

Preliminary Report
Gene Environment Interactions and Exposure to JP8 Jet Fuel

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Introduction:

The characterization of genetic polymorphisms in genes that signal the production of key enzymes in the metabolism of components in jet fuel is being conducted to describe gene-environment interactions in personnel in the jet fuel study. Stratifying personnel by allele specific genotypes can help explain variability in health effects in personnel with similar levels of exposure, and to increase correlations between exposure to toxicants and a variety of measures of biological effect. Polymorphic enzymes may be more important at low-level exposures, since at high-level exposures, both high activity and low activity enzymes may be saturated.

Several of the genes involved in production of enzymes that activate and detoxify components in jet fuel are polymorphic. In the jet fuel study, allelic variants in three polymorphic genes, GSTT1, CYP2E1, and NQO1, are being characterized (genotyped). Relationships with health effects and other biological indicators of exposure are under investigation.

It has been reported that people vary in susceptibility to adverse health effects of chemicals such as benzene, a neurotoxic and hematotoxic component in jet fuel, partly because of interindividual variations in metabolic enzymes that activate and detoxify the toxicant (Wiencke et al, 1997, Snyder and Hedli, 1996). Metabolism plays a critical role in benzene toxicity. The polymorphic enzyme CYP2E1 converts benzene to benzene oxide, which is spontaneously rearranged to phenol. Phenol is oxidized via CYP2E1 to hydroquinone and other hydroxy metabolites, which are converted in the bone marrow by myeloperoxidase to genotoxic and hematotoxic benzoquinones. Polymorphic NAD(P)H quinone oxidoreductase (NQO1) catalyzes the conversion of benzoquinones to less reactive metabolites. In addition, benzene oxide is detoxified by conjugation to glutathione via the polymorphic glutathione S-transferase (GST). High CYP2E1 activity, low NQO1 activity and/or low GST activity may alter the production of toxic metabolites. This can alter interactions with critical macromolecules and affect toxic outcomes.

Approximately 50% of healthy people carry the C to T transition at base pair 609 of exon 6 in the NQO1 gene. There is a three-fold decrease in enzyme activity associated with this allele in the heterozygous state, and a total loss of activity in the homozygous state (Rothman et al, 1997, Wiencke et al, 1997). Approximately 30% of the US population does not produce the GSTT1 enzyme because of a homozygous deletion of the GSTT1 gene (Ketterer et al, 1992). The minor DraI allele C in the CYP2E1 gene is present in about 10-14% of the population and provides a high activity enzyme. Association of the CYP2E1 DraI allele C with DNA adducts was found to be greatest in individuals with low-level exposures (Kato et al, 1995).

Methods:

Assays to characterize CYP2E1, GSTT1, and NQO1 genotypes were determined following gene amplification using polymerase chain reaction (PCR) methodology. DNA was isolated at Texas Tech University. Genotyping was performed on DNA isolated from blood donated both before (pre) and after work (post). CYP2E1 Dra1 was identified by methods described by Kato et al, 1994. GSTT1 genotyping was performed by a slight modification to methods described in Pemble et al, 1994. NQO1 genotyping was performed according to methods described in Wiencke et al, 1997.

Chi-squared tests were used to determine if the proportion of genotypes varied across exposure categories. Linear models were used to determine if genotype interacted with exposure on endocrine, liver, and kidney function. Separate sets of models were used for exposure categories and for continuous measures of exposure.

Results:

Genotype analysis for CYP2E1, GSTT1, and NQO1 was completed for the 316 participants who provided blood for DNA preparation. Results using DNA isolated from pre and post blood had complete concordance. All 316 participants were characterized for the three genes.

CYP2E1 Genotype Analysis

The CYP2E1 DD genotype was found in 85.4 percent of participants. The CYP2E1 CD was found in 13.3 percent of participants. The CYP2E1 CC genotype was present in 1.3 percent of participants. Distribution of the CYP2E1 genotypes across the three exposure categories (low, moderate, high) is shown in Table 1. The frequency of genotypes did not vary significantly across the exposure categories ($p=0.3546$).

Table 1: Distribution of CYP2E1 Genotypes in Exposure Categories in 316 Participants

Genotype	Low	Moderate	High
CYP2E1 DD	132	40	98
CYP2E1 CD	22	3	17
CYP2E1 CC	1	0	3

GSTT1 Genotype Analysis

The deletion of the GSTT1 gene was found in 21.8 percent of 316 participants. The GSTT1 gene was present in 78.2 percent of participants. Distribution of the genotypes across the three exposure categories is shown in Table 2. The frequency of genotypes did not vary significantly across exposure categories ($p=0.5985$).

Table 2: Distribution of GSTT1 Genotypes in Exposure Categories in 316 Participants

Genotype	Low	Moderate	High
GSTT1 +	121	36	90
GST1 null	34	7	28

NQO1 Genotype Analysis

The NQO1 CC genotype was present in 64.6 percent of the 316 participants. The NQO1 CT genotype was present in 31.3 percent of participants. The NQO1 TT genotype was present in 4.1 percent of the participants. Distribution of the NQO1 genotypes across the three exposure categories is shown in Table 3. The frequency of genotypes did not vary significantly across exposure categories ($p=0.4751$).

Table 3: Distribution of NQO1 Genotypes in Exposure Categories in 316 Participants

Genotype	Low	Moderate	High
NQO1 CC	104	29	71
NQO1 CT	47	11	41
NQO1 TT	4	3	6

Analysis of Gene Environment Interactions

CYP2E1, GSTT1, and NQO1 genotype data were analyzed to determine if genotypes interacted with exposure and α -GST ($n=292$). α -GST in serum is an indicator of early hepatic damage. There were no statistically significant effects indicating an interaction of genotype with exposure in the 292 participants.

CYP2E1, GSTT1, and NQO1 genotype data were analyzed to determine if genotype interacted with exposure and urinary α -GST ($n=304$). Urinary α -GST is an indicator of early damage to the proximal tubules in the kidney. There were no statistically significant effects indicating an interaction of genotype with exposure in the 304 participants.

CYP2E1, GSTT1, and NQO1 genotype data were analyzed to determine if genotype interacted with exposure and urinary π -GST ($n=307$). Urinary π -GST is an indicator of early damage to the distal tubules in the kidney. There were no statistically significant effects indicating an interaction of genotype with exposure in the 307 participants.

CYP2E1, GSTT1, and NQO1 genotype data were analyzed to determine if genotype interacted with exposure and endocrine function measured by serum endocrine concentrations in 130 men. Interactions were examined with levels of serum luteinizing hormone, follicle stimulating hormone, prolactin, free and total testosterone, estradiol, inhibin B, and cortisol. For endocrine function, there were no statistically significant effects indicating an interaction of genotype and exposure.

Summary/Conclusions:

Final analysis of gene-environment interactions in the JP8 jet fuel study will be completed as soon as data are available on all outcome measures. Analysis will be simplified because results of the genotype analysis in the 316 participants have shown that there is no significant difference in distribution of genotypes across exposure categories. Exposure will be refined by including measures of dermal exposure and blood levels of JP8 jet fuel components. Gene environment interactions will be examined in the postural sway test, an assessment of a

participant's control mechanism for governing balance and in the conditional eyeblink response, which can identify cognitive deficits. In addition, gene interactions will be evaluated in the GASH/BARS system that is designed to measure motivation, response speed, coordination, grip strength, complex mental functioning, memory, and attention. Furthermore, interactions with passive benzene and naphthalene exposure, exhaled and urinary benzene and naphthalene, and molecular adducts of naphthalene may be described.

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JP-8 Final Risk Assessment

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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.				
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