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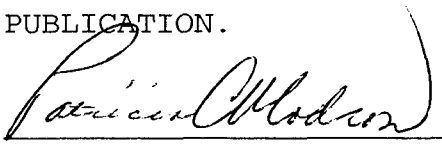

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13. ABSTRACT ***(Maximum 200 Words)*** Certain aliphatic and aromatic hydrocarbons in fuels are known or suspected human reproductive or developmental toxicants. The primary purpose of this study was to evaluate possible menstrual and reproductive endocrine effects of exposure to jet fuel. Eligible military and civilian women (n=170) were recruited from 10 U.S.A.F. bases. Internal dose of fuel components measured in exhaled breath was used to characterize exposure, including: total C6-C16 ("aliphatic hydrocarbons"); and, total benzene, ethyl-benzene, toluene, and m,p,o-xylenes ("BTEX"). Four endocrine endpoints linked to conceptive cycles and internal dose measurements were available for a subset of 63 participants. An inverse relationship ($p=0.007$) between preovulatory LH and breath aliphatic hydrocarbons levels was found, i.e., as levels of compounds in fuels increased, preovulatory LH levels decreased. Women in occupations involving fuel handling did not have significantly ($p \leq 0.05$) higher odds of menstrual disorders in adjusted analyses, although life event stress was associated with dysmenorrhea, hypermenorrhea, and abnormal cycle length. African American participants had lower follicular phase Pd3G levels and LH:FSH ratios, and lower rates of periovulatory increase than Caucasians. In conclusion, exposure to aliphatic hydrocarbons may be associated with hormonal changes, but these effects may not be related to effects on the menses.

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TABLE OF CONTENTS:

INTRODUCTION:

This report summarizes a four-year study of potential female reproductive effects of exposure to fuels at USAF bases. Jet fuel (JF) constitutes at least two thirds of the turbine fuels used by the Department of Defense (DoD). It consists of a variable mixture of hydrocarbon compounds, and it is one of the most common chemical exposures at all Air Force (AF) bases. JP-8 turbine engine fuel is a kerosene-based distillate with a higher flash point, higher chain hydrocarbons and lower benzene that its JP-4 predecessor. JP-8 is, therefore, presumed to be safer to use. Potential reproductive and developmental toxicity of the complex streams that comprise fuels have not yet been established. The literature does, however, contain both animal and human studies of exposure to various fuels and primary fuel components. Certain organic compounds in fuels and emissions are known or suspected human reproductive or developmental toxicants[1-5]. The purpose of this study was to explore possible reproductive endocrine and menstrual effects of exposure to jet fuel, and to determine if racial differences affected exposure or reproductive responses to exposure. As reflected by the technical objectives, the scope of the study was to: 1) identify and recruit fuel exposed and unexposed women group-matched with respect to race and age; 2) characterize workplace exposures; 3) determine if hormonal patterns differed significantly between the exposed and unexposed groups and to determine if there are differences between racial groups and; 4) ascertain if prevalences of menstrual disorders differed significantly between the exposed and unexposed group; and determine if there were differences between racial groups.

BODY:

Statement of Work and Participation Summary: Year 01 through Year 04 activities outlined in the "Statement of Work" (see Appendix I) are concluded with the submission of this report. As documented within our previous reports, our objectives have been met with the exception of attaining our recruitment goal (n=200). In an effort to reach that goal, we visited a total of ten AF bases (Table 1, Appendix II), i.e., six bases in addition to the four that were originally proposed. A total of 170 women ultimately participated in the baseline questionnaire interview. Tables 1-5 (see Appendix II) describe recruitment and participation. Attempts were made to contact 996 subjects for recruitment (Table 2). Of these 996, 711 were reached, either by phone or in-person. Eligibility status could not be ascertained for 285 of these women as they were not available at the work-site due to deployment, leaves or illness. Of the remaining 711 women, 376 did not meet one or more eligibility requirements. Women on hormonal contraceptives were excluded, as were those pregnant within the last six months, currently pregnant, or over age 41. Also excluded were those women with any of the following diagnosed disease conditions: endometriosis, chronic pelvic inflammatory disease, vaginal, cervical, uterine, or ovarian cancer, systemic lupus erythematosus, hypopituitarism, Cushing's syndrome, sarcoidosis, pituitary tumor, acute hepatitis, HIV or AIDS, cirrhosis of the liver, hypothyroidism, hyperthyroidism, multiple sclerosis, tuberculosis, or diabetes. Women that had one or both of their ovaries removed or women that had a hysterectomy were also excluded from the study. Of the 335 who

were eligible, 170 (51%) completed the baseline questionnaire interview. Daily diaries were also collected for 120 (71%) of the 170 participants (Table 3). The average number of recorded diary days per participant was 46 (range 3 to 103). Of the 170 subjects who completed the baseline questionnaire, 112 provided urine samples for hormonal analysis, and 108 provided both urine samples and diaries (Table 4). Valid breath samples, used to characterize internal dose of compounds in fuels, were available for 96 subjects. Four of 10 AF bases were revisited to recollect breath samples after detection and correction of laboratory equipment failure. Comparison of internal dose of hydrocarbons in fuel and hormonal outcomes was possible for 65 subjects for whom valid urine and workweek breath samples plus diaries were available (Table 4). Some subjects who provided properly collected diaries did not do so for the urine samples and visa-versa. Table 5 describes the reasons for incomplete diary and urine data.

RESULTS AND DISCUSSION OF SOW ACTIVITIES (YEAR 04):

SOW Item #1: **Conduct final statistical analysis for menstrual, hormonal and jet fuel data (months 37-39).**

Final statistical analyses have been completed for three publications, and one abstract. The abstract, entitled "Internal dose of Benzene, Ethyl-benzene, Toluene, Xylenes, & Fuel Components and Effects on Reproductive Hormones in Women" was presented at the Society for Epidemiological Research, June, 2000. The second abstract, entitled "Differences in Urinary Reproductive Hormone Levels between African American and Caucasian Women of Reproductive Age" will be presented at the June, 2001 meeting of the Congress of Epidemiology.

SOW Item #2: **Distribute preliminary report for review and comments (months 39-41).**

This report and the study publications were submitted to co-investigators for review and comments. Results of the study have been distributed to the participants, each Base Commander and the Bio-Environmental Engineering Flight Chief at each base (see Appendix IV). Attached publications have been distributed to U.S. Air Force contacts, Col. Gibson and Col. Neal.

SOW Item #3: **Begin preparation of papers for publication and scientific presentation (months 39-43).**

Effects of fuel exposure on hormonal patterns and menstrual function have been addressed in three separate papers (Appendix III)[6,7]. Preliminary findings of the hormonal subanalysis were also presented at the (2000) Annual Society for Epidemiological Research meeting [8]. In the three papers, we addressed the primary null hypothesis of this study was that there would be no statistically significant difference in hormonal patterns and menstrual function between women exposed to jet fuel and an unexposed group. Effects of fuel on menstrual function were explored in an analysis published in the September 2000 issue of the Journal of Occupational and Environmental Medicine entitled "Menstrual Disorders and Occupational, Stress, and Racial

Factors Among Military Personnel" (see Appendix III)[7]. Fuel contact, based on reported job exposure category, was available for all (n=170) participants and was used to classify exposure in this analysis. ***Women in occupations involving fuel handling did not have significantly ($p \leq 0.05$) higher odds of menstrual disorders in adjusted analyses.*** Life-event stress, however, was found to be significantly ($p \leq 0.05$) related to dysmenorrhea (odds ratio [OR], 2.20), abnormal cycle length (OR, 3.42) and hypermenorrhea (OR, 2.99). Non-Caucasians had significantly ($p \leq 0.001$) increased risks of hypermenorrhea (OR 4.99) and abnormal cycle length (OR, 4.12). The interaction of life-events and race was also significant ($p \leq 0.001$), as reporting at least one stressful life event increased the risk of abnormal cycle lengths among non-Caucasian, but not Caucasian women (OR, 6.52). The proportion of non-Caucasian and Caucasian women reporting multiple events and specific event items was similar. The most frequently reported life events were work changes (40%), taking examinations (24%), interpersonal problems related to work (22%), and moving (21%).

Our second paper is entitled "Relationship between Exposure to Fuels and Solvents and Endocrine Disruption" is in preparation for submission to Environmental Health Perspectives (see Appendix III) and has been distributed for NIOSH review. For this analysis, preovulatory LH (LH), mid-luteal estrone-3-glucuronide ($E_1$3G), and mid-luteal and follicular pregnanediol-3-glucuronide were selected *a-priori* as key study endpoints, as optimum levels of these four hormones have been linked to fertile menstrual cycles (Baird et al., 1999). Exposure level was ascertained based on the internal dose of compounds, as measured by breath analysis. Total C6-C16 (**aliphatic hydrocarbons**) and total aromatic hydrocarbons (HCs): benzene, toluene, ethyl-benzene, and xylenes ("**BTEX**") were chosen to characterize exposure for this analysis. Subjects included 63 of the 65 women for whom breath data and one or more of the four endocrine endpoints were available (Table 4, Appendix II). ***The major finding of this study was the inverse relationship between preovulatory LH and breath aliphatic hydrocarbons levels ($p=0.007$), i.e., as levels of compounds in fuels increased, preovulatory LH levels decreased*** (see Appendix III). This relationship remained significant when the alpha level was adjusted for multiple testing (actual $\alpha = 0.013$ per endpoint, adjusted $\alpha=0.05$ / four endocrine endpoints). Lower levels of LH and mid-luteal PD3G have been linked to infertile cycles[9-11], but it is unknown if the decrements observed in the present study are of sufficient magnitude to reduce fertility.

Our secondary null hypothesis was that there would be no significant racial differences in reproductive endocrine response to jet fuel exposure. Thirteen of 63 participants included in the second paper (described above) were African Americans. Race was included in the univariate regression models in the analysis described above; neither race alone, nor race x aliphatic hydrocarbon or race x BTEX interactions, were significant ($p \leq 0.05$) in any of the final models ($p > 0.05$) after adjustment for aliphatic HCs, BTEX and other covariates. The relationship between fuel and hormone levels was also addressed in our third paper, entitled "Differences in Urinary Reproductive Hormone Levels between African American and Caucasian Women of Reproductive Age" (see Appendix III). This paper is being prepared for submission to Fertility and Sterility. In this paper, relationships between additional endocrine

endpoints and race were examined, controlling for other covariates, including, among others, aliphatic HCs, BTEX, and race x aliphatic and race x BTEX interactions. ***Breath level of BTEX was inversely related to mid-follicular $E_1$3G levels (B=-0.04; p=0.0006)*** but the magnitude of change in this hormone was slight. Also, ***total aliphatic HC levels were inversely related to mid-luteal FSH levels (B=- 0.89; p=0.01). A race x aliphatic HC interaction was related to early follicular $E_1$3G levels (B=-0.97; p=0.001),*** with an increase in this hormone among Caucasions, and a decrease among African Americans in the high exposure group.

Only 7.7% of African Americans *versus* 28.6% of Caucasians reported working in exposed job categories. It should be noted that levels of the analytes were generally low with a few exceptions (Table 6). Further, exposures to compounds under study may also have occurred during other activities, including: cigarette smoking, auto refueling, lawn mowing, degreasing, painting, using nail polish remover, refinishing, and eating grilled food.

An additional goal achieved by the project was to develop a portable method for obtaining breath samples. Many improvements were made in the analytical system during the course of the study, the most notable being the data collection by the EZ Chrom data system and the optimization of the FID detector. Introduction of the analytes of interest onto the chromatographic column needed to be refined, however. The Purge and Trap setup required considerable maintenance and numerous calibration runs were performed before each batch could be analyzed. Sample introduction at the column-transfer line interface is critical and could be vastly improved by shortening the transfer line and using deactivated, high temperature fused silica tubing to transport the sample to the analytical column. This should improve the peak shape of the compounds of interest and the ability of the data system to accurately integrate these compounds.

We also examined salivary hormone levels and compared them to urinary hormone levels. Pearson correlation coefficients were calculated between salivary progesterone (P4) values and urinary pregnandiol 3-glucuronide (Pd3G) values. Correlations were computed for pairs collected on the same day and lagged. The correlation between salivary P4 and urinary Pd3G with no lag is 0.552 ($P<0.0001$; n=4197 pairs). A summary draft of the publication in progress is included in Appendix III, entitled "A Comparison of Salivary and Urinary Progesterone Levels in Women".

SOW Item #4: **Send results to bases and participating subjects (months 42-44).**

Individualized notification letters addressing menstrual symptoms and hormonal patterns were sent to participants in 1999. In August 2000, individualized letters were also mailed reporting breath fuel and solvent analyte levels. Each Base Commander received a letter and a complete summary of the exposure findings. Copies of example letters are included in Appendix IV. Letters regarding breath levels at each base (sans names, bases coded) were also sent to Bioenvironmental Engineering Chiefs.

SOW Item #5: **Present study to bases as requested (months 42-48).**

On several occasions, we have communicated with the USAF Chief Epidemiology Consultant and base Bioenvironmental Engineering personnel regarding the methods, results and interpretation of this study to facilitate distribution of our findings by the Air Force. Tables of results were provided to each Base Commander and are provided in Appendix IV. Each of the Base Commanders was provided with their own base code for review of base exposure levels, but confidentiality was maintained among the bases.

SOW Item #6: **Prepare and distribute final report (months 45-54).**

Year 04 preparation and distribution of the final report is completed by the submission of this document. A no-cost extension was granted through May 31, 2000. During this extended time-period, we examined relationships between race and other reproductive endocrine endpoints, and prepared the publication entitled "Differences in Urinary Reproductive Hormone Levels between African American and Caucasian Women of Reproductive Age" (See Appendix III). An abstract by the same title has been submitted to the American Congress of Epidemiology (acceptance pending).

KEY RESEARCH ACCOMPLISHMENTS:

- Implemented research activities, including: gaining permission from 10 base commanders; identified a potential cohort of ~1,000 women working for the USAF; recruited 170 eligible participants; completed analyses of menstrual symptom disorders from the baseline questionnaire, and found life-event stress to be positively associated with dysmenorrhea; published the menstrual disorder findings in JOEM , 42(9):871-861, 2000.

- Developed and implemented a portable method for obtaining breath samples and completed analyses of hormone level data from urine samples. Demonstrated an inverse relationship between urinary preovulatory LH levels and internal dose of aliphatic hydrocarbons found in fuels and solvents.

- Presented the jet-fuel related hormonal findings at the Society of Epidemiologic Research, June 2000, Seattle, Washington with a published abstract. The paper for publication of these findings, entitled "Relationship between Exposure to Fuels and Solvents and Endocrine Disruption", is currently under review.

- Notified the Participants and the AF Base Commanders and BioEnvironmental Engineering Chiefs of study results, requiring three months of full-time effort.

- An abstract of the race related hormonal findings has been accepted for presentation at the American Congress of Epidemiology. The paper for publication of these findings, entitled "Differences in Urinary Reproductive Hormone Levels between African American and Caucasian Women of Reproductive Age", is currently under final review.

- Measured indices of variation for urinary pregnanediol 3-glucuronide and salivary progesterone (see Appendix II).

REPORTABLE OUTCOMES:

- Manuscripts, abstracts, presentations: References for study publications are listed in the Bibliography (Appendix V). To date, one paper has been published in the Journal of Occupational and Environmental Medicine [7] and an abstract was presented at the Society for Epidemiological Research and published in the American Journal of Epidemiology[8]. Two additional articles, entitled "Relationship between Exposure to Fuels and Solvents and Endocrine Disruption" and "Differences in Urinary Reproductive Hormone Levels between African American and Caucasian Women of Reproductive Age", have been circulated to co-authors for final review in preparation for submission (See Appendix III). The abstract has been accepted for presentation at the American Congress of Epidemiology (June, 2001).

Gordley's masters thesis, entitled "Stress Factors and Menstrual Disorders in Military Personnel". Ms. Gordley was first author on the publication (JOEM, 2000; 42:871-881) and is now a second-year medical student.

- Doctoral Students in Epidemiology supported by this award: Angela Booth-Jones, M.S., Hwa-chung Yiin, M.P.H, Susan Simpson Reutman, Ph.D.. This study was Dr. Reutman's dissertation topic, entitled "Effects of Race & Exposure to Fuels & Solvents On On Female Reproductive Endocrine Outcomes".

- Development of cell lines, tissue or serum repositories: None

- Informatics such as databases and animal models, etc.: None

- Funding applied for based on work supported by this award: Major Audry Gayle Rhodes, M.D., U.S.A.F., an Occupational Medicine resident in the Department of Environmental Health, was funded by the University of Cincinnati Educational Resource Center Pilot Project Program to conduct a study entitled "The Effects of JP-8 on the Immune System of Tank Entry Workers" (See Appendix VI). James Kesner (National Institute of Occupational Safety and Health) has received NIOSH support to evaluate hormonal changes in workers (primarily male) exposed to JP-8. Dr. Ranjan Deka (UC) collaborated with Susan Simpson Reutman on a female reproductive endocrine fellowship application to the American Diabetes Association, entitled "A Study of Endocrine Levels In Polycystic Ovarian Syndrome", however, this application did not receive funding.

- Employment and/or research opportunities applied for and/or received on training or experiences supported by this award: Two of the project programmers (Lu and Zivkovich), and one of the epidemiology students (Booth-Jones), have subsequently obtained full-time employment at the Centers for Disease Control's National Institutes for Occupational Safety and Health. Another doctoral quantitative epidemiology student programmer on the project (Yiin), was awarded a fellowship at NIOSH.

CONCLUSIONS:

This study population of both military and civilian employees of the U.S. Air Force represents a unique group. Menstrual disorders may be sentinel conditions that reveal a woman's adjustment to her environment. The menstrual study results have shown that these women have adapted mechanisms equipping them to deal with the day-to-day job stress but atypical life events, including those correlated with employment, may cause more of a physiological response. The significance of the life event findings suggests that menstrual function is altered during times of major life crises. The significant findings of race as a risk factor for hypermenorrhea and abnormal cycle length need further investigation from both a biological and psychological perspective to determine possible etiology for these differences. While our menstrual study found that women who handled fuel reported two times more dysmenorrhea than those reporting no contact with fuels, other studies have found that solvent exposure had no effect on the menstrual cycle; hence this finding remains unclear. With almost half of the current workforce consisting of women, the menstrual effects of physical, chemical, and psychological occupational exposures need evaluation as the costs in relationship to productivity, financial loss and personal health is great.

Future study of chemical mixtures found in fuels and solvents is essential, as these compounds are ubiquitous in our environment. Gender differences in the relationship between these chemicals and hormones may be anticipated based not only on divergent endocrine factors, but also on male *versus* female variation in the metabolism, storage and excretion of these primarily lipophilic compounds[12]. The focus of the current investigation was on hormones that appear to alter female fertility, however, future studies examining both men and women are urged. Solvent exposure of men in the military has been reported to decrease sperm motility[4] to, equivocally, increase sperm anomalies in men, to reduce fertility and increase menstrual disorders in women[13] and to have acute cytotoxic effects[14]. Exposures that may chronically alter the endocrine environment are of importance in that these not only impact reproduction, but can potentially impact risks for morbidity and mortality from diseases linked to hormonal factors, including osteoporosis, cancer, and heart disease. In conclusion, it appears that the internal dose of compounds found in fuels may alter reproductive hormone levels, specifically in reproductive aged women.

Future research is needed to clarify several scientific questions identified during these three analyses. Our findings with regard to fuel handling, race and menstrual disorders, and race and endocrine differences may have clinical significance. The generalizability of our results concerning fuel exposures, race, life-events, and their impact on menstrual function, could be determined by additional study in non-military populations. In the hormonal study, the significant association between fuels and preovulatory LH generated hypotheses about the potential for endocrine disruption from low-level exposure in the general population, effects in men, potential neuroendocrine mechanisms, and possible reproductive and immune implications of lowered LH. Also, a multivariate relationship between fuels and LH, $E_1$3G, Pd3G that was marginally significant, and the borderline significance of BTEX with $E_1$3G and Pd3G, are

indications to further explore these trends. Our collection of daily urine samples allowed us to detect these trends, as we were able to detect daily hormone levels throughout a menstrual cycle. Although lowered preovulatory LH and mid-luteal Pd3G have been linked to menstrual cycles in which conception did not occur, it is uncertain whether the magnitude of decrease that we found among fuel-exposed women has clinical effects. And despite the challenges of collecting and analyzing biological marker data, we must stress the importance of using biomarkers, such as hormone levels and internal dose of toxicants, in order to avoid misclassification. Extramural collaborations in which trained AF personnel collect samples on-site may increase the feasibility of conducting exploratory studies with limited budgets.

Appendix I: Statement of Work and Abbreviations

STATEMENT OF WORK:

Year 01: (*Completed*)*

1. Develop questionnaires for collecting menstrual and occupational histories (months 1-4).
2. Adapt portable breath analysis system (months 1-6).
3. Develop protocols for breath analysis, industrial hygiene sampling, biological sampling (months 3-9).
4. Pilot test questionnaires on a representative sample of women (months 4-6).
5. Train personnel in use of breath analysis equipment, in teaching participants how to collect urine and saliva samples (months 4-5).
6. Recruit four military bases for participants in study (months 5-9).
7. Characterize the female populations within each selected base that are exposed to jet fuel and that are not exposed [comparison group]; determine the expected number of study participants (months 5-9).
8. Determine the optimal logistical approaches for distributing, monitoring, and collecting samples and supportive material (months 5-9).
9. Perform pilot study air sampling analysis at Hill AFB (months 6-8).
10. Conduct pilot study breath analysis sampling at Hill AFB (months 6-8).
11. Pilot test administration of occupational and menstrual history questionnaires and menstrual diaries at WPAFB (months 6-7).
12. Pilot test collection of collect daily urine and saliva samples at from University of Cincinnati pilot test volunteers: (months 6-7).
13. Collection of daily urine and saliva pilot test samples at the University of Cincinnati pilot test volunteers (months 7-9).
14. Perform formal study air sampling at Base 1 (months 9-11).
15. Conduct breath analysis sampling at Base 1 (months 9-11).
16. Administer occupational and menstrual history questionnaires. Implement menstrual diaries at Base 1 (months 9-12).
17. Collect daily urine and saliva samples at Base 1 on approximately 50 women (months 9-11).
18. Ship samples to NIOSH; perform laboratory analysis of IH and biological samples collected at Base 1; inventory and organize urine samples; store/conduct urinary LH & FSH fluoroimmunoassays; store/conduct urinary $E_1$3G and Pd3G fluoroimmunoassays; store/conduct creatinine assays (months 10-13).
19. Prepare year 01 summary report (months 10-12).
20. Perform items 15-19 at Base 2 on approximately 50 women (months 11-14).

Year 2 (*Completed*)*

1. Ship samples to NIOSH; perform laboratory analysis of IH and biological samples collected at Base 2; inventory and organize urine samples; store/conduct urinary LH & FSH fluoroimmunoassays; store/conduct urinary $E_1$3G and Pd3G fluoroimmunoassays; store/conduct creatinine assays (months 13-16).
2. Perform items 15-19 at Base 3 on approximately 50 women (months 15-18).
3. Ship samples to NIOSH; perform laboratory analysis of IH and biological samples collected at Base 3; inventory and organize urine samples; conduct urinary LH & FSH fluoroimmunoassays; conduct urinary $E_1$3G and Pd3G fluoroimmunoassays; conduct creatinine assays (months 16-19).
4. Abstract military personnel and occupational history data for validity subanalysis (month 16).
5. Conduct validity subanalysis: questionnaire vs. military records and prepare valididty subanalysis report (months 18-21).
6. Perform items 15-19 at Base 4 on approximately 50 women (months 21-24).
7. Prepare year 02 summary report (months 22-24).
8. Ship samples to NIOSH; perform laboratory analysis of IH and biological samples collected at Base 4; inventory and organize urine samples; conduct urinary LH & FSH fluoroimmunoassays; conduct urinary $E_1$3G and Pd3G fluoroimmunoassays; conduct creatinine assays (months 23-25).

** Recruited a total of 170 out of our goal of 200 women during Years 01 & 02 after expanding our field locations from four to ten AFBs (see Tables 1-5, Appendix II).*

Year 3 (*Completed*):

1. Data management: Standardize and computerize data collected from air sampling, biological sampling and breath analysis at four military bases; reduce data onto spreadsheets importable for statistical and graphic analyses; generate graphic depictions of data; conduct preliminary statistical analyses, preparatory to subsequent, complex analyses (months 25-36).
2. Prepare preliminary report (months 34-36).

Year 04 *(Completed):*

1. Conduct final statistical analysis for menstrual, hormonal and jet fuel data (months 37-39).
2. Distribute preliminary report for review and comments (months 39-41).
3. Begin preparation of papers for publication and scientific presentation (months 39-43).
4. Send results to bases and participating subjects (months 42-44).
5. Present study to bases as requested (months 42-48).
6. Prepare and distribute final report (months 45-48).

Abbreviations:

LH	**Luteinizing hormone**
$E_1$3G	**Estrone-3-glucuronide**
Pd3G	**Pregnanediol-3-glucuronide**
BTEX	**Sum of benzene, toluene, ethyl-benzene and m,p and o-xylene**
WPAFB	**Wright Patterson Air Force Base**
BMI	**Body mass index**

Appendix II: Report Tables

Table 1:

Participation of Eligible Subjects By Base & Reported Exposure Status:

AF Base:	State:	Exposed:	Non-exposed
Davis	AZ	10	5
Hill	UT	4	17
Luke	AZ	10	13
Langley	VA	5	12
Moody	GA	2	12
Nellis	NV	6	2
Pope	NC	2	3
Robins	GA	12	21
Seymour Johnson	NC	4	17
Shaw	SC	2	11
Total (10 Bases)		**57**	**113**

Table 2

Recruitment Status of Potential Subjects by Recruitment Outcome Category:

Recruitment Outcome:	**Number**
Eligible, participated:	170
Eligible, declined participation:	135
Scheduled, but no shows:	30
Ineligible:	376
Unavailable (absent or gone with eligibility undetermined):	285
Totals	**996**

Table 3

Number of Baseline Questionnaires, and Daily Diaries Completed by Exposed and Unexposed Status

Completed Study Items:			
Questionnaires:	57	113	170
Diaries:*	33	87	120

* Diaries = # of subjects who returned ≥ 1 day of diary information

Table 4

Number of Biological Samples from 170 Participants

Completed Study Items	**Exposed:**	**Non-Exposed:**	**Total:**
Urine:	29	83	112
Breath:	29	67	96
Questionnaire, Diaries & Urine:	29	79	108
Questionnaire, Diaries, Urine & Breath:	16	49	65*

Urine = # of subjects who returned urine samples from whom at least one study endocrine endpoint was obtained.

* 63 subjects had one of the four endocrine endpoints examined in the paper (ref) (Appendix III)

Table 5

Number of Subjects with Incomplete Diary and Urine Sample Participation during Follow-up Period by Data Type, Reason and Fuel Exposure (Job Category)

Instrument:	Reasons for Incomplete Data:	Exposed:	Nonexposed:	Total:
Diary:	Quit post-questionnaire; no diaries returned to study; no known exclusions:	15	18	33
	Diaries reportedly "lost" post-completion, prior to study receipt:	9	8	17
	Diaries received but later excluded from analysis for medical reasons.	4	2	6
Total:	**# diaries not received/out for analysis:**	**28**	**28**	**56**
Urine:	Quit post-questionnaire; no samples returned to study; no known exclusions:	18	18	36
	Samples reportedly "lost" post-completion, prior to study receipt:	7	6	13
	Apparent wrongful aliquoting:	3	6	9
	Samples received by study, but *out* for hormone analysis because pregnancy/ medical exclusion found (post-questionnaire):	2	2	4
Total:	**# urines not received/out for analysis:**	**30**	**32**	**62**

Appendix III: Publications

Paper One: Menstrual Disorders and Occupational, Stress, and Racial Factors Among Military Personnel

Paper Two: Relationship between Exposure to Fuel and Endocrine Disruption

Paper Three: Differences in Urinary Reproductive Hormone Levels between African American and Caucasian Women of Reproductive Age

Paper Four: A Comparison of Salivary and Urinary Progesterone Levels in Women (in preparation)

Abstract One: Internal Dose of Benzene, Ethyl-benzene, Toluene and Xylenes, and Fuel Components and Effects on Reproductive Hormones in Women

Abstract Two: Differences in Urinary Reproductive Hormone Levels between African American and Caucasian Women of Reproductive Age

Menstrual Disorders and Occupational, Stress, and Racial Factors Among Military Personnel

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Few studies have assessed multiple stress factors as a potential risk for menstrual disorders. This study evaluated whether work-related stress or life event stress was associated with alterations in menstrual function of military personnel. The study is unique in that it evaluated the association between race and three job factors—job stress, handling chemical mixtures, and being a military or civilian employee of the US Air Force. A comprehensive questionnaire was administered to 170 healthy, premenopausal employed women to examine the relationship between work-related or life event stress and menstrual disorders. Multiple logistic regression analyses showed no statistically significant association between work-related stress and menstrual disorders, whereas life event stress was significantly associated with dysmenorrhea (odds ratio [OR], 2.20; 95% confidence interval [CI], 1.08 to 4.50) abnormal cycle length (OR, 3.42; CI, 1.12 to 10.50), and hypermenorrhea (OR, 2.99; 95% CI, 1.20 to 7.42). Having one or more menstrual disorders was significantly associated with life events by race interaction (OR, 6.52; 95% CI, 2.45 to 17.36). Non-Caucasians had significantly increased risks of hypermenorrhea (OR, 4.99; 95% CI, 2.07 to 12.05) and abnormal cycle length (OR, 4.12; 95% CI, 1.47 to 11.55). The prevalence of menstrual disorders in this military population was 31.2% for dysmenorrhea, 17.9% for hypermenorrhea, and 12.0% for abnormal cycle length. This study suggests that women in the military report less day-to-day job stress but more atypical life events, including those related to their jobs, and that these life events are associated with adverse menstrual consequences. (J Occup Environ Med. *2000;42:871–881*)

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In past studies, numerous physiological and psychological factors have been associated with menstrual disorders. Although many of the physiological factors have been extensively researched, the impact that various forms of psychological factors may have on menstrual disorders has been neglected. In the United States alone, 4 million office visits, or one-fifth of all visits for diseases of the female genital tract, are made annually for disorders of menstruation.[1] These disorders can cause serious disruption to schooling, work, or home lives for 1 to 3 days each month. The total US cost of missed workdays associated with menstrual dysfunction is estimated at between 94 and 308 million dollars per day missed.[2] Therefore, menstrual disorders may not only reduce a woman's quality of life but may also cause serious economic losses related to lost workdays and decreased productivity.

The purpose of this study was to examine the relationship of stress factors and their association with menstrual disorders. Specifically, the role of job strain and life events as two distinct types of stress were examined to determine if stress in the workplace or stress related to situations outside of the workplace was more predominantly associated with menstrual disorders. A second purpose of this study was to determine if the prevalence of menstrual disorders varied among racial groups.

Background

Several physiological risk factors for menstrual disorders have been thoroughly researched. Earlier age at menarche has been associated with

an increased occurrence, duration, and severity of pain.[3,7] Studies have shown that menstrual blood loss is higher in parous women when compared with nulliparous women[8-12] and that nulliparous women are at a greater risk for amenorrhea and dysmenorrhea.[3,4,6,7,13] Prolonged bleeding,[8] frequent periods,[8] irregular periods,[8,12,14] dysmenorrhea,[4,7,8,12,15] increase in blood clots,[16] intermenstrual bleeding,[8] and heavy bleeding[8] were reported more frequently in smokers than in non-smokers. Although vigorous exercise has been associated with amenorrhea and oligomenorrhea,[17] there has been no association between the prevalence and severity of dysmenorrhea and the frequency of physical exercise.[7] Increasing age has been significantly associated with an increase in menstrual blood loss and a decrease in the prevalence of dysmenorrhea, whereas the risk for irregular cycles is the greatest immediately after menarche and near menopause.[17]

Several studies have reported differences in menstrual patterns among racial groups in adolescents, yet few have examined adults. Earlier studies found that African-American adolescent girls reach maturation at an earlier age than do Caucasians, even though Caucasians have an earlier age at menarche,[18-20] but more recent studies have shown that Caucasians have a later age at menarche.[21-23] Harlow and Campbell,[23] in a study of African-American and European-American girls aged 12 to 14, found ethnicity to be the strongest determinant of duration and heavy flow of menstrual bleeding. In contrast, Jamieson and Steege[24] found no association between race and dysmenorrhea in adult women. However, a study by Woods et al[25] found that African-American women were significantly less likely ($P = 0.04$) than Caucasians to experience menstrual cramps. These discrepant findings could be related to other risk factors such as age, parity, or the definition for dysmenorrhea. On a global scale, racial differences in duration of menstrual bleeding have been found, with Mexican and Latin-American women bleeding for a mean of 4.5 days compared with a mean of 5.9 days for European women.[26-28] No data exist on the menstrual characteristics of adult African-Americans, and few studies have attempted to discern differences in bleeding patterns among racial groups. Some studies have, however, examined differences in hygiene practices among racial groups.[29]

Stress (measured by life event or perceived stress scales) has been associated with variations in menstrual cycle length,[30] duration and amount of bleeding,[23] and anovulation.[31] Harlow and Matanoski[30] found that although personal gains (eg, starting a new job) were associated with variations in cycle length, personal losses (eg, deaths) had no affect on length of the menstrual cycle. These findings refute Drew's[32] hypothesis that loss and separation are important components of stressors that perturb menstrual function. Meanwhile, other studies have reported that psychological stress and life events or life satisfaction were not associated with menstrual dysfunction.[33,34]

A few studies have evaluated the relationship between work-related psychological stress and menstrual function. Previous studies found no association between level of stress reported and menstrual dysfunction.[34-36] A study by Fenster et al,[37] however, found that although stressful work was not related to an increased risk for anovulation or cycle variability, women working in stressful jobs versus those in non-stressful jobs had double the risk for short cycle length.

Some studies have examined the association of menstrual disorders with specific occupations. A study by Jeyaseelan and Rao[38] found that long menstrual cycles were twice as frequent among active workers (housewives, farmworkers, craftswomen) than inactive workers (beedi worker, clerical) ($P < 0.01$). Women working in the production of electrical insulating materials have reported a greater frequency of polymenorrhea, dysmenorrhea, and irregular cycles than controls.[39-41] A review by Lemasters[42] summarized occupational exposures and risk factors associated with menstrual disorders, including the menstrual effects of solvents, petrol, antineoplastics, hormones, and physical factors such as cold, vibration, noise, and shift work.[16,39-41,43-46] Benzene, toluene, and xylene have been linked to menstrual disorders.[41,45,47] The major use of toluene (90%) is as a conductivity additive of fuels.[48] Fuel exposure is one of the most common chemical exposures at all military bases for both military and civilian women. Specifically, jet fuel, gasoline, diesel fuels, and the products of their complete and incomplete combustion are among the sources of fuel exposure encountered.[49] Nearly 60 billion gallons of jet fuel are produced yearly; the US Department of Defense uses approximately 3.5 billion gallons of jet propellant fuel (JP-8), with the US Air Force as the largest user.[50]

This study is unique in that it evaluated the association between two distinct forms of stress, work-related and life-event, and job factors such as handling chemical mixtures and military versus civilian employment in the Air Force. The study hypothesis was that after adjusting for other risk factors, high levels of job strain as measured by an adapted version of the Job Content Questionnaire (JCQ),[51,52] or life events as measured by the Life Events Questionnaire (LEQ),[53] will be significantly associated ($P < 0.05$) with menstrual abnormalities (dysmenorrhea, hypermenorrhea, or abnormal cycle length) in women employed by the US Air Force.

Methods

Air Force Base and Subject Recruitment

The employees recruited were both military and civilian women

from 10 Air Force Bases: Davis-Monthan, Hill, Langley, Luke, Moody, Nellis, Pope, Warner-Robins, Seymour Johnson, and Shaw. The Air Force bases participating in this study were involved in an expanded study investigating the hormonal effects of exposure to jet fuel in women. Recruitment at these Air Force Bases involved the preliminary identification of a contact person at each base and mailing letters requesting study approval to each base commander. Recruiting and scheduling bases involved follow-up activities, such as confirming permission, scheduling base visits around exercises and deployments, identifying office space, arranging briefings, and accessing phone recruitment lists.

Potential participants were contacted by telephone and in person at each base both prior to and during each base visit to ascertain personal interest and eligibility status and to inform the women of the voluntary nature of the study. The women were given a brief overview of the study with emphasis placed on the study requirements, which for the expanded study included collection of daily urine samples. Women who were on hormonal contraceptives, had been pregnant within the previous 6 months, or were currently pregnant were excluded from the study. Also excluded were women with any of the following diagnosed conditions: endometriosis; chronic pelvic inflammatory disease; vaginal, cervical, uterine, or ovarian cancer; systemic lupus erythematosus; hypopituitarism; Cushing's syndrome; sarcoidosis; pituitary tumor; acute hepatitis; HIV or AIDS; cirrhosis of the liver; hypothyroidism; hyperthyroidism; multiple sclerosis; tuberculosis; or diabetes. Women who had had one or both of their ovaries removed or those who had had a hysterectomy were also excluded. Appointments were scheduled for those considered eligible after the initial screening. At the appointment, participation requirements and eligibility criteria were discussed and informed consent was obtained.

Baseline Interviews

During the personal interviews, all administrative forms along with the questionnaires were completed and all instructions for data collection were given. The study participants completed a questionnaire that collected information on the study population demographics, overall menstrual function, and reproductive history. Also, the height and weight of each study participant was measured.

Acquisition of Data

Independent variables. One stress factor, job strain, was measured by using an adaptation from the JCQ developed by Karasek et al.[51,52] The JCQ is a questionnaire-based instrument designed to measure the content of a respondent's work tasks in a general manner and is applicable to all jobs and jobholders in the United States.

The JCQ questionnaire has been used to predict job-related stress and coronary heart disease in the United States and Sweden.[52] The most common procedure in cardiovascular disease research studies has been to create a dichotomous "job strain" variable. Thus, participants were classified as having high-strain jobs if they are above the median or mean on demands and also below the median on decision latitude. In most studies in which scale reliability is reported, coefficient alpha for job demands and job decision latitude is above 0.70.[52] The job strain instrument used in this study consisted of 12 statements that participants responded to as being either true or false. Their responses to the 12 statements were analyzed using two approaches. The first was based on the scoring methods proposed by Karasek for the JCQ.[51,52] This method divided the 12 statements into three different categories—Demand, Control, and Social Support at work. Of the 12 questions that measured job strain, three measured job demands, five measured job control, and four measured social support at work. A score of high or low job demands and a score of high or low job control was calculated for each study participant. The second approach was to calculate a strain score for each participant. This score consisted of a number from 0 to 12 based on the participant's answers to the 12 statements. A score of 12 was the maximum level of job strain, whereas a score of 0 meant that a participant had no job strain.

The second stress factor measured was related to life events. These stressful life events were defined as any major life changes that disrupt everyday normal activities.[54] Holmes and Rahe offer the most commonly used definition, which "views life events in terms of the changes or 'readjustments' which are required in one's ongoing lifestyle".[55] There are several different instruments used to evaluate these life events. The life events in this study were measured by using the LEQ developed by Horowitz et al.[53] A study comparing four life event scales found the LEQ to be a significantly better predictor of adjustment for women than other scales.[54] The LEQ measures life stress with events including both desirable (eg, job promotion) as well as undesirable (eg, fired from job) events. The life event measurement, therefore, focuses on change regardless of whether the change is construed as being positive or negative. In this study, only events happening within the past 30 days were considered because those events were the most likely to have an acute affect on current menstrual cycle characteristics. The life event score was calculated for each study participant by summing the number of events that each woman reported from the list of 34 possible events. The third stress variable considered in this study examined two non-work situations occurring in the previous 12 months. Women responded either yes or no when asked whether or not

they had had an accidental injury while away from work or if they had had primary responsibility for childcare duties in the past 12 months.

Also included in the questionnaires was information on possible risk factors for menstrual disorders. Potential covariates, including marital status, age, educational level, smoking status, sidestream smoke exposure at work or home, race and ethnicity, military versus civilian employment status, and occupational jet fuel exposure, were all derived from personal initial interview data. The fuel exposure risk factor was ascertained by asking women to define themselves as having a job either handling or not handling fuel, such as aircraft maintenance and refueling operations. Weight in pounds and BMI (BMI = weight in kilograms per height square meters) were also included. Reported exercise, defined as mean hours of moderate or greater activity per day and as mean miles walked or run per day, was included for the subset of participants (n = 104) who completed and returned daily diaries after the personal initial interview.

Dependent variables. Descriptive menstrual function data collected included age at menarche and last menstrual period. Reproductive history included information on infertility, number of pregnancies and their outcomes, a history of female genital tract disorders such as polyps, uterine fibroids, pelvic infection, sexually transmitted diseases, and any other reproductive abnormalities or surgeries.

The dichotomous outcomes measured in this study were menstrual abnormalities. Menstrual dysfunction can be divided into three broad categories: (1) cycle length or rhythm; (2) hypermenorrhea, or excessively profuse or prolonged menstruation; and (3) dysmenorrhea, or presence of pain. If a woman's interval between menses is outside the limits considered normal, she may have either polymenorrhea or oligomenorrhea. Polymenorrhea is defined as menstrual cycles with fewer than 24 days between menses, and oligomenorrhea is defined as menstrual cycles with more than 35 days between menses.[56] The literature in the area of cycle length is ambiguous, and there are no precise definitions on what is considered less than normal or abnormal. Based on a review of the literature, the lower limit was either 23, 24, or 25 days.[56,57] A correlation coefficient was calculated to determine which lower limits were most highly correlated with women reporting their periods as "irregular." These results showed that less than 24 days was the most highly correlated (r = 0.24) with this response and was therefore chosen as our lower limit for normal interval length.

Women were asked about their menstruation patterns over the previous 3 months. Abnormal cycle lengths consisted of those intervals less than 24 or greater than 35 days. Information on cycle length was obtained from the survey item ("... how many days usually passed from the start of one period to the start of the next?"). The second outcome, hypermenorrhea, described abnormal bleeding patterns, either in duration or amount of menstrual flow. Subjects were queried with regard to bleeding duration ("... how many days have your periods usually lasted?") and amount ("... describe the amount of bleeding during your typical menstrual period... spotting, light, moderate or heavy?"). Normal duration of menses was defined as between 3 and 7 days.[17] Therefore, hypermenorrhea was defined as either menses excessive in duration (>7 days) or as amount of menstrual bleeding reported as "heavy" rather than "spotting," "light," or "moderate." The third menstrual outcome, primary dysmenorrhea, is the presence of pain and is among the most common of all gynecological complaints. Women were excluded if they had any of the pathological conditions contributing to secondary dysmenorrhea. Dysmenorrhea is generally recognized as a condition severe enough to warrant women to cease their daily activities, such as loss of time from work or school,[17] and was defined as ever having the need for bedrest or missing work because of menstrual pain, and menstrual or premenstrual symptoms. This variable was derived from responses to the questions ("... did you miss work... need to lie down... due to [menstrual or premenstrual] symptoms?").

Statistical Analyses

The first step of this analysis was to obtain frequency counts on all variables of interest, including exposure variables, explanatory variables, and outcome measures. Next, single associations between stress factors and outcome measures were examined. SAS analysis evaluated the correlations among the main effects and found that none of the exposure factors or explanatory variables remaining in the final model was highly correlated.

Multiple logistic regression was the primary analysis method used to model the relationship between the binary outcome response variables, exposure factors, and other explanatory variables known or suspected of being associated with menstrual disorders. The following variables were evaluated in bivariate logistic regressions but were not significant so were excluded from any further analyses: age (<30 = 0, ≥30 = 1), income (≥$30,000 = 0, <$30,000 = 1), marital status (partner = 0, no partner = 1), number of children (none = 0,0; one child = 1,0; two or more children = 0,1 vs one or more children = 1), weight above and below median (<148.5 lbs = 0, weight ≥ 148.5 lbs = 1), lean versus non-lean (BMI < 25 = 0, BMI ≥ 25 = 1), BMI above and below top quartile (BMI < 27.3 = 0 and BMI ≥ 27.3 = 1), current smoking status (non-smoker = 0, smoker = 1). The main effects remaining in the models included: race (Caucasian = 0, non-Caucasian =

1), two education level variables (some high school or high school and technical training = 0,0; some college or associates degree = 1,0; bachelors or masters degree = 0,1), military employee status (civilian employee = 0, military employee = 1), fuel handling (non-fuel handling = 0, fuel handling = 1), and passive smoke exposure (non-exposed = 0, exposed = 1). Daily-reported exercise, defined as total miles run and walked per day (<0.47 miles = 0, ≥ 0.47 miles = 1), also remained in the model for the subset of participants (n = 104) from whom diary data were obtained.

The stress exposure variables were life events (no life events = 0, life events = 1), non-work activities (no non-work activities = 0, non-work activities = 1), and job strain. The exposure variable job strain was modeled several ways. In the first analysis, job strain was divided into two categories based on the calculated strain score (no/low strain = 0 and high = 1 defined as below, or equal to or above, the median value of 4). In the second analysis, job strain was treated as continuous and the actual strain scores, which ranged from 0 to 12, were entered into the model for the job strain variable. Next, an alternative way of scoring job strain was done by scoring only questions related to job demands. A score of 0 or 1 on this section represented low demands, and a score of 2 or 3 on this section represented high demands (low-demand = 0, high-demand = 1). Another analysis used the questions related to demands (scored the same) and the questions related to control. A score of 0 to 2 represented low control and a score of 3 to 5 represented high control, therefore coding high-demand, low-control = 1 and all other combinations (high-demand, high-control; low-demand, low-control; or low-demand, high-control) equal to 0. These models were proposed to evaluate the different scoring strategies used for the variable job strain. The three binary outcome response variables—dysmenorrhea, hypermenorrhea, and abnormal cycle length—were modeled separately along with the outcome of a positive response to any of the three menstrual disorders.

A backward elimination approach was used, and a significance level of 0.10 was specified for inclusion in the model. All variables not meeting this specified level were then dropped. Adjusted odds ratios (OR) and the 95% confidence interval (CI) of the ORs were calculated for factors remaining in the model.

Results

Subjects

There were 335 preliminarily eligible military and civilian women employed at 10 US Air Force bases. A total of 202 of the 335 eligible women (60.3%) agreed to participate in all aspects of the total study (ie, provide breath samples, 2-month daily diaries, and urine and saliva samples through two menstrual cycles for future analyses). Of this group, 170 completed the baseline questionnaire used in this report.

As Table 1 shows, the 170 participants in this study ranged in age from 18 to 41 years old with a mean age of 29.4 years. The mean age at menarche for this population was 12.7 years and ranged from 9 to 17 years of age. Mean values for weight and BMI were, respectively, 152.3 lb (range, 97 to 291.5 lb) and 25.2 (range, 17.7 to 51.2 BMI units). Over half of the participants, 61.8% (n = 105) were Caucasian. Most, 56.8% (n = 96) were either married or had a permanent partner and were well educated, with 75.9% (n = 129) having had some college education. Half the population (50.6%, n = 85) had a net family income of $30,000 or higher. Only 8.2% (n = 14) of the study group were cigarette smokers, an original exclusion criteria; however, 29.4% (n = 50) of the subjects, including most (n = 11) of the smokers, reported passive cigarette smoke exposure at home and/or at work. Over half of the group, 58.3% (n = 98) had children. The majority of this population, 82.4% (n = 140) were in the military, and 38.8% (n = 66) reported handling fuels as part of their work detail. Subjects worked, on average, 8.6 hours per day (range, 5.1 to 13.0 hours per day), and most (71%) worked exclusively during the day.

Stress Exposures

Based on the responses of the 170 participants to the JCQ job strain section, 55.3% (n = 94) scored "high" (above the median). Using a definition of job strain defined by Karasek et al[51,52] as "high demand, low control, and low social-support," only 6.5% (n = 11) of the group met this definition, whereas 17.7% (n = 30) reported "high demands and low control" and 39.4% (n = 67) reported "high demands" in their job position. About half (54.7%, n = 93) reported one or more life event(s) in the 30 days before the interview date, and similarly, about 94 subjects (53.3%) reported having an accidental injury or having primary child care duties.

Outcome Measures

The prevalence rates of menstrual outcomes were calculated and are shown in Table 2. Of the 170 participants, 46.1% (n = 77) reported having one or more menstrual disorders. Dysmenorrhea was reported by 31.2% (n = 53 of 170), all of whom needed to lie down and 8.8% (n = 15 of 170) also missed work. Medication was taken for relief of menstrual symptoms by most 86.8% (n = 46 of 53) reporting dysmenorrhea defined by disability as above, and by 62.9% (n = 107 of 170) of all study participants. Hypermenorrhea was reported by 17.9% and abnormal cycle length data was reported by 12.0%.

Because participants were obtained from an investigation of fuel exposure, we first examined if there were any significant differences among the non-fuel handlers and the fuel-handlers with respect to the stress factors, and there were no

TABLE 1
Demographic Characteristics of the Participating Population

Demographic Characteristics	%	n	Mean ± SD	Range
Age (yr)	-	170	29.4 ± 6.4	18–41
Age at menarche (yr)	-	170	12.7 ± 1.6	9–17
Weight (lb)*	-	163	152.3 ± 30.7	97–291.5
Body mass index*	-	163	25.2 ± 5.0	17.7–51.2
Race				
Caucasian	63.8	105		
Non-Caucasian				
African-American	32.8	54		
Hispanic	4.7	8		
Other	1.8	3		
Marital status*				
Never married	26.0	44		
Married or have permanent partner	56.8	96		
Widowed, divorced, or permanently separated	17.2	29		
Education				
Some HS/HS or GED†/HS & tech training	24.1	41		
Some college	61.8	105		
College degree	14.1	24		
No. of Children*				
0	41.7	70		
1	26.2	44		
2+	32.1	54		
Family Income*				
<$30,000	49.4	83		
≥$30,000	50.6	85		
Cigarette exposure				
Smoker	8.2	14		
Non-smoker	91.8	156		
Passive tobacco smoke exposure				
Exposed	29.4	50		
Non-exposed	70.6	120		
Job Category				
Military	82.4	140		
Civilian	17.6	30		
Fuel exposure				
Fuel handling	38.8	66		
Non-fuel handling	61.2	104		

* Marital status missing for one person; income and no. of children missing for two people, subject's weight and body mass index missing for seven people.
† GED refers to high school (HS) equivalency test diploma.

TABLE 2
Association Between Stress Variables and Menstrual Outcomes

Menstrual Outcome	Prevalence (%)	Job Strain		Life Events		Non-Work Activities	
		χ^2	P	χ^2	P	χ^2	P
Dysmenorrhea (n = 53)	31.2	1.51	0.22	5.43	0.02	0.01	0.92
Hypermenorrhea (n = 30)*	17.9	0.33	0.57	4.55	0.03	2.11	0.15
Abnormal cycle length (n = 20)*	12.0	0.26	0.61	3.77	0.05	2.15	0.14
Any menstrual disorders (n = 77)*	46.1	0.65	0.42	8.76	0.003	1.47	0.23

* Two missing from hypermenorrhea analysis, three missing from menstrual disorder analysis, and four missing from abnormal cycle length analysis.

significant differences. Therefore, both groups were combined. The results of evaluating associations between each stress factor and the menstrual abnormalities are shown in Table 2. Only stress associated with life events was significantly associated with all menstrual outcomes: dysmenorrhea (chi-squared = 5.43, $P = 0.02$), hypermenorrhea (chi-squared = 4.55, $P = 0.03$), abnormal cycle length (chi-squared = 3.77, $P = 0.05$), and having one or more menstrual disorders (chi-squared = 8.76, $P = 0.003$). There was no association between any of the menstrual outcomes and job strain or non-work activities.

Multiple logistic regression analysis again showed that job strain was not significantly associated with any menstrual outcomes, regardless of the scoring method. Non-work activities, age, education, income, smoking status, and BMI did not approach significance ($P < 0.10$) in final models. Table 3 displays the multiple logistic regression results. As Table 3 shows, life events were significantly associated with dysmenorrhea (OR, 2.2; 95% CI, 1.08 to 4.50), hypermenorrhea (OR, 3.0; 95% CI, 1.2 to 7.4), and abnormal cycle length (OR, 3.4; 95% CI, 1.1 to 10.5). Race was also significantly associated with hypermenorrhea (OR, 5.0; 95% CI, 2.1 to 12.1) and abnormal cycle length (OR, 4.1; 95% CI, 1.5 to 11.6). We also examined a potential interaction between life events (high vs low) and racial group (Caucasian vs non-Caucasian). Because of small cell size for individual outcomes, numbers were only sufficient to examine the effect of this interaction on two outcomes: dysmenorrhea and having at least one menstrual disorder. In the logistic model, the interaction of race and life events, adjusted for other covariates, was significant (OR, 6.5; 95% CI, 2.4 to 17.4) for having one or more menstrual disorder(s) but nonsignificant for dysmenorrhea. Reports of one or more menstrual disorder(s) were also significantly

TABLE 3
Adjusted Logistic Regression Odds Ratios for Life Events and Menstrual Disorders[a]

Outcome	Life Event	Race	Fuel-Handling	Military	Passive Smoking	Exercise[b]	Life Events × Race
Dysmenorrhea (n = 53)	2.20†		1.83*		2.03*		
	(1.08–4.50)		(0.90–3.70)		(0.98–4.18)		
	0.79		0.60		0.71		
Hypermenorrhea (n = 30)[c]	2.99†	4.99§		4.12*			
	(1.20–7.42)	(2.07–12.05)		(0.89–19.1[illegible])			
	1.09	1.61		1.42			
Abnormal cycle length (n = 20)[c]	3.42†	4.12§	0.29*				
	(1.12–10.50)	(1.47–11.55)	(0.08–1.06)				
	1.23	1.42	−1.24				
Menstrual disorders (n = 77)					2.50†	2.75†	6.52§
					(1.23–5.09)	(1.10–6.87)	(2.45–17.36)
					0.92	1.01	1.88

[a] Results shown are odds ratios, 95% confidence intervals in parentheses, and coefficients. All variables that were significant at the 0.10 level are included in the table. Full model included job strain, life events, non-work activities, race, age, fuel exposure, education level, military employee, body mass index, smoking status, and passive smoke exposure status.

[b] Exercise was included only in a subanalysis of subjects who returned diaries (n = 104); passive smoking and life events × race also remained in that model.

[c] Two missing from abnormal bleeding patterns analysis, and four missing from abnormal cycle length analysis.

* $P \leq 0.10$; † $P \leq 0.05$; § $P \leq 0.001$.

TABLE 4
Characteristics of Menstrual Patterns by Racial Status

	Caucasian (n = 105)[a]	Non-Caucasian[b] (n = 65)[c]
% Dysmenorrhea	31.4	30.8
% Hypermenorrhea***	9.7	30.8
Mean ± 1 SD days periods last	5.0 ± 1.2	5.2 ± 2.7
% Typical amount of bleeding reported		
Not heavy	90.3	73.8
Heavy**	9.7	26.2
% Abnormal cycle length (<24 or >35 days)*	6.8	20.6
Mean (± 1 S.D.) of cycle length in days	28.9 ± 4.5	28.0 ± 6.4
% Reporting at least 1 menstrual disorder*	38.5	58.7
% Reporting regular periods 3 months before interview	88.1	89.2
% Reporting stressful life events 1 month before interview	59.1	47.7
Mean ± 1 SD age at menarche in years	12.9 ± 1.6	12.5 ± 1.6
Mean ± 1 SD age in years	29.8 ± 6.1	29.0 ± 6.9
Mean ± SD weight in pounds	150.0 ± 22.9	156.2 ± 40.0
BMI ± 1 SD	24.7 ± 3.5	26.1 ± 6.7
% Children		
None	37.5	47.7
Children	62.5	52.3

[a] One missing for one or more menstrual disorders and no. of children; two missing for typical amount of bleeding and abnormal cycle length; five missing mean weight.

[b] Non-Caucasian group consisted of 54 African-Americans, 8 Hispanics, and 3 who reported race as "Other."

[c] Two missing for abnormal cycle length, one or more menstrual disorders, and mean weight.

* $P \leq 0.01$; ** $P \leq 0.005$; *** $P \leq 0.001$.

associated with passive smoke exposure (OR, 2.5; 95% CI, 1.2 to 5.1) and with exercise (OR, 2.8; 95% CI, 1.1 to 6.9).

Based on the results of race as a risk factor for hypermenorrhea and abnormal cycle length and the interaction of race and life events as a risk factor for any menstrual disorders, further post-hoc exploration was undertaken. As Table 4 shows, both Caucasians and non-Caucasians reported the same prevalence of dysmenorrhea, 31.4% and 30.8%, respectively. Compared with Caucasians, non-Caucasians reported a significantly greater prevalence of hypermenorrhea (30.8% vs 9.7%), which was related to the report of "heavy" bleeding (26.2% vs 9.7%). Race was significantly associated with abnormal cycle length and report of any menstrual disorder. Both groups reported the mean length of their period as approximately 5 days. Prevalence of abnormal cycle length (<24 or >35 days) was higher in non-Caucasians (20.6%) than in Caucasians (6.8%). Although both Caucasians and non-Caucasians had a mean cycle length close to 28 days (28.9 and 28.0, respectively) the variation was greater for non-Caucasians (6.4 days) compared with Caucasians (4.5) days. Of the non-Caucasians, 58.7% reported at least one of the three menstrual disorders compared with 38.5% Caucasians.

As shown in Table 4, both groups had approximately the same percent-

ages reporting regular periods in the 3 months before their interview date. The mean age at menarche for the non-Caucasian group (12.5 years of age) was similar to that of the Caucasian group (12.9 years of age). The mean age and weight of both groups were similar; however, the non-Caucasian group was more likely to be childless and to have reported fewer recent life events (Table 4).

Discussion

When considering menstrual disorders, comparison of studies is difficult because there are no widely accepted definitions for determining normal ranges. Also, there is no universal method of collecting and analyzing menstrual data, which contributes to wide variations in the prevalence rates of abnormalities. In this study, menstrual disorders are defined by the subject's characterization of her own patterns and are affected by perception and recall errors. The potential for self-selection is also present, relative to the degree that menstrual abnormalities and stress affected our subjects' decisions to participate. Subjects were recruited into a study of fuel handling rather than stress, however. Further, similar mean cycle lengths[16,58] and frequencies of severe dysmenorrhea, defined as absenteeism due to menstrual pain during at least half of their menses,[4,59] have been reported in other populations, suggesting that self-selection was likely minimal. In addition, the mean number of life events reported within 6 months of the current study (2.5 of 33 LEQ items) was comparable with the number cited (2.9 per 33 "Schedule of Recent Life Events" items) within a 7-month period by female military health care workers in another investigation.[60]

Most of our participants (82.4% within 6 months) reported at least one life event, the most frequent being work changes (40%), examinations (24%), work interpersonal problems (22%), and moving (21%); for military personnel, moves and examinations are likely related to their career. Work changes[30,61] and moving[30] have been cited as the predominant stressors among women in other settings. The proportion of participants with high job strain in the present study (17.6%), however, was lower than rates reported among the general US working population (23% to 27%)[62,63] and for specific groups, such as nurses (24%)[64] and teachers (45.7%).[65]

Thus, job stress, as measured by the adapted JCQ, did not show an effect on menstrual function in this military population. Regardless of the scoring method used, job stress was not associated with any menstrual outcome, and this finding is in agreement with others.[34,35] The lack of association between job stress and menstrual dysfunction in our study may be related to the population of Air Force employees. The amount of job strain reported by the participants was low overall; the highest possible score was 12, and this population had a median score of 4. The stress measure stemming from major life events, however, was significantly associated with all three of the menstrual outcomes—dysmenorrhea, hypermenorrhea, and abnormal cycle length. Of the 77 women who met the definition for one or more menstrual disorders, 33.8% (n = 26) reported no life event stress and 66.2% (n = 51) gave 85 positive responses. Of those 85 responses, 43.5% (n = 37) were related directly to work or training, whereas 29.4% (n = 25), 11.8% (n = 10), 8.2% (n = 7), and 7.1% (n = 6) were related, respectively, to a change in a relationship; legal or financial problems; a move; or injury, illness, or death of a loved one. Thus, although job stress, per se, was not associated with menstrual dysfunction, almost half of the stressful life events reported were related to military work or training. The adapted JCQ stress measure was based on a woman's perception of how stressful her job typically is, whereas the life event section focused only on the immediate specific life events that had occurred within the past 30 days. Therefore, it is possible that this population of military workers typically reports little job stress, accounting for the low job strain scores, but that 43.5% had an atypically job-related stressful situation that had occurred within 30 days of the interview (eg, new work activity, getting moved to another area of the base) that likely contributed to a menses disorder. Hence, the lack of association between job stress and any of the menstrual disorders as opposed to life events associated with all menstrual disorders may be related to this distinct working population that consisted of 82% active duty military and 18% civilian employees. The subjects likely have more regimented and disciplined environments than most other groups and may be more psychologically equipped and less vulnerable to job stress. Many enlisted in the Air Force soon after high school, and because their average age was 30 they have had several years to adjust to this particular lifestyle. Because these women are stationed all over the country, however, they may lack social support and be more affected by life events over which they have little control. They may therefore be less equipped emotionally to deal with life events than job stress, which could account for why so few reported job strain. We did observe that 20% of the military employees reported 4 times the amount of hypermenorrhea compared with civilian employees (6.7%).

Besides job stress and military status, one other work-related factor was assessed: handling fuel. Women who reported handling fuel in their work activity reported twice the amount of dysmenorrhea compared with women having no fuel contact. Of the fuel handlers, 40.9% (n = 26) had dysmenorrhea, whereas 26.0% (n = 27) of non-fuel handlers had dysmenorrhea. It cannot be certain if this finding is related to exposure or other related work factors such as

physical activity. Work activities that involved jet fuel (JP-8) exposure included such jobs as fuel cell maintenance, aircraft maintenance, and fuel injection on the flightline. JP-8 consists of alkanes, cycloalkanes, alkylbenzenes, naphthalenes, dicycloparaffins, tetralins, and olefins, along with several additives including toluene and other solvents.[66,67] The major use of toluene is as a fuel additive, but it is also prevalent in other occupational settings in solvents and paints. Ng et al[46] found that alterations in frequency and severity of dysmenorrhea and prevalence of severe dysmenorrhea were more common in female employees of an audio speaker manufacturing factory highly exposed to toluene when compared with an external community control group ($P > 0.001$). Therefore, this particular finding warrants further investigation.

In this military population, race was a risk factor for hypermenorrhea. Non-Caucasians, who were primarily African-Americans (83.1%) were 5 times more likely than Caucasians to report having "heavy" periods. Hartz et al[68] studied waist-to-hip girth ratios and found that greater fat distribution in the upper body was associated with irregular menstrual cycle and oligomenorrhea in women aged 20 to 39. Compared with other racial backgrounds, female African-Americans have significantly greater appendicular skeletal muscle, bone mineral and total body potassium,[69,70] skeletal mass, and upper body fat distribution,[71] independent of body weight, height, percent body fat,[72–74] and education.[73] In the present study, the mean BMI for non-Caucasians (BMI, 26.1) was slightly higher than for Caucasians (BMI, 24.7). Differences in fat distribution have been shown to cause increased aromatization, resulting in increased endogenous estrogen levels, and may contribute to differences in menstrual cycle characteristics.[75]

Non-Caucasians were 4 times more likely than Caucasians to report their menstrual cycle as being abnormal in length, primarily less than 24 days. Race and life events, however, interacted to alter risk of abnormal cycle length inasmuch as reporting at least one life event significantly increased the risk of abnormal cycle length among non-Caucasian, but not Caucasian, women. We postulated that perhaps the nature or frequency of life events among those who experienced at least one event might differ according to racial group. The proportion of non-Caucasian and Caucasian women reporting multiple events and specific event items was similar, however. Alternatively, it is possible that unexplained sociocultural or biological influences within each group could modulate the relationship between life events and cycle length.

Differences between groups may also be related to measurement error. For instance, the term heavy is subjective and reports may differ depending on the perception of bleeding. Perception differences may exist because racial groups obtain information regarding their reproductive health from different sources. A study of racial differences in menopause information found that the primary source of menopause-related information for Caucasian women was the media, whereas African-American women reported that their family was the primary source.[76] In addition to perception, variations may also be explained by differences in personal hygiene patterns. A study by Gustafson et al[77] found that Caucasian women used tampons more frequently (85%) than did African-American women (50%) and were almost 3 times as likely to use tampons continuously (day and night) during a menses than were African-American women. Therefore, additional studies need to distinguish whether these racial differences are related to biological or cultural differences.

In contrast to the above differences, the prevalence rates of dysmenorrhea were similar in both Caucasians (31.4%) and African-Americans (30.8%) and are in agreement with others. Jamieson and Steege[24] also found no racial differences in the reporting of dysmenorrhea in 581 women aged 18 to 45, although 90% of their population reported having dysmenorrhea, defined as "pain with menstrual periods." Woods et al,[25] after administering the Moos Menstrual Distress Questionnaire to 179 nonpregnant women, found that 8.6% of African-American women compared with 20.2% of Caucasian women reported experiencing "menstrual cramps." Although our study had a more stringent definition of dysmenorrhea than those used by either the Jamieson or Woods studies (pain severe enough to "lie down or miss work" vs "pain" with menstrual periods or "menstrual cramps"), our prevalence rates for dysmenorrhea fell between theirs. These discrepancies in definitions and prevalence rates illustrate the need for standardization and universal methods of collecting menstrual data.

In conclusion, this study population of both military and civilian employees of the US Air Force represents a unique group. Menstrual disorders may be sentinel conditions that reveal a woman's adjustment to her environment. The results have shown that these women have adapted mechanisms equipping them to deal with day-to-day job stress but that atypical life events may cause more of a physiological response. The significance of the life event findings suggests that menstrual function is altered during times of major life crises. The significant findings of race as a risk factor for hypermenorrhea and abnormal cycle length need further investigation from both a biological and psychological perspective to determine the possible etiology for these differences. Although this study found that women who handled fuel reported 2 times more dysmenorrhea than those reporting no contact with fuel, other studies have found that solvent exposure had no effect on the menstrual cycle; hence, this finding remains unclear. With almost half of the current workforce consisting of women, the menstrual effects of physical,

chemical, and psychological occupational exposures need evaluation, because the costs in relation to productivity, financial loss, and personal health are great.

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PAPER TWO: RELATIONSHIP BETWEEN EXPOSURE TO FUEL AND ENDOCRINE DISRUPTION. REUTMAN SS, LEMASTERS GK, KNECT EA, SHUKLA R, LOCKEY JE, BURROUGHS GE, MEYER DM, KESNER JS.

Background:

Evidence is accumulating that hydrocarbons (HCs) in fuels and solvents are reproductive toxicants. For example, reductions in female fertility have been identified in occupational groups who are exposed to organic solvents containing benzene (1), toluene (2), and mixtures of solvents (3,4). Fuels, solvents, and their constituent chemicals are ubiquitous exposures. Contact may occur during routine home or workplace activities, such as automobile refueling, lawn mowing, painting, refinishing, or degreasing. There is particular concern for individuals with frequent, chronic, work-related exposure. Among those occupational populations at risk of high level exposures to fuels and solvents are farmers, mechanics, maintenance workers, printers, petroleum refinery workers, metal cleaners, painters, and aircraft maintenance personnel. Approximately 116,000 women work for the USAF as officers, enlisted personnel, and civilians (5). Women hold jobs in the USAF that involve handling fuel, maintaining jets and ground vehicles, and working on the flight-line. These jobs potentially expose women to solvents, fuels, and products of fuel combustion. Furthermore, gender differences in exposure, toxicokinetics, and physiological responses may affect susceptibility. Pharmacokinetic modeling reveals that women metabolize 23-26% more benzene than men under the same exposure conditions and, therefore, may have different responses to exposure than men (6). Historically, over three billion

gallons of jet fuel have been issued annually to United States Department of Defense (7). The principal jet fuel used by the USAF, JP-8, is a mixture of petroleum distillates composed primarily of aliphatic and aromatic HCs in approximately a 6:1 ratio (8,9). Among the constituent HCs common to both JP-8 and other, more commonly used solvents are aliphatic HCs such as hexane, and aromatic HCs such as toluene and xylenes (10-12). Fuel and solvents may be encountered separately or as mixtures during job activities such as aircraft maintenance.

The purpose of this study is to assess the potential effects of fuel and solvent exposure on menstrual cycle function. Specific endocrine endpoints that are predictive of conceptive menstrual cycles (13) were monitored as sub-clinical markers of female reproductive dysfunction to identify early, subtle reproductive effects of low-dose exposures to solvents and fuels.

MATERIALS AND METHODS:

Study population:

Female USAF employees are the study population. Potential participants were recruited for initial interviews by phone and in-person at ten USAF bases. There were 335 civilian and active military women employed at these ten USAF bases who were preliminarily eligible during recruitment screening. Of these women, 51% (n=170) consented to participate by maintaining daily diaries and collecting daily urine samples, and were confirmed as eligible during the baseline interview. Eligibility criteria included: age 18-42 years; had not used hormonal medications, oral contraceptives or hormone replacement for 3 months; had not used an intrauterine device during the past three months; no surgery on reproductive tissues (tubal

ligation accepted); had not been pregnant or breast feeding for three months; had not been diagnosed with any of the following: chronic pelvic inflammatory disease; endometriosis; vaginal, cervical, uterine, or ovarian cancer; systemic lupus erythematosus; hypopituitarism; Cushing's syndrome; sarcoidosis; pituitary tumor; acute hepatitis; HIV or AIDS; cirrhosis of the liver; hypothyroidism; hyperthyroidism; multiple sclerosis; tuberculosis; or diabetes. Non-smokers were targeted, however, a small subset of smokers were also included.

Of the 170 women who completed the baseline questionnaire, 100 participants provided completed diaries and urine samples. There were 53 women who did not return either diaries and/or urine samples, and 13 women who returned samples that were inadequate to evaluate the four key endocrine endpoints. Four participants were excluded retrospectively because of pregnancies (two), oral contraceptive use (one), and symptomatic endometriosis (one) which were reportedly present during sample collection. Breath samples to yield exposure data were also available for 63 of these 100 compliant subjects. Compensation for time and inconvenience was $50 for daily diaries, $25 for urine samples, and $25 for breath samples.

Initial Subject Interview and Diary Collection:

During the initial interview, potential participants were told the study procedures, eligibility criteria, and the voluntary nature of participation; informed consent was obtained. Next, the baseline questionnaire was administered to collect information about their work, socioeconomic status, pregnancy, lifestyle, and reproductive and menstrual histories. Results of

the menstrual history are reported elsewhere (14). Instructions were given for collecting daily urine samples and for completing daily diaries. Weight and height were measured.

Subjects were asked to immediately begin maintaining their diaries daily, and to continue through the end of their second post-interview menstrual period. The diary requested menstrual, psychosocial, lifestyle, work, chemical and physical exposures, and sample collection information. Diary items used for preliminary analyses in this report include: menstrual bleeding/spotting (yes/no); number of cigarettes smoked; hours slept; hours of second-hand smoke exposure; any episodes of illness and/or fever > 101° (yes/no); ounces of caffeinated drinks (coffee, tea, soda); number of alcoholic drinks; number of hours worked; number of shifts worked; hours of smelling fuel, solvents, or pesticides; hours of skin contact with fuel, solvents, or pesticides; number of miles run; number of miles walked; duration of light-to-moderate and heavy physical activity at home and work; and weekly job strain (true/false questions). Job strain was measured using an adaptation of the Job Content Questionnaire developed by Karasek (15,16). Subjects mailed their diaries to investigators upon completion.

Endocrine Data Analyses:

First morning urine samples were collected daily concurrent to maintaining the diaries. Participants stored the samples in home freezers; samples contained 7% glycerol to prevent loss of hormonal activity (17). Participants shipped frozen samples with freezer packs to the NIOSH laboratory by next-day courier. Samples were stored in the laboratory at -80° C until assayed.

Menstrual periods were derived from the participants' daily records of vaginal bleeding based on a modification of a previously described menstrual algorithm (18). The first day of the menstrual cycle was the first of two consecutive days of bleeding, only one of which could be spotting. After day two of the period, 1-2 day interruptions in bleeding (non-bleeding or spotting) were counted together with bleeding days as part of the menses. Menses were preceded and followed by three or more consecutive days of non-bleeding or spotting. Participants with missing diary menstrual bleeding entries were contacted immediately regarding menses dates, and reported menses dates were accepted up to 14 days retrospectively.

Urinary endocrine measurements and menses dates were used to derive the four key endocrine endpoints using established algorithms (13,19). Baird et al. (1999) reported that nonconception during ovulatory cycles is associated with elevated levels of follicular pregnanediol 3-glucuronide (Pd3G), and reduced levels of preovulatory luteinizing hormone (LH), mid-luteal Pd3G, and possibly mid-luteal estrone 3-glucuronide ($E_1$3G). Therefore, these endpoints were selected *a priori* for analysis.

The major urinary metabolites of estradiol and progesterone, i.e., $E_1$3G and Pd3G, were assayed using competitive, double-antibody time-resolved fluoroimmunoassays (20). Urinary LH was assayed using a commercial non-competitive, two-site, time-resolved immunofluorometric assays (21,22). Creatinine was measured spectrophotometrically (23). Each urinary endocrine value was divided by the creatinine concentration to adjust for urine dilution. All samples for each subject were measured in the same assay. Intra- and inter-assay coefficients of variation, respectively, for urinary endocrine measurements were: 6.2% and 4.6%

for LH; 15.4% and 10.1% for $E_1$3G; 11.6% and 8.4% for Pd3G; and 0.97% and 3.4% for creatinine.

Internal Dose Exposure Measures:

We previously demonstrated that relatively low internal doses of aromatic HCs in solvents could be measured with greater sensitivity in breath than in blood or urine (24). Therefore, internal doses of aliphatic and aromatic HCs from solvents and fuels were estimated in breath samples collected from 63 subjects. In order to estimate analyte levels in the vessel-rich tissue compartment, breath samples were collected 1.4 hours (SD=2.2), on average, after subjects left the work-site (25) on the 2nd to 5th consecutive workday. For one additional subject with no workday sample, a Monday (1st workday) sample was substituted as a proxy measurement for this analysis (total n=63). Breath samples were collected through desiccant filters into Tedlar® bags and then suctioned into sorbent charcoal tubes 1.5 hours (SD=1.4), on average, after collection.

In the laboratory, breath sample analytes were concentrated by thermal desorption of the sorbent tube contents onto the charcoal bed of a Tekmar 3000 Purge and Trap. The collected analytes were then flash-heated to 225° C, and released to the heated nickel transfer line under a constant back-pressure. The transfer line was directly connected to the column (DB-VRX, J & W Scientific) with a zero dead volume union. Analysis was accomplished using a Hewlett-Packard Model 5890 Series 2 Gas Chromatograph, equipped with a FID detector optimized for detection of aromatic HCs, including benzene, toluene, ethyl-benzene, m,p, and o-xylenes (i.e.,

BTEX). Quantitation was performed using a Hewlett-Packard Model 3396 Integrator and by an EZ Chrom data system (Scientific Software Inc., Pleasanton, CA).

The samples were analyzed in two batches and aliphatic C_6H_{14}-$C_{16}H_{34}$ (C_6-C_{16}) and aromatic (BTEX) HC levels were quantified as area-under-the-curve (AUC) for all 63 samples. Sample concentrations for AUC corresponding to 0.5 ppb were calculated, although for any individual sample, there was a 1% statistical chance that background could have been at the 1 ppb level. Conversion factors for transforming the EZ Chrom output from AUC to ppb of each BTEX analyte were derived from calibration samples for all study samples (n=63). These conversion factors demonstrated adequate linearity for the aromatic analytes; goodness of fit ranged between r^2=0.88 to 0.98 based on 10, 25 and 50 ppb calibration samples. The BTEX exposure variable examined as a continuous ppb variable and as a dichotomous variable above and below the median ppb value in statistical analyses. After publication of a report describing JP-8 volatile fraction "fingerprint compounds" (8), we derived ppb conversion factors for aliphatic HCs for a subset of the study samples (n=22). A standard gas was developed in the laboratory to create conversion factors for the aliphatic HCs; goodness of fit was adequate (r^2=0.87 to 0.99 at 10, 25 and 50 ppb) for all analytes except dodecane (C_{12}) (r^2=0.10) and tetradecane (C_{14}) (r^2=0.55). Therefore, aliphatic HC levels (except C_{12} and C_{14}) were converted to ppb for the second analysis batch of 22 samples. Only AUC measurements were used in the statistical analysis of aliphatic HCs for the 63 breath samples; the aliphatic HC parts ppb levels were used only to present range of exposure. The total AUC for aliphatic HCs was dichotomized for each of the two analysis batches (above or below the median AUC for each batch) and then

the two batches were combined into a single dichotomous aliphatic HC variable. Since C_6-C_{16} measurements were only available in ppb units for 22 participants, analysis with a combined breath exposure variable (aliphatic HCs + BTEX) was not explored.

For quality control, duplicate breath samples were collected in immediate succession from thirteen participants. These samples were analyzed and the duplicate measurements compared. Intra- and inter-subject sample coefficients of variation, respectively, for exhaled breath measurements in ppb were: 3.6 and 0.0% for benzene, 1.8 and 0.8% for toluene, 5.1 and 0.0% for ethyl-benzene, 3.5 and 0.0% for m,p-xylene, and 1.2 and 1.0% for o-xylene. The duplicate, end-of-shift breath sample levels from the 13 subjects were assigned to the same aliphatic HC and BTEX exposure groups (high/low) approximately 70% of the time, indicating relatively high comparability among samples.

STATISTICAL ANALYSIS:

Statistical analyses of the potential relationship between endocrine outcomes (preovulatory LH, follicular Pd3G, mid-luteal PD3G, and mid-luteal $E_1$3G) and exposure variables were conducted on two groups of participants: 1) the 100 subjects with endocrine and recorded diary data and 2) for 63 of the 100 subjects with breath analysis data. For the bivariate analysis of data from the women (n=100) who reported diary and baseline questionnaire information, exposure was defined using three reported exposure variables: 1) mean weekly hours reported as smelling or having skin contact with fuels; 2) mean weekly hours reported as smelling or having skin contact with solvents, and; 3) and job category (exposed vs. non-

exposed) based on self-report during the initial interview and review of job titles, job codes, and descriptions by AF industrial hygiene personnel. Variables used to characterize the internal dose of HCs measured in the 63 breath samples were aliphatic $C_6 - C_{16}$ (AUC) and BTEX (ppb), each dichotomized above and below their respective medians.

Square root transformation optimally transformed the hormonal outcomes. Bivariate associations between each of the four endocrine endpoints (transformed and non-transformed) and other potential covariates were examined. Those approaching marginal significance ($p<0.15$) were retained for regression models in subsequent analyses. Potential interactions between breath exposure variables and alcoholic beverages, and breath exposure variables and race did not approach significance. When inter-correlation between candidate covariates was present, or when two or more covariates represented the similar constructs, the variable with the most significant association with the outcome(s) was used.

Covariates retained for regression models containing *reported* hours of fuel or solvent exposure included: age at interview (years), body mass index (BMI = weight in kg / [height in m^2]), race (Caucasian, non-Caucasian), alcoholic beverages (number of drinks per day / kg body weight), coffee consumption (estimated mean mg of caffeine per day from coffee), caffeine consumption (estimated mean mg total caffeine in coffee, tea, and soda per day), running (mean miles run per day), sleep (mean hours per day), any episodes of illness and/or fever > 101° (yes/no), maximum weekly job strain score (high vs. low), and currently smoking cigarette (yes/no). Separate regression analyses were conducted for each *reported* exposure variable, and each exposure variable was forced to remain in the final model. Multiple regression analysis of

each endocrine outcome was conducted separately using backward stepwise elimination of covariates, with significant ($p \leq 0.05$) covariates retained in the final models.

Covariates retained for regression models containing *breath analyte* exposure variables dichotomized about the median for aliphatic ($C_6 - C_{16}$) and BTEX included: illness and/or fever > 101°, alcoholic beverages, and maximum job strain, race group, and age. Multivariate regression analysis of each endocrine outcome was conducted as described above, with both breath exposure variables forced to remain in the regression models.

RESULTS:

Demographic and Reproductive Characteristics:

Table 1 describes demographic and reproductive history characteristics for the 100 eligible participants who provided questionnaires, diaries and daily urine samples, and for the 63 subjects with breath sample data. In both groups, the average age of respondents was approximately 31 years. In both groups, respondents were predominantly Caucasian, military, married, had children, had attended some college, had annual household incomes of at least $30,000, and most had no history of irregular menses or dysmenorrhea within three months of the interview. Characteristics of the low *versus* high exposure groups for $C_6 - C_{16}$ and for BTEX also were similar, and none of these differences were statistically significant.

Internal Dose (Breath) Analysis:

Individual $C_6 - C_{16}$ and BTEX HCs measured in post-shift breath samples, grouped by

low (n=32) *versus* high (n=31) analysis category, are presented in Table 2. Mean internal doses for the high BTEX category were highest for m,p-xylene (37.3 ppb), followed by benzene (13.0 ppb), o-xylene (11.3 ppb), toluene (9.0 ppb) and ethyl-benzene (3.0 ppb). When high and low BTEX categories were combined, toluene was the most frequently detected among the BTEX analytes, present in 71.4% of all breath samples with levels ranging from below detection to 52.0 ppb. Xylenes were detectable in roughly half of all samples with m,p-xylene (57.1%) and o-xylene (47.6%), followed in frequency by benzene (30.2%) and ethylbenzene (22.2%).

Within the high $C_6 - C_{16}$ category (n=22), the mean internal dosage for decane (159.1 ppb) was highest, followed by hexane (51.4 ppb), heptane (35.4 ppb), undecane (28.7 ppb), nonane (4.5 ppb), and octane (0.6 ppb). Both decane and hexane were virtually ubiquitous, and heptane was present in most (63.6%) of the samples in the high and low $C_6 - C_{16}$ category groups. Many samples also contained octane, nonane and undecane in both high and low $C_6 - C_{16}$ category groups.

Endocrine Assessment:

Mean levels of the four study endocrine endpoints for all the 100 subjects who provided both diaries and daily hormonal data were similar between each of the reported exposure variables. Furthermore, there was no significant ($p \leq 0.05$) difference in endocrine levels between self-reported exposed *versus* non-exposed subjects when examined bivariately or in multi-variable regression models including potential confounders and covariates.

Table 3 presents mean urinary endocrine levels by low and high breath levels of $C_6 - C_{16}$

and BTEX, the primary exposure variables. Mean preovulatory LH levels were significantly lower in participants with high breath $C_6 - C_{16}$ (15.4 *vs.* 22.6 mIU LH / mg creatinine; p=0.01) and BTEX (15.8 *vs.* 22.0 mIU LH / mg creatinine; p=0.03) in the bivariate analysis. The difference between the high and low BTEX groups also approached bivariate significance for mid-luteal Pd3G (8.5 *vs.* 12.0 ug/mg creatinine; (p=0.06).

Each of the four endocrine outcomes were modeled in separate multiple regressions, and the results are presented in Table 4. To aid in interpretation, values reported for regression coefficients in Table 4 were obtained by applying the same models with non-transformed outcomes. High versus low exposure to $C_6 - C_{16}$ HCs was inversely related (β= - 7.34, p=0.007) to preovulatory LH after adjusted for age and exposure to BTEX. The association between LH and $C_6 - C_{16}$ category remained significant ($p \leq 0.013$) after Bonferroni correction for four tests. $C_6 - C_{16}$ and BTEX categories were not significantly associated with changes in mid-luteal E_13G, or with follicular or midluteal Pd3G levels.

Illness and/or fever > 101° was associated with elevated mid-luteal E_13G (β=8.93, p=0.01). Other potential covariates and confounders were not associated ($p \geq 0.05$) with any of the hormonal outcomes after adjustment. When analyzed as continuous variables, neither total BTEX nor toluene, a component of BTEX, were significantly ($p \leq 0.05$) associated with any of the hormonal levels. However, toluene approached significance (β=-0.03, p=0.058) with preovulatory LH in a model together with $C_6 - C_{16}$ (β=-0.77, p=0.01) and age (β=0.06, p=0.04).

DISCUSSION:

Four urinary endocrine endpoints (preovulatory LH, mid-luteal $E_1$3G, follicular PD3G, and mid-luteal PD3G) were selected based on heuristic evidence that they are jointly predictive of the probability of conception in women within a given ovulatory menstrual cycle (13). We found that preovulatory LH in urine was lower in healthy, reproductive aged women who had higher internal doses of aliphatic HCs in exhaled breath.

In an examination of ovulatory cycles, Baird et al. (13) reported that non-conceptive *versus* conceptive cycles had urinary preovulatory mean LH levels of 13.4 *versus* 15.3 mIU/mg creatinine, respectively. Our high *versus* low aliphatic HC exposure groups had urinary LH levels of 15.4 *versus* 22.6 mIU/mg creatinine, respectively. And while the same assay was used to measure LH in both of these studies, they are not directly comparable quantitatively. The urine samples from the current study contained 7% glycerol to preserve LH activity (17), while those from the Baird study (13) had been stored frozen for many years without preservative. So while qualitative comparisons are valid within the Baird study (13), the LH values in the Baird study would be expected to be lower than those in the present study.

The mechanism by which aliphatic HCs could lower LH levels is unknown. Luteinizing hormone levels could potentially be lowered by effects on the pituitary gland, hypothalamus, or extrahypothalamic central nervous system inputs. Evidence derived from animal experiments demonstrates that exposure to high doses of aromatic HCs alters levels of hypothalamic neurotransmitters, including noradrenaline, and dopamine (26, 27), which are involved in regulating pituitary hormone secretions. Andersson et al. (1980) reported that toluene-exposed

mice had increased hypothalamic noradrenaline and dopamine with a concomitant non-significant ($p>0.05$) decrease in LH secretion.

Few published studies have examined the effects of exposure to low levels of fuels and mixed solvents on the human neuroendocrine system, although neurological (9,10,28-30) and sensory-neural (31,32) effects of solvents have been documented at low and high analyte levels. Sub-clinical and clinical CNS effects have been reported to manifest at levels as low as .07 to 5 ppm (29). The only human studies of the effects of HCs on gonadotrophins, to our knowledge, were of toluene exposure (33-35). In the current study, breath toluene approached ($p=0.058$), but did not reach a significant inverse relationship with LH. However, significantly lowered LH levels have been reported among toluene-exposed male printers (33). This effect was reversed after a four-week non-working period after which blood toluene levels dropped and FSH and LH rebounded 37.5% and 29.9%, respectively (34). Results from these studies, mostly of men, are salient since LH controls the secretion of sex hormones in both genders. In women, LH is essential for ovulation and luteinization.

While the primary exposure variables for these analyses were breath levels of the aliphatic and BTEX HCs in fuels and solvents, a secondary source of exposure information was self-report in participants' daily diaries and baseline questionnaires. Reported hours of exposure were consistent with job categories, as women in fuel handling, flightline, and maintenance jobs reported more hours of exposure than women in "non-exposed" jobs. However, reported hours of exposure were similar for women with low and high levels of aliphatic and BTEX HCs in exhaled breath. One explanation is that, while hours of exposure were similar across exposed

and nonexposed job categories, the intensity of exposure was greater in exposed job categories. Accordingly, a higher percentage of women in exposed job categories were in the high breath aliphatic and BTEX groups. The discrepancy between reported and breath data could also be due, in part, to poor recall or directional recall bias. Underreporting of hours of exposure for women in contact with fuels at work could have resulted if workplace exposures were too low for subjects to perceive, or if they became desensitized to the odor. Conversely, low levels of exposure may have been common and perceptible, with resultant over- or underestimation of self-reported hours of exposure among those with higher fuel and solvent contact. Mean breath aliphatic and aromatic levels among self-reported non-exposed subjects in the current study were generally higher than for AF workers with solvent and fuel contact in other studies (8,11) and, therefore, may have been detectable by some participants. Median aliphatic HC levels among the women (n=22) who provided breath samples and reported low fuel exposure were similar to those reported in the general U.S. population (TEAM study) (36), except for decane, which was higher in our subgroup.

Gender differences in the effects of these hydrocarbons on the neuroendocrine system may be anticipated based on differences in the neuroendocrine axes and reproductive organs, as well as differences in metabolism, storage and excretion of lipophilic hydrocarbons (12). The focus of the current investigation was on hormones that affect female fertility. Future studies should examine both men and women. Solvent exposure has been reported to decrease sperm motility (37) and may increase the rate of sperm anomalies in men.

In conclusion, the internal dose of compounds in fuel is associated with reduced LH

levels prior to ovulation in reproductive aged women. Several other caveats must be considered with regard to interpretation of these results. It is likely that the aliphatic and aromatic compounds we chose to represent exposure are also markers of exposure to the complex mixture of other compounds found in fuels, including additives and byproducts of combustion. The design was cross-sectional, and exposures or endocrine measurements assessed during the study cycle may or may not represent past exposure and/or endocrine levels. However, if HC exposures chronically alter LH levels, this effect could impact LH-dependent processes and thereby compromise reproduction.

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Table 1: Demographic & Reproductive Characteristics of Subjects With Endocrine Data (n=100), and With Endocrine and Breath Data (n=63), by Aliphatic HC Group:

		Endocrine & Breath Data (n=63):		
Participant Characteristic:	**Endocrine Data (n=100):**	**Low Aliphatic:‡**	**High Aliphatic:‡**	**Combined:**
Mean age at interview (years):	30.9	30.5	31.7	31.1
Mean age at menarche (years):	2.7	12.8	12.8	12.8
Race (%):				
Caucasian:	64.0	80.7	75.0	77.8
African-American:	30.0	16.1	21.9	19.0
Hispanic:	4.0	0.0	3.1	1.6
Other:	2.0	3.2	0.0	1.6
Education (%):				
HS* &/or technical training:	20.0	19.4	18.8	19.1
Some college or associates degree:	61.0	64.5	59.4	61.9
Four year degree or more:	19.0	16.1	21.9	19.1
Family Income (%):†				
<$15,000:	9.1	12.9	0.0	6.4
$15,000 < $30.000:	32.3	35.5	34.4	34.9
≥$30.000:	58.6	51.6	65.6	58.7
Personnel Status (% Military):	84.0	87.1	71.9	79.4
Marital Status (%):†				
Currently married or partner:	61.6	54.8	68.8	61.9
Widowed, divorced , or separated:	16.2	12.9	15.3	14.3
Never married:	23.2	32.3	15.6	23.8
One or more children (%):	63.0	64.5	75.0	69.8
Irregular menses past 3 months (%):	11.0	16.1	6.3	11.1
Dysmenorrhea past 3 months (%):	30.0	22.6	34.4	28.6

* HS = High School

† One subject with missing information for income and marital status (n=99)

‡ No significant differences ($p \leq 0.05$) between above groups by low and high fuels category

Table 2: Breath Levels of Aromatic and Aliphatic HCs by Analyte and Exposure Group:*

BTEX Analyte:	Low BTEX: (n=32):	High BTEX: (n=31):	Total BTEX (n=63):
Benzene (C_6H_6):	0.5 ± 1.6 [ND]	13.0 ± 27.5 [ND]	6.6 ± 20.2 [ND]
	(ND – 8.6)	(ND – 97.5)	(ND – 97.5)
	18.8%	41.9%	30.2%
Toluene ($C_6H_5CH_3$):	1.3 ± 2.2 [0.1]	9.0 ± 12.3 [5.1]	5.1 ± 9.5 [1.5]
	(ND – 7.5)	(ND – 52.0)	(ND – 52.0)
	50.0%	93.5%	71.4%
Ethyl-benzene:	1.0 ± 0.5 [ND]	3.0 ± 6.9 [ND]	1.5 ± 5.0 [ND]
($C_6H_5C_2H_5$)	(ND – 2.7)	(ND – 35.7)	(ND – 35.7)
	9.4%	35.5%	22.2%
M,p-xylene:	0.8 ± 1.2 [ND]	37.3 ± 85.6 [3.8]	18.7 ± 62.3 [0.8]
$C_6H_4(CH_3(2))$	(ND – 4.4)	(ND – 400.9)	(ND – 400.9)
	40.6%	74.2%	57.1%
O-xylene:	1.0 ± 2.0 [ND]	11.3 ± 15.0 [7.2]	6.1 ± 11.7 [ND]
$C_6H_4(CH_3(2))$	(ND – 6.9)	(ND – 67.3)	(ND-67.3)
	28.1%	67.7%	47.6%
Total BTEX:	3.8 ± 3.8 [2.9]	73.5 ± 86.2 [32.4]	38.1 ± 69.6 [11.7]
C_6H_6-$C_6H_4(CH_3(2))$	(ND - 11.7)	(11.8 – 415.1)	(ND-415.1)
	81.3%	100.0%	90.5%
Aliphatic Analyte:	**Low Aliphatic (n=11):**	**High Aliphatic (n=11):**	**Total Aliph.(n=22):**
Hexane (C_6H_{14}):	17.6 ± 8.6 [19.6]	51.4 ± 62.9 [35.9]	34.5 ± 47.1 [25.9]
	(ND – 28.5)	(11.7 – 238.7)	(ND-238.7)
	90.9%	100.0%	95.4%
Heptane (C_7H_{16}):	3.7 ± 9.8 [0.3]	35.4 ± 75.3 [4.4]	19.5 ± 54.9 [0.6]
	(ND – 33.0)	(ND – 248.7)	(ND – 248.7)
	63.6%	63.6%	63.6%
Octane (C_8H_{18}):	0.2 ± 0.4 [ND]	0.6 ± 1.8 [ND]	0.4 ± 1.3 [ND]
	(ND – 1.2)	(ND-6.0)	(ND – 6.0)
	18.2%	27.3%	22.7%
Nonane (C_9H_{20}):	5.3 ± 10.9 [ND]	4.5 ± 9.1 [ND]	4.9 ± 9.8 [ND]
	(ND – 29.2)	(ND – 27.0)	(ND – 29.2)
	36.4%	45.4%	40.9%
Decane ($C_{10}H_{22}$):	34.6 ± 23.5 [28.4]	159.1 ± 237.4 [14.8]	96.9 ± 176.6 [23.9]
	(12.8 – 76.8)	(2.8 – 659.7)	(2.8 – 659.7)
	100.0%	100.0%	100.0%
Undecane ($C_{11}H_{24}$):	8.8 ± 14.6 [ND]	28.7 ± 68.9 [ND]	18.7 ± 49.7 [ND]
	(ND – 45.0)	(ND – 226.7)	(ND – 226.7)
	36.4%	36.4%	36.4%
Total C_6H_{16} to $C_{11}H_{24}$:	70.1 ± 31.3 [63.4]	279.6 ± 276.2 [170.8]	174.9 ± 219.8 [66.6]
	(28.6 – 128.0)	(39.5 – 765.1)	(28.6 – 765.1)
	100.0 %	100.0 %	100%

* Mean ± standard deviation in ppb; [ ]=median in ppb; ( ) = range; % = percentage of samples with detectable levels of each analyte within each breath group; ND=non-detectable.

Table 3: Unadjusted Endocrine Outcomes: Means by Breath Aliphatic & BTEX* HC Exposure Groups:

Endocrine Outcome:	Exposure:		Level (Mean):	Standard Deviation:	Range:
Preovulatory LH (mIU/mg Cr.):	Aliphatics:†	Low:	22.6	12.0	4.8-55.3
		High:	15.4	8.2	3.0-39.0
	BTEX: ‡	Low:	22.0	12.2	4.3-55.3
		High:	15.8	8.2	3.0-38.7
Follicular Pd3G (ug/mg Cr):	Aliphatics:	Low:	1.2	0.7	0.01-2.7
		High:	1.2	0.8	0.04-3.6
	BTEX:	Low:	1.2	0.7	0.3-3.6
		High:	1.1	0.8	0.01-3.5
Mid-luteal Pd3G (ug/mg Cr):	Aliphatics:	Low:	10.0	6.3	0.1-24.5
		High:	10.5	7.4	2.2-37.9
	BTEX:§	Low:	12.0	8.1	0.3-37.9
		High:	8.5	4.9	0.1-18.8
Mid-luteal $E_1$3G (ng/mg Cr):	Aliphatics:	Low:	27.2	13.6	9.5-82.5
		High:	24.9	13.1	2.1-58.8
	BTEX:	Low:	27.3	13.4	11.7-82.5
		High:	24.8	13.3	2.1-58.8

* BTEX=benzene + toluene + ethyl-benzene + m,p,o-xyxlene
† Significance level: (p=0.01) between high and low categories in unadjusted, bivariate analysis
‡ Significance level: (p=0.03) between high and low categories in unadjusted, bivariate analysis
§ Significance level: (p=0.06) between high and low categories in unadjusted bivariate analysis

Table 4: Results of Regression of Each of the Endocrine Outcomes:

		Covariates:[*,†,‡]			
Endocrine Outcomes:	**Model:[§,¶]**	**Aliphatics:**	**BTEX:**	**Age:**	**Illness /Fever:**
Preovulatory LH (n=58):	F=5.28 p=0.003	β=-7.34 p= 0.007	β=-4.61 p= 0.10	β=0.49 p=0.05	-
Follicular Pd3G (n=62):	F=0.46 p=0.64	β= 0.04 p= 0.89	β=-0.10 p= 0.34	-	-
Mid-Luteal Pd3G (n=58):	F=1.68 p=0.20	β= 1.04 p= 0.51	β=-3.59 p= 0.08	-	-
Mid-luteal $E_1$3G (n=58):	F=2.79 p=0.05	β=-2.79 p= 0.34	β=-2.73 p= 0.32	-	β=8.93 p=0.01

* BTEX=benzene + toluene + ethyl-benzene + m,p,o-xyxlene

† Aliphatics and BTEX retained in each model; other covariates retained only if $p \leq 0.05$.

‡ P-values generated from transformed models, betas (β) from untransformed models.

§ Dash indicates non-significance ($p>0.05$)

¶ Adjusted model R2: LH=0.18, E13G=0.09; follicular Pd3G=0.00; mid-luteal Pd3G=0.02.

PAPER THREE: DIFFERENCES IN URINARY REPRODUCTIVE HORMONE LEVELS BETWEEN AFRICAN AMERICAN AND CAUCASIAN WOMEN.

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Background:

Normative values for reproductive hormones are generally used as guides for clinical assessments. It is important, therefore, to determine if normative values based on samples from predominantly Caucasian populations are appropriate when applied as norms for patients of other races. Alternatively, stratification of normal ranges by race may be indicated for some reproductive hormones. Serum progesterone levels have been shown to be similar among premenopausal African American and Caucasian women in several smaller studies, (1,2) while estrogens (estrone and estradiol) were similar in most, (1-3) but not all investigations (4). We are unaware of any comparison of mean serum LH levels and FSH levels between reproductive-aged African American and Caucasian women.

Measuring hormones in urine is a non-invasive method to collect daily samples throughout the menstrual cycle to evaluate the status of menstrual cycle function. The purpose of this report is to compare urinary levels of reproductive hormones between African American (n=33) and Caucasian (n=65) women of reproductive age. We assessed two gonadotrophins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), which are secreted by the pituitary gland and which control ovarian function, including follicular and luteal development and ovulation. We also

evaluated levels of estrone 3-glucuronide ($E_1$3G) and pregnanediol 3-glucuronide (Pd3G), the two primary urinary metabolites of estradiol and progesterone, respectively. $E_1$3G and Pd3G measurements permit follicular development and luteal function to be monitored during the menstrual cycle. Both urinary metabolites are well correlated with circulating levels of their parent hormones (5).

MATERIALS AND METHODS:

Population:

Subjects for the current study are a subset of participants from a larger DoD-funded study of Air Force (AF) women. Analysis results of endocrine effects of fuel and solvent exposure (6), and stress and menstrual disorders (7) are published elsewhere. Subjects in this subset contributed daily diary information and urine samples from one or more menstrual cycles. There were 335 preliminarily eligible civilian and active duty military women employed at ten United States AF bases who were contacted during recruitment screening. Of these women, 170 (51%) consented to participate, to maintain daily diaries and collect daily for one menstrual cycle, and were confirmed to be eligible during the baseline interview. Eligibility criteria included: being under age 42 years; not on hormonal medications including oral contraceptives or hormone replacement; no surgery on their reproductive organs except for tubal ligation; not using an intrauterine devises; having not been pregnant within six months of the baseline interview; having not been diagnosed with chronic pelvic inflammatory disease, endometriosis, vaginal, cervical, uterine, or ovarian cancer, systemic lupus erythematosus, hypopituitarism, Cushing's

syndrome, sarcoidosis, pituitary tumor, acute hepatitis, HIV or AIDS, cirrhosis of the liver, hypothyroidism, hyperthyroidism, multiple sclerosis, tuberculosis, or diabetes. Though non-smokers were initially targeted, a small number of smokers (n=19) were also included. Of 170 eligible women who completed the baseline questionnaire, 53 did not return either diaries and/or urine samples, and 9 returned samples that were inadequate for measurement of any of the endocrine endpoints in the current study. In addition, four participants were excluded retroactively due to conceptions (two), use of oral contraceptive (one), or ongoing, symptomatic endometriosis (one) during sample collection. Six of the remaining 104 participants of Hispanic (n=5) or Native American (n=1) ancestry with no African American or Caucasian admixture were excluded. Caucasian and African American participants (n=98) were included. Monetary reimbursement for home data collection activities was provided: $50 for all daily diaries, $25 for all urine samples.

Potential participants were recruited for intake interviews by phone and in-person at each USAF base. During the interview, study subject procedures, eligibility criteria, and the voluntary nature of participation, were discussed, then informed consent was obtained. The baseline questionnaire was then administered to collect socioeconomic, reproductive health, lifestyle, and work history information. Instructions were given for collecting daily urine samples and completing daily diaries. Weight and height were also measured.

Daily Diaries:

Subjects were asked to complete daily diaries beginning on the day following the initial interview through the last day of their second menstrual period. The diary obtained menstrual, psychosocial, lifestyle, work, chemical and physical exposures, and sample collection information. Diary items used for preliminary, bivariate analyses included daily menstrual bleeding/spotting (yes/no), and potential covariates and confounders, including: number of cigarettes smoked, hours slept, hours of side-stream smoke exposure, illness or fever > 101° (yes/no), ounces of caffeinated drinks, number of alcoholic drinks, number of hours and shift(s) worked, hours that fuel, solvents or pesticides were smelled, hours of skin contact with fuel, solvents or pesticides, number of miles run, number of miles walked, duration of light-to-moderate and heavy physical activity (minutes at home and work), and weekly job strain questions (true/false).

Job strain was measured using an adaptation of the Job Content Questionnaire developed by Karasek (8,9). Diaries were mailed to investigators upon completion.

Endocrine Data Collection and Analyses:

Daily first morning urine samples were collected concurrently with the diaries. Participants stored the samples in home freezers; 7% glycerol was added to the samples to prevent loss of hormonal activity. Participants shipped frozen samples with freezer packs to the National Institute for Occupational Safety and Health laboratory by next-day courier. Samples

were stored in the laboratory at –80° C. Menses dates were derived from the participants' daily records of vaginal bleeding using an algorithm defined as follows (10): the first day of the menstrual cycle began on the first of 2 consecutive days of bleeding, only one of which was spotting. Menstruation was preceded and followed by ≥ 3 consecutive days of non-bleeding or spotting. After day 2 of menstruation, < 2 day interruptions in bleeding (non-bleeding or spotting) were counted together with bleeding days as part of the menstrual period. Retrospectively reported menses were accepted up to 14 days after data collection for participants with missing diary menstrual bleeding entries. Reported menses dates and follicular, ovulatory and luteal-phase LH, FSH, $E_1$3G, Pd3G measurements were used to derive 17 endocrine endpoints by applying algorithms (see Appendix 13).

Hormonal Measures:

Urinary LH and FSH were assayed in duplicate using non-competitive, two-site time-resolved immunofluorometric assays (11,12). Estrone 3-glucuronide ($E_1$3G) and pregnanediol 3-glucuronide (Pd3G) were assayed in triplicate using competitive, double-antibody time-resolved fluoroimmunoassays (13). Creatinine was measured spectrophotometrically (14), and all endocrine values were divided by the respective sample's creatinine concentration to adjust for urine dilution (11). Coefficients of variation (CVs) for urinary endocrine measurements compared favorably with CVs reported by the manufacturers. Intra- and inter-assay coefficients were, respectively: 6.2% & 4.6% for LH; 2.8% and 3.5% for FSH; 15.4% and 10.1% for $E_1$3G; 11.6% and 8.4% for Pd3G; 0.97% and 3.4% for creatinine. All samples for each subject were

measured in the same assay. The algorithms by which hormonal variables were defined and the units of measurement for each hormone are described in the Appendix.

Internal Dose Measures:

The importance of controlling for the potential effects of fuel and solvent exposure in the current study was evident based on prior significant findings of an inverse relationship between aliphatic hydrocarbon exposure and LH levels among a subgroup of 63 participants in the current study (6). Previously, breath measurement of internal dose of solvents was found by our laboratory to be more sensitive than measurements using blood or urine when levels of exposure were relatively low (15). Therefore, exhaled breath samples were used to estimate internal dose of fuel and solvent components, including BTEX (total benzene, ethyl-benzene, toluene, & m,p,o-xylenes) and aliphatic hydrocarbons (total C6-C16). Breath samples, available for 63 of 104 subjects, were collected after two to five workdays approximately one to two hours (mean=1.2 hours) after leaving the worksite. Cite article

STATISTICAL METHODS:

The 98 subjects included in this study were categorized as either African American or Caucasian. Racial admixture was common, however, in both the African American (64% admixed) and Caucasian (26% admixed) analysis groups. Racial categories, based on the reported race(s) of subjects' great-grandparents, were developed to define race for women of mixed ancestry. Women with admixed lineages were categorized as Caucasian if they reported

some Caucasian and no African American ancestry, and as African American if they reported some African American ancestry. In almost every case, these assigned racial categories matched the racial category reported by the admixed women.

Distributions of the endocrine endpoints were reviewed and, if needed, transformed to normalize the distribution of continuous data. Bivariate relationships between transformed endocrine endpoints, racial group, and other potential covariates were examined using SAS (SAS Institute, Inc.). Correlations (Pearson and Spearman) , t-tests, or Wilcoxon Rank Sum tests were used depending on the type and distribution of the outcomes (endocrine endpoints) and candidate covariates. Bivariate relationships were also assessed between covariates and race. Then, possible confounders and other potential covariates that approached bivariate significance ($p<0.15$) with a given endpoint were entered into the full regression model for that endpoint. Race was entered into each regression model, regardless of its bivariate significance. Based on this strategy, the following covariates were entered into regression models for one or more endpoints: racial group (Caucasian=0, African American=1); age at interview (continuous years, and age tertile); age at first menses; number of reported pregnancies (gravida) and ever pregnant (no=0, yes=1); body mass index at interview (BMI = weight in kilograms/ height in meters ^{2}); military status (civilian=0, military=1); annual household income group ($\geq$\$30,000=0, <\$30,000=1); maximum job strain (maximum cumulative score over a week for 12 questionnaire items) (8,9); major life events (no life events=0, life events=1) (16); non-work stressors, including accidental injury or primary responsibility for child care (0, $\geq$1); and, average number of: alcoholic drinks per day, caffeine in drinks per day in milligrams (mg), hours of sleep per day,

proportion of ill or febrile days, proportion of days exposed to "very cold" temperature, hours of moderate to heavy activity per day, miles running or walking per day; cigarettes or cigars per day, hours of second-hand cigarette smoke exposure per day, reported hours of fuel exposure per week (smelled or skin contact), and reported hours of solvent exposure per week (smelled or had skin contact). High vs. low breath levels of aliphatic hydrocarbons and BTEX (below median=0, above median=1), and total BTEX in ppb were also examined among the subset for whom these were available (n=63). The interactions of racial group with breath aliphatic HCs, BTEX and alcohol were also examined. Multiple regression analysis of each endocrine outcome was conducted separately by backward stepwise elimination of covariates ($p>0.05$) using SAS; racial group was always retained.

RESULTS:

Demographics:

Demographic differences between the racial groups in crude bivariate analyses included a higher proportion of single ($p=0.05$) and childless ($p=0.02$) African American versus Caucasian women (Table 1). African American women were slightly younger at menarche ($p=0.04$). Household incomes were also lower ($p=0.004$) among African Americans, however, this difference was only significant among married women ($p=0.01$) in stratified analysis. There was no significant difference ($p\leq0.05$) in mean BMI between the racial groups when all ages were combined; however, young African American participants aged 18 to 29 years were leaner ($p=0.03$) than their Caucasian counterparts (21.6 versus 23.4, respectively). Among the women

in the 30 to 41 year age group, there was a non-significant ($p>0.05$) trend for this BMI relationship to be reversed (28.7 versus 25.7, respectively). The two racial groups were similar in age, and the percentages of military versus civilian women in each group were similar. Occupational and non-occupational exposure variables which differed significantly ($p\leq0.05$) between the racial groups in the crude bivariate analyses are shown in Table 2.

Bivariate Analyses:

Unadjusted bivariate significance levels were examined for all candidate covariates, and are presented for racial group for the endocrine endpoints in Table 3. Relative to Caucasians, African American women had significantly ($p\leq0.05$) lower follicular LH:FSH ratios, slope of periovulatory Pd3G, and mid-luteal Pd3G levels. Anovulatory cycles were detected in a similar proportion of African Americans and Caucasians (7.7% and 7.9%, respectively).

To explore our hypothesis that women with varying degrees of admixed ancestry will have intermediate hormone levels, participants were separated into three groups by proportion of reported African American ancestry (0.0 - 33.3%, 33.4 - 66.6%, 66.7 – 100%) and mean hormone levels were compared bivariately. Inverse relationships ($p\leq0.05$) were found between the tertile for proportion of African American ancestry and the ratio of LH:FSH (1.0, 0.8, 0.5), slope of periovulatory Pd3G (1.0, 0.7, 0.5), mid-luteal Pd3G (11.3, 8.8, 7.1), follicular phase Pd3G (1.2, 1.0, 0.8), follicular LH (6.5, 5.0, 3.9), and early follicular Pd3G (1.6, 1.5, 1.1), respectively.

Multivariable Regression:

Final multivariable regression models of the relationships between significant ($p \leq 0.05$) covariates and individual gonadotrophin and gonadal hormone endpoints (transformed, if needed) with covariate significance levels are described in Table 4. All final models were adjusted for African American vs. Caucasian racial group. Potential covariates (bivariate p-values ≤ 0.15) that were entered in the full model for at least one endpoint, but did not reach significance ($p > 0.05$) in any final model, are footnoted separately for gonadotrophins and gonadal hormones. The practical significance of a given covariate in the final models may be interpreted, in part, by observing the estimated change in each endpoint accompanying a one-unit change in a covariate, controlling for other covariates in the model i.e., the regression coefficient (B). Values for the regression coefficients were derived from the final models by substituting non-transformed endpoints into the models in order to improve their interpretability (see Table 4). Relative to Caucasian women, African American women had significantly ($p \leq 0.05$) lower follicular phase ratios of LH:FSH (mean 7.0 vs. 1.0; B=-0.23, p=0.03), follicular phase Pd3G levels (mean 1.0 vs. 1.2 μg/mg Cr; B=-0.33, p=0.05) and slope of periovulatory Pd3G (mean 0.5 vs. 1.0 μg/mg Cr; B=-0.48, p=0.02).

Multiple significant ($p \leq 0.05$) relationships between endocrine endpoints and other covariates were also found and are described in Table 4. Breath level of BTEX reach significance (with mid-follicular $E_1$3G) after downward adjustment of the alpha level to correct for multiple testing (α of 0.05 / 17 tests = α of 0.0029 / test), but the magnitude of change in that hormone level was very slight (B=-0.04). Significant ($p \leq 0.05$) covariates that produced

relatively large shifts in the values of the endocrine endpoint(s) included military membership, which had a substantial inverse relationship to the rise in FSH before menses (B=-0.48, p=0.01). Hours of sleep per day was directly related to the slope of $E_1$3G:Pd3G (B=17.82, p=0.04), and mid-luteal Pd3G (B=2.00, p=0.03). In addition, breath aliphatic hydrocarbons at the median had a large effect on preovulatory LH levels (B=-8.003, p=0.003) and a noteworthy effect on mid-luteal FSH (B=-0.89, p=0.01). Other covariates were also significant for some endpoints in Table 4, but the estimates of effect associated with these variables corresponded to smaller shifts in endpoint values.

DISCUSSION:

Few studies have compared sex steroid hormone levels between African American and Caucasian women and, to our knowledge, an analysis of differences in levels of the gonadotrophins, LH and FSH, has not been published. In contrast to our findings of a lower follicular phase urinary LH:FSH ratio among African American women, Kitabchi (17) and coworkers (1999) reported no significant difference in the ratio of serum LH:FSH levels between the two groups. However, their negative study was small (n=14 per group), and the study population was obese women. Obesity has been linked to decreased LH:FSH ratios by others (18), which may explain the difference between Kitabchi's results and ours. While, to our knowledge, racial differences in the ratio of serum hormone: urinary metabolite levels for either LH or FSH have not been demonstrated, such potential differences could also account for the inconsistency between studies. Two other small studies (n=18 - 26) (1,2) also found no

significant racial differences in follicular and luteal phase serum progesterone levels. In contrast, in our study, African Americans had significantly lower follicular phase Pd3G levels, and slope of periovulatory Pd3G. We detected no significant racial difference in follicular or luteal phase urinary $E_1$3G measurements, which is consistent with equivalent circulating estradiol levels among the races in several other studies (1-3), but not all other investigations (4). Race was not significantly associated with the other endocrine outcomes in adjusted analyses.

We also examined interactions between racial group and alcoholic drinks per day, as racial differences in alcohol dehydrogenase associated metabolism have been documented (19). Significant race x alcohol interactions were seen for three $E_1$3G endpoints. The direction of the change in $E_1$3G with increased alcohol was inconsistent for the three $E_1$3G endpoints. Neither race, nor its interactions, however, remained significant for any of the outcomes after stringent downward adjustment of the alpha level to correct for multiple testing.

We speculated that the endocrine endpoints would potentially vary between racial groups due to endogenous factors, such as potential differences in hormone metabolism, and due to environmental factors, such as cultural and lifestyle differences. Accordingly, environmental covariates were also significantly ($p \leq 0.05$) associated with endocrine outcomes by regression analyses and produced substantial shifts in endocrine values (Table 4). Non-work stressors were associated with elevated mid-luteal FSH. Having high maximum job strain was also associated with a subtle increase in mid-luteal FSH, as well as slight decreases in mid-follicular $E_1$3G and 3-day periovulatory $E_1$3G. Another psycho-social stress index, the occurrence a major life event, was not associated with any endocrine changes in our adjusted analyses. Other relatively large

differences in endocrine values observed for work-related covariates included lower values for the FSH rise before menses among military vs. civilian women, and lower preovulatory LH levels (6) and mid-luteal FSH levels among women with higher breath levels of the aliphatic hydrocarbons found in fuels. The positive relationship between the amount of alcohol consumed and LH surge peak is consistent with findings from studies of alcohol's acute, but not chronic effects (20).

Although civilians were included in the study, subjects were predominantly military personnel, and so there are caveats to be considered before generalizing our findings to other populations. For instance, fewer study women (23.5%) were overweight (defined as BMI $\geq$27.3) than in the general population (21). And in contrast with other investigations (3,22) young African American participants were slightly leaner (mean BMI=21.6) than their Caucasian counterparts (mean BMI =23.4). Almost equal percentages of African Americans (77.8%) and Caucasians (86.2%) reported some exposure to side-stream cigarette smoke at work or at home. The low number of smokers, compared to those exposed to passive smoke may offer one explanation as to why passive smoking, but not mainstream smoke exposure, reached significance with some endocrine endpoints. Patterns of caffeine (mg. per day) and alcohol (drinks per week) consumption among our participants overall were similar to those reported elsewhere for reproductive age working women (23), and less consumption of both was reported by African Americans than Caucasians. Use of daily diary data to construct the majority of reported covariates should have reduced memory errors. Subjects were aware the study was of reproductive health, and so the potential for self-selection due to menstrual symptoms or fertility

problems was present. Reanalysis excluding women who reported irregular menses, or infertility, however, did not substantially alter the study findings. Thus, it appears that potential self-selection based on these conditions was not a major factor. Multiple statistical tests were performed to explore endocrine endpoints that may differ between subgroups. Therefore, we recommend that our significant results be interpreted cautiously as preliminary evidence of associations.

In summary, we have described endocrine endpoints in a population of healthy, working, reproductive age African American and Caucasian women. The data were derived from daily urine samples, a method that is more acceptable and feasible than collecting blood, especially in population studies. We also conducted hypothesis generating, adjusted analyses of racial differences between the groups, and found African Americans to have lower follicular phase LH:FSH ratios, follicular phase Pd3G levels, and rate of peri-ovulatory Pd3G rise compared to Caucasians. These findings of racial differences are exploratory and await confirmation in future studies to determine if they have clinical significance.

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Table 1: Selected Characteristics of Participants by Racial Category:

	African Americans: (n=33):	Caucasians: (n=65):
Participant Characteristics:		
Education:		
GED, HS graduate, or HS & tech. training:	8 (24.2%)	12 (18.5)
Some college or associates degree:	20 (60.6%)	39 (60.0%)
Four year degree or more:	5 (15.2%)	14 (21.5%)
Family Income:* †		
<$15,000:	6 (18.8%)	3 (4.6%)
$15,000<$30.000:	14 (43.8%)	19 (29.2%)
≥$30.000:	12 (37.5%)	43 (66.2%)
% in Military:	28 (84.9%)	55 (84.6%)
Marital Status (%):*†		
Never Married:	12 (37.5%)	11(16.9%)
Currently married or partner:	14 (43.8%)	44 (67.7%)
Widowed, divorced, or separated:	6 (18.8%)	10 (15.4%)
One or more children (%):†	15 (45.4%)	45 (69.2%)
Mean number of pregnancies: ‡	1.2 (SD=1.6)	1.6 (SD=1.3)
Mean age at interview (years):	29.5 (SD=7.3)	31.4 (SD=5.7)
Mean age at menarche (years): †	12.3 (SD=1.4)	12.9 (SD=1.6)
Mean weight (pounds):	150.5 (SD=38.9)	150.0 (SD=21.4)
(kilograms):	68.3 (SD=17.6)	68.1 (SD=9.7)
Mean BMI: Overall:	25.5 (SD=6.8)	24.8 (SD=3.5)
Age < 30: †	21.6 (SD=2.7)	23.4 (SD=3.1)
Age ≥ 30:	28.7 (SD=7.5)	25.7 (SD=3.4)

*** = One observation missing**

† = Significant ($p \leq 0.05$) difference between African Americans and Caucasians in bivariate analysis

‡ SD = Standard deviation

Table 2: Environmental and Occupational Exposures Associated with Racial Category by Bivariate Analysis:

	African Americans (n=33) :	Caucasians (n=65) :	P-value:
Mean alcohol intake (# drinks/day):	**0.2 (SD=0.3)**	**0.5 (SD=0.8)**	**0.005**
Mean caffeinated beverage intake (mg/day):* †	**63.0 (SD=48.1)**	**124.8 (SD=112.7)**	**0.009**
Cigarette smokers (%):	**3.0**	**27.7**	**0.004**
Breath BTEX above the median (%):‡	**76.9**	**40.8**	**0.02**
Reported non-work stressors(%):	**42.2**	**64.6**	**0.04**

* **Total intake of caffeine in coffee (~ 10.01 mg/oz) , tea (~ 4.29 mg/oz), and soda (~ 3.50 mg/oz).**

† **SD = Standard deviation**

‡ **BTEX = total benzene, toluene, ethyl-benzene, m,p,o-xylenes in exhaled breath.**

Table 3: Unadjusted Means, Standard Deviations, and Ranges of Endocrine Endpoints by Race:

	African Americans (n=33):			Caucasians (n=65):		
Gonadotrophin Endpoints						
	Mean:	**SD:**	**(Range):**	**Mean:**	**SD:**	**(Range):**
Follicular LH:	4.8	2.2	(1.0 – 9.1)	6.5	4.5	(2.1– 29.6)
Preovulatory LH:	15.3	9.8	(2.4 – 35.8)	19.1	11.7	(4.0 – 55.4)
Level of LH surge peak:	42.8	19.6	(14.5 – 88.7)	46.2	20.5	(10.0 – 101.6)
Early follicular FSH:	6.5	2.8	(2.2 – 12.8)	7.0	3.8	(0.7 – 18.2)
Follicular LH:FSH ratio: †	0.7	0.4	(0.1 – 2.3)	1.0	0.6	(0.3 – 3.2)
Mid-luteal FSH:	3.3	1.7	(1.5 – 8.4)	3.5	2.3	(1.1 – 14.0)
FSH rise before menses:	0.4	0.7	(-1.0 – 2.4)	0.4	0.6	(-2.0 – 2.1)
Gonadal Hormone Endpoints						
Early follicular $E_1$3G:	13.5	8.6	(5.3 – 38.8)	11.9	9.4	(3.2 – 77.4)
Mid-follicular $E_1$3G:	16.9	6.7	(6.2 – 33.9)	17.9	11.0	(3.7 – 86.9)
Periovulatory $E_1$3G peak:	46.1	23.1	(15.3 – 112.8)	42.7	17.8	(6.8 – 92.5)
Mid-luteal $E_1$3G:	30.8	21.9	(2.1 – 89.1)	26.0	11.0	(7.5 – 58.8)
Early follicular Pd3G:	1.4	1.0	(0.4 – 4.6)	1.6	0.9	(0.2 – 4.1)
Follicular Pd3G:	1.0	0.5	(0.2 – 2.7)	1.2	0.8	(0.01 – 3.8)
Slope of periovulatory Pd3G:†	0.5	0.7	(-0.1 – 3.6)	1.0	1.2	(-0.0 – 6.8)
$E_1$3G:Pd3G on the DLT:	35.3	25.3	(1.3 – 96.2)	29.8	32.6	(4.3 – 223.7)
Slope of $E_1$3G: Pd3G:	-37.4	89.9	(-472.1-5.5)	-21.1	44.2	(-295.9- -2.3)
Mid-luteal Pd3G: †	8.0	5.1	(2.9 – 26.9)	11.5	7.3	(0.1 – 37.9)

* LH and FSH levels in mIU/mg Cr; E13G levels in ng/mg Cr; Pd3G levels in µg/mg Cr.

† Significant difference ($p \leq 0.05$) between Caucasians and African Americans in bivariate analysis using t-tests; transformations applied to non-normal endpoint distributions.

Table 4: Final Adjusted Regression Models of Endocrine Endpoints & Covariates:*

Gonadotrophin Endpoints:	Significant Covariates:	Beta (p-value):
Follicular LH	Hours of passive smoke / day	0.37 (0.02)
Preovulatory LH	Age	0.58 (0.02)
	Breath aliphatic HCs at median	-8.09 (0.003)
Level of LH surge peak	Alcohol / day	7.06 (0.04)
Follicular LH:FSH	Racial group	-0.23 (0.03)
	Hours of passive smoke / day	0.04 (0.05)
	Average activity / day	- 0.04 (0.02)
FSH rise before menses	Body mass index	- 0.04 (0.02)
	Military status	-0.48 (0.01)
Mid-luteal FSH	Age at first menses	0.36 (0.05)
	Breath aliphatic HCs at median	- 0.89 (0.01)
	Maximum job strain	0.17 (0.03)
	Non-work stressors	1.39 (0.03)
Gonadal Hormone Endpoints:		
Early follicular $E_1$3G	Racial group x alcohol/day	-1.36 (0.03)
	Racial group x breath aliphatic HCs	-0.97 (0.001)
Mid-follicular $E_1$3G	Breath BTEX in ppb	-0.04 (0.0006)
	Maximum job strain	-0.60 (0.02)
3-day periovulatory $E_1$3G	Racial group x alcohol/day	2.52 (0.004)
Mid-luteal $E_1$3G	Racial group x alcohol/day	2.22 (0.006)
Follicular Pd3G	Racial group	-0.33 (0.05)
	Age	-0.02 (0.03)
Slope of periovulatory Pd3G:	Racial group	-0.48 (0.02)
E13G:Pd3G on the DLT	Age	1.08 (0.01)
Slope of $E_1$3G:Pd3G	Hours of sleep/day	17.82 (0.04)
Mid-luteal Pd3G	Number of pregnancies	1.05 (0.04)
	Miles ran & walked / day	0.84 (0.02)
	Hours of sleep / day	2.00 (0.03)

* Notes regarding above regression analyses:

- Final regression results for each endpoint were adjusted for race and listed (significant at $p \leq 0.05$) covariates
- No significant predictors in final models for early follicular FSH, & early follicular Pd3G

A Comparison of Salivary and Urinary Progesterone Levels in Women (Publication in Progress)

Methods:

Women collected about 2 ml whole saliva samples daily during a complete menstrual cycle. Participants used Nabisco Care-Free Sugarless Peppermint gum to stimulate saliva production to collect saliva samples in the morning after washing their mouth with water, but before eating, drinking, or brushing their teeth. Samples were expectorated into 5 ml polypropylene cryogenic vials (Corning Prod No. 03-374-25). Participants stored their saliva samples in their home freezers until their collection period was complete, at which time they shipped the samples frozen to the NIOSH laboratories, packed with frozen ice packs, by overnight courier. Samples were stored at -80EC until assayed.

Salivary progesterone (P4) was assayed in duplicate by Aeron Laboratories using a modification of a competitive double antibody radioimmunoassay (Diagnostic Systems Laboratory; Prod No. 3400). Three pre-processed whole saliva quality control pools, covering the range of the standard curve (6-600 pg/ml), were assayed in duplicate at the beginning and again at the end of every assay.

Samples were examined visually for coloration and tested for blood contamination using Hemastix (Bayer Corp Prod. No. 2190). Samples exhibiting a positive response to Hemastix were diluted 1:20 with water and retested. Samples with a positive reaction to this diluted test were estimated to have sufficient blood contamination to bias the salivary P4 measurement.

Results:

With rare exceptions, P4 values of saliva samples that significantly colored the Hemastix showed no indication of being outliers relative to their salivary P4 neighbors or to the urinary Pd3G profile. Accordingly, these values have not been excluded from the database.

The standard curves for the 153 salivary P4 assays were fitted to both linear logarithmic (LL) and 2 parameter logistic (2PL) models. Based on visual inspection of the fitted curves and based on a comparison of the residual variances, the fit of the 2PL model was better than that of the LL model. That is, the residual variance for the 2PL model was statistically less ($P<0.05$) or tended to be less than that of the LL model 56 and 71 times, respectively, whereas the residual variance for the LL model was never statistically less than the 2PL model and tended to be less only 26 times. Therefore, data derived from standard curves fit to the 2PL model were used.

Values and Precision for QC Salivary P4 QC Pools					
	Number	Segments	Mean	Assay Prec. (%CV)	
LEVEL	of Assays	per Assay	(pg/ml)	Within	Among
Low	150	1	43.35	10.87	17.7
Medium	150	1	114.43	4.98	7.12
High	150	1	244.77	4.87	9.37

Pearson correlation coefficients were calculated between salivary P4 values and urinary Pd3G values (adjusted for creatinine levels). Correlations were computed for pairs collected on the same day and lagged. Linear models were used to estimate among- and within-woman variances and means to calculate the coefficients of variation .

In general, salivary P4 patterns paralleled those of urinary pregnanediol 3-glucuronide (Pd3G). There are, however, some cases in which the correlation between these two measures is strikingly different. In some cases, this persists for most of a menstrual cycle, while in other cases the disparity is brief and isolated.

The correlation coefficient between salivary P4 and urinary Pd3G with no lag is 0.552 ($P<0.0001$; $n=4197$ pairs). This relationship improved slightly by lagging the urinary Pd3G values by 1 day ($r=0.565$) or 2 days ($r=0.562$), and then deteriorated thereafter. The relationship was also weaker if salivary P4 values lagged after the urinary Pd3G values. Correlation coefficients were comparable, though just slightly lower when salivary P4 values were derived from a 2PL fit of the standard curve.

To compare within- and among-woman variation between salivary P4 and urinary Pd3G values, three endpoints were calculated:

Follicular Phase Mean Baseline: Geometric mean from cycle day 5 through the third day before the day of ovulation (day of luteal transition or day of LH surge onset), or days 6-10. Omit if < 2 values present. Must have start menses.

Luteal Phase Area-Under-the-Curve: Area-under-the-curve from the day after the day of ovulation through the last day of the menstrual cycle. Extrapolate missing values; omit if >3 missing values or consecutive missing values. Must have end menses.

Luteal Phase 3-Day Mean Peak: Maximum 3-day arithmetic mean to include the peak Pd3G value of the cycle. Omit if any missing values.

Results of this analysis is presented in the following table:

Indices of Variation (% Coefficients of Variation) for Urinary Pd3G and Salivary P4			
	Follicular Phase Mean Baseline	**Luteal Phase Area-Under-the-Curve**	**Luteal Phase 3-Day Mean Peak**
Within Woman:			
Urinary Pd3G	32.3%	21.8%	28.1%
Salivary P4	35.1%	20.5%	28.8%
Among Women:			
Urinary Pd3G	61.6%	55.0%	85.5%
Salivary P4	75.0%	43.8%	38.7%

These results suggest that within-woman variation is similar between urinary and salivary progestin measurements. On the other hand, there is considerably more variation among women for urinary Pd3G levels than for salivary P4 values. This difference may reflect variations in the manner that steroid hormones are cleared. If true, salivary P4 may provide better resolution to assess the progestin status for a population, or for an individual.

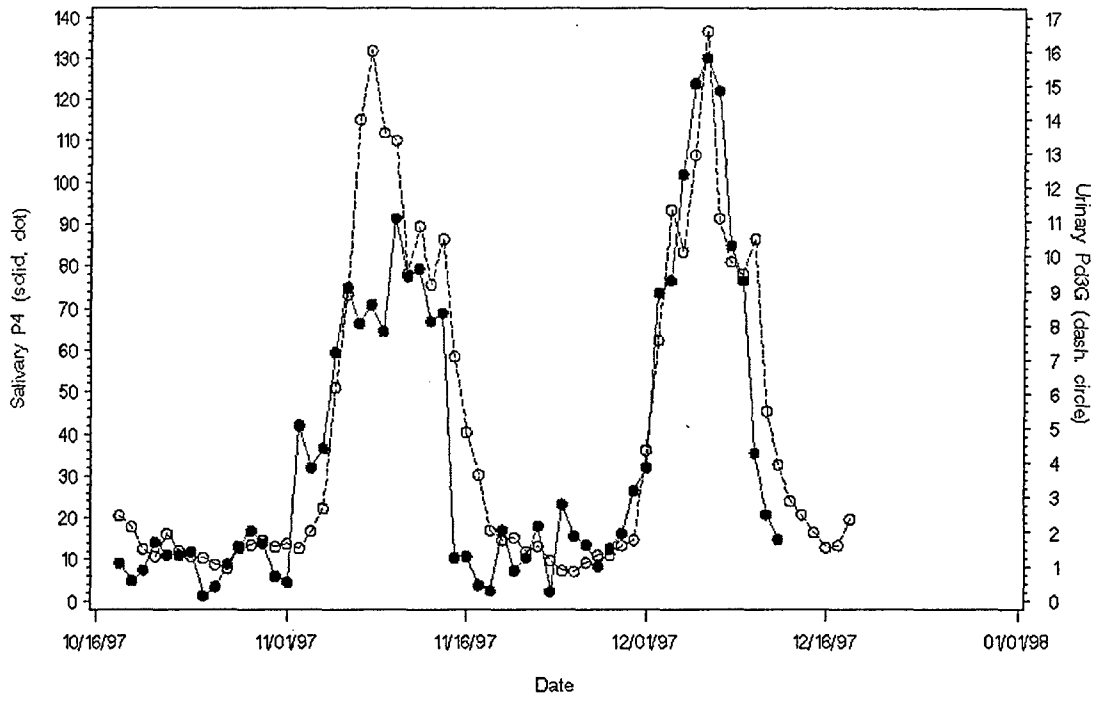

Figure 1. Salivary progesterone (solid lines & filled symbols) and urinary pregnanediol 3-glucuronide (dashed lines & open symbols) concentrations correlate well for most study cycles, as exemplified throughout the 2 menstrual cycles for this subject #109.

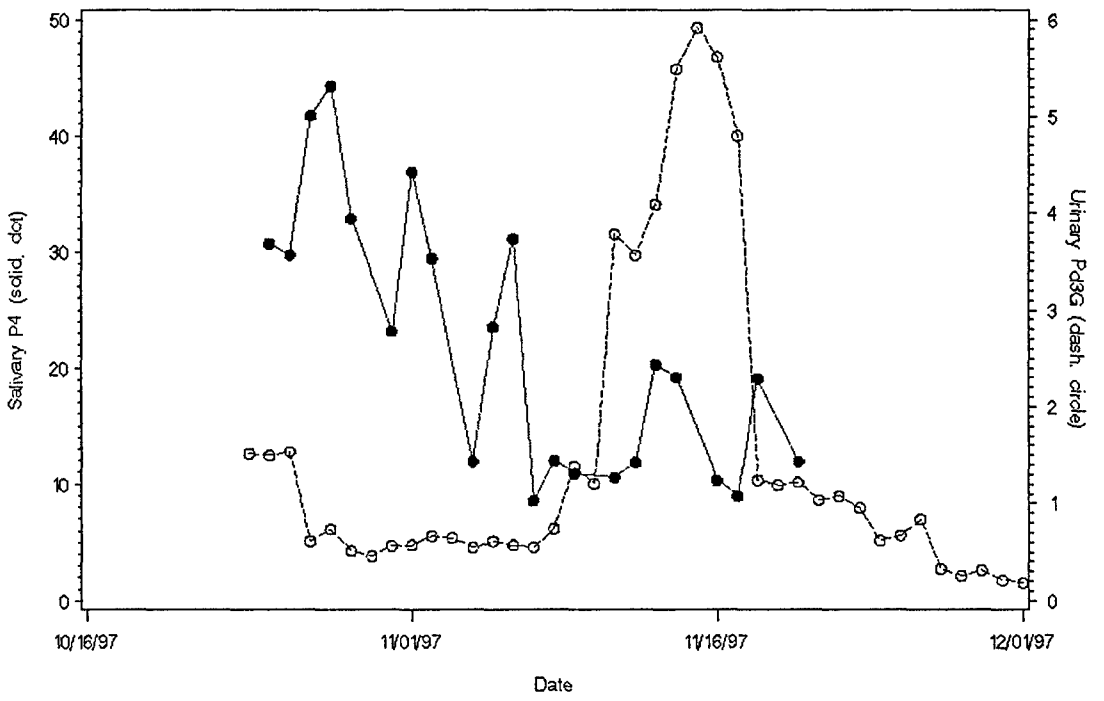

Figure 2. Salivary progesterone (solid lines & filled symbols) and urinary pregnanediol 3-glucuronide (dashed lines & open symbols) concentrations do not correlate well in a few cases, as represented by subject #118. In most cases, the explanation for the poor correlation is not known. In this cycle, salivary progesterone levels are inexplicably high during the follicular phase of this cycle.

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ECOLOGIC BIAS IN ESTIMATING ECOLOGIC EFFECTS. Sander Greenland (Department of Epidemiology, UCLA School of Public Health, Los Angeles, CA 90095-1772)

A number of authors have attempted to defend ecologic health and social research by claiming that the goal of that research is estimation of ecologic (group-level) effects rather than individual-level effects. Critics of these attempts point out that ecologic effect estimates are inevitably used as estimates of individual effects, despite disclaimers. A more subtle problem is this: The defenders of ecologic studies usually seem to presume that ecologic bias (bias from failure to take account of individual-level distributions) cannot affect estimates of ecologic effects from ecologic data. This presumption is incorrect. The author shows how ecologic variation in the distribution of individual effects can bias ecologic estimates of ecologic effects, especially under standard epidemiologic assumptions. It is argued that the conditions leading to this bias are plausible and perhaps even common in studies of ecosocial factors and health outcomes.

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A CASE-CONTROL STUDY OF PESTICIDES AND FETAL DEATH DUE TO CONGENITAL ANOMALIES. Erin M. Bell,* Irva Hertz-Picciotto, and James J. Beaumont (Department of Epidemiology, University of North Carolina, Chapel Hill, NC 27599-7400)

While animal studies have shown several agricultural pesticides to be teratogenic, epidemiologic studies have been inconclusive. A case control study of fetal death (pregnancy loss at greater than 20 weeks gestation) was conducted in ten agricultural counties of California to evaluate the association between fetal death due to congenital anomalies (cases = 73, controls = 608) and maternal residential proximity to commercial pesticide applications. A statewide database that recorded all applications of restricted pesticides was linked to maternal address to determine daily exposure status for all cases and controls. Individual pesticides were grouped into classes, with phosphates, pyrethroids, halogenated hydrocarbons, carbamates and endocrine disruptors chosen for analysis. Multivariate logistic regression models adjusted for maternal age and county of residence showed several of these classes to be associated with an elevated risk of fetal death due to congenital anomalies. Furthermore, a consistent pattern was found with respect to timing of exposure; the largest risks for fetal death due to congenital anomalies were from pesticide exposure during the 3rd-8th week of pregnancy. For those exposed within 9 square miles of their residence, Odds Ratios (OR) ranged from a low of 1.4 (95% confidence interval 0.8,2.4) for phosphates, carbamates and endocrine disruptors to 2.2 (1.3,3.9) for halogenated hydrocarbons. When exposure was restricted to within 1 square mile of residence, ORs increased, ranging from 2.0 (0.8,4.9) for pyrethroids to 3.0 (1.4,6.5) for phosphates. Major strengths of this study include the objective measures of exposure, and our ability to look at exposure for relevant biological time periods and specific pesticide classes.

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EXPOSURE TO MAGNETIC FIELDS (MF) DURING PREGNANCY AND THE RISK OF SPONTANEOUS ABORTION (SAB). D-K. Li,* R. Odouli, S. Wi, T. Janevic, I. Golditch, D. Bracken, R. Senior, D. Rankin, and R. Iriye (Kaiser Permanente, Oakland, CA 94611)

To determine the effect of prenatal MF exposure on the risk of SAB, we conducted a prospective cohort study in the population of Kaiser Permanente Medical Care Program of the Northern California region. During 1996 to 1998, all pregnant women in the San Francisco area who had a positive pregnancy test and who decided to carry the pregnancy to term were eligible for the study. Among the 2,729 eligible women we contacted, 1,390 (50.6%) agreed to participate in the study. The median gestational age at entry into the study was 40 days. In addition to an in-person interview, each participant was asked to wear an EMDEX-II meter to measure her personal MF exposure for 24 hours and to keep a diary to record activities during this period. Participants' residences were also measured for MF levels and assigned a wire-code based on their residential power line configuration. Pregnancy outcomes for all participants were ascertained. The Cox proportional hazard regression was used to take into account variation in gestational age at entry and to control for confounders while examining the effect of MF on the risk of SAB. Although the average MF exposure level was not associated with the risk of SAB, any exposure to MF $\geq$ 1.6 μT (717 subjects) was associated with an increased risk of SAB (adjusted rate ratio (aRR)=1.8, 95% confidence interval (CI): 1.2-2.7). The risk continued to increase slightly with either increased duration or total amount of the exposure above 1.6 μT; the trend test for dose-response effect of both exposure measurements was significant ($p < 0.05$). The MF effect was stronger during early pregnancy (aRR = 2.2, 95% CI: 1.2-4.0 for gestation < 10 weeks; aRR = 1.3, 0.8-2.5 for gestation $\geq$ 10 weeks). The effect was also stronger among women with a history of multiple SABs or sub-fertility (susceptible populations): aRR = 3.1, 95% CI: 1.3-7.7. Our study suggests a potential adverse effect on reproductive outcomes associated with a relatively high level of peak MF exposure during early pregnancy.

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INTERNAL DOSE OF BENZENE, ETHYL-BENZENE, TOLUENE & XYLENES & FUEL COMPONENTS AND EFFECTS ON REPRODUCTIVE HORMONES IN WOMEN: Susan Simpson,* Grace Lemasters, James Kesner, James Lockey, Rakesh Shukla, Edwin Knect, and Edward Krieg (University of Cincinnati, Cincinnati, OH 45267)

Background: Exposures to hydrocarbons from sources such as solvents and fuels are ubiquitous and have been associated with adverse pregnancy outcomes. Few studies, however, have addressed potential reproductive hormonal effects. Lower baseline pregnanediol 3-glucuronide (PD3G), and higher mid-luteal PD3G, preovulatory luteinizing hormone (LH), and mid-luteal estrone-3 glucuronide ($E_1$3G) have been linked to successful conceptions. Therefore, these endocrine measures were chosen as outcomes. Our hypothesis tested whether these endocrine outcomes were affected by solvent and/or fuel exposure among reproductive-aged women. US Air Force personnel (n = 63) recorded daily solvent/fuel contact, collected daily urine, and provided end-of-shift breath samples. Breath samples were analyzed by gas chromatography. Total benzene, toluene, ethyl-benzene, and m,p,o-xylenes (BTEX) were measured in ppb (parts per billion) and examined both continuously and dichotomously (no-to-low versus high). Fuel (C6-C16) was only quantified dichotomously. Breath levels of BTEX, and fuel, and potential confounders and covariates were regressed with untransformed and transformed endocrine outcomes. Results: LH level was inversely related to breath fuel ($p = 0.02$) and BTEX ($p = 0.05$) analyzed simultaneously as dichotomous exposure variables. BTEX, when analyzed as a continuous variable (range=0.0 to 415.0 ppb), was non-significant ($p = 0.26$), but fuels remained significant ($p = 0.01$). The adjusted mean LH level for the no-to-low fuels group was 22.6 mIU LH/mg creatinine (range=19.6-24.9), and for the high group it was 15.4 mIU LH/mg creatine (range=13.1-18.4). Results were consistent with and without outcome transformation and during multivariate analysis of the four endocrine outcomes together. Conclusion: Compounds found in fuels appear to alter reproductive hormone levels.

ABSTRACT:

Differences in Urinary Reproductive Hormone Levels between African American and Caucasian Women of Reproductive Age. *S.R. Reutman, G.K. Lemasters, J.S. Kesner, E.F. Krieg Jr., E.A. Knecht, R. Shukla, and J.E. Lockey (University of Cincinnati and NIOSH Cincinnati, OH, 45267-0056 and 45226).

Few studies have evaluated whether reproductive hormone ranges that are normal for Caucasians are applicable as norms for other races. The purpose of this report is to compare urinary levels of reproductive hormones between African American (n=33) and Caucasian (n=65) women of reproductive age. Participants recorded vaginal bleeding information in daily diaries and collected daily first-morning urine samples throughout one menstrual cycle. Menstrual periods and endocrine endpoints were defined by established algorithms. Menstrual cycle phase lengths and endocrine endpoints were derived from menstrual periods and urinary luteinizing hormone (LH), follicle stimulating hormone (FSH), pregnanediol 3-glucuronide (Pd3G), and estrone 3-glucuronide ($E_1$3G) concentrations, measured by fluoroimmunoassays and adjusted for urinary creatinine levels. Racial differences were examined using regression analyses with models including potential confounders and covariates. These analyses revealed that, relative to Caucasians, African American women have lower follicular phase LH:FSH ratios (mean $\pm$ SD: 0.7$\pm$0.4 vs. 1.0$\pm$0.6; Beta (B)=-0.23, p=0.03), follicular phase Pd3G levels (1.0$\pm$0.5 vs. 1.2$\pm$0.8 μg/mg Creatinine (Cr); B=-0.33, p=0.05), and periovulatory Pd3G rates of increase (0.5$\pm$0.7 vs. 1.0$\pm$1.2 μg/mg Cr; B=-0.48, p=0.02). These analyses are exploratory; the findings of racial differences need to be confirmed; and the practical significance of these differences needs to be evaluated.

Appendix IV: Letters Sent to Participants and U.S.A.F.

University of Cincinnati
Medical Center

Department of Environmental Health
Occupational and Environmental Medicine Divison
University of Cincinnati
PO Box 670056
Cincinnati OH 45267-0056

Delivery Address:
3223 Eden Avenue
Cincinnati OH 45267

DATE

NAME
STREET ADDRESS
CITY, STATE, ZIP

Dear _____,

Thank you for participating in the study on "Female Reproductive Effects of Exposure to Jet Fuel at US Air Force Bases" conducted by the University of Cincinnati Department of Environmental Medicine, the National Institute for Occupational Safety and Health, and the United States Air Force. Your participation provided valuable information for evaluating the effect of the workplace on the menstrual cycle. We are including some of your results in this letter.

Our laboratory analyzed your urine samples by measuring the four hormones that regulate the menstrual cycle: estrogen, progesterone, luteinizing hormone, and follicle stimulating hormone. These results are being used to determine if your workplace conditions disrupt the menstrual cycle in USAF personnel.

As you may know, the menstrual cycle begins on the first day of your period and ends on the first day before your next period. For most women, their menstrual cycle is 24-35 days long and their period is 3-7 days long. Your menstrual cycle(s) was/were XXX days long and your period(s) was/were XXX days long.

INSERT A

Please understand that most women occasionally experience menstrual cycles that do not result in ovulation, that are long or short, or in other ways are not typical. Your results combined with those of all the other participants are useful for evaluating the reproductive health of the large population of women studied.

Your results may or may not reflect your overall reproductive health. Should you have more specific questions concerning your menstrual cycle or reproduction, you should seek the attention of your personal physician. If you have questions concerning the study or testing sites, please feel free to contact Grace Lemasters, Ph.D. at 513-558-0045. Questions concerning your hormone values should be directed to James Kesner, Ph.D. at 513-533-8202. James Lockey, M.D. can be contacted for other related medical questions at 513-558-0030.

INSERT B

You also provided us with information in the initial questionnaire that you completed, in the daily diaries, and during conversations with our study personnel.

INSERT C

Your breath samples are currently being analyzed to look for some of the chemicals generally found in jet fuel. These data are not yet available and will be provided to you at a future date. At this time, we also have not analyzed the saliva samples. Generally saliva hormone samples are similar to urinary hormone values and may be analyzed at a future date.

Once again, thank you for participating in this program. Your participation has provided valuable information on the female menstrual cycle. It is through participation such as yours that we can learn to better understand the female body to improve medical care and

workplace conditions. We will send you the final results of the study in approximately a year from now. If you have any questions, please feel free to call us at your convenience.

With kindest regards,

Grace Lemasters, Ph.D.

James Kesner, Ph.D.

Jim Lockey, M.D., M.S.

University of Cincinnati
Medical Center

Department of Environmental Health
Occupational and Environmental Medicine Divison
University of Cincinnati
PO Box 670056
Cincinnati OH 45267-0056

Delivery Address:
3223 Eden Avenue
Cincinnati OH 45267

DATE:

BASE COMMANDER
ADDRESS

RE: "Female Reproductive Effects of Exposure to Jet Fuel at U.S. Air Force Bases"
(Funded by the Department of Defense)

Dear Colonel _____,

This letter and enclosure are to provide you with a summary of our study. We would like to express our gratitude to you for the participation by ____ AFB personnel in the above referenced study. The information we obtained is central to our objectives of evaluating the potential hormonal effects of jet fuel exposure to females. Implementation of our study would not have been possible without the generous time contributions made by the participants.

The University of Cincinnati conducted the breath sampling for the study over 10 Air Force bases between September 1997 and October 1998. The women provided an end of shift breath sample and some provided an additional breath sample on the following Monday (coded as pre shift in the table). The breath samples were analyzed by thermal desorption and gas chromatography. In Table 1 you will find the results of the breath analysis for individual participants identified by a number only. Values are reported for all bases that participated in the study. *The results for Shaw Air Force Base can be located as base code "__" in the attached table.* If available, we have reported each participant's AFSC or Occupational series.

The acceptable limits (biological exposure indices) based on breath levels are only available for one of the compounds that we measured (benzene, 80 ppb) and were only reported by the American Conference of Governmental Industrial Hygienists (ACGIH) in the 1995-96 handbook. Therefore, we have provided Table 2, which shows breath values of components of fuels in another Air Force population and an unexposed group for comparison purposes.

While fuels and solvents may be a major source of exposure to volatile organic compounds (VOC's) there are other sources of exposure that are commonly encountered in the general population. Contact with VOC's in fuels and solvents can occur during routine activities, such as automobile refueling, lawn mowing, degreasing, painting, using nail polish remover, refinishing, inhaling cigarette smoke either directly or passively, and eating grilled foods. These other sources are likely reflected in the levels seen among participants' Monday morning breath samples. You will note that for some employees Monday pre-shift samples were higher than workweek post shift samples. Also note that some AFSC/OS codes that would be considered non-fuel exposed jobs such as Base *A* ID *212* had higher values of fuel components when compared to AFSC/OS codes indicating fuel exposed jobs. Their high values may be attributable to indirect contact at the work site or contact with compounds from non-work activities such as smoking. As with most exposures, individuals may exhibit variation in sensitivity to the components of jet fuels and exhausts. Adherence to work practices which limit exposure by inhalation, dermal and ingestion routes help to ensure the safety of workers across the range of potential sensitivities. The employees will be informed of their individual results in approximately two to three weeks. Also enclosed please find a copy of the abstract entitled "Internal Dose of Benzene, Ethyl-benzene, Toluene & Xylenes & Fuel Components and Effects on Reproductive Hormones in Women" that has been published and a copy of the abstract of the article entitled "Menstrual Disorders and Occupational, Stress, and Racial Factors Among Military Personnel" that has been accepted for publication. If you have any questions please feel free to contact the investigators at the following number, 513-558-0030.

Again, thanks for your kind assistance.

Sincerely,

Grace Lemasters, PhD

James Lockey, M.D.

Susan Simpson, MS

cc: Flt Chief, _____

University of Cincinnati
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Delivery Address:
3223 Eden Avenue
Cincinnati OH 45267

DATE:

Participant Address Here

Dear Ms. *Participant Name Inserted Here,*

I am writing to update you with regard to your participation in the study conducted at _____ Air Force Base entitled, "Female Reproductive Effects of Exposure to Jet Fuel at U.S. Air Force Bases." Last year you received a letter describing your results in the health study regarding the menstrual and hormonal findings. Four reproductive hormones were evaluated: luteinizing hormone, midluteal estrogen, midluteal progesterone, and follicular progesterone. We found that women with higher levels of compounds found in fuels had slightly lower levels of luteinizing hormone.[1] We also examined stress among women in the military. The results suggest that job stress is lower among women in the military compared with stress in other occupational groups.[2] However, significant life events reported by this study population, including getting married, death of a family member, or job changes were associated with abnormal lengths of time between periods, painful periods, and long or heavy periods. We also found a small but significant association between reported working with fuels and painful periods. Non-Caucasians had a significantly increased risk of periods lasting longer than 7 days, heavy bleeding, and an abnormal length of time between periods. This study entitled, "Menstrual Disorders and Occupational, Stress and Racial Factors Among Military Personnel" has been accepted for publication in the Journal of Occupational and Environmental Medicine and will be published in the September or October 2000 issue.

Since our last report to you, we have analyzed levels of selected compounds found in fuels and solvents in the exhaled breath samples. Results of the analysis of your breath sample(s) are presented in Table 1. You are ID # _____. Please note that Table 1 contains participants' ranges in the current study, while average values from another study are located in Table 2.

While fuels and solvents may be a major source of exposure to these compounds there are other sources of exposure that are commonly encountered in the general population. Contact with the compounds in fuels and solvents can occur during routine activities, such as automobile refueling, lawn mowing, degreasing, painting, using nail polish

remover, refinishing, inhaling cigarette smoke either directly or passively, and eating grilled foods. These other sources are likely reflected in the levels seen among participants' Monday morning breath samples. You will note that, for some employees, Monday pre-shift samples were higher than workweek post shift samples. Also note that some AFSC/OS codes that would be considered non-fuel exposed jobs such as Base *A* ID *212* had higher values of fuel components when compared to AFSC/OS codes indicating fuel exposed jobs. Their high values may be attributable to indirect contact at the work site or contact with compounds during non-work activities such as smoking. Breath levels of the compounds listed in the table that are measured on one or two occasions may or may not reflect typical levels in your breath. As with most exposures, individuals may differ in sensitivity to these compounds. Adherence to work practices which limit exposure by inhalation, dermal and ingestion routes help to ensure the safety of workers across the range of potential sensitivities.

Thank you again for your participation. Our study would not have been possible without your generous assistance. Enclosed please find copies of the abstracts that were referenced above. If you have any additional questions please feel free to contact the investigators at the following number, 513-558-0030.

Sincerely,

Grace Lemasters, PhD

James Lockey, M.D.

Susan Simpson, MS, RN

[1] Simpson S, Lemasters G, Kesner J, Lockey J, Shukla R, Knecht E, Krieg E. Internal Dose of Benzene, Ethyl-Benzene, Toluene & Xylenes & Fuel Components and Effects on Reproductive Hormones in Women. *Am J Epidemiol.* 2000; 151(11):S70.

[2] Gordley L, Lemasters G, Simpson S, Yiin J. Menstrual Disorders and Occupational, Stress and Racial Factors Among Military Personnel. *J Occup Environ Med.* September or October 2000.

Base Code	ID	Shift†	AFSC	Reported Exposure to Cigarette Smoke	Benzene	Ethylbenzene	Toluene	*m,p* -Xylene	o -Xylene	Hexane	Heptane	Octane	Nonane	Decane	Undecane	Dodecane	Tridecane	Tetradecane	Pentadecane	Hexadecane
H	171	Post	65F0X1	Passive	6.79	2.31	5.13	220.17	ND	H	H	M	ND	H	H	H	ND	>ND	ND	H
08/98	190	Post	2A651A	Smoker	ND	2.33	6.88	30.91	13.33	M	L	ND	M	H	H	H	ND	>ND	ND	H
	191	Post	NAF2	Passive	ND	ND	ND	ND	1.87	L	M	M	L	L	M	M	ND	>ND	ND	M
	192	Post	1471	Passive	12.62	35.68	27.67	27.31	32.15	M	L	H	M	M	H	L	ND	>ND	ND	L
	192	Pre	1471	Passive	ND	21.62	6.27	15.82	7.44	M	M	H	H	M	H	ND	ND	>ND	ND	H
	195	Post	4A0X1	Non-smoker	ND	ND	ND	ND	ND	L	ND	ND	ND	M	L	ND	ND	ND	ND	ND
	197	Post	0101	Non-smoker	ND	4.89	0.66	2.44	0.22	H	ND	L	ND	M	M	L	ND	ND	ND	ND
	198	Post	4Y051	Passive	ND	2.72	1.37	0.48	6.78	L	M	L	ND	M	H	H	ND	>ND	ND	H
	198	Pre	4Y051	Passive	0.49	6.32	13.44	0.76	3.27	H	ND	L	ND	M	H	L	ND	>ND	ND	L
	202	Post	4Y032	Passive	ND	0.53	3.30	1.25	1.62	M	L	M	L	H	M	H	ND	>ND	ND	H
	205	Post	2F051	Non-smoker	ND	ND	0.36	157.67	ND	H	ND	ND	ND	H	M	L	ND	>ND	ND	M
	205	Pre	2F051	Non-smoker	ND	6.12	13.41	34.03	3.65	M	H	ND	ND	H	H	H	ND	>ND	ND	M
	230	Post	1W0S1	Non-smoker	ND	4.47	4.15	6.28	4.98	H	ND	L	L	M	M	H	ND	>ND	ND	L
	230	Pre	1W0S1	Non-smoker	ND	2.56	10.57	ND	ND	H	ND	L	ND	M	H	ND	ND	ND	ND	ND
	232	Post	15W3	Non-smoker	ND	5.56	0.60	3.56	7.52	M	ND	ND	ND	L	L	M	ND	>ND	ND	H
	243	Post	2A632	Smoker	ND	3.66	5.26	2.77	6.15	L	ND	L	ND	H	H	H	ND	>ND	ND	M
	243	Pre	2A632	Smoker	0.81	17.45	5.99	22.12	25.30	L	L	ND	H	H	H	M	ND	>ND	ND	L

‡ H = "high", M = "medium", L = "low", and ND = "Non-detect" are used to divide the participants into tertiles where conversion factors for PPB values were not available

† "Pre" shift refers to the Monday sample that was taken prior to the workday and "post" shift refers to the workweek sample that was taken postshift midweek

* Indicates AFSC or Occupational Series information missing

Base Code	ID	Shift†	AFSC	Reported Exposure to Cigarette Smoke	Benzene	Ethylbenzene	Toluene	*m,p* -Xylene	*o* -Xylene	Hexane	Heptane	Octane	Nonane	Decane	Undecane	Dodecane	Tridecane	Tetradecane	Pentadecane	Hexadecane
G	226	Pre	0610	Non-smoker	ND	ND	0.12	0.86	ND	76.63	30.91	ND	ND	11.26	ND	ND	ND	ND	ND	ND
06/98	227	Post	3806	Non-smoker	ND	ND	0.45	2.25	ND	L	L	M	ND	L	M	M	ND	ND	ND	ND
10/98	266	Post	2A654	Smoker	25.44	ND	16.48	ND	12.14	26.39	23.81	ND	0.70	9.31	ND	ND	ND	ND	ND	ND
	266	Pre	2A654	Smoker	1.04	ND	3.58	2.77	5.74	29.01	0.75	ND	ND	44.29	ND	ND	ND	ND	ND	ND
	823	Post	*	Passive	ND	ND	ND	4.24	ND	M	ND	ND	ND	L	L	ND	ND	ND	ND	ND

Base Code	ID	Shift†	AFSC/OS	Reported Exposure to Cigarette Smoke	Benzene	Ethylbenzene	Toluene	*m,p* -Xylene	*o* -Xylene	Hexane	Heptane	Octane	Nonane	Decane	Undecane	Dodecane	Tridecane	Tetradecane	Pentadecane	Hexadecane
G	164	Post	690	Passive	9.92	ND	52.01	0.14	2.47	H	M	ND	ND	L	ND	ND	ND	ND	ND	ND
06/98	164	Pre	690	Passive	ND	ND	0.31	ND	ND	38.29	16.53	ND	ND	16.12	40.14	ND	ND	ND	ND	ND
10/98	168	Post	2A676	Passive	ND	ND	5.60	6.16	ND	M	ND	M	ND	H	M	H	ND	ND	ND	ND
	168	Pre	2A676	Passive	8.02	5.47	46.33	5.40	31.21	H	H	H	H	M	ND	ND	ND	ND	ND	ND
	172	Post	3806	Non-smoker	ND	ND	18.32	1.01	ND	H	ND	ND	L	L	L	ND	ND	ND	ND	ND
	172	Pre	3806	Non-smoker	ND	ND	ND	ND	ND	30.45	ND	ND	ND	41.39	37.67	M	ND	ND	ND	ND
	174	Post	4V071	Non-smoker	ND	ND	0.34	ND	ND	L	ND	ND	ND	L	M	ND	ND	ND	ND	ND
	174	Pre	4V071	Non-smoker	0.56	ND	26.58	7.08	13.56	167.19	54.82	7.89	ND	120.15	141.99	ND	ND	ND	ND	ND
	179	Post	4C031	Passive	ND	ND	ND	1.54	ND	ND	ND	ND	ND	L	L	L	ND	ND	ND	ND
	179	Pre	4C031	Passive	ND	ND	6.95	2.57	ND	H	ND	ND	L	M	L	L	ND	ND	ND	ND
	180	Post	2A4X1	Non-smoker	ND	ND	0.12	ND	ND	H	ND	ND	ND	L	M	M	ND	ND	ND	ND
	180	Pre	2A4X1	Non-smoker	12.09	ND	0.99	ND	4.55	38.42	10.49	ND	ND	24.54	ND	ND	ND	ND	ND	ND
	181	Post	*	Passive	24.42	ND	24.71	2.10	ND	H	ND	ND	ND	L	ND	ND	ND	ND	ND	ND
	182	Post	4N071	Passive	ND	ND	ND	ND	ND	H	ND	ND	M	L	L	L	ND	ND	ND	ND
	182	Pre	4N071	Passive	0.18	ND	ND	ND	ND	22.24	0.67	ND	ND	72.88	18.63	ND	ND	ND	ND	ND
	183	Post	4N031	Passive	ND	ND	4.28	0.15	ND	M	ND	ND	ND	L	L	ND	ND	ND	ND	ND
	183	Pre	4N031	Passive	28.05	ND	0.43	3.39	2.89	246.88	170.00	ND	ND	390.61	ND	ND	ND	ND	ND	ND
	184	Post	2A671A	Passive	ND	ND	2.95	1.45	ND	L	ND	ND	ND	L	L	ND	ND	ND	ND	ND
	184	Pre	2A671A	Passive	ND	ND	8.02	2.41	ND	M	ND	M	ND	M	M	M	ND	ND	ND	ND
	185	Post	51J3	Non-smoker	ND	ND	6.41	2.74	ND	M	ND	ND	ND	L	L	L	ND	ND	ND	ND
	185	Pre	51J3	Non-smoker	ND	ND	ND	ND	ND	20.29	1.86	ND	ND	53.37	16.15	ND	ND	ND	ND	ND
	188	Post	2A553A	Non-smoker	ND	ND	ND	3.46	ND	M	ND	ND	ND	L	L	L	ND	ND	ND	ND
	188	Pre	2A553A	Non-smoker	ND	7.20	ND	ND	1.82	22.33	2.56	ND	ND	19.18	7.6	ND	ND	ND	ND	ND
	189	Post	2A772	Passive	8.04	ND	7.36	3.75	ND	M	ND	ND	ND	L	L	ND	ND	ND	ND	ND
	189	Pre	2A772	Passive	10.95	ND	4.97	ND	ND	43.45	8.30	ND	41.12	9.27	ND	ND	ND	ND	ND	ND
	222	Post	3806	Non-smoker	ND	ND	ND	ND	ND	M	ND	ND	ND	H	L	L	ND	ND	ND	ND
	222	Pre	3806	Non-smoker	ND	ND	ND	ND	ND	ND	ND	ND	ND	11.50	ND	ND	ND	ND	ND	ND
	224	Post	0318	Smoker	ND	ND	ND	ND	ND	H	ND	ND	ND	L	H	ND	ND	ND	ND	ND
	224	Pre	0318	Smoker	ND	ND	48.84	2.14	4.91	L	M	H	H	M	H	H	ND	ND	ND	ND
	226	Post	0610	Non-smoker	ND	ND	0.70	0.13	ND	M	ND	ND	ND	L	L	ND	ND	ND	ND	ND

Base Code	ID	Shift†	AFSC/OS	Reported Exposure to Cigarette Smoke	Benzene	Ethylbenzene	Toluene	*m,p* -Xylene	o -Xylene	Hexane	Heptane	Octane	Nonane	Decane	Undecane	Dodecane	Tridecane	Tetradecane	Pentadecane	Hexadecane
E	244	Post	2A551J	Non-smoker	ND	ND	ND	ND	22.04	38.82	ND	ND	1.70	2.76	7.92	M	ND	ND	ND	131.98
08/98	244	Pre	2A551J	Non-smoker	ND	10.54	15.17	5.41	15.59	H	ND	H	ND	H	H	H	ND	>ND	ND	M
	246	Post	3S051	Passive	ND	9.52	2.32	3.83	10.54	M	ND	L	ND	M	H	M	ND	>ND	ND	M
	246	Pre	3S051	Passive	ND	2.32	1.00	0.24	0.69	H	ND	ND	ND	L	H	L	ND	>ND	ND	L
	250	Post	2A654	Non-smoker	ND	ND	7.68	3.70	7.41	M	M	ND	H	H	M	ND	ND	ND	ND	ND
	250	Pre	2A654	Non-smoker	ND	5.33	5.13	1.46	3.62	H	ND	L	M	M	H	M	ND	>ND	ND	L
	847	Post	2F0X1	Smoker	ND	ND	ND	ND	12.44	33.67	ND	ND	ND	43.61	32.68	M	ND	>ND	ND	ND
	848	Post	2F071	Non-smoker	ND	ND	9.87	ND	8.40	15.62	ND	ND	ND	62.84	25.68	M	ND	ND	ND	105.48
	848	Pre	2F071	Non-smoker	2.29	0.50	2.43	3.64	8.96	M	ND	H	M	M	H	H	ND	>ND	ND	M
F	143	Post	4R051B	Non-smoker	ND	ND	1.86	ND	6.93	19.56	0.97	ND	ND	60.48	ND	L	ND	ND	ND	ND
10/98	148	Post	2A651A	Non-smoker	78.01	ND	1.05	ND	5.78	238.66	4.43	ND	ND	14.78	ND	ND	ND	ND	ND	ND
	148	Pre	2A651A	Non-smoker	ND	ND	ND	ND	0.66	18.79	ND	ND	ND	5.45	ND	ND	ND	ND	ND	ND
	155	Post	2S071	Non-smoker	ND	ND	4.73	6.72	14.73	49.02	248.74	ND	26.92	26.93	ND	H	ND	ND	ND	ND
	155	Pre	2S071	Non-smoker	ND	ND	ND	ND	1.64	30.24	ND	ND	ND	33.71	ND	ND	ND	ND	ND	ND
	267	Pre	26AX4	Smoker	ND	ND	ND	ND	3.70	35.88	ND	ND	ND	3.66	ND	ND	ND	ND	ND	ND
	280	Post	2A671A	Smoker	2.29	0.45	0.26	ND	4.13	40.59	6.78	0.06	2.50	13.51	ND	ND	ND	ND	ND	ND
	280	Pre	2A671A	Smoker	ND	ND	2.60	ND	0.37	32.29	ND	ND	7.97	31.96	4.70	M	ND	ND	ND	ND

Base Code	ID	Shift†	AFSC	Reported Exposure to Cigarette Smoke	Benzene	Ethylbenzene	Toluene	*m,p* -Xylene	o -Xylene	Hexane	Heptane	Octane	Nonane	Decane	Undecane	Dodecane	Tridecane	Tetradecane	Pentadecane	Hexadecane
D	247	Post	1C052	Non-smoker	5.33	ND	0.22	ND	ND	32.84	1.62	0.15	5.94	6.50	ND	ND	ND	ND	ND	ND
09/98	249	Post	2F071	Non-smoker	0.18	ND	ND	ND	ND	26.99	ND	ND	ND	28.40	ND	ND	ND	ND	ND	ND
	249	Pre	2F071	Non-smoker	ND	ND	ND	ND	ND	46.31	ND	ND	0.73	607.10	ND	ND	ND	ND	ND	ND
	251	Post	3S051	Smoker	3.33	ND	1.13	ND	ND	21.33	0.33	0.75	ND	17.57	23.45	ND	ND	ND	ND	ND
	251	Pre	3S051	Smoker	ND	ND	1.15	ND	2.01	245.00	ND	ND	ND	54.20	ND	ND	ND	ND	ND	ND
	253	Post	3S051	Non-smoker	ND	ND	ND	ND	ND	20.55	1.47	ND	ND	6.48	ND	ND	ND	ND	ND	ND
	253	Pre	3S051	Non-smoker	ND	ND	8.47	ND	33.80	53.74	ND	ND	ND	48.74	ND	ND	ND	ND	ND	ND
	254	Post	1C0X2	Smoker	ND	ND	9.38	ND	2.35	48.80	90.44	ND	ND	261.24	24.96	ND	ND	ND	ND	ND
	255	Post	3S071	Passive	ND	ND	ND	ND	ND	31.49	0.83	0.36	17.69	11.36	ND	ND	ND	ND	ND	ND
	255	Pre	3S071	Passive	ND	ND	ND	ND	ND	39.54	ND	1.14	6.06	23.76	ND	ND	ND	ND	ND	ND
	256	Post	2F071	Smoker	8.56	ND	ND	ND	ND	21.66	ND	ND	24.96	76.78	ND	ND	ND	ND	ND	ND
	256	Pre	2F071	Smoker	22.34	ND	5.34	ND	ND	56.01	ND	ND	ND	15.35	ND	ND	ND	ND	ND	ND
	257	Post	5R031	Non-smoker	0.04	ND	0.37	ND	ND	27.85	7.25	ND	41.33	62.76	ND	ND	ND	ND	ND	ND
	258	Post	3E432	Passive	15.64	ND	ND	ND	ND	25.37	ND	ND	0.71	13.93	ND	ND	ND	ND	ND	ND
	258	Pre	3E432	Passive	ND	ND	3.27	ND	ND	40.93	2.22	ND	ND	7.81	ND	ND	ND	ND	ND	ND
	259	Post	2S051	Non-smoker	ND	4.20	ND	ND	ND	26.04	0.50	ND	72.78	6.58	23.52	ND	ND	ND	ND	ND
	259	Pre	2S051	Non-smoker	ND	ND	ND	ND	0.55	21.75	78.06	ND	ND	7.85	ND	ND	ND	ND	ND	ND
	260	Post	2A651A	Smoker	0.40	ND	ND	ND	ND	28.47	1.60	ND	3.74	12.75	ND	ND	ND	ND	ND	ND
	261	Post	3P051B	Passive	35.03	ND	9.16	ND	ND	17.56	15.92	ND	ND	33.71	18.44	ND	ND	ND	ND	ND
	262	Post	3N131A	Non-smoker	ND	ND	0.96	ND	ND	7.84	0.03	ND	29.24	20.93	14.14	ND	ND	ND	ND	ND
	262	Pre	3N131A	Non-smoker	ND	ND	6.30	ND	ND	24.54	2.18	ND	1.54	104.76	ND	ND	ND	ND	ND	ND
	263	Post	3N171A	Non-smoker	ND	ND	ND	ND	ND	13.36	0.45	ND	ND	69.24	44.96	ND	ND	ND	ND	ND
	263	Pre	3N171A	Non-smoker	ND	ND	0.30	ND	2.32	72.77	11.83	ND	3.20	26.47	ND	ND	ND	ND	ND	ND
	264	Post	2A651A	Smoker	9.64	ND	10.81	ND	ND	13.84	3.82	1.15	ND	37.12	13.93	ND	ND	ND	ND	ND
	852	Post	3S0X1	Non-smoker	ND	ND	ND	ND	1.02	17.53	ND	0.32	4.42	17.53	ND	ND	ND	ND	ND	ND
	865	Post	3A031	Non-smoker	12.66	ND	21.34	ND	4.45	34.99	3.47	ND	ND	129.70	32.48	ND	ND	ND	ND	ND

Base Code	ID	Shift†	AFSC/OS	Reported Exposure to Cigarette Smoke	Benzene	Ethylbenzene	Toluene	m,p -Xylene	o -Xylene	Hexane	Heptane	Octane	Nonane	Decane	Undecane	Dodecane	Tridecane	Tetradecane	Pentadecane	Hexadecane
C	229	Post	*	Non-smoker	2.14	8.55	1.94	60.01	ND	M	ND	ND	ND	L	M	ND	ND	ND	ND	ND
06/98	229	Pre	*	Non-smoker	ND	ND	2.39	6.86	ND	H	ND	ND	ND	L	ND	ND	ND	ND	ND	ND
	231	Post	3N051	Smoker	0.78	5.07	18.91	31.60	10.75	L	ND	H	ND	H	L	ND	ND	ND	ND	ND
	233	Post	44051	Passive	ND	ND	4.97	11.62	ND	L	ND	ND	ND	L	ND	ND	ND	ND	ND	ND
	233	Pre	44051	Passive	ND	0.95	9.67	1.39	150.33	M	ND	H	L	H	H	H	ND	ND	ND	ND
	234	Post	2A6X6	Passive	ND	11.23	7.85	134.44	2.89	L	ND	H	ND	L	H	ND	ND	ND	ND	ND
	234	Pre	2A6X6	Passive	ND	1.22	2.01	ND	154.94	ND	H	H	ND	L	L	ND	ND	ND	ND	ND
	235	Post	4T071	Non-smoker	0.08	4.43	1.61	400.86	8.08	L	ND	M	ND	M	ND	ND	ND	ND	ND	ND
	235	Pre	4T071	Non-smoker	25.55	ND	3.60	9.37	ND	H	ND	H	ND	H	H	M	ND	ND	ND	ND
	236	Post	2W131F	Non-smoker	ND	ND	7.48	ND	ND	M	ND	ND	ND	L	L	ND	ND	ND	ND	ND
	236	Pre	2W131F	Non-smoker	4.50	ND	8.66	6.15	ND	M	L	M	L	M	H	L	ND	ND	ND	ND
	237	Post	4A051	Non-smoker	ND	9.49	7.24	ND	ND	L	ND	M	M	H	L	L	ND	ND	ND	ND
	237	Pre	4A051	Non-smoker	ND	8.38	12.77	6.23	5.64	H	ND	ND	ND	M	L	ND	ND	ND	ND	ND
	238	Post	*	Smoker	ND	2.10	8.38	88.53	ND	L	ND	ND	ND	H	H	H	ND	>ND	ND	ND
	238	Pre	*	Smoker	3.86	ND	24.14	5.73	48.01	H	ND	H	M	H	M	L	ND	ND	ND	ND
	239	Post	42B3	Non-smoker	ND	ND	ND	1.08	ND	L	ND	ND	ND	L	ND	ND	ND	ND	ND	ND
	239	Pre	42B3	Non-smoker	ND	ND	ND	8.67	ND	M	ND	ND	ND	L	L	ND	ND	ND	ND	ND
	240	Post	4N051	Non-smoker	ND	ND	0.68	0.29	1.12	L	ND	ND	ND	M	M	M	ND	>ND	ND	ND
	240	Pre	4N051	Non-smoker	ND	6.60	7.21	106.20	2.73	M	ND	ND	ND	H	L	ND	ND	ND	ND	ND
	241	Post	3A071	Non-smoker	ND	ND	0.33	1.74	2.65	L	ND	ND	ND	H	H	M	ND	ND	ND	ND
	241	Pre	3A071	Non-smoker	ND	ND	3.27	7.71	ND	M	ND	ND	ND	M	L	ND	ND	ND	ND	ND
	242	Post	2S051	Smoker	ND	0.08	2.62	1.37	39.05	M	L	L	ND	M	M	ND	ND	ND	ND	ND
	252	Post	350	Passive	ND	ND	2.68	0.35	6.58	L	ND	ND	ND	M	ND	ND	ND	ND	ND	ND
	252	Pre	350	Passive	ND	28.29	20.46	7.56	4.82	H	ND	ND	M	H	ND	ND	ND	ND	ND	ND

Base Code	ID	Shift†	AFSC/OS	Reported Exposure to Cigarette Smoke	Benzene	Ethylbenzene	Toluene	*m,p* -Xylene	*o* -Xylene	Hexane	Heptane	Octane	Nonane	Decane	Undecane	Dodecane	Tridecane	Tetradecane	Pentadecane	Hexadecane
B	123	Post	2A672	Non-smoker	43.57	ND	1.48	ND	39.06	ND	33.00	ND	ND	30.00	ND	ND	ND	ND	ND	ND
10/98	123	Pre	2A672	Non-smoker	ND	ND	94.94	54.50	3.92	42.38	11.09	ND	116.33	65.66	40.66	ND	ND	ND	ND	ND
	140	Post	*	Non-smoker	ND	ND	3.71	0.80	27.96	11.73	17.15	5.99	ND	659.72	70.49	ND	ND	ND	ND	ND
	140	Pre	*	Non-smoker	ND	ND	194.07	2.40	18.38	86.86	491.51	ND	ND	18.97	ND	ND	ND	ND	ND	ND
	142	Post	2W151	Non-smoker	ND	ND	5.18	ND	30.66	15.10	ND	ND	ND	13.55	ND	H	ND	ND	ND	ND
	142	Pre	2W151	Non-smoker	ND	ND	ND	ND	42.06	25.45	ND	ND	ND	33.48	ND	H	ND	ND	ND	ND
	144	Post	4N031	Passive	94.96	ND	44.51	2.47	3.50	21.08	ND	ND	ND	469.79	226.71	ND	ND	ND	ND	ND
	144	Pre	4N031	Passive	0.14	ND	4.10	ND	22.69	52.32	ND	ND	ND	92.17	46.81	ND	ND	ND	ND	ND
	145	Post	4N051	Passive	ND	ND	ND	ND	1.60	38.35	87.44	ND	ND	410.69	ND	ND	ND	ND	ND	ND
	145	Pre	4N051	Passive	ND	ND	ND	ND	47.95	25.34	163.69	ND	ND	3.21	ND	ND	ND	ND	ND	ND
	146	Post	4N051	Passive	ND	ND	ND	ND	3.10	33.02	ND	ND	ND	127.35	10.39	L	ND	ND	ND	ND
	146	Pre	4N051	Passive	ND	ND	ND	ND	ND	54.83	ND	ND	ND	145.07	ND	ND	ND	ND	ND	ND
	147	Post	4N031	Non-smoker	ND	ND	ND	ND	23.44	14.72	2.33	ND	ND	116.97	74.87	ND	ND	ND	ND	ND
	147	Pre	4N031	Non-smoker	ND	ND	7.76	ND	21.43	13.86	ND	ND	ND	18.83	ND	ND	ND	ND	ND	ND
	282	Post	2A631	Non-smoker	ND	ND	1.33	ND	10.93	20.65	ND	ND	ND	11.66	49.70	ND	ND	ND	ND	ND
	285	Post	*	Non-smoker	ND	ND	ND	ND	6.23	28.86	675.33	ND	334.08	69.64	ND	ND	ND	ND	ND	ND
	285	Pre	*	Non-smoker	ND	0.67	ND	ND	43.94	23.38	ND	ND	ND	2.98	ND	ND	ND	ND	ND	ND
	286	Post	4N051	Non-smoker	ND	ND	ND	ND	3.43	42.80	252.07	ND	239.92	227.32	77.29	ND	ND	ND	ND	ND
	286	Pre	4N051	Non-smoker	ND	ND	16.46	ND	1.92	56.63	ND	ND	145.17	297.96	ND	ND	ND	ND	ND	ND
	287	Post	4N071	Smoker	ND	ND	6.76	ND	0.80	26.60	ND	ND	ND	156.44	ND	ND	ND	ND	ND	ND
	287	Pre	4N071	Smoker	ND	ND	ND	ND	37.48	41.48	ND	ND	ND	85.18	ND	ND	ND	ND	ND	ND

Table 1 - Breath levels by Air Force Base Per Subject for Analytes Measured in Parts Per Billion Found in Fuel and Solvents[‡]

Base Code	ID	Shift[†]	AFSC/OS	Reported Exposure to Cigarette Smoke	Benzene	Ethylbenzene	Toluene	*m,p* -Xylene	*o* -xylene	Hexane	Heptane	Octane	Nonane	Decane	Undecane	Dodecane	Tridecane	Tetradecane	Pentadecane	Hexadecane
A	006	Post	0203	Smoker	0.15	ND	5.78	1.50	ND	L	ND	ND	ND	L	ND	H	ND	ND	ND	H
05/98-06/98	203	Post	1702	Non-smoker	ND	ND	1.10	ND	5.63	L	ND	ND	L	ND	ND	ND	ND	ND	ND	ND
	203	Pre	1702	Non-smoker	ND	ND	ND	11.72	18.37	L	ND	M	ND	M	ND	ND	ND	ND	ND	ND
	207	Post	*	Non-smoker	ND	ND	1.01	2.74	ND	H	ND	ND	ND	ND	M	H	ND	ND	ND	H
	207	Pre	*	Non-smoker	0.86	1.43	23.10	0.38	17.29	L	ND	ND	ND	M	M	H	ND	>ND	ND	L
	208	Post	2A353B	Non-smoker	0.33	ND	0.76	0.15	ND	L	M	L	ND	ND	M	H	ND	ND	ND	M
	208	Pre	2A353B	Non-smoker	ND	ND	1.96	1.05	ND	L	ND	M	ND	L	ND	L	ND	ND	ND	L
	210	Post	3S051	Non-smoker	ND	ND	1.62	2.04	ND	L	ND	ND	ND	M	L	M	ND	ND	ND	ND
	210	Pre	3S051	Non-smoker	ND	ND	0.64	10.96	5.27	H	M	L	M	M	H	M	ND	ND	ND	ND
	211	Post	0611	Smoker	0.83	ND	5.10	1.73	3.67	L	L	ND	H	L	M	M	ND	ND	ND	ND
	211	Pre	0611	Smoker	ND	ND	30.87	2.58	4.02	M	ND	L	ND	H	M	M	ND	ND	ND	ND
	212	Post	0318	Non-smoker	ND	ND	7.35	4.36	ND	L	ND	ND	ND	H	M	M	ND	ND	ND	ND
	212	Pre	0318	Non-smoker	ND	0.70	31.50	3.52	0.67	H	ND	H	H	H	M	M	ND	ND	ND	ND
	213	Post	0318	Non-smoker	ND	ND	6.00	6.92	16.46	M	ND	M	H	H	H	H	ND	ND	ND	ND
	213	Pre	0318	Non-smoker	ND	ND	19.24	10.01	ND	H	ND	ND	ND	H	M	L	ND	ND	ND	ND
	214	Pre	5J071	Non-smoker	ND	ND	20.21	1.78	0.27	H	ND	ND	L	M	M	L	ND	ND	ND	ND
	215	Post	0318	Passive	ND	ND	1.29	4.50	7.18	L	ND	ND	ND	L	H	H	ND	ND	ND	ND
	215	Pre	0318	Passive	ND	ND	98.03	8.65	28.72	H	H	ND	M	H	M	M	ND	ND	ND	ND
	216	Post	2T151	Non-smoker	ND	ND	2.69	5.80	12.00	M	ND	M	H	H	L	H	ND	ND	ND	ND
	216	Pre	2T151	Non-smoker	ND	1.00	6.00	1.41	ND	H	ND	ND	L	H	L	L	ND	ND	ND	ND
	217	Post	2T071	Passive	97.47	ND	0.42	0.43	67.31	L	ND	ND	H	M	L	L	ND	ND	ND	ND
	217	Pre	2T071	Passive	26.88	ND	11.18	2.96	ND	H	H	L	ND	H	L	L	ND	ND	ND	ND
	220	Post	11473	Smoker	ND	ND	0.74	2.02	ND	M	ND	ND	ND	M	H	M	ND	ND	ND	ND
	220	Pre	11473	Smoker	0.09	ND	43.72	5.66	6.39	L	H	ND	ND	M	M	H	ND	ND	ND	ND
	221	Post	3806	Passive	ND	ND	12.41	10.37	ND	M	ND	ND	ND	H	M	M	ND	ND	ND	ND
	221	Pre	3806	Passive	17.79	ND	ND	ND	ND	61.31	22.64	ND	ND	91.09	ND	ND	ND	ND	ND	ND

Table 2 – Breath Values of Fuel Components from a Study Comparing Levels of Analytes in the Breath of Air Force Employees Exposed to JP-8 Jet Fuel With the Levels of Analytes in Breath Samples Taken From a Control Population.*‡

Compounds	Controls' Values	Fuel Workers' Values
Benzene	0.60	3.03
Toluene	1.02	6.13
Ethylbenzene	0.09	2.11
m,p-Xylene	0.15	3.11
o-Xylene	0.10	4.00
Hexane	1.11	0.84
Heptane	0.22	1.48
Octane	0.08	2.77
Nonane	0.17	36.13
Decane	0.12	41.38
Undecane	0.16	15.59
Dodecane	3.33	8.86

* Pleil et al. Personal Exposure to JP-8 Jet Fuel Vapors and Exhaust at Air Force Bases. Environ Health Perspect 108(3): 183-192 (2000).

‡ Control values were based on control data from EPA studies of ambient levels from the Los Angeles basin (Asuza, CA) as an indicator of urban exposure, and from Research Triangle Park as an indicator of suburban/rural exposure.

Appendix V: Bibliography of Publications and Abstracts

Bibliography of Publications and Abstracts:

Gordley LB, Lemasters GK, Simpson SR, Yiin. "Menstrual Disorders and Occupation Stress and Racial Factors Among Military Personnel." JOEM, 2000;42(9):871-881.

Simpson SR, Lemasters GK, Kesner J, Lockey J, Shukla R, Knect E, Krieg E. "Internal dose of Benzene, Ethyl-benzene, Toluene, Xylenes, & Fuel Components and Effects on Reproductive Hormones in Women". American Journal of Epidemiology (suppl) 151(11): S70 (June, 2000)

Reutman SS, Lemasters GK, Knect EA, Shukla R, Lockey JE, Burroughs GE, Meyer DM, Kesner JS. Relationship between Exposure to Fuel and Endocrine Disruption (in preparation).

Reutman SS, Lemasters GK, Kesner JS, Krieg EF Jr., Knect EA, Shukla R, Lockey JE. Differences In Urinary Reproductive Hormone Levels between African American and Caucasian Women (in preparation).

Kesner JS, Lemasters GK. A Comparison of Salivary and Urinary Progesterone Levels in Women (in preparation).

Appendix VI: Proposals

AA

Grant Application

LEAVE BLANK — FOR OFFICIAL USE ONLY

Review Group	Number
Council/Board *(Month, Year)*	Date Received

1. TITLE OF PROJECT
The Effects of JP-8 Jet Fuel on the Immune System of Tank Entry Workers

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT
Number: 98045A Title: University of Cincinnati Pilot Project Program

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR — New Investigator ☐ YES

3a. NAME *(Last, first, middle)*
RHODES. Audry Gayle

3b. DEGREE(S)
Doctor of Medicine

3c. POSITION TITLE
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3d. MAILING ADDRESS *(Street, city, state, zip code)*
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Erlanger, KY 41018

3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT
Environmental Health, College of Medicine

3f. MAJOR SUBDIVISION
Occupational Medicine

3g. TELEPHONE AND FAX (Area code, number and extension)
TEL: 859-426-7861
FAX: 513-558-6272

E-MAIL ADDRESS:
arhodes@uh.healthbridge.org

4. HUMAN SUBJECTS ☐ No ☐ Yes X
4a. If "Yes," Exemption no. or IRB approval date pending
☐ Full IRB or ☒ Expedited Review
4b. Assurance of compliance no.

5. VERTEBRATE ANIMALS ☒ NO ☐ YES
5a. If "Yes," IACUC approval date
5b. Animal welfare assurance no.

6. DATES OF PROPOSED PERIOD OF SUPPORT *(MONTH, DAY, YEAR - MM/DD/YY)*
From 7/15/00 Through 4/30/01

7. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT
7a. Direct Costs ($) 9,818
7b. Total Costs ($) 9,818

8. APPLICANT ORGANIZATION
Name: Department of Environmental Health
University of Cincinnati College of Med
Address: 3223 Eden Ave. Cincinnati, OH 45267-0458

9. TYPE OF ORGANIZATION
Public: ⟶ ☐ Federal ☒ State ☐ Local
Private: ⟶ ☐ Private Nonprofit
Forprofit: ⟶ ☐ General ☐ Small Business

10. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name
Title James Lockey M.D., M.S.
Address Chairman, Div of Occ Med
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Cincinnati, OH 45267
Telephone
Fax 513-558-0030
E-mail 513-558-6272

11. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name
Title Grace K. Lemasters, Ph.D. FACE
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Cincinnati, OH 45267
Telephone
Fax 513-558-0030
E-mail 513-558-6272

12. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE:
I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF PI/PD NAMED IN 3a. *(In ink. "Per" signature not acceptable.)*
A. Gayle Rhodes

DATE
4 Jun 00

13. APPLICANT ORGANIZATION CERTIFICATION & ACCEPTANCE:
I certify that the statements herein are true, complete and accurate to the best of my knowledge and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF PI/PD NAMED IN 11. *(In ink. "Per" signature not acceptable.)*
Grace Lemasters

DATE
June 5, 00

1. SPECIFIC AIMS: My hypothesis is that exposure of aircraft tank entry workers to JP-8 jet fuel alters the number of circulating immune cells as compared to unexposed controls. The specific aims are to:

A. Identify exposed and unexposed workers at selected Air Force bases.
B. Determine immune cell counts in the peripheral blood.
C. Perform an epidemiological analysis of each subject group.

2. BACKGROUND: The worldwide consumption of jet fuel approaches 60 billion gallons annually.[1] The U.S. Department of Defense uses Jet Propellant fuel type eight (JP-8) at a rate of 3.5 billion gallons yearly, of which, the Air Force is the largest consumer. JP-8 is the battlefield fuel for all U.S. military operations well beyond the year 2025.[2]

JP-8 is a kerosene based fuel similar to commercial aviation fuel, but has military additives which include antioxidants, static inhibitors, corrosion inhibitors, fuel system icing inhibitors. lubrication improvers, biocides, and thermal stability improvers. In 1996 JP-8 replaced JP-4, a volatile, explosive, gasoline based fuel containing significant amounts of benzene. an A-1 carcinogen.[3]

In the Air Force, persons at most risk of high exposure to JP-8 are tank entry personnel. These persons must enter aircraft fuel tanks periodically for inspection or repair. Reticulated polyurethane foam is present in some tanks to prevent explosion of fuel/air mixtures that could be caused by electrical arcing, lightening strikes, or static electricity. The fuel tanks with foam are less likely to explode if struck by small arms ground fire. The foam prevents fuel sloshing within tanks and reduces the amount of fuel spray when a tank is ruptured in a crash. Fuel entry personnel wear respirators when inside the confined space of the tanks but wear cotton clothing rather than impermeable garments which are a risk in generating static electricity. Fuel, left as residual within tanks or released from foam during handling, is readily absorbed and deposited onto the skin of tank entry workers.

The Occupational Safety and Health Administration (OSHA) has not developed a permissible exposure limit but the Air Force has occupational exposure limits of 350 mg/cubic meter, Time Weighted Average (TWA) over 8 hours and 1,800 mg/cubic meter for short term exposures over 15 minutes. Tank entry personnel handling foam have been found to have exposures as high as 1,304 mg/cubic meter for an 8-hour TWA and 10,295 mg/cubic meter for a 15 min short-term exposure. [1,4]

After its introduction fuel handlers complained of objectionable odors, skin irritation, dizziness and the persistent taste of jet fuel long after exposure. A reference report, published in 1998, by the Center for Disease Control's Agency for Toxic Substances and Disease Registry, indicated the toxicities of jet fuel are not well defined. Data gaps exist in many areas to include the effects on the immune system.[5]

The immune system is responsible for regulatory responses to infection, cancer, autoimmune disease, and allergens. B-cells form antibodies. T-cells that consist of CD4 helper cells and CD8 suppressor cells modulate cell-mediated immunity. Natural Killer (NK) cells attack cancer cells.

The immunological effect of jet fuel has been studied in mice. Exposure to inhaled benzene produced a decreased ratio and absolute number of T and B-lymphocytes in the blood and spleen. The effect was dose dependent and resulted in a suppressed ability to form antibodies. Subpopulations of T-cells were not studied and jet fuel as a complex mixture was not evaluated.[6] The short term effects of mice exposed to JP-8, by inhalation, were a dose response

decrease in weights of the primary immunological organs (spleen and thymus), and a reduction in T-cell subpopulations in the lymph nodes. A decrease in circulating immune cells at low and high concentrations was noted whereas at medium concentrations the number of cells increased. T-cells were noted to decrease substantially in the peripheral blood but subpopulations were not determined.[7] Long-term effects of inhaled JP-8 were studied out to 28 days post-exposure. The weights of the spleen and thymus, initially decreased, then returned to normal, and finally increased. At an exposure of 2,500 mg/cubic meters, immune cell numbers in the peripheral blood were substantially decreased at 1, 7 and 21 days, but were not noted to be statistically different at 14 and 28 days. Again the subpopulations of immune cells were not delineated.[8] In experiments with mice exposed to JP-8, by dermal absorption, impairment in the induction of contact sensitivity and the generation of delayed-type hypersensitivity was noted when the mice were later challenged by antigens. Splenic T-cells were noted to have decreased proliferation rates when stimulated, indicating a reduction in the functional capacity of the immune system.[9]

In humans, few immunotoxicity studies have been reported. Lead exposed workers were evaluated with a comprehensive panel of immune system parameters and no major differences were noted in CD3 cells, CD4 T-cells, CD8 T-cells, B-cells or NK cells when compared to nonlead exposed workers.[10] In a pilot study of exposed and unexposed workers, during the conversion of JP-4 to JP-8, differences in the hematopoietic system were noted. Mean corpuscular hemoglobin and mean corpuscular volume were significantly lower in the exposed group while white blood cell counts and percent lymphocyte counts were not significantly different. The sample size was small (18 exposed and 18 unexposed) and the lymphocyte subpopulations were not studied.[11]

3. RELEVANCE TO NORA: JP-8 is a complex fuel that represents the most common mixed exposure among members of the Armed Forces. Immunotoxic effects have been demonstrated in mice but have not been adequately studied in humans. Information gathered in this study would help the National Institute of Occupational Safety and Health (NIOSH) recommend exposure limits not only for the military but also for civilian aviation.

4. METHODS AND DATA ANALYSIS: The study will be a cross-sectional design to examine the association between jet fuel exposure and alterations in the immune system. It will be an addendum to a larger Air Force study "Risk Assessment of Acute Exposure to Jet Fuel" which began in February 2000. This larger study is obtaining data on body burden, postural sway, neurocognition, and DNA damage and has participation from NIOSH, the Air Force, the Navy, the Universitites of Texas, North Carolina and Cincinnati. Dr. Grace Lemasters, my University of Cincinnati mentor, is one of the consultants for this larger Air Force study.

4.1 Description of the population – Exposed workers will be 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 will consist of Air Force personnel who do not routinely work with or have exposure (other than incidental) to fuels or solvents. Groups will be matched by gender and base of assignment. Exclusion criteria are history of autoimmune disease, cancer, diabetes, immune altering medication, and pregnancy.

4.2 Method for sampling the population – Sampling will be done by convenience. Volunteers will be chosen from those meeting eligibility criteria and will receive 50 dollars.

4.3 Sample size calculation – Calculations are based on a test of two proportions using independent, dichotomous samples taken from a study that found a 40% decrease in proportions

of CD3 cells of mice exposed to JP-8.8 Using a two sided alpha of .05, beta of .20, p_1(control) of .75 and p_2(exposed) of .44, the following formula is used to calculate sample size:

$$n = \frac{p_1(1-p_1) + p_2(1-p_2)}{(p_1-p_2)^2} \quad (Z_{\alpha/2} + Z_\beta)^2 \qquad (Z_{\alpha/2} + Z_\beta)^2 = 7.849$$

A sample size (n) of 36 will be required. Protocols for the larger study of which this is a part will provide 72 subjects per group. This will serve to increase the power to 95% or improve the p value to < .005. Females will represent a smaller proportion of subjects and will be matched two unexposed for each one exposed.

4.4 Description of independent and dependent variables – The independent variables are exposure to JP-8, gender and age. The dependent variables will be proportions and total counts of peripheral blood immune cells: B-cells (CD19), Natural Killer cells (CD56), T-cells (CD3). Helper T-cells (CD4) and Suppressor T-cells (CD8).

4.5 Method for data collection - Three Air Force bases will participate in the study, each one visited for four days over three consecutive months. Data will be collected at the beginning of the first worker shift. Twelve subjects will be processed each of four days, Monday through Thursday. Phlebotomy will be performed and 5 ccs of venous blood will be collected in EDTA tubes for flow cytometry studies. Specimens will be randomly numbered for blinded analysis. The tubes of blood will be kept at room temperature and shipped for testing to occur within 24 hours of collection. A questionnaire will be completed to ascertain epidemiological data and medical records will be reviewed to code applicable diagnoses.

4.6 Reliability issues – Specimens must be analyzed within 24 to 36 hours before cells can lyse. Flow cytometry requires intact cells as each cell is counted individually. Specimens will be shipped in approved containers overnight to an arranged lab that is certified to perform flow cytometry. The lab will process the specimens immediately.

Diurnal variations in immune parameters can occur. By limiting the blood draws to a two hour window at the same time each day for all participates this problem should be eliminated.

4.7 Validity issues – The parameters being studied are the same as those followed in persons with Acquired Immune Deficiency Syndrome (AIDS). AIDS is the classic immune disease and lymphocyte counts, particularly CD4, are the gold standard in accessing this disease.

4.8 Bias issues – Selection bias can occur when sampling is by convenience. Exposed personnel with perceived health problems may be more likely to volunteer and give an over-representation of the exposed group.

Confounders are age, race, smoking, alcohol, marijuana, more than 5 hours of sun exposure in the past week, loss of sleep, cold or allergy symptoms within the past week and use of medications that can alter the immune system. Several confounders will be eliminated by restriction in the design phase (see exclusion criteria). Data on those remaining will be collected by questionnaire and evaluated by stratification in the analysis of data.

4.9 Methods for data analysis – The student t test will be used to test the hypothesis of differences in proportions and means of the populations. Multivariate analysis will be used to examine the role of risk factors and multiple regression to correlate dose of JP-8 with immune cell counts.

4.10 Limitations: Cross-sectional designs can discover associations but cannot provide proof of causation. This design, however, can provide significant findings in a timely manner. Convenience sampling will be used rather than the usually preferred random sampling. It is

important, particularly at a military base, to use volunteers rather than give the appearance of forced participation. Immune cell counts will be measured but their functional capabilities will not be tested.

5.0 EXPECTED RESULTS: I expect to see a decrease in total T-cells and CD4 cells. In comparing body burden information gathered during the larger Air Force study I anticipate being able to construct a dose response curve.

6.0 FUTURE DIRECTION: As a follow-up study immune function will be evaluated. New recruits to the Air Force slotted for fuel handling will have baseline data gathered before being exposed to jet fuel. The ability to create antibodies to periodic required immunizations such as influenza and anthrax will be determined and compared with controls.

7.0 TIMETABLE:

10-14 July 2000	Collect data at Pope AFB, NC
14-18 Aug 2000	Collect data at Little Rock AFB, AR
11-15 Sep 2000	Collect data at Eglin AFB, FL
31 Dec 2000	Complete analysis of data
31 Mar 2000	Complete write-up of project

REFERENCES

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2. Smith, L.B. "Risk Assessment of Acute Exposure to Jet Fuel", Proposal # F-BR-200-0016-H, IERA/RSHI, Brooks AFB, TX, 3 Jan 2000.
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5. ATSDR. "Toxicological Profile for Jet Fuels JP-5 and JP-8", Agency for Toxic Substances and Disease Registry, CDC, Atlanta GA, 1998.
6. Aoyama, K. "Effects of Benzene Inhalation on Lymphocyte Subpopulations and Immune Response in Mice", *Toxicology and Applied Pharmacology* 85, 92-101 (1996).
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MENTOR-BASED POSTDOCTORAL FELLOWSHIP PROGRAM APPLICATION - 1999

1. APPLICANT INVESTIGATOR

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POSITION: Associate Professor

INSTITUTION: University of Cincinnati

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2. Social Security #: 167-68-1881

3. Citizen: Yes X No
If not a U.S. citizen, do you have permanent resident status? Yes No

4. Are you a member of ADA's Professional Section? Yes X No

5. Name, title, address, telephone number and signature of responsible financial officer to whom funds should be sent, and who will keep a full account of disbursements:

Name, Title: John Michnowicz, Director, Office of Sponsored Programs

Signature: 9/25/98

Address: University of Cincinnati
PO Box 670053
Cincinnati, OH 45267-0553

Telephone Number: (513) 558-3683 Fax Number: (513) 558-3954

ABSTRACT:

Polycystic ovarian syndrome (PCOS) is a highly prevalent (~5-10% of women) and poorly understood condition associated with (diabetes type II and gestational), dyslipidemias, obesity, and endometrial cancer, with equivocal evidence for increased rates of miscarriages, hypertension, coronary artery disease, ovarian and breast cancer. As many as 40 % of these women develop type II diabetes, and up to 52% of diabetic women fit the PCOS profile. Affected women who manifest both insulin resistance and hyperandrogenism (30 - 75 %) are also believed to be at greatest risk for health complications. Observational and intervention study evidence links insulin resistance to the etiology of most PCOS cases, and hyperandrogenism has been called the *sine qon non* of the syndrome. Despite consensus regarding a strong genetic component to the risk of PCOS, and the apparently key roles of both insulin resistance and hyperandrogenism, only one investigation of five families has published both insulin (twenty-one female and seven male relatives) and androgen levels (in women) among family members. Poor characterization of these parameters among relatives is an impediment to genetic studies of PCOS. The fellow's primary aim, therefore, will be to describe endocrine (glucose, insulin, androgen) and clinical phenotypic features among 1st degree, female and male relatives of PCOS probands and controls, and to contrast their profiles in preparation for a future genetic study. The primary hypothesis is that insulin and testosterone levels will be significantly ($p \leq 0.05$) elevated among the PCOS probands and their 1st degree relatives compared with control women and their relatives.

Appendix VII: Report References

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Appendix VIII: Paid Personnel

List of Paid Personnel (1996-2000):

Staff:
Grace Lemasters, Ph.D., P.I.
James Lockey, M.D., M.S., Co-investigator
Donna Olsen, Ph.D., Research Associate
Susan Reutman, M.P.H., Project Coordinator/Student
Hwa-Chung Yiin, M.S., Programmer/Student
John Lu, M.S., Programmer
Zachary Zivkovich, Programmer

Graduate Student Helpers/Assistants:
Lori Gordley, M.S.
Angela Booth-Jones, M.S.
Kelly Worley

Student Assistants:
Mark Mercurio
Abhirup Bhattacharya
Patrice Flowers
Huifang Chen
Kimberly Rickard
Wenzhong Tang
Traci Walker

Contractors:
Dennis Meyers, MS, Analysis Contractor
Three Interview Contractors

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
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REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 1 Apr 03

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession document numbers be changed to "Approved for public release; distribution unlimited." Copies of these reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

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