Final Report
Sensitive early indicators of hepatic and kidney damage in workers exposed to Jet Fuel
Risk Assessment of Acute Exposure to Jet Fuel

Introduction:

Sensitive assays for liver and kidney damage provide an opportunity to evaluate early hepatic and renal damage in workers exposed to JP8 jet fuel. In this study, an assay for the cytoplasmic enzyme, plasma alpha-glutathione S-transeferase (alpha-GST), tested the association between JP8 exposure and hepatic damage. Over 85% of alpha-GST in humans is found in hepatocytes and is released into the blood upon hepatic damage. As a result of its short half-life, plasma alpha-GST rises rapidly when hepatocytes are damaged and returns rapidly to baseline when damage is repaired. Plasma alpha-GST is a more sensitive indicator of damage than alanine aminotransferase and aspartate aminotransferase (Clarke et al, 1997; Rees et al, 1995). In an earlier study, JP8 workers had no significant changes in liver function measured by alanine aminotransferase and aspartate aminotransferase. However, some have questioned the use of these measurements for assessing hepatocyte damage since levels may be normal in some people with chronic liver disease (Clarke et al, 1997).

Two additional tests which measure urinary alpha or pi glutathione S-transferase (GST) are more sensitive for detection of early kidney damage than serum creatinine and blood urea nitrogen assays. The latter assays require a considerable loss of renal capacity before test values are significantly elevated. In earlier work, there were no significant differences in kidney function measured by BUN and creatinine in JP8 exposed and unexposed workers(Gould, 1998). . In the kidney, alpha-GST is located only in the epithelial cells in the proximal tubule; pi-GST is localized within the epithelial cells in the distal tubules (Sundberg et al, 1994). Monitoring the urinary levels of these enzymes has proven valuable in monitoring kidney transplant patients to differentiate between transplant rejection and nephrotoxicity without the necessity of a biopsy (Sundberg et al, 1994).

For this research, enzyme-immunoassays were used to measure hep alpha-GST in subject plasma to monitor early signs of hepatic damage; neph alpha- and pi-GST in urine were used for the detection of early kidney tubule damage.

Methods:

Study population: Exposed workers were tank-entry personnel with at least nine months of persistent exposure to jet fuel, i.e., one-hour entry, twice a week, validated against shop records. The unexposed group consisted of US Air Force personnel who do not routinely work with or have significant exposure to fuels or solvents. Participants were chosen from among those meeting eligible criteria from six USAF bases: Davis Monthan AFB, AZ, Seymour Johnson AFB, NC, Langley AFB, VA, Pope AFB, NC, Little Rock AFB, AR, and Hurlbert Field, FL.. Exclusion criteria are history of autoimmune disease, cancer, diabetes, and immune altering medication. Participants received $50 for their time and inconvenience.

Participants completed a questionnaire to provide job, exposure, medical, and demographic information.

Sample Collection: Venous blood samples were collected from 107 individuals using 10 ml EDTA tubes. Pre- and post-shift samples were collected for all subjects. Blood samples were shipped on ice to the NIOSH laboratory by next day courier. At the NIOSH laboratories, samples were spun in a centrifuge to separate plasma from packed cells and plasma was transferred to screw-top polypropylene cryovials (Cat no. 60-542, Sarstedt, Inc.) approximately 24 hours after blood collection, and kept frozen at –80°C until assayed. Pre- and post-shift urine samples were collected from subjects and shipped on ice to the NIOSH laboratory by next day courier. At the NIOSH laboratories samples were divided into aliquots for respective studies. Samples destined for determination of neph alpha- and pi-GST were prepared for storage by the addition of stabilizing buffer (Biotrin International, 20% urine volume) and samples were frozen at –80° C until thawed for assay.

All samples were randomly numbered for blinded analysis. Plasma samples were thawed and assayed in duplicate for Hep alpha GST using commercial immunoassay kits (Biotrin International, Cat # BIO60HEPA). Urine samples were thawed and assayed for both neph alpha- and pi-GST using commercial immunoassay kits (Biotrin International, BIO66NEPHA and BIO69NEPHPI, respectively).

Statistical Analyses:

Pearson correlation coefficients were derived for each of the enzyme variables as well as pre- and post-shift change against the following variables:

Age, Base, Mthjob, Hisp, Pexert, Mental, Natlogpass, Natlogpre_n, Natlogpost_n, height, weight, BMI, Smoker, Alcohol, Alcdown, Alcbout, Alcuse, Alcsitng, Physwrk, Physntwk, Analyco24, and Noalcdurstudy.

Univariate analyses of variance were conducted for each enzyme variable against the following variables:

Race, Hisp, Category, Smoker, Alcohol, Analyco24, and Noalcdurstudy.

Status:

Analysis for hep alpha-GST for plasma and neph alpha- and pi-GST in urine samples from all subjects have been completed and statistical analysis conducted as described above.

Findings:

Assessment of liver toxicity; Levels of serum hep alpha-GST in the study subjects were well within the normal range for this measure; no differences were observed indicative of hepatic changes attributable to any of the variables examined (Table 1).

Assessment of kidney toxicity; Levels of urinary neph alpha- and pi-GST in the study subjects fell within the normal range for healthy subjects; no differences were observed indicative of renal changes attributable to any of the variables examined (Table2) .

Assessment of urine creatinine; Creatinine was used to normalize neph alpha- and pi-GST to correct for urinary dilution. One finding of this study was that individuals from exposure category 3 had significantly higher levels of urinary creatinine in their post shift samples. While the mean values are within normal ranges (0.25-4.0 mg/ml) for this measure in healthy

individuals and are not indicative of clinical disease, they are evidence of more concentrated urine (Table 3).

Discussion/Conclusions:

The study group represents a very healthy segment of the population. Sensitive measures for liver and kidney damage did not detect any adverse effects in specimens from this study group. Evidence of elevated creatinine in the mean post-shift samples of exposure category 3 was seen. However, while these values are within normal clinical ranges, they are consistent with concentrated urine indicative of mild dehydration. Because of the elevated urine creatinine levels in post-shift samples of exposure category 3, there is an apparent decrease in pre- vs. post-shift levels of Neph alpha- and pi-GST in these samples. This is an artifact of the concentrated creatinine. When levels of urinary GSTs are expressed on a per volume basis, levels remain unchanged between pre- and post-shift samples.

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Table 1. Effect of JP8 Exposure Category on Hepatic Alpha Glutathione Transferase (Hep Alpha GST).

Exposure Category	Hep Alpha GST (µg/L)	Hep-Alpha GST Normal Range (µg/L)	Hep-Alpha GST Pre-Post Difference (µg/L)
1 Pre	2.82 ± 2.46	0-11	-------
2 Pre	3.01 ± 2.32	0-11	-------
3 Pre	2.73 ± 2.34	0-11	-------
1 Post	2.81 ± 4.94	0-11	-0.01
2 Post	2.96 ± 2.08	0-11	-0.05
3 Post	2.67 ± 2.50	0-11	-0.06

Table 2. Effect of JP8 Exposure Category on Kidney Proximal Tubule Glutathione Transferase (Neph Alpha GST) and Kidney Distal Tubule Glutathione Transferase (Neph Pi GST).

Exposure Category	Neph-Alpha GST (μg/mg creatinine)	Neph-Alpha GST Normal Range (μg/mg creatinine)	Neph-Alpha GST Pre-Post Difference (μg/mg creatinine)	Neph-Pi GST (μg/mg creatinine)	Neph-Pi GST Normal Range (μg/mg creatinine)	Neph-Pi GST Pre-Post Difference (μg/mg creatinine)
1 Pre	2.13 ± 5.03	0-10	-------	4.71 ± 5.61	0-12	-------
2 Pre	1.43 ± 2.86	0-10	-------	5.00 ± 5.12	0-12	-------
3 Pre	1.02 ± 2.39	0-10	-------	4.32 ± 4.95	0-12	-------
1 Post	2.44 ± 5.74	0-10	0.31	5.71 ± 7.65	0-12	+1.0
2 Post	1.46 ± 2.67	0-10	0.03	5.07 ± 8.05	0-12	+0.07
3 Post	0.71 ± 2.83	0-10	-0.29	2.93 ± 3.20	0-12	-1.39

Table 3. Effect of JP8 Exposure Category on Urine Creatinine Levels.

Exposure Category	Urine Creatine (mg/ml)	Urine Creatinine Normal Range (mg/ml)	Urine Creatine Pre-Post Difference (mg/ml)
1 Pre	1.82 ± 1.01	0.25-4.0	-------
2 Pre	1.71 ± 0.96	0.25-4.0	-------
3 Pre	1.96 ± 0.96	0.25-4.0	-------
1 Post	1.62 ± 0.90	0.25-4.0	-0.20
2 Post	1.84 ± 0.97	0.25-4.0	+0.13
3 Post	2.54 ± 1.15	0.25-4.0	+0.58

IOH-RS-BR-SR-2005-0003

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JP-8 Final Risk Assessment

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August 2001

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REPORT DOCUMENTATION PAGE	Form Approved OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 2001	Final

4. TITLE AND SUBTITLE
JP-8 Final Risk Assessment

5. FUNDING NUMBERS
F41624-99-2-0002

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Air Force Institute for Operational Health
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Brooks City-Base, TX 78235-5116

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
IOH-RS-BR-SR-2005-0003

11. SUPPLEMENTARY NOTES
Prepared in cooperation with The Institute of Environmental and Human Health (TIEHH) for the Air Force Institute for Operational Health (AFIOH).

12a. DISTRIBUTION AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*
The JP-8 Final Risk Assessment is a collection of several studies assessing the health and performance effects from acute exposure to Jet Propellant type 8 (JP-8) jet fuel. Exposed subjects were active duty Air Force personnel who worked with or were routinely exposed to JP-8 in the performance of their duties. Non-exposed subjects were active duty Air Force personnel who did not have routine contact with JP-8 during the performance of their duties. Assessments included JP-8 exposure in the immediate personal environment, JP-8 body burden, neurologic effects, hormonal effects, immunological effects, liver/renal function, cytotoxic/genotoxic effects, glutathione-S-transferase activity, and self-reported health status.

14. SUBJECT TERMS	15. NUMBER OF PAGES
JP-8, Jet Fuel, Human Risks, Occupational Health	186
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	UL