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14. ABSTRACT Jet propulsion fuel 8 (JP-8) has recently been recognized by the Department of Defense 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 adverse neurological health in military personnel. The research objective include 1) determination of the individual service member's level of exposure to JP-8 components while carrying 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 neuroepidmiology field study to examine predicted dose-response relationships. The field study is being carried out with military (Air Force) personnel. The Tier I data collection phase has been completed; the Tier II phase is currently being planned.					
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Introduction

Jet propulsion fuel 8 (JP-8) has recently been recognized by the Department of Defense 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 adverse neurological health 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.

Body

Due to administrative delays that occurred at the beginning of this project, a request to modify the timeline of the study SOW was submitted in March 2007. Thus, the current, approved SOW (Table 1) represents modifications that more accurately reflect the timeline of required tasks. The period of performance for this project now ends 30 June 2008.

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.
Year 3	Months 25-32	Task 8	-Initiate Tier II phase.
		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. 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. Also, progress on **Tasks 2 & 3** was described.

The progress made on tasks outlined in the modified SOW during this funding period (30 June 2006 – 30 June 2007) is described below. This funding period corresponds to Months 9 through 20 (and **Tasks 2-6**) of the project SOW.

Task 2: Conduct of study planning steps- COMPLETED

During the Fall of 2006, the planning steps of the project (**Task 2**) were completed. This included two visits to the Tier I phase study site (Grand Forks Air Force Base (GFAFB), ND). Specifically, the PI (Dr. Proctor) visited GFAFB on 6 December, 2006 to brief the Wing

commander on the study and discuss logistics regarding the implementation of the study protocol. Also, Dr. Mike McClean (leader of the Exposure Assessment Methodology Working Group) visited GFAFB the third week in December 2006 to collect test samples for the confirmation of sample collection and analyses feasibility and methods. (The feasibility study procedures were approved as a separate study protocol by the USARIEM Research Office (ARIEM #H06-17).)

Task 3: Convene Working Groups- COMPLETED

During this funding period, the establishment of the Working Groups was completed (**Task 3**). The Exposure Assessment Methodology and the Data Management and Logistics Working Groups have met on a weekly-to-monthly basis during this period. The Neurological Outcome Assessment Working Group has been organized and a meeting schedule established.

Task 4: Conduct Tier I phase- COMPLETED

The Tier I data collection phase of this project was completed in January 2007 (**Task 4**).

The objective of the Tier I phase of the project is to fully characterize the levels of exposure to JP8 (and its component compounds) among participants working a variety of work tasks within a set of 6-8 different job titles. The information collected as part of Tier I will be used to more accurately identify the appropriate personnel to recruit for participation in the planned Tier II field study.

For this phase, five members of the study team went to GFAFB for a 2-week period to carry out the data collection. Prior (1-5 days) to the study start date, potential volunteers were briefed by the PI and consent for participation was obtained from 24 participants. By design, the 24 participants represented 4-5 broad Air Force Specialty Codes (AFSCs) and approximately 10 different specific job titles reflective of varying experience levels and daily work activities. As part of the protocol, area air, personal air, dermal, exhaled breath, and urine samples were collected from participants over the course of three consecutive work days. A baseline and daily pre- and post-shift questionnaires were completed by study participants to assess demographic information, work history, risk factors, and potential confounders. All 24 Air Force personnel participants completed the Tier I phase. Nine of the 24 participants who worked in fuel systems maintenance jobs were *a priori* defined as the high exposure group, 9 who worked in the fuel lab or in refueling, storage, or fuel distribution jobs were categorized as the moderate exposure group, and 6 who worked in primarily health-related office jobs (in the medical clinic building) represented the no/low exposure group. During the times of day when Tier I data collection was on-going, the ambient outdoor temperature ranged between -10 to 28 degrees F.

Table 2 summarizes the group characteristics (n=24). The majority of the group are male (n=21, 87.5%). As reported at baseline, 14 (58.3%) of the group described their health as either excellent or very good. Four (16.7%) described having felt sick, dizzy, or off-balance because of an experience at work at some point in the past 6 months, 4 (16.7%) described having difficulty concentrating because of an experience at work at some point in the past 6 months, and 3 (12.5%) reported difficulty paying attention because of an experience at work at some point in the past 6 months.

Table 2. JP-8 Baseline Survey Descriptive Statistics (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%)				

Task 5: Analyses of environmental/biological samples from Tier I phase - COMPLETED

All samples collected during the Tier I phase were sent (Express mail from ND) to the Harvard University School of Public Health's Organic Chemistry Laboratory (HOL) for analysis. As of 30 June 2007, all sample analyses have been completed and the sample analyte results have been provided to the PI. The draft of the Final Report document from HOL containing the technical information regarding the instrument and method limits of detection parameters has been received, but we are awaiting the Final version.

In summary, to assess personal exposure levels to JP8 components, personal air and dermal samples were collected. 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 following primary analytes of interest were measured: total hydrocarbons, benzene, toluene, ethylbenzene, xylene, and naphthalene.

In summary, 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 following primary analytes of interest were measured: total hydrocarbons, 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 the naphthalene levels present in the JP8 exposure.

All of the collected samples (personal as well as field blanks) were transported successfully from the field to the laboratory for analyses.

JP8 represents a mixture of over 200 aliphatic and aromatic hydrocarbon components (with thousands of isomers), and the exact composition varies from batch to batch. In the Tier I phase, we measured total hydrocarbon levels of personal air (and 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.)

Table 3 summarizes the results for the different sample types from the 24 participants, across all 3 work days and pre- and post-shift samples together. The results are presented in this manner to provide the range of JP8 exposure and absorbed dose levels detected during the Tier I phase.

As described below, under **Task 6**, we are currently in the process of analyzing and reviewing the environmental and biological results across exposure groups and across work-days, in concert with the method limits of detection parameters and in combination with the questionnaire and log sheets describing individual work practices during the study (such as, smoking status and co-exposure to gasoline due to vehicle re-fueling). (See below for further summary of the Tier I data.)

Table 3. 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.

Task 6: Data management tasks and data integration processes– In PROGRESS

All Tier I phase-collected data (questionnaire responses, daily exposure and work practices log sheet information, and lab results for the Tier I samples) have been entered into individual datasets. Data integration and analyses are in progress. An abstract describing aspects of the Tier I phase urine results has been accepted for presentation at the EPICOH meeting in October 2007 (see below).

Our preliminary analyses of the levels of the different components detected among the participants pre- and post-shift and across the 3 workdays, indicate:

- the *a priori* designated high, moderate, and low exposure groupings do distinguish personal degree of exposure to JP8, as **Table 4** presents results for total hydrocarbons, benzene, and naphthalene air levels for each work day; results for toluene, ethylbenzene, and xylenes indicate similar group differences,
- there is variability in exposure across work days within the *a priori* exposure groups related to degree of direct work task exposure to JP8, particularly within the high exposure group, and
- there are observed differences between absorbed doses pre- to post- shift across workdays (see **Figure 1**).

Table 4. 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.

Task 7: Convene Exposure Assessment Workshop– In PROGRESS

As part of the March 2007 request to modify the study SOW, we submitted a supplemental funding request to convene an Exposure Assessment Workshop (**Task 7**). The request has been approved, with supplemental funds awarded 14 June 2007. We anticipate holding this Workshop in Natick, MA in the fall of 2007.

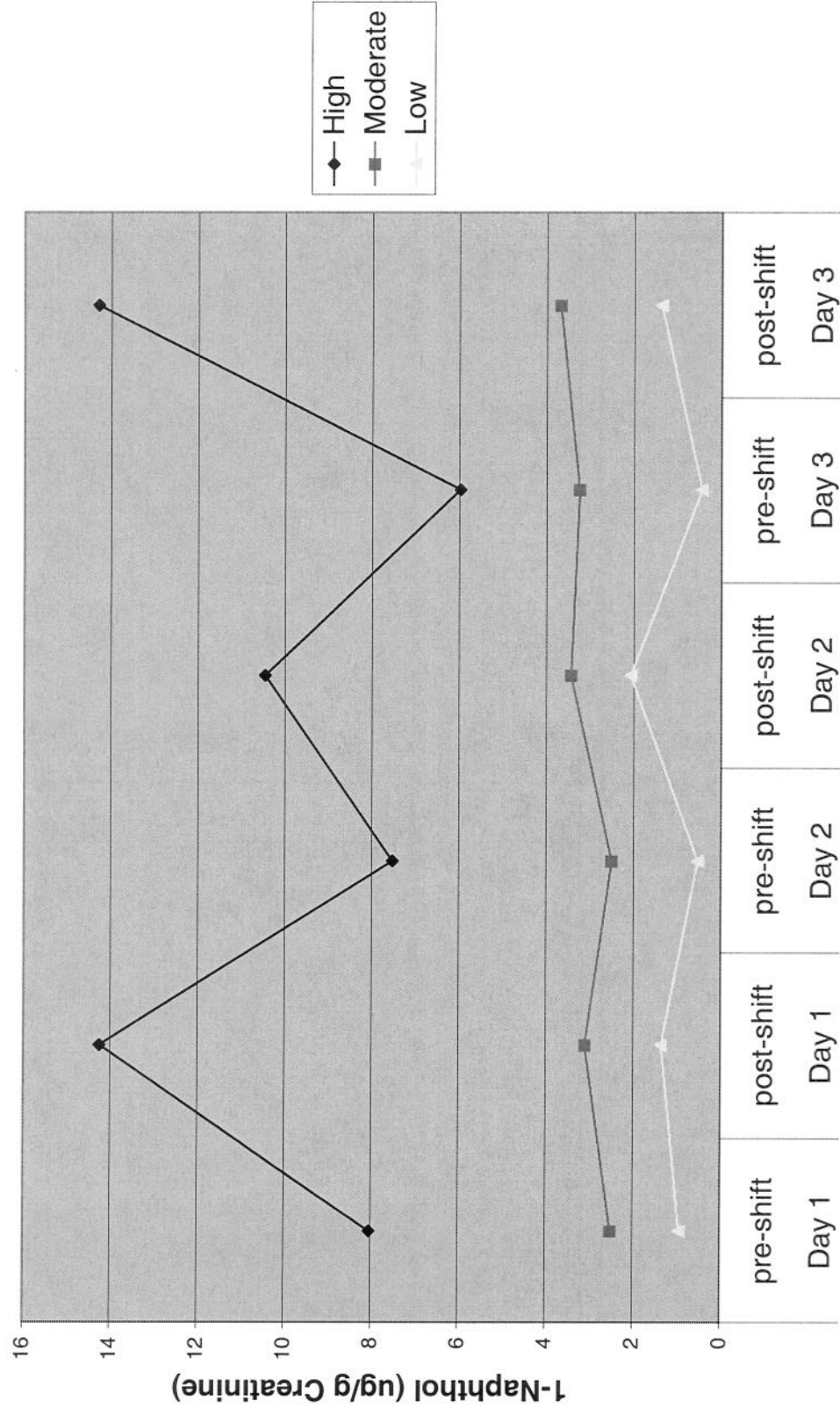
Task 8: Initiate Tier II phase– In PROGRESS

Planning for the Tier II phase is in progress and will continue throughout the summer and fall of 2007. The Exposure Assessment Methodology Work Group is currently reviewing the data from Tier I and, coupled with advice garnered at the upcoming Workshop, will make recommendations regarding the most appropriate exposure markers for the Tier II phase. The Neurological Outcome Assessment Work Group is discussing several logistical and scientific issues pertaining to the appropriate neurobehavioral test battery for this field study during their

regularly planned meetings. In May, a preliminary training session took place regarding the use of the SwayStar software and equipment for postural sway assessment. Prior to the Tier II phase, a final protocol will be submitted to the USARIEM and AFRL Research Offices for review and approval. Anticipated implementation of the Tier II phase is planned to occur in early 2008.

Figure 1.

Mean Urinary 1-Naphthol Concentration by Exposure Category



Key Research Accomplishments

Below is a bulleted list of the accomplishments over this study period:

- ❑ The Exposure Assessment Methodology Working Group and the Data Management and Logistics Working Group met on a weekly to monthly basis, and completed the planning required for the Tier I phase of the project.
- ❑ The PI briefed the Wing Commander on the study in early December 2006.
- ❑ The PI and the leader of the Exposure Assessment Methodology Working Group visited GFAFB in December of 2006 to evaluate the study site and collect pilot samples for the feasibility study.
- ❑ Tier I was completed January of 2007.
- ❑ Baseline demographic data that was collected via questionnaire during Tier I has been analyzed.
- ❑ Samples collected during Tier I were sent to the Harvard University Organics Lab, and analyses are completed. A final report of methods and results is currently being compiled.
- ❑ Preliminary analysis of the combined Tier I analyte and questionnaire data has begun. An abstract focused on the urine biomarker results has been accepted for presentation at the upcoming EPICOH meeting.
- ❑ Continuing Review Reports have been reviewed and approved by the USARIEM Human Use Research Committee (3 May 2007) and the AFRL/Wright Site IRB (13 April 2007).

Reportable Outcomes

1. Reports, manuscripts, abstracts

- An Abstract has been accepted for presentation at the 19th International Conference on Epidemiology in Occupational Health to be held in Banff, Alberta, Canada in October, 2007. (See Appendix.)

2. Degrees and research training opportunities

- Three Boston University School of Public Health doctoral students have received funding support over the past year and are currently working on this project.

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

- Henk C. Trap, BSc, from TNO Defense Security and Safety (The Netherlands) has a current USAMRMC-funded project titled "Profiling Jet Fuel on Neurotoxic Components with 'Comprehensive Two-Dimensional GC'". In this project, samples of jet fuel are 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 are being performed to monitor the vapor concentration time profile of a maximum of 20 compounds of interest specifically in JP-8. On the basis of these results, exploration will proceed to determine whether this information can be used to estimate a 'toxic load' for several neurotoxic compounds following exposure to JP-8 vapor under realistic conditions. Additional real-life exposure

scenarios are also being explored, that is under different climatic conditions. The PI serves as a collaborator on this proposed project, helping to advise on aspects related to neurotoxicity. Samples of JP8 fuel, collected in ND, were provided to TNO.

3. Related projects and collaborations initiated

- On 15 September, 2006, the PI participated and presented (via teleconference) in 1-Day Workshop, "Naphthalene: Exposure, Epidemiology, Human Effects & Cancer"; Brooks City Base, San Antonio, Texas. During the meeting, she briefed COL Gibson and other invitees 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 on 18 June, 2007. 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-dimensional GC (see above). Future collaborations were also discussed.

Conclusions

The work on this funded project is on-going. There has been substantial progress over this funding period and the project timetable is on schedule. When completed, the study will provide important occupational health and exposure assessment information concerning JP8.

As stated in the recent report (National Research Council, 2003), 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 will contribute significantly to the knowledge of the subclinical effects of both acute and chronic exposure to occupational solvent exposures.

References

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.

Appendix

Accepted ABSTRACT

To be presented at the 19th International Conference on Epidemiology in Occupational Health (EPICOH); Oct 2007, Banff, Canada.

Repeated measures of urinary 1- and 2-naphthol among jet fuel exposed Air Force personnel

Smith KW, Allen JG, Proctor SP, McClean MD.

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 6-8 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: 6 workers with administrative or office roles; moderate: 9 workers with fuel distribution jobs, and high: 9 workers from fuel systems maintenance). In January 2007, urine samples (n=144) were collected pre- and post-shift over three consecutive workshifts and analyzed 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 analyzed 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 7-fold higher than in the moderate group (p=0.0005) and 9-fold higher than in the low group (p=0.0004). Similarly, the mean urinary 2-naphthol measurements in the high exposure group were 4-fold 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.

Conclusions: 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.