The Effects of JP8 Jet Fuel on Serum Endocrine Concentrations in Men: Risk Assessment of Acute Exposure to Jet Fuel

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Introduction:

Evidence from animal and human studies suggest that components of JP8 jet fuel can disrupt the neuroendocrine axes that control or impact the reproductive and immunologic systems [1-12]. Human studies have demonstrated reduced secretion of luteinizing hormone (LH) associated with toluene and JP8 exposure [9-12]. LH is critical for reproductive performance, but also effects immunologic functions by increasing interleukins involved in natural killer cell activation and B-cell differentiation [1]. Adrenocorticopic hormone, follicle stimulating hormone (FSH), prolactin, corticosterone, testosterone, and estradiol also regulate immune function and are affected by compounds in fuels and exhaust [1-12].

JP8 represents the most common chemical exposure among members of the Armed Forces. The specific objective of this aspect of the study is to **determine endocrine levels in the peripheral blood and whether these endocrine concentrations are associated with JP8 exposure or other work conditions. Information gathered in this study would help the National Institute for Occupational Safety and Health (NIOSH) establish recommended exposure limits for military and civilian aviation.**

Methods:

Exposed workers were male 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 three USAF bases: Pope, Little, and Hurlburt. 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. In addition, venous blood samples were collected from 153 individuals using 10 ml Serum Separation Tubes with clot activator and twice the polymer barrier (Cat no. 367985, Becton, Dickinson and Co.). Samples were collected in the morning at about the same time for all subjects, to minimize the confoundment of diurnal rhythms. Blood samples were inverted 5 times and allowed to set for 30-60 minutes at room temperature to allow the blood to clot before centrifugation at 1,000-1,300 g for 10 minutes. Separated samples were immediately refrigerated and shipped on ice to the NIOSH laboratory by next day courier.

At the NIOSH laboratories, serum was transferred to screw-top polypropylene cryovials vials (Cat no. 60-542, Sarstedt, Inc.) approximately 24 hours after blood collection, and kept frozen at -80°C until assayed.

Serum samples were randomly numbered for blinded analysis. Serum samples were assayed in duplicate for each hormone; inhibin-B duplicates were drawn from a single pre-treatment aliquot. Quality control serum pools (2-3 levels) were assayed at the beginning and end of each assay.

FSH & LH were measured using DELFIA noncompetitive, microtiter immunofluorometric assays (cat. no. A017-201 & A031-101, respectively; PerkinElmer-Wallac). Inhibin-B was measured using a noncompetitive, microtiter enzyme immunoassay (cat. no. MCA1312KZZ; Serotec, Inc); inhibin-B values were adjusted to correct for a slight shift across the microtiter plate. Prolactin was measured using a tube-based noncompetitive immunoradiometric assay (cat. no. DSL-4500; Diagnostic Systems Laboratories, Inc (DSL). Cortisol was measured using a coated tube, competitive radioimmunoassay (cat. no. DSL-2100; DSL). Estradiol was measured using a sensitive, double antibody, competitive, ultra-sensitive radioimmunoassay (cat. no. DSL-4800; DSL). Total and free testosterone were measured using coated tube, competitive radioimmunoassays (cat. no. TKTT & TKTF, respectively; Diagnostic Products Corp.).

Of the 153 individuals who provided morning blood samples, 17 were excluded who were women and 2 were excluded who provided only afternoon blood samples.

Pearson correlation coefficients were derived for each of the 8 hormone endpoints 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.*

In addition, linear models were used to assess the effect of exposure (*Cat2*) and months-on-the-job (*MthJob*) and their interaction (*Cat2* x *MthJob*), while controlling for age, smoking, and alcohol-use. MthJob, age, smoking, and alcohol-use were continuous variables, exposure was a classification variable. A separate model was conducted for each hormone.

Status:

All venous blood samples collected for this aspect of the study have been analyzed for endocrine concentrations. Statistical analyses have been conducted as described herein.

Findings:
Correlations that statistical significance at the P < 0.01 level:

Correlates Variable Vs. Endpoint	Correlation Coefficient	P-Value
MthJob vs. Total	r = - 0.263	P = 0.002
MthJob vs. Free	r = - 0.278	P = 0.001
Age vs. FSH	r = 0.347	P =
Age vs. Free Testosterone	r = - 0.263	P = 0.002
Smoker vs Prolactin	r = 0.227	P = 0.009
Alcdown vs Total	r = - 0.350	P <
Alcbout vs Total	r = - 0.272	P = 0.002
Alcsitng vs Total	r = - 0.254	P = 0.004

For FSH, the main effect of exposure was significant (p = 0.03), though none of the adjusted means were significantly different from each other. The Cat2 x MthJob interaction approached significance (p = 0.06): while the slopes describing the relationship between months-on-the-job and low (b = 0.0019, p = 0.7) and moderate (b = -0.0086, p = 0.2) exposure groups were not different from zero, the slope for the high exposure group tended to be greater than zero (b = 0.015, p = 0.055). FSH level was also directly related to age (b = 0.10, p = 0.005).

The main effect of exposure was significantly (p = 0.035) related to inhibin B levels. Adjusted serum levels of the high exposure group (205 mIU/ml) were significantly greater than that for the moderate exposure group (167 mIU/ml).

Increased smoking was significantly related to reduced prolactin levels (b = 0.59, p = 0.013) and tended to be associated with reduced total testosterone levels (b = 0.23, p = 0.059). Estradiol levels decreased with increased alcohol use (b = 0.21, p = 0.028).

There was no indication that naphthalene exposure or month-on-the-job affect serum levels of testosterone, estradiol, LH, prolactin, or cortisol. There were no significant main effects or interactions for LH, cortisol, or free testosterone.

Endocrine Endpoint	Effect	P-Value	Description
FSH	Cat2	p = 0.03	Adjusted means are not different.
	Cat2 x	p = 0.06	Only MthJob x High Exp slope tended to differ
	Age	p =	FSH may increase with age (b=0.10).
Inhibin B	Cat2	p =	Inhibin B levels may be higher for High Exp
Prolactin	Smoking	p =	Prolactin may decrease with smoking
Total	Smoking	p =	TT tends to decrease with smoking (b=0.23).
Estradiol	Alcohol	p =	Estradiol may decrease with alcohol use

Discussion/Conclusions:

These preliminary statistical analyses reveal statistical trends suggesting that FSH levels may be higher in AF personnel who have worked for longer duration in jobs with higher naphthalene exposure. These results also suggest that men with high naphthalene exposure experience elevated inhibin B levels. Inasmuch as inhibin B exerts negative feedback on FSH secretion, this scenario is consistent with an exposure effect either stimulating FSH secretion leading to elevated inhibin B levels, and/or a relative desensitization of the feedback setting.

Month-on-the-job was also inversely correlated with testosterone levels. This association, however, disappeared upon including age in the multivariate model.

Preliminary analyses would suggest that heavy smokers might experience reduced prolactin and testosterone levels. Preliminary analyses reveals that the heavy alcohol consumption is associated with reduced prolactin levels. There wis also a direct correlation between the amount of alcohol consumed and testosterone levels, however this relationship was not apparent with multivariate analyses.

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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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