Evidence of Reproductive Endocrine Effects in Women with Occupational Fuel and Solvent Exposures

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Hydrocarbons (HCs) found in fuels and solvents are ubiquitous in the environment, yet we know little about their effects on the endocrine system. The objective of this study was to assess the potential reproductive endocrine effects of low-dose HCs encountered by female U.S. Air Force personnel with fuel (primarily JP-8 jet fuel) and solvent exposures (n = 63). We estimated the internal dose of HCs in fuels and solvents by measuring their levels in exhaled breath, including the sum of aliphatic HCs (C_6H_{14}–$C_{16}H_{34}$) and the sum of aromatic HCs (benzene, ethylbenzene, toluene, and *m,p,o*-xylenes). Adverse outcome measures included urinary endocrine markers that have been associated with nonconceptive (vs. conceptive) menstrual cycles in ovulatory women: lower preovulatory luteinizing hormone (LH) and mid-luteal phase pregnanediol 3-glucuronide (Pd3G) and estrone 3-glucuronide, and higher follicle phase Pd3G. We also obtained reproductive and exposure information from baseline questionnaires and daily diaries. Toluene was the most frequently found analyte in the breath, with values up to 52.0 ppb, and benzene breath levels were up to 97.5 ppb. Regression analysis revealed that preovulatory LH levels were significantly lower (p = 0.007) among women whose total aliphatic HC levels were above the median. The relationship between elevated aliphatic HC exposure and lowered preovulatory LH levels in the present study suggests that compounds in fuels and some solvents may act as reproductive endocrine disruptors. Confirmation of these findings is needed, not only to determine if fuel and solvent exposure may impact other LH-dependent physiologic functions but also to examine effects of fuels and solvents on conception. *Key words*: epidemiology, estrogen, fuel, hydrocarbon, luteinizing hormone, military, progesterone, reproduction, solvent. *Environ Health Perspect* 110:805–811 (2002). [Online 25 June 2002]
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Evidence has accumulated that hydrocarbons (HCs) in fuels and solvents are reproductive toxicants. For example, reductions in female fertility have been identified in occupational groups exposed to organic solvents containing benzene (Cho et al. 2000), toluene (Plenge-Bönig and Karmaus 1999), and mixtures of solvents (Sallmén et al. 1995; Smith et al. 1997a). Fuels, solvents, and their constituent chemicals are ubiquitous exposures. Contact may occur during routine home or workplace activities, such as refueling automobiles, mowing lawns, or painting, refinishing, or degreasing. Among those occupational populations at risk of high-level exposures to fuels and solvents were farmers, mechanics, maintenance workers, printers, petroleum refinery workers, metal cleaners, painters, and aircraft maintenance personnel.

Approximately 116,000 women work for the U.S. Air Force (USAF) as officers, enlisted personnel, and civilians (Air Force Personnel Center, Randolph AFB, 2000). Women have held 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 fuels such as JP-8 (jet fuel) and diesel; a variety of solvents, including toluene and xylene; and products of fuel combustion. Furthermore, gender differences in exposure, toxicokinetics, and physiologic responses may affect susceptibility to the potential effects of fuel and solvent exposure. Pharmacokinetic modeling revealed that women metabolize 23–26% more benzene than do men under the same exposure conditions and therefore may have different responses to exposure than do men (Brown et al. 1998). Historically, over 3 billion gallons of jet fuel have been issued annually to the U.S. Department of Defense (Directorate of Resources Management 1999). 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 (Pleil et al. 2000; Smith et al. 1997b). 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 (ATSDR 1993; Ikeda 1992; Lemasters et al. 1997). Fuel and solvents may be encountered separately or as mixtures during job activities such as aircraft maintenance.

The purpose of this study was to assess the potential effects of fuel and solvent exposure on menstrual cycle function. We monitored specific endocrine end points that are predictive of conceptive menstrual cycles (Baird et al. 1999) as subclinical markers of female reproductive dysfunction to identify early, subtle reproductive effects of low-dose exposures to solvents and fuels.

Materials and Methods

Study population. The study population consisted of female USAF employees. We obtained approval from the University of Cincinnati and the USAF prior to initiation of recruitment. We recruited potential participants for initial interviews by phone and in person at 10 USAF bases. Eligibility criteria included age between 18 and 42 years and requirements that the subject had not used hormonal medications, oral contraceptives, or hormone replacement for 3 months; had not used an intrauterine device during the past 3 months; had no surgery, other than tubal ligation, on reproductive tissues; had not been pregnant or breast-feeding for 3 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. We targeted nonsmokers; however, we also included a small subset of smokers (n = 8). Of the civilian and active military women employed at the 10 USAF bases who were contacted, 335 were preliminarily eligible during recruitment screening. Of these women, 51% (n = 170) provided informed consent and participated by maintaining daily diaries and collecting daily urine samples, and were confirmed as eligible during the baseline interview.

Initial participant interview and diary collection. During the initial interview, we

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explained the study procedures, eligibility criteria, and the voluntary nature of participation to the potential participants and we obtained informed consent. Next, we administered the baseline questionnaire to collect information about their work, socioeconomic status, pregnancy, lifestyle, major life events (Horowitz et al., 1977), and reproductive and menstrual histories. Results of the menstrual history are reported elsewhere (Gordley et al. 2000). We provided instructions for collecting daily urine samples and for completing daily diaries starting the day after the initial interview. We also measured weight and height.

We asked participants to immediately begin maintaining their diaries daily and to continue through the end of their second postinterview menstrual period. The diary requested menstrual, psychosocial, lifestyle, work, chemical and physical exposures, and sample collection information. We measured job strain within the diaries using an abbreviated adaptation of the Job Content Questionnaire developed by Karasek et al. (1998). Participants mailed their diaries to investigators upon completion.

Initial interview and daily diary information abstracted to construct potential covariates for preliminary analyses included age, income group, marital status, education, race, body weight, body mass index (BMI = weight in kilograms ÷ height in square meters), alcoholic beverage consumption, coffee and caffeine consumption, cigarettes smoked, second-hand smoke exposure, episodes of illness and/or fever > 101°F, major life events, job strain, hours worked, shifts worked, miles run and walked, hours slept, physical activity at home and work, exposure to cold temperatures, and fuel and solvent exposure.

Endocrine data analyses. We collected first morning urine samples daily concurrent to maintaining the diaries. Participants stored the samples in home freezers; samples contained 7% glycerol to prevent loss of hormonal activity (Kesner et al. 1995). Participants shipped frozen samples with freezer packs to the National Institute for Occupational Safety and Health (NIOSH) laboratory by next-day courier. We stored samples in the laboratory at –80°C until assayed.

We ascertained menstrual periods from the participants' daily records of vaginal bleeding based on a modification of a previously described menstrual algorithm (Hornsby 1991). The first day of the menstrual cycle was the first of 2 consecutive days of bleeding, only one of which could be spotting. We counted 1–2 day interruptions in bleeding (nonbleeding or spotting) that occurred after day 2 of the period together with bleeding days as part of the menses. Menses were preceded and followed by 3 or more consecutive days of nonbleeding or spotting. We contacted participants with missing diary menstrual bleeding entries immediately regarding menses dates and accepted reported menses dates up to 14 days retrospectively.

We used urinary endocrine measurements and menses dates to derive the four key endocrine end points using established algorithms (Baird et al. 1999; Baird 1999). 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), midluteal Pd3G, and possibly midluteal estrone 3-glucuronide ($E_1$3G). Therefore, we selected these end points *a priori* for analysis.

We assayed the major urinary metabolites of estradiol and progesterone (i.e., $E_1$3G and Pd3G) using competitive, double-antibody time-resolved fluoroimmunoassays (Kesner et al. 1994b). We assayed urinary LH using a commercial noncompetitive, two-site, time-resolved immunofluorometric assay (Kesner et al. 1998, 1994a). Detailed characteristics of these assays, including specificity, sensitivity, and precision, are described in their respective references (Jaffe 1886; Kesner et al. 1994a, 1994b, 1998). We measured creatinine spectrophotometrically (Jaffe 1886) and divided each urinary endocrine value by the creatinine concentration to adjust for urine dilution. We measured all samples for each participant in the same assay. Intra-assay and interassay coefficients of variation for urinary endocrine measurements were, respectively, 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 (Lemasters et al. 1999a). Therefore, we estimated internal doses of aliphatic and aromatic HCs from solvents and fuels in mixed-exhaled breath samples collected from 63 participants. To estimate analyte levels in the vessel-rich tissue compartment, we collected a breath sample during the initial interview, 1.4 hr (SD = 2.2), on average, after participants left the work site (Pellizzari et al. 1992) on the second to fifth consecutive workday. For one additional participant with no workday sample, we substituted a Monday (first workday) sample as a proxy measurement for this analysis (total n = 63). We collected breath samples through desiccant filters into Tedlar bags (SKC Inc., Eighty Four, PA) and then suctioned them into sorbent charcoal tubes 1.5 hr (SD = 1.4), on average, after collection.

In the laboratory, we concentrated breath sample analytes by thermal desorption of the sorbent tube contents onto the charcoal bed of a Tekmar 3000 Purge and Trap (Tekmar-Dohrmann, Mason, OH). We then flash-heated the collected analytes to 225°C and released to the heated nickel transfer line under a constant back-pressure. We directly connected the transfer line to the column (DB-VRX, J&W Scientific, Folsom, CA) with a zero dead volume union. We analyzed the material with a Hewlett-Packard Model 5890 Series 2 gas chromatograph (Hewlett-Packard, Wilmington, DE), equipped with a flame ionization detector optimized for detection of aromatic HCs, including benzene, toluene, ethylbenzene, and *m,p,o*-xylenes (BTEX). We performed quantitation using a Hewlett-Packard Model 3396 Integrator and an EZ Chrom data system (Scientific Software Inc., Pleasanton, CA).

We analyzed the samples in two batches and quantified aliphatic C_6H_{14}–$C_{16}H_{34}$ (C_6–C_{16}) and aromatic (BTEX) HC levels as area under the curve (AUC) for all 63 samples. We calculated sample concentrations for AUC corresponding to 0.5 ppb, although any individual sample had a 1% statistical chance that background could have been at the 1 ppb level. We derived conversion factors for transforming the EZ Chrom output from AUC to parts per billion of each BTEX analyte from calibration samples for all study samples (n = 63). These conversion factors demonstrated adequate linearity for the aromatic analytes; goodness of fit ranged from an r^2 of 0.88–0.98 based on 10, 25, and 50 ppb calibration samples. We examined the total BTEX exposure variable as a continuous parts per billion variable and as a dichotomous variable above and below the median parts per billion value in statistical analyses. After publication of a report describing JP-8 volatile fraction "fingerprint compounds" (Pleil et al. 2000), we began deriving parts per billion conversion factors for aliphatic HCs and obtained parts per billion values for a subset of study samples (n = 22). We developed a standard gas in the laboratory to create conversion factors for the aliphatic HCs; goodness of fit was adequate (r^2, 0.87–0.99 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, we converted aliphatic HC levels (except C_{12} and C_{14}) to parts per billion for the second analysis batch of 22 samples. We used only AUC measurements, not parts per billion, in the statistical analysis of aliphatic HCs for the 63 breath samples; we used the aliphatic HC parts per billion levels only to present range of exposure. We dichotomized the total AUC for aliphatic HCs for each of the two analysis batches (above or below the

median AUC for each batch) and then combined the two batches into a single dichotomous aliphatic HC variable. Because C_6–C_{16} measurements were only available in parts per billion units for a convenience sample of 22 participants, we did not explore analysis with a combined breath exposure variable (aliphatic HCs + BTEX).

For quality control, we collected duplicate breath samples in immediate succession from 13 participants. We analyzed these samples and compared the duplicate measurements. Intra- and interparticipant sample coefficients of variation for exhaled breath measurements in parts per billion were, respectively, 3.6% and 0.0% for benzene, 1.8% and 0.8% for toluene, 5.1% and 0.0% for ethylbenzene, 3.5% and 0.0% for *m,p*-xylene, and 1.2% and 1.0% for *o*-xylene. We assigned the duplicate, end-of-shift breath sample levels from the 13 participants to the same aliphatic HC and BTEX exposure groups (high/low) approximately 70% of the time, indicating relatively high comparability among samples.

Statistical analysis. We conducted statistical analyses of the potential relationship between endocrine outcomes (preovulatory LH, follicular Pd3G, midluteal Pd3G, and midluteal $E_1$3G) and exposure variables on two groups of participants: *a*) the 100 participants with endocrine and recorded diary data and *b*) for 63 of the 100 participants with breath analysis data. For the bivariate analysis of data from the women who reported diary and baseline questionnaire information (n = 100), we defined exposure using three reported exposure variables: *a*) mean weekly hours reported as smelling or having skin contact with fuels, *b*) mean weekly hours reported as smelling or having skin contact with solvents, and *c*) job category (exposed vs. nonexposed) based on self-report during the initial interview and review of job titles, job codes, and descriptions by USAF 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 (parts per billion), each dichotomized above and below their respective medians.

Square root transformation optimally transformed the hormonal outcomes. The variable selection strategy for regression analysis involved examining bivariate associations between each of the four endocrine end points (transformed and nontransformed) and other potential covariates. We assessed covariates that approached significance ($p \leq 0.15$) with at least one endocrine level in unadjusted analysis bivariately as potential confounders with fuel and BTEX exposure variables. We retained those approaching marginal significance ($p < 0.15$) with exposure variables 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 intercorrelation between candidate covariates was present, or when two or more covariates represented similar constructs, we used the variable with the most significant association with the outcome(s).

Covariates retained for regression models containing reported hours of fuel or solvent exposure included: age at interview (years), BMI, race (white, nonwhite), alcoholic beverages (number of drinks per day per kilogram body weight), coffee consumption (estimated mean milligrams of caffeine per day from coffee), caffeine consumption (mean milligrams total caffeine estimated from mean ounces of coffee, tea, and soda recorded per day in diary), running (mean miles run per day), sleep (mean hours per day), any episodes of illness and/or fever > 101°F (yes/no), maximum weekly job strain score (high vs. low), and currently smoking cigarettes (yes/no). We conducted separate regression analyses for each reported exposure variable, and we forced each exposure variable to remain in the final model. We conducted multiple regression analysis of each endocrine outcome 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°F, alcoholic beverages, maximum job strain, race group, and age. We conducted multiple regression analysis of each endocrine outcome as described above, with both breath exposure variables forced to remain in the regression models.

Results

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

Demographic and reproductive characteristics. Table 1 describes demographic and reproductive history characteristics for the 100 eligible participants who provided

Table 1. Demographic and reproductive characteristics of participants with endocrine data (n = 100) and with endocrine and breath data (n = 63/100) by aliphatic HC group.

		Endocrine and breath data (n = 63)		
Participant characteristic	Endocrine data (n = 100)	Low aliphatic (n = 31)[a]	High aliphatic (n = 32)[a]	Combined (n = 63)
Mean age at interview (years)	30.9 ± 6.2 (18.0–41.0)	30.5 ± 5.7 (20.0–39.0)	31.7 ± 5.5 (20.0–40.0)	31.1 ± 5.6 (20.0–40.0)
Mean age at menarche (years)	12.7 ± 1.5 (9.0–17.0)	12.8 ± 1.6 (10.0–17.0)	12.8 ± 1.5 (9.0–16.0)	12.8 ± 1.5 (9.0–17.0)
Race (%)				
White	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 (%)				
High school and/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 (%)[b]				
< $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
Military personnel status (%)	84.0	87.1	71.9	79.4
Marital status (%)[b]				
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

Values shown for age variables are mean ± SD (range). Chi-square tests were used except for age variables, which were examined using *t*-tests.
[a]No significant differences ($p \leq 0.05$) between above groups by low and high fuels category. [b]One participant had missing information (n = 99).

questionnaires, diaries, and daily urine samples and for the 63 participants with breath sample data. In both groups, the average age of respondents was approximately 31 years. In both groups, respondents were predominantly white, military, and married, had children, had attended some college, and had annual household incomes of at least $30,000, and most had no history of irregular menses or dysmenorrhea within 3 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 was statistically significant.

Internal dose (breath) analysis. Tables 2 and 3 present individual C_6–C_{16} and BTEX HCs measured in postshift breath samples, grouped by low (n = 32) versus high (n = 31) analysis categories. 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 ethylbenzene (3.0 ppb). When we combined the high- and low-BTEX groups, 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 one-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} groups. Many samples also contained octane, nonane, and undecane in both high and low C_6–C_{16} groups. This convenience sample of 22 contained a higher proportion of women reportedly in exposed jobs (40%) than did the larger group of 63 (23%) and therefore was most representative of a more highly exposed setting.

Endocrine assessment. Mean levels of the four study endocrine end points for all the 100 participants who provided both diaries and concurrent daily hormonal data during the study menstrual cycle were similar above and below the median for each of the reported exposure variables. Furthermore, we found no significant ($p \leq 0.05$) difference in endocrine levels between self-reported exposed versus nonexposed participants when examined bivariately or in multivariable regression models including potential confounders and covariates.

Figure 1 illustrates mean endocrine levels across the menstrual cycle for participants' high and low aliphatic HC (C_6–C_{16}) levels and highlights the four study end points. Table 4 presents mean urinary levels of these four end points 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 significance in bivariate analysis for midluteal Pd3G (8.5 vs. 12.0 µg/mg creatinine; p = 0.06).

We modeled each of the four endocrine outcomes in separate multiple regressions (Table 5). To aid in interpretation, we obtained values reported for regression

Table 2. Breath levels (ppb) of aromatic HCs by analyte and exposure group.

BTEX analyte	Low (n = 32)	High (n = 31)	Total (n = 63)
Benzene (C_6H_6)			
Mean ± SD (median)	0.5 ± 1.6 (ND)	13.0 ± 27.5 (ND)	6.6 ± 20.2 (ND)
Range	ND–8.6	ND–97.5	ND–97.5
Percent of samples	18.8	41.9	30.2
Toluene ($C_6H_5CH_3$)			
Mean ± SD (median)	1.3 ± 2.2 (0.1)	9.0 ± 12.3 (5.1)	5.1 ± 9.5 (1.5)
Range	ND–7.5	ND–52.0	ND–52.0
Percent of samples	50.0	93.5	71.4
Ethylbenzene ($C_6H_5C_2H_5$)			
Mean ± SD (median)	1.0 ± 0.5 (ND)	3.0 ± 6.9 (ND)	1.5 ± 5.0 (ND)
Range	ND–2.7	ND–35.7	ND–35.7
Percent of samples	9.4	35.5	22.2
m,p-Xylene [$C_6H_4(CH_3)_2$]			
Mean ± SD (median)	0.8 ± 1.2 (ND)	37.3 ± 85.6 (3.8)	18.7 ± 62.3 (0.8)
Range	ND–4.4	ND–400.9	ND–400.9
Percent of samples	40.6	74.2	57.1
o-Xylene [$C_6H_4(CH_3)_2$]			
Mean ± SD (median)	1.0 ± 2.0 (ND)	11.3 ± 15.0 (7.2)	6.1 ± 11.7 (ND)
Range	ND–6.9	ND–67.3	ND–67.3
Percent of samples	28.1	67.7	47.6
Total BTEX [C_6H_6–$C_6H_4(CH_3)_2$]			
Mean ± SD (median)	3.8 ± 3.8 (2.9)	73.5 ± 86.2 (32.4)	38.1 ± 69.6 (11.7)
Range	ND–11.7	11.8–415.1	ND–415.1
Percent of samples	81.3	100.0	90.5

ND, nondetectable was assigned the value of zero for calculation of means and SDs.

Table 3. Breath levels (ppb) of aliphatic HCs by analyte and exposure group.

Aliphatic analyte	Low (n = 11)	High (n = 11)	Total (n = 22)
Hexane (C_6H_{14})			
Mean ± SD (median)	17.6 ± 8.6 (19.6)	51.4 ± 62.9 (35.9)	34.5 ± 47.1 (25.9)
Range	ND–28.5	11.7–238.7	ND–238.7
Percent of samples	90.9	100.0	95.4
Heptane (C_7H_{16})			
Mean ± SD (median)	3.7 ± 9.8 (0.3)	35.4 ± 75.3 (4.4)	19.5 ± 54.9 (0.6)
Range	ND–33.0	ND–248.7	ND–248.7
Percent of samples	63.6	63.6	63.6
Octane (C_8H_{18})			
Mean ± SD (median)	0.2 ± 0.4 (ND)	0.6 ± 1.8 (ND)	0.4 ± 1.3 (ND)
Range	ND–1.2	ND–6.0	ND–6.0
Percent of samples	18.2	27.3	22.7
Nonane (C_9H_{20})			
Mean ± SD (median)	5.3 ± 10.9 (ND)	4.5 ± 9.1 (ND)	4.9 ± 9.8 (ND)
Range	ND–29.2	ND–27.0	ND–29.2
Percent of samples	36.4	45.4	40.9
Decane ($C_{10}H_{22}$)			
Mean ± SD (median)	34.6 ± 23.5 (28.4)	159.1 ± 237.4 (14.8)	96.9 ± 176.6 (23.9)
Range	12.8–76.8	2.8–659.7	2.8–659.7
Percent of samples	100.0	100.0	100.0
Undecane ($C_{11}H_{24}$)			
Mean ± SD (median)	8.8 ± 14.6 (ND)	28.7 ± 68.9 (ND)	18.7 ± 49.7 (ND)
Range	ND–45.0	ND–226.7	ND–226.7
Percent of samples	36.4	36.4	36.4
Total C_6H_{16}–$C_{11}H_{24}$			
Mean ± SD (median)	70.1 ± 31.3 (63.4)	279.6 ± 276.2 (170.8)	174.9 ± 219.8 (66.6)
Range	28.6–128.0	39.5–765.1	28.6–765.1
Percent of samples	100.0	100.0	100

ND, nondetectable was assigned the value of zero for calculation of means and SDs.

coefficients in Table 5 by applying the same models with nontransformed outcomes. High versus low exposure to C_6–C_{16} HCs was inversely related (β = –7.34; p = 0.007) to preovulatory LH adjusted for age (β = 0.49; p = 0.05) and exposure to BTEX. C_6–C_{16} and BTEX categories were not significantly associated with changes in midluteal $E_1$3G, or with follicular or midluteal Pd3G levels.

Illness and/or fever > 101°F was associated with elevated midluteal $E_1$3G (β = 8.93; p = 0.01). Other potential covariates and confounders were not associated ($p \geq 0.05$) with any of the hormonal outcomes after adjustment. When analyzed as continuous variables, neither total BTEX nor toluene, a component of BTEX, was significantly ($p \leq 0.05$) associated with any of the hormonal levels. However, toluene approached significance (β = –0.19; p = 0.058) with preovulatory LH in a model together with C_6–C_{16} (β = –7.17; p = 0.01) and age (β = 0.51; p = 0.04).

Discussion

We selected four urinary endocrine end points (preovulatory LH, midluteal $E_1$3G, follicular Pd3G, and midluteal Pd3G) based on heuristic evidence that they are jointly predictive of the probability of conception in women within a given ovulatory menstrual cycle (Baird et al. 1999). We found that preovulatory LH in urine was lower in healthy, reproductive-age women who had higher internal doses of aliphatic HCs in exhaled breath. Although we performed multiple statistical tests, the association between LH and aliphatic HCs remained significant ($p \leq 0.013$) after we applied a Bonferroni correction for the four separate hormone models.

In an examination of ovulatory cycles, Baird et al. (1999) reported that nonconceptive versus conceptive cycles had urinary preovulatory mean LH levels of 13.4 versus 15.2 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. Although we used the same assay to measure LH in both of these studies, they are not directly comparable quantitatively. The urine samples from the present study contained 7% glycerol to preserve LH immunoreactivity (Kesner et al., 1995), whereas those used by Baird et al. (1999) had been stored frozen for many years without preservative. So although qualitative comparisons are valid within Baird et al.'s study, the LH values in that study are expected to be lower than those in the present study. Because these preovulatory LH levels cannot be directly compared, whether the lower levels seen among women in the high

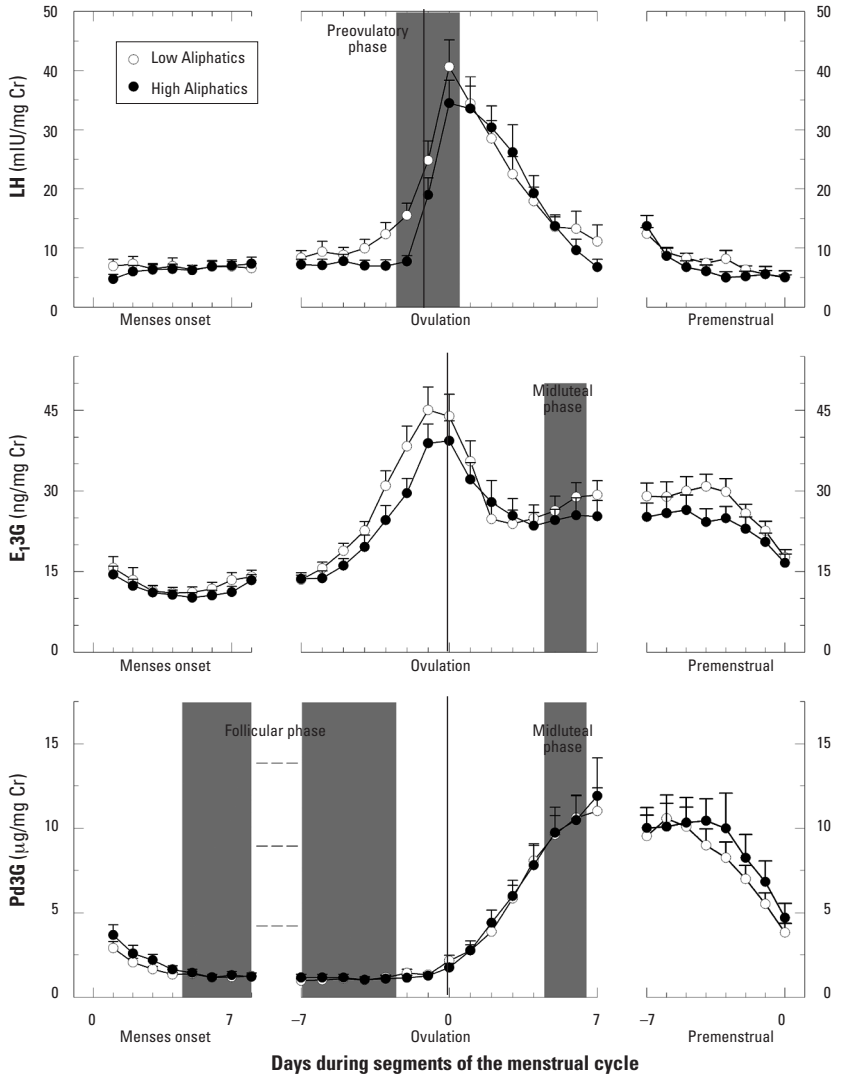

Figure 1. Crude concentrations of urinary LH, $E_1$3G, and Pd3G (creatinine-adjusted) during the menstrual cycles of female USAF personnel with low (n = 31) and high (n = 32) total aliphatic HC exposure. Cr, creatinine. Values (means ± SE) are plotted relative to menses onset and the estimated day of ovulation (blue line) as defined in "Materials and Methods." Shaded regions highlight the four endocrine end points examined. (Although follicular-phase Pd3G represents a series of contiguous days, the shaded region is graphically split across segments). The low and high aliphatic groups include women with breath aliphatic HC levels below and above the median for the AUC. Regression analysis revealed that preovulatory LH levels were lower (p = 0.007) among women with high aliphatic HC levels.

Table 4. Unadjusted endocrine outcomes: means by breath aliphatic and BTEX HC exposure groups.

Endocrine outcome/ exposure	Endocrine level Mean ± SD	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 (µg/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
Midluteal Pd3G (µg/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
Midluteal $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

Cr, creatinine.
*Significance (p = 0.01) between high and low categories in unadjusted, bivariate analysis (t-test). **Significance (p = 0.03) between high and low categories in unadjusted, bivariate analysis (t-test). #Significance (p = 0.06) between high and low categories in unadjusted bivariate analysis (t-test).

aliphatic HC exposure group were sufficiently low to affect conception is unclear. We did not design the present study to detect conceptions, and it did not have the power to do so. Studies designed to detect potential effects of aliphatic HC exposure on the ability to conceive and maintain pregnancies are indicated.

The mechanism by which aliphatic HCs could lower LH levels is unknown. LH 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 (Andersson et al. 1980, 1981), 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 nonsignificant ($p > 0.05$) 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 neurologic (ATSDR 1993; Grasso 1988; Knave et al. 1979; Langman 1994; Smith et al. 1997b) and sensory-neural (Kaufman 1998; Morata et al. 1997) effects of solvents have been documented at low and high analyte levels. Subclinical and clinical central nervous system effects have been reported to manifest at ambient levels as low as 0.07–5 ppm (Langman 1994). The only human studies of the effects of HCs on gonadotropins, to our knowledge, were of toluene exposure (Luderer et al. 1999; Svensson et al. 1992a, 1992b). In the present 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 (Svensson et al. 1992b). This effect was reversed after a 4-week nonworking period, after which blood toluene levels dropped and follicle-stimulating hormone and LH rebounded 37.5% and 29.9%, respectively (Svensson et al. 1992a). Results from these studies, mostly of men, are salient because LH controls the secretion of sex hormones in both genders. In women, LH is essential for ovulation and luteinization.

Although 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 because women in fuel handling, flight line, and maintenance jobs reported more hours of exposure than women in "nonexposed" 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, although 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 participants 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 nonexposed participants in the present study were generally higher than for USAF workers with solvent and fuel contact in other studies (Lemasters et al. 1997; Pleil et al. 2000) 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) (Wallace et al. 1996), except for decane, which was higher in our subgroup. Differences in reported exposure and breath analyte levels could also be attributed to using a single breath sample to represent typical exposure levels. This discrepancy could also be due to interindividual differences in analyte retention times; however, retention times are unlikely to differ systematically between the breath exposure groups.

Overrepresentation of women with concerns about their reproductive health in the final study population could have resulted in bias due to self-selection and nonresponse. We noted no apparent evidence of such bias-however. We observed no significant differences between women in exposed versus nonexposed jobs regarding the proportion of women who reported preexisting reproductive symptoms (dysmenorrhea, irregular cycles, infertility), although only large differences in these nonhormone variables would be detectable statistically, given our small group sizes. Further, only one of the women reported having a reproductive condition (fibroids), and none reported endometriosis or polycystic ovarian syndrome. Self-reported conditions should be interpreted with caution because women are sometimes unaware of early, often subclinical, pregnancy losses and undiagnosed reproductive abnormalities. Prevalences of severe dysmenorrhea (31%) and abnormal (< 24 or > 35 days) cycle lengths (12%) were previously reported to be similar between the larger study group of 170 participants and the general population (Gordley et al. 2000). The subset of 63 of the 170 participants had comparable prevalences of severe dysmenorrhea (37%) and abnormal cycle lengths (13%).

Gender differences in the effects of these HCs 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 HCs (Brown 1998). The focus of the present 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 (Lemasters et al. 1999b) and may increase the rate of sperm anomalies in men.

Table 5. Results of multiple regression of each of the endocrine outcomes.

Endocrine outcomes	Model[b]	Covariates[a]						
		Aliphatics	BTEX	Age	Race group	Illness/fever	Alcohol	Job strain
Preovulatory LH ($n = 58$)	$F = 5.28$ $p = 0.003$	β = –7.34 (2.60)[c] $p = 0.007$	β = –4.61 (2.59) $p = 0.10$	β = 0.49 (0.24) $p = 0.05$	—	—	—	—
Follicular Pd3G ($n = 62$)	$F = 0.46$ $p = 0.64$	β = 0.04 (0.20) $p = 0.89$	β = –0.10 (0.20) $p = 0.34$	—	—	—	—	—
Midluteal Pd3G ($n = 58$)	$F = 1.68$ $p = 0.20$	β = 1.04 (1.79) $p = 0.51$	β = –3.59 (1.79) $p = 0.08$	—	—	—	—	—
Midluteal $E_1$3G ($n = 58$)	$F = 2.79$ $p = 0.05$	β = –2.79 (3.18) $p = 0.34$	β = –2.73 (3.38) $p = 0.32$	—	—	β = 8.93 (3.37) $p = 0.01$	—	—

[a]Aliphatics and BTEX were retained in each final model; other covariates in the full models (age, maximum job strain, illness/fever > 101°F, alcoholic beverages, race group) were not retained in any of the final models because $p > 0.05$ (—); p-values were generated from transformed models, and β-values from untransformed models. [b]Adjusted model r^2: LH = 0.18, $E_1$3G = 0.09; follicular Pd3G = 0.00; midluteal Pd3G = 0.02. [c]SEs of the β-values are shown in parentheses.

Conclusions

Internal dose of compounds in fuel is associated with reduced LH levels prior to ovulation in women of reproductive age. Several other caveats must be considered regarding interpretation of these results. The aliphatic and aromatic compounds we chose to represent exposure likely are also markers of exposure to the complex mixture of other compounds found in fuels, including additives and by-products 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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