{WW}) variance estimates from intercept-only model

[d]n=68; 2 missing air samples (high exposure group); 1 excluded air sample (moderate exposure group); 1 missing post-shift creatinine sample (low exposure group)

Table 4. Final models evaluating the effect of exposure group and previous day breathing-zone naphthalene on pre-shift 1N and 2N levels (all participants)

	Pre 1-Naphthol (ln(µg/L))		Pre 2-Naphthol (ln(µg/L))	
Parameters	β (SE)	P-values	β (SE)	P-values
Model 1[a]				
Intercept	-1.85 (0.43)		-0.54 (0.33)	
Exposure Group		<0.0001		0.27
High	1.77 (0.41)		0.49 (0.30)	
Moderate	0.78 (0.41)		0.25 (0.31)	
Low	0 (Ref)		0 (Ref)	
Smoking status		<0.0001		<0.0001
Yes	1.40 (0.35)		1.13 (0.26)	
No	0 (Ref)		0 (Ref)	
Creatinine (g/L) (pre-shift)	0.75 (0.13)	<0.0001	0.83 (0.10)	<0.0001
Variance estimates				
σ^2_{BW} (Full model)[b]	0.41 (0.19)		0.21 (0.11)	
σ^2_{WW} (Full model) [b]	0.52 (0.11)		0.33 (0.07)	
σ^2_{BW} (Intercept only)[c]	1.33 (0.47)		0.42 (0.20)	
σ^2_{WW} (Intercept only)[c]	0.79 (0.16)		0.74 (0.15)	
Model 2[d]				
Intercept	-0.79 (0.45)		-0.17 (0.33)	
Naphthalene (µg/m^3) (previous day)	0.56 (0.27)	0.04	0.16 (0.20)	0.42
Smoking status		0.002		0.0002
Yes	1.37 (0.44)		1.14 (0.31)	
No	0 (Ref)		0 (Ref)	
Creatinine (g/L) (pre-shift)	0.54 (0.18)	0.003	0.71 (0.14)	<0.0001
Variance estimates				
σ^2_{BW} (Full model)[b]	0.60 (0.32)		0.25 (0.17)	
σ^2_{WW} (Full model) [b]	0.66 (0.21)		0.41 (0.13)	
σ^2_{BW} (Intercept only)[c]	1.11 (0.47)		0.47 (0.28)	
σ^2_{WW} (Intercept only)[c]	0.87 (0.25)		0.74 (0.22)	

[a]n=72; days 1 – 3
[b]between-worker (σ^2_{BW}) and within-worker (σ^2_{WW}) variance estimates from full model
[c]between-worker (σ^2_{BW}) and within-worker (σ^2_{WW}) variance estimates from intercept-only model
[d]n=45; 2 missing air samples (high exposure group); 1 excluded air sample (moderate exposure group); days 2 and 3

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Table 5. Final model evaluating job-related predictors of post-shift 1N and 2N (high exposure group)[a]

	1-Naphthol (ln(µg/L))		2-Naphthol (ln(µg/L))	
Parameters	β (SE)	P-values	β (SE)	P-values
Intercept	0.91 (0.36)		1.70 (0.55)	
Entrant		<0.0001		0.0001
Yes	1.11 (0.27)		1.01 (0.27)	
No	0 (Ref)		0 (Ref)	
Repaired leak		0.01		0.02
Yes	0.60 (0.24)		0.63 (0.26)	
No	0 (Ref)		0 (Ref)	
Direct skin contact		0.08		0.02
Any	0.55 (0.31)		1.10 (0.46)	
None	0 (Ref)		0 (Ref)	
Wore gloves during shift		0.11		0.0005
Yes	-0.68 (0.42)		-1.86 (0.53)	
No	0 (Ref)		0 (Ref)	
Smoking status		<0.0001		0.02
Yes	1.28 (0.31)		1.09 (0.47)	
No	0 (Ref)		0 (Ref)	
1N or 2N (µg/L) (pre-shift)	0.007 (0.007)	0.33	0.005 (0.02)	0.83
Creatinine (g/L) (post-shift)	0.22 (0.13)	0.10	0.23 (0.15)	0.14
Variance estimates				
σ^2_{BW} (Full model)[b]	0.01 (0.09)		0.25 (0.28)	
σ^2_{WW} (Full model) [b]	0.26 (0.10)		0.23 (0.10)	
σ^2_{BW} (Intercept only)[c]	0.75 (0.49)		0.83 (0.52)	
σ^2_{WW} (Intercept only)[c]	0.67 (0.22)		0.63 (0.21)	

[a]n=27
[b]between-worker (σ^2_{BW}) and within-worker (σ^2_{WW}) variance estimates from full model
[c]between-worker (σ^2_{BW}) and within-worker (σ^2_{WW}) variance estimates from intercept-only model

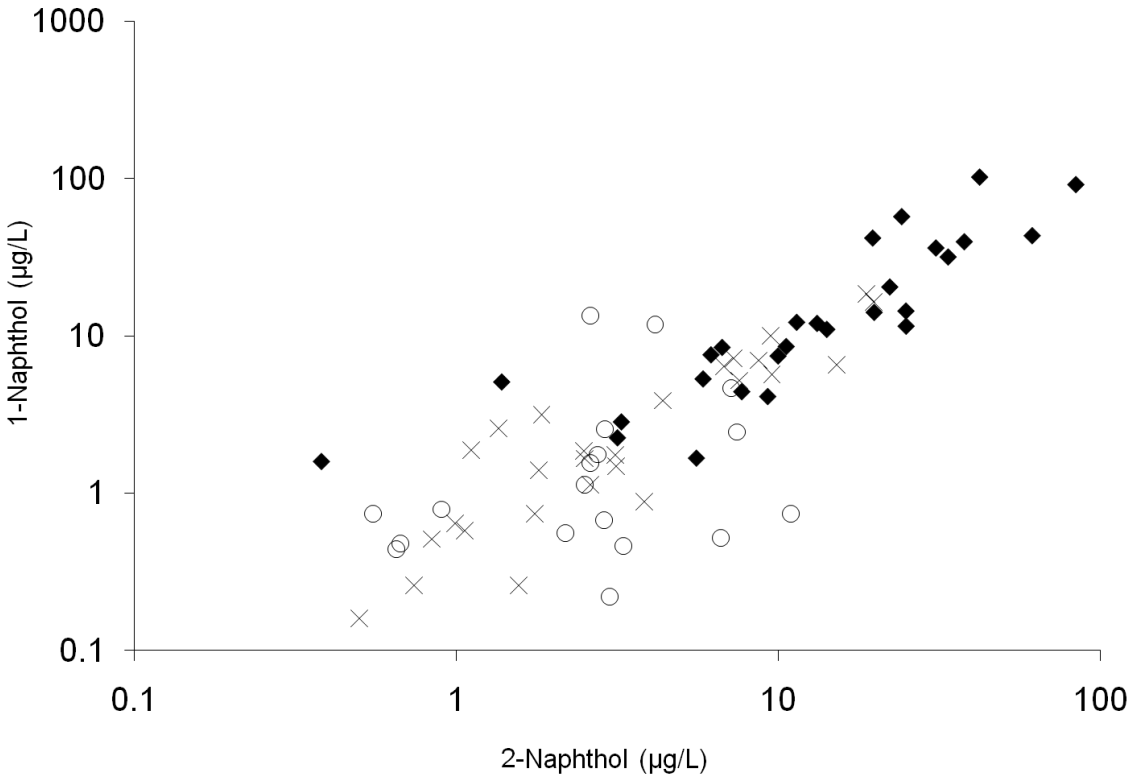

Figure 1. Scatter plot of post-shift 1N and 2N by exposure group (log scale)[a]

[a]Solid diamond=High exposure group (r=0.9); X=Moderate exposure group (r=0.9); Open circle=Low exposure group (r=0.3); Overall (r=0.8)

The Occupational JP8 Exposure Neuroepidemiology Study (OJENES): Repeated workday exposure and central nervous system functioning among US Air Force personnel.

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ABSTRACT

One of the most prevalent workplace chemical exposures historically and currently confronting the global military and civilian workforce is jet propellant (JP) fuel (e.g., JP4, JP5, JP8, jet A1), a complex mixture of numerous hydrocarbon compounds and additives. To date, numerous protective and preventive strategies (e.g., federal exposure limits, workplace procedure protocols, protective gear such as goggles, respirator use, gloves, and coveralls) have been put in place to minimize acutely toxic exposure levels. However, questions remain regarding the effect of repeated exposures at lower (than regulated) levels of JP fuel. The Occupational JP8 Exposure Neuroepidemiology Study (OJENES) was designed to examine the relationships between occupational JP8 exposure over multiple, repeated workdays and specific aspects of central nervous system (CNS) functioning among Air Force (AF) personnel. In this report, we present the OJENES methodology, descriptive findings related to participant characteristics, JP8 exposure levels observed over a work week among higher and lower exposure groups, and neuropsychological task performances at the first study assessment. Results indicated minimal differences between participants in the high and lower exposure groups in terms of descriptive characteristics, other than daily JP8 exposure levels ($p<.001$). In addition, neuropsychological task performances for most task measures were not found to be significantly different from reported reference ranges. These findings demonstrated that confounding and misclassification of exposure and outcome status are not major concerns for the study. Therefore, future OJENES analyses targeting the more focused research questions regarding associations between JP8 exposure and CNS functioning are likely to provide valid conclusions, as they will be less influenced by these research biases.

Keywords: JP8; jet fuel; central nervous system; exposure assessment; military

1. INTRODUCTION

One of the most prevalent workplace chemical exposures historically and currently confronting the global military and civilian workforce is jet propellant (JP) fuel (e.g., JP4, JP5, JP8, jet A1), a complex mixture of numerous hydrocarbon compounds and additives. Recent estimates indicate that over 2 million commercial and military airline workers are exposed to jet fuels each year (Ritchie, 2003). Currently in widespread use, JP8 is similar in composition to kerosene and contains over 200 aliphatic and aromatic compounds (in the C9-C17+ range, such as benzene, toluene, ethylbenzene, xylene, and naphthalene) including thousands of isomeric forms and several nonhydrocarbon performance additives (ATSDR 1998; NRC 2003; Ritchie, 2003; Zieger and Smith, 1998). The current American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) for kerosene and jet fuel vapor in air is 200 mg/m^3, measured as an 8-hour Time Weighted Average (TWA) of total hydrocarbons (THC) (ACGIH, 2003). This level is also the current occupational exposure limit (OEL) for JP8 recommended by the US Air Force (AF) for 8 hour exposure (although there is no enforced AF-wide exposure standard).

To date, numerous risk management strategies, relating to exposure level regulations, workplace procedure protocols, and the use of protective gear such as goggles, gloves, and coveralls, have been put in place to minimize negative health effects associated with toxic exposure levels (e.g., ACGIH, 2003). The rationale for these strategies have been based largely on animal and *in vitro* toxicological research, which indicates adverse effects on immune, respiratory, and nervous systems at JP8 exposure concentrations near 350 mg/m^3 (NRC, 2003). However, the human health hazards of JP8 in workplace settings have not been well characterized. Key questions remain regarding variability in day-to-day occupational exposures

to JP8, factors moderating JP8 exposure in workplace settings, and the effects of repeated acute (days, weeks) and chronic (years) low-level exposures (i.e., lower than regulated levels) on central nervous system (CNS) functioning.

Although no one epidemiology study can address all potential human health consequences pertaining to JP8, the Occupational JP8 Exposure Neuroepidemiology Study **(**OJENES) was designed to address critical knowledge gaps related to JP8 exposure in the workplace, in particular the relationships between acute occupational exposure to JP8 and aspects of CNS functioning. Due to the widespread use of JP8 and comparable jet fuel mixtures (e.g., commercial airline fuel, JetA), the knowledge gained by examining the relationship between occupational JP8 exposure and CNS functioning will be directly relevant to the health and safety of US military personnel as well as civilian airline workers.

1.1. Why Focus on CNS Functioning as the Primary Outcome of Interest?

Neurotoxic disorders rank among the ten leading work-related diseases and injuries in the United States (CDC, 1986). In fact, almost one-third of the acute exposure standards for workplace chemicals have been based on neurotoxicant effects (Anger, 1984).

Historically, animal and human occupational studies involving exposure to solvent mixtures indicate that the nervous system (both central and peripheral) is one of the primary targets. Also, chronic, long-term (17 years on average) occupational exposure to jet fuel estimated to be 300mg/m^3 resulted in significant neurological symptoms (neurasthenia), signs of polyneuropathy, and attentional difficulties and motor speed slowing on neuropsychological tests (Knave et al., 1976, 1978, 1979).

In 2000, a large-scale study of AF personnel was conducted at six different US installations and designed to examine jet fuel exposures and health effects across a range of body systems including the CNS (NRC, 2003; TIEHH, 2001). The study involved 340 AF personnel who were monitored during a 4 hour workshift. Suggestive subclinical neuropsychological performance differences were noted between high and low (or control) exposed workers (Anger and Storzbach, 2001). Specifically, findings indicated that high JP8 exposure groups performed more poorly at pre-shift on tasks involving number sequence recall, psychomotor coding speed, and motor skills, suggesting possible chronic solvent effects involving the executive (reasoning) and psychomotor functional domains. When examining the differences over a four-hour workday period, significant changes in visuospatial memory task performances were related to higher categories of naphthalene exposure. In two other recent studies involving AF (Olsen et al., 1998) and Air National Guard personnel (Tu et al., 2004), high exposure groups showed subtle neurobehavioral effects in functional tasks involving reaction time and response accuracy when compared to lower exposure groups.

Postural sway (balance) markers, a function of both central and peripheral nervous system functions, were associated with naphthalene air levels in preliminary reports from the 2000 AF study (Bhattacharya, 2001). And, in a cross-sectional study of aircraft maintenance personnel by Smith and colleagues (1997), postural sway was significantly associated with higher cumulative JP8 exposure and its constituents (toluene, benzene and xylene), suggesting subtle functional impairment of vestibular and proprioceptive systems with longer exposure.

Although there are data to indicate that CNS functioning may be impacted at levels below current exposure limits, there is limited human epidemiologic research examining the impact of repeated workplace exposures to JP8 and/or its constituent components on CNS functional

outcomes (such as cognitive or balance changes). Preclinical assessment of CNS functioning or performance fulfills a critical occupational health research requirement, not only to identify early indicators of neurologic disease and work performance difficulties but to illuminate and minimize potential safety and injury hazards.

1.2. Knowledge Gaps in the Occupational Health Literature Pertaining to Solvent Mixtures

1.2.1. Repeat Measures of Personal Exposure and Absorbed Dose

Repeated JP8 exposure in both military and civilian occupational settings can occur through exposure to raw fuel, vapor phase, aerosol phase, fuel combustion exhaust, or some combination of these scenarios, depending on individual job responsibilities. Additionally, JP8 exposure through different exposure scenarios may consist of hydrocarbon constituent mixtures with varying composition (Pleil et al., 2000; Puhala et al., 1997). JP8 differs from earlier jet fuel formulations (such as J4 and JP5) as it was designed to provide a safer and less hazardous fuel source than earlier sources, namely by containing less benzene (a known carcinogen), having a higher flashpoint, and being less volatile (NRC, 2003). However, this latter factor implies JP8 and its residues may remain on surfaces (e.g., skin and clothes) following a spill for longer periods than other jet fuels, which may increase the duration of exposure, and dose, among workers directly handling the solvent and persons working in close proximity (Pleil et al., 2000).

1.2.2. Objective Measurement of Individual Exposure and Response Endpoints

Self-reported information about workplace exposures or categorizing workers based on job title, job matrices, or work task descriptions can provide useful surrogate measures of exposure. However, quantification of personal exposure via dermal and inhalation routes combined with biological markers of internal dose provide more accurate estimates of exposure,

thereby minimizing the potential for misclassification biases. Similarly, although self-reported symptoms concerning cognition and balance provide valuable information regarding individual perceptions of functional status and performance, such subjective outcome data may be vulnerable to reporting biases and not always correspond with objective measures of functioning.

1.2.3. Inclusion of the Impact of Potential Modifying Risk and Protective Factors

Numerous factors have the potential to modify JP8 exposure and resulting health outcomes. The interactions between jet fuel exposure and environmental conditions (e.g., temperature, humidity) are known to influence individual exposure and thus personal dose (e.g., Gordon, 2005), while other conditions (e.g., noise) may interact with exposure to influence nervous system functioning (e.g., hearing) (Morata et al., 1993; Odkvist et al., 1986). Lifestyle factors, such as smoking and alcohol use and genetic variability in terms of enzyme polymorphisms (Sodervist et al., 1996), also can interact to modify potential JP8 exposure dose and CNS functioning.

Limited human epidemiologic research has investigated the relationships between objectively and individually-measured repeated measures of JP8 exposure dose and critical CNS functional capabilities (such as cognitive and motor speed abilities and balance parameters). Existing studies lack prospective (repeated measures) designs that could inform about the potential for cumulative doses and/or day-to-day changes in functioning that affect work performance. Recent studies have examined inhalation and dermal exposure combined with biological indicators of JP8 dose measured in urine and exhaled breath samples (e.g., Serdar et al 2004; Egeghy et al 2003). However, few published studies to date have incorporated concurrent, standardized objective measurement of CNS performance measures and potential environmental, lifestyle, and genetic moderating factors.

1.3. Occupational JP8 Exposure Neuroepidemiology Study (OJENES)

The primary research objectives of the OJENES (**Table 1**) were 1) to characterize JP8 exposure and biological dose in an occupational setting over a typical workweek 2) to evaluate the impact of JP8 exposure on CNS functioning, specifically by examining neuropsychological and neurophysiologic (postural sway) performances, and 3) to identify potential modifiers of occupational JP8 exposure including those that may alter exposure levels (i.e., use of personal protective equipment), and those that may modify the relationship between JP8 exposure and CNS functioning (i.e. lifestyle behaviors (smoking status) and genetic polymorphisms). Two studies were carried out as part of the OJENES project (**Figure 1**). In Study 1, we completed a comprehensive exposure assessment to characterize JP8 exposure and biological dose over three consecutive days across a number of AF occupational job categories and work environments (Smith et al. 2010; Smith et al. in press). Study 1 was designed to understand the relationships between repeated exposure to JP8 and both personal exposure and absorbed dose levels. Using the information garnered about high and low exposure job-type categories characterized in Study 1, a second study (Study 2) was conducted using a neuroepidemiology field study design to prospectively (using repeated measures) assess JP8 exposure and CNS functioning over a 6-day work schedule.

This paper outlines and provides the rationale for the OJENES research approach undertaken to examine JP8 exposure and its impact on CNS functioning in an occupational setting. Specifically, we describe the OJENES participant characteristics, examine the variation in JP8 personal exposure levels observed over the work day schedule, and evaluate the initial neuropsychological task performances compared to reference group functional performance ranges. Our focus is Study 2, with the inclusion of several methodological aspects pertaining to

Study 1 where necessary, to present a cohesive overview of the project. Specifically, we have focused attention on descriptive analyses to rule out major sources of bias that could contribute major sources of errors when addressing the OJENES research objectives. As such, this paper lays the foundation for future reports that will target discrete research questions pertaining to exposure-outcome associations within the larger OJENES project.

As a result of our study design and inclusion/exclusion criteria, we anticipated minimal opportunity for participant selection biases; specifically, we hypothesized participants categorized in the higher exposure and lower exposure groups would not differ substantially other than in the degree of JP8 exposure. As observed in Study 1, we predicted personal exposure to JP8 over the study workweek schedule would be markedly different between those participants from higher and lower exposure jobs. Also, as the focus of the OJENES design was to examine relationships between JP8 exposure and CNS functioning (as measured by task performances) rather than overt clinical neurological outcomes, we sought to compare performances from the initial administration of study neuropsychological tasks with accepted reference ranges for those tasks.

2. MATERIAL and METHODS

Research approvals were obtained from human subjects review boards of the Army (US Army Research Institute of Environmental Medicine), AF (AF Research Laboratory at Wright Patterson AF Base (AFB), VA (VA Boston Healthcare System), and Boston University. All participants provided written informed consent prior to participation.

2.1. Study Design and Population

A total of 89 Active Duty AF personnel, who were currently performing job activities of interest, were invited to learn more about the study and 74 (83%) consented to participate in Study 2. Participants were from three US AFB study sites (n=21 at A, n=20 at B, n=33 at C) and each had worked in their current AF job for a period of at least 6 months prior to the study. The study excluded persons with self-reported medical histories of loss of consciousness greater than 20 minutes or known neurological or psychological disorder(s), based on anonymous eligibility screening done at the time of recruitment. The predominant reason for nonparticipation was work scheduling conflicts, as 13 of the nonparticipants were not able to participate in the 6-day study due to their work-leave schedules. One person screened ineligible and another declined to participate.

The recruitment process for both Studies 1 and 2 was designed so that the study sample population included personnel with higher and lower levels of JP8 exposure as part of normal work activities. In Study 1, *a priori* participants were grouped as high, moderate, or low based on AF job codes, job titles and frequency of job-related activities involving JP8. The results from Study 1 suggest that regular job task groupings rather than reliance on AF job codes or job titles provided the best correlations with measured JP8 exposure (Smith et al., 2010). Therefore, in Study 2, we refined our grouping categories into two *a priori* grouping relying primarily on job activities. Participants categorized as in the high exposure group included those in jobs with observed routine exposure to JP8 such as fuel cell repair, maintenance, and fuel handling. In Study 2, 38 participants performed job tasks that involved regular and routine individual personal exposure to JP8 (high *a priori* exposure group) and 36 individuals worked in jobs with little-to-no regular direct exposure to JP8 (low *a priori* exposure group) as part of their work activities.

2.2. Procedures and Methods

At each of the three AFBs, the data collection schedule began post-shift on a Friday afternoon (Day 1) and continued Monday morning through Friday morning of the following workweek, (Day 2 - Day 6). The types of data collected in Study 2 encompass three broad categories: questionnaires and records, JP8 exposure assessment, and assessment of nervous system functioning. These are described in greater detail below.

2.2. 1. Questionnaires and Records

Participants completed a self-administered baseline questionnaire on the first day of the study assessing demographic, health, and military service information. Demographic information included age, sex, height, weight, ethnicity, and education. Military service information included rank, AF Specialty Code (AFSC, Air Force Personnel Center, 2008), time in service and current AF job. Additionally, the questionnaire asked about overseas deployment and combat exposure history, work routine and support, CNS health history (learning disorder diagnoses, head injury with loss of consciousness, acoustic trauma) and patterns of alcohol, coffee, and tobacco use. General health and physical and mental functional health status was measured by the Veterans RAND 12 Item Health Survey (VR-12) (formerly named SF12V; Jones et al., 2001; Kazis et al., 1999). Also, three questions were adapted from those administered in the six-site 2000 AF study (TIEHH, 2001) to provide a descriptive summary of self-reported neurologic health symptoms. Participants were asked to indicate the number of times they had experienced neurologic symptoms (dizziness, concentration, and ability to pay attention) due to work related factors within the past 6 months. Response options were ‘never’, ‘once’, ‘2-5 times’, ‘6-10 times’ or ‘11 times or greater’. If they indicated experiencing the

symptom, they were asked to describe the perceived nature of the contributory work event or experience.

At the start of each workshift on each day in Study 2 (Days 2-6), participants completed a brief survey to itemize any non-occupational exposure to chemicals over the prior 16 hours. This information was collected for the *post hoc* adjustment of possible confounding influences affecting correlations between biological samples (absorbed dose) and measure JP8 exposure levels. These included exposures to other petroleum products (i.e., personal exposure to gasoline as a result of filling car), and tobacco intake (Serdar et al., 2003). Factors (i.e., use of caffeine and alcohol) known to affect performances on neuropsychological tests (Lezak et al. 2004) were also queried. At the end of each workshift, participants also completed a survey to describe specific exposures during their work routine, protective equipment used, and their level of tobacco use and coffee intake during that workday. Respirator fit-test results for those participants who wear a respirator as part of their job activities were obtained from the specific AFB occupational safety office.

2.2.2. JP8 Exposure Assessment

A description of JP8 exposure assessment methods involving air, dermal and urine samples are described below. Although this description focuses on Study 2 procedures, similar methods for collection and analysis were used in both Studies 1 and 2. For specific details on complete Study 1 exposure assessment methods, please see Smith et al. (2010) and Smith et al. (in press).

2.2.2.1. Area and Personal Air Monitoring. On each full study day (Days 2-5), air samples were collected from the participants' personal breathing zone and work areas via active sampling methods. For breathing zone samples, participants wore battery-operated personal air sampling

pumps on a belt around their waist or in a shoulder pouch and sorbent tubes were clipped to the lapel of each participant near their personal breathing zone. For area samples, pumps and sorbent tubes were placed in the work environment and sorbent tubes were clipped to tripods at a height of approximately five feet. Both a coconut shell charcoal and a Chromosorb 106 sorbent tube were used since the Chromosorb 106 tube was found to provide a better measure of naphthalene in air. Flow rates of each sorbent tube were calibrated to 0.2 L/min at the start of each workday; at the end of each sampling day, the flow rates were measured again to derive the mean flow rates for each individual sample collected. In addition, air temperature and relative humidity measurements specific to each worker were obtained via a HOBO® data logger (Onset Computer Corporation, Bourne, MA) that was attached to each pump and collected these data in 15-minute intervals throughout each workshift.

Air samples in Study 2 were collected following NIOSH methods 1550 and 1501 (NIOSH 2003; NIOSH, 1994) for THC and benzene, toluene, ethylbenzene, m, p-xylene, o-xylene (BTEX) and OSHA 35 for naphthalene (OSHA, 1982). Extraction and analyses methods for these compounds followed the same methods as performed in Study 1.

The 8 hr TWA exposure levels were computed for each participant for each study day.

2.2.2.2. Dermal Exposure. Dermal samples were collected post-shift on Day 5 (Thursday) of the study using a tape-stripping method described by Chao and colleagues (2004; 2005) and Mattorano et al. (2004). These samples were analyzed for THC, BTEX, and naphthalene, via methods described above for air samples.

2.2.2.3. Exhaled Breath. Exhaled breath samples were collected on one work day (Thursday, Day 5). Following step-by-step verbal instructions from study team personnel, each worker provided pre- and post-shift exhaled breath samples in 75 ml glass bulbs equipped with threaded, plastic

end caps (Egeghy et al., 2000; Egeghy et al., 2003). In brief, breath samples were self-collected by first, removing the end caps; completely and forcibly exhaling through the glass bulb; replacing one end of the cap while the bulb was still in their mouth, and then quickly replacing the other end cap. Breath samples were analyzed for BTEX and naphthalene by gas chromatography mass spectrometry (GC/MS) in selective ion monitoring mode (SIM).

2.2.2.4. Urine Samples. Pre- and post-shift urine samples were collected from each participant on each workday (Day1-6) and analyzed for 1- and 2-naphthol, by GC/MS in SIM mode using a modification of a previously described method (Serdar et al., 2003). As the concentration of compounds measured in urine vary between and within individuals as a result of the kidney output and hydration, urinary creatinine was measured and included as a covariate in statistical models. Additionally, samples were analyzed for: 2-,3-, & 9- hydroxyfluorene; 1-,2-,3-, & 4-hydroxyphenanthrene; and 1-hydroxypyrene (Li et al., 2006, 2008) and volatile organic compounds (VOC) mercapurates including N-acetyl-S-(benzyl)-L-cysteine (parent compound: toluene), N-acetyl-S-(phenyl)-L-cysteine (parent compound: benzene) (Ding et al., 2009). Cotinine and other nicotine analytes (Bernert et al., 2005) were also collected to complement self-reported smoking history information.

2.2.2.5. Blood Samples. Blood was collected post-shift on one work day (Thursday, Day 5) and analyzed for trace level amount (ppt) quantification of VOC fuel components including benzene, ethyl benzene, m-/p-/o- xylenes, and toluene, using the solid-phase microextraction (SPME) (Blount et al., 2006). The presence of glutathione-S-transferase (GST) enzyme polymorphisms (Schwartz et al. 2005), specifically deletion of GSTM1, was examined as a potential marker of susceptibility of exposure (Nan et al., 2001) and neurotoxic response (Soderkvist et al., 1996).

As another potential marker of exposure, levels of peripheral blood DNA methylation patterns were determined using methods described by Bollati et al. (2007).

2.2.3. Assessment of Neurological Functioning

All participants underwent a brief baseline neurologic screening examination performed by a trained military professional (occupational or physical therapist) to rule out the presence of gross neurologic impairment(s). The neurologic examination assessed handedness, cranial nerve function, motor and sensory function, and reflexes (Feldman and Travers, 1984; White et al., 1992). Body weight and height of each participant were recorded to calculate his/her weight-to-height ratio for use in the assessment of balance functioning.

Neuropsychological testing was conducted at the end of shift on the first day of the study (Day 1 Battery) and subsequently at the start of shift on Day 2, Day 4, and Day 6 (Repeated Day Battery). The test batteries (**Table 2**) were designed to be feasible in a field study environment (given time and environmental constraints) and to provide appropriate and reliable measurements of performance in a repeat testing scenario (White and Proctor, 1992; White et al., 1994). To increase experimenter reliability and facilitate administration and data management efficiency, the battery included several tasks of attention, reaction time, psychomotor speed and efficiency administered in a computer-assisted format using the Automated Neuropsychological Assessment Metrics (version 4, ANAM4™) test battery (C-SHOP, 2007). These tasks included tests of spatial processing and visual-spatial memory (Match to Sample-M2S) and simple reaction time (Simple Reaction Time-SRT), which are described more fully in Vincent et al. (2008). In addition, the ANAM4 Standard Continuous Performance Task (CPT) and ANAM4 Finger Tapping Test were included to assess sustained attention and motor speed, respectively. Other traditional examiner-administered neuropsychological tasks were included to allow

examination of particular functional domains of interest (e.g., general academic abilities) that were not measured via the computer-assisted tasks. Participants completed the Positive and Negative Affect Scale (PANAS, Watson et al., 1988) to assess current mood state and the ANAM4 Sleepiness Scale to assess current alertness on each day of neuropsychological testing. On Day 1, all participants were administered trial 1 of the Test of Memory Malingering (TOMM, Tombaugh, 1996). The TOMM is a simple 50-item visual memory test that was administered for the purpose of excluding persons from the analyses who exhibit low levels of engagement in the objective cognitive tests. Insufficient effort was defined as scores below 38 on Trial 1 of the TOMM (O'Bryant et al., 2007).

Balance testing using the SwayStar™ Balance System (Allum et al., 2001; Gill et al., 2001) was conducted pre- and post shift during a work day (either Tuesday or Thursday) in a subset of high and low exposure group participants to assess visual, vestibular, and proprioceptive systems as measures of CNS integrity. SwayStar™ consists of a belt mounted device that rests against the subject's lower back and contains two digitally based angular velocity transducers that measure pitch (anterior/posterior movement) and roll (lateral movement). The amount of sway, or deviation of the body center, as quantified by the SwayStar™ Balance System was assessed under four different task paradigms (standing bare floor without shoes with eyes open, standing bare floor without shoes with eyes closed, standing on a 4-inch (10 cm) thick piece of foam with eyes open, and standing on a 4-inch (10 cm) thick piece of foam with eyes closed).

2.3. Data Analyses

Descriptive characteristics of the high and low exposure groups were compared via Student's t-test for continuous variables or Chi-square statistics for categorical analyses. The measurement of THC levels in air provided an overall estimate of the level of JP8, and units for computed 8- hour TWA for THC are reported as mg/m^3. All values were blank corrected as appropriate using the mean of the field blanks and all values less than the limit of detection (LOD) were replaced with LOD/2. THC air values exhibited a log-normal distribution and were natural log-transformed prior to analyses. All analyses involving personal air were conducted using SAS statistical software (SAS v 9.1.3). To compare between-group THC levels measured for each separate study day, Wilcoxon rank sum tests were performed. Linear mixed-effects models were used to examine differences in repeated THC air levels over the 4 consecutive work days (Days 2-5) when personal air sampling was performed. Specifically, exposure levels groupings (high, low) and study day (2,3,4,5) were examined as predictors of THC air levels, along with the interaction between exposure group and day to determine whether the two exposure groups differed over time.

Neuropsychological task performances on the Day 1 Battery tasks and those from the first assessment period for the Repeated Battery (Day 2) were examined. Two sample t-test analyses were performed to compare neuropsychological task performances of the study group overall to reported normative values for these tasks. The normative, reference group data were obtained groups of healthy adults as reported in the associated clinical test manuals or published studies for those specific neuropsychological tasks (references provided below as part of Table 4). The reference group values reported for the select ANAM4 tasks were from a study of Active Duty military (Army). Neuropsychological performance means for age-specific subgroups within the OJENES study were compared to appropriate age-adjusted reference

values, when available and provided there were greater than n=12 in the study age group. Statistical significance was defined as $p<.05$ for all analyses.

3. RESULTS

All participants were enlisted AF personnel, with 39% ranked Staff Sergeant (E5) or higher. No significant differences were observed in demographic (e.g., age, rank, gender) or lifestyle habits (e.g., recent sleep, alcohol consumption, current smoking status) variables (**Table 3**) between the high and low JP8 exposure groups. Significantly fewer females were included in the higher exposure compared to the lower exposure group ($p <0.001$). There were no significant differences between the JP8 exposure groups in their reported histories of hobbies or activities outside of work that included exposure to chemicals with potential neurological effects, such as model building, painting/silk screening/art work, furniture refinishing, woodworking, home carpentry, house painting, automobile restoration or mechanics, and plumbing. A total of 21 (28%) reported some hobby work currently or in the past which had the potential for exposure to neurotoxicants, with 13 (34%) from the high group and 8 (22%) from the low group. There were no significant differences in reports of general health or specific neurologic health symptoms between the high and low exposure groups. Workers in higher exposure jobs compared to those in lower exposure jobs were more likely to report a higher prevalence of feeling dizzy because of a work experience, though this difference was not statistically significant. When asked to describe the contributing experience, those in the higher group reported exposure to fuel or other workplace chemicals, while those in the low group reported it being the result of stomach virus, stress at work, or intense computer screen work.

During the study period, 8 hr TWA THC levels ranged between 0.24-22.01 mg/m^3 and 0.24-73.93 mg/m^3 in the low and high exposure group, respectively. And, the 8 hr TWA THC exposure among those persons working in *a priori* high exposure jobs demonstrated at least a 2-fold or greater increase over the study work-week schedule, from Monday to Thursday (**Figure 2)**. When comparing the JP8 exposure between the high and low exposure groups on each of the 4 full study days, significantly higher daily levels were observed on each day in the high group compared to low exposure group (all $p<0.0001$). And, also, when taking into account the repeated measures, THC levels were significantly higher in the high compared to the low exposure groups ($p<0.0001$). As the 4-day study period progressed through the work week, THC levels were significantly higher ($p =0.015$). However, no significant interaction effect was found ($p=0.31$); that is, the increase in exposure levels over the 4 study days was not found to be statistically significantly different in the high compared to the low group.

None of the participants demonstrated impairment(s) on the brief baseline neurologic screening examination. When administered on Day 1, no evidence of insufficient task engagement was observed on the TOMM, as no participant scored below 38 (mean=48.05 (SD=2.37). No significant differences between OJENES neuropsychological performances and reported reference values were found for any of the specific tasks in the Day 1 and Repeated Day Battery administration on Day 2 (**Table 4**), except for among the 20-29 year old group on the Total Recall, Delayed Recall, and Retention tasks on the Hopkins Verbal Learning Test-Revised (HVLT-R). Specifically, the means (SD) among the 43 OJENES participants in the 20-29 year age group were 25.5 (3.6) on Total Recall, 9.1 (2.0) on Delayed Recall, and 90.1 (10.4) on the Retention tasks and significant differences (significant differences were $p<0.001$, $p<0.001$ and $p<0.004$ for these tasks, respectively).

4. DISCUSSION

Although occupational epidemiologic and toxicologic research over the years has led to increased understanding of potential impacts of mixed solvent exposures (such as JP8) on CNS functioning, many knowledge gaps still exist, particularly in terms of repeated, lower (than regulated standards) levels of exposure to chemical mixtures. In this set of foundation analyses for the OJENES, we found minimal descriptive differences between the participants comprising the high versus the low exposure groups other than their degree of exposure to JP8. We documented an increase in JP8 exposure levels as the workweek progressed, with a 2-fold or higher increase within the high exposure group. No statistical difference was noted between the exposure groups over the week, most likely due to the wide range of exposures measured in the high group on any given study day (see plotted error bars reflecting 95% confidence intervals in **Figure 2**). In addition, neuropsychological task performances did not differ from normative reference values with the exception of total score on learning trials and delayed recall and the % retention score on the HVLT-R for those in the 20-29 year old group. These findings demonstrated limited presence of confounding influences in that the higher and lower exposure groups do not differ in terms of descriptive characteristics. Also, minimal misclassification of exposure and outcome status was present. In summary, these results provide evidence of sufficient range of JP8 exposure from low to higher relative levels and reinforce the study design's capability to provide valid conclusions when testing further hypotheses focused on research questions pertaining to the association between JP8 exposure and CNS functional health in an occupational setting.

To our knowledge the OJENES is the first study to examine of JP8 exposure in a military setting using a prospective, repeated workday design and combine objective measurement of both environmental and personal indicators of JP8 exposure within individuals with objective, repeated measurements of CNS functioning (i.e., neurocognitive changes over a workweek schedule and postural sway changes over a workshift). Findings from OJENES Study 1 (Smith et al., 2010) support earlier work identifying those AF job tasks with higher levels of JP8 exposure to include fuel tank entry, maintenance, and refueling activities (e.g. TIEHH 2001; Egeghy et al., 2003). With the OJENES, we have extended the scope of earlier studies in order to fully document differences in the degree of exposure and personal dose, both between and within personnel present during consecutive work days depending on workshift job task assignments and use of protective gear. In addition, the OJENES design has incorporated the individual assessment of lifestyle habits (smoking status, alcohol use), other workplace exposures, and genetic characteristics that could potentially impact repeat exposure to JP8 in a workplace setting and its relationship with CNS functioning.

In terms of study limitations, the OJENES was conducted in AF occupational settings and thus may not necessarily reflect the JP8 exposure scenarios present in current operational or deployment scenarios. While the study is able to examine some aspects of the health consequences of historical (chronic) exposure to jet fuels (based on modeling estimates of exposure using years of AF service and years worked in current AF job), its strength focuses on better understanding consecutive, acute workday exposures to JP8 and specific CNS functioning endpoints. That said, there are limits to the study's ability to measure task-specific, short-lived spikes in exposures, as the OJENES utilizes day-specific, 8- hour TWAs and not real-time exposure level (minutes or hours) changes.

Nonetheless, OJENES builds on existing knowledge concerning exposure assessment from the 2000 AF study (NRC, 2003; TIEHH, 2001) and extends the state of the science to evaluate the influence of repeated, consecutive workday exposure to JP8 on CNS functioning measured via standardized objective measures. The OJENES design directly addresses the NRC (2003) report's recommendation for the conduct of field research studies that combine the in-depth assessment of on-the-job ambient concentrations of JP8 and its constituent compounds, determine body burden via assessment of target biomarkers, and correlate these exposure and dose levels with objective performance endpoints. Future papers will address those additional research questions outlined in **Table 1** regarding relationships between individual-level, repeated occupational exposures to JP8 and objective CNS functioning.

Conflicts of Interest Statement

The authors declare that there are no conflicts of interest.

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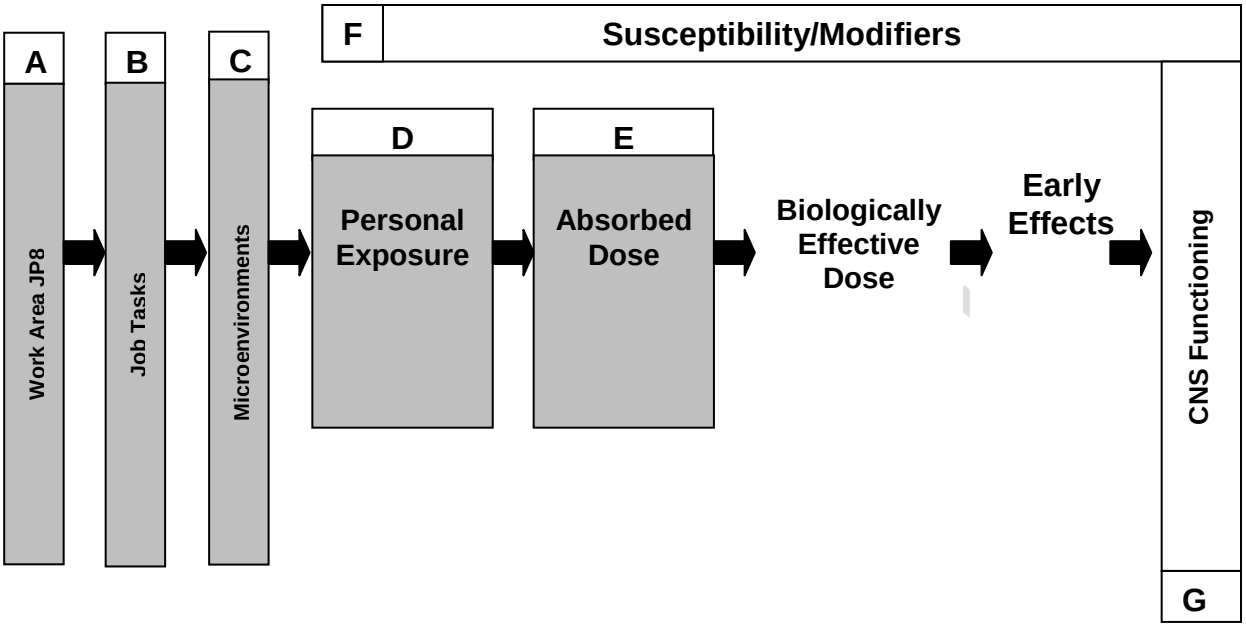
Figure 1: Overview of Study Design



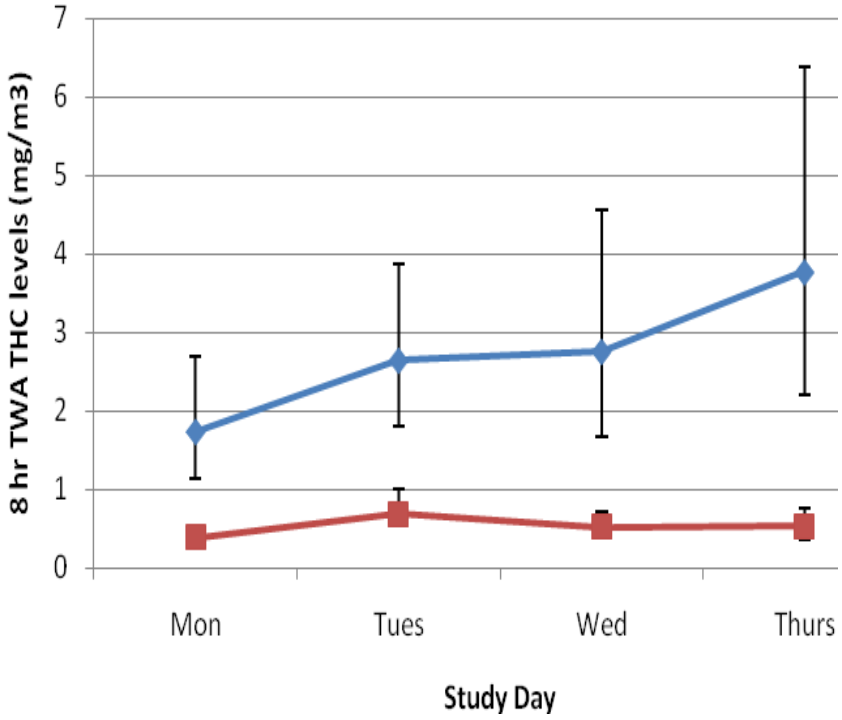
A Exposure Groups: High and Low-none
B Specific Job Tasks
C Job Task work area microenvironments, in terms of exposure, temperature and humidity
D Personal Exposure measures: Breathing space air and dermal samples
E Absorbed Dose measure: Exhaled breath, urine, blood
F Lifestyle factors (smoking), use of protective equipment (gloves, respirator, etc.)
G Neuropsychological and postural sway (balance) performances

TABLE 1. Overview of OJENES Primary Study Objectives and Research Questions

Study Objective 1: **Characterize exposure to JP8 (Study 1 and Study 2)**
1. What is the variability in JP8 exposure over consecutive workdays? 2. What levels of personal exposure to JP8 fuel and its constituents are experienced by different AF job categories? 3. What is the impact of repeated exposure to JP8 across consecutive workday periods on body burden? 4. What is the association between measured personal JP8 exposure and biological indicators (i.e. urinary biomarkers)?
Study Objective 2: **Evaluate relationship between JP8 exposure and CNS functioning (Study 2)**
1. How do study participants' functional performances compare to normative/reference group levels? 2. What is the relationship between level of AF occupational exposure to JP8 and neuropsychological functioning? 3. What is the impact of repeated exposure to JP8 on changes in neuropsychological functioning over a workweek schedule? 4. Is workday exposure to JP8 associated with postural sway (balance) performance changes?
Study Objective 3: **Identify potential risk and protective factors (Study 1 and Study 2)**
1. What demographic, occupational, or lifestyle (i.e. smoking) risk and protective factors impact the degree of JP8 exposure? 2. Are there demographic, occupational, lifestyle (i.e. smoking), or genetic risk and protective factors that significantly influence the relationship between exposure to JP8 exposure and CNS functioning?

Bold, italicized questions are the focus of this report.

Figure 2. Geometric mean 8 hr -TWA THC levels measured in personal air for each of the four work-week study days, by *a priori* exposure group.



Error bars represent 95% confidence intervals.
High exposure Low exposure

Table 2. OJENES Neuropsychological Batteries

	Domain Assessed	**Task Reference**
Day 1 Battery		
Shipley Institute of Living Scale, Vocabulary	General Academics	(Shipley, 1946)
Hooper Visual Organization Test	Visual Memory	(Hooper 1958)
Hopkins Verbal Learning Test – Revised	Verbal Learning and Memory	(Brandt et al. 1998; Brandt and Benedict 2001)
Repeated Day Battery		
Auditory Consonant Trigrams	Executive function, memory	(Stuss 1987)
ANAM4 Match to Sample	Visuospatial ability, memory	(C-SHOP 2007)
ANAM4 Simple Reaction Time	Attention, psychomotor ability	(C-SHOP 2007)
ANAM4 Continuous Performance Test	Sustained attention	(C-SHOP 2007)
ANAM4 Finger Tapping	Psychomotor speed	(C-SHOP 2007)
WAISIII Digit Span	Attention	(The Psychological Corp, 1997)
Grooved Pegboard	Fine motor abilities	(Matthews and Klove, 1964)

Table 3. Characteristics of OJENES Tier II Study Participants (n=74)

	Overall Group (n=74)	Low Exposure Group (n=36)	High Exposure Group + (n=38)	
8 hr TWA THC (mg/m^3), GM(SD)	1.22 (3.64)	.53 (2.8)	2.65 (4.2)	***
Demographics				
Age, mean years (SD) [range]	25.8 (6.25) [18.6-43.0]	26.18 (6.33)	25.4 (6.23)	
Education, mean years (SD) [range]	12.5 (1.36) [12.0-20.0]	12.69 (1.72)	12.3 (0.88)	
Male, n (%)	62 (83.8)	25 (69.4)	37 (97.4)	***
White, Caucasian, n (%)	53 (71.6)	26 (71.2)	27 (71.1)	
Currently married, n (%)	40 (54.1)	20 (55.6)	20 (52.6)	
AF service, mean years (SD) [range]	5.8 (5.35) [0.5-20.0]	6.09 (5.68)	5.6 (5.07)	
Lifestyle Characteristics				
Handedness, n (% left or ambidextrous)	8 (10.8)	2 (5.6)	6 (15.8)	
Current smoker, n (%)	32 (43.2)	13 (36.1)	19 (50.0)	
Chew tobacco, n (% yes)	12 (16.2)	3 (8.3)	9 (23.7)	
Drink alcohol, n (% yes)	51 (68.9)	25 (69.4)	26 (68.4)	
Live on base, n (%)	35 (47.3)	11 (30.6)	24 (63.2)	**
Body mass index, mean (SD) [range]	26.2 (3.52) [17.8-34.4]	26.2 (3.57)	26.1 (3.52)	
Experienced head injury with LOC[a], n (%)	11 (14.9)	6 (16.7)	5 (13.2)	
Hours of sleep, mean/day in past week (SD) [range]	6.68 (1.09) [3-9]	6.63 (1.22)	6.72 (0.96)	
Familiarity with computers, n (% moderately, very familiar)	59 (79.7)	31 (86.1)	28 (73.7)	
Health Status				
General health rating, n (% excellent, very good)	41 (55.4)	21 (58.3)	20 (52.6)	
dizziness, n (% at least twice in 6 mos.)	7 (9.6)	2 (5.6)	5 (13.2)	
difficulty concentrating, n (% at least twice in 6 mos.)	12 (16.2)	5 (13.9)	7 (18.4)	
difficulty paying attention, n (% at least twice in 6 mos.)	10 (13.6)	5 (13.9)	5 (13.2)	

+ High and Low exposure groups from *a priori* categorizations based on job-type activities
Comparison between high and low exposure groups: ** p<.01 *** p<.001
TWA: Time weighted average THC: total hydrocarbons
GM: geometric mean SD: standard deviation
[a] Persons with brain injury defined as self-reported consciousness (LOC) >20 minutes were excluded from being eligible for study participation

Table 4. Summary Outcome Measures of Interest: Neuropsychological Task Performances

	Outcomes of Interest	Possible Score Range	Normative/ Reference Mean (SD)	OJENES Mean (SD)+
DAY 1 BATTERY				
Shipley Institute of Living Scale, Vocabulary [Zachary 2006]				
	Mean # of correct responses	0-40	20-24y: 29.4 (6.3) 25-34y: 29.7 (5.3)	29.1(3.1)
Hooper Visual Organization Test [WPS, 2004]				
	Mean # of correct responses	0-30	25.8 (4.8)	26.2(1.9)
Hopkins Verbal Learning Test –Revised [Brandt & Benedict 2001]				
Total Recall	Total # correct, trials 1-3	0-36	20-29y: 28.8 (3.9) 30-39y: 28.0 (4.4)	25.1(3.8)
Delayed Recall	Total # correct, trial 4	0-12	20-29y: 10.5 (1.6) 30-39y: 9.9 (2.0)	9.0(1.9)
Retention (%)	Delayed Recall/(Higher of recall score from trial 2 or 3)*100	0-100	20-29y: 96.1 (11.1) 30-39y: 91.2 (13.1)	89.3(11.0)
Recognition Discrimination Index	Total # True Positives – Total # False Positives	0-12	20-29y: 10.9 (1.4) 30-39y: 11.0 (1.3)	10.4(1.4)
REPEATED DAY BATTERY				
Auditory Consonant Trigrams [Stuss et al., 1987; 1988]				
36 sec delay	# correct	0-15	16-29y: 9.4 (2.7) 30-49y: 9.9 (3.0)	8.7(3.0)
Total	sum of # correct from 0, 9,18,36 sec delay trials	0-60	NA	43.0(7.1)
ANAM4 [Vincent et al., 2008]				
Match to Sample	Throughput score		34.2 (11.8)	36.8(10.5)
Simple Reaction Time	Throughput score		235.1 (44.8)	241.8(26.4)
Continuous Performance Test Mean RT # commission errors (raw) # omission errors (raw)			NA *	 403.2(41.5) 0.38(1.5) 0.26(1.56)
Finger Tapping				
Dominant (D) hand	Mean # of taps/10s, 2 trials		NA	63.1(1.5)
Non-dominant (ND) hand	Mean # of taps/10s, 2 trials		NA	55.7(8.2)
WAISIII Digit Span [Wechsler, 1997]				
Forward (F)	Mean # span correct	0-16	20-24y: 10.2 (3.0) 24-29y: 10.0 (3.1)	10.9(2.0)
Backward (B)	Mean # span correct	0-14	NA	6.9(2.0)
Grooved Pegboard [Ruff and Parker, 1993]				
Dominant (D) hand	Mean time to complete	0-300	D: 67.3 (12.0)	66.7(12.0)
Non-dominant (ND) hand	Mean time to complete	0-300	ND: 73.2 (13.3)	72.9(11.7)

+ Mean (standard deviation, SD) are computed for Day 1 Battery tasks and tasks completed during the 1st session assessment (Day 2) for the Repeated Battery
y=years
RT=Response time
WAISIII= Wechsler Adult Intelligence Scale III
NA= Reference values not available
NA*=CPT ANAM4 task administration differed for OJENES (longer duration) compared to task reported as reference

REFERENCES

American Conference of Governmental Industrial Hygienists (ACGIH). Kerosene/jet fuels: TLV® Chemical Substances 7th Edition Documentation. Cincinnati, OH: ACGIH; 2003.

Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for JP-5 and JP-8. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 1998.

Air Force Personnel Center. Air force enlisted classification directory; the official guide to the air force enlisted classification codes. [Internet] 2008 [Accessed 2009 May]. Available at: http://ask.afpc.randolph.af.mil/.

Allum JHJ, Adkin AL, Carpenter MG, Held-Ziolkowska M, Honegger F, Peirchala K. Trunk sway measures of postural stability during clinical balance tests: effects of unilateral vestibular deficit. Gait Posture 2001;14:227-37.

Anger WK. Neurobehavioral testing of chemicals: impact on recommended standards. Neurobehav Toxicol Teratol 1984;6:147-153.

Anger WK, Storzbach D. Results and discussion: neurobehavioral – interim report. In: JP-8 final risk assessment. The Institute of Environmental and Human Health (TIEHH). Lubbock, TX; 2001. p. 65-67.

Benedict RHB, Schretlen D, Groninger L, Brandt J. The Hopkins Verbal Learning Test-Revised: Normative data and analysis of interform and test-retest reliability. The Clin Neuropsychol 1998; 12:43-55.

Bernert JT, Harmon TL, Sosnoff CS, McGuffey JE. Use of cotinine immunoassay test strips for preclassifying urine samples from smokers and nonsmokers prior to analysis by LC-MA-MS. J Anal Toxicol 2005;29:814-18.

Bhattacharya A. Postural sway measurements. Risk assessment of acute exposure to jet fuel. In: JP-8 final risk assessment. The Institute of Environmental and Human Health (TIEHH). Lubbock, TX; 2001. p. 72-75.

Blount BC, Kobelski RJ, McElprang D, Ashley DL, Morrow JC, Chambers DM, et al. Quantification of 31 volatile organic compounds in whole blood using solid-phase microextraction and gas chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2006;832(2):292-301.

Bollati V, Baccarelli A, Hou L, Bonzini M, Fustinomi S, Cavello D, et al. Changes in DNA methylation patterns in subjects exposed to low-dose benzene. Cancer Res 2007;67:876-80.

Brandt J., Benedict R. Hopkins Verbal Learning Test-Revised. Lutz, FL.:Psychological Assessment Resources, Inc.; 2001.

Center for the Study of Human Operator Performance (C-SHOP). ANAM4: software user manual. University of Oklahoma, Norman, OK; 2007.

Centers for Disease Control and Prevention (CDC). Leading work related diseases and injuries-United States. MMWR Morb Mortal Wkly Rep 1986;35:113-116, 121-123.

Chao YC, Nylander-French LA. Determination of keratin protein in a tape-stripped skin sample from jet fuel exposed skin. Ann Occup Hyg 2004;48:65-73.

Chao YC, Gibson RL, Nylander-French LA. Dermal exposure to jet fuel (JP-8) in US Air Force personnel. Ann Occup Hyg 2005;49:639-645.

Ding YS, Blount BC, Valentin-Blasini L, Applewhite HS, Xia Y, Watson CH, et al. Simultaneous determination of six mercapturic acid metabolites of volatile organic compounds in human urine. Chemical Res Toxicol 2009;22:1018-25.

Egeghy PP, Hauf-Cabalo L, Gibson R, Rappaport SM. Benzene and naphthalene in air and breath as indicators of exposure to jet fuel. Occup Environ Med 2003;60:969-76.

Egeghy PP, Nylander-French L, Gwin KK, Hertz-Picciotto I, Rappaport SM. Self-collected breath sampling form monitoring low level benzene exposures among automobile mechanics. Ann Occup Hyg 2002;46:489-500.

Egeghy PP, Tornero-Velez R, Rappaport SM. Environmental and biological monitoring of benzene during self-service automobile refueling. Environ Health Perspect 2000;108:1195-202.

Feldman RG, Travers PH. Environmental and occupational neurology. In: Feldman RG (ed.) Neurology: a physician's guide. New York: Thieme Stratton; 1984. p. 191-213.

Gill J, Allum JH, Carpenter MG, Held-Ziolkowska M, Adkin AL, Honegger F, et al. Trunk sway measures of postural stability during clinical balance tests: effects of age. J Gerontol A Biol Sci Med Sci 2001;56:M438-M47.

Gordon CJ. Environmental Stress. In: Temperature and toxicology: an integrative, comparative, and environmental approach. New York: CRC Press; 2005. p. 195-231.

Hooper HE. The Hooper visual organization test manual. Los Angeles, CA: Western Psychological Press; 1958.

Jones D, Kazis L, Lee A, Rogers W, Skinner K, Cassar L, et al. Health status assessments using the Veterans SF-36 and SF-12: methods for evaluating outcomes in the Veterans Health Administration. J Ambul Care Manage 2001;24:68-86.

Kazis LE, Ren XS, Lee A, Skinner K, Rogers W, Clark J, et al. Health status and outcomes of veterans: physical and mental component summary scores (Veterans SF-12). 1998 National Survey of Hospitalized Patients: Executive Report. Washington, DC: Department of Veterans Affairs, Veterans Health Administration Office of Performance and Quality, 1999.

Knave B, Mindus P, Struwe G. Neurasthenic symptoms in workers occupationally exposed to jet fuel. Acta Psychiatr Scand 1979;60:39-49.

Knave B, Olson BA, Elofsson S, Gamberale F, Isaksson A, Mindus P, et al. Long term exposure to jet fuel. II. A cross-sectional epidemiologic investigation on occupationally exposed industrial workers with special reference to the nervous system. Scand J Work Environ Health 1978;4:19-45.

Knave B, Persson HE, Goldberg M, Westerholm P. Long-term exposure to jet fuel: an investigation on occupationally exposed workers with special reference to the nervous system. Scand J Work Environ Health 1976;2:152-64.

Lezak MD, Howieson DB, Loring DW. Neuropsychological Assessment. Fourth edition. New York NY: Oxford University Press; 2004.

Li Z, Romanoff LC, Trinidad DA, Hussain N, Jones RS, Porter EN, et al. Measurement of urinary mono-hydroxy polycyclic aromatic hydrocarbons using automated liquid-liquid extraction and gas chromatography/isotope dilution high resolution mass spectrometry. Anal Chem 2006;78:5744-51.

Li Z, Sandau CD, Romanoff LCS, Caudill SP, Sjodin A, Patterson Jr. DG. Concentrations and profiles of urinary polycyclic aromatic hydrocarbon metabolites in the general U.S. population, 2001-2002. Environ Res 2008;107:320-31.

Matthews CG, Klove K. Instruction manual for the adult neuropsychological test battery. Madison, WI: University of Wisconsin Medical Center; 1964.

Mattorano DA, Kupper LL, Nylander-French LA. Estimating dermal exposure to jet fuel (naphthalene) using adhesive tape strip samples. Ann Occup Hyg 2004;48:139-146.

Morata T, Dunn D, Kretschmer L, Lemasters G, Keith R. Effects of occupational exposure to organic solvents and noise on hearing. Scand J Work Environ Health 1993;19:245-54.

Nan H, Kim H, Lim H, Choi JK, Kawamoto T, Kang J, Lee C, Kim Y, Kwon EH (2001). Effects of occupation, lifestyle and genetic polymorphisms of CYP1A1, CYP2E1, GSTM1 and GSTT1 on urinary 1-hydroxypyrene and 2-naphthol concentrations. Carcinogenesis 22: 787-793.

National Research Council (NRC). Toxicologic assessment of jet-propulsion fuel 8. Washington, DC: The National Academies Press; 2003.

National Institute for Occupational Safety and Health (NIOSH). Hydrocarbons, aromatic: method 1501. In: NIOSH manual of analytical methods (NMAM). 4th ed. Cincinnati, OH: Centers for Disease Control and Prevention; 2003.

National Institute for Occupational Safety and Health (NIOSH). Naphthas: Method 1550. In: NIOSH manual of analytical methods (NMAM). 4th ed. Cincinnati, OH: Centers for Disease Control and Prevention; 1994.

O'Bryant S, Engel L, Kleiner J, Vasterling JJ, Black F. Test of memory malingering (TOMM) trial 1 as a screening measure for insufficient effort. Clin Neuropsychol 2007;21:511-21.

Odkvist LM, Arlinger SD, Edling C, Larsby B, Bergholtz LM. Audiological and vestibula-oculomotor findings in workers exposed to solvents and jet fuel. Scand Audiol 1986;16:75-81.

Olsen DM, Mattie DR, Gould WD, Witzmann F, Ledbetter M, et al. A pilot study of occupational assessment of Air Force personnel exposure to jet fuel before and after conversion to JP-8. Wright-Patterson Air Force Base, OH: United States Air Force Research Laboratory, 1998.

Occupational Safety and Health Administration (OSHA). Method 35. Analytical methods manual. Salt Lake City, UT: OSHA Organic Methods Evaluation Branch; 1982.

Pleil J, Smith L, Zelnick S. Personal exposure to JP-8 jet fuel vapors and exhaust at Air Force bases. Environ Health Perspect 2000;108:183-92.

The Psychological Corporation. WAISIII-WMSII Technical manual. 1997; available through www.harcourtassessment.com

Puhala E, Lemasters G, Smith L, Talaska G, Simpson S, Joyce J, et al. Jet fuel exposure in the United States Air Force. Appl Occup Environ Hyg 1997;12:606-10.

Ritchie G. Biological and health effects of exposure to kerosene-based jet fuels and performance additives. J Toxicol Environ Health B Crit Rev 2003;6:357-451.

Ruff RM, Parker SB. Gender and age-specific changes in motor speed and ey-hand coordination in adults: Normative values for Finger Tapping and Grooved Pegboard tests. Percept Motor Skills 1993;76:1219-1230.

Schwartz J, Park SK, O'Neill MA, Vokonas PS, Sparrow D, Weiss S, et al. Glutathione-S-transferase M1, obesity, statins and autonomic effects of particles. Am J Respir Crit Care Med 2005;172:1529-33.

Serdar B, Egeghy PP, Waidyanatha S, Gibson R, Rappaport SM. Urinary biomarkers of exposure to jet fuel (JP-8). Environ Health Perspect 2003;111:1760-64.

Serdar B, Egeghy PP, Gibson R, Rappaport SM (2004). Dose-dependant production of urinary naphthols among workers exposed to jet fuel (JP-8). American Journal of Industrial Medicine 46: 234-244.

Shipley WC Institute of Living Scale. Los Angeles, CA: Western Psychological Services; 1946.

Smith KW, Proctor SP, Ozonoff A, McClean MD. Inhalation exposure to jet fuel (JP8) among U.S. Air Force personnel. J Occup Environ Hyg 2010;7:563-72.

Smith KW, Proctor SP, Ozonoff A, McClean MD. Urinary biomarkers of occupational jet fuel (JP8) exposure among U.S. Air Force personnel. JESEE *in press.*

Smith L, Bhattacharya A, Lemasters G, Succop P, Puhala E, Medvedovic M, et al. Effect of chronic low-level exposure to jet fuel on postural balance of US Air Force personnel. J Occup Environ Med 1997;39:623-32.

Soderkvist P, Ahmadi A, Akerback A, Axelson O, Flodin U. Glutathione S-transferase M1 null genotype as a risk modifier for solvent-induced chronic toxic encephalopathy. Scand J Work Environ Health 1996;22:360-3.

Stuss DT. Contribution of frontal lobe injury to cognitive impairment after closed head injury: Methods of assessment and recent findings. In: HS Levin, J GRafman, and HM Eisenberg (Eds) Neurobehavioral recovery from health injury. New York: Oxford University Press; 1987.

Stuss DT, Stethem LL, Poirier CA. Comparison of three tests of attention and rapid information processing across six age groups. Clin Neuropsychol 1987;1:139-152.

Stuss DT, Stethem LL, Pelchat G. Three tests of attention and rapid information processing: An extension. Clin Neuropsychol 1988;2:246-250.

The Institute of Environmental and Human Health (TIEHH). JP-8 final risk assessment. Lubbock, TX; 2001.

Tombaugh TN. Test of memory malingering. North Tonawanda, New York: Multi-Health Systemc, Inc; 1996.

Tu R, Mitchell C, Kay G, Risby T. Human exposure to jet fuel, JP-8. Aviat Space Environ Med 2004;75:49-59.

Vincent A, Bleiberg J, Yan S, Ivins B, Reeves DL, Schwab K, Gilliland K, Schlegel R, Warden D. Reference data from the Automated Neuropsychological Assessment Metrics for use in traumatic brain injury in an active Duty military sample. Mil Med 2008;173:836-852.

Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS Scale. J Pers Soc Psychol 1988;54:1063-70.

Wechsler D. WAIS III administration and scoring manual. The Psychological Corp; 1997; available through www.harcourtassessment.com

Western Psychological Services (WPS). Hooper Visual Organization Test (VOT) Manual-1983 Edition with Norms for Children and Adults. Los Angeles: Western Psychological Services; 1983, and reprinted 2004.

White RF, Feldman RG, Proctor SP. Chapter 1. Neurobehavioral effects of toxic chemicals. In: White RF (ed.) Clinical syndromes in adult neuropsychology. New York: Elsevier, 1992.

White R, Gerr F, Cohen RF, Green R, Lezak MD, Lybarger J, et al. Criteria for progressive modification of neurobehavioral batteries. Neurotoxicol Teratol 1994;16:511-24.

White RF, Proctor SP. Research and clinical criteria for the development of neurobehavioral test batteries. J Occup Med 1992;34:140-48.

Zachary R. Shipley Institute of Living Scale Revised manual. Los Angeles: Western Psychological Services; 2006.

Zeiger E, Smith L. The first international conference on the environmental health and safety of jet fuel. Environ Health Perspect 1998;106:763-64.