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

Susan R. Reutman,1 Grace Kawas LeMasters,1 Edwin A. Knecht,2 Rakesh Shukla,1 James E. Lockey,1 G. Edward Burroughs,2 James S. Kesner2

1Department of Environmental Health, University of Cincinnati, Cincinnati, Ohio, USA; 2National Institute for Occupational Safety and Health, Cincinnati, Ohio, USA

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 (C₆H₁₄–C₄H₁₀) and the sum of aromatic HCs (benzene, ethylbenzene, toluene, and m,p-xylene). 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 (PDG).

Fuel and solvents may be encountered separately or as mixtures during jet 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 endpoints 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...
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 the laboratory by next-day courier. We stored the samples in home freezers; samples collected from 63 participants. To ascertain menstrual periods from participants’ daily records of vaginal bleeding we counted 1–2 day interruptions of 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 progesterone (Pd3G) and reduced levels of preovulatory luteinizing hormone (LH), midluteal Pd3G, and possibly midluteal estrone 3-glucuronide (E1G). Therefore, we selected these endpoints as a priori for analysis. We assayed the major urinary metabolites of estradiol and progesterone (i.e., E1G 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 E1G, 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,x-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 C6–C16, aromatic C6–C16, 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% 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 variable and as a dichotomous variable above and below the median parts per billion 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 (C12; $r^2 = 0.10$) and tetradeacne (C14; $r^2 = 0.55$). Therefore, we converted aliphatic HC levels (except C12 and C14) to parts per billion for the second analysis batch of 22 samples. We used only AUC measurements, not parts per billion, in the statistical analyses of aliphatic HCs for the 63 breath samples; we used the aliphatic HC parts per billion levels to present range of exposure. We dichotomized the total AUC for aliphatic HCs for each of the two analysis batches (above or below the
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 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 report either daily diaries and/or urine samples, and 13 women returned samples that were inadequate to evaluate the four key endocrine endpoints. 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

| Participant characteristic | Endocrine data (n = 100) | Endocrine and breath data (n = 63) |
|----------------------------|--------------------------|-----------------------------------|
| Age at menarche (years)    | 12.7 ± 1.5               | 12.8 ± 1.6                        |
| Midluteal LH (pg/mL)       | 1.8 ± 0.8                | 2.0 ± 0.8                         |
| FSH (mIU/mL)               | 1.2 ± 0.8                | 1.3 ± 0.8                         |
| Total body weight (kg)     | 69.1 ± 7.6               | 69.9 ± 7.7                        |
| Caffeine consumption (mg/d) | 127 ± 72                | 132 ± 72                         |
| Alcohol consumption (g/d)  | 21 ± 16                 | 22 ± 16                           |
| Smoking status             | Current smoker           | 34%                               |
| Marital status             | Married                  | 66%                               |
| Education level            | High school or/and tech training | 16%     |
| Income level               | < $15,000                | 91%                               |
| Occupation                 | Blue collar              | 84%                               |
| Employment status          | Full-time                | 79%                               |
| Employment status          | Part-time                | 68%                               |
| Employment status          | Self-employed            | 30%                               |
| Employment status          | Unemployed               | 12%                               |
| Employment status          | Retired                  | 11%                               |
| Employment status          | Other                    | 3%                                |
| Employment status          | No job                   | 4%                                |
| Employment status          | No employment            | 1%                                |
| Employment status          | Self-employed            | 90%                               |
| Employment status          | Unemployed               | 10%                               |
| Employment status          | Retired                  | 10%                               |
| Employment status          | Other                    | 10%                               |
| Employment status          | No job                   | 10%                               |
| Employment status          | No employment            | 10%                               |
| Employment status          | Self-employed            | 90%                               |
| Employment status          | Retired                  | 10%                               |
| Employment status          | Unemployed               | 10%                               |
| Employment status          | Other                    | 10%                               |
| Employment status          | No job                   | 10%                               |
| Employment status          | No employment            | 10%                               |
| Employment status          | Self-employed            | 90%                               |
| Employment status          | Retired                  | 10%                               |
| Employment status          | Unemployed               | 10%                               |
| Employment status          | Other                    | 10%                               |
| Employment status          | No job                   | 10%                               |
| Employment status          | No employment            | 10%                               |
| Employment status          | Self-employed            | 90%                               |
| Employment status          | Retired                  | 10%                               |
| Employment status          | Unemployed               | 10%                               |
| Employment status          | Other                    | 10%                               |
| Employment status          | No job                   | 10%                               |
| Employment status          | No employment            | 10%                               |
| Employment status          | Self-employed            | 90%                               |
| Employment status          | Retired                  | 10%                               |
| Employment status          | Unemployed               | 10%                               |
| Employment status          | Other                    | 10%                               |
| Employment status          | No job                   | 10%                               |
| Employment status          | No employment            | 10%                               |
| Employment status          | Self-employed            | 90%                               |
| Employment status          | Retired                  | 10%                               |
| Employment status          | Unemployed               | 10%                               |
| Employment status          | Other                    | 10%                               |
| Employment status          | No job                   | 10%                               |
| Employment status          | No employment            | 10%                               |
| Employment status          | Self-employed            | 90%                               |
| Employment status          | Retired                  | 10%                               |
| Employment status          | Unemployed               | 10%                               |
| Employment status          | Other                    | 10%                               |
| Employment status          | No job                   | 10%                               |
| Employment status          | No employment            | 10%                               |
| Employment status          | Self-employed            | 90%                               |
| Employment status          | Retired                  | 10%                               |
| Employment status          | Unemployed               | 10%                               |
| Employment status          | Other                    | 10%                               |
| Employment status          | No job                   | 10%                               |
| Employment status          | No employment            | 10%                               |
| Employment status          | Self-employed            | 90%                               |
| Employment status          | Retired                  | 10%                               |
| Employment status          | Unemployed               | 10%                               |
| Employment status          | Other                    | 10%                               |
| Employment status          | No job                   | 10%                               |
| Employment status          | No employment            | 10%                               |
| Employment status          | Self-employed            | 90%                               |
| Employment status          | Retired                  | 10%                               |
| Employment status          | Unemployed               | 10%                               |
| Employment status          | Other                    | 10%                               |
| Employment status          | No job                   | 10%                               |
| Employment status          | No employment            | 10%                               |
| Employment status          | Self-employed            | 90%                               |
| Employment status          | Retired                  | 10%                               |
| Employment status          | Unemployed               | 10%                               |
| Employment status          | Other                    | 10%                               |
| Employment status          | No job                   | 10%                               |
| Employment status          | No employment            | 10%                               |
| Employment status          | Self-employed            | 90%                               |
| Employment status          | Retired                  | 10%                               |
| Employment status          | Unemployed               | 10%                               |
| Employment status          | Other                    | 10%                               |
| Employment status          | No job                   | 10%                               |
| Employment status          | No employment            | 10%                               |
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 menstrues or dysmenorrhea within 3 months of the interview. Characteristics of the low- versus high-exposure groups for C6–C16 and for BTEX also were similar, and none of these differences was statistically significant.

**Internal dose (breath) analysis.** Tables 2 and 3 present individual C6–C16 and BTEX HCx 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 C6–C16 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 C6–C16 groups. Many samples also contained octane, nonane, and undecane in both high and low C6–C16 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 ≥ 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.

Table 4 presents mean urinary levels of these four end points by low and high breath levels of C6–C16 and BTEX, the primary exposure variables. Mean preovulatory LH levels were significantly lower in participants with high breath C6–C16 (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

| BTEX analyze | Low (n = 32) | High (n = 31) | Total (n = 63) |
|--------------|-------------|--------------|---------------|
| Benzene (C6H6) | 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 (C6H5CH3) | 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 | 32.5 | 71.4 |
| Ethylbenzene (C6H5C2H5) | 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 (C6H4(CH3)2) | 0.6 ± 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 (C6H4(CH3)2) | 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 (C6H6–C6H4(CH3)2) | 3.8 ± 3.8 (2.9) | 73.5 ± 86.2 (32.4) | 38.1 ± 69.6 (11.7) |
| Range | ND–11.7 | ND–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 (C6H12) | 17.6 ± 9.6 (19.6) | 51.4 ± 62.9 (25.9) | 34.5 ± 47.1 (25.9) |
| Mean ± SD (median) | ND–28.6 | 11.7–238.7 | ND–238.7 |
| Range | 90.9 | 100.0 | 95.4 |
| Percent of samples | Heptane (C7H16) | 3.7 ± 9.8 (0.3) | 35.4 ± 75.3 (4.4) | 19.5 ± 54.9 (0.6) |
| Mean ± SD (median) | ND–33.0 | ND–248.7 | ND–248.7 |
| Range | 63.6 | 63.6 | 63.6 |
| Percent of samples | Octane (C8H18) | 0.2 ± 0.4 (ND) | 0.6 ± 1.8 (ND) | 0.4 ± 1.3 (ND) |
| Mean ± SD (median) | ND–1.2 | ND–6.0 | ND–6.0 |
| Range | 18.2 | 27.3 | 22.7 |
| Percent of samples | Nonane (C9H18) | 5.3 ± 10.9 (ND) | 4.5 ± 9.1 (ND) | 4.9 ± 9.8 (ND) |
| Mean ± SD (median) | ND–29.2 | ND–27.0 | ND–29.2 |
| Range | 36.4 | 45.4 | 40.9 |
| Percent of samples | Decane (C10H22) | 34.6 ± 23.5 (28.4) | 159.1 ± 237.4 (14.8) | 96.9 ± 176.0 (23.9) |
| Mean ± SD (median) | 12.8–76.8 | 2.8–659.7 | 2.8–659.7 |
| Range | 100.0 | 100.0 | 100.0 |
| Percent of samples | Undecane (C11H22) | 8.8 ± 14.6 (ND) | 28.7 ± 69.9 (ND) | 18.7 ± 49.7 (ND) |
| Mean ± SD (median) | ND–45.0 | ND–226.7 | ND–226.7 |
| Range | 36.4 | 36.4 | 36.4 |
| Percent of samples | Total C6H16–C11H24 | 70.1 ± 31.3 (63.4) | 279.6 ± 276.2 (170.8) | 174.9 ± 219.8 (66.6) |
| Mean ± SD (median) | 28.6–129.0 | 39.5–765.1 | 28.6–765.1 |
| Range | 100.0 | 100.0 | 100.0 |

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 C6–C16 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. C6–C16 and BTEX categories were not significantly associated with changes in midluteal E13G, or with follicular or midluteal Pd3G levels.

Illness and/or fever > 101°F was associated with elevated midluteal E13G (β = 8.93; p = 0.007) among women with high aliphatic HC levels. Although follicular-phase Pd3G represents a series of contiguous days, the shaded region is nine. Values (means ± SE) are plotted relative to menses onset and the estimated day of ovulation (blue β = 0.01). Other potential covariates and confounders were not associated (p ≥ 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 ≤ 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 C6–C16 (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 E13G, 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 ≤ 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 concepitive 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

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  |

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 = 0.08) 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 (Andersen et al. 1980, 1981), which are involved in regulating pituitary hormone secretions. Andersen 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 toluene exposure (Langman 1994). The only human studies of toluene exposure, were of toluene exposure (Langman 1994). The only human studies of toluene exposure (Langman 1994). The only human studies of toluene exposure (Langman 1994). The only human studies of toluene exposure (Langman 1994). The only human studies of toluene exposure (Langman 1994). The only human studies of toluene exposure (Langman 1994). The only human studies of toluene exposure (Langman 1994). The only human studies of toluene exposure (Langman 1994). The only human studies of toluene exposure (Langman 1994). The only human studies of toluene exposure (Langman 1994).

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

| Endocrine outcomes | Model | Aliphatics | BTEX | Age | Race group | Illness/fever | Alcohol | Job strain |
|--------------------|-------|------------|------|-----|------------|--------------|---------|-----------|
| Preovulatory LH (n = 58) | \( f = 5.28 \) | \( \beta = -7.34 (2.60) \) | \( \beta = -4.61 (2.59) \) | \( p = 0.003 \) | \( p = 0.007 \) | \( p = 0.10 \) | \( p = 0.49 (0.24) \) | | |
| Follicular Pd3G (n = 62) | \( f = 0.46 \) | \( \beta = 0.04 (0.20) \) | \( \beta = -0.10 (0.20) \) | | | | | | |
| Midluteal Pd3G (n = 58) | \( f = 0.64 \) | \( \beta = 1.04 (1.79) \) | | | | | | | |
| Midluteal E13G (n = 58) | \( f = 0.20 \) | \( \beta = 2.79 (3.18) \) | | | | | | | |
| Midluteal E23G (n = 58) | \( f = 0.79 \) | \( \beta = 0.05 \) | \( p = 0.34 \) | \( p = 0.32 \) | \( p = 0.01 \) | |

\( \beta \)-values from untransformed models. \( \beta \)-values were generated from transformed models, and \( \beta \)-values from untransformed models. \( \beta \)-values from untransformed models, and \( \beta \)-values from untransformed models.
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.

References and Notes
Air Force Personnel Center, Randolph AFB. Personnel Statistics Website. Available: http://www.afpc.randolph.af.mil [cited 31 October 2000].
Andersson K, Fuxe K, Nilsson OG, Tofftärd R, Eneroth P, Gustafsson J-A. 1981. Production of discrete changes in dopamine and noradrenaline levels and turnover in various parts of the rat brain following exposure to xylene, ortho-, meta-, and para-xylene, and ethylbenzene. Toxicol Appl Pharmacol 66:535-548.
Andersson K, Fuxe K, Tofftärd R, Nilsson OG, Eneroth P, Gustafsson J-A. 1980. Toluene-induced activation of certain hypothalamic and median eminence catecholamine nerve terminal systems of the male rat and its effects on anterior pituitary hormone secretion. Toxicol Lett 5:393-398.
ATSDR. 1993. Toxicological Profile for Stoddard Solvent (Draft). Air Force Personnel Center, Randolph AFB. Personnel Statistics and thereby compromise reproduction.
Baird DD, Weinberg CR, Zhou H, Kamel F, McConnaughey ER, Horowitz M, Schaefer C, Hiroto D, Wilner N, Levin B. 1997. Life event questionnaires for measuring presumptive stress. Psychosom Med 59:413-431.
Ikeda M. 1992. Public health problems of organic solvents. Toxicol Lett 64/65(Spec No):191-201.

Articles • Endocrine effects in women with occupational hydrocarbon exposures